CAN EATING AND BODY ATTITUDES AFFECT PHYSIOLOGICAL HEALTH OUTCOMES IN PREMENOPAUSAL WOMEN? PROSPECTIVE 2-YEAR CHANGES IN BONE, AND RELATIONSHIPS WITH OVULATION, CORTISOL, AND BLOOD PRESSURE

by

Jennifer Lynn Bedford
BSNH, Acadia University, 2003

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
(Human Nutrition)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)
April 2010

© Jennifer Lynn Bedford, 2010
Cognitive dietary restraint (CDR) is the perception that one is limiting food intake in an effort to achieve/maintain a perceived ideal body weight. Cross-sectional studies suggest CDR is associated with an increased frequency of subclinical ovulatory disturbances (%SOD; anovulation and luteal phase <10 days long) and lower bone mass, possibly mediated by cortisol, a stress hormone. This research was conducted to prospectively examine relationships among CDR, %SOD, 24-hour urinary free cortisol (UFC) and 2-year areal bone mineral density change (ΔaBMD) in non-obese, regularly-menstruating women, aged 19-35. To monitor %SOD, least-squares quantitative basal temperature (LS-QBT) analysis was used. LS-QBT was first further validated against urinary pregnanediol glucuronide (PdG), an indirect indicator of ovulation (n=40, Chapter 2). Relative to PdG, LS-QBT showed excellent detection of ovulatory cycles (97%) but poor detection of anovulatory cycles (25%). Estimated day of luteal onset was correlated between methods (r=0.8, P<0.001). Chapter 3 presents prospective findings (n=123). Women with higher CDR had higher %SOD (56% versus 34%, P<0.001) and higher UFC (28.0 µg/day versus 24.0 µg/day, P=0.021). ΔaBMD did not differ by CDR level. Women with higher %SOD had less positive lumbar spine (L1-4; 0.7% versus 1.9%, P=0.034) and hip (-0.6% versus 0.9%, P=0.001) ΔaBMD, and higher CDR scores (8.7 versus 7.1, P=0.04). UFC was not associated with %SOD or ΔaBMD. Whether eating/body attitudes (EBA) were associated with 12-hour daytime ambulatory blood pressure (ABP) was explored as a secondary objective (n=120, Chapter 4). Women with negative EBA had higher diastolic ABP and mean arterial pressure, independently of weight loss effort. Finally, at baseline (n=137, Chapter 5), UFC was inversely associated with total body bone mineral content (BMC; r= -0.30, P<0.001) and aBMD (r= -0.27, P=0.003); L1-4 aBMD (r= -0.19, P=0.035) and BMC (r= -0.18, P=0.049); and hip BMC (r= -0.23, P=0.011), after adjustment for potential confounders. In summary, findings suggest CDR and other negative EBA may be associated with adverse health outcomes including higher ABP and %SOD. Furthermore, more frequent SOD, which are not apparent to women, were associated with less positive ΔaBMD. However, cortisol may not be the only or most important mediator of these relationships.
# TABLE OF CONTENTS

**ABSTRACT** ........................................................................................................................................ii

**TABLE OF CONTENTS** ..................................................................................................................iii

**LIST OF TABLES** ............................................................................................................................ix

**LIST OF FIGURES** ............................................................................................................................xi

**LIST OF ABBREVIATIONS** .................................................................................................................xii

**PREFACE** ..........................................................................................................................................xiv

**ACKNOWLEDGEMENTS** .....................................................................................................................xv

**CO-AUTHORSHIP STATEMENT** .......................................................................................................xvii

**Chapter 1: Introduction** ...................................................................................................................1

1.1 Background and rationale ....................................................................................................................2

1.2 Literature review ..................................................................................................................................5

1.2.1 Introduction .....................................................................................................................................5

1.2.2 Cognitive dietary restraint ..............................................................................................................5

1.2.2.1 Background and assessment .....................................................................................................5

1.2.2.2 Behavioural versus perceptual aspects of CDR .........................................................................8

1.2.3 Stress, cortisol and CDR .................................................................................................................10

1.2.3.1 Chronic stress and neuroendocrine function ..............................................................................10

1.2.3.2 Assessment of cortisol and general stress perception ....................................................................11

1.2.3.3 Cortisol and CDR .......................................................................................................................13

1.2.3.4 Perception of psychosocial stress and CDR ................................................................................15

1.2.4 Ovulatory function ..........................................................................................................................15

1.2.4.1 Physiology of the menstrual cycle ...............................................................................................15

1.2.4.2 Disturbances in ovulatory function .............................................................................................16

1.2.4.3 Monitoring ovulatory function .....................................................................................................16

1.2.4.4 Ovulatory function and the HPA axis .........................................................................................18

1.2.4.5 Ovulatory disturbances and CDR ...............................................................................................18

1.2.5 Bone and cortisol .............................................................................................................................21

1.2.6 Bone and ovulatory function .........................................................................................................24

1.2.7 Bone and CDR ...............................................................................................................................26

1.3 Gaps in our current understanding .....................................................................................................28

1.4 Study purpose ....................................................................................................................................29

1.4.1 Objectives .......................................................................................................................................30
Chapter 4: Negative eating and body attitudes are associated with higher daytime ambulatory blood pressure in healthy young women

4.1 Introduction

4.2 Methods

4.2.1 Participants

4.2.2 Procedure

4.2.3 Questionnaires

4.2.3.1 Eating and body attitudes

4.2.3.2 General stress

4.2.3.3 Weight loss effort

4.2.4 Urine analysis

4.2.5 ABP measurement

4.2.6 Statistical analyses

4.3 Results

4.3.1 Participant characteristics

4.3.2 Correlation analyses

4.3.3 Differences by Eating/Body Attitudes and weight loss effort.

4.4 Discussion

4.5 References

Chapter 5: The relationship between 24-hour urinary cortisol and bone in healthy young women

5.1 Introduction

5.2 Methods

5.2.1 Participants

5.2.2 Procedure

5.2.3 Questionnaires

5.2.4 Urine analysis

5.2.5 ABP measurement

5.2.6 Statistical analyses

5.3 Results

5.3.1 Participant characteristics

5.3.2 Correlation analyses

5.3.3 Differences by Eating/Body Attitudes and weight loss effort.

5.4 Discussion

5.5 References
5.1 Introduction ............................................................................................................ 109
5.2 Methods ................................................................................................................ 109
  5.2.1 Participants ....................................................................................................... 109
  5.2.2 Questionnaires ................................................................................................. 110
  5.2.3 Dietary intake .................................................................................................... 111
  5.2.4 Urine collection and analysis .............................................................................. 111
  5.2.5 Anthropometrics and body composition ............................................................... 111
  5.2.6 Statistical analyses ........................................................................................... 112
5.3 Results ................................................................................................................... 112
  5.3.1 Participant characteristics .................................................................................. 112
  5.3.2 Associations with aBMD, BMC and bone area .................................................... 114
  5.3.3 Associations with 24-hour urinary free cortisol .................................................... 114
  5.3.4 Associations with PSS score .............................................................................. 115
  5.3.5 Associations between 24-hour urinary free cortisol and aBMD, BMC and bone area ......................................................................................................................... 115
5.4 Discussion .............................................................................................................. 117
5.5 References .............................................................................................................. 121

Chapter 6: Conclusion ................................................................................................. 126
  6.1 General conclusion ............................................................................................... 127
  6.2 General discussion ............................................................................................... 130
  6.3 Strengths and limitations ..................................................................................... 137
  6.4 Future directions .................................................................................................. 142
  6.5 References .......................................................................................................... 145

Appendix 1: Recruitment Materials for Quantitative Basal Temperature (QBT) Validation Study ......................................................................................................................... 151
Appendix 2: QBT Validation Study Letter of Initial Contact (via email) ......................... 153
Appendix 3: QBT Validation Study Eligibility Phone Script ........................................... 155
Appendix 4: QBT Validation Study Questionnaires ....................................................... 158
Appendix 5: QBT Validation Study Temperature Calendar ........................................... 162
Appendix 6: QBT Validation Study Instructions for Daily Urine Sample Collection ...... 164
Appendix 7: QBT Validation Study Ethics Approval Certificate ..................................... 169
Appendix 8: QBT Validation Study Letter of Consent .................................................. 170
Appendix 9: QBT Validation Study Transportation Reimbursement Receipt .................. 174
Appendix 10: QBT Validation Study Gift Card Receipt.................................175
Appendix 11: QBT Validation Study Individual Results .................................176
Appendix 12: Sensitivity and specificity of least-squares quantitative basal
temperature analysis (LS-QBT) methods in determining luteal
phase length (LPL) relative to Kassam’s urinary pregnanediol
glucuronide (PdG) algorithm (n=35) .............................................................181
Appendix 13: Recruitment Materials for 2-year Prospective Bone Study ........183
Appendix 14: 2-year Prospective Bone Study Letter of Initial Contact (via email) ........185
Appendix 15: 2-year Prospective Bone Study Eligibility Phone Script ..............188
Appendix 16: 2-year Prospective Bone Study Letter of Consent ....................192
Appendix 17: 2-year Prospective Bone Study Ethics Approval Certificate ..........197
Appendix 18: 2-year Prospective Bone Study Transportation Reimbursement
Receipt ...........................................................................................................198
Appendix 19: 2-year Prospective Bone Study Gift Card Receipt .....................199
Appendix 20: 2-year Prospective Bone Study 24-hour Urine Collection
Instructions .................................................................................................200
Appendix 21: 2-year Prospective Bone Study Temperature Calendar ..............202
Appendix 22: 2-year Prospective Bone Study Bone Density Scan Instructions ....204
Appendix 23: 2-year Prospective Bone Study Annual Questionnaire Package ......206
Appendix 24: List of Validated Questionnaires Included in Annual Questionnaire
Package .......................................................................................................236
Appendix 25: 2-year Prospective Bone Study Daily Stress Inventory ..............237
Appendix 26: 2-year Prospective Bone Study 12-hour Ambulatory Blood
Pressure Monitoring Instructions and Diary .............................................240
Appendix 27: Correlations of Cognitive Dietary Restraint and Subclinical
Ovarian Disturbances with General Stress Questionnaires .....................243
Appendix 28: Comparison of Least-squares Basal Temperature Analysis Method
Relative to Other Non-invasive Methods to Detect Ovulation
Regarding Cost, Participant Acceptability, Ease-of-use and
Accuracy in Detecting the Day of Luteal Onset .......................................245
Appendix 29: Partial Correlations of 24-hour Urinary Free Cortisol (UFC) and
Average Perceived Stress Scale (PSS) Scores with Questionnaire
Scores .......................................................................................................251
Appendix 30: Cognitive Dietary Restraint Score, General Stress Score, Subclinical Ovulatory Disturbances, 24-hour Urinary Free Cortisol and 2-year ΔaBMD by Ethnicity (n=123) ..............................................................254

Appendix 31: Cross-sectional Examination of Differences in 24-hour Urinary Free Cortisol by Ethnicity and Level of Cognitive Dietary Restraint (CDR) and the Ethnicity-by-CDR Interaction ..................................................255

Appendix 32: Pearson’s Partial Correlations of 12-hour Average Daytime Ambulatory Blood Pressure (ABP, mm Hg) and Eating and Body Attitude Questionnaire Scores At First Follow-up (n=120) ........................................256

Appendix 33: Email Correspondence with Participants of the 2-year Prospective Bone Study ..................................................................................................................................257

Appendix 32: 2-year Prospective Bone Study Temperature Calendar Individual Results .....................................................................................................................................266

Appendix 35: 2-year Prospective Bone Study Ambulatory Blood Pressure Individual Results ...........................................................................................................................................268

Appendix 36: 2-year Prospective Bone Study Letter Accompanying Bone Density Results .............................................................................................................................................270

Appendix 37: Pearson’s Correlations Between the Duration of Hormone Use and 2-year ΔaBMD (n=123) .......................................................................................................................................274

Appendix 38: 24-hour Urinary Free Cortisol at Baseline and Follow-ups and Level of Significant Difference Between Values by Repeated Measures General Linear Model (n=116) ......................................................................................................275
LIST OF TABLES

Table 2.1  Descriptive characteristics of the sample (n=40) ................................................................. 54

Table 2.2  Sensitivity and specificity of least-squares quantitative basal temperature analysis (LS-QBT) methods in determining evidence of luteal activity (ELA) relative to Kassam’s urinary pregnanediol glucuronide (PdG) algorithm ......................................................................................................................... 55

Table 2.3  Predictive value and accuracy of least-squares quantitative basal temperature analysis (LS-QBT) methods in determining evidence of luteal activity relative to Kassam’s urinary pregnanediol glucuronide (PdG) algorithm ......................................................................................................................... 55

Table 3.1  Mean questionnaire scores and energy intakes, and partial correlation coefficients of the Three Factor Eating Questionnaire subscales and 24-hour urinary free cortisol in healthy premenopausal women (n=123) ................................................................. 72

Table 3.2  Physical measurements at baseline, 2-year follow-up and the 2-year percent change in healthy premenopausal women (n=123) .......................................................................................................................... 74

Table 3.3  Differences between healthy premenopausal women with higher and lower cognitive dietary restraint (by median split) in baseline anthropometrics, Δanthropometrics questionnaire scores, energy intakes, menstrual cycle characteristics, 24-hour urinary free cortisol and 2-year ΔaBMD (n=123) .......................................................................................................................... 75

Table 3.4  Differences between healthy premenopausal women with higher and lower percentage of cycles with subclinical ovulatory disturbances (median split) in menstrual cycle characteristics, age, anthropometrics, Δanthropometrics, questionnaire scores, 24-hour urinary free cortisol and 2-year ΔaBMD (n=114) .......................................................................................................................... 78

Table 4.1  Mean age, body mass index, questionnaire scores, energy intakes, 24-h urinary free cortisol (UFC) and 12-h daytime ambulatory blood pressure; and adjusted correlates of Eating/Body Attitude Z-score, General Stress Z-score, and UFC in healthy premenopausal women (n=120) .......................................................................................................................... 96

Table 4.2  Main and interactive effect of Eating/Body Attitude level and weight loss effort on age, body mass index, questionnaire scores, energy intakes, 24-h urinary free cortisol, and 12-h daytime ambulatory blood pressure (n=120) ......................................................................................................................... 98

Table 5.1  Physical activity and questionnaire scores, reported nutrient intakes, 24-hour urinary free cortisol excretion, anthropometrics and DXA measurements for all participants and differences by ethnicity ................................................................. 113

Table 5.2  Correlations of aBMD, BMC and bone area with anthropometrics, perceived stress, physical activity, duration of previous oral contraceptive use, calcium/kcal intake, 24-hour urinary free cortisol excretion and 24-hour urine volume ......................................................................................................................................................... 116

Table 5.3  Partial correlation models of the relationship between aBMD, BMC and bone area and 24-hour urinary free cortisol excretion ......................................................................................................................................................... 117
Table 6.1  Summary of results with regard to specific hypotheses .................................127
LIST OF FIGURES

Figure 1.1  Hypothesis guiding this PhD research programme juxtaposition with the physiological stress response ................................................................. 4

Figure 2.1  Correlation of the day of LS-QBT temperature rise versus day of sustained PdG rise by Kassam algorithm: All temperatures................................. 56

Figure 2.2  Correlation of the day of LS-QBT temperature rise versus day of sustained PdG rise by Kassam algorithm: Royston wake-time adjusted............. 57

Figure 2.3  Correlation of the day of LS-QBT temperature rise versus day of sustained PdG rise by Kassam algorithm: 2-hour average wake time temperatures ........................................................................................................... 57

Figure 2.4  Correlation of the day of LS-QBT temperature rise versus day of sustained PdG rise by Kassam algorithm: Expert reviewed temperatures .......... 58

Figure 3.1  Model driving our hypothesis of cognitive dietary restraint and bone density juxtaposition with the physiological stress response ......................... 66

Figure 3.2  Flow diagram depicting study recruitment, participation and data collection at baseline and first and final follow-up assessments ................................. 67
LIST OF ABBREVIATIONS

aBMD: areal bone mineral density (g/cm$^2$)
ACTH: adrenocorticotropic hormone
ABP: 12-hour daytime mean ambulatory blood pressure (mm Hg)
ABP-activity: continuous score for concurrent activity during ABP (sum of diary codes for each reading ABP [0=sedentary, 1=active] divided by total number of readings per participant)
BMC: bone mineral content (g)
BMD: bone mineral density (g/cm$^2$)
BMI: body mass index (kg/m$^2$)
BP: blood pressure
BUA: broadband ultrasonic attenuation
CDR: cognitive dietary restraint (equivalent to dietary restraint)
CRH: corticotropin releasing hormone
CVD: cardiovascular disease
DEBQ: Dutch Eating Behaviour Questionnaire
DEBQ-R: DEBQ Restraint subscale
DHQ: Diet History Questionnaire
DLT: day of luteal transition
DSI: Daily Stress Inventory
DXA: dual energy X-ray absorptiometry
EBA: eating and body attitudes
EDE: Eating Disorder Exam
EDI: Eating Disorder Inventory-2
ELA: evidence of luteal activity
FFQ: Food Frequency Questionnaire
FHA: functional hypothalamic amenorrhea
FSH: follicle stimulating hormone
GLM: General Linear Model
GnRH: gonadotropin-releasing hormone
HPA: hypothalamic-pituitary-adrenal
HPG: hypothalamic-pituitary-gonadal
kcal: kilocalorie
L1-4: lumbar spine vertebrae 1 through 4, inclusive
LH: luteinizing hormone
LPL: luteal phase length
LS-QBT: least-squares method of quantitative of basal temperature analysis
mm Hg: millimetres of mercury
n: number of participants
NPV: negative predictive value
p: page
P: probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true
PdG: pregnanediol glucuronide
PPV: positive predictive value
PSS: Perceived Stress Scale
QBT: quantitative methods of basal temperature analysis
QCT: quantitative computed tomography
r: Pearson’s correlation coefficient
REE: resting energy expenditure
RS: Restraint Scale
SD: standard deviation
SOD: subclinical ovulatory disturbances
**SOS**: speed of sound  
**SPSS**: Statistical Package for the Social Sciences  
**TFEQ**: Three Factor Eating Questionnaire or Eating Inventory  
**TFEQ-R**: TFEQ Restraint subscale (questionnaire to assess CDR)  
**UFC**: 24-hour urinary free cortisol (µg/day)  
**VGH**: Vancouver General Hospital  
**Z-score**: how many standard deviation units away from the mean a particular value of data lies
PREFACE

I prepared this dissertation according to the University of British Columbia Faculty of Graduate Studies requirements for a manuscript-based thesis. Therefore, Chapters 2 to 5 are elaborated versions of manuscripts that have been published, accepted for publication or submitted for publication to scientific journals. Although some overlap may occur, each chapter is designed to stand alone, and these chapters can be read in any order. Chapter 2 includes a validation study that was conducted prior to commencement of the main study. The findings of the main research question (two year prospective data) are presented in Chapter 3. Chapters 4 and 5 include data collected at the first follow-up and baseline, respectively, as secondary objectives. Additional analyses that were not included in the manuscripts due to space constraints are presented as appendices.
ACKNOWLEDGEMENTS

I could not have completed this research project and the resulting manuscripts and dissertation without the support and hard work of many others. I would like to take this time to formally acknowledge their contributions to the project and my personal experience.

First and foremost, I would like to thank my supervisor, Dr. Susan Barr. Susan, I have no words to adequately express what your unwavering support, guidance and dedication to your students and integrity as a scientist have meant to my professional development. I am the scientist that stands before you today because of you. Your humour, kindness and friendship have made the past six years an enjoyable experience. Beyond the work, your generosity and genuine concern about me as a person has touched me to the very depths of my soul. Thank you Susan. I look forward to a continued friendship and future collaborations.

Completion of this project would not have been possible without the 140 women who earnestly contributed their time and effort to my study. It was a pleasure to meet each of you and to be a part of your lives for those two years. Thank you to the Canadian Institute of Health Research who provided both the project operating grant and supported me personally for three years. Thanks also to funders of my other scholarships including the Michael Smith Foundation for Health Research, the National Science and Engineering Research Council, and the University of British Columbia particularly the Faculty of Land and Food Systems.

I am appreciative of the contributions of my supervisory committee members Drs. Jerilynn Prior, Wolfgang Linden and Kathy Keiver. Thank you for providing me the opportunity to learn a small piece of your area of expertise under your guidance and support. As well, thank you to Dr. Christine Hitchcock for your assistance with the Maximina program and the QBT validation paper. You have helped improve my precision while writing and have taught me to think critically about what I say and what I mean to say.

To my mentors Oonagh Holmes, Dr. Shanthi Johnson, Dr. Elizabeth Johnston and Debbie Zibrik. Your guidance, encouragement and support have helped steer me to where I am today. Thank you for sharing your experiences with me.

Thank you to the wonderful women (and their staff) that conducted the various analyses for the project including Ellie Brindle, Romi Chan, Darlene Christopher and Nazneen. Also thanks to the staff and faculty of the Food, Nutrition and Health department particularly Tram Nguyen, Patrick Leung and Karol Traviss.

My deepest gratitude to my research assistant Amandeep Ghuman for her hard work and dedication to the project. Anu, only you could make inputting over 1500 temperature calendars fun. Beyond the project, thank you for being such a sincere friend and for adopting me into your wonderful family.

From the bottom of heart, I thank my extended family including all my Aunts, Uncles and cousins. Your encouragement and love have made it possible for me to get through the past decade of university. Thank you Gramma for all of your cross-country visits and hand written letters. I am so grateful that you taught me to knit and bake as it has allowed me to be more balanced during this process. Deepest thanks to my Aunt Carrol and Uncle Jeff for allowing me to stay with you in Arizona and giving me the space to breathe – it saved my life. Thank you to my Uncle Phil for pointing me in the right direction and for your reassuring emails over the years. It was always nice to hear from someone that truly understands the academic struggle. Uncle David and Michelle, thank you for making the transition to BC easier and for welcoming me and Andrew into your family for those few years. To my friends that are like family, Jessica
Kelly and Brian Milroy. Thank you for your friendship regardless of the distance between us over the past decade.

And last, but certainly not least, my immediate family.

Andrew Nichols, I could not have made it through a single day of this process without you beside me. Thank you for reminding me to breathe; for keeping me warm; for rubbing my back, massaging my head and wiping my tears. Thank you for loving me. Thank you for tolerating more mood swings than I’m sure you ever thought were humanly possible. Thank you for moving from Nova Scotia to British Columbia so that I could achieve my goals. Thank you for believing in me: my potential and my abilities. Thank you for picking me up from the floor when I could no longer go on and for carrying me through. This degree and dissertation were truly a teamwork effort – I hope that you are proud of our accomplishments. I promise to be saner now that this is finished. I look forward to new adventures together.

Melissa, my sister and best friend. Thank you for allowing me to be who I am, and for understanding that like no one else. Thank you for looking up to me when we were kids-knowing that you were watching my every act, made me want to be the best I could. I am sorry it took me so many years (and fights!) to realize it. Thank you for being silly with me, for truly listening and caring, and for taking on ‘the big sister’ responsibilities at home when I was unable. Most of all, thank you for moving to Vancouver—it has changed my life. To be able to do all those small sister things so easily is truly the best gift I have ever received.

To my Dad. Thank you for always encouraging my child-like enthusiasm, for wanting me to be happy, for giving me the space to be a silly-carefree-redneck, for bragging about me to near strangers, and for teaching me things beyond the textbook. Your enthusiasm and hard work on my early science projects sparked my passion for research! Thank you for working over-time in the heat and the cold so that I could spend a decade in school without having to eat Kraft Dinner once. Thank you for protecting me. Even though I am Dr. Bedford now, I will always be your little girl.

And finally, to my Mum, for making all of this possible. Every paper I have published, every scholarship I received, every ‘A’ on my transcript is because of YOU. Because you told me it was better to be smart than pretty when the kids teased me for being a ‘browner’; because you made up tests for me to complete during the summer when I was little; because you read to me, bought me thousands of books (Baby Sitters Club!) and allowed me to become lost in the stories; because you helped me study for high school tests when you got home from work at 9 o’clock at night; because you were willing to proof-read everything I ever wrote (including this entire dissertation!); because you drove me everywhere, signed me up for everything and opened every door you could for me; because you taught me how to work hard, be a kind person, manage my time and stay focused. I had the courage and strength to finish this because you encouraged me and you believed in me when I could no longer believe in myself. You are my one and only bosom friend—a true kindred spirit. Although it is far from adequate, I dedicate this dissertation to you with all my love.
CO-AUTHORSHIP STATEMENT

Chapters 2 through 5 are manuscripts that have been published (Chapter 2), are accepted for publication (Chapter 5) or are currently under review (Chapters 3 and 4). For each manuscript, I identified the research question, conceived of the study design, recruited all participants, completed all data collection and management, planned and conducted the data analyses, presented the findings and wrote and edited the manuscript. My co-authors made significant contributions as follows. Dr Susan Barr, my research supervisor, was the Principal Investigator for the Canadian Institutes of Health Research Operating Grant that funded this work. Committee members Drs Linden and Prior were co-applicants. Drs Barr, Keiver (additional committee member), Linden and Prior contributed to study design and implementation. Dr Linden stimulated discussion of results and provided editorial input for Chapter 4. Dr Prior stimulated discussion of results and provided editorial input for Chapters 2 and 3. For Chapter 2, Dr Christine Hitchcock stimulated discussion of results and provided editorial input. For each manuscript, Dr Susan Barr contributed continuously to data collection, management and analysis, results discussion and key editorial input.
Chapter 1:

Introduction
1.1 Background and rationale

Thinness has become a well established cultural norm for women in North America [1-3]. In response to societal pressure to be thin, many women experience body image dissatisfaction [1-3]. This may lead to unhealthy behaviours such as extreme dieting and exercising [e.g. 4-9] and, in a small number, clinical eating disorders such as anorexia and bulimia [10]. For many women, behaviours do not necessarily change, but rather a disordered relationship and preoccupation with food and weight may develop. One such attitude that has emerged as being experienced by many young women [11-12] is cognitive dietary restraint. Cognitive dietary restraint (CDR) is the perception that one is limiting food intake in an effort to achieve or maintain a perceived ideal body weight [13]. Evidence suggests that CDR is perceptual in nature, reflecting habitual monitoring of food intake and body weight preoccupation, rather than a behaviour such as dieting where food intake is limited in an effort to reduce weight. For example, several studies report no difference between women with higher and lower CDR in energy intakes, relative body mass or weight change over time [12,14-15].

The experience of CDR may negatively influence young women’s physiological health, possibly mediated by the stress response. With the experience of any psychosocial stress, the hypothalamic-pituitary-adrenal (HPA) axis is activated initiating a sequence of events that results in increased production of the stress hormone cortisol [16]. We and others have hypothesised that the habitual monitoring and preoccupation with food and body weight experienced by women with higher levels of CDR may act as a subtle but chronic stressor that is sufficient to activate the HPA axis. Indeed elevated cortisol and higher CDR are associated in some studies [17-21]. Cortisol at high levels has direct negative affects on bone density by disturbing bone turnover and calcium balance [22]. Indirectly, cortisol may adversely influence bone by disrupting the normal cyclic secretion of the reproductive hormones [23]. Furthermore, elevations in cortisol are also associated with higher blood pressure and greater accumulation of abdominal fat [24]. Whether modestly elevated, yet physiologically normal cortisol levels, such as those occurring as a result of psychosocial stressors are capable of affecting health outcomes is not yet known.

There is some evidence to suggest the possibility. First, an inverse relationship between cortisol and bone density has been observed among healthy older adults [25-28]. Furthermore, higher cortisol levels have been shown to be associated with lower bone density among clinical samples of young women with eating disorders and major depression [29-35], though not consistently [36-37]. Secondly, various life stresses are associated with infertility and evidence suggests it may be related to the physiological stress response [38]. Correspondingly, women with higher levels of CDR are more likely to report menstrual cycle irregularities [12,39-40] and to experience subclinical disturbances in ovulatory function [41-43]. Subclinical ovulatory
disturbances (anovulatory cycles and/or cycles with short luteal phase duration) are not apparent to women yet indicate deficiencies or imbalances of the reproductive hormones. In addition to fertility, the reproductive hormones, estradiol and progesterone, are crucial to achieving and maintaining peak bone mass in premenopausal women [44]. There is some evidence to suggest that women who experience more frequent subclinical ovulatory disturbances have reduced bone density [45-49]. However, these findings are not conclusive as others have reported no associations among subclinical ovulatory disturbances and bone [50-51]. Nevertheless, more frequent disturbances in menstrual cycle and ovulatory function represent an additional mechanism by which CDR may negatively affect young women’s health. In fact, a direct relationship between higher CDR and reduced bone mineral content and/or bone density has been reported in some [40,49,52-54] but not all studies [41,55-56]. At the time the research described herein was proposed, no study had examined these relationships prospectively, and to date, only one prospective study has been published [49]. The cross-sectional studies [12,17,40,43,48-50,52-56] are limited by insufficient power to detect differences in bone density due to small sample sizes and the considerable inter-individual variability in both bone density and menstrual cycle and ovulatory characteristics.

In summary, several cross-sectional studies suggest that women with higher CDR are more likely to experience menstrual cycle and ovulatory disturbances and to have higher levels of cortisol than women with lower CDR. In turn, disturbances in menstrual cycle and ovulatory function and elevated cortisol have the potential to negatively impact bone density. To date, there is only one study that has prospectively examined associations among CDR, subclinical ovulatory disturbances and bone and no one study has prospectively examined these relationships in conjunction with assessment of cortisol.

Increasingly the role of psychosocial characteristics in the development of chronic disease has been recognised; however, the majority of research to date has focused on the health outcomes of middle-aged men [57]. The potential association between CDR-related stress and bone is relevant in regard to future risk of osteoporosis. This condition, characterised by low bone mass and increased bone fragility, is experienced by one in four postmenopausal women in Canada [58]. If fractures occur, osteoporosis is associated with reduced quality of life and considerably increased health care expenditures [58]. A key factor in osteoporosis prevention is thought to be achieving and maintaining peak bone mass during younger years [59]. It is therefore critical that we have a comprehensive understanding of the factors that influence young women’s bone health.

Thus, the primary objective of this PhD research project, as depicted in Figure 1.1, was to prospectively investigate potential relationships among CDR, cortisol, subclinical ovulatory disturbances, and change in bone density in healthy premenopausal women over two years. In
order to conduct this research, a method of monitoring ovulatory function that was inexpensive, accurate and acceptable to women was required. Therefore, a validation study was conducted prior to commencement of the main study to do this. Additionally, associations among eating- and body attitudes, cortisol and blood pressure were explored as secondary objectives. In order to place the current study hypotheses and objectives in context of the current state of knowledge, a review of the literature will be presented that will focus on the primary purpose. For the secondary objectives, the relevant literature will be described in the introduction and discussion sections of the corresponding manuscript.

**Figure 1.1** Hypothesis guiding this PhD research programme juxtaposition with the physiological stress response

Variables shown as ovals were assessed in my PhD study. The dashed line (1) between CDR and chronic psychosocial stress reflects the hypothesis that CDR acts as a subtle chronic stressor capable of activating the HPA axis. Solid black lines indicate well established mechanisms of the stress response including: (2) increased secretion of cortisol, which has a direct negative effect on bone density, and (3) inhibition of the hypothalamic-pituitary-gonadal (HPG) axis resulting in deficiencies or imbalances of the reproductive hormones and therefore menstrual cycle and ovulatory disturbances. Grey lines represent hypothesised but inconclusive relationships previously reported including: (4) the possibility that subclinical ovulatory disturbances can have detrimental effects on bone density; (5) an association between higher CDR and elevated cortisol; and (6) an association between higher CDR and the occurrence of menstrual cycle and ovulatory disturbances. This leads to the primary hypothesis guiding this project (grey dashed line (7)) that CDR may result in less positive changes in bone density. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; E, estradiol; FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone; P, progesterone.
1.2 Literature review

1.2.1 Introduction

Several areas of literature will be reviewed in order to place my PhD research project in the context of current knowledge. Gaps in our current understanding of how women’s eating attitudes may affect physiological health will also be highlighted. First, cognitive dietary restraint (CDR) will be discussed, including the history and assessment of CDR. The relationship between CDR and dieting will also be addressed in order to support the hypothesis that it is the experience of dietary restraint (rather than only behavioural changes) that is associated with negative health outcomes. Broadly, my research was designed to examine whether CDR is associated with cortisol and subclinical ovulatory disturbances, and the association of each of these variables with change in bone density. Therefore, neuroendocrine function will be discussed focusing on the HPA axis, cortisol and psychosocial stress, including the operationalisation of cortisol and perceived stress. The potential relationship between cortisol and CDR will be reviewed. Next, the physiology of the menstrual cycle will be reviewed including disturbances in normal function, monitoring of ovulatory function, the relationship between ovulatory function and the HPA axis and cortisol, and the relationship between CDR and ovulatory function. Lastly, factors affecting bone, specifically CDR, cortisol, and subclinical ovulatory disturbances, will be reviewed. This chapter will conclude by highlighting the gaps in our current understanding of the problem, and the purpose, hypotheses and objectives of this project.

1.2.2 Cognitive dietary restraint

1.2.2.1 Background and assessment

Eating behaviour is the result of internalised multidimensional constructs that include behavioural, cognitive and affective elements [60]. The theory of dietary restraint attempts to synthesize these elements in order to understand and assess the complex picture of eating behaviour [61]. Over the past three decades the concept of CDR, its meaning, and our understanding of its potential relationship with physiological health outcomes have evolved from their original presentation. Currently in the literature, there are three unique operational definitions of dietary restraint each with a questionnaire-based assessment tool [13,62-63]. For the purposes of this research project, CDR will be defined as an attitude towards eating and food and a preoccupation with body size, shape and weight, that may or may not result in abnormal eating behaviours [64]. Women with high levels of CDR perceive that they are attempting to limit their food intake in order to achieve or maintain their ideal body weight [13], allowing cognitive processes rather than physiological systems, such as hunger and satiety, to govern eating behaviour [65]. A brief review of the history of this construct is essential to
understanding current operational definitions, the definition and assessment tool chosen as appropriate for my PhD research, and how this work will contribute to the current body of knowledge as to whether the experience of CDR affects women’s physiological health.

The theory of restrained eating originally developed from studies by Schachter [66-67] and Nisbett [68] purporting to describe and explain differences in the eating behaviours of obese and normal-weight persons. Schachter and colleagues performed a series of experiments in which they found that obese individuals were more likely to respond to external than internal food cues compared to normal-weight persons [66-67,69]. When viewed from the current CDR framework, Schachter’s work demonstrates disinhibition, a tendency to overeat when restraint is removed. Nisbett proposed the set point theory as an alternative explanation [68]. He hypothesised that the number of fat cells in the body is a physiological set point that the body will defend and when individuals fall below this weight, they will be more responsive to external cues [68].

The set point theory was extended to normal-weight persons by Herman and coworkers [62,70] who suggested that a subgroup of normal-weight individuals may have obese set points but restrain their eating to maintain a lower weight. In one of their experiments normal-weight and obese college-aged women completed a questionnaire to measure restraint and were randomly assigned to receive zero, one or two servings of a milkshake preload [70]. As the size of the milkshake preload increased, low restraint eaters consumed less ice cream, while those with high restraint consumed larger quantities of ice cream as the size of the preload increased [70]. Termed “counter-regulation”, it was suggested that external cues trigger additional eating when restraint is removed [70]. Subsequent studies confirmed that anxiety [62], depression [71] and alcohol [72-73] could also cause counter-regulation or disinhibition among restrained eaters.

From this work, the 10-item Restraint Scale (RS) was developed consisting of two subscales, Weight Fluctuations and Concern for Dieting [62,70,74]. The RS was the most widely used psychometric tool to operationalise dietary restraint [75]. The current version of the RS has moderate internal consistency with Cronbach alpha scores ≥0.75 [75]. However, the predictive and construct validity of the RS was questioned following repeated observation of an association between RS score and severity of overweight [76-79]. As well, evidence suggested that the factorial composition of the RS differed between obese and normal-weight populations [60]. Subsequently, psychometric studies found that the RS was strongly correlated with weight fluctuation [76,80-83], which may have more to do with obesity than restraint. The RS was also correlated with scores of social desirability scales among obese but not normal-weight individuals [81,84].
Over time, it was also observed that not all individuals with high dietary restraint exhibit disinhibition and these constructs are confounded within the RS [85]. Therefore, to truly understand eating behaviour, disinhibition would need to be assessed separately. Stunkard and Messick [13] developed a series of questions based on the RS [62], Pudel’s Latent Obesity Questionnaire [86] and clinical experience resulting in the 51-item Three Factor Eating Questionnaire (TFEQ) or Eating Inventory. The TFEQ assesses three unique aspects of eating attitudes that may influence eating behaviour: Restraint, which indicates the level of cognitive control of eating behaviour (TFEQ-R); Disinhibition, which assesses the tendency to overeat when restraint is removed; and Hunger, which measures the susceptibility to hunger and food cravings. Since then, many studies support the separation of disinhibition and restraint [60, 86-90]. Around the same time, the 33-item Dutch Eating Behaviour Questionnaire (DEBQ) was developed consisting of three similar subscales: Restraint, External Eating and Emotional Eating [63]. The purpose of the 10-item Restraint subscale (DEBQ-R) is to describe intentions to restrict food intake for weight reasons [63]. The DEBQ-R (Cronbach alpha score ≥0.9) [64] and TFEQ-R (Cronbach alpha score 0.79 to 0.93) [64] have good internal consistency and test-retest reliability. The TFEQ-R however is more widely used to assess restraint in the literature. As well, the validity and reliability of the TFEQ is well established [13,64,75,91].

Despite the good psychometric properties of the TFEQ-R, its factor structure has been investigated [75,92]. Westenhoefer [92] suggested that while restraint was necessary for disinhibition, it did not always result in disturbed eating behaviours as some women with higher restraint do not display disinhibition [93]. Furthermore, there are inconsistencies in the direction of correlations between the TFEQ-R and disinhibition subscales [13,94-95]. Findings from a large sample of overweight and obese persons with higher and lower TFEQ disinhibition scores participating in a weight reduction programme revealed two sources of variation within the TFEQ-R [92]. These were termed Flexible Control, an adaptable and accommodating approach to food and weight that is associated with lower disinhibition, and Rigid Control, an “all-or-nothing” approach associated with higher disinhibition [92,96]. To assess these two distinct factors, additional items were added to the TFEQ-R resulting in the 12-item Flexible Control and 16-item Rigid Control subscales [96]. These subscales have moderate reliability (Cronbach alphas 0.77 to 0.79) and good predictive validity [96]. The negative relationship between Flexible Control and Disinhibition and positive relationship between Rigid Control and Disinhibition have been observed by others [96-97]. As the rigid and flexible control dimensions of CDR were identified in a sample of overweight individuals who were actively dieting, their relevance to those who are not overweight or actively dieting is not certain.

In summary, each of the three assessment tools is based on different operational definitions of CDR and thus measure distinct aspects of the construct. For the purposes of the
current research programme, the operational definition of the TFEQ-R is most relevant. Furthermore, the TFEQ-R is frequently used to classify women with higher and lower restraint in similar studies, allowing for more appropriate comparisons of findings.

1.2.2.2 Behavioural versus perceptual aspects of CDR

Although the RS, DEBQ and TFEQ are highly inter-correlated [64], it has been suggested the scales do not measure the same behavioural tendencies or actual energy restriction. Heatherton and coworkers [85] suggested that the DEBQ and TFEQ measure successful dieting and the RS is designed to identify dieting. Moreover, factor analysis reveals that although all three scales share a restraint factor, only the TFEQ also assesses behavioural restraint, only the RS also assesses weight fluctuations [75], the RS is a measure of unsuccessful dieting [91], and the DEBQ and TFEQ measure successful dieting behaviour [91]. Additionally, only the RS also assesses binge eating behaviour [98]. As a result of these inconsistencies, the operational definition of CDR in research has become confused with the behavioural aspect of actual energy restriction or dieting. The distinction between CDR and dieting is important when attempting to assess whether it is the perceptual experience of CDR that is associated with health outcomes or the effects of negative energy balance achieved through dieting. Several lines of evidence, described below, establish that CDR should not be considered to be indicative of dieting.

Many women with higher CDR do not self-identify as dieters [11, 99-100]. Moreover, while dieting behaviour is sporadic, CDR appears to be a relatively stable perceptual construct [49, 64, 101]. That is, while women with higher CDR levels are chronically concerned with and aware of the amount and types of foods that they eat in an attempt to control dietary intake, it does not appear that this results in consistently reduced energy intake or lower body weight, indicators of more “successful dieting”. There are some studies that have found lower self-reported energy intake among women with higher CDR levels [43, 53, 91, 102-105] or an inverse correlation between self-reported energy intake and TFEQ-R score [91, 106-108]. Yet, several studies have observed no difference in energy consumption by level of CDR [41, 87, 109-110]. In a large population-based study, restrained eaters (assessed by the DEBQ) were more likely to underreport energy intake than those with lower restraint [111]. Thus, self-reported dietary intake may not be an accurate means of examining differences by level of CDR.

More substantial evidence using objective measures of energy intake suggests that TFEQ-R score is not associated with short-, moderate- or longer term energy intake [15, 112]. Furthermore, in a sample of 84 physically active university-aged women of normal and stable weight with no history of an eating disorder, there was no difference by level of CDR (TFEQ-R score ≥9 or below 9) in resting energy expenditure (REE) measured by indirect calorimetry, the
ratio of predicted REE by Harris Benedict equation (pREE) to measured REE or the proportion of women with an energy deficit (pREE:REE <0.90) [40]. It could be that dietary restraint does not have a consistent effect on energy intake but instead has a highly variable effect. Under certain conditions, CDR may have a large on energy intake yet no effect under other conditions [113].

Further support for the concept that elevated CDR is not synonymous with dieting is provided by studies in which relative body mass or body mass index (BMI; kg/m²) does not differ by level of CDR among normal-weight and obese women [40,42-43,53-54,34,87,91,103,110, 114-116]. In a recent study of over 1000 postmenopausal women, BMI was 1.0 kg/m² (1 BMI unit) lower in women with higher versus lower TFEQ-R scores, and dieters had BMI that was 4.1 BMI units higher than non-dieters [100]. However, no interaction between dieting status and TFEQ-R score was apparent [100]. The association between BMI and dieting status was much stronger than that between BMI and TFEQ-R score providing further support that CDR and dieting are largely independent.

Prospective studies also suggest that change in weight is more strongly associated with dieting than CDR. Among women, a history of dieting but not dietary restraint scores predicted weight gain during the first year of college [14]. Similarly, in a study of 163 middle-aged women, those who identified as dieters had a higher BMI at baseline and gained significantly more weight over six years than non-dieters [117]. On the other hand, CDR was not associated with baseline BMI or weight change [117]. Furthermore, although baseline TFEQ-R score moderated the association between disinhibition and weight, the direction of the relationship was different by dieting status [117]. Similarly, in a study of adolescents and young adults, CDR was not associated with two-year change in BMI, although dieting status was not assessed [101]. On the other hand, in a 6-year study of 283 healthy adults aged 18 to 64, those with higher restraint scores (TFEQ-R >8) gained 1 kg more than those with lower restraint after adjusting for potentially confounding variables [118]. As well, those with higher CDR were 26% more likely to experience a weight gain of ≥5 kg and were 18% more likely to develop obesity [118]. The discrepancy of findings from this study may be related to the inclusion of men and that approximately one half of participants had an obese BMI at baseline. In a prospective study of women with BMI values ranging from 17 to 40 kg/m², TFEQ-R score was positively associated with BMI [49]. The change in weight by level of CDR was not reported in that study although neither BMI nor percent lean mass changed over time among all participants [49].

Despite the general lack of association between CDR and either BMI or weight change measured longitudinally, several studies have reported an increased frequency of past weight fluctuations in women with higher CDR [12,31,91,97,105,119]. This finding has been used as evidence to confirm that higher CDR scores reflect dieting behaviour. However, as these data
were collected retrospectively, the preoccupation and habitual monitoring of weight among women reporting higher CDR scores may heighten their awareness of actual weight changes or even the perception of weight change compared to women with lower CDR. Therefore, prospective studies are warranted to examine weight changes both by level of CDR and by dieting status. In order to distinguish between health outcomes associated with the experience of CDR and those associated dieting behaviours, it would be important to monitor energy intake, weight changes and dieting status in prospective studies.

1.2.3 Stress, cortisol and CDR
1.2.3.1 Chronic stress and neuroendocrine function

Stress, whether inflammatory, traumatic or psychosocial, triggers a neuroendocrine response by the central nervous system and its peripheral components [23]. One response is activation of the hypothalamic-pituitary-adrenal (HPA) axis. As this is the focus of my PhD research project, it is the only aspect of the stress response discussed further in this review. The activation of the HPA axis is one of the body’s main allostatic mediators allowing homeostasis to be maintained during stressful conditions by adaptive responses [120]. As shown in Figure 1.1, HPA axis activation stimulates release of corticotropin releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus and in turn adrenocorticotropic hormone (ACTH) secretion from the pituitary [16]. Cortisol, a glucocorticoid stress hormone, is subsequently released by the adrenal cortex [16]. Cortisol maintains homeostasis during acute stress by activating short-term behavioural and physical changes that improve the chance of survival [23]. Examples include increased alertness, inhibition of hunger and increased respiratory rate and cardiovascular tone [23]. Cortisol excretion is highly variable throughout the day and is associated with the diurnal pattern of circadian rhythm [23]. During nocturnal sleep, cortisol is low and then increases during the second half of the night, peaking shortly after waking (acrophase), and then steadily declining over the course of the day [23]. Under normal circumstances, cortisol levels are regulated via negative feedback: high levels of circulating cortisol inhibit CRH and ACTH, thus decreasing cortisol synthesis [23]. However, dysregulated allostasis can occur, leading to chronically elevated cortisol [120]. McEwen terms this “wear-and-tear” on the body’s systems allostatic overload [120]. The continuous secretion of CRH and cortisol, as seen with Cushing’s syndrome, can adversely affect growth and development, thyroid function, reproduction, metabolism, gastrointestinal function and immune function [23].

Chronic psychosocial stress is associated with many negative health outcomes including anxiety, depression, infertility, hypertension, obesity, type 2 diabetes mellitus, atherosclerosis, neurovascular degenerative disease, osteoporosis and sleep disorders [16]. Evidence suggests that the relationship between adverse health conditions and chronic psychosocial stress may be
related to dysregulation of HPA axis activation [16]. For example, higher cortisol levels have been observed among middle-aged women reporting greater financial strain [121], higher job demands [122], higher job strain [123] and more marital stress [124]. Among young women, studies suggest a relationship between cortisol and eating attitudes, discussed subsequently.

1.2.3.2 Assessment of cortisol and general stress perception

Cortisol is a well established biological indicator of HPA axis activation resulting from stress [125]. In the blood, cortisol circulates both in free form and bound to corticosteroid-binding globulin [125]. The Free Hormone Hypothesis assumes that only free cortisol is biologically active and therefore relevant in determining HPA axis activity related to stress. There is debate as to the biological activity of both free and bound cortisol as well as the best method of assessing cortisol levels in relation to chronic stressors [125]. Cortisol levels can be determined using samples of urine, saliva and plasma. Each method measures a unique aspect of the HPA axis response to stress and has its own strengths and limitations.

In plasma, free cortisol levels are calculated from total cortisol and either of corticosteroid-binding globulin binding capacity or corticosteroid-binding globulin, as no kit currently exists to measure free plasma cortisol [125]. There is potential for high variability in cortisol levels using immunoassays, as cortisol is capable of cross-reacting with other steroids [125]. Plasma cortisol is useful in the clinical setting to diagnosis disease states by comparing levels to the normal range. However, the use of plasma cortisol in research has many limitations including the need for medical staff and specialised equipment as well as high costs and subject burden [125]. Furthermore, some participants may find blood sampling stressful, potentially elevating cortisol levels artificially [125]. Due to the diurnal rhythm of cortisol, the timing of plasma sampling is also an important consideration [125]. Multiple measures of plasma cortisol over time are useful for determining the response to stressful stimuli or to determine whether there is dysregulation of the 24-hour rhythm of cortisol production under various conditions [125]. Single assessments are used to examine whether cortisol levels are associated with physiological or affective state characteristics [125].

Salivary cortisol represents free cortisol that has entered the salivary glands by passive diffusion [125]. Cortisol in saliva is assayed using the same kits for total serum cortisol adjusted for sensitivity [125]. Determining cortisol levels in the saliva has become increasingly popular since the 1980s. It is highly correlated with plasma cortisol ($r= 0.71-0.96$) and has many advantages over plasma assessment, for example, salivary collection is very useful for research outside of the laboratory as sampling is non-invasive and can occur quickly and frequently [125]. Moreover, salivary cortisol is stable at room temperature, does not require specialised staff or equipment and has a lower processing cost [125]. Similarly to plasma assessment of
cortisol, the timing of sampling is an important consideration. Other limitations include problems of participant compliance and the potential contaminating effect of food, drink or blood [125]. Salivary cortisol may be a useful diagnostic tool and may be particularly useful for examining the stress response outside the laboratory.

In 1995, a sharp rise in cortisol levels 20-45 minutes after waking was discovered in addition to the previously described diurnal pattern, termed the cortisol awakening response: the difference in salivary cortisol measured immediately upon waking and 30 minutes later [126]. Evidence suggests that it is a distinct occurrence, a reliable indicator of HPA axis activity and is associated with psychiatric, autoimmune and cardiovascular disorders [126]. However, great variability has been documented and potential confounders include age, smoking status, time of wakening, day of the week and participant compliance [126]. Although the physiological role has not been clearly defined, the cortisol awakening response does appear to be associated with various psychosocial stressors including work overload, social stress, lack of social recognition and perceived stress [126]. Based on these and other findings, it is hypothesised that the cortisol awakening response may represent expectation of the demands of the approaching day [126].

Urinary free cortisol excretion is generally measured over 24-hours and is a useful index of 24-hour plasma cortisol [125]. Determination of 24-hour urinary free cortisol (UFC) is currently recognised as the gold standard in diagnosis of hypercortisolism [127]. Traditionally, UFC was determined using immunoassay methods adapted from serum cortisol methods [127]. These methods overestimate UFC as cortisol metabolites, such as cortisone (the inactive form of cortisol), interfere with the immunoassays used [127]. Recently, more specific methods have been developed based on liquid chromatography-tandem mass spectrometry [127]. These methods allow for quantification of cortisone and have reduced interference for cortisol quantification, resulting in increased sensitivity and specificity relative to previous methods [127].

The stress associated with CDR would likely occur mostly when women were awake and actively involved in eating behaviour decisions. Therefore, using salivary samples at various points during the day and/or overnight blood or urine sampling to assess cortisol levels may not capture the persistent activation of the HPA axis associated with chronic stressors such as CDR. Additionally, evidence suggests that cortisol is highly variable and is associated with the occurrence of everyday minor stressful events [128-129]. Therefore, repeated measures of UFC may give a more accurate depiction of “usual” stress-induced HPA axis activation. Thus, multiple 24-hour urine samples assessed for UFC using high-throughput liquid chromatography-tandem mass spectrometry will be used as an indicator of stress induced activation of the HPA axis in the present study.
Assessment of the perception of stress is also important in understanding the relationship between physiological and psychosocial health. The most commonly used measure of stress perception in the literature is the 14-item Perceived Stress Scale (PSS) [130]. The PSS measures one’s feelings of stress in various life situations during the previous month [130]. The PSS is an indicator of the level of stress one feels in various life situations, as opposed to determining the presence or frequency of particular stressful events [131]. It is widely used in the CDR literature and is reported to be both reliable and valid with Cronbach alpha scores ranging from 0.80 to 0.86 [130-132]. In addition to assessing the perception of “usual” stress by employing the PSS, checklists are often used to determine the frequency of stressful events that occurred over a defined period time. One such checklist is the Daily Stress Inventory (DSI) [133] which includes two subscales, Impact and Frequency. The DSI queries the frequency of 58 everyday minor stressful events which may have occurred (Frequency score) as well as a ranking of the intensity of stressful events (Impact score) that occurred on a scale of 1 (“not at all stressful”) to 7 (“caused me to panic”) [133]. The DSI has good internal consistency with Cronbach alphas ranging from 0.83 to 0.87 and adequate convergent and discriminant validity [128,133]. Assessment of both usual and acute stressors is likely important when examining the association between a particular stressor (such as CDR) and cortisol levels.

1.2.3.3 Cortisol and CDR

We and others have hypothesised that the constant monitoring of food intake and preoccupation with body weight experienced by women with higher CDR may act as a chronic daily stressor that is sufficient to activate the HPA axis (Figure 1.1). The majority of cross-sectional studies investigating this relationship support this hypothesis. Previous work from the Barr lab found significantly higher 24-hour urinary cortisol and cortisol:creatinine ratios among those with a high versus low (TFEQ-R ≥13 versus ≤5 or less) level of CDR [19]. This study included 62 regularly menstruating university-aged women with no previous eating disorders who did not identify as dieters and had normal activity levels and normal BMI values [19]. These results were confirmed in a similar study of 77 healthy postmenopausal women of normal and stable weight [17]. Among 85 premenopausal university students, salivary cortisol was higher in those with higher CDR (TFEQ-R scores ≥8) [18]. Salivary cortisol was also significantly correlated with TFEQ-R score in that study [18]. However, it is noteworthy that saliva samples were collected after participants were weighed and had completed the TFEQ-R and RS, activities that may have acted as a stressor for women with higher CDR and thus acutely elevated cortisol levels in this group.

Conversely, the first study that examined CDR and neuroendocrine function did not observe differences in cortisol by level of CDR in 22 university-aged women using overnight
blood samples obtained by venous catheter (every 30 minutes) [103]. The lack of an association may be related to several study design issues including the very small sample size and timing of the cortisol assessment. As described in the Assessment section (1.2.3.2), CDR-related stress may only activate the HPA axis when women are awake and actively involved in eating behaviour decisions. Therefore, the stress associated with CDR may not be captured by overnight assessment of cortisol levels. Two recent studies further indicate that the timing of cortisol assessment in relation to CDR is an important consideration. Serum cortisol was sampled for five hours (during which time breakfast and lunch were provided) in 38 normal-weight women [21]. After the sampling period, a dexamethasone suppression test was performed [21], which assesses the integrity of the HPA axis negative feedback loop. Dexamethasone is a synthetic glucocorticoid which under normal conditions suppresses CRH and thus ACTH and subsequently, cortisol [134]. Higher cortisol levels after a suppression test indicate decreased cortisol feedback functioning, a characteristic that is associated with hypercortisolism [134]. Women with higher CDR (TFEQ-R score ≥9) had higher cortisol levels than women with lower CDR over the 5-hour sampling period and after the suppression test [21]. Furthermore, in a sample of 170 university-aged women, TFEQ-R score was significantly associated with afternoon but not waking salivary cortisol [20]. These three studies suggest that the timing of cortisol assessment is highly important when considering if CDR may influence the HPA axis.

Two other studies did not find associations between CDR and cortisol [56,135]. Among 65 women with characteristics similar to previous studies, no difference was observed in waking salivary cortisol by the median split TFEQ-R score of nine [56]. Furthermore, there was no correlation between TFEQ-R score and waking salivary cortisol collected within 1.5 hours of waking [56]. As wake-time was not specifically accounted for in that study, it is possible that the peak that occurs 30 minutes after waking may have been captured in some but not all of the samples. This may have caused significant variability in cortisol levels; however, salivary cortisol values are not reported [56]. In a small study of 28 women, the cortisol awakening response (averaged from three samples provided over two months) was not correlated with TFEQ-R scores but was inversely associated with other eating attitude questionnaire scores including Rigid Control of restraint, Disinhibition and Hunger [135]. Participants in this study were significantly older (mean 37 years) and had a higher BMI (mean 29 kg/m²) than those studied previously. Though the study included similar eligibility criteria to previous work and a sound experimental procedure, in a sample this small a single outlier would be sufficient to pull correlations one way or the other. Unfortunately, despite a detailed description of their experiment, the authors do not describe whether outliers were examined or how they were dealt with.
1.2.3.4 Perception of psychosocial stress and CDR

The experience of other psychosocial stresses may be important to consider in the relationship between CDR and cortisol: some women with higher CDR may perceive greater stress in all aspects of their lives. Studies examining the relationship between perceived stress and CDR in women are inconclusive with some reporting no difference in PSS scores by level of CDR [17,43] and others finding higher PSS scores in women with higher versus lower dietary restraint [12,50]. Significant correlations between PSS score and scores on the TFEQ-R [20] and other questionnaire scores reflecting disordered eating attitudes and behaviours, and body dissatisfaction [20,81,136-137] have been reported. As it is not clear if general stress is associated with CDR, it is important to assess and control for this potentially confounding variable.

1.2.4 Ovulatory function

1.2.4.1 Physiology of the menstrual cycle

The menstrual cycle is the result of the coordinated activity of the following hormones: gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus, luteinizing hormone (LH) and follicle stimulating hormone (FSH) secreted from the pituitary, and estradiol and progesterone secreted from the stimulated follicle within the ovaries [138]. The menstrual cycle occurs on average over 28 days and is divided into two phases: the follicular phase and the luteal phase. At the onset of menstrual flow, the first day of the cycle, estradiol and progesterone levels are low, allowing for release of GnRH, which then triggers the production and release of LH and FSH [138]. This stimulates the growth and maturation of ovarian follicles, which begin to secrete estradiol that peaks at midcycle and inhibits FSH [138]. The majority of stimulated ovarian follicles are then degraded and resorbed but the most mature and now dominant follicle continues to release large amounts of estradiol [138]. This results in thickening of the endometrial lining of the uterus and stimulation of the LH surge [138]. The surge in LH functions to: (i) inhibit estradiol release by the follicles, (ii) stimulate rupture of the dominant follicle and ovulation by releasing the ovum, and (iii) transform the ruptured follicle into the corpus luteum [138]. The corpus luteum then releases some estradiol and very high levels of progesterone, the predominant hormone in the luteal phase of the cycle [138]. If fertilization does not occur, the corpus luteum regresses and estradiol and progesterone levels decline, resulting in the shedding of the thickened endometrial lining as menstrual flow and thus, the beginning of a new menstrual cycle [138].
1.2.4.2 Disturbances in ovulatory function

Disturbances in menstrual cycle length and cycle characteristics as the result of various physiologic and psychosocial stressors may occur during the reproductive years [38]. Some of these disturbances are apparent to women including amenorrhea (the absence of menstrual flow for six or more months) and oligomenorrhea (long cycles of 36 to 180 days in length). It is well established that these disturbances, indicators of insufficient estradiol and progesterone which are important in achieving and maintaining peak bone mass, are associated with reduced bone density [139]. Other cycle disturbances, including anovulation and short luteal phase length (LPL), are not apparent to women yet evidence is mounting that these subclinical disturbances in ovulatory function are also associated with bone loss [45-49]. Subclinical disturbances of the menstrual cycle are difficult to monitor in research studies, as women are unaware of these disturbances. Therefore survey methods cannot be used and a physiological indicator is necessary. Furthermore, the menstrual cycle shows considerable intra-individual variability, particularly in LPL, and therefore long-term monitoring of the menstrual cycle is required to properly identity women with subclinical ovulatory disturbances [140-142]. As most methods to detect ovulatory function are expensive and burdensome procedures, a non-invasive inexpensive method to assess these subclinical ovulatory disturbances over long periods of time and that is acceptable to women, is required.

1.2.4.3 Monitoring ovulatory function

The current gold standard for directly determining if and when ovulation has occurred is observation of collapse of the dominant follicle with corpus luteum formation by daily transvaginal ultrasound of the ovaries. During the follicular phase, developing follicles and the dominant follicle can be observed and during the luteal phase, the corpus luteum can be seen [143]. In addition to being costly, and needing a probe in the vagina, this method requires extensive training in its operation and results interpretation [144]. Indirect methods of assessing ovulatory function, referred to as “evidence of luteal activity” (ELA) include: (i) histomorphometric examination of endometrium biopsy to monitor cellular characteristics that change in response to estradiol and progesterone; (ii) repeated blood samples to monitor normal cyclic patterns of estradiol and progesterone levels; (iii) a single blood sample obtained during days 18 to 22 of the cycle to determine if estradiol and progesterone are above baseline values; (iv) urine samples collected between cycle days 12 and 16 for detection of the LH surge or to monitor the change in the ratio of estradiol to progesterone metabolites [145]; (v) measurement of salivary progesterone levels every three to four days; and (vi) monitoring the ratio of daily urinary progesterone metabolites to identify a sustained rise [146]. However, these
methods are also costly and burdensome to women that are not motivated to achieve conception or who have infertility.

Analysis of daily basal temperature records can also be used to determine ELA. Progesterone has a thermic effect on the hypothalamus that leads to an increase of approximately 0.3°Celsius from the follicular phase, when progesterone is low, to the luteal phase, when progesterone peaks [147]. Basal temperature records have been used for decades in combination with changes in cervical mucous as an established fertility-awareness based method of contraception to aid conception [148]. In the past, basal temperature was charted by women and ovulatory function was determined using qualitative analysis methods. Qualitative methods include identification of a basal temperature nadir (low point) at the estradiol peak one to two days prior to the LH surge [149] and visual determination of a biphasic (and thus ovulatory) basal temperature graph by reproductive medical experts. Numerous studies have found these qualitative methods to be inaccurate in the documentation of ovulation relative to ultrasound [150-154], the LH surge [155-159] or the ratio of estradiol to progesterone over the cycle [160]. As well, experts do not always agree on whether or not the same plotted temperatures are biphasic [161], even when using uniform analysis criteria [162].

Quantitative methods of basal temperature analysis (QBT) may be more accurate at determining ELA than previous qualitative methods. However, little work has been done to validate them against other established methods of ovulation detection. There are currently three QBT methods described in the literature. The first is the Vollman averaging method, for which the average cycle temperature is computed and then compared with the recorded temperatures to determine at which day the temperature rises higher and is maintained above the average until flow begins [163]. The second method, the cumulative sum method, involves calculating a baseline average, the average temperature of cycle days five to eleven, and then determining which cycle day the recorded temperature is more than 0.35°Celsius above the baseline [164]. The third and most recently developed method is a computerised least-squares analysis of quantitative basal temperature (LS-QBT), the Maximina© programme [165]. LS-QBT detects ELA by dividing the cycle into two phases by least squares criterion and determining if the mean temperature difference between the phases is statistically significant [165]. The day of luteal onset identified by all three QBT methods has been assessed relative to peak serum LH concentration in 24 ovulatory cycles [165]. Both the Vollman averaging ($r=0.89$, $P<0.001$) and LS-QBT methods ($r=0.88$, $P<0.001$) showed excellent correlation [165]. However, further validation of these methods and against an indicator more clearly and reliably related to ovulation, such as progesterone, is necessary to increase their acceptability.
1.2.4.4 Ovulatory function and the HPA axis

Various life stresses have been found to be associated with disturbed ovulatory function among young women [38]. Although the underlying mechanism is not fully understood, evidence suggests that it may be related to the physiological stress response [38]. Stress-induced HPA axis activation triggers the release of CRH from the hypothalamus suppressing pulsatile GnRH release, as shown in Figure 1.1. As described above, GnRH is responsible for the secretion of LH and FSH from the pituitary, which in turn, stimulates the secretion of ovarian estradiol and progesterone. There is a substantial amount of evidence that HPA axis activation is related to disturbed menstrual cycle and ovulatory function [23,38]. For example, in several studies, women with functional hypothalamic amenorrhea (FHA) have been found to have elevated cortisol, lower urinary metabolites of progesterone and reduced 24-hour LH pulse frequency (indicating suppression of GnRH) than ovulatory women or women with organic forms of anovulation [38]. Additionally, in a small longitudinal study, increased cortisol was associated with lower progesterone levels between days four and 10 after ovulation [166].

1.2.4.5 Ovulatory disturbances and CDR

Evidence suggests that menstrual cycle and ovulatory disturbances may be more common among women with higher CDR, including both disturbances in cycle length (e.g. irregular cycles, oligomenorrhea or amenorrhea) and of subclinical characteristics (anovulation and short LPL). In an exploratory study of 334 female university students not using oral contraceptives and with no history of eating disorders, 33% of women with higher CDR (TFEQ-R score ≥13) reported irregular menstrual cycles [12]. This was significantly more than the 16% of women with lower levels of restraint (TFEQ-R score ≤5) [12]. Furthermore, TFEQ-R score was the only measured variable that significantly differentiated between women reporting regular versus irregular cycles [12]. Among 38 athletic women, 50% of those with high CDR scores had oligo- or amenorrhea versus 25% of those with lower scores [39]. As well, when divided by the TFEQ-R median score of nine, self-reported oligomenorrhea was higher among those with higher versus lower CDR (50% versus 26%) in a sample of 84 physically active university-aged women of normal and stable weight with no history of an eating disorder [40].

Schweiger and colleagues [43] were the first to observe the relationship between CDR and subclinical disturbances in ovulatory function in a sample of 22 young, normal-weight, normally active, regularly menstruating women [43]. Those with high dietary restraint had shorter menstrual cycle lengths, lower mean luteal phase progesterone levels and shorter LPL than women with lower dietary restraint [43]. Shortened LPL assessed by LS-QBT was also observed among normal-weight, regularly menstruating, ovulatory women participating in various levels of physical activity with higher versus lower dietary restraint [42]. These findings
were confirmed in a six-month prospective study, also using LS-QBT, in which normal-weight, regularly menstruating women with higher CDR had more anovulatory cycles and shorter LPL [41]. Furthermore, among 33 female athletes those with menstrual disturbances (determined by salivary progesterone and estradiol levels) reported higher TFEQ-R scores than those with normal menstrual cycles despite similar BMI and exercise levels between groups [167]. Similarly, in a sample of 48 university-aged, non-dieting women of normal and stable weight with no prior history of eating disorders, TFEQ-R scores were significantly higher among exercising amenorrheic women than either exercising or sedentary ovulatory women [168]. Additional support that eating and body stresses can lead to ovulatory disturbances comes from studies that have observed associations using measures other than CDR. Higher scores on the Eating Attitudes Test [169], and the Drive For Thinness and Bulimia subscales of the Eating Disorder Inventory [170] have been reported in women with FHA versus women with organic causes of amenorrhea and/or regularly menstruating women [171-175].

Since my PhD research was proposed, a 2-year prospective study examined the relationship between CDR and ovulatory function [49]. The sample included 189 healthy, regularly menstruating women that were not using oral contraceptives, with a mean age of 32.4 and a mean BMI of 24.3 [49]. Ovulatory function was monitored by salivary progesterone and commercial ovulation kits (mean of 9.8±3.4 cycles were monitored, maximum 12) [49]. Classified by tertiles of TFEQ-R, there was no difference by level of CDR in mean menstrual cycle length, mean LPL, mean luteal salivary progesterone or the percentage of women with three or more cycles with ovulatory disturbances (anovulation or LPL <10 days) [49]. As well, there were no differences by CDR tertiles in serum estradiol or testosterone on cycles days three to five in one of the monitored cycles [49]. There are several possible reasons as to why a null relationship between CDR and ovulatory function was observed in that study. First, the highest CDR tertile included women with TFEQ-R scores >9.4, the median score observed in several other studies. Second, it is unclear why the authors defined more than three cycles with anovulation and/or short LPL as their categorization of higher subclinical ovulatory disturbances. Furthermore, it is not clear why correlations coefficients between the percentage of cycles with subclinical ovulatory disturbances and TFEQ-R scores were not reported.

The authors of that study suggest that the association between subclinical ovulatory disturbances and CDR observed in previous studies among normal- or under-weight women is related to caloric restriction and/or other dieting behaviours such as over exercising. In their study, TFEQ-R scores were associated with higher physical activity levels and women in the highest CDR tertile had higher sport activity levels and BMI than women in the lowest tertile [49]. Based on this, the authors suggest that women with higher CDR were overweight women that were attempting to lose weight by dieting and exercising [49]. However, weight loss
attempts, change in weight or BMI, and energy intakes were not reported. The larger sample size of this study would have allowed for examination of the relationship between CDR and subclinical ovulatory disturbances among normal- versus over-weight women to support their hypothesis. However, this analysis was not reported.

It is unlikely that women with higher CDR in the above-cited studies [41-43,49,167-168,171-172,174] were trying to lose weight or were “successful restrainers/dieters” because participants were weight-stable, did not report current dieting and those with current or past eating disorders were excluded. As well, with the exception of three studies [41,173,175], BMI and exercise levels did not differ between groups. Therefore, it is doubtful that an energy deficit in women with higher CDR caused ovulatory disturbances. In fact, in a study of normally menstruating women, LH pulsatility during the follicular phase was not disrupted until energy availability was <30 kilocalories per kg lean mass [176]. Energy intakes that low would be unlikely in samples of healthy, normal weight women. Moreover, literature exists to indicate that physical activity per se does not cause ovulatory disturbances [46,177-179]. Nevertheless, energy intake, physical activity, and changes in anthropometric measurements would be important variables to monitor when assessing disturbances in ovulatory function.

The authors of the only other prospective study also note that their sample had a higher mean age than previous work suggesting that participants would have reached gynaecologic maturity and therefore their cycles would be less likely to be affected by psychosocial stresses [49]. The much lower prevalence of subclinical ovulatory disturbances that was reported in that study (33.3% of women experienced at least one during their study) versus previous work (67 to 80%) provides support for this hypothesis. The criterion for cycles with short LPL used in that study may be an additional reason for the low frequency of subclinical ovulatory disturbances observed. In that study [49], ELA was determined by commercial ovulation kits, which detect the urinary LH surge. The serum LH peak is a well established indirect indicator of ovulation, and occurs 16 to 48 hours (average 24 hours) prior to documentation of follicular collapse (ovulation) by ultrasound [180]. The lag between the serum and urinary LH peak is less than eight hours (average two to three hours) [181]. Therefore, using urinary LH peak as the estimated day of luteal transition, the criterion for short LPL should be <11-12 days rather than <10 days, the cut off used in that study [49]. Ten days or less is used as the criterion for short LPL by LS-QBT analysis as the significant rise in basal temperature occurs approximately 2.4 days following serum peak LH [165].

In summary, although a recent prospective study did not detect an association between CDR and ovulatory function, the majority of research supports such associations. As CDR appears to be associated with elevated cortisol, which can impair ovarian function it could be
that the relationship between CDR and ovulatory disturbances is mediated by the physiological stress response.

### 1.2.5 Bone and cortisol

Glucocorticoids such as cortisol have negative effects on bone via direct and indirect mechanisms. Cortisol acts directly to disrupt bone via adverse effects on the bone-forming osteoblast cells, by suppressing their formation and activity, as well as by supporting apoptosis [22]. Synthesis of the bone matrix is inhibited as glucocorticoids decrease the synthesis of type 1 collagen, and alter the expression of messenger ribonucleic acid encoding matrix components including osteopontin, fibronectin, beta-integrin and bone sialoprotein [182]. Although the role of the glucocorticoids on the osteoclasts is less clear, bone resorption is increased via an anti-apoptotic effect resulting in increased osteoclast number [183]. As well, osteoclast activity may increase as glucocorticoids are associated with decreases in serum osteoprotegerin, a cytokine that inhibits osteoclast differentiation [184-185].

Indirectly, glucocorticoids may impact bone density via impaired calcium metabolism by: (i) reducing intestinal calcium absorption possibly by inhibiting active transcellular calcium transport; (ii) decreasing the synthesis of calcium binding protein and/or increasing the rate of degradation of active vitamin D at its mucosal binding site; and (iii) decreasing renal calcium reabsorption as evidenced by increased urinary calcium excretion [22]. Among premenopausal women, the disruption of the normal cyclic patterns of the reproductive hormones is another indirect means by which cortisol may affect bone density. The importance of the reproductive hormones to bone is discussed in the next section of this literature review.

Excess endogenous cortisol or hypercortisolism, such as in Cushing’s syndrome, has long been known to increase the risk of osteoporosis. A recent review indicates that Cushing’s syndrome patients have reduced bone formation, lower bone density and an increased incidence of osteoporosis and fractures [186]. Subclinical hypercortisolism, as may occur with an adrenal adenoma, shows similar patterns [186]. The effect of subtle increases in cortisol within the physiological normal range on bone is less certain. Currently, there are several lines of evidence, discussed in detail subsequently, that suggest that slight elevations in cortisol may negatively influence bone. Correlations between higher cortisol and lower bone density have been reported in healthy samples of older adults, clinical samples of premenopausal women with anorexia nervosa or major depressive disorder, and in studies of women with higher and lower CDR.

The first line of evidence is available from studies suggesting an inverse relationship between cortisol levels and bone density in healthy older adults. In a sample of 37 healthy men aged 43 to 73, a significant negative correlation was observed between lumbar spine areal bone
mineral density (aBMD) and fasting waking cortisol levels ($r=-0.33$) [25]. As well, backward regression analysis indicated that cortisol (along with testosterone and BMI) was a significant predictor of lumbar spine aBMD [25]. In a sample of 45 healthy, normal-weight, postmenopausal women not using hormone therapy, salivary cortisol level assessed at 11 p.m. was negatively correlated with lumbar spine aBMD ($r=-0.20$) [27]. However, night salivary cortisol was not associated with aBMD at the femoral neck or trochanter, and salivary cortisol collected at 7 a.m. was not associated with aBMD at any site [27]. That study also included 130 healthy men among whom morning salivary cortisol was negatively correlated with lumbar spine aBMD ($r=-0.31$) [27]. Unexpectedly, night time cortisol was positively correlated with trochanter aBMD ($r=0.18$) and radial aBMD ($r=0.21$). There is no obvious reason for the discrepant findings.

The most convincing evidence of the potential for elevated cortisol to negatively impact bone in healthy individuals comes from two prospective studies. The first study included 34 healthy older men who completed measures of aBMD at baseline and four years later, as well as baseline 24-hour serum cortisol assessment [26]. After adjusting for potentially confounding variables, a significant positive correlation was observed between trough cortisol level and the rate of bone loss at the lumbar spine ($r=0.38$), femoral neck ($r=0.47$) and trochanteric region ($r=0.41$) [26]. This suggests that those with higher cortisol levels at the lowest point in the diurnal cycle experienced greater bone loss over four years. The second study involved 151 men and 96 women who had aBMD assessed at baseline and again four years later [28]. Cortisol levels were assessed by 24-hour urinary cortisol as well as a dynamic suppression-stimulation test of the HPA axis [28]. After adjustment for potentially confounding variables, elevated peak plasma cortisol at activation was correlated with lumbar spine bone loss in men ($r=0.22$) and femoral neck bone loss in women ($r=0.24$) [28]. However, aBMD change was not associated with 24-hour urinary cortisol or cortisol levels following the suppression test [28]. Findings from this study suggest that HPA axis sensitivity but not dysregulation of the negative feedback mechanism may be related to the rate of bone loss. The effect of cortisol on bone has also been investigated by evaluating fracture risk. A large prospective study evaluated the influence of cortisol on 8-year fracture risk in a sample of 684 generally healthy men and women, aged 70 to 79 [187]. Logistic regression analysis adjusted for confounders found those in the highest quartile of baseline 24-hour urinary free cortisol had a significantly greater risk of fracture (odds ratio 5.38) than those in the lowest quartile [187].

In premenopausal women, cortisol has the potential to negatively influence bone directly and indirectly via associations with reproductive hormone deficiencies or imbalances. As discussed subsequently, the reproductive hormones are crucial to achieving and maintaining bone mass. Yet, few studies have examined the relationship between physiologically normal elevations in cortisol levels and bone in healthy women. Women with anorexia nervosa and
major depressive disorder tend to have higher cortisol levels but do not present with hypercortisolism [188-189]. Among women with major depressive disorder, correlations have been reported between waking serum cortisol and aBMD in some [29,31], but not all studies [36-37]. Similarly, findings from studies of anorexia nervosa patients suggest an association between cortisol and bone mineral content (BMC) and/or aBMD [30-31,33]. However, as amenorrhea and very low body weight are part of the diagnostic criteria for anorexia, the independent effect of cortisol on bone is difficult to determine from these studies. Therefore the generalisability of findings from clinical samples to healthy young women is limited due to the presence of other conditions in these disorders (e.g. amenorrhea, muscle atrophy, immune dysfunction) that have significant effects on bone density.

Three of the studies examining CDR, cortisol and bone in small samples of healthy young women have also reported on the cross-sectional relationship between cortisol and aBMD. Among 62 regularly menstruating women with either higher or lower dietary restraint, the 24-hour urinary cortisol:creatinine ratio was negatively correlated with total body BMC [53]. However, the 24-hour urinary cortisol:creatinine ratio was not a significant independent predictor of total body BMD in a multiple regression [53]. Salivary cortisol assessed within 1.5 hour of waking was not associated with aBMD measured at any site (total body, lumbar spine, non-dominant hip & forearm) in 65 regularly menstruating university-aged women [56]. However, as mentioned previously, the timing of the cortisol assessment limits the interpretation of these findings. Finally, in a sample of 78 middle-aged obese women with a history of chronic dieting, waking serum cortisol was not related to BMC or aBMD at any site (total body, lumbar spine, right femur) [52]. Furthermore, when women were categorised as those with low and normal bone density (Z-score ≤-1.0 versus >-1.0), no differences in cortisol were apparent [52].

The possible association between cortisol and bone has also been assessed in a study that used broadband ultrasonic attenuation (BUA) and speed of sound (SOS) as indices of bone strength [190]. The cortisol awakening response was positively associated with calcaneal BUA and SOS in a sample of 36 healthy, normally active, regularly menstruating, non-obese women [190]. As well, women with peak cortisol above the median had higher BUA and SOS than women with cortisol levels below the median [190]. The very small sample size and the relevance of ultrasound bone measures to bone strength in premenopausal women [191] limit the significance of these findings. Additional examination of this question, particularly through longitudinal studies, is warranted in order to determine if subtle elevations in cortisol have a small but persistent negative influence on bone change over time.
1.2.6 Bone and ovulatory function

Disturbances in menstrual cycle and ovulatory function result in reduced exposure to estradiol and progesterone. It is well established that estradiol deficiency, regardless of the aetiology, results in increased bone loss as estradiol prevents bone resorption [44]. Amenorrhea and oligomenorrhea are menstrual cycle disturbances that are known to result in increased bone loss [139]. It has been suggested that progesterone deficiency may also result in bone loss, as progesterone appears to promote bone formation [44,192]. Progesterone peaks during the luteal phase of the menstrual cycle and thus women who experience luteal phase disturbances (anovulation and short LPL) are exposed to lower levels of progesterone.

Several studies by Prior and colleagues support an association between subclinical ovulatory disturbances and bone. In the first study, 66 normal-weight, regularly menstruating women were screened to be initially normally ovulatory in two consecutive cycles with normal estradiol levels [46]. Significant associations were observed with 1-year change in spinal cancellous BMD and both LPL (r=0.48) and luteal phase progesterone levels (r=0.25) [46]. As well, LPL explained 20% of the variance in annual cancellous BMD change [46]. These data were further analyzed by dividing women into two groups (runners versus normally active women) to evaluate exercise-related effects separately from those of ovulatory function: both exercise and LPL had independent positive effects on spinal cancellous BMD [45]. In a subsample that continued to have regular cycles, 1-year LPL was correlated with 5-year BMD change [47]. However, 1-year LPL was not a predictor of BMD change in regression analyses [47]. Finally, in a 1-year randomised, double-blind, placebo-controlled trial among 61 active women with various menstrual cycle disturbances (amenorrhea, oligomenorrhea, anovulatory cycles or short LPL cycles), supplementation with cyclic medroxyprogesterone (10 mg/day for 10 days/month) resulted in significant gains in lumbar spine aBMD [193].

Support is also available from a nested case-control study of healthy, regularly menstruating women with normal (50 to 75th percentile) versus low (<10th percentile) aBMD who were similar in age of menarche, annual number of periods, smoking history, and calcium and energy intakes [48]. The women with lower aBMD had significantly lower urinary estradiol and progesterone metabolites and a less pronounced LH response than women with normal aBMD [48]. Finally, a prospective study provides convincing evidence of an association between subclinical ovulatory disturbances and less positive changes in aBMD, though perhaps not mediated by progesterone [49]. In this 2-year study, 189 healthy women with regular menstrual cycles, aged 21 to 40, had their cycles monitored two times per year for three consecutive cycles (maximum of 12 cycles, average 9.8±3.4) by daily salivary progesterone and urinary LH surge [49]. Serum estradiol and testosterone for cycle days three to five were assessed during one of the monitored cycles [49]. Subclinical ovulatory disturbances were
experienced by 33.3% of participants during the study [49]. Women with three or more disturbed cycles did not differ in physical activity level or lifestyle characteristics than those with fewer than three disturbed cycles [49]. Women with three or more disturbed cycles had significantly less positive changes in lumbar spine aBMD than those with less than three disturbed cycles. Baseline aBMD and neither femoral neck nor total body aBMD change differed by subclinical ovulatory disturbances [49]. Furthermore, in expanded predictive models of 2-year aBMD change, having three or more disturbed cycles resulted in a significantly decreased rate of change (-0.0109 g/cm²) in lumbar spine aBMD but not femoral neck or total body aBMD [49]. As salivary progesterone levels were not associated with change in aBMD in this study [49], the mechanism mediating the relationship between subclinical ovulatory disturbances and bone loss is uncertain.

Conversely, others have reported no associations between indicators of subclinical ovulatory disturbances and changes in aBMD among healthy, normal-weight, regularly menstruating women. In a study of 53 sedentary women who collected daily urine samples for a mean of 4.1 cycles (maximum six, minimum not reported) and were then monitored for aBMD over 17.5 months, neither mean LPL nor average urinary progesterone metabolites were associated with baseline aBMD or aBMD change [51]. Similarly, daily urine samples were collected over three menstrual cycles before aBMD was assessed in small samples of sedentary ovulatory women (n=9), active ovulatory women (n=14) and active women with luteal defects (n=10) [50]. Although there were differences in luteal phase function, there were no differences between groups in aBMD or markers of bone turnover [50]. This study included few women for a cross-sectional study of bone density. In a more recent cross-sectional study of 242 women, aged 30-40, with BMIs ranging from underweight to obese, two menstrual cycles were assessed for serum estradiol and progesterone between cycle days 20 to 24 [194]. Neither mean estradiol nor progesterone levels were associated with hip or spine aBMD [194].

Differences in study design may provide some explanation for the contradictory results. First, the length of time that menstrual cycles are monitored is critical as characteristics of the menstrual cycle show considerable intra-individual variation, particularly for LPL [140-142]. Indeed the majority of studies that monitored cycles for a short duration of time (three to six months) did not observe associations between subclinical ovulatory disturbances and aBMD [50-52,194]. On the other hand, studies that monitored more cycles (9.8 to 12 cycles over one to two years) did find that ovulatory disturbances were associated with less positive changes in bone [45-46,49]. Therefore, continuous or long-term monitoring is critical to correctly identify women with luteal phase defects and thus reduced exposure to estradiol and progesterone.

Second, some investigated aBMD via dual energy X-ray absorptiometry (DXA) cross-sectionally [50-51,194] and/or assessed aBMD prior to evaluation of ovulatory function [50-51]. On the
other hand, others assessed ovulatory function at the same time as changes in BMD using quantitative computed tomography (QCT) [45-47] or 2-year aBMD change by DXA [49]. Examining ovulatory function and bone density at the same time is important as it has been shown that ovulatory characteristics change over time [47,193], and even change cycle by cycle within women [46-47,193]. The method of bone density assessment is also significant to consider. DXA assesses both cancellous and cortical bone and QCT assesses only cancellous bone which turns over more rapidly than cortical bone. This may be important when monitoring bone density over shorter periods of time.

1.2.7 Bone and CDR

Many studies have found that clinical eating disorders, most notably anorexia nervosa, have a substantial negative effect on bone [195]. However, very few studies have examined the effect of non-clinical disordered eating attitudes and behaviours, which are encountered frequently in the general population of young women [1-3]. Given the above associations (between CDR and each of cortisol and ovulatory function, between cortisol and bone, and between ovulatory function and bone), it is logical to ask whether high levels of CDR are associated with lower bone mass or density. The small number of cross-sectional studies that have compared BMC and/or aBMD between women with higher and lower restraint report conflicting results. Due to the large inter-individual variation in bone density and considering the small influence that eating attitudes would exert on bone, cross-sectional studies would require very large sample sizes, and the cross-sectional studies conducted to date are likely insufficiently powered.

Barr and coworkers were the first to examine CDR and bone in a cross-sectional study of 27 regularly menstruating, ovulatory women of normal and stable weight categorised to upper and lower tertiles of TFEQ-R score [42]. There was no difference in aBMD of the lumbar spine assessed by either QCT or DXA [42]. Barr and colleagues followed that study by examining CDR and bone in a sample of 62 normally active, regularly menstruating women of normal and stable weight [53]. Women with higher CDR had significantly lower total body BMC than those with lower CDR [53]. As well, in multiple regression analysis, TFEQ-R score was a significant predictor of both total body BMC and aBMD, explaining approximately 5.5% of variation [53]. Dietary restraint did not enter the multiple regression equation for lumbar spine aBMD, although its effect approached significance (P=0.070) [53]. In a larger study (n=185) with greater power to detect differences in bone, premenopausal women with higher restraint levels were found to have lower total body BMC (but not aBMD) than those with lower dietary restraint in three of four body weight groups [54]. The same group found a significant correlation between TFEQ-R score and femoral BMC (r= -0.24) among 78 generally healthy, premenopausal obese women
with very high RS scores [52]. However, when grouped as normal versus osteoporotic aBMD, there was no difference in TFEQ-R score between groups [52].

Further evidence of a relationship between CDR and bone comes from a study of women runners [196]. Those with a history of stress fracture had significantly higher TFEQ-R scores than those without stress fractures, yet ran similar distances and were of similar relative weight [196]. Support for the relationship between CDR and bone is also available from studies examining this relationship using other tools to assess different aspects of eating attitudes. Competitive women runners with high levels of weight preoccupation (assessed using the Eating Disorder Inventory-2 subscale scores for Drive For Thinness, Bulimia and Body Dissatisfaction) had lower spinal aBMD than those with normal scores [197]. Adolescent women runners with higher restraint scores (determined by the Eating Disorder Exam (EDE) questionnaire Eating Restraint subscale) had lower lumbar spine BMC and aBMD than runners with elevated Weight or Shape Concern (additional subscales) or normal EDE scores, after controlling for potentially confounding variables [55]. However, findings from that study are difficult to interpret due to inclusion of girls with menstrual cycle irregularities, which were more common among those with high restraint. Lastly, in a study of 100 regularly menstruating, university-aged women, those with DEBQ-R scores above the median and who were not using oral contraceptives had lower tibial SOS by quantitative ultrasound and lower markers of bone formation than women with lower restraint [198]. Other bone turnover markers and SOS at the radius did not differ by CDR level [198].

Three recent cross-sectional studies have examined bone in relation to TFEQ-R scores. The first study included 65 regularly menstruating, university-aged women with normal and stable weight, normal activity levels and no history of eating disorders [56]. While TFEQ-R scores were not associated with BMC or aBMD at any site measured, TFEQ-R score was inversely associated with markers of bone turnover [56]. This suggests a reduced rate of bone turnover, which if maintained throughout adulthood, could potentially impact BMC or aBMD in later life. In a sample of 77 normal-weight post-menopausal women not using hormone therapy, there was no difference in aBMD measurements by level of CDR [17]. However, this study was not powered to detect a difference in bone [17]. The most recent study involved 84 physically active, university-aged women of normal and stable weight with no history of an eating disorder [40]. Lower total body and lumbar spine aBMD was found among women with higher versus lower CDR [40]. Furthermore, an inverse correlation between TFEQ-R scores and aBMD at the total body, lumbar spine, total hip and femoral neck was observed [40]. However, interpretation of these findings is complicated by the inclusion of women with oligomenorrhea, as abnormal cycle length was significantly more prevalent in the high CDR group [40].
By examining bone prospectively, a smaller sample size may be required to detect a difference in BMC/aBMD. This is because the inter-individual variability resulting from the many genetic and lifestyle variables that affect bone, is reduced. At the time my research project was proposed, only one study had prospectively examined eating attitudes in relation to bone [199]. In this 2-year study of bone mineral acquisition in 45 healthy premenarcheal girls, those with high scores on the Children’s Eating Attitude Test Oral Control subscale had lower total body BMC at baseline and lower total body and spinal aBMD at two years, when height, weight and Tanner breast maturation stage were included as covariates [199]. Multiple regression analysis, controlling for the same covariates, found that Children’s Eating Attitude Test Oral Control score predicted baseline, 2-year and 2-year change in total body and spinal BMC, explaining 0.9% to 7.6% of the variance [199]. Oral control reflects the perception of being able to control or limit food intake. Although not completely synonymous with CDR, the data support an association between eating attitudes and bone health starting at a young age, and before most were menstruating. In contrast, the prospective study examining associations among CDR, ovulatory function and bone described in detail above (Bone & Ovulatory function section) found that CDR had no significant effect on baseline aBMD or 2-year aBMD change in mixed-model analyses after adjustment for BMI and activity levels [49].

Taken together, available data are suggestive of a relationship between CDR and BMC/aBMD in healthy young women but are far from conclusive. Additional prospective studies may provide clarification as to the possibility of a direct association between CDR and bone.

1.3 Gaps in our current understanding

The experience of dietary restraint may act as a subtle but chronic stressor among young women that is capable of activating the physiological stress response including the HPA axis. Elevated yet physiologically normal cortisol levels may have the potential to negatively influence ovulatory function and bone density. Prospective examination of these relationships is required for several reasons. First, cortisol secretion is highly variable and is affected by everyday minor stressful events. Therefore, repeated longitudinal assessment is crucial to correctly capturing individuals’ “usual” levels. Most of the previous cross-sectional studies to date have used single cortisol assessments (some which are also limited by the timing of collections) and a few used two assessments three to six months apart. Secondly, it is well recognised that there is considerable within-person variability in the characteristics of the menstrual cycle. For that reason, long-term monitoring of ovulatory function is essential before women can be categorised as to their experience of subclinical ovulatory disturbances. The majority of studies to date monitoring ovulatory function concurrently with bone density have observed two to four cycles and only two have assessed a maximum of 12 cycles. As most
methods of monitoring ELA are expensive and have high participant burden, a method that is inexpensive, accurate and acceptable to women is required for long-term observation in large numbers of women. Finally, the majority of studies completed to date were cross-sectional in nature and often included a small number of participants. Therefore, these studies were likely not powered to see associations between bone density and either cortisol levels or ovulatory function due to the high inter-individual variability in bone density.

Only one study to date has prospectively examined CDR, ovulatory function and change in bone density [49]. The study included a sample of women with higher relative body weight that was considerably older and more gynaecologically mature than groups studied in previous work–factors which may affect bone and the characteristics of the menstrual cycle. Moreover, this study monitored a maximum of 12 menstrual cycles and did not assess cortisol levels, which is hypothesised to be the mediator in the relationship between both CDR and ovulatory function, and CDR and bone. My PhD study was designed to prospectively examine the associations among CDR, ovulatory function, cortisol and change in bone density over two years. Findings addressed the gaps in our current understanding outlined above, providing additional evidence as to whether or not the experience of CDR influences young women’s health outcomes. Secondary objectives included examination of the associations among eating and body attitudes, cortisol and blood pressure, which are discussed in more detail in the appropriate chapters.

1.4 Study purpose

The primary purpose of this research project was to prospectively explore relationships among CDR, UFC and, subclinical ovulatory disturbances, and the association of each of these variables with change in bone density over two years in healthy premenopausal women. Before conducting the 2-year prospective study, it was necessary to further validate LS-QBT against progesterone, an established indirect indicator of ovulation which may also be associated with bone density. If valid, this method would be used to document ovulatory function in a large number of women in relation to bone density over two years. This validation study is discussed in detail in Chapter 2, prior to presentation of the 2-year study in Chapter 3. As chronic psychosocial stress may also detrimentally affect blood pressure, associations among eating and body attitudes, cortisol and blood pressure were also examined, and are reported in Chapter 4. Baseline findings regarding the relationships among eating attitudes, cortisol and bone density are described in Chapter 5.
1.4.1 Objectives

1.4.1.1 Objectives for Chapter 2

1. To compare computerised least-squares analysis of quantitative basal temperature (LS-QBT) to urinary pregnanediol glucuronide (PdG) for detecting evidence of luteal activity, as reflected by menstrual cycles classified as ovulatory versus anovulatory.

2. To compare LS-QBT to PdG in estimating the day of luteal phase onset as reflected by the day of significant temperature rise relative to the day of a sustained PdG rise.

3. To evaluate whether editing temperatures based on wake-time variation prior to LS-QBT improves the performance of the method relative to PdG classification of cycles as ovulatory or anovulatory and estimation of the day of luteal phase onset.

4. To evaluate whether assessment and editing of temperature records by a reproductive expert prior to LS-QBT improves the performance of the method relative to PdG classification of cycles as ovulatory or anovulatory and estimation of the day of luteal phase onset.

1.4.1.2 Objectives for Chapter 3

1. To examine potential relationships among the following study outcome variables in healthy, non-obese, regularly menstruating women:
   a. CDR score (TFEQ-Restraint score averaged from assessments at baseline and both follow-ups);
   b. Dietary intake of bone-related nutrients and physical activity averaged from assessments at baseline and both follow-ups;
   c. 24-hour urinary free cortisol (UFC) averaged from assessments at baseline and both follow-ups;
   d. The frequency of subclinical ovulatory disturbances (%SOD, anovulation and/or luteal phase <10 days long) by LS-QBT analyses;
   e. Anthropometric measurements and 2-year change in anthropometrics (Δanthropometrics); and
   f. 2-year change in areal bone mineral density (ΔaBMD; g/cm$^2$) measured at the lumbar spine, both total hips and whole body.

2. To examine whether energy intake, physical activity, General Stress (based on standardised Z-scores of the Perceived Stress Scale and Daily Stress Inventory completed on the days of urine collection), anthropometric measurements, Δanthropometrics, UFC, %SOD, and ΔaBMD differ between healthy, non-obese, regularly menstruating women with higher and lower CDR (median split).
3. To examine whether energy intake, physical activity, General Stress, CDR, anthropometric measurements, Δanthropometrics, UFC and ΔaBMD differ between healthy, non-obese, regularly menstruating women with a higher versus lower %SOD (median split).

4. To examine the interactive effect of CDR-by-ethnicity on UFC, %SOD and ΔaBMD, and the interactive effect of %SOD-by-ethnicity on UFC and ΔaBMD.

1.4.1.3 Objectives for Chapter 4

All objectives for Chapter 4 are based on data collected at the first follow-up, approximately six to 12 months (average seven) after the baseline assessment.

1. To examine the main and interactive effects of Eating/Body Attitude level (based on standardised Z-scores of the Three Factor Eating Questionnaire subscales, Body Shape Questionnaire, Beliefs About Appearance Questionnaire, and the Drive For Thinness and Bulimia subscales of the Eating Disorder Inventory-2) and current weight loss effort among young, non-obese, regularly menstruating women on:
   a. BMI, energy intake, physical activity and General Stress (based on standardised Z-scores of the Perceived Stress Scale and Daily Stress Inventory completed on the days of urine collection and blood pressure monitoring);
   b. UFC; and
   c. 12-hour daytime average mean arterial pressure and systolic and diastolic ambulatory blood pressure (ABP).

2. To examine potential associations among Eating/Body Attitudes, General Stress, UFC and 12-hour ABP measures, after adjustment for potentially confounding variables.

1.4.1.4 Objectives for Chapter 5

All objectives for Chapter 5 are based on data collected at the baseline assessment.

1. To examine potential cross-sectional associations among aBMD, bone mineral content (BMC, g), and bone area (cm²) measured at the lumbar spine, both total hips and whole body and the following outcome variables in healthy, non-obese, regularly menstruating women:
   a. CDR;
   b. Perceived stress, physical activity, the duration of previous oral contraceptive use, age, age of menarche, reported intake of bone-related nutrients and anthropometric measurements; and
   c. UFC, before and after adjustment for potentially confounding covariates.
2. To examine potential cross-sectional correlations among UFC and the following outcome variables in healthy, non-obese, regularly menstruating women:
   a. CDR, perceived stress and physical activity; and
   b. Anthropometric measurements.

3. To examine potential cross-sectional correlations among perceived stress and the following outcome variables in healthy, non-obese, regularly menstruating women:
   a. CDR and physical activity; and
   b. Anthropometric measurements.

1.4.2 Hypotheses

Hypotheses are stated in the null form rather than directional and will be tested using two-tailed P-values.

1.4.2.1 Hypotheses for Chapter 2

1. There will be no relationship between LS-QBT and PdG in terms of the proportion of cycles classified as ovulatory versus anovulatory.
2. There will be no relationship between LS-QBT and PdG for the estimated day of luteal phase onset.
3. Editing temperatures based on waking time will have no effect on the performance of LS-QBT relative to PdG in terms of detecting ovulatory versus anovulatory cycles, or in estimation of the day of luteal phase onset.
4. The assessment and editing of temperatures by a reproductive expert will have no effect on the performance of LS-QBT relative to PdG in terms of detecting ovulatory versus anovulatory cycles, or in estimation of the day of luteal phase onset.

1.4.2.2 Hypotheses for Chapter 3

1. There will be no relationships among CDR, intakes of bone-related nutrients or physical activity, UFC, %SOD, Δanthropometrics and ΔaBMD at any measured site and UFC.
2. Women with higher and lower CDR will not differ with regard to energy intake, physical activity, General Stress, anthropometrics, Δanthropometrics, UFC, %SOD and ΔaBMD.
3. Women with higher and lower %SOD will not differ with regard to energy intake, physical activity, General Stress, anthropometrics, Δanthropometrics, UFC, CDR and ΔaBMD.
4. There will be no ethnicity-by-CDR interactive effect on UFC, %SOD or ΔaBMD, or ethnicity-by-%SOD interactive effect on UFC or ΔaBMD.
1.4.2.3 Hypotheses for Chapter 4

1. There will be no main or interactive effects of Eating/Body Attitude level or current weight loss effort on BMI, energy intake, physical activity, General Stress, UFC and 12-hour daytime average ABP measures.

2. There will be no cross-sectional relationships among Eating/Body Attitudes, General Stress, UFC and 12-hour daytime average ABP measures after adjustment for potentially confounder variables.

1.4.2.4 Hypotheses for Chapter 5

1. There will be no cross-sectional relationships among aBMD, BMC and bone area measured at any site and the following outcome variables: CDR, perceived stress, physical activity, the duration of previous oral contraceptive use, age, age of menarche, reported intake of bone-related nutrients, anthropometric measurements and UFC.

2. There will be no cross-sectional relationships among UFC and the following outcome variables: CDR, perceived stress, physical activity and anthropometric measurements.

3. There will be no cross-sectional relationships among perceived stress and the following outcome variables: CDR, physical activity and anthropometric measurements.
1.5 References


Chapter 2:

Detecting evidence of luteal activity by least-squares quantitative basal temperature analysis against urinary progesterone metabolites and the effect of wake-time variability¹

¹ A version of this chapter has been published:
2.1 Introduction

Variability in ovulation frequency and luteal phase duration are characteristics of ovulatory function related to progesterone that are not apparent to women but may be important to health. Beyond implications for fertility, research suggests that normal ovulation and cyclic progesterone levels may benefit bone [1-4]. However, the ability to examine this relationship is limited by the need for inexpensive and minimally demanding methods to estimate ovulation over long periods. The current ‘gold standard’, daily transvaginal ultrasound, is costly, has a high subject burden and requires extensive training [5]. Repeated blood or urine samples to assess normal cyclic reproductive hormone patterns can be used to indirectly determine if ovulation has occurred by detecting evidence of luteal activity (ELA). However, these methods are costly for large, long-term studies. Therefore, accurate and reliable methods of determining ovulatory function that are inexpensive, easy-to-use and acceptable to participants are required.

For decades basal temperature records have been used in combination with changes in cervical mucous as an established fertility-awareness based method of contraception [6]. Basal temperature is an indirect measure of ovulation as a result of the progesterone-induced temperature increase of approximately 0.3°Celsius from the follicular phase, when progesterone is low, to the luteal phase, when progesterone peaks following ovulation [7]. Previous studies that have examined basal temperature as an ovulation indicator have labelled it an inaccurate method relative to determination by ultrasound [8-11], the luteinizing hormone (LH) surge [12-15], or estradiol:progesterone [16]. However, these studies used non-validated visual methods including identification of a nadir prior to the LH surge and subjective determination of a biphasic basal temperature graph by reproductive experts. As experts do not always agree on visual classification [17], even when using uniform criteria [18] and experience is required for interpretation, these methods are not accessible to all researchers examining the role of ovulatory function in women’s health.

Quantitative methods of basal temperature analysis (QBT) are inexpensive and easy-to-use. QBT methods may also be more accurate at predicting the day of luteal transition (DLT) than previous visually determined methods, though little validation work exists. The three QBT methods used by most computerised systems are the Vollman averaging method [19], Royston’s cumulative sum method [20] and least-squares analysis (LS-QBT) [21]. Previously we observed excellent correlations between the DLT identified by both LS-QBT and Vollman’s averaging methods, compared with serum LH peak concentration [21]. Because the progesterone rise is an indicator of ovulatory function that may be important to bone [1-4], validation of LS-QBT against progesterone is needed to confirm it is appropriate for use in scientific studies.
Another concern with basal temperature methods is the ability to control variables that may alter temperature such as illness and wake-time variation [22]. The LS-QBT method prompts participants to describe illness and sleep disturbances and to record wake-time. Whether a reproductive expert is required to interpret this information has not been examined. Thus, the purpose of the present study was (1) to assess LS-QBT against urinary progesterone metabolites (pregnanediol glucuronide or PdG), and (2) to assess whether LS-QBT is stable to modest wake-time variations.

2.2 Methods
2.2.1 Participants
Fifty-three healthy, normal-weight (body mass index 18.5-25 kg/m$^2$) women aged 19 to 34 were recruited from university classes (Appendix 1). Interested women contacted the investigator for details regarding participation (Appendix 2) and were screened for eligibility including: self-reported regular menstrual cycles (menses every 21-35 days), consistent sleep patterns (wake up and go to bed at approximately the same time most days), no use of hormones in the previous six months and no medical conditions that would interfere with study measurements (Appendix 3). All were nulliparous and non-smoking.

2.2.2 Procedures
Eligible participants met with an investigator to receive study materials and instructions, complete a demographic questionnaire (Appendix 4) and have anthropometrics measured. Verbal and written instructions were provided for completing daily temperature records (Appendix 5) and collecting a portion of first void urine daily beginning the first day of flow and continuing until menstrual flow began for the next cycle (Appendix 6). The university’s Clinical Research Ethics Board approved the study protocol (Appendix 7), and all participants provided written informed consent (Appendix 8). Participants were provided travel compensation (Appendix 9), a $20 gift card for their participation (Appendix 10), and their personal results upon completion of study (Appendix 11).

Each morning, immediately upon awakening, participants recorded their temperature using a digital thermometer (524052, Becton Dickinson, NJ, USA). They also recorded wake-time, flow status and any illness. Sleep quality was rated on a scale of zero to four. A daily sample of first morning urine was obtained with a labelled sponge vial and stored in the participant’s freezer. After one full menstrual cycle, participants returned completed materials.
2.2.3 Determination of evidence of luteal activity

We did not observe ovulation directly by daily ovarian ultrasound and therefore cannot describe cycles as “ovulatory” or “anovulatory”. Several indirect measures of ovulation have been proposed and we compared two of these: (1) the increase in progesterone production by the corpus luteum from the follicular to the luteal phase [7], and (2) the resulting increase in basal temperature. As previously reported [23], we use the term “evidence of luteal activity” (ELA) to clarify that both measures are indirect ovulation indicators. Cycles showing ELA are referred to as ELA+ and cycles that do not show ELA are referred to as ELA-.

2.2.3.1 Urinalysis

Samples were stored at -20°C Celsius until shipment to the University of Washington where aliquots were taken from thawed specimens, preserved with 17 mg/mL boric acid and refrigerated (4°C Celsius) until assayed in duplicate for PdG using competitive enzyme immunoassays [24-25]. The inter- and intra-assay coefficients of variation were 10.3% and 9.2% [24]. PdG concentration was estimated from optical density (Dynex MR7000 MicroPlate Reader, test wavelength 405 nm, reference 570 nm) using a four-parameter logistic model in Biolinx 2.0 Software (Dynex Laboratories, Inc., Chantilly, VA) and was corrected for hydration status by specific gravity [26]. Urinary PdG is highly correlated (r=0.98) with previous day serum progesterone [24].

The Kassam algorithm for determining ELA compares a daily 5-day moving average of PdG to a minimum 5-day baseline PdG level [27]. ELA+ cycles are those with PdG for ≥3 consecutive days at ≥3-times baseline [28]. Cycles are not analysed when ≥3 consecutive samples in the second half of the cycle are missing. This method of determining ELA has been shown to have 100% sensitivity and specificity versus visual classification by reproductive experts using daily urinary reproductive hormones in 52 menstrual cycles [28]. The LH surge occurs approximately 3 days before the sustained urinary PdG rise [27-28]. Therefore, cycles were classified as having a short luteal phase length (LPL) if the luteal phase was <10 days in duration and normal LPL if ≥10 days.

2.2.3.2 Basal temperature analysis

Temperature records were used to determine ELA using computerised LS-QBT, which divides the cycle into two phases using least-squares criterion in a two-step function to maximize the mean difference [21]. A cycle is classified as ELA+ if the mean temperature difference between the phases is statistically significant (P<0.05) [21]. Cycles are not analysed if febrile illness occurs for ≥5 days or at any point mid-cycle, if ≥33% of temperatures are missing, or if ≥3 days are missing at mid-cycle [21]. The LH surge occurs approximately 2.4
days before the day of significant temperature rise [21]. Therefore, cycles were classified as having a short LPL if the luteal phase was <10 days in duration and normal LPL if ≥10 days.

To determine the impact of wake-time variation and whether a reproductive expert is required to evaluate LS-QBT, the analysis was repeated for each cycle using:

A. All temperatures: all recorded temperatures were included except for febrile illness (temperatures ≥37°Celsius with note of illness).
B. Royston-adjusted [22]: all recorded temperatures were adjusted by subtracting 0.1°Celsius/hour from the earliest wake-time (e.g., if earliest wake-time was 6 a.m., temperatures recorded at 8 a.m. would have 0.2°Celsius subtracted).
C. 2-hour average wake-time: temperatures recorded >1-hour before or after the determined average wake-time were removed (e.g., for a 7 a.m. average wake-time, records before 6 a.m. or after 8 a.m. were excluded).
D. Expert reviewed: temperatures were removed based on interpretation by a reproductive endocrinologist.

2.2.3.3 Statistical analyses

Data were coded, verified and entered into SPSS software (SPSS version 15 Inc., 2006, Chicago, IL) and crosschecked for accuracy. Variables were examined for normality. Descriptive statistics were used to characterise the sample. Sensitivity and specificity of LS-QBT for ELA were determined relative to Kassam’s PdG algorithm. Sensitivity is the number of cycles classified as ELA+ by LS-QBT divided by the number of ELA+ cycles by PdG (true ELA+). Specificity is the number of cycles classified as ELA- by LS-QBT divided by the number of ELA- cycles by PdG (true ELA-). Positive predictive value (PPV) is true ELA+ cycles divided by true and false ELA+ cycles. Negative predictive value (NPV) is true ELA- cycles divided by true and false ELA- cycles. Accuracy is the number of true ELA+ and true ELA- cycles divided by the total number of cycles. Pearson’s correlations of the first day of sustained PdG rise and the day of a significant temperature increase by the four LS-QBT methods were also calculated. Although the Kassam PdG algorithm was not designed to assess LPL, it does estimate the day of luteal transition which can then be used to estimate LPL. We conducted sensitivity and specificity analyses of LS-QBT in detecting cycles with short versus normal LPL relative to Kassam’s PdG algorithm (Appendix 12). The significance level for all analyses was $p \leq 0.05$. 

53
2.3 Results

2.3.1 Participant characteristics

Of the 53 women recruited to the study, 48 returned completed materials and 40 had sufficient data. There were no differences in descriptive characteristics between the 40 participants included in the analysis and the 13 excluded.

Table 2.1 presents descriptive characteristics of the 40 participants. The majority were currently students (90%) and single (85%) and 100% had completed some post-secondary education. Most were either Caucasian (n=17) or Chinese (n=13); others were South/West Asian (n=5), Japanese (n=3) or Latin American (n=2). Method C resulted in removal of 0-14 recorded temperatures. For method D, 0-28 recorded temperatures were removed. This resulted in too many missing values for n=4 and n=6, respectively, and thus exclusion from analysis. As shown in Table 2.1, the mean difference between the earliest and latest wake-time was 4.6±1.6 hours and the within-person standard deviation of wake-time variation was 1.1±0.3 hours.

Table 2.1 Descriptive characteristics of the sample (n=40)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.3 ± 3.7</td>
</tr>
<tr>
<td>Age of menarche (years)</td>
<td>12.3 ± 1.3</td>
</tr>
<tr>
<td>Gynaecologic age (years)</td>
<td>12.0 ± 3.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.7 ± 1.9</td>
</tr>
<tr>
<td>Adult amenorrhea</td>
<td>7.5% (3)</td>
</tr>
<tr>
<td>Previous therapy with progesterone/progestin</td>
<td>2.5% (1)</td>
</tr>
<tr>
<td>Previous use of oral contraceptives</td>
<td>47.5% (19)</td>
</tr>
<tr>
<td>Study cycle length (days)</td>
<td>29.2 ± 3.1</td>
</tr>
<tr>
<td>Difference between earliest and latest wake-time (hours)</td>
<td>4.6 ± 1.6</td>
</tr>
<tr>
<td>Within-person variation in wake-time (hours)</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation for continuous variables or proportion (%) (n) for categorical variables.

2.3.2 Sensitivity, specificity, predictive values and accuracy

Table 2.2 shows the ability of LS-QBT to classify cycles as ELA+ relative to our reference standard, Kassam’s PdG algorithm. The reference method classified 36 of 40 cycles as ELA+ of which LS-QBT detected 35 (methods A and B), 33 of 34 (method C) and 30 of 31 (method D). Of the four cycles classified as ELA- by the reference, methods A and B detected one and methods C and D detected none. Table 2.3 shows the predictive values and accuracy of the LS-QBT methods. PPV ranged from 91% to 92% and accuracy ranged from 88% to 90%. NPV was 50% for methods A and B and 0% for methods C and D.
Table 2.2  Sensitivity and specificity of least-squares quantitative basal temperature analysis (LS-QBT) methods in determining evidence of luteal activity (ELA) relative to Kassam’s urinary pregnanediol glucuronide (PdG) algorithm

<table>
<thead>
<tr>
<th>LS-QBT method</th>
<th>ELA+ by PdG (sensitivity)</th>
<th>ELA- by PdG (misclassified)</th>
<th>ELA+ by PdG (misclassified)</th>
<th>ELA- by PdG (specificity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All temperatures(^a)</td>
<td>97% (35)</td>
<td>3% (1)</td>
<td>75% (3)</td>
<td>25% (1)</td>
</tr>
<tr>
<td>Royston adjusted(^b)</td>
<td>97% (35)</td>
<td>3% (1)</td>
<td>75% (3)</td>
<td>25% (1)</td>
</tr>
<tr>
<td>2-hour average wake-time(^c)</td>
<td>97% (33)</td>
<td>3% (1)</td>
<td>100% (3)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>Expert reviewed(^d)</td>
<td>97% (30)</td>
<td>3% (1)</td>
<td>100% (3)</td>
<td>0% (0)</td>
</tr>
</tbody>
</table>

Data are presented as proportion (% (n)).

a. All recorded temperatures were included except for febrile illness (n=40).
b. All recorded temperatures were adjusted by 0.1°Celsius/hour from earliest wake-time (n=40).
c. Temperatures recorded >1 hour before or after the average wake-time were removed. Three cycles could no longer be analysed because of the number of temperature values removed (n=37).
d. Temperatures were removed based on interpretation by a reproductive endocrinologist. Six cycles could no longer be analysed because of the number of temperature values removed (n=34).

Table 2.3  Predictive value and accuracy of least-squares quantitative basal temperature analysis (LS-QBT) methods in determining evidence of luteal activity relative to Kassam’s urinary pregnanediol glucuronide (PdG) algorithm

<table>
<thead>
<tr>
<th>LS-QBT method</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>All temperatures(^a)</td>
<td>92% (35/38)</td>
<td>50% (1/2)</td>
<td>90% (36/40)</td>
</tr>
<tr>
<td>Royston adjusted(^b)</td>
<td>92% (35/38)</td>
<td>50% (1/2)</td>
<td>90% (36/40)</td>
</tr>
<tr>
<td>2-hour average wake-time(^c)</td>
<td>92% (33/36)</td>
<td>0% (0/1)</td>
<td>89% (33/37)</td>
</tr>
<tr>
<td>Expert reviewed(^d)</td>
<td>91% (30/33)</td>
<td>0% (0/1)</td>
<td>88% (30/34)</td>
</tr>
</tbody>
</table>

Data are presented as proportion (% (n)).

a. All recorded temperatures were included except for febrile illness (n=40).
b. All recorded temperatures were adjusted by 0.1°Celsius/hour from earliest wake-time (n=40).
c. Temperatures recorded >1 hour before or after the average wake-time were removed. Three cycles could no longer be analysed because of the number of temperature values removed (n=37).
d. Temperatures were removed based on interpretation by a reproductive endocrinologist. Six cycles could no longer be analysed because of the number of temperature values removed (n=34).
2.3.3 Correlation of luteal onset: sustained PdG rise versus LS-QBT temperature increase

Figures 2.1-2.4 show the relationships between the day of a significant temperature increase by LS-QBT relative to the first day of a sustained PdG rise. All correlations were \( P<0.001 \). Inspection of Bland-Altman plots revealed good agreement with no problems in terms of proportional error or variation that depends on the magnitude of the measurement (data not shown).

Figure 2.1 Correlation of the day of LS-QBT temperature rise versus day of sustained PdG rise by Kassam algorithm: All temperatures

\[ N = 35 \]
\[ R = 0.803 \]
\[ p<0.001 \]

\[ \text{a All recorded temperatures were included except for febrile illness.} \]
Figure 2.2  Correlation of the day of LS-QBT temperature rise versus day of sustained PdG rise by Kassam algorithm: Royston wake-time adjusted\(^a\)

\[ N = 35 \]
\[ R = 0.741 \]
\[ p<0.001 \]

\(^a\) All recorded temperatures adjusted by Royston’s adjustment of 0.1°Celsius/hour from earliest wake time.

Figure 2.3  Correlation of the day of LS-QBT temperature rise versus day of sustained PdG rise by Kassam algorithm: 2-hour average wake time temperatures\(^a\)

\[ N = 33 \]
\[ R = 0.651 \]
\[ p<0.001 \]

\(^a\) Temperatures recorded >1 hour before or after the average wake time were removed. Two cycles could no longer be analysed because of the number of temperature values removed.
2.4 Discussion

Women have recorded basal temperature for decades as part of fertility awareness-based methods for contraception [6]. Basal temperature is also an inexpensive and accessible method of estimating ovulation in scientific research. Qualitative analysis of temperature records is unreliable and inaccurate [8-18]; however, little information exists on the validity of computerised quantitative methods, such as LS-QBT. Relative to PdG, an established indirect marker of ovulation [28], LS-QBT classification of cycles as ELA+ was excellent, but classification as ELA- was poor. This may, at least in part, be explained by the small number of cycles (10%) classified as ELA- by our reference method. Determination of the day of significant temperature increase by LS-QBT correlated well with the day of sustained PdG rise, as was previously observed relative to the LH surge [21]. Our results suggest that LS-QBT can be used to determine ELA and still retain the positive aspects of being inexpensive and noninvasive.

Despite efforts to recruit women with consistent sleep patterns, considerable wake-time variability was observed in our sample, which has been reported to affect basal temperature [22]. We assessed whether selected expert or systematic removal of temperatures improved LS-QBT performance. None of these methods improved performance relative to PdG, indicating that LS-QBT is robust to wake-time variation. Accordingly, LS-QBT is an inexpensive and accessible method for all researchers in women's health as reproductive experts are not
required. However, our conclusions apply to women with reasonably regular diurnal schedules and may not extend to extreme changes in sleep patterns such as with rotating shift work.

We are aware of only two studies that assessed QBT relative to clinical measures of ovulation [8, 21]. Ecochard and coworkers compared the averaging method to ultrasound and urinary LH determination of ovulation [8]. A temperature rise occurred in 98% of cycles classified as ovulatory by ultrasound, although this occurred within one day of the LH surge in only 20% of cycles, leading the authors to suggest that the averaging method is unreliable [8]. However, the progesterone rise that causes temperatures to increase follows the LH peak by 24-48 hours, and temperature increases require some duration of progesterone exposure. Therefore, one would not expect temperature to increase within one day of the urinary LH peak. Data from Prior and coworkers detected temperature rises 2.7 days after the serum LH surge using the averaging method and 2.4 days after the LH surge using LS-QBT [21]. Ecochard and coworkers’ distribution of lag times between the LH surge and the temperature rise show that most cycles were within the expected two to three days following the LH surge [8]. Similarly, Prior and colleagues found that using either the averaging or LS-QBT method, approximately 75% of cycles had a temperature rise within three days of the LH surge [21]. Taken together, it appears that LS-QBT and the averaging method detect a temperature rise approximately three days following the LH surge. Lack of exact concordance between the days of LH surge and temperature rise does not invalidate these two QBT methods as indices of luteal activity.

A limitation of our study is the absence of a direct measure of ovulation such as sequential transvaginal ultrasound measurements. We chose Kassam’s PdG algorithm as our indirect reference method because it reflects follicular to luteal change in progesterone, and the increase in basal temperature during the luteal phase results from the thermogenic effects of progesterone [7]. Kassam’s algorithm was reported to have 100% sensitivity and specificity relative to visual classification of daily urinary reproductive hormones by experts; however, it should be noted that 10% of the cycles were classified as indeterminate and excluded [28]. It is conceivable that some of the misclassified cycles in our study may have been “indeterminate”.

Despite lack of direct ovulation observation, our data comparing LS-QBT to PdG, combined with previous work comparing temperature rise with the serum LH surge [21], suggest that LS-QBT is a useful tool that performs reasonably well in detecting ELA+ cycles and estimating the DLT. Longitudinal epidemiology studies examining ovulatory function relative to other aspects of health require accurate, inexpensive methods that are acceptable to study participants. Because temperature recording is noninvasive and as our findings suggest, robust to greater wake-time variability than previously thought, it does not require expert interpretation or other time consuming or expensive adjustments. Subject burden is low and although cervical mucous assessment is more accurate than basal temperature methods in determining DLT
[8,10,14,29], it is less acceptable to participants [30] and may be less suitable for long-term use in epidemiological studies. Further validation of LS-QBT, using daily transvaginal ultrasound as the gold standard and in a population with more anovulatory and irregular cycles, is required.
2.5 References


Chapter 3:

A prospective exploration of cognitive dietary restraint, subclinical ovulatory disturbances, cortisol and change in bone density over two years in healthy young women

1 A version of this chapter has been accepted for publication:
Bedford JL, Prior JC, Barr SI. A prospective exploration of cognitive dietary restraint, subclinical ovulatory disturbances, cortisol and change in bone density over two years in healthy young women. Journal of Clinical Endocrinology & Metabolism.
Date of Acceptance: March 2010. Copyright © The Endocrinology Society.
3.1 Introduction

Cognitive dietary restraint (CDR) is the perception that one is limiting food intake in an effort to achieve or maintain a perceived ideal body weight [1]. Different from dieting, a behaviour where energy intake is limited in an intent to lose weight, CDR is a psychosocial construct reflecting habitual monitoring of food intake and body weight preoccupation. The perceptual nature of CDR is reflected by the lack of clear evidence that energy intake, relative body mass or weight change differs by restraint level among young women [e.g. 2-4].

Evidence suggests that the experience of higher CDR may detrimentally affect physiological health including menstrual cycle and ovulatory function and bone. Young women with higher CDR are more likely to report menstrual cycle irregularities [2,5] and to unknowingly experience subclinical ovulatory disturbances [6-8]. Subclinical ovulatory disturbances, such as short luteal phases and anovulation, indicate reproductive hormone inadequacies and may influence bone mass [9]. It is well established that overt ovarian disturbances such as amenorrhea detrimentally affect bone [10]. Whether subclinical ovulatory disturbances are associated with lower bone mineral density (BMD) or increased bone loss is controversial [11-17]. As well, a direct cross-sectional relationship between higher CDR and reduced BMD and bone mineral content (BMC) has been reported by some [5,18-21] but not all [7,22] studies. In the only prospective study to date, CDR was not associated with subclinical ovulatory disturbances or BMD change; although BMD change was lower in women with more subclinical ovulatory disturbances [17].

The relationship between CDR, ovulatory function and bone may be mediated by the physiological stress response. The constant monitoring and attempts to control food intake may act as a stressor capable of activating the hypothalamic-pituitary-adrenal (HPA) axis. As shown in Figure 3.1, stress activation of the HPA axis triggers a cascade of events resulting in increased cortisol, and concurrent inhibition of the hypothalamic-pituitary-gonadal (HPG) axis, leading to disturbed menstrual cycles and ovulatory function [23]. Reports of higher cortisol levels among women with higher CDR [24-28] suggest that restraint may be a subtle but chronic stressor, resulting in modest but persistent elevations in cortisol within the physiological range. Cortisol has well established direct effects on bone, and clinical hypercortisolism is consistently associated with reduced BMD [23]. Whether cortisol elevations within the normal range influence bone in young healthy women is unclear [5,7,18-22,29]. Therefore we hypothesised that women with higher CDR would have increased 24-hour urinary free cortisol, more frequent subclinical ovulatory disturbances and less positive change in bone density over two years (Figure 3.1).
Figure 3.1  Model driving our hypothesis of cognitive dietary restraint and bone density juxtaposition with the physiological stress response

Variables shown as ovals were measured in the present study. The dashed line (1) between dietary restraint and chronic psychosocial stress reflects our hypothesis that restraint acts as a subtle chronic stressor capable of activating the hypothalamic-pituitary-adrenal (HPA) axis. Solid black lines indicate well established mechanisms of the stress response including: (2) increased secretion of cortisol, which has a direct negative effect on bone density, and (3) inhibition of the hypothalamic-pituitary-gonadal (HPG) axis resulting in deficiencies or imbalances of the reproductive hormones and therefore menstrual cycle and ovulatory disturbances. Grey lines represent hypothesised but inconclusive relationships that we and others have observed including: (4) the possibility that subclinical ovulatory disturbances can have detrimental effects on bone density; (5) an association between higher dietary restraint and elevated cortisol; and (6) an association between higher restraint and the occurrence of menstrual cycle and ovulatory disturbances. This leads to our hypothesis, indicated by the grey dashed line (7), that dietary restraint may result in less positive changes in bone density. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; E, estradiol; FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone; P, progesterone.

3.2 Methods
3.2.1 Participants

Participants were recruited from University of British Columbia classes and the Vancouver (British Columbia, Canada) community for a 2-year study on potential correlates of bone density (Appendix 13). No reference was made to eating/body attitudes in recruitment materials. Interested women contacted the investigators for additional study details (Appendix 14). Potential participants were interviewed by phone to determine eligibility (Appendix 15).
including: 19-35 years of age, no pregnancy or breastfeeding currently or within 12 months, regular menstrual cycles (self-reported menses every 21-35 days in the previous ≥6 months), non-obese (self-reported body mass index (BMI) 18-30 kg/m²), consistent sleep patterns (wake up and go to bed at approximately the same time most days) and absence of any medical conditions (previous or current diagnosis of hirsutism, eating disorder, polycystic ovarian syndrome, Cushing’s syndrome, inflammatory conditions, hyperthyroidism) or use of medications (oral contraceptives, progesterone, glucocorticoids currently or within the past six months) that could affect study variables. Of 148 women assessed, 142 were eligible (Figure 3.2). A final convenience sample of 140 provided written informed consent (Appendix 16) and was oriented to the study. Data collection was completed by 137 at baseline, 127 at first follow-up and 123 at final follow-up. Data are reported for these 123 individuals. The study protocol was approved by the university’s Clinical Research Ethics Board (Appendix 17). Participants were provided with travel compensation (Appendix 18) and $90 in gift cards for their participation (Appendix 19).

Figure 3.2  Flow diagram depicting study recruitment, participation and data collection at baseline and first and final follow-up assessments

Excluded  n=6
Ineligible because:
Oral contraceptive user  n=3
Shift work  n=1
BMI <17 kg/m²  n=1
Glucocorticoid user  n=1

n=2 did not attend orientation
n=3 came for materials and instructions but did not complete the procedures.

Losses to follow up (n=14)
Reasons:
Moved  n=4
Did not respond  n=2
No longer interested  n=3
Pregnant  n=3
Androgen excess  n=1
Thyroid cancer  n=1

N=148 assessed for eligibility from August to December 2006

N=142 eligible

N=137 consented to participate and completed the baseline procedures: questionnaires, 24-h urine collection, anthropometric measurements, dual energy X-ray absorptiometry (DXA) scan

N =127 participated in first follow-up (~1 year after baseline): questionnaires, 24-h urine collection, anthropometric measurements.

N = 123 participated in final follow-up (~2 years after baseline): questionnaires, 24-h urine collection, anthropometric measurements, DXA scan.

Basal body temperature recorded every day during 2-year study for menstrual cycle and ovulatory monitoring
3.2.2 Data collection

This 2-year prospective cohort study included data collection at baseline and two follow-ups at 6-12 months (mean 7) and 1.5-2.5 years (mean 2) after baseline (Figure 3.2). At each of the three data collections, participants met with an investigator to complete anthropometric measurements and to be oriented to study procedures (materials and detailed written and oral instructions). At each data collection, participants completed the following procedures at-home: a questionnaire package, a food frequency questionnaire, and 24-hour urine collections (Appendix 20). Every day during the 2-year study, participants were also asked to record their basal temperature in a provided temperature calendar (Appendix 21). Dual energy X-ray absorptiometry (DXA) scans were conducted at Vancouver General Hospital (VGH) at baseline and 2-year follow-up (Appendix 22).

3.2.3 Questionnaires

The questionnaire package (completed at baseline and both follow-ups, Appendix 23) included validated self-report questionnaires (Appendix 24) as well as questions to elicit demographic information (age, ethnicity, education, employment) and health information (cigarette use, medical/menstrual history and changes).

The well-validated Three Factor Eating Questionnaire (TFEQ) was used to examine stress related to eating and the body [1]. The questionnaire includes three subscales that assess dimensions of eating attitudes that may influence eating behaviour including: Restraint with higher scores indicating higher perceived dietary restraint; Disinhibition with higher scores indicating a greater tendency to overeat when restraint is removed; and Hunger for which higher scores indicate increased susceptibility to hunger and food cravings [1].

To examine the role of general psychosocial stress the Perceived Stress Scale was completed at each data collection to determine stress perception over the previous month [30] and the Daily Stress Inventory was completed after each 24-hour urine collection (Appendix 25) to determine the frequency and impact of stressful events [31].

The Baecke Questionnaire of Habitual Physical Activity [32] was completed at each assessment to measure participants’ usual activity levels at work, in sport, and during leisure.

At the 2-year follow-up only, any reproductive hormone use was documented and the Eating Disorder Examination (EDE) Questionnaire [33] was completed to confirm absence of clinical eating disorders. The EDE includes four subscales (Restraint, Eating Concern, Weight Concern and Shape Concern) and an average Global score to assess body attitudes that are concurrent with eating disorder pathology over the previous four weeks. Global scores ≥4 (possible range 0-6) are considered “clinically significant” but not diagnostic [34-35].
3.2.4 Food frequency questionnaire (FFQ)

The Diet History Questionnaire (version 1.0, National Institutes of Health, Applied Research Program, National Cancer Institute, 2002) was completed at baseline and both follow-ups and analysed using a Canadian version of the programme [36]. Energy intakes of <600 or >3500 kcal were deemed biologically implausible [36]. This resulted in removal of four FFQs at baseline and two FFQs at each of the first and second follow-ups. Complete FFQ data were available for 119 at baseline and first follow-up and 120 at the second follow-up. All 123 participants had data from at least one FFQ available for use.

3.2.5 Ovulatory function

Ovulatory function can be observed indirectly by determining whether or not basal temperature increases from the follicular to luteal phase of the cycle as a result of increased progesterone production. Every day during the 2 year study, participants were asked to record their temperature immediately upon waking. Temperatures were recorded in provided temperature calendars using a digital thermometer (Becton Dickinson, Franklin Lakes, NJ, product number 524052). Time of waking, menstrual flow status and any illness were recorded and sleep quality was rated from 0-4. Completed temperature calendars were returned to investigators at each follow-up. Temperatures collected during hormonal contraceptive use were not analyzed.

Temperatures were entered into a computer programme (Maximina ©) which uses least-squares quantitative basal temperature analysis (LS-QBT) to determine evidence of luteal activity by identifying a statistically significant difference by least squares criterion in temperature values to divide the cycle into two phases [37]. Cycles are not analysed if exogenous hormones were used, if a febrile illness occurs for ≥5 days or at any point mid-cycle, if ≥33% of temperature readings for a cycle are missing, or if ≥3 days are missing at mid-cycle. This method has been validated against established markers of ovulation: the serum peak luteinizing hormone (LH) concentration [37] and the rise in urinary progesterone metabolites [38].

Cycles are classified as having evidence of luteal activity or being “ovulatory”, if the maximum mean temperature difference between the phases is statistically significant [37]. If no temperature increase occurs, the cycle is classified as “anovulatory”. Luteal phase length (LPL) was calculated as the number of days from the day of significant temperature rise until the day before menstrual flow began [37]. As the LH surge occurs approximately 2.4 days prior to the temperature rise [37], LPL is classified as “short” if <10 days or “normal” if ≥10 days. The percentage of cycles with subclinical ovulatory disturbances was calculated by adding the number of anovulatory and/or short LPL cycles and dividing by the total number of cycles.
analysed. To examine differences in outcome variables, participants were classified with a higher or lower percentage of disturbed ovulation by median split.

3.2.6 Urine collection and analyses

Within several weeks of meeting with investigators at each data collection, participants’ chose a ‘normal day’ free of any unusual physical or mental stresses to complete the 24-h urine collection. Participants discarded their first urine void, recorded the time this occurred and then collected all subsequent voids for 24 hours including a void at the recorded time the following morning. After their last void, participants completed the Daily Stress Inventory described above [34]. At the VGH Laboratory, urine volume was measured and aliquots were frozen and stored prior to analysis of urinary free cortisol (UFC, µg/24-hour) by high-throughput liquid chromatography and tandem mass spectrometry [39]. Six participants completed two urine collections and 117 completed three.

3.2.7 Physical measurements

Physical measurements were made in duplicate at each data collection point. Weight was measured while wearing light indoor clothing without shoes, to the nearest 0.1 kg using an electronic scale. Using a stadiometer (model 214; Seca, Hamburg, Germany), height without shoes was measured to the nearest 0.1 cm at full inspiration. Body mass index (BMI; kg/m\(^2\)) was calculated from these data. Measurements were made in duplicate. If differences occurred, a third measurement was made and the two closest measurements were averaged.

At baseline and final follow-up (1.95±0.14 years after baseline), DXA scans of the lumbar spine (L1-4), both total hips and whole body were completed. Total body bone-free lean mass (kg), fat mass (kg), percent body fat and areal bone mineral density (aBMD, g/cm\(^2\)) were measured on a Lunar Prodigy machine with enCORE software (General Electric Healthcare, Madison, WI). Daily quality assurance tests were conducted using a spine phantom scan and densitometric calibration. Repeat aBMD measurements fall within ±0.01 g/cm\(^2\) for L1-4 and ±0.012 g/cm\(^2\) for the proximal femur according to the manufacturer. The in-house coefficient of variation for aBMD at L1-4 averaged 0.94% (0.82–1.10%) and the coefficient of variation for total proximal femur averaged 0.70% (0.65-0.76%).

3.2.8 Statistics

Data were coded, verified and entered into SPSS software (version 17, SPSS Inc., 2008, Chicago, IL) and crosschecked for accuracy. Physiologic variables were examined for outliers (mean ± >4 standard deviation (SD)) and none were present.
Repeated measures General Linear Model (GLM) with least significant difference post-hoc analysis was used to examine changes over time. Because reported nutrient intakes, questionnaire scores (including CDR), UFC and urine volume did not change, averages were calculated and used in analyses.

A General Stress Z-score was calculated from the average Perceived Stress Scale and Daily Stress Inventory Impact and Frequency scores. Questionnaire Z-scores ([(participant score – questionnaire mean)/questionnaire SD]) were then summed and divided by three.

The second DXA scan was completed 1.95±0.14 years after the baseline scan. For all physical measurements, the percentage of change over two years was calculated and annualised ([(observed percent change * 2-year)/[duration between Time 1 and Time 2]]) and is hereafter referred to as change (Δ).

Descriptive statistics were used to characterise the sample. Pearson’s correlations were conducted to identify potentially confounding covariates of study outcome variables (Restrain score, UFC, ovulatory function, ΔaBMD). Comparisons between groups (lost to follow-up, ethnicity, study hormone use, number of cycles analysed) were examined by Chi-square for categorical data and by independent t-tests or GLM with appropriate covariates for continuous variables. Women were classified by median split for subclinical ovulatory disturbances (≥38.8% versus <38.8% of cycles) and for CDR (Restrain score ≥7.7 versus <7.7). Comparisons were made between groups for study outcome variables using independent t-tests and GLM adjusted for appropriate covariates. Because steroid metabolism may differ between Asians and Caucasians [40], interactions between ethnicity and CDR were examined with regard to UFC, ovulatory disturbances and ΔaBMD. The significance level for all analyses was P≤0.05 and all cases were excluded pairwise.

3.3 Results
3.3.1 Sample
Consistent with the UBC student body, the sample included Asians (63%) and Caucasians, who did not differ in study outcome variables (data not shown). Mean age at baseline was 22.1±3.3 years and gynaecological age was 9.7±3.7 years. Almost all had completed some post-secondary education (96%), were single (92%), non-smokers (98%) and nulliparous (98%). During the study, 18 women took oral contraceptives for 1-22 months (mean 8.2±6.2), two used hormonal intrauterine systems for 8-18 months, and two used progesterone cream for 0.3-3 months. Participants who used hormones (n=22) did not differ in outcome variables versus non-users (data not shown). Thus, all participants were included and the duration of study hormone use was examined as a potential confounder. The EDE subscale and
Global scores (data not shown) were lower than published norms [34,41] and only one participant had a clinically significant Global score of 4.5.

3.3.2 Questionnaires

Average questionnaire scores and energy intake, and partial correlation coefficients of the Three Factor Eating Questionnaire subscales are shown in Table 3.1. CDR was not associated with energy intake, General Stress or physical activity. Disinhibition was negatively associated with Baecke total and occupational activity score and was positively with Hunger and General Stress. Hunger was positively associated with General Stress.

Table 3.1 Mean questionnaire scores and energy intakes, and partial correlation coefficients of the Three Factor Eating Questionnaire subscales and 24-hour urinary free cortisol in healthy premenopausal women (n=123)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Rp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td></td>
</tr>
<tr>
<td></td>
<td>participants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CDR</td>
<td>Disinhibition</td>
</tr>
<tr>
<td>CDR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9 ± 4.1</td>
<td>---</td>
</tr>
<tr>
<td>Disinhibition&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0 ± 2.9</td>
<td>0.24</td>
</tr>
<tr>
<td>Hunger&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 2.3</td>
<td>-0.09</td>
</tr>
<tr>
<td>General Stress Z-score&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0 ± 0.8</td>
<td>-0.01</td>
</tr>
<tr>
<td>Physical Activity&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.8 ± 1.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Occupational</td>
<td>2.4 ± 0.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Sport</td>
<td>2.5 ± 0.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Leisure</td>
<td>2.9 ± 0.6</td>
<td>0.04</td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>1556 ± 478</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Data are reported as mean ± standard deviation. Values are reported as averages from assessments at baseline and both follow-ups as values did not change over time by repeated measured General Linear Modelling. CDR, Cognitive dietary restraint; Rp, partial correlation coefficients; UFC, 24-hour urinary free cortisol. Correlation significant at * P<0.05, ** P≤0.01; *** P≤0.001.

a. Three Factor Eating Questionnaire subscales scores: CDR (possible score 0-21); Disinhibition (0-16); and Hunger (0-14).
b. Adjusted for body mass index (kg/m<sup>2</sup>).
c. Adjusted for urine volume (L/24-hour).
d. Z-score of the Perceived Stress Scale and Daily Stress Inventory Impact and Frequency subscales assessed on the days of urine collection.
e. Baecke Habitual Physical Activity Questionnaire, possible scores for subscales 1-5 and total 3-15.

3.3.3 Urine volume and UFC

Urine volume (mean±SD 1.8 ± 0.8 L/24-hour) and UFC (mean±SD 25.7 ± 9.5 µg/24-hour) were correlated (r=0.34, P<0.001). This did not change after controlling for height and/or
weight (data not shown). Therefore UFC was adjusted for urine volume in all analyses including the partial correlations presented in Table 3.1. UFC was positively correlated with General Stress but was not associated with other questionnaire scores (including CDR), or with baseline or Δ anthropometrics (data not shown). Volume-adjusted UFC did not differ by ethnicity (P=0.858).

3.3.4 Menstrual cycle and ovulatory function

114 women provided 1-28 cycles (mean±SD=13.6±7.0) sufficient for analysis. There were no differences in demographics or outcome variables between participants who provided ≤10 cycles (n=42), ≤5 cycles (n=17) and ≤3 cycles (n=6) versus those that provided more cycles for each cut-off. The number of cycles analysed was not correlated with the percentage of cycles with subclinical ovulatory disturbances. Therefore, all 114 participants were included in further analyses and the number of cycles analysed was examined as a potential confounder.

Study cycle length was 30.8±4.1 days with 14 women experiencing oligomenorrhea (cycle lengths of 36-90 days) and one experiencing amenorrhea (>180 days between cycles). Cycle length was inversely associated with age (r=-0.25, P=0.007), gynaecological age (r=-0.19, P=0.042), height (r=-0.20, P=0.035), weight (r=-0.23, P=0.015) and total, leisure and sport (r=-0.25-0.32, P<0.01) activity scores. Cycle length was not associated with BMI, Δanthropometrics, energy intake, the number of cycles analysed or the percentage of subclinical ovulatory disturbances (data not shown). There were no differences in study outcome variables between those with longer versus normal cycle lengths after adjusting for appropriate covariates (data not shown).

The mean percentage of cycles with subclinical ovulatory disturbances was 43.7±32.0%. Sixty-one percent of women had ≥1 anovulatory cycles and 82% ≥1 cycles with short LPL. Age (r=-0.25, P=0.008), gynaecological age (r=-0.29, P=0.002) and BMI (r=0.20, P=0.031) were associated with subclinical ovulatory disturbances. Examination of a scatterplot suggested that two women with BMI >29 and high percentage of subclinical disturbances may have driven the correlation, as their removal resulted in the association becoming nonsignificant (r=0.15, P=0.124). No other anthropometric (baseline or Δ) were associated with subclinical ovulatory disturbances.

After adjustment for baseline gynaecological age and BMI, the percentage of cycles with subclinical ovulatory disturbances was positively associated with CDR score (r=0.22, P=0.018), but not with physical activity, energy intake or UFC (urine volume as additional covariate; data not shown).
3.3.5 Physical measurements

Table 3.2 describes participants’ baseline, 2-year and annualised percent change in anthropometrics and aBMD. Height, weight and total body and L1-4 aBMD increased significantly during the study. ΔaBMD did not differ by ethnicity (P=0.311-0.398).

Baseline height, weight and lean mass were not associated with ΔaBMD, and. Hip ΔaBMD was inversely associated with Δfat mass (r= -0.18, P=0.047) and Δ%body fat (r= -0.20, P=0.026), and positively with baseline BMI (r=0.23, p=0.012), fat mass (r=0.23, p=0.01) and %body fat (r=0.19, p=0.038). Total body ΔaBMD was positively associated with Δweight (r=0.21, P=0.018), ΔBMI (r=0.18, P=0.049) and Δlean mass (r=0.18, P=0.048). L1-4 ΔaBMD was not associated with Δanthropometrics (data not shown). No dietary intake variables were associated with ΔaBMD except that calcium/kcal was negatively associated with total hip ΔaBMD (r= -0.19, P=0.036). Examination of a scatterplot revealed a participant with a calcium intake of 1.04 mg/kcal and total hip ΔaBMD of -5.94% and her removal made the association nonsignificant (r= -0.13, P=0.164).

Volume-adjusted UFC was not associated with ΔaBMD at the hip (r=0.099, P=0.279), L1-4 (r=0.008, P=0.933) or total body (r=0.04, P=0.658). Adjusted for Δlean mass, baseline gynaecological age and BMI, only hip ΔaBMD was significantly associated with the percentage of cycles with subclinical ovulatory disturbances (r = -0.29, p=0.002).

Table 3.2 Physical measurements at baseline, 2-year follow-up and the 2-year percent change in healthy premenopausal women (n=123)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>2-year</th>
<th>% 2-year changea</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heightc (cm)</td>
<td>163.0 ± 7.2</td>
<td>163.1 ± 7.2</td>
<td>0.001 ± 0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.9 ± 8.8</td>
<td>58.4 ± 9.0</td>
<td>1.2 ± 5.5</td>
<td>0.036</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.8 ± 2.5</td>
<td>21.9 ± 2.6</td>
<td>0.7 ± 5.6</td>
<td>0.198</td>
</tr>
<tr>
<td>Bone free lean mass (kg)</td>
<td>37.8 ± 5.0</td>
<td>38.0 ± 5.1</td>
<td>0.7 ± 3.7</td>
<td>0.051</td>
</tr>
<tr>
<td>Bone free fat mass (kg)</td>
<td>16.8 ± 5.6</td>
<td>17.3 ± 5.7</td>
<td>4.1 ± 17.7</td>
<td>0.053</td>
</tr>
<tr>
<td>Bone free body fat (%)</td>
<td>30.3 ± 6.6</td>
<td>30.7 ± 6.5</td>
<td>2.2 ± 12.8</td>
<td>0.169</td>
</tr>
<tr>
<td>Total body aBMD</td>
<td>1.136 ± 0.077</td>
<td>1.147 ± 0.078</td>
<td>1.1 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lumbar spine aBMD</td>
<td>1.183 ± 0.121</td>
<td>1.196 ± 0.122</td>
<td>1.2 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip aBMD</td>
<td>1.025 ± 0.120</td>
<td>1.027 ± 0.122</td>
<td>0.2 ± 2.2</td>
<td>0.380</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. aBMD, areal bone mineral density (g/cm²).

a 2-year measurements were conducted 1.95 ±0.14 year after baseline. Measurements before or after the 2-year time point were corrected to 2 year percent change.

b Level of significance of differences between baseline and 2-year values by repeated measures General Linear Model.

c Height increased significantly over the 2 year period. This is likely due to measurement error and that many participants were young (36% ≤20 years of age at baseline) and may have still been growing.
3.3.6 Differences by CDR median split

Differences in study variables are presented in Table 3.3. The following variables did not differ by level of CDR: age, ethnicity, height, lean mass, waist circumference, Δanthropometrics, the number of cycles analysed, and the frequency and duration of study hormone use. Women with higher CDR had higher baseline weight, BMI, fat mass, %body fat, BMI-adjusted energy intakes and Disinhibition scores. Physical activity, Hunger and General Stress did not differ.

After adjusting for baseline BMI and gynaecological age (Table 3.3), subclinical ovulatory disturbances were more frequent in women with higher CDR. The ethnicity effect and the ethnicity-by-CDR interaction were not significant.

Women with higher CDR had significantly higher UFC (Table 3.3). There was no effect of ethnicity, but there was a significant ethnicity-by-CDR interaction: Caucasians but not Asians with higher CDR had higher UFC, and among Caucasians, CDR and UFC tended to correlate (r=0.29, p=0.056). For ΔaBMD, there were no main effects of CDR (F=0.032-1.167, P=0.282-0.859) or ethnicity (F=0.635-1.264, P=0.263-0.427) and no interaction (F=0.029-0.263, P=0.609-0.866).

Table 3.3 Differences between healthy premenopausal women with higher and lower cognitive dietary restraint (by median split) in baseline anthropometrics, Δanthropometrics questionnaire scores, energy intakes, menstrual cycle characteristics, 24-hour urinary free cortisol and 2-year ΔaBMD (n=123)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Higher CDR&lt;sup&gt;a&lt;/sup&gt; (n=60)</th>
<th>Lower CDR&lt;sup&gt;b&lt;/sup&gt; (n=63)</th>
<th>P value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.9 ± 3.3</td>
<td>22.4 ± 3.4</td>
<td>0.399</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td>0.827</td>
</tr>
<tr>
<td>Caucasian</td>
<td>33.3</td>
<td>41.3</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>66.7</td>
<td>58.7</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 ± 1.0</td>
<td>162.8 ± 6.6</td>
<td>0.921</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.5 ± 1.2</td>
<td>56.3 ± 1.0</td>
<td>0.041</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>22.4 ± 0.3</td>
<td>21.2 ± 0.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>66.4 ± 0.8</td>
<td>64.4 ± 0.7</td>
<td>0.058</td>
</tr>
<tr>
<td>Bone free fat mass (kg)</td>
<td>18.0 ± 0.8</td>
<td>15.7 ± 0.6</td>
<td>0.025</td>
</tr>
<tr>
<td>Bone free lean mass (kg)</td>
<td>38.1 ± 0.7</td>
<td>37.5 ± 0.6</td>
<td>0.470</td>
</tr>
<tr>
<td>Bone free body fat (%)</td>
<td>31.6 ± 0.9</td>
<td>29.1 ± 0.8</td>
<td>0.033</td>
</tr>
<tr>
<td>Δ Height (%)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.349</td>
</tr>
<tr>
<td>Δ Weight (%)</td>
<td>0.7 ± 0.7</td>
<td>1.6 ± 0.7</td>
<td>0.332</td>
</tr>
<tr>
<td>Δ BMI (%)</td>
<td>0.2 ± 0.6</td>
<td>1.3 ± 0.8</td>
<td>0.297</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.9 ± 3.3</td>
<td>22.4 ± 3.4</td>
<td>0.399</td>
</tr>
<tr>
<td>Category</td>
<td>Higher CDR&lt;sup&gt;a&lt;/sup&gt; (n=60)</td>
<td>Lower CDR&lt;sup&gt;b&lt;/sup&gt; (n=63)</td>
<td>P value&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-------------------------------</td>
<td>--------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Δ Waist circumference (%)</td>
<td>2.8 ± 0.6</td>
<td>3.4 ± 0.6</td>
<td>0.473</td>
</tr>
<tr>
<td>Δ Bone free fat mass (%)</td>
<td>2.5 ± 2.0</td>
<td>5.6 ± 2.5</td>
<td>0.339</td>
</tr>
<tr>
<td>Δ Bone free lean mass (%)</td>
<td>0.9 ± 0.5</td>
<td>0.6 ± 0.5</td>
<td>0.663</td>
</tr>
<tr>
<td>Δ Bone free percent body fat (%)</td>
<td>1.0 ± 1.4</td>
<td>3.3 ± 1.8</td>
<td>0.309</td>
</tr>
<tr>
<td>Total physical activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.9 ± 0.2</td>
<td>7.7 ± 0.2</td>
<td>0.478</td>
</tr>
<tr>
<td>Sport activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>0.388</td>
</tr>
<tr>
<td>Disinhibition&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.8 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>0.006</td>
</tr>
<tr>
<td>Hunger&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.2 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>0.665</td>
</tr>
<tr>
<td>General stress Z-score&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.04 ± 0.1</td>
<td>-0.05 ± 0.1</td>
<td>0.536</td>
</tr>
<tr>
<td>Energy intake&lt;sup&gt;g&lt;/sup&gt; (kcal)</td>
<td>1676 ± 61</td>
<td>1443 ± 60</td>
<td>0.009</td>
</tr>
<tr>
<td>Study hormone users (%)</td>
<td>13.3</td>
<td>15.9</td>
<td>0.690</td>
</tr>
<tr>
<td>Duration study hormone use (months)</td>
<td>7.8 ± 2.6</td>
<td>8.2 ± 1.5</td>
<td>0.894</td>
</tr>
<tr>
<td>Number of cycles analysed</td>
<td>13.4 ± 0.9</td>
<td>13.8 ± 0.9</td>
<td>0.758</td>
</tr>
<tr>
<td>Δ Waist circumference (%)</td>
<td>2.8 ± 0.6</td>
<td>3.4 ± 0.6</td>
<td>0.473</td>
</tr>
<tr>
<td>Δ Bone free fat mass (%)</td>
<td>2.5 ± 2.0</td>
<td>5.6 ± 2.5</td>
<td>0.339</td>
</tr>
<tr>
<td>Δ Bone free lean mass (%)</td>
<td>0.9 ± 0.5</td>
<td>0.6 ± 0.5</td>
<td>0.663</td>
</tr>
<tr>
<td>Δ Bone free percent body fat (%)</td>
<td>1.0 ± 1.4</td>
<td>3.3 ± 1.8</td>
<td>0.309</td>
</tr>
<tr>
<td>Total physical activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.9 ± 0.2</td>
<td>7.7 ± 0.2</td>
<td>0.478</td>
</tr>
<tr>
<td>Sport activity&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>0.388</td>
</tr>
<tr>
<td>Disinhibition&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.8 ± 0.4</td>
<td>5.3 ± 0.4</td>
<td>0.006</td>
</tr>
<tr>
<td>Hunger&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.2 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>0.665</td>
</tr>
<tr>
<td>General stress Z-score&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.04 ± 0.1</td>
<td>-0.05 ± 0.1</td>
<td>0.536</td>
</tr>
<tr>
<td>Energy intake&lt;sup&gt;g&lt;/sup&gt; (kcal)</td>
<td>1676 ± 61</td>
<td>1443 ± 60</td>
<td>0.009</td>
</tr>
<tr>
<td>Study hormone users (%)</td>
<td>13.3</td>
<td>15.9</td>
<td>0.690</td>
</tr>
<tr>
<td>Duration study hormone use (months)</td>
<td>7.8 ± 2.6</td>
<td>8.2 ± 1.5</td>
<td>0.894</td>
</tr>
<tr>
<td>Cycle length&lt;sup&gt;h&lt;/sup&gt; (days)</td>
<td>31.5 ± 0.5</td>
<td>30.1 ± 0.5</td>
<td>0.060</td>
</tr>
<tr>
<td>Subclinical ovulatory disturbances&lt;sup&gt;i&lt;/sup&gt; (%)</td>
<td>55.8 ± 4.0</td>
<td>34.1 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caucasian</td>
<td>64.5 ± 6.5</td>
<td>33.3 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>47.1 ± 4.7</td>
<td>34.9 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>UFC&lt;sup&gt;i&lt;/sup&gt; (µg/24-hour)</td>
<td>28.0 ± 1.2</td>
<td>24.0 ± 1.1</td>
<td>0.021</td>
</tr>
<tr>
<td>Caucasian</td>
<td>32.0 ± 2.1</td>
<td>22.6 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>25.8 ± 1.4</td>
<td>25.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Total body ΔaBMD&lt;sup&gt;j&lt;/sup&gt; (%)</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.424</td>
</tr>
<tr>
<td>L1-4 ΔaBMD&lt;sup&gt;j&lt;/sup&gt; (%)</td>
<td>1.0 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>0.323</td>
</tr>
</tbody>
</table>
Higher CDR\(^a\) (n=60) & Lower CDR\(^b\) (n=63) & P value\(^c\) \\
Hip ΔaBMD\(^d\) (%) & -0.1 ± 0.2 & 0.4 ± 0.3 & 0.292

Data are presented as mean ± standard error. Questionnaire scores, energy intakes and UFC values are averages from assessments at baseline, and both follow-ups because values did not change over time by repeat measures General Linear Model. CDR; cognitive dietary restraint; UFC, 24-hour urinary free cortisol; ΔaBMD, annualised 2-year percent change in areal bone mineral density (g/cm\(^2\)); L1-4, lumbar vertebrae 1 to 4.

a. Women with Three Factor Eating Questionnaire Restraint subscale scores higher than or equal to the median (7.7) score.
b. Women with Three Factor Eating Questionnaire Restraint subscale scores below the median (7.7) score.
c. Level of significance of difference between women with higher and lower CDR by independent t-test or General Linear Model adjusted for covariates.
d. Baecke Habitual Physical Activity Questionnaire, possible scores for sport 1-5 and total 3-15.
e. Three Factor Eating Questionnaire subscale scores: Disinhibition (0-16); and Hunger (0-14).
f. Z-score of the Perceived Stress Scale and Daily Stress Inventory Impact and Frequency subscales assessed on the days of urine collection.
g. Adjusted for body mass index (kg/m\(^2\)).
h. N=114; Adjusted for weight (kg) and gynaecological age.
i. N=114; Adjusted for baseline gynaecological age and body mass index (kg/m\(^2\)). Interactive effect of ethnicity-by-CDR: F=3.103, P=0.081. Main effect of ethnicity: F=1.930, P=0.168.
j. Adjusted for urine volume (L/24 hour). Interactive effect of ethnicity-by-CDR: F=4.5866, P=0.034. Main effect of ethnicity: F=0.218, P=0.641.

### 3.3.7 Differences by subclinical ovulatory disturbances median split

Participants were classified by median split as those with higher (≥38.8% of cycles had a short luteal phase or were anovulatory) or lower (<38.8%) subclinical ovulatory disturbances as shown in Table 3.4. There were no differences in energy intake, number of cycles analysed, General Stress score or physical activity level. Women with a higher frequency of subclinical ovulatory disturbances were younger, had lower gynaecological age, more positive Δlean mass, and BMI tended to be higher. Other baseline or Δ anthropometrics, and the frequency or duration of study hormone use did not differ. Findings were consistent when analyses were repeated including only those with normal BMI levels (18.5-24.9 kg/m\(^2\)) and when those having >10, >5 and >3 cycles of available data were analysed (data not shown).

After adjusting for baseline gynaecological age and BMI (and Δlean mass for ΔaBMD, Table 3.4), women with more frequent ovulatory disturbances reported higher CDR scores and had less positive hip and L1-4 ΔaBMD. Other questionnaire scores, total body ΔaBMD and UFC (urine volume as additional covariate) did not differ. No study outcome variables showed a significant main effect of ethnicity or an ethnicity-by-ovulatory disturbances interaction (data not shown).
Table 3.4 Differences between healthy premenopausal women with higher and lower percentage of cycles with subclinical ovulatory disturbances (median split) in menstrual cycle characteristics, age, anthropometrics, Δanthropometrics, questionnaire scores, 24-hour urinary free cortisol and 2-year ΔaBMD (n=114)

<table>
<thead>
<tr>
<th></th>
<th>Higher Subclinical Ovulatory Disturbances&lt;sup&gt;a&lt;/sup&gt; (n=57)</th>
<th>Lower Subclinical Ovulatory Disturbances&lt;sup&gt;b&lt;/sup&gt; (n=57)</th>
<th>P value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles analysed</td>
<td>12.8 ± 0.9</td>
<td>14.5 ± 0.9</td>
<td>0.193</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>30.9 ± 0.5</td>
<td>30.7 ± 0.6</td>
<td>0.754</td>
</tr>
<tr>
<td>Study hormone users (%)</td>
<td>17.5</td>
<td>15.8</td>
<td>0.802</td>
</tr>
<tr>
<td>Duration study hormone use (months)</td>
<td>8.9 ± 2.2</td>
<td>8.5 ± 2.1</td>
<td>0.889</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.4 ± 0.4</td>
<td>22.9 ± 0.5</td>
<td>0.011</td>
</tr>
<tr>
<td>Gynaecological age (years)</td>
<td>8.5 ± 0.4</td>
<td>10.8 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td>0.847</td>
</tr>
<tr>
<td>Caucasian</td>
<td>38.6</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>61.4</td>
<td>63.2</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.5 ± 1.0</td>
<td>163.2 ± 0.8</td>
<td>0.602</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.6 ± 1.1</td>
<td>57.1 ± 1.2</td>
<td>0.360</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>22.3 ± 0.3</td>
<td>21.4 ± 0.3</td>
<td>0.085</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>65.8 ± 0.8</td>
<td>64.7 ± 0.8</td>
<td>0.342</td>
</tr>
<tr>
<td>Bone free fat mass (kg)</td>
<td>17.5 ± 0.8</td>
<td>16.1 ± 0.7</td>
<td>0.195</td>
</tr>
<tr>
<td>Bone free lean mass (kg)</td>
<td>37.6 ± 0.6</td>
<td>38.0 ± 0.7</td>
<td>0.725</td>
</tr>
<tr>
<td>Bone free percent body fat (%)</td>
<td>31.3 ± 0.9</td>
<td>29.2 ± 0.9</td>
<td>0.092</td>
</tr>
<tr>
<td>Δ Height (%)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Δ Weight (%)</td>
<td>1.1 ± 0.6</td>
<td>1.3 ± 0.9</td>
<td>0.830</td>
</tr>
<tr>
<td>Δ BMI (%)</td>
<td>0.5 ± 0.6</td>
<td>1.0 ± 0.9</td>
<td>0.625</td>
</tr>
<tr>
<td>Δ Waist circumference (%)</td>
<td>3.1 ± 0.8</td>
<td>3.0 ± 0.6</td>
<td>0.950</td>
</tr>
<tr>
<td>Δ Bone free fat mass (%)</td>
<td>2.9 ± 1.9</td>
<td>5.5 ± 2.8</td>
<td>0.441</td>
</tr>
<tr>
<td>Δ Bone free lean mass (%)</td>
<td>1.6 ± 0.5</td>
<td>0.0 ± 0.4</td>
<td>0.018</td>
</tr>
<tr>
<td>Δ Bone free percent body fat (%)</td>
<td>0.7 ± 1.5</td>
<td>3.7 ± 2.0</td>
<td>0.227</td>
</tr>
<tr>
<td>Total physical activity&lt;sup&gt;de&lt;/sup&gt;</td>
<td>7.9 ± 0.2</td>
<td>7.7 ± 0.2</td>
<td>0.621</td>
</tr>
<tr>
<td>Sport activity&lt;sup&gt;de&lt;/sup&gt;</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>0.963</td>
</tr>
<tr>
<td>Restraint&lt;sup&gt;e&lt;/sup&gt;</td>
<td>8.7 ± 0.5</td>
<td>7.1 ± 0.5</td>
<td>0.040</td>
</tr>
<tr>
<td>Disinhibition&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.3 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>0.425</td>
</tr>
<tr>
<td>Hunger&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.5 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>0.431</td>
</tr>
</tbody>
</table>
### Table 3.3

<table>
<thead>
<tr>
<th></th>
<th>Higher Subclinical Ovulatory Disturbances&lt;sup&gt;a&lt;/sup&gt; (n=57)</th>
<th>Lower Subclinical Ovulatory Disturbances&lt;sup&gt;b&lt;/sup&gt; (n=57)</th>
<th>P value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>General stress Z-score&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
<td>0.868</td>
</tr>
<tr>
<td>Energy intake&lt;sup&gt;e&lt;/sup&gt; (kcal)</td>
<td>1588 ± 67</td>
<td>1516 ± 67</td>
<td>0.468</td>
</tr>
<tr>
<td>UFC&lt;sup&gt;b&lt;/sup&gt; (µg/24-hour)</td>
<td>25.8 ± 1.3</td>
<td>25.6 ± 1.3</td>
<td>0.894</td>
</tr>
<tr>
<td>Total body ΔaBMD&lt;sup&gt;i&lt;/sup&gt; (%)</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>0.775</td>
</tr>
<tr>
<td>L1-4 ΔaBMD&lt;sup&gt;i&lt;/sup&gt; (%)</td>
<td>0.7 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>0.034</td>
</tr>
<tr>
<td>Hip ΔaBMD&lt;sup&gt;i&lt;/sup&gt; (%)</td>
<td>-0.6 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error. Questionnaire scores, energy intakes and UFC values are averages from assessments at baseline and both follow-ups because values did not change over time by repeat measures General Linear Model. UFC, 24-hour urinary free cortisol; ΔaBMD, annualised 2-year percent change in areal bone mineral density (g/cm<sup>2</sup>); L1-4, lumbar vertebrae 1 to 4.

a. Menstrual cycles were anovulatory and/or had a luteal phase length <10 days by least squares quantitative basal temperature analysis ≥38.8% of the time.
b. Menstrual cycles were anovulatory and/or had a luteal phase length <10 days by least squares quantitative basal temperature analysis <38.8% of the time.
c. Level of significance of difference between women with higher and lower percentage of cycles with subclinical ovulatory disturbances by independent t-test or General Linear Model adjusted for covariates.
d. Baecke Habitual Physical Activity Questionnaire, possible scores for sport 1-5 and total 3-15.
e. Adjusted for baseline gynaecological age and body mass index (kg/m<sup>2</sup>).
f. Three Factor Eating Questionnaire subscales scores: Restraint (possible score 0-21); Disinhibition (0-16); and Hunger (0-14).
g. Z-score of the Perceived Stress Scale and Daily Stress Inventory Impact and Frequency subscales assessed on the days of urine collection.
h. Adjusted for urine volume, and baseline gynaecological age and body mass index (kg/m<sup>2</sup>).
i. Adjusted for change in lean mass, and baseline gynaecological age and body mass index (kg/m<sup>2</sup>).

### 3.4 Discussion

The purpose of this study was to examine whether the frequency of subclinical ovulatory disturbances and UFC differed by level of CDR, and if these variables affected change in aBMD over two years in healthy young women. We confirmed previous reports of an increased frequency of subclinical ovulatory disturbances and higher UFC among women with higher CDR (Table 3.3). We also confirmed that less positive aBMD changes occurred in women with more frequency subclinical ovulatory disturbances. However, UFC did not differ by the percentage of subclinical ovulatory disturbances (Table 3.4) and there was no difference in aBMD change by CDR level (Table 3.3). Additionally, UFC was not associated aBMD change. Consequently, whether cortisol mediates the relationship between CDR, ovulatory disturbances and aBMD (Figure 3.1) still remains to be established.

The most noteworthy finding of the current study was the confirmation of less positive aBMD changes at lumbar spine among women with more frequent subclinical...
ovulatory disturbances [12-15,17]. We also found less positive hip aBMD change in
women with more frequent ovulatory disturbances. It is well established that overt
menstrual cycle abnormalities lead to bone loss [10]. Yet, whether anovulation and short
LPL are associated with bone loss remains controversial [9]. Our findings suggest that
they are, although their impact is modest. One potential reason for conflicting findings
regarding bone and ovulatory disturbances could be the duration of ovulatory
observations. As ovulatory function is highly variable [42], long-term monitoring is critical
to correctly identify women with subclinical disturbances. The studies which did not
observe associations between ovulatory disturbances and bone monitored two to four
cycles [11,16]. In contrast, the current study and most others that did see a relationship
monitored nine to 14 cycles [13,15,17].

Our results corroborate that ovulatory disturbances are more common among women
reporting higher CDR [6-8,43-44]. The only other prospective study of CDR and ovulatory
function did not find a difference by CDR level in the proportion of women with ≥3 subclinical
ovulatory disturbances cycles [17]. The null relationship between CDR and subclinical ovulatory
disturbances in that study is most likely due to the very low proportion of women with ≥3 cycles
with subclinical ovulatory disturbances (approximately 7%), making detection of a difference
more difficult. The authors did not describe why they classified women on that basis, but the low
prevalence of subclinical ovulatory disturbances may be related to their sample’s greater
gynaecological maturity, such that psychosocial stress would be less likely to affect cycles.
Furthermore, the definition of short LPL in that study (<10 days by urinary LH surge detection)
may underestimate the prevalence of cycles with prevalence. Urine LH peaks before follicular
collapse by ultrasound [45], whereas the significant rise in basal temperature detected with LS-
QBT occurs ~2 days after the LH peak [37]. To equate the two methods, the criterion for short
LPL based on urinary LH would be <11-12 days, rather than <10 DAYS used with LS-QBT.

Additional support that eating and body stresses can lead to menstrual cycle and
ovulatory disturbances comes from studies using measures other than Restraint. Higher scores
on the Eating Attitudes Test, and the Drive For Thinness and Bulimia subscales of the Eating
Disorder Inventory have been reported in women with functional hypothalamic amenorrhea
(FHA) versus women with organic causes of amenorrhea and/or regularly menstruating women
[46-48].

It has been suggested that normal- or under-weight women with higher CDR experience
more frequent subclinical ovulatory disturbances due to caloric restriction and other dieting
behaviours [17]. However, examination of data from studies, which observed relationships
among ovulatory disturbances and CDR or similar eating attitudes [6-8,43-44,46-48], suggest
that mechanism is unlikely. In our current sample, for example, physical activity did not differ by
CDR level and women with higher CDR actually had higher BMI values and energy intakes (Table 3.3). Moreover, we used the EDE questionnaire to establish that participants did not exhibit clinical eating disorders [34-35,41]. Therefore, it is unlikely that an energy deficit in women with higher CDR caused ovulatory disturbances in the current study. Finally, while various life stresses are associated with anovulation and short LPL cycles [49], among our participants general stress was not associated with subclinical ovulatory disturbances and did not differ by CDR level. In fact the only measured variable that differed by the frequency of ovulatory disturbances in our sample was CDR score and ΔaBMD.

Contrary to our hypothesis, we did not confirm that cortisol plays a role in mediating the relationships among CDR, ovulatory disturbances and change in bone density. While UFC was higher among women with higher CDR, as previously reported [24-28], it was not correlated with CDR score in the entire group, and did not differ by %SOD level. It is generally accepted that stress-induced HPA axis activation is related to menstrual cycle and ovulatory disturbances [49]. Corticotropin-releasing hormone alters pulsatile gonadotropin-releasing hormone (GnRH) release (Figure 3.1), this leads to impaired secretion of reproductive hormones and a spectrum of disturbances of decreasing severity from amenorrhea to oligomenorrhea, to regular cycles with anovulation or short luteal phases [23]. However, it could be that eating and body stress impacts ovulatory function via neuroendocrine pathways that do not involve the HPA axis. The secretion of GnRH can be affected by numerous neurotransmitters and neuropeptides of which several relate to appetite control [49]. This may be relevant to CDR in which to women attempt to override physiological cues to hunger.

Furthermore, we found that UFC was elevated in Caucasians with higher CDR (and that UFC and CDR tended to correlate in Caucasians), but UFC did not differ by CDR level among Asians. In a study of young, healthy, regularly menstruating women, Asian women had 6-beta-hydroxycortisol: cortisol ratios that were two to three times lower than Caucasians [40]. This is significant as the 6-beta-hydroxycortisol: cortisol ratio is an indirect indicator of cytochrome P450 3A4 activity, an enzyme that is involved in the metabolism of steroids including cortisol, estradiol and progesterone [40]. However, as has also been reported by others [50-51], we did not see a difference in UFC by ethnicity. Moreover, Asians with higher CDR, despite having similar UFC as Asians with lower CDR, had more frequent subclinical ovulatory disturbances. Taken together, this suggests that cortisol may not mediate the association between CDR and ovulatory function.

That UFC differed by level of CDR among Caucasians but not Asians is an interesting finding. There were no differences in CDR, general stress, the frequency of subclinical ovulatory disturbances, UFC or aBMD change by ethnicity. If cortisol is metabolised more rapidly by Asians [40], we would still expect to see the same pattern of difference by CDR, although lower
absolute levels. It could be that despite similar CDR scores, the qualitative experience of eating and body-related stress differs between Asians and Caucasians. The influence of ethnicity on the experience of CDR as a stressor has not yet been explored.

We also did not find an association between UFC and change in aBMD. Although reduced BMD is observed in hypercortisolism [23], it is less clear if this occurs in healthy young women, when cortisol is elevated yet remains within the normal range [5,7,18-22,29]. Estradiol may mediate the relationship between cortisol and bone: women who continue to menstruate, such as our participants, would likely have normal estradiol levels. A major negative effect of cortisol on bone may be prevented by estrogen’s antiresorptive effects [52]. This may explain why studies including women with oligomenorrhea found an association between higher restraint and lower aBMD [5,19]. It could also be that the association of bone change with elevated cortisol within the normal range is relatively subtle, and difficult to detect over two years. In fact, we observed a modest association between higher UFC and lower aBMD and BMC in this sample at baseline [29]. Although differences in the rate of bone loss between those with slightly elevated versus lower cortisol may be relatively small, over time the accumulated affects could substantially impact aBMD and fracture risk.

This study was not without limitations. Our sample was relatively homogeneous and our findings are generalisable only to those with similar characteristics. We did not account for osteoporosis family history or physical activity during adolescence. Both may be associated with aBMD in healthy premenopausal women. Twenty-two women used hormonal contraceptives during the study, however, these did not influence ΔaBMD. Moreover, in our study women initiated hormone use for contraception, not because of menstrual abnormalities. Although we screened for polycystic ovarian syndrome based on clinical symptoms, androgen levels were not measured. Our method of observing ovulatory function is not as accurate as cyclic determinations of reproductive hormones. However, the quantitative basal temperature method we used, LS-QBT, has been validated [37-38], is inexpensive and is acceptable to women. It should be noted that our method differs from previous qualitative methods in which basal temperature was plotted and each chart was visually inspected to determine if a shift is apparent. We used a computer programme to conduct quantitative analyses where a least squares criterion is used to determine ovulation if the maximum mean temperature difference between the first and latter parts of the cycle is statistically significant [37]. Furthermore, given within-person variability in ovulatory function, especially LPL [42], accurate characterisation requires that cycles be monitored over a long period. This method allowed us to accomplish this.
The use of DXA to measure aBMD may also be a limitation. DXA assesses bone mass rather than bone strength. Additional prospective studies examining eating attitudes, ovulatory function and bone would be improved by using quantitative computed tomography, which differentiates between cortical and trabecular bone or other measures that can document bone micro-architecture.

Our study contributes to the emerging field of research linking psychosocial and physiological health, a field that is “changing what it means to be healthy” by defining well-being not only by our behaviours but our attitudes [53]. Interestingly, cognitive behavioural therapy has been shown to improve ovulatory function among women with FHA [53]. Whether cognitive behavioural therapy may help young women with disordered eating attitudes warrants investigation.

In summary, we confirmed that healthy premenopausal women higher CDR experienced more frequent subclinical ovulatory disturbances and that a higher occurrence of these disturbances resulted in less positive changes in bone density over two years. Although the magnitude of the effect on bone was modest, subclinical ovulatory disturbances may have a persistent negative influence on bone in young women [15]. Contrary to our hypothesis, although UFC was higher in women with greater CDR, we did not confirm that cortisol played a role in mediating associations among CDR, subclinical ovulatory disturbances and change in aBMD. Future studies would be improved by examining other potential mechanisms including neuropeptides. The high variability in BMD and ovulatory function indicate the need for a large sample with longer follow-up in order to firmly establish whether young women’s eating attitudes can affect bone.
3.5 References


Chapter 4:

Negative eating and body attitudes are associated with higher daytime ambulatory blood pressure in healthy young women.\(^1\)

\(^1\) A version of this chapter has been submitted for publication:
Bedford JL, Linden W, Barr SI. Negative eating and body attitudes are associated with elevated daytime ambulatory blood pressure in healthy young women.
Date of Submission: April 2010.
4.1 Introduction

For more than 100 years, cardiovascular disease (CVD) has been the leading cause of death among American adults [1]. High blood pressure (BP) or hypertension (systolic BP >140 or diastolic BP >90 mm Hg) is one of the strongest CVD risk factors [2] and affects nearly one in three Americans [1]. Although the prevalence of hypertension among young adults is low [1], BP while young is associated with BP later in life [3]. Additionally, young adult BP is positively correlated with atherosclerosis [4] and also predicts carotid intima-media thickness [5], another CVD risk marker. Furthermore, young adults with prehypertension (systolic BP 120-139 or diastolic BP 80-89 mm Hg) were shown to have an increased risk of coronary calcium atherosclerosis 15-20 years later, after adjustment for other risk factors including current BP [6]. Thus, BP level even in the young and healthy is related to future CVD risk, accentuating the need to fully understand factors that influence BP in this population.

Evidence is accumulating that the subjective experience of stress may influence cardiovascular outcomes, mediated by the physiological stress response [7]. When a stressor is perceived, the central nervous system and peripheral components are activated, seeking to maintain homeostasis via adaptive responses to deal with the perceived threat [8]. Two allostatic mediators of the stress response are activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased secretion of the stress hormone cortisol, and stimulation of the sympathetic nervous system which causes BP to rise [8]. Increased cortisol and BP are beneficial during acute stress; however, continuous elevations can lead to allostatic overload, causing “wear-and-tear” on body systems [8]. High cortisol levels may also be independently associated with increased BP [9].

Exposure to laboratory stressors clearly elevates BP and cortisol in otherwise healthy adults [10]. It is less clear whether chronic psychosocial stressors of limited salience are sufficient to increase BP and cortisol [7]. When evaluating the physiological effects of chronic stress, the measurement of clinical BP may be of limited relevance. Perceived stress that is encountered over the course of the day, rather than discrete events or laboratory tasks, may not be captured by a single measurement of BP. Therefore, ambulatory BP (ABP) monitoring while performing the activities of daily living may be a more sensitive tool. After controlling for CVD risk factors including clinical BP, ABP is independently associated with cardiovascular morbidity and mortality [11-13].

Occupational stressors have received the most attention in the investigation of stress, cortisol and ABP. The results among middle-aged women are inconsistent such that some [14-18] but not all [15,19-21] studies report higher ABP and cortisol in women reporting higher occupation-related stress. It could be that women experience other stressors that either interact with work stress or are more relevant to stress perception [22-24]. Other chronic stressors
associated with ABP and cortisol identified among women include financial stress [25-26], family/marital stress [15,27-29] and lack of social support [30-31]. Given that the majority of women report negative attitudes towards food and body [32-34], we and others have hypothesised that eating/body attitudes may be subtle but chronic daily stressors sufficient to activate the physiological stress response, and potentially lead to negative health outcomes.

A large number of psychometric scales have been developed to assess eating/body attitudes [35], and some studies have detected associations between increasing scores on these scales and cortisol in healthy women [36-41]. Most work in the area of eating/body stress has employed the Three Factor Eating Questionnaire (TFEQ) Restraint subscale to assess cognitive dietary restraint: the *perception* that one is constantly monitoring and attempting to limit food intake in an effort to achieve or maintain a perceived ideal body weight [42].

Generally, there are no or only minimal differences by level of dietary restraint in women’s self-reported energy intakes, relative weight, weight changes or dieting behaviour [34,43-44]. This suggests that some negative eating/body attitudes are not necessarily indicative of dieting or disordered eating behaviours. Taken together, these studies also support the idea that the *experience* of negative eating/body attitudes could be associated with the physiological stress response. It therefore seems reasonable to ask whether or not these attitudes are also associated with BP. The authors are aware of only one study that examined eating attitudes in relation to ABP (Koo-Loeb et al., 2000).[45]. In that study, no differences in 24-hour ABP were observed between healthy university-aged women with very high or very low scores on the Eating Disorder Inventory Bulimia subscale who did not meet the diagnostic criteria for eating disorders; although 24-hour urinary cortisol was higher in women with higher scores [45].

Participants completed the ABP and urine assessments after administration of the diagnostic interview for bulimia nervosa; answering psychosocial questionnaires; and performing the laboratory stress test [45]. Thus, findings do not reflect participants' “usual” cortisol and ABP, which was the goal of the current study.

Therefore, given that psychosocial stressors are capable of elevating ABP in healthy middle-aged women [14-16,22-25,27-31], and that negative eating/body attitudes are common among women [32-34], it is reasonable to postulate that eating/body attitudes may be a source of subtle but chronic stress with the potential to elevate BP. This relationship could be most evident among university-aged women, since others stressors (i.e. occupational, family) would be less significant for most. Thus, the objective of this study was to examine whether women with negative versus neutral/positive eating/body attitudes had higher 24-hour urinary free cortisol (UFC) and daytime ABP. To fully conceptualise the experience of eating/body stress, several body image and eating attitude questionnaires, which have previously been associated
with cortisol [36-41], were included because we hypothesised that they may also be associated with BP, an additional health outcome of chronic physiological stress.

In order to differentiate stress that is specific to eating and body image from “general stress”, chronic perceived stress and stressful events for the days of cortisol and ABP monitoring were assessed. We hypothesised that those with negative eating/body attitudes were not highly stressed people in general, but experienced stress specific to food and weight. Finally, in order to distinguish between the potential effect of cognitive versus behavioural aspects of eating/body attitudes on ABP and cortisol, current weight loss effort was also examined. We hypothesised that cortisol and ABP would not differ by weight loss effort, supporting our hypothesis that it is the subjective experience of stress related to food and weight, rather than behaviours, that are associated with negative health outcomes.

4.2 Methods
4.2.1 Participants

Potential participants were recruited between August and December 2006 for a 2-y bone density study from university classes and using poster advertisements (Appendix 13). Eligibility was assessed by telephone interview in 148 interested women (Appendix 15). Criteria included: age 19-35, no pregnancy/breastfeeding currently or within 12 months, regular menstrual cycles (menses every 21-35 days in the previous ≥6 months), non-obese (self-reported body mass index (BMI) 18-30 kg/m²), consistent sleep patterns (arise and retire at approximately the same time most days) and absence of medical conditions (current or previous diagnosis of eating disorder, polycystic ovarian syndrome, Cushing’s syndrome, inflammatory conditions, hypertension, hyperthyroidism or hirsutism) or use of medications (oral contraceptives, progesterone or glucocorticoids currently or within the past 6 months) that could affect study variables. Of the 142 eligible women screened, 140 were oriented to the study. Data collection for the cross-sectional study presented here occurred 6-12 months following enrollment. During that time interval, seven participants moved, four no longer wanted to participate and two became ineligible. Results are reported for the 120 women with complete data. The study protocol was approved by the university’s Clinical Research Ethics Board (Appendix 17), and written informed consent was obtained from all participants (Appendix 16). Participants were provided with travel compensation (Appendix 18) and a $30 gift card for their participation (Appendix 19).

4.2.2 Procedure

Participants met with an investigator at UBC for study orientation. A questionnaire package (Appendix 23) was given to complete at home, which included a food frequency
questionnaire and validated self-report instruments (Appendix 24), as well as questions to elicit demographic information and weight loss effort. Height and weight were measured in duplicate. From these data, BMI was calculated.

Participants were fitted with an ABP monitor including demonstration of cuff placement on the non-dominant arm over the brachial artery. A sample reading was performed to familiarise them with the process. Monitoring for 12-h was completed within three days on a “normal day”, avoiding any unusual physical or mental stresses, or heavy physical activity while wearing the monitor. Detailed written instructions similar to those provided verbally were given for review prior to starting the procedure (Appendix 26). The monitor was programmed to take blinded measurements of ABP and heart rate every 30 minutes. Participants were instructed to keep their arm still during readings and, if possible, to be seated. If there was too much movement, the monitor was programmed to abort and re-try one minute later. Immediately after each reading, participants recorded their concurrent activity in a provided diary (Appendix 26). After 12 hours, participants removed the monitor and completed the Daily Stress Inventory [46] (Appendix 25).

Materials and oral and written instructions for home completion of a 24-hour urine collection were reviewed (Appendix 20). Participants were instructed to complete the urine collection within several weeks of the meeting, on a different “normal day”, after reviewing written instructions. On the day of collection, participants discarded their first urine void, recorded the time this occurred and then collected all subsequent voids for 24 hours including a void at the recorded time the following morning. After their last void, participants completed the Daily Stress Inventory [46] (Appendix 25).

4.2.3 Questionnaires
4.2.3.1 Eating and body attitudes

The TFEQ pertains to three dimensions of eating attitudes: Cognitive Dietary Restraint, the perception that one is constantly monitoring and attempting to limit food intake to achieve a perceived ideal body weight; Disinhibition, which is the tendency to overeat when restraint is removed; and Hunger, which assesses susceptibility to hunger [42]. Two subscales from the Eating Disorders Inventory were included: Drive for Thinness with higher scores indicating extreme concern with weight, dieting and the intense pursuit of thinness, and Bulimia which assesses one’s tendency to think about and engage in uncontrolled overeating [47]. The shortened Body Shape Questionnaire was used to measure participants’ body dissatisfaction caused by feelings of being fat [48]. The Beliefs About Appearance Scale assesses the degree of agreement with beliefs about the perceived importance of appearance for relationships, achievement, self-view and feelings [49]. These beliefs are thought to underlie the desire to
restrict eating, criticise the body and focus on appearance-related stimuli [49]. From these seven questionnaires/subscales, a single standardised “Eating/Body Attitude” Z-score was calculated.

4.2.3.2 General stress

The Perceived Stress Scale was used to evaluate participants’ perception of stress during the previous month [50]. To account for everyday minor stressful events, the Daily Stress Inventory [46] (Appendix 25) was completed after ABP and cortisol assessments. Participants indicated the frequency of 58 everyday minor stressful events which may have occurred during the day (Frequency score). They also ranked the intensity (Impact score) on a scale of 1 (“not at all stressful”) to 7 (“caused me to panic”). From these three questionnaires/subscales, a single standardised “General Stress” Z-score was calculated.

4.2.3.3 Weight loss effort

Participants were asked “are you currently trying to lose weight?” and grouped as those reporting and not reporting current weight loss attempts. To determine energy intake, the Diet History Questionnaire (version 1.0, National Institutes of Health, Applied Research Program, National Cancer Institute, 2002) was completed. Scannable questionnaires were analysed with a Canadian version of the programme [51]. All reported energy intakes were within range considered biologically plausible (600-3500 kcal).

4.2.4 Urine analysis

At the Vancouver General Hospital Laboratory, 24-hour urine volume was measured in duplicate, and aliquots were frozen and stored prior to analysis of urinary free cortisol (UFC, µg/24 hour) by high-throughput liquid chromatography and tandem mass spectrometry [52].

4.2.5 ABP measurement

The Spacelabs 90207 ABP monitor (Redmond, WA) measured 12-hour average systolic BP), diastolic BP, mean arterial pressure and heart rate. Monitoring for 12 hours relative to 24 hours avoids discomfort during sleep [53] and provides meaningful data regarding stress during participants’ typical activities. We have found that 8-hour and 24-hour measurements correlated with r>0.90 (unpublished observation).

Participants’ data were reviewed following the modified Casadei criteria [54]. Readings were considered artifactual if: systolic BP <70 or >240 mm Hg, diastolic BP <40 or >140 mm Hg, or heart rate <40 or >125 beats per minute. When any of these criteria were met, all data for that time point were excluded. This resulted in 18 participants having one reading excluded and
three participants having two readings. Paired t-tests revealed that ABP before and after data editing were not significantly different (data not shown). After editing, the mean ± standard deviation (SD) number of readings per participant was 23.4±1.5, range 18-26.

To account for physical activity during ABP monitoring, participants recorded their activity in a diary at the time of each ABP measurement. Each entry was coded as either sedentary and given a score of one (e.g. sitting in class/work, watching television, studying/reading) or active and given a score of two (e.g. laundry, cooking, walking). A continuous score for activity during ABP (ABP-activity) was derived by summing the diary codes and dividing by the number of readings available for each participant. As physical fitness may also be associated with ABP, the Baecke Questionnaire of Habitual Physical Activity was used to determine usual activity levels at work, in sport, and during leisure [55].

4.2.6 Statistical analyses

Data were coded, verified, entered into SPSS software (version 17, SPSS Inc., 2008) and crosschecked for accuracy. Physiologic variables were examined for outliers (mean ± >4SD) and none were present. Descriptive statistics were used to characterise the sample.

Single standardised “Eating/Body Attitude” and “General Stress” Z-scores were calculated. For each questionnaire, participants’ scores were subtracted from the corresponding mean and divided by the SD. The questionnaire Z-scores were then summed and divided by the number of scales/subscales included. Because higher scores on the eating and body attitude questionnaires reflect more negative eating/body attitudes, Z-scores were inverted so that women were classified as having either negative (Z-score <0) or neutral/positive (Z-score ≥0) attitudes towards food and body. Higher General Stress Z-score reflects higher levels of perceived psychosocial stress.

Pearson’s correlations were used to identify correlates that could potentially confound analyses of ABP and UFC. Partial correlations adjusted for potential confounders were conducted between Eating/Body Attitude Z-score, General Stress Z-score, UFC and ABP. Chi-square for categorical variables and independent t-tests and General Linear Modeling (with appropriate covariates) for continuous variables were used to examine differences between women with negative versus neutral/positive Eating/Body Attitudes, and between those reporting and not reporting current weight loss attempts. Interactive effects were also examined in order to differentiate between the cognitive and behavioural aspects of Eating/Body Attitudes. As cortisol metabolism may differ between Asians and Caucasians [56], interactions between ethnicity and Eating/Body Attitudes were examined with regard to UFC. For all analyses, cases were excluded pairwise and the significance level for all analyses was P≤0.05.
4.3 Results

4.3.1 Participant characteristics

All women were normotensive (systolic BP ≤135 and diastolic BP ≤85 mm Hg). Most participants were currently students (86%) and single (91%). All had completed some post-secondary education. Similar to the student population of UBC, 62.5% of the sample was Asian and the remainder was Caucasian. Current weight loss attempts were reported by 41%. Six women (5%) started using oral contraceptives between eligibility screening and completing the study procedures. Given this small number and that they had used them for ≤3 months, we did not discard their data. Table 4.1 describes participants’ mean age, BMI, questionnaire scores, energy intake, UFC and ABP.

4.3.2 Correlation analyses

As shown in Table 4.1, more negative Eating/Body Attitudes were associated with higher BMI and General Stress, and lower physical activity level. ABP was not associated with BMI, energy intakes or physical activity level (data not shown). Age was associated with diastolic BP \( (r=0.21, p=0.023) \). ABP-activity was associated with diastolic BP \( (r=0.18, p=0.046) \) and mean arterial pressure \( (r=0.22, p=0.015) \), and tended to be associated with systolic BP \( (r=0.18, p=0.053) \). Age and ABP-activity were therefore included as ABP covariates. After adjustment, there were still no relationships among ABP and BMI, physical activity level or energy intakes (data not shown). However, as BMI is associated with ABP in the literature and was associated with Eating/Body Attitudes, BMI was also included as an ABP covariate.

UFC was correlated with the volume (L) of urine collected \( (r=0.27, P=0.004) \), and was therefore included as a covariate. Volume-adjusted UFC was not associated with Eating/Body Attitudes. UFC was positively correlated with General Stress and ABP (with age, BMI and ABP-activity added as covariates). More negative Eating/Body Attitudes were associated with higher diastolic BP and mean arterial pressure, after controlling for age, BMI and ABP-activity. Higher General Stress was associated with higher BMI-adjusted energy intakes but was not associated with ABP after adjusting for covariates.
Table 4.1  Mean age, body mass index, questionnaire scores, energy intakes, 24-h urinary free cortisol (UFC) and 12-h daytime ambulatory blood pressure; and adjusted correlates of Eating/Body Attitude Z-score, General Stress Z-score, and UFC in healthy premenopausal women (n=120)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All participants</td>
<td>Eating/Body Attitudes Z-score</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.8 ± 3.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>21.8 ± 2.5</td>
<td>-0.35***</td>
</tr>
<tr>
<td>Physical Activity^b</td>
<td>7.7 ± 1.5</td>
<td>0.19*</td>
</tr>
<tr>
<td>General stress Z-score</td>
<td>0.0 ± 0.8</td>
<td>-0.23†</td>
</tr>
<tr>
<td>Energy intake^c (kcal)</td>
<td>1531 ± 513</td>
<td>-0.15</td>
</tr>
<tr>
<td>UFC^a (µg/24-h)</td>
<td>25.6 ± 1.3</td>
<td>-0.11</td>
</tr>
<tr>
<td>Systolic ABP^d (mm Hg)</td>
<td>115.2 ± 6.5</td>
<td>-0.10</td>
</tr>
<tr>
<td>Diastolic ABP^d (mm Hg)</td>
<td>71.8 ± 5.6</td>
<td>-0.24**</td>
</tr>
<tr>
<td>Mean arterial pressure^d (mm Hg)</td>
<td>86.2 ± 5.4</td>
<td>-0.22†</td>
</tr>
</tbody>
</table>

Correlation is significant at p<0.05 (*) or p<0.01 (**). ABP; mean 12-h daytime ambulatory blood pressure; Eating/Body Attitudes Z-score; inverted Z-score derived from the Three Factor Eating Questionnaire Restraint, Disinhibition and Hunger subscales, the Eating Disorder Inventory-2 Drive For Thinness and Bulimia subscales, the Body Shape Questionnaire and the Beliefs About Appearance Scale; General Stress Z-score; Perceived Stress Scale, and Daily Stress Inventory Frequency and Impact subscales collected on the days of cortisol and ABP assessment; SD, standard deviation; UFC; 24-h urinary free cortisol.

a. Partial correlation adjusted for urine volume (L/24-h).

b. Baekke Habitual Physical Activity Questionnaire, possible score 3-15.

c. Partial correlation adjusted for body mass index (kg/m^2).

d. Partial correlation adjusted for age, body mass index (kg/m^2) and activity during ABP monitoring.

4.3.3 Differences by Eating/Body Attitudes and weight loss effort

Women with more negative Eating/Body Attitudes were more likely to report current weight loss attempts than those with neutral/positive attitudes (63% versus 21%, P<0.001).

The main and interactive effects of Eating/Body Attitude level and weight loss effort on study outcome variables are presented in Table 4.2. There was a significant main effect of current weight loss effort on BMI, such that women currently trying to lose weight had significantly higher BMI values. There was no main effect on BMI of Eating/Body Attitude nor was there an Eating/Body Attitude-by-weight loss effort interaction.

No main or interactive effects of Eating/Body Attitudes or weight loss effort were observed on age, physical activity level, General Stress Z-score, BMI-adjusted energy intakes
or volume-adjusted UFC. Ethnicity did not have a main or interactive (with Eating/Body Attitude or weight loss effort) effect on UFC (data not shown).

A significant main effect of Eating/Body Attitude on ABP was detected, such that higher diastolic BP and mean arterial pressure were seen in those with more negative Eating/Body Attitudes. There was no main effect of weight loss effort or a weight loss effort-by-Eating/Body Attitude interaction on ABP. Differences in diastolic BP ($P=0.023$) and mean arterial pressure ($P=0.054$) by Eating/Body Attitude level also remained after the inclusion of physical activity as a covariate.
Table 4.2  Main and interactive effect of Eating/Body Attitude level and weight loss effort on age, body mass index, questionnaire scores, energy intakes, 24-h urinary free cortisol, and 12-h daytime ambulatory blood pressure (n=120).

<table>
<thead>
<tr>
<th></th>
<th>Negative Eating/Body Attitudes&lt;sup&gt;a&lt;/sup&gt; (n=57)</th>
<th>Neutral or Positive Eating/Body Attitudes&lt;sup&gt;b&lt;/sup&gt; (n=63)</th>
<th>Main effect</th>
<th>Trying to Lose Weight&lt;sup&gt;c&lt;/sup&gt; (n=49)</th>
<th>Not Trying to Lose Weight&lt;sup&gt;d&lt;/sup&gt; (n=71)</th>
<th>Main effect</th>
<th>Interactive effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.9 ± 0.5</td>
<td>22.8 ± 0.5</td>
<td>0.890</td>
<td>22.8 ± 0.6</td>
<td>22.8 ± 0.5</td>
<td>0.984</td>
<td>0.917</td>
</tr>
<tr>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>22.4 ± 0.3</td>
<td>21.8 ± 0.3</td>
<td>0.180</td>
<td>23.1 ± 0.4</td>
<td>21.1 ± 0.3</td>
<td>&lt;0.001</td>
<td>0.639</td>
</tr>
<tr>
<td>Physical activity&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.3 ± 0.2</td>
<td>7.8 ± 0.2</td>
<td>0.087</td>
<td>7.7 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>0.382</td>
<td>0.150</td>
</tr>
<tr>
<td>General Stress Z-score</td>
<td>0.15 ± 0.11</td>
<td>-0.07 ± 0.12</td>
<td>0.182</td>
<td>0.03 ± 0.13</td>
<td>0.05 ± 0.10</td>
<td>0.895</td>
<td>0.353</td>
</tr>
<tr>
<td>Energy intake&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1618 ± 74</td>
<td>1425 ± 83</td>
<td>0.086</td>
<td>1504 ± 91</td>
<td>1539 ± 70</td>
<td>0.766</td>
<td>0.579</td>
</tr>
<tr>
<td>UFC&lt;sup&gt;g&lt;/sup&gt; (µg/24-hour)</td>
<td>27.4 ± 1.7</td>
<td>25.6 ± 1.8</td>
<td>0.518</td>
<td>28.1 ± 2.0</td>
<td>24.8 ± 1.6</td>
<td>0.291</td>
<td>0.319</td>
</tr>
<tr>
<td>Systolic ABP&lt;sup&gt;h&lt;/sup&gt; (mm Hg)</td>
<td>115.5 ± 0.9</td>
<td>114.5 ± 1.0</td>
<td>0.440</td>
<td>115.5 ± 1.2</td>
<td>114.5 ± 0.9</td>
<td>0.529</td>
<td>0.245</td>
</tr>
<tr>
<td>Diastolic ABP&lt;sup&gt;h&lt;/sup&gt; (mm Hg)</td>
<td>73.2 ± 0.7</td>
<td>70.3 ± 0.8</td>
<td>0.011</td>
<td>71.8 ± 1.0</td>
<td>71.7 ± 0.7</td>
<td>0.967</td>
<td>0.510</td>
</tr>
<tr>
<td>Mean arterial pressure&lt;sup&gt;h&lt;/sup&gt; (mm Hg)</td>
<td>87.3 ± 0.7</td>
<td>84.9 ± 0.8</td>
<td>0.032</td>
<td>86.1 ± 0.9</td>
<td>86.1 ± 0.7</td>
<td>0.958</td>
<td>0.401</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error. P values represent main effect of Eating/Body Attitude level and weight loss effort, and the Eating/Body Attitude-by-weight loss effort interactive effective by General Linear Modeling. ABP; mean 12-h daytime ambulatory blood pressure; Eating/Body Attitudes; Z-score derived from the Three Factor Eating Questionnaire Restraint, Disinhibition and Hunger subscales, the Eating Disorder Inventory-2 Drive For Thinness and Bulimia subscales, the Body Shape Questionnaire and the Beliefs About Appearance Scale; General Stress Z-score; Perceived Stress Scale, and Daily Stress Inventory Frequency and Impact subscales collected on the days of cortisol and ABP assessment; UFC; 24-h urinary free cortisol.

a. Eating/Body Attitudes Z-score <0.
b. Eating/Body Attitudes Z-score ≥0.
c. Participants reported current weight loss effort.
d. Participants reported no current weight loss effort.
e. Baecke Habitual Physical Activity Questionnaire, possible score 3-15.
f. Adjusted for body mass index (kg/m<sup>2</sup>)
g. Adjusted for 24-h urine volume (L/24-h).
h. Adjusted for age, body mass index (kg/m<sup>2</sup>) and activity during ABP monitoring.
4.4 Discussion

Our findings suggest that the *experience* of negative eating/body attitudes, rather than specific weight loss efforts, may be a stressor capable of activating the physiological stress response in healthy young women. In this study, we found that women with negative eating/body attitudes had higher diastolic BP and mean arterial pressure than women with more positive attitudes. Several of our findings suggest that the differences in ABP may be related to the cognitive aspects of eating/body attitudes rather than behaviours. First, BMI, energy intakes and physical activity level did not differ by eating/body attitude level, indicating that differences in ABP were not related to physiological stress due to energy deprivation or conversely, to excessive body weight. Secondly, although women with more negative attitudes were more likely to report current weight loss attempts, ABP did not differ by weight loss effort. Thirdly, there was no interactive effect between eating/body attitudes and weight loss effort for any variables measured in this study.

The difficulty in defining and measuring specific psychosocial stressors, and relating them to physiological indicators of the stress response, outside of the laboratory setting is well recognised [7]. This may partly explain why it is not clear whether the association between higher ABP and more negative eating/body attitudes is specific to stress concerning food and weight or psychosocial stress in general. Our measure of general stress was significantly associated with UFC, our indicator of stress-induced HPA axis activation, and tended to be association with diastolic BP (P=0.052) and mean arterial pressure (P=0.088). Therefore, both eating/body attitudes and general stress were associated with indicators of the physiological stress response, and it does appear that we were able to operationalise the experience of “usual” stress.

It could be that women with more negative eating/body attitudes perceive more stress in other aspects of life and are highly stressed individuals, although this is also not clear from our findings or the literature. In the current study, more negative eating/body attitudes were associated with higher general stress. Associations between perceived stress and eating/body attitude measures have also been observed among large groups of university-aged women [33-34,38,57-58]. However, no difference in perceived stress was found among women with high versus low scores on eating/body attitude measures, despite higher 24-hr urinary cortisol among women with more negative eating/body attitudes [39,45].

Surprisingly, we did not confirm that negative eating/body attitudes were associated with UFC. The majority of previous studies that were adequately powered and of strong experimental design, have found higher cortisol in women with more negative eating/body attitudes [36-41,45]. As these studies examined individual questionnaires (in most cases, cognitive dietary restraint), we also performed correlations between individual questionnaire
scores and volume-adjusted UFC, and found no significant relationships (Rp= -0.03 to 0.14, P=0.140-0.731). It could be that eating and body stress resulted in preferential activation of the sympathetic nervous system. It has been suggested that increased cortisol may result from negative affect, lack of control, distress or misery; while sympathetic nervous system activation (indicated by catecholamine levels) may be associated with higher subjective effort under stress or mental arousal [20]. Levels of catecholamines and affective state should be examined in relation to eating/body attitudes and ABP in future studies.

In this study, UFC was modestly associated with ABP. Although the mechanism linking cortisol and BP is not conclusively established, it is well documented that conditions of hypercortisolism, such as Cushing’s syndrome, are also associated with hypertension [9]. It is not currently known whether higher cortisol within physiologically normal levels is associated with higher BP: associations between higher 24-hour UFC and 24-hour ABP measures have been observed in some [59] but not all [60] studies of healthy middle-aged adults. As well, higher 24-hour UFC has been found in middle-aged adults with untreated hypertension versus matched normotensives [61-62]. Our findings add support to the hypothesis that slightly higher cortisol may negatively impact BP in healthy young individuals.

Future studies examining young women’s eating/body attitudes and the stress response will be improved by addressing our limitations. In most studies, ABP and urinary stress hormones are assessed concurrently. That we did not do this may be a limitation although the purpose of our study differed from previous work as we were not seeking to determine whether particular situations resulted in activation of the stress response. Instead, we sought to examine whether long-standing, intrinsic beliefs and values about eating and body activated the stress response. We felt that concurrent ABP and urine assessments would increase subject burden and perhaps result in artifactual elevations. However, conducting these measurements on separate days would be expected to attenuate their relationship, so the fact that we observed associations provides evidence of their ongoing relationship. Another issue is that participants chose when to complete the procedures. This likely resulted in choice of a less active day, as participants appear to reduce their activity levels during ABP procedures [63]. Related to this was our inability to objectively verify physical activity during ABP monitoring by accelerometers [64-65]. Our participants recorded their concurrent activity which, although not perfect, appeared to capture general activity during monitoring as it was associated with ABP. Lastly, this study is limited by our relatively small, homogenous sample.

Despite these potential limitations, our findings of an association between more negative eating/body attitudes and higher ABP contribute to the limited knowledge of variables that influence the BP of healthy, non-obese, young women. Given that young adulthood BP is independently associated with future CVD risk [3-6], it is important that we understand the
correlates of BP while young, as even small influences appear to have a cumulative effect on BP over time. Moreover, as negative eating/body attitudes are almost normative among young women today [32-34], our findings of higher ABP among these women may be meaningful. However, we recognize that they have limited generalisability and hope that this exploratory study stimulates additional research. We recommend that future research, in addition to addressing the limitations outlined, include young working women, women with children and overweight/obese individuals. In conclusion, the subjective experience of negative eating/body attitudes was associated with increased ABP independent of weight loss effort in these healthy young women.
4.5 References


Chapter 5:
The relationship between 24-hour urinary cortisol and bone in healthy young women

1 A version of this chapter has been accepted for publication:
Date of Online Publication: October 3 2009. Copyright © Informa Healthcare
5.1 Introduction

Stress, whether inflammatory, traumatic or psychological, activates the hypothalamic-pituitary-adrenal (HPA) axis triggering an increase in cortisol, a glucocorticoid stress hormone, which over time may have implications for various body systems [1]. Cortisol may negatively affect bone density by altering bone turnover, impairing intestinal absorption and renal reabsorption of calcium, and, in premenopausal women, by inhibiting reproductive hormones [2]. A recent review strongly suggests that patients with Cushing’s syndrome, a condition of hypercortisolism, have reduced bone formation, lower bone density and an increased incidence of osteoporosis and fractures [2]. Subclinical hypercortisolism, as found with adrenal adenoma, shows similar patterns [2]. Whether variation in cortisol within the normal range can also have adverse effects on bone density is less clear.

Studies among healthy older adults report an inverse association between cortisol and bone density [3-6] and a positive association between cortisol and fracture risk [7]. Among premenopausal women, evidence suggestive of an inverse relationship between cortisol and bone density comes from clinical samples of patients with major depression and eating disorders [8-13]. However, the findings are not consistent [14-16], possibly due to the presence of other bone-related factors in these disorders such as amenorrhea, immune dysfunction and medication side effects. Only three cross-sectional studies have assessed whether cortisol is related to bone density in healthy young women, all of which included women with high versus low levels of cognitive dietary restraint [17-19]. Dietary restraint represents the perception that one is constantly monitoring and attempting to limit food intake in an effort to achieve a perceived ideal body weight, and may be a subtle but chronic stressor, as women with high restraint have been reported to have higher salivary or 24-hour urinary cortisol than women with low restraint [17, 20-22]. However, due to small sample sizes, inconsistent findings, and selection of women with particular eating attitudes, the findings related to bone density and cortisol from these studies are inconclusive [17-19]. Thus, the purpose of the current study was to assess the relationship between 24-hour urinary free cortisol excretion and bone density in healthy young women.

5.2 Methods
5.2.1 Participants

From August to December 2006, participants were recruited using announcements in University of British Columbia classes and poster advertisements in the Vancouver, British Columbia community (Appendix 13). Women were asked to participate if they were: 19-35 years of age, regularly menstruating (menses every 21-35 days), non-obese (self-reported body
mass index (BMI) of 18-30 kg/m$^2$), and in general good health (Appendix 14). Women were excluded if they reported any medical conditions (eating disorder, polycystic ovarian syndrome, Cushing’s syndrome, inflammatory conditions and thyroid disorders) or use of any medications, currently or within the previous six months, that would affect the HPA axis or bone density (oral contraceptives, progesterone, glucocorticoids). After 148 interested women were screened for eligibility by phone (Appendix 15), 142 were eligible: women were ineligible as a result of oral contraceptive use (n=3), shift work (n=1), BMI of <17 kg/m$^2$ (n=1) and glucocorticoid use (n=1). Two women did not attend their study orientation and were unavailable to reschedule. Therefore, 140 participants provided written informed consent (Appendix 16) and met with an investigator (JB) to have anthropometric measurements made and to receive materials and instructions on completion of a questionnaire package (Appendix 23), food frequency questionnaire (FFQ), 24-hour urine collection (Appendix 20) and a dual energy X-ray absorptiometry (DXA) scan (Appendix 22). Data collection occurred from August 2006 to February 2007. The study protocol was approved by the university’s Clinical Research Ethics Board (Appendix 17).

5.2.2 Questionnaires

Participants completed a questionnaire package which included questions to elicit information about some variables known or thought to be associated with bone density including the following: demographics (e.g. age, ethnicity), cigarette use, age of menarche, pregnancies and previous use of progesterone and oral contraceptives. To assess the influence of physical activity on bone density, the widely used Baecke Questionnaire of Habitual Physical Activity (Appendix 24) was used to measure occupational, sport, non-sport leisure and total physical activity [23]. The 16 items are scored on a five-point Likert scale. Higher scores reflect higher physical activity levels, with possible scores of 1-5 for each domain and 3-15 for total score. To evaluate the psychosocial aspects of stress we used the following well-validated and widely used questionnaires. The Perceived Stress Scale (PSS, Appendix 24) includes 14 items, each scored on a five-point Likert scale, to determine the perception of stress in the previous month [24]. Possible scores range from 0-56 with higher scores indicating elevated stress perception. The 21 item Restraint subscale of the Three Factor Eating Questionnaire (TFEQ, Appendix 24) assesses the level of cognitive dietary restraint. Possible scores range from 0 to 21, and higher scores suggest increased awareness and concern with weight, shape and eating [25]. As well, participants answered the Daily Stress Inventory (DSI) to assess the potential impact of 58 everyday minor stressful events which may have occurred during the 24-hour urine collection (Appendix 25). Events that did not occur are scored as zero, and those that occurred are
scored on a scale of 1 (not at all stressful) to 7 (caused me to panic) [26]. Accordingly, higher scores indicate increased general stress for the day of the urine collection.

5.2.3 Dietary intake

The FFQ used in this study was the Diet History Questionnaire (DHQ, V. 1.0 National Institutes of Health, Applied Research Program, National Cancer Institute, 2002). The DHQ has been adapted for use with the Canadian Nutrient File [27]. Energy intakes of <600 or >3500 kcal are deemed biologically implausible and removed [27], resulting in removal of three questionnaires >3500 kcal. Reported nutrient intakes are from food and supplements.

5.2.4 Urine collection and analysis

At home, participants completed a 24-hour urine collection on a "normal day" avoiding any unusual physical or mental stresses. On the day of collection, participants discarded their first urine void, recorded the time this occurred and then collected all subsequent voids for 24 hours (including a void at the recorded time the following morning). Completed samples were delivered to the Vancouver General Hospital (VGH) Laboratory by courier. Volume was measured and aliquots were frozen and stored prior to analysis. Twenty-four-hour urinary free cortisol excretion (UFC; µg) was measured by high-throughput liquid chromatography and tandem mass spectrometry [28].

5.2.5 Anthropometrics and body composition

Anthropometric measurements were made at study entry. Waist circumference was measured at the narrowest point between the iliac crest and lowest rib to the nearest 0.1 cm using an inelastic, flexible measuring tape. Height without shoes was measured to the nearest 0.1 cm at full inspiration using a stadiometer (model 214; Seca, Hamburg, Germany). While wearing light indoor clothing without shoes, weight was measured to the nearest 0.1 kg using an electronic scale. From these data, BMI (kg/m²) was calculated. Measurements were made in duplicate. If differences occurred, a third measurement was made and the two closest measurements were averaged.

At VGH, areal bone mineral density (aBMD, g/cm²), bone mineral content (BMC, g) and bone area (cm) at the lumbar spine (L1-4), both hips and total body were measured using DXA (Lunar Prodigy, enCORE software; General Electric Healthcare, Madison, WI). As well, total body bone-free lean and fat mass (kg) and percent body fat were determined. Daily quality assurance tests were conducted using a spine phantom scan and densitometric calibration. Repeat aBMD measurements fall within ±0.01 g/cm² for L1-4 and ±0.012 g/cm² for the proximal femur according to the manufacturer. The in-house coefficient of variation for aBMD at the
lumbar spine averaged 0.94% (range 0.82–1.10%) and the coefficient of variation for total proximal femur averaged 0.70% (range 0.65-0.76%).

5.2.6 Statistical analyses

Data were coded, verified and entered into SPSS (version 15) software (SPSS Inc., 2006) and crosschecked for accuracy. Data were examined for outliers (mean ± >4 standard deviation (SD)) and none were present except one extreme UFC outlier (7.4 SD above the mean) which was removed from all analyses. Descriptive statistics were used to characterise the sample. Pearson’s correlations were used to identify variables associated with aBMD, BMC, bone area, UFC and PSS score. Relationships between UFC and aBMD, BMC and bone area were then examined using partial correlations to adjust for potentially confounding affects of urine volume, ethnicity, height, lean mass and duration of previous oral contraceptive use as well as lifestyle variables (physical activity, perceived stress, calcium intake). For calcium intake, correlations were examined as calcium intake per unit of energy (mg/kcal). When independent variables were highly inter-correlated (e.g., weight and lean mass; total activity and sport activity), the variable with the highest univariate correlation with aBMD and BMC was included in partial correlations. Differences between Caucasians and Asians were examined by independent sample t tests and general linear model adjusting for covariates. For all analyses, cases were excluded pairwise. As this was an exploratory study, P values <0.10 are reported.

5.3 Results

5.3.1 Participant characteristics

Of the 140 women who were oriented to the study, 137 completed a DXA scan and 135 returned completed questionnaire packages. A urine collection was completed by 139 participants and UFC data were available for 134 as the lab lost four samples. The data presented in this paper are from the 132 women for whom both UFC and DXA measurements were available. Participants were 22.3 ± 3.6 years of age; almost all were students (94%) and single (92%); 41% were Caucasian and 59% were Asian; and 3% currently smoked cigarettes. Mean age at menarche was 12.6 ± 1.4 years, 98.5% were nulliparous, 32% reported previous use of oral contraceptives and 8% reported previous progesterone use. Table 5.1 provides descriptive statistics of physical activity and questionnaire scores, intake of bone-related nutrients, urine analysis, anthropometrics and DXA measurements. Asians were significantly younger (21.5±3.0 versus 23.5±3.8, P=0.001), smaller (height, weight, waist circumference, lean mass, fat mass and BMI), less physically active and had lower intakes of calcium and alcohol than Caucasians. Asians were less likely to report previous progesterone (2.5% versus 11%, P=0.042) or oral contraceptive (10% versus 66%, P<0.001) use and among those who
did, for a significantly shorter period of time (11.4±5.3 versus 37.2±31.3 months, P<0.001. However, dietary restraint, age of menarche (12.4±1.4 versus 12.8±1.4, P=0.075), percent body fat and intake of energy, protein, vitamin D or caffeine did not differ by ethnicity.

**Table 5.1** Physical activity and questionnaire scores, reported nutrient intakes, 24-hour urinary free cortisol excretion, anthropometrics and DXA measurements for all participants and differences by ethnicity

<table>
<thead>
<tr>
<th></th>
<th>All participants (n=132)</th>
<th>Caucasian (n=55)</th>
<th>Asian (n=80)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical activity scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupational activity</td>
<td>2.4 ± 0.6</td>
<td>2.4 ± 0.6</td>
<td>2.4 ± 0.6</td>
<td>0.757</td>
</tr>
<tr>
<td>Sport activity</td>
<td>2.7 ± 1.3</td>
<td>3.2 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Non-sport leisure activity</td>
<td>3.0 ± 0.7</td>
<td>3.2 ± 0.7</td>
<td>2.8 ± 0.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Total physical activity</td>
<td>8.0 ± 1.8</td>
<td>8.8 ± 1.3</td>
<td>7.5 ± 1.3</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td><strong>Questionnaire scores</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perceived stress scale</td>
<td>27.0 ± 6.5</td>
<td>24.6 ± 6.4</td>
<td>28.7 ± 6.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cognitive dietary restraint</td>
<td>8.1 ± 4.5</td>
<td>7.6 ± 4.0</td>
<td>8.1 ± 4.7</td>
<td>0.510</td>
</tr>
<tr>
<td>Daily stress inventory</td>
<td>31.1 ± 18.0</td>
<td>32.3 ± 19.0</td>
<td>30.4 ± 17.2</td>
<td>0.558</td>
</tr>
<tr>
<td><strong>Nutrient intakes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1648 ± 525</td>
<td>1684 ± 514</td>
<td>1639 ± 520</td>
<td>0.626</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>66.4 ± 26.9</td>
<td>67.5 ± 24.2</td>
<td>66.1 ± 28.8</td>
<td>0.773</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>862 ± 409</td>
<td>1006 ± 449</td>
<td>764 ± 343</td>
<td>0.001</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>90 ± 143</td>
<td>95 ± 141</td>
<td>81 ± 142</td>
<td>0.570</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td>143 ± 195</td>
<td>155 ± 177</td>
<td>137 ± 214</td>
<td>0.619</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>3.5 ± 5.3</td>
<td>6.8 ± 6.9</td>
<td>1.3 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Urine analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour UFC (μg/day)</td>
<td>27.3 ± 13.6</td>
<td>27.3 ± 13.4</td>
<td>27.6 ± 13.7</td>
<td>0.877</td>
</tr>
<tr>
<td>24-hour urine volume (L)</td>
<td>1.8 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>1.7 ± 0.9</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Anthropometric measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.3 ± 7.2</td>
<td>167.1 ± 6.8</td>
<td>160.6 ± 6.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.1 ± 8.5</td>
<td>62.7 ± 8.2</td>
<td>54.8 ± 7.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.7 ± 2.4</td>
<td>22.4 ± 2.6</td>
<td>21.2 ± 2.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>65.2 ± 5.8</td>
<td>67.5 ± 6.5</td>
<td>63.7 ± 4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>DXA measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone-free lean mass (kg)</td>
<td>38.1 ± 5.0</td>
<td>41.1 ± 3.9</td>
<td>35.8 ± 4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bone-free fat mass (kg)</td>
<td>16.8 ± 5.4</td>
<td>18.2 ± 6.1</td>
<td>15.8 ± 4.7</td>
<td>0.012</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.1 ± 6.5</td>
<td>30.0 ± 6.8</td>
<td>30.3 ± 6.1</td>
<td>0.831</td>
</tr>
</tbody>
</table>
All participants (n=132) | Caucasian (n=55) | Asian (n=80) | P value
--- | --- | --- | ---
**DXA measurements continued**
Total body aBMD (g/cm²) | 1.136 ± 0.075 | 1.166 ± 0.073 | 1.116 ± 0.070 | <0.001
Total body BMC (g) | 2392 ± 365 | 2578 ± 326 | 2259 ± 334 | <0.001
Lumbar spine aBMD (g/cm²) | 1.184 ± 0.123 | 1.224 ± 0.138 | 1.156 ± 0.101 | <0.001
Lumbar spine BMC (g) | 62.00 ± 11.33 | 66.55 ± 13.18 | 58.59 ± 9.27 | <0.001
Lumbar spine area (cm²) | 52.11 ± 5.25 | 54.06 ± 6.23 | 50.52 ± 4.92 | <0.001
Total hip aBMD (g/cm²) | 1.026 ± 0.120 | 1.071 ± 0.126 | 0.997 ± 0.102 | <0.001
Total hip BMC (g) | 30.24 ± 5.04 | 32.98 ± 5.07 | 28.29 ± 3.98 | <0.001
Total hip area (cm²) | 29.38 ± 2.45 | 30.75 ± 24.6 | 28.23 ± 1.91 | <0.001

aBMD, areal bone mineral density; BMC, bone mineral content; UFC, urinary free cortisol. Data are presented as mean ± standard deviation. P values indicate differences between ethnicity by independent sample t-tests.
a. Baecke Questionnaire of Habitual Physical Activity, subscales score range 1-5 and total 3-15.
b. Perceived Stress Scale, score range 0-56.
c. Three Factor Eating Questionnaire Restraint subscale, score range 0-21.
d. Daily Stress Inventory, score range 0-406.
e. n=129 for reported dietary intake from food and supplements.

5.3.2 Associations with aBMD, BMC and bone area

Table 5.2 shows Pearson’s correlation coefficients for the variables that were significantly associated with bone parameters (aBMD, BMC and bone area). All bone parameters were positively associated with height, weight, waist circumference, lean mass, physical activity, sport activity, leisure activity (except total hip aBMD) and energy-adjusted calcium intake (for total body aBMD, P=0.055). All bone parameters were negatively associated with PSS score except L1-4 area. Fat mass was related to total body aBMD and BMC, L1-4 BMC and total hip and L1-4 area. Total body and total hip parameters were positively correlated with duration of previous oral contraceptive use. All bone parameters were significantly lower among Asians (Table 5.1) but did not differ by ethnicity after controlling for height, lean mass, sport activity, prior oral contraceptive use and calcium/kcal intake. No other variables (including age or age of menarche) or nutrients of relevance to bone health were correlated with bone parameters.

5.3.3 Associations with 24-hour urinary free cortisol

The volume of urine collected was related to UFC (r=0.174, P=0.046). Urine volume was positively associated with height, weight, LBM, BMI, waist circumference, reported energy intake and sport activity (r=0.18 to 0.37, P<0.05) but not with fat mass or scores of dietary restraint, PSS or DSI. Controlling for weight and/or height did not change the relationship.
between UFC and urine volume and therefore, urine volume was included as a covariate in all further analyses. In univariate and partial correlation adjusting for urine volume, UFC was not correlated with age, any questionnaire scores or anthropometric measurements including waist circumference, fat and lean mass (data not shown). UFC did not differ by ethnicity before (Table 5.1) or after adjusting for urine volume (P=0.523). As dietary restraint was positively correlated with BMI (r=0.32, P<0.001), the analysis was repeated with BMI as an additional covariate however; the relationship between restraint and UFC remained nonsignificant.

5.3.4 Associations with PSS score

PSS score was negatively correlated with physical activity (r= -0.37, P<0.001), sport activity (r= -0.39, P<0.001), non-sport leisure activity (r= -0.17, P=0.048), weight (r= -0.27, P=0.002), lean mass (r= -0.30, P<0.001), BMI (r= -0.24, P=0.006) and waist circumference (r= -0.23, P=0.006). An inverse relationship between PSS score and age (r= -0.17, P=0.056) approached significance. Positive associations were observed between PSS and DSI scores recorded on the day of urine collection (r=0.24, P=0.006).

5.3.5 Associations between 24-hour urinary free cortisol and aBMD, BMC and bone area

As shown in Table 5.2, UFC was negatively correlated with total body aBMD and approached significance with total body BMC (P=0.057) and L1-4 aBMD (P=0.074) in univariate analyses. Urine volume was positively related to BMC and area at all sites and hip aBMD. Partial correlations between UFC and bone parameters are shown in Table 5.3. Adjusting for urine volume (Model 1), UFC was negatively correlated with total body aBMD and BMC, L1-4 aBMD and total hip BMC. The inverse relationship between UFC and L1-4 BMC approached significance (P=0.061). After controlling for variables identified as being related to bone parameters in univariate analyses (Model 2: urine volume, ethnicity, height, lean mass, duration of previous oral contraceptive use), significant inverse associations were observed between UFC and total body BMC and aBMD, total hip BMC, and L1-4 aBMD and BMC. The relationships between UFC and total hip aBMD (P=0.076) and area (P=0.092) approached significance. These relationships between UFC and bone parameters did not change meaningfully after additional adjustment for lifestyle variables (Model 3: calcium/kcal intake, sport activity and PSS score) except that L1-4 aBMD (P=0.059) and BMC (P=0.070) became nonsignificant. The association between UFC and total hip area (P=0.09) also did not reach significance in the final adjusted model.
Table 5.2  Correlations of aBMD, BMC and bone area with anthropometrics, perceived stress, physical activity, duration of previous oral contraceptive use, calcium/kcal intake, 24-hour urinary free cortisol excretion and 24-hour urine volume

<table>
<thead>
<tr>
<th></th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Lean mass (kg)</th>
<th>Fat mass (kg)</th>
<th>Waist circ (cm)</th>
<th>PSS score</th>
<th>Total activity score</th>
<th>Sport activity score</th>
<th>Leisure activity score</th>
<th>Prior OC use</th>
<th>Calcium (mg/kcal)</th>
<th>UFC (µg/day)</th>
<th>Urine Volume (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body aBMD</td>
<td>0.38***</td>
<td>0.52***</td>
<td>0.58***</td>
<td>0.19'</td>
<td>0.35***</td>
<td>-0.27**</td>
<td>0.34***</td>
<td>0.40***</td>
<td>0.18'</td>
<td>0.18'</td>
<td>0.17'</td>
<td>-0.19'</td>
<td>0.13</td>
</tr>
<tr>
<td>Total body BMC</td>
<td>0.75***</td>
<td>0.79***</td>
<td>0.79***</td>
<td>0.42***</td>
<td>0.51***</td>
<td>-0.28**</td>
<td>0.36***</td>
<td>0.43***</td>
<td>0.17'</td>
<td>0.20'</td>
<td>0.23'</td>
<td>-0.17'</td>
<td>0.22'</td>
</tr>
<tr>
<td>L1-4 aBMD</td>
<td>0.38***</td>
<td>0.42***</td>
<td>0.47***</td>
<td>0.16'</td>
<td>0.24'</td>
<td>-0.21'</td>
<td>0.27'</td>
<td>0.30***</td>
<td>0.19'</td>
<td>0.13'</td>
<td>0.18'</td>
<td>-0.16'</td>
<td>0.11</td>
</tr>
<tr>
<td>L1-4 BMC</td>
<td>0.64***</td>
<td>0.57***</td>
<td>0.67***</td>
<td>0.21'</td>
<td>0.30***</td>
<td>-0.21'</td>
<td>0.32'</td>
<td>0.35***</td>
<td>0.23'</td>
<td>0.15'</td>
<td>0.21'</td>
<td>-0.13'</td>
<td>0.19'</td>
</tr>
<tr>
<td>L1-4 area</td>
<td>0.75***</td>
<td>0.58***</td>
<td>0.70***</td>
<td>0.23''</td>
<td>0.30***</td>
<td>-0.15'</td>
<td>0.29***</td>
<td>0.30***</td>
<td>0.21'</td>
<td>0.15'</td>
<td>0.19'</td>
<td>-0.06'</td>
<td>0.23''</td>
</tr>
<tr>
<td>Total hip aBMD</td>
<td>0.28***</td>
<td>0.39***</td>
<td>0.50***</td>
<td>0.09</td>
<td>0.25''</td>
<td>-0.27''</td>
<td>0.32''</td>
<td>0.40***</td>
<td>0.13</td>
<td>0.21'</td>
<td>0.19'</td>
<td>-0.10'</td>
<td>0.19'</td>
</tr>
<tr>
<td>Total hip BMC</td>
<td>0.61***</td>
<td>0.60***</td>
<td>0.76***</td>
<td>0.17'</td>
<td>0.35***</td>
<td>-0.30***</td>
<td>0.42***</td>
<td>0.50***</td>
<td>0.18'</td>
<td>0.28'</td>
<td>0.25'</td>
<td>-0.12'</td>
<td>0.26''</td>
</tr>
<tr>
<td>Total hip area</td>
<td>0.80***</td>
<td>0.62***</td>
<td>0.78***</td>
<td>0.20'</td>
<td>0.33***</td>
<td>-0.19'</td>
<td>0.37***</td>
<td>0.42***</td>
<td>0.18'</td>
<td>0.27'</td>
<td>0.21'</td>
<td>-0.09'</td>
<td>0.27''</td>
</tr>
</tbody>
</table>

Data are presented as Pearson’s (R) coefficients. Exact n varied by comparison as cases were excluded pairwise. Waist circ, circumference of narrowest point between lowest rib and iliac crest; PSS, Perceived Stress Scale; Prior OC use, Duration of oral contraceptive use prior to the study (non users= 0); UFC, 24-hour urinary free cortisol excretion; aBMD, areal bone mineral density (g/cm²); BMC, bone mineral content (g); area, bone area (cm²); L1-4; lumbar spine vertebra 1-4. t; Correlation approached significance at p<0.09. Correlation is significant at:  *P<0.05, **P<0.01, ***P<0.001.
Table 5.3  Partial correlation models of the relationship between aBMD, BMC and bone area and 24-hour urinary free cortisol excretion

<table>
<thead>
<tr>
<th></th>
<th>UFC model 1</th>
<th>UFC model 2</th>
<th>UFC model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body aBMD</td>
<td>-0.21*</td>
<td>-0.27**</td>
<td>-0.25**</td>
</tr>
<tr>
<td>Total body BMC</td>
<td>-0.21*</td>
<td>-0.30***</td>
<td>-0.28**</td>
</tr>
<tr>
<td>Lumbar spine aBMD</td>
<td>-0.18*</td>
<td>-0.19*</td>
<td>-0.17†</td>
</tr>
<tr>
<td>Lumbar spine BMC</td>
<td>-0.16†</td>
<td>-0.18*</td>
<td>-0.17†</td>
</tr>
<tr>
<td>Lumbar spine area</td>
<td>-0.10</td>
<td>-0.07</td>
<td>-0.08</td>
</tr>
<tr>
<td>Total hip aBMD</td>
<td>-0.13</td>
<td>-0.16†</td>
<td>-0.14</td>
</tr>
<tr>
<td>Total hip BMC</td>
<td>-0.18*</td>
<td>-0.23†</td>
<td>-0.21*</td>
</tr>
<tr>
<td>Total hip area</td>
<td>-0.15†</td>
<td>-0.15†</td>
<td>-0.16†</td>
</tr>
</tbody>
</table>

UFC, 24-hour urinary free cortisol excretion (µg); aBMD, areal bone mineral density (g/cm²); BMC, bone mineral content (g); area, bone area (cm²).
Data are presented as Pearson’s (R) and partial (Rp) coefficients adjusting for variables identified as cofounders in univariate analyses.
Exact n varied by comparison as cases were excluded pairwise.
* Correlation approaches significance at P<0.10.
Correlation is significant at: * P<0.05, ** P<0.01, *** P<0.001.
Model 1: Adjusted for 24-hour urine volume (L).
Model 2: Partial correlations adjusting for 24-hour urine volume, height, lean mass, ethnicity and previous duration of oral contraceptive use (zero for non-users).
Model 3: Partial correlations adjusting for 24-hour urine volume, height, lean mass, ethnicity, previous duration of oral contraceptive use, sport activity, calcium/kcal intake and Perceived Stress Scale score.

5.4 Discussion

Our findings suggest that cortisol within the normal range is negatively associated with bone density in healthy young women, after adjusting for other variables, consistent with reports from samples of healthy older adults [3-6]. We found that 24-hour urinary free cortisol excretion (UFC) was modestly associated with total body and lumbar spine aBMD and BMC and total hip BMC.

Previously, the majority of data concerning cortisol and bone density in young women came from studies using samples with clinical depression and eating disorders. Some studies observed negative associations between cortisol and bone density [8-13], although others did not [14-16]. These discrepancies are perhaps related to disease conditions and/or treatments which may have a greater impact on bone density. The authors are aware of only three studies that examined associations between cortisol and bone density in healthy young women, all of which included only women with high and low dietary restraint, and found no significant relationships between aBMD and fasting serum, salivary or 24-hour urinary cortisol [17-19]. The small sample sizes (n=62-78) in these studies and recruitment of women with particular eating attitudes may have limited their power to detect a potential association between cortisol and
bone density. As well, the method of determining cortisol may be important when assessing this relationship.

As cortisol secretion is characterised by marked diurnal variation, fasting single measurements, overnight sampling or sampling during hospitalisation may not reflect usual cortisol levels. Using a 24-hour urine collection captures all daily cortisol excretion and for the most part participants are able to go about their normal activities. This method may therefore more accurately reflect usual cortisol levels resulting from stress-induced activation of the HPA axis. Nevertheless, we acknowledge that our method of assessing cortisol also has limitations. Though detailed instruction and support were provided, the collection of urine for 24 hours may be imprecise if, for example, the collection period is not exactly 24 hours or if some voids are not collected. As well, we did not account for participants’ menstrual cycle phase, and the diurnal rhythm of cortisol appears to differ during the follicular and luteal phases of the menstrual cycle [29-33]. However, 24-hour quantitative measures do not differ between phases [29-33], so this is unlikely to have affected our results.

In addition to cortisol, our indicator of HPA axis activity, we examined psychosocial indicators of stress. Interestingly, UFC was not related to perceived stress over the previous month (PSS score), the amount of stress encountered on the day of the UFC measurement (DSI score), or to concern related to eating and body image (as assessed by dietary restraint). Several other groups report no relationship between salivary cortisol and PSS scores among young women leaving the welfare system [34], undergraduate students [21, 35], white-collared working men [36-37], post-menopausal women [20] or healthy middle-aged [38] and older adults [39]. The effect of perceived stress on cortisol may be mediated by other psychosocial variables, such as mood [37, 39]. Assessing these variables was beyond the scope of this study.

Furthermore, UFC was not associated with lean or fat mass, % body fat or waist circumference. Obesity, particularly the accumulation of visceral fat, is a well-known characteristic of hypercortisolism and some evidence suggests the potential for cortisol to lead to positive energy balance and abdominal obesity among healthy individuals [1]. There is little evidence of an association between cortisol and indicators of body fat in healthy, normal-weight women and therefore prospective data are required to determine if cortisol is associated with gains in visceral fat or overall weight over time in these women.

We speculated that dietary restraint may be a stressor for young women sufficient to increase cortisol, which over time may cause adverse effects on health including bone density. However, dietary restraint was not correlated with PSS score, UFC or aBMD and BMC at any site in the current study before or after adjustment for confounders. Our previous work where UFC and BMC in young women [17] and UFC in post-menopausal women [20] differed by
dietary restraint level involved prescreening women for either high (TFEQ Restraint subscale score ≥13) or low (score ≤5) dietary restraint. It is possible that there may be a threshold for an effect of dietary restraint, such that in the current study we did not have enough women with very high scores (n=23 with high restraint) to detect an association between restraint and UFC or bone density. Studies similar to ours in sample size and characteristics that examined bone density over a spectrum of dietary restraint scores also reported no relationship with aBMD [40-41], although one study noted lower total body BMC with higher restraint in three out of four body weight groups [41]. In addition, two studies of teens and young women with high activity levels reported lower aBMD among those with high versus low dietary restraint [42, 43], although the inclusion of women with oligo-amenorrhea in those studies complicates interpretation. Thus, whether dietary restraint has an independent effect on bone density remains to be established.

One of the strengths of the present study is that we assessed and accounted for other variables thought to affect bone density in young women including ethnicity, calcium intake, previous oral contraceptive use and physical activity. Consistent with the literature, we found that bone density did not differ between Asians and Caucasians after adjustment for differences in body size [45] and we observed modest positive associations between aBMD and BMC and energy-adjusted calcium intake [45] and the duration of previous oral contraceptive use [46]. Physical activity is highly important in maintaining bone density [47] and we observed correlations between physical activity, particularly sport activity, and aBMD, BMC and bone area at all sites. This is consistent with findings of greater differences in bone density between athletes and controls than between physically active and normally active women [48].

Interestingly, PSS score was negatively associated with aBMD and BMC at all sites (Table 5.2), though the correlations were no longer significant after controlling for sport activity ($r = -0.035$ to $-0.16$, $P=0.063$ to 0.686). Stress relief is promoted as a benefit of physical activity and a relationship between higher physical activity and lower perceived stress has been reported in the literature [49-53], and was also observed in our sample. However, neither physical activity nor PSS score was associated with our indicator of HPA axis activity, UFC. Intense exercise is a stress condition in which cortisol secretion is elevated and there is much research on cortisol during acute exercise [54]. Few studies however have examined the relationships between cortisol, perceived stress and usual physical activity. A few small intervention trials have observed a reduction in cortisol after an 8-week jogging programme in 49 young women with mild depression [55] and after an 18-week Tai Chi programme in nine young adults [56]. Additionally, PSS scores were reduced among 10 older adults from an assisted living community after a 10-week exercise programme, though serum cortisol levels did not change significantly over time or differ from 10 controls [52]. It could be that in addition to
the well established mechanical role, physical activity may help to maintain bone density by limiting stress-induced elevations in cortisol. Further investigation as to whether stress reduction programmes that incorporate physical activity can reduce cortisol levels among young healthy women with high levels of perceived stress may be warranted.

In summary, study findings suggest that endogenous cortisol within the normal range is negatively associated with bone density in young women with characteristics similar to the study sample. Therefore, these study findings are generalisable to other healthy, regularly menstruating, non-obese, non-smoking women (only 3% of participants current smokers compared to smoking rates of 8% among women aged 20-24 in the health region of the sample population [57]). We are not suggesting that cortisol within the normal range is a major determinant of bone density, but rather that cortisol may be one of the many factors that have a small, but persistent influence on bone density over time. Longitudinal data examining the relationship between cortisol and changes in aBMD are needed to further establish this relationship in healthy young women.
5.5 References


Chapter 6:

Conclusion
6.1 General conclusion

Overall, this research provided insight into how psychosocial constructs, such as eating and body attitudes and general stress, may impact physiological health outcomes in healthy young women. This study was the first to prospectively examine the relationships among cognitive dietary restraint (CDR), subclinical ovulatory disturbances, 24-hour urinary free cortisol (UFC) and change in bone density over two years in healthy, non-obese, university-aged women. Beyond the major research question, the relationship between eating and body attitudes and 12-hour daytime ambulatory blood pressure (ABP) was also examined. The extent to which the experience of CDR, as opposed to current weight loss effort contributes to the potential health outcomes was also explored. This chapter first presents a summary of results with regard to the hypotheses presented in Chapter 1 in Table 6.1. A general discussion of the findings and their interpretation follows. Strengths and limitations of the research are discussed, and the chapter concludes with a discussion of directions for future research.

Table 6.1 Summary of results with regard to specific hypotheses

<table>
<thead>
<tr>
<th>Hypotheses in null form</th>
<th>Summary of relevant findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 2</strong></td>
<td></td>
</tr>
<tr>
<td>1. There will be no relationship between LS-QBT and PdG in terms of the proportion of cycles classified as ovulatory versus anovulatory.</td>
<td>1. Relative to PdG, LS-QBT showed excellent detection of ovulatory cycles (97% sensitivity) but poor detection of anovulatory cycles (25% specificity).</td>
</tr>
<tr>
<td>2. There will be no relationship between LS-QBT and PdG for the estimated day of luteal phase onset.</td>
<td>2. The correlation between the estimated day of luteal onset by LS-QBT relative to PdG was r=0.803, P&lt;0.001.</td>
</tr>
<tr>
<td>3. Editing temperatures based on waking time will have no effect on the performance of LS-QBT relative to PdG in terms of detecting ovulatory versus anovulatory cycles, or in estimation of luteal phase onset.</td>
<td>3. Editing of temperatures based on wake-time did not affect the performance of LS-QBT relative to PdG in terms of detecting ovulatory cycles. The performance of detecting anovulatory cycles was reduced to 0% specificity. The correlation of the estimated day of luteal onset by wake-time adjusted LS-QBT relative to PdG was slightly reduced (r=0.651 and 0.741, P&lt;0.001).</td>
</tr>
<tr>
<td>4. The assessment and editing of temperatures by a reproductive expert will have no effect on the performance of LS-QBT relative to PdG in terms of detecting ovulatory versus anovulatory cycles, or in estimation of luteal phase onset.</td>
<td>4. Editing of temperatures by a reproductive expert did not affect the performance of LS-QBT relative to PdG in terms of detecting ovulatory cycles. The performance of detecting anovulatory cycles was reduced to 0% specificity. The correlation of the estimated day of luteal onset by wake-time adjusted LS-QBT relative to PdG was slightly reduced (r=0.747, P&lt;0.001).</td>
</tr>
<tr>
<td>Hypotheses in null form</td>
<td>Summary of relevant findings</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><strong>Chapter 3</strong></td>
<td><strong>Chapter 3</strong></td>
</tr>
<tr>
<td>1. There will be no relationships among CDR, intake of bone-related nutrients, physical activity, UFC, %SOD, Δanthropometrics, or ΔaBMD.</td>
<td>1. CDR was not associated with energy intake, General Stress or physical activity. UFC was not correlated with baseline or Δanthropometrics or questionnaire scores (including CDR) with the exception of General Stress. %SOD was positively associated with CDR score (r=0.22, P=0.018), but not with physical activity, energy intake or UFC. There was no relationship between ΔaBMD at any measured site and UFC, physical activity or General Stress. Hip ΔaBMD was associated with Δfat mass (r= -0.18, P=0.047) and Δ%body fat (r= -0.20, P=0.026). Total body ΔaBMD was positively associated with Δweight (r=0.21, P=0.018), ΔBMI (r=0.18, P=0.049) and Δlean mass (r=0.18, P=0.048). No other anthropometric or dietary intake variables were associated with ΔaBMD except that calcium/kcal was negatively associated with total hip ΔaBMD (r= -0.19, P=0.036). Only hip ΔaBMD was significantly associated with %SOD (r =-0.29, p=0.002).</td>
</tr>
<tr>
<td>2. Women with higher and lower CDR will not differ with regard to energy intake, physical activity, General Stress, anthropometrics, Δanthropometrics, UFC, %SOD and ΔaBMD.</td>
<td>2. Women with higher CDR had higher baseline weight, BMI, fat mass, %body fat and BMI-adjusted energy intakes. %SOD was higher in women with higher CDR (56% versus 34%, P&lt;0.001). Women with higher CDR had significantly higher UFC (28.0 µg versus 24.0 µg, P=0.021). Physical activity, General Stress, Δanthropometrics and ΔaBMD did not differ by CDR level.</td>
</tr>
<tr>
<td>3. Women with higher and lower %SOD will not differ with regard to energy intake, physical activity, General Stress, anthropometrics, Δanthropometrics, UFC, CDR and ΔaBMD.</td>
<td>3. Women with higher %SOD had higher baseline BMI and more positive Δlean mass. Other baseline or Δanthropometrics did not differ. Energy intake, General Stress, physical activity and UFC did not differ. Women with higher %SOD reported higher CDR scores (8.7 versus 7.1 P=0.04) and had less positive hip ΔaBMD (-0.6% versus 0.9%, P=0.001) and L1-4 ΔaBMD (0.7% versus 1.9%, P=0.034).</td>
</tr>
<tr>
<td>Hypotheses in null form</td>
<td>Summary of relevant findings</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td><strong>Chapter 3 continued</strong></td>
<td><strong>Chapter 3 continued</strong></td>
</tr>
<tr>
<td>4. There will be no ethnicity-by-CDR interactive effect on UFC, %SOD or ΔaBMD, or ethnicity-by-%SOD interactive effect on UFC or ΔaBMD.</td>
<td>4. There was a significant ethnicity-by-CDR interactive effect on UFC, such that UFC was higher in those with higher versus lower CDR among Caucasians (32.0 µg versus 22.6 µg) but not Asians (25.8 µg versus 25.4 µg). There was no ethnicity-by-CDR interactive effect on %SOD or ΔaBMD. There was no ethnicity-by-%SOD effect on UFC or ΔaBMD.</td>
</tr>
<tr>
<td><strong>Chapter 4</strong></td>
<td><strong>Chapter 4</strong></td>
</tr>
<tr>
<td>1. There will be no main or interactive effects of Eating/Body Attitude level or current weight loss effort on BMI, energy intake, physical activity, General Stress, UFC and 12-hour daytime average ABP measures.</td>
<td>1. There was no main effect of Eating/Body Attitude on BMI, energy intake, physical activity, General Stress or UFC. Significant main effects of Eating/Body Attitude on ABP were detected such that diastolic BP and mean arterial pressure were higher among women with more negative Eating/Body Attitudes. There was a significant main effect of weight loss effort on BMI such that women currently trying to lose weight had higher BMI. There were no main effects of weight loss effort on energy intake, physical activity, General Stress, UFC or 12-hour ABP measures. There was no Eating/Body Attitude-by-weight loss effort interaction on BMI, energy intake, physical activity, General Stress, UFC or 12-hour ABP.</td>
</tr>
<tr>
<td>2. There will be no cross-sectional relationships among Eating/Body Attitudes, General Stress, UFC and 12-hour daytime average ABP after adjustment for potentially confounder variables.</td>
<td>2. More negative Eating/Body Attitudes were associated with higher diastolic ABP (r= -0.24, p&lt;0.01), mean arterial pressure (r= -0.22, p&lt;0.01) and General Stress (r= -0.23, p&lt;0.05), but not UFC. General Stress was not associated with ABP measures but was associated with UFC (r=0.25, p&lt;0.01). UFC was positively associated with systolic ABP (r=0.24, P=0.009), diastolic ABP (r=0.26, P=0.005) and mean arterial pressure (r=0.26, P=0.005).</td>
</tr>
<tr>
<td>Hypotheses in null form</td>
<td>Summary of relevant findings</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td><strong>Chapter 5</strong></td>
<td><strong>Chapter 5</strong></td>
</tr>
<tr>
<td>1. There will be no cross-sectional relationships among aBMD, BMC and bone area measured at any site and the following outcome variables: CDR, Perceived Stress Scale (PSS) score, physical activity, previous oral contraceptive use, age, age of menarche, reported intake of bone-related nutrients, anthropometric measurements and UFC.</td>
<td>1. CDR, age and age of menarche were not associated with bone parameters. All bone parameters were positively associated with height, weight, waist circumference, lean mass, energy-adjusted calcium intake and physical, sport and leisure activity (except hip aBMD) and were negatively associated with PSS score (except L1-4 area). Fat mass was related to total body aBMD and BMC, L1-4 BMC, and bone area of the hips and L1-4. Total body and hip parameters were positively correlated with the duration of previous oral contraceptive use. UFC was negatively correlated with total body aBMD and BMC, L1-4 aBMD and hip BMC. After adjustment for ethnicity, height, lean mass, the duration of previous oral contraceptive use, significant inverse associations were observed between UFC and total body BMC and aBMD, hip BMC, and L1-4 aBMD and BMC. These relationships did not change meaningfully after addition of lifestyle covariates (calcium/kcal intake, sport activity and PSS score) except that L1-4 aBMD ($P=0.059$) and BMC ($P=0.070$) became nonsignificant.</td>
</tr>
<tr>
<td>2. There will be no cross-sectional relationships among UFC and the following outcome variables: CDR, perceived stress, physical activity and anthropometric measurements.</td>
<td>2. UFC was not correlated with CDR, perceived stress, physical activity or anthropometric measurements.</td>
</tr>
<tr>
<td>3. There will be no cross-sectional relationships among perceived stress and the following outcome variables: CDR, physical activity and anthropometric measurements.</td>
<td>3. Perceived stress score was negatively correlated with physical activity, sport activity, non-sport leisure activity, weight, lean mass, BMI and waist circumference. Perceived stress score was not associated with CDR.</td>
</tr>
</tbody>
</table>

**6.2 General discussion**

In this study, women with higher CDR experienced more subclinical ovulatory disturbances (anovulation and/or luteal phases <10 days long) and these disturbances were associated with less positive changes in bone density over two years (Chapter 3). There was no direct relationship apparent between CDR and areal bone mineral density (aBMD) at baseline (Chapter 5) or 2-year change in aBMD (Chapter 3). Findings indicate that the hypothesis that
stress-induced elevations in cortisol were mediating the relationship between CDR and adverse health outcomes is plausible, but not conclusively documented. On one hand, UFC was not associated with CDR at baseline (Chapter 5) or first follow-up (Chapter 4). Also, average UFC did not differ by level of subclinical ovulatory disturbances (Chapter 3). Yet, average UFC (from the three collections over two years) was higher in women with higher CDR (Chapter 3). Moreover, study findings do indicate the capacity for elevated cortisol within the normal range to affect health outcomes. First, a modest inverse relationship was observed between UFC and aBMD at baseline (Chapter 5). Second, at the first follow-up, UFC was positively associated with ABP (Chapter 4), an additional physiologic indicator of the stress response. However, average UFC was not significantly associated with change in aBMD (Chapter 3). Finally, study results suggest that the association between eating and body attitudes and negative health outcomes is a consequence of the experience of stress, and was not associated with energy intakes, physical activity or relative body weight (Chapters 3 and 4). Specifically, women with negative eating and body attitudes had elevated ABP versus those with neutral/positive attitudes independent of weight loss effort (Chapter 4). Similarly, CDR was the only measured variable to differ by level of subclinical ovulatory disturbances and these disturbances were associated with less positive bone change. Taken together, findings from my PhD research suggest that eating and body attitudes may be associated with adverse health outcomes including subclinical ovulatory disturbances, reduced bone density and higher blood pressure. Furthermore, findings indicate that these relationships may be somewhat mediated by stress-induced hypothalamic-pituitary-adrenal (HPA) axis allostatic overload. However, cortisol does not appear to be the most important or only mediator of these relationships.

When this study was proposed, there were no prospective data regarding the relationship between CDR and bone density. Though inadequately powered to detect associations with bone density, several small cross-sectional studies suggested that women with higher CDR may have lower aBMD and/or bone mineral content (BMC) [1-4], possibly resulting from a higher frequency of menstrual cycle or ovulatory disturbances [4-10]. Long-term observation of ovulatory function is required to accurately classify women as those with higher and lower subclinical ovulatory disturbances due to high intra-individual variability in ovulation and luteal phase length (LPL). Therefore, concurrent longitudinal observation of ovulatory function and aBMD in relation to CDR was the main research question of my PhD project. In the only other prospective study to date, women with more subclinical ovulatory disturbances (defined as anovulation and/or LPL <10 days in three or more cycles out of 12 monitored by urinary luteinizing hormone peak) experienced less positive 2-year change in lumbar spine aBMD [11]. However, these disturbances did not differ by level of CDR in that study [11]. This study included a large sample of healthy, somewhat older premenopausal women with varying
CDR levels and body weights. In my study, when participants were grouped by the median percentage of cycles with subclinical ovulatory disturbances (anovulatory and/or short LPL for ≥38.8% versus <38.8% of cycles by LS-QBT), women with more disturbances had less positive 2-year changes in aBMD at the lumbar spine and total hip. Similarly, previous longitudinal studies report less positive changes in lumbar spine bone density with anovulation and shorter LPL [12-13]. Overt disturbances in menstrual cycle function, such as amenorrhea, are known to be associated with bone loss [14]. The concept that disturbances during the luteal phase of the cycle -- when progesterone is the predominant reproductive hormone -- are also related to bone is controversial [15]. Findings from this study provide substantial support to the hypothesis that progesterone, in addition to estradiol, is one of many important factors in bone density maintenance in premenopausal women [16].

We also confirmed that women with higher CDR experienced more subclinical ovulatory disturbances than women with lower CDR (56% versus 34% of cycles with disturbances; Chapter 3). The relationship between elevated CDR and both menstrual cycle irregularities [4, 8-9] and subclinical ovulatory disturbances [5,6,10,17] has been consistently reported among adolescents and young adult women. Some have suggested that the relationship between CDR and menstrual cycle or ovulatory disturbances results from dieting behaviours that may be associated with elevated CDR including energy deficit, high levels of physical activity, disordered eating behaviours and/or lower body mass or percent body fat [11]. This was not the case in my study. Women with higher CDR (Three Factor Eating Questionnaire Restraint (TFEQ-R) scores >7.7) actually had somewhat higher body mass index (BMI; kg/m²), percent body fat and BMI-adjusted energy intakes than women with lower CDR, and physical activity levels did not differ. As well, BMI tended to be higher among those with more subclinical ovulatory disturbances and neither physical activity nor BMI-adjusted energy intakes differed by the level of ovulatory disturbances. The frequency of disordered eating behaviours (bingeing, purging, compulsive exercising and laxative use) determined by the Eating Disorder Examination (EDE) Questionnaire [18] was not associated with TFEQ-R score or the percentage of ovulatory disturbance. Furthermore, mean EDE scores, a questionnaire used to assess body attitudes that are concurrent with eating disorder pathology, were below norms [19-20]. As numerous life stressors have been associated with menstrual cycle disturbances, it could also be suggested that women with higher CDR or those with more subclinical ovulatory disturbances are highly stressed in other aspects of life. Yet none of the following indicators of stress were associated with CDR or subclinical ovulatory disturbances: life events stress over the 2-year study; depression, anxiety, or perceived stress over the previous month; or the frequency or perceived impact of every day minor stressful events determined on the days of urine collection (Appendix 27).
There are several possibilities that may explain why CDR was not associated with subclinical ovulatory disturbances in the other prospective study [11]. First, it is unclear why anovulation and/or LPL <10 days by urinary luteinizing hormone (LH) peak in three or more cycles was chosen as the cut-off value to classify women with higher and lower subclinical ovulatory disturbances. As well, when the urinary LH peak is used as an indirect indicator of ovulatory function, the criterion for short LPL should be <11-12 days not <10 days. This is because the peak in serum LH occurs approximately 24 hours prior to the documentation of ovulation by ultrasound LH [21] and the peak in urinary LH is detectable within eight hours [22]. Defining short LPL as <10 days by urinary LH would therefore result in fewer women being classified with subclinical ovulatory disturbances. Indeed only 16 of the 189 women in the study experienced anovulation and/or short LPL in three or more cycles out of a maximum of 12 (average 9.8) monitored cycles [11]. Such disparity in group size makes the interpretation of differences between groups difficult. Correlation analysis of the percentage of cycles with subclinical ovulatory disturbances and TFEQ-R score or classifying groups by median split may have been more appropriate. Another possibility put forth by that study’s authors may be related to the gynaecological maturity of their sample compared to the age of previous study samples, as the reproductive system becomes more robust with age and menstrual cycle characteristics are less affected by lifestyle variables [11]. The much lower prevalence of subclinical ovulatory disturbances that was reported in that study (33.3% of women experienced at least one during their study) versus the current study (82%) and previous work (67-80%) provides support for that hypothesis.

That the menstrual cycle characteristics of older premenopausal women may be less affected by CDR-related stress does not diminish the significance of our findings. It has been suggested that attainment of peak bone mass is more important to future fracture risk than the degree of bone loss that occurs with age [23]. In the current study, at baseline, 82% of participants were <25 years old and 50% were <21 years old, and total body and lumbar spine aBMD increased significantly during the study. This suggests that many were still accruing bone and may not have yet achieved their peak bone mass. It should be noted that correlation does not imply causation and there is a possibility that unmeasured variables were mediating the relationship between CDR and subclinical ovulatory disturbances. Nevertheless there is now a substantial amount of indirect evidence indicating that the experience of CDR is associated with menstrual cycle and ovulatory disturbances, and these disturbances negatively affect bone density in young women. As both changes in bone density and subclinical ovulatory disturbances are not outwardly perceptible to women, these findings are an important contribution to the literature.
My PhD research project documented ovulatory function in more menstrual cycles per participant than previous work in relation to bone density [11-13,24-28]: 114 participants in this study provided up to 28 cycles (average 13.6) for analyses versus a maximum of 12 in other studies [13]. Furthermore, 64 women provided 12 or more cycles over the 2-year period. This was accomplished using least-squares quantitative basal temperature analysis (LS-QBT). Findings from my PhD project suggest that this method of monitoring ovulatory characteristics is both acceptable to women and cost-effective for use in large, prospective studies (Chapter 2, Appendix 28). The LS-QBT method was previously validated against the midcycle peak in serum LH, an established indirect indicator of ovulation [29]. Before commencing the 2-year study, further validation of LS-QBT was conducted against the sustained rise in urinary metabolites of progesterone (Chapter 2). Monitoring of urinary metabolites of progesterone over the cycle is an established indirect indicator of ovulation and reflects levels of a reproductive hormone that may be important in bone maintenance in premenopausal women [15-16]. Findings also indicate that LS-QBT is easy-to-use for both participants and researchers. The accuracy of LS-QBT in detecting evidence of luteal activity was not significantly affected by moderate wake-time variation, meaning participants do not need to adhere to a strict wake-sleep cycle. As well, expert screening/interpretation of raw data did not improve LS-QBT performance, suggesting that this method can be used by investigators with various backgrounds (Chapter 2). Although monitoring of cervical mucous is also an inexpensive and easy-to-use method to document ovulation, it is less acceptable to women [30], and this may be particularly true for women who are not trying to become pregnant. Furthermore, although other computerised methods exist that indicate the fertile period of a cycle, these methods are more costly than LS-QBT, have not been validated against other established indicators of ovulation and are not designed to identify the day of luteal onset (Appendix 28). In summary, although not conclusive, findings from my PhD work suggest that LS-QBT is inexpensive, easy-to-use and acceptable to young women not seeking conception, and performs reasonably well in detecting whether cycles are ovulatory and in estimating the day of luteal onset. The method did not perform particularly well in identifying anovulatory cycles. But as discussed in Chapter 2, this may be related to the very small number of anovulatory cycles (four of out 40 cycles) monitored amongst the validation study sample. Though further validation is required, particularly relative to daily transvaginal ultrasound, this method shows promise as a tool in large representative studies and could be used to examine whether subclinical ovulatory disturbances are related to other health outcomes such as cardiovascular disease risk, depression and dementia [31].

The second part of the primary research question was to explore the hypothesis that the relationships among CDR, ovulatory function and bone are mediated by the physiological stress
response. Previous work has indicated that the habitual monitoring of food intake and a preoccupation with body weight experienced by women with higher CDR may act as a stressor capable of activating the physiological stress response resulting in elevated cortisol [32-36]. That elevated cortisol can detrimentally affect health is based on the hypothesis of McEwen [37], who suggests that repeated exposure to cortisol as a result of chronic stress results in allostatic overload affecting many body systems [37]. The majority of the evidence linking chronic psychosocial stress to detrimental health outcomes has been conducted among men and/or those with clinically elevated cortisol. Few studies have examined whether elevated cortisol within the normal range can lead to adverse health outcomes among healthy young women. In this sample of healthy, non-obese, regularly menstruating women, a modest inverse relationship between UFC and total body aBMD and BMC, lumbar spine aBMD and total hip BMC was observed at baseline (Chapter 5). After adjustment for potentially confounding variables (urine volume, ethnicity, height, lean mass, previous oral contraceptive use duration, calcium intake, sport activity and perceived stress), UFC continued to explain 4-8% of the variation in total body BMC and aBMD and total hip BMC. However, average UFC was not significantly associated with ovulatory disturbances or 2-year change in aBMD (Chapter 3). As evidence from older adults suggests that the effect on bone of elevated cortisol within the normal range is subtle [38-41], a longer period of observation may be required to see differences in bone density in young women. Further support that cortisol is capable of influencing health outcomes in healthy young women is presented in Chapter 4. A positive association between UFC and ABP, an additional physiologic indicator of the stress response, was observed at the first follow-up. As this study was not designed or powered to detect an association with ABP, these findings are intriguing. These findings are meaningful as blood pressure during young adulthood has been shown to be related to indicators of cardiovascular disease (CVD) risk later in life [42-45].

In cross-sectional analysis, UFC was not associated with CDR at baseline (Chapter 5) or first follow-up (Chapter 4). As well, UFC averaged from the three collections was not associated with average CDR or average scores for any other eating attitude questionnaires (Appendix 29). The only psychosocial variables that were associated with average UFC in this study were Bulimia score from the Eating Disorder Inventory-2 (EDI) [46] (completed at baseline and first follow-up), 2-year Eating Concern (from the EDE) [18], 2-year Depression Anxiety and Stress Scale score including the Depression and Anxiety subscales [47], 2-year Life Events Scale [48] score for the Personal, School and Social section, average Perceived Stress Scale [49] and average Daily Stress Inventory Impact scores [50] (Appendix 29). This was somewhat surprising as other studies have reported associations between cortisol and CDR [34] as well as several other measures of eating attitudes [34,51].
Yet, it is still plausible that cortisol is one of the mediators in the relationship between CDR and negative health outcomes, although perhaps not the only or most important mediator. Women with higher average CDR in this study had higher average UFC (Chapter 3) and several other studies have found higher cortisol among women with higher CDR [32-33,35-36]. The metabolism of steroids (including cortisol, progesterone and estradiol) may be greater among Asians than Caucasians due to differences in cytochrome P450 3A4 activity [52]. Therefore, the main effect of ethnicity and the interactive effect of ethnicity and CDR were examined (Chapter 3). Findings revealed that UFC was elevated among Caucasians with higher CDR but, among Asians, UFC did not differ by CDR level. Yet CDR, general stress, the frequency of subclinical ovulatory disturbances, UFC and ΔaBMD did not differ by ethnicity (Appendix 30). Reanalysis of cross-sectional data showed a significant ethnicity-by-CDR interaction with UFC at baseline, and trends at first follow and final follow-up (Appendix 31). There was no main effect of ethnicity for any data collection point (Appendix 31). There was a main effect of CDR level at first follow-up, and a trend was apparent at final follow-up (Appendix 31).

Ethnicity may reflect cultural differences as well as genetic or racial differences. In this study, for example, we speculate that there may be differences in the qualitative experience of CDR among those with differing ethnicities, and that these differences could be culturally mediated. Conversely, differences in steroid metabolism by ethnicity likely relate to physiological aspects related to genetic differences in the activity of the P450 3A4 enzyme. In assessing our participants’ ethnicity we did not distinguish between the genetic and cultural aspects of ethnicity. Participants in this study were asked “With what race/ethnic group do you identify?” (Question 5, Appendix 23) and were then grouped as Caucasian (Caucasian, Latin American) or Asian (Chinese, South Asian, Arab/West Asian, Filipino, South East Asian, Japanese, Korean) in order to examine differences that may relate to both physiology and culture. As we did not distinguish between physiology and culture, we describe differences and similarities between Asians and Caucasians in the preceding Chapters that could relate to both aspects. Therefore, whether the experience of CDR as a stressor differs among Asian and Caucasian women is not clear from my PhD research.

There are numerous pathways that can disrupt ovulatory function that do not involve the HPA axis [31]. In fact several of the neuropeptides that have been identified in causing dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis are related to appetite control including corticotropin releasing hormone, ghrelin, leptin and neuropeptide Y [53]. This may be particularly relevant to the experience of CDR, as one of the characteristics of CDR is an attempt to ignore physiological hunger. Asians with higher CDR in my PhD study had more subclinical ovulatory disturbances than women with lower CDR, although UFC did not differ by CDR level among Asians. Additionally, negative eating and body attitudes were associated with
elevated ABP but not with UFC (Chapter 4). Though CDR specifically was not associated with ABP in that study, other questionnaires reflecting negative eating attitudes and body dissatisfaction were, including the Disinhibition and Hunger subscales of the TFEQ [54], the EDI-Bulimia subscale [46] and the Body Shape Questionnaire [55] (Appendix 32). Taken together, evidence indicates that cortisol may not be the only or the most important mediator in the association between CDR and adverse health outcomes.

Finally, study results suggest that the association among eating and body attitudes and negative health outcomes are consequences of the experience of stress specific to eating/body rather than to general stress or to disordered eating behaviours (Chapters 3 and 4). As a whole, my PhD research findings indicate that eating and body attitudes may act as a chronic stressor possibly leading to adverse health outcomes due to allostatic overload including impaired ovulatory function and elevated blood pressure.

6.3 Strengths and limitations

My PhD research was the second study to prospectively examine the relationships among CDR, subclinical ovulatory disturbances and 2-year change in aBMD in healthy young women. And it was the first study to examine whether cortisol, an indicator of the physiological stress response, was a mediator of the above relationships. The greatest contribution of this study was the documentation of ovulatory function over two years and its association with change in aBMD. Previous studies that have examined the association between bone density and the menstrual cycle have monitored two to four cycles in relation to one-time bone density measures [24-25,27-28], or a maximum of 12 cycles in relation to change in bone density over one- to two-years [11-12,26]. In my PhD study, 114 women provided usable daily basal temperature recordings for 1 to 28 cycles (average 13.6 cycles) over two years. Therefore, it is more likely that women were correctly classified as those with a higher and lower percentage of cycles with subclinical ovulatory disturbances than occurred in much of the previous work.

However, the assessment of ovulatory function should also be recognised as a study limitation. Optimally, ovulation would have been monitored by the cyclic patterns of reproductive hormones via daily salivary, serum or urinary samples of LH, estradiol and progesterone. Due to cost constraints and consideration of subject burden, and therefore participant retention, ovulatory function was monitored using LS-QBT. Although it is less accurate than monitoring cyclic reproductive hormone patterns, it has been validated against established indirect indictors of ovulation including peak serum LH [29] and the sustained rise in urinary metabolites of progesterone (Chapter 2). The inclusion of estradiol and progesterone levels over the menstrual cycle would have allowed for stronger conclusions to be made regarding the relationship between CDR and subclinical ovulatory disturbances as these hormones affect bone turnover.
Furthermore, the credibility of our data would have improved with their inclusion as our method of documenting ovulatory function is currently not widely accepted.

One-hundred and twenty-three women were followed for two years (12% lost to follow up) allowing original power estimates requiring 100 women in order to observe associations with two-year change in bone to be maintained. One hundred women provided 80% power to detect a relationship between CDR and change in aBMD at $P<0.05$, if the true rate of change in aBMD is 0.33% per unit increment in CDR score. This was based on the assumption that the standard deviation of CDR was 5.5 [9] and that the standard deviation of 3-year aBMD change in young adult women is 7% (from unpublished data from the CaMOS study). A considerable amount of time and care was taken in ensuring as many participants as possible were retained and that their collection of data was conducted appropriately. A strong personal relationship developed between each participant and myself over the 2-year study making this possible. I ensured that participants knew I was always available for questions about study procedures and data collection including 24-hour support by email and telephone during the urine collection and ABP monitoring procedures. As well, participants were emailed and contacted by telephone with friendly reminders for daily temperature recording, appointments and the return of completed questionnaires (Appendix 33). In order to encourage continued participation, participants were provided with updates on overall study progress and their personal data over the course of the study including temperature calendar analyses (Appendix 34), ABP monitoring (Appendix 35) and change in bone density (Appendix 36). Travel reimbursements and small financial incentives (annual $30 gift cards) were also provided to increase retention.

Our high retention rate is also somewhat attributed to the convenience sampling method used. This likely resulted in self-selection bias, as women interested in participating in a study of this nature may differ in various aspects from those who do not volunteer to participate. In particular, the participants were likely highly health-motivated as they committed their time over two years, including several data collection requirements that were somewhat invasive and time consuming such as daily temperature recording and three 24-hour urine collections. Participants in this study were not limited to women characterised with either very high or very low CDR levels, as most previous work in the area, somewhat increasing the generalisability of the findings in that regard. Yet, study findings are generalisable only to women with characteristics similar to the sample: Caucasian or Asian, young, healthy, non-obese, non-smokers (3% of sample smoked), consistent sleep patterns (no shift work), with post-secondary education (most were current university students and all had completed some post-secondary education) and no children (only two participants had children) or spouse/partner (92% were single).

It is also important to note that at study enrollment, these young women had no previous eating disorders or other health conditions or current medication use that could affect study
outcomes, including oral contraceptives or other hormones. According to Statistics Canada, approximately 18% of women aged 15-49 years report current oral contraceptive use, and oral contraceptive use is associated with being young, unmarried, sexually active, having prescription drug insurance and relatively high education [56]. That 22 women began using hormones during the study may also be a limitation, as the affect of contraceptive use on bone density is controversial [57]. However, 2-year study analyses were repeated excluding these 22 participants and findings did not change. Furthermore, the duration of total and study hormone use was not associated with 2-year change in bone (Appendix 37). The duration of previous oral contraceptive use was associated with baseline aBMD and BMC at the total hip and whole body (Chapter 5). Finally, volume-adjusted UFC was not associated with either the duration of total (r= -0.091, P=0.320) or study (r= -0.002, P=0.980) hormone use. It appears that oral contraceptives have more influence on assessment of bound cortisol rather than free cortisol [58-59], particularly 24-hour UFC [60] which was measured in this study. It is also important to note that participants initiated hormone use for contraception, not because of menstrual cycle abnormalities or low hormone levels. Therefore, the hormone levels attained in those on contraceptives may not have differed dramatically from non-users.

In order to enroll a diverse group of women, recruitment occurred within the local community and from university classes covering several Faculties (Applied Science, Science, Arts, Health, Pharmacy and Graduate Studies). An effort was also made to limit recruitment within the Food, Nutrition and Health department in order to avoid over sampling young women who may have a heightened awareness about food and their body. As well, no reference was made to body image or eating attitudes or behaviours in recruitment material to avoid recruitment of women who may have previous or current eating disorders. This may have resulted in inadequate representation of women with elevated CDR. Our mean and median CDR scores were lower than scores previously reported in similar samples [5,9,61-62]. Also, very few participants were classified as “very high” CDR based on previously used cut-offs (n=14 with Three Factor Eating Questionnaire Restraint scores ≥13 at the two-year follow-up). Future longitudinal studies may benefit from determining CDR scores during recruitment, as well as other relevant demographics, in order to guide recruitment with the goal of achieving a more diverse sample of young women, reflecting a broad spectrum of CDR levels.

The measures of dietary intake, physical activity, general stress, eating attitudes and behaviours and daily temperature recordings relied on self-report measures. This was the only feasible means of obtaining this information; however, it should be recognised as a limitation of the study. The reporting of measures relating to eating and weight, such as reported dietary intake, physical activity and previous weight fluctuations, may have differed between those with higher versus lower CDR. For example, women with higher CDR who weigh themselves more
frequently may report more episodes of weight fluctuation than those who weigh themselves less frequently, simply because they are more aware of changes in their weight.

Three 24-hour urine collections analysed for UFC were used to operationalise “usual” activation of the HPA axis. In order to determine “usual” cortisol levels, the three collections were averaged for analysis with subclinical ovulatory disturbances and ΔaBMD that occurred over the same time. Although UFC did not change consistently among subjects over time by repeated measures General Linear Model (Appendix 38), it is well established that cortisol varies considerably from day-to-day as the result of everyday minor stressful situations [63-64]. Therefore, additional measures would have provided a better picture of participants’ “usual” cortisol levels and how that relates to eating attitudes and general stress perception.

Furthermore, though detailed written and verbal instruction was provided, along with access to continuous support during the 24-hour urine collections, it is possible that errors may have occurred. For example, the collection period may not have been exactly 24 hours or some voids may not have been collected. However, I do not have reason to believe that these issues interfered with the validity of data collected by participants in my PhD study. Continuous contact with my participants during the procedures suggests that the collections were complete and appropriately timed. As well, participants’ menstrual cycle phase was not accounted for, and the diurnal rhythm of cortisol appears to differ during the follicular and luteal phases of the menstrual cycle; however, 24-hour measures appear to be less affected [65-67].

Another limitation is that participants were allowed to choose the days to complete the urine collections and ABP monitoring. Based on communication with participants, many of them completed these procedures when they would be at home, mostly resting, rather than a regular school or work day. It is also reported that participants in ABP studies report lower activity levels on monitoring days versus control days [68]. In an attempt to account for the type of day participants encountered during these procedures, the Daily Stress Inventory was completed to assess the frequency and perceived impact of 58 everyday minor stressful events that may have occurred during the procedures. Whether DSI scores reflected the level of stress participants encountered during the completion of these procedures is not clear: DSI scores were not associated with ABP measures (Chapter 4). The average DSI Impact score, but not Frequency, was associated with average UFC (r=0.21, P=0.024). The documentation of “usual” physiologic indicators of stress would have been improved by having participants complete the procedures on days when they were engaging in their usual activities such as school and employment.

Related to this, activity during ABP was not verified with accelerometers, and both activity and posture during ABP assessment is known to significantly affect readings [69-70]. In order to account for physical activity during the monitoring procedures, participants recorded
their concurrent activity for each reading. These activities were coded as either sedentary or active and then summed and averaged for the number of readings provided. Although not perfect, activity during blood pressure monitoring score was significantly associated with ABP measures (Chapter 4). This suggests that we were somewhat successful at controlling for activity during ABP monitoring by this method. Similar to the urine collections, we did not account for participants’ menstrual cycle phase, which may affect HPA axis reactivity to acute stressors but does not appear to influence 24-hour ABP in a meaningful way among normotensive women [71-72].

By examining bone prospectively, a smaller sample size may be required to detect a difference in ΔaBMD as inter-individual variability related to genetics and lifestyle behaviours affecting bone is reduced. However, two years is a relatively short period of time to observe bone density changes in young healthy women. Based on the literature, we estimated that a sample size of 100 at a minimum would be sufficient to observe changes in aBMD by dual energy X-ray absorptiometry (DXA). Although aBMD changed significantly over the two years (Chapter 3), a longer period of observation may have been warranted. However, it would be difficult to retain university students over the age of 19 years at baseline (the age at which they can legally provide informed consent) for more than two years. Most students in this study were in their second or third year when they enrolled in the study and therefore would have completed their programmes within two years, potentially resulting in less participant retention over a longer time period. Although DXA has good precision and reproducibility in measuring aBMD [73], errors in repeat measures can occur. For example, if different nuclear technicians perform the scan small differences in the same person may occur. The percentage aBMD change from Time 2 to Time 1 would need to differ by >2.8% in order to detect a real change if the precision error rate was 1% [74]. Therefore, it could also be that measurement error in aBMD was as great as the change in aBMD over time.

Finally, as this study was a prospective cohort design, rather than experimental, the relationships presented in previous chapters reflect correlation rather than causation. This is particularly relevant given the modest differences between groups and small Pearson’s correlation coefficients that were observed in this study. As well, factors that were not examined in this study (e.g. acculturation in regard to the experience of CDR as a stressor, social support and coping in regard to ABP, and adolescent physical activity and family history of osteoporosis in regard to bone) may have had a significant influence on study outcome variables for which these data do not account.
6.4 Future directions

Although a considerable amount of work has been conducted in the area of young women’s eating attitudes, particularly CDR (Chapter 1), the majority of studies are cross-sectional. Therefore, many questions remain as to whether young women’s eating attitudes are associated with negative health outcomes over time. One of the clinically relevant questions is whether subclinical ovulatory disturbances, which are not apparent to women because their menstrual cycle lengths and flow are normal, are associated with bone loss. Osteoporosis is a major health care issue in Canada resulting in considerable health care spending and reduced quality of life for those that experience fractures [75]. It is of concern that negative eating and body attitudes are so common among young women and may be associated with unseen subclinical ovulatory disturbances, which in turn may be related to bone loss.

Ideally, eating attitudes, ovulatory function and changes in aBMD would be monitored in a larger sample of women over a longer period of time. In order to retain a sufficient sample size, a large number of women would need to be recruited in order to account for the social and geographical instability at this time of life. For example, young women may experience unexpected pregnancy and on the other hand, hormone-based contraceptive use may occur as well as frequent moving for jobs or schooling. Therefore, a study of this type may require multiple site sampling, particularly outside of the university setting. Although monitoring of cycles using daily transvaginal ultrasound or the cyclic pattern of reproductive hormones (LH, estradiol and progesterone) to detect ovulation would be optimal, this would require significant investment of resources and represent a high level of subject burden. Further validation of the LS-QBT method against daily transvaginal ultrasound, the LH surge and cyclic levels of estradiol and progesterone is thus warranted. This is particularly important to do in women with irregular cycles (who may be more likely to experience anovulation) and those who are more reflective of the general population, for example inclusion of women that are overweight or obese. This would increase the acceptability of this method in the research community. As well, the ease of use of Maximina © computer programme used to analyse temperature recordings could be improved. And if the programme was web-based, it would allow for multiple researchers to analyse a large amount of data. Further validation and acceptance of the LS-QBT method would also allow for examination of ovulatory function in other stress-related conditions encountered by young women including depression and anxiety.

Additional prospective studies examining the relationships among eating attitudes, ovulatory function and bone would also be improved by using quantitative computed tomography (QCT) to document change in bone density rather than DXA. DXA assesses both cancellous and cortical bone and QCT assesses only cancellous bone. Cancellous bone is found at sites where osteoporotic fractures are common and turns over more rapidly. Thus,
observation of cancellous bone may require a shorter follow-up period when considering the relationship between eating attitudes and subclinical ovulatory disturbances, as well as cortisol, on bone density. As QCT does result in higher radiation exposure, new methods such as peripheral QCT measuring bone at the forearm, wrist or heel may be more useful and acceptable. Although QCT is not validated for osteoporosis diagnosis, it may be useful in tracking within-individual changes in bone density over time. Use of QCT would also allow for exploration of other aspects of bone strength, such as bone internal architecture and structure, which are not detected by DXA but could potentially be influenced by exposure to elevated cortisol or insufficient estradiol or progesterone. From an evolutionary prospective, bone strength may be more important to survival than bone mass [76], and therefore whether cortisol and subclinical ovulatory disturbances affect bone structure may be important.

Previous research is not entirely consistent as to whether cortisol levels, an indicator of the physiological stress response, are associated with CDR and other negative eating and body attitudes. As 24-hour measures of cortisol appear to be a good indicator of CDR-related stress, future studies would be improved by having participants complete multiple 24-hour urine collections on days in which they are engaged in their “usual” activities of daily living. As this would increase subject burden, improved methods of collection should be investigated. Ethnicity should also be taken into account as the metabolism of cortisol may differ between Asians and Caucasians [52].

This was the first study to examine whether cortisol mediated the relationships among CDR, ovulatory disturbances and bone; yet the findings in this regard were inconclusive. The interactive effect of CDR and ethnicity on UFC (Chapter 3) suggests that future studies should explore the potential for ethnicity to affect the experience of CDR as a stressor. Validation of the Three Factor Eating Questionnaire among English-speaking Asians should be the first step. Assessing level of acculturation may also be relevant when evaluating the experience of eating and body stress. In particular, qualitative studies among young women of differing ethnicities and/or levels of acculturation may be used to examine the meaning and experience of high CDR and other eating attitudes, as well as the experience of stress in general and specific to eating and the body. As well, future studies should consider other aspects of the stress response in relation to CDR including the indicators of stress-activation of the sympathetic nervous system, such as epinephrine and norepinephrine. Exploration of other potential mechanisms linking CDR and subclinical ovulatory disturbances are also warranted. Research among women with functional hypothalamic amenorrhea (FHA) suggests that various neurotransmitters and neuropeptides are capable of affecting gonadotropin-releasing hormone pulsatile section and therefore ovulatory function [53]. Some of these neuropeptides, such as corticotropin releasing hormone, ghrelin, leptin and neuropeptide Y, are also involved in appetite
control and may therefore be particularly relevant to CDR where women try to override physiological hunger cues. Use of QCT, for reasons mentioned previously, may provide additional information as to whether elevated cortisol within the normal range is associated with bone loss in otherwise healthy young women.

To examine other aspects of the stress response in relation to CDR, we included a measure of 12-hour daytime ABP. When this study was proposed, there was very little information regarding normative ABP values among young healthy women and therefore this study was not powered to detect associations among psychosocial constructs and ABP. There was very little variation in ABP in this group of healthy young women indicating that a very large sample size would be required to observe an association with eating attitudes. Yet modest associations between negative eating attitudes and elevated ABP were evident (Chapter 4), suggesting the opportunity for future research in this area. As young adult blood pressure is associated with future CVD risk [42-45] and CVD is the number one killer of Canadians [77], research in this area is relevant from a public health perspective. Similarly to the measures of UFC, future studies examining ABP in relation to eating attitudes would be improved by having participants complete multiple days of ABP monitoring in which they are engaged in “usual” activities.

Although not conclusive, evidence is accumulating that negative eating attitudes, particularly CDR, are associated with adverse physiological health outcomes. For that reason, the next step may be to determine if interventions can reduce eating and body stress in women presenting with high levels of CDR in order to prevent or reduce the impact of chronic stress on physiological health. Research among women with FHA suggests the possibility of improvement in ovulatory function with cognitive behavioural therapy. Women with FHA experience amenorrhea of unknown aetiology: these women present with normal weight, normal activity levels and adequate dietary intake [31]. The majority of evidence suggests that FHA is related to stress-induced activation of the HPA axis and suppression of the HPG axis [31]. In a 20-week study of 16 women with FHA randomised to either cognitive behavioural therapy or observation, six women in the experimental group resumed ovulating versus one in the observation group [31]. Furthermore, ovarian recovery was not associated with weight gain [31]. Whether cognitive behavioural therapy may help young women with disordered eating attitudes in regards to menstrual cycle and ovulatory function warrants investigation.
6.5 References


Appendix 1: Recruitment Materials for Quantitative Basal Temperature (QBT) Validation Study

Flyer given to students of University of British Columbia classes and posted around the university and local Vancouver community.

Are you a WOMAN between 19-35 years?

Want to learn more about your menstrual cycle?

*We are inviting you to participate in a research study conducted by Dr. Susan Barr at the University of BC!*

**Who Can Participate?** You are eligible to participate in this study if you are: (1) female, (2) between 19 and 35 years of age, (3) regularly menstruating, (4) at a normal body weight defined as body mass index (an index of your weight in kilograms divided by your height in metres squared) between 18.5 and 25, (5) in good health and not suffering from any chronic diseases that may affect hormones, and (6) able to read and understand English.

**Who Should Not Participate?** You are not able to participate in this study if you: (1) do not meet inclusion criteria, (2) use oral contraceptives (birth control pills), receive Depo-Provera injections or use other drugs that affect menstruation, (3) have been diagnosed with or treated for an eating disorder, (4) are pregnant or lactating, or (5) work at night or have an inconsistent sleep pattern.

**What is involved?**
1. A telephone survey to determine if you are eligible to participate (~15 mins).

2. Study orientation at the Nutrition department at UBC to receive materials & instructions, answer a brief questionnaire, have your height, weight and waist circumference measured and answer a question regarding symptoms associated your menstrual cycle (~ 45 minutes).

3. Keeping a daily record of your morning body temperature (at home) - we will provide a calendar and digital thermometer (~3 mins per day for one menstrual cycle, ~21-35 days).

4. Daily collection of a sample of your first urination (at home) - we will provide small sponge vials to collect a small portion of your first morning urination which you will store in your freezer. As needed, you will return the frozen samples to UBC using small coolers and icepacks which we will provide (~5 mins per day for one menstrual cycle, ~21-35 days).

**What’s in it for me!?**
We will reimburse all travel expenses and at the end of the study you will be provided with a gift certificate. You will be provided with information about your menstrual cycle and a free digital thermometer.

For more information please call Jennifer at 604-616-4676
OR e-mail jbedford@interchange.ubc.ca
• Are you a normal-weight, healthy woman aged 19 to 35 who doesn’t use birth control pills?
• Would you like to learn more about your menstrual cycle/fertility?

If so, we would like to invite you to participate in a research study, conducted by Dr. Susan Barr at the University of British Columbia. Please call or e-mail Jennifer (see below) to determine if you are eligible to participate.

The study will involve the following: coming to UBC to be oriented to the study procedures, complete a brief questionnaire and have your height, weight and waist measured; at your home recording your body temperature and collecting a sample of your first urination each morning for one menstrual cycle; returning the frozen samples to UBC.

We provide ALL materials and will reimburse you for travel expenses. After completing the study, you will be given information on your menstrual cycle and will also receive a gift certificate.

Please contact Jennifer at (604) 616-4676 or e-mail jbedford@interchange.ubc.ca for more information. We look forward to hearing from you.
Appendix 2: QBT Validation Study Letter of Initial Contact (via email)

Hi [name]! Thanks for your interest in our study!

I've included more detailed information about the study procedures and eligibility requirements at the bottom of this email.

Read the information over and let me know if you have any questions or require any additional info or clarification.

If you are still interested in participating, email me back and we can set up a time that I can call you to answer a few questions about yourself (age, health, etc) to make sure you are eligible. This takes about 15 minutes and we can do it anytime that is convenient for you - day or night. When you email me back, let me know what number I can reach you at and some convenient days/times for me to call.

If you are able to participate, we can set up a time to meet at UBC for about 45 minutes where I will orient you to the study - again at your convenience, and then you can complete the procedures during a menstrual cycle that is convenient for you!

Let me know what you think and please contact me again if you have any questions or require further clarification and if you want to set up a time for me to call!

Thanks again for your interested and please feel free to pass the info included in this email onto any of your friends/family/coworkers who may be interested in participating :)

Jen

We are inviting regularly menstruating, normal-weight women aged 19 to 35 years who are in general good health to participate in this research study being conducted by Dr. Susan Barr at the University of BC.

Characterising the menstrual cycle determines whether different parts of the cycle are normal. Measuring body temperature at the time of awakening is a non-invasive way to characterise a women's menstrual cycle. However, this method needs to be compared to other laboratory-based methods to demonstrate that it is accurate. Therefore, the purpose of this study is to access how well a computerized method of evaluating body temperature, measured upon awakening, characterises the menstrual cycle. Body temperature records will be compared to hormone levels in the urine, which is an established laboratory-based method of characterising the menstrual cycle.

**Who Can Participate?** You are eligible to participate in this study if you are: (1) female, (2) between 19 and 35 years of age, (3) regularly menstruating, (4) at a normal body weight defined as body mass index (an index of your weight in kilograms divided by your height in metres squared) between 18.5 and 25, (5) in good health and not suffering from any chronic diseases that may affect hormones, and (6) able to read and understand English.

**Who Should Not Participate?** You are not able to participate in this study if you: (1) do not meet inclusion criteria, (2) use oral contraceptives (birth control pills), receive Depo-Provera injections or use other drugs that affect menstruation, (3) have been diagnosed with or treated for an eating disorder, (4) are pregnant or lactating, or (5) work at night or have an inconsistent sleep pattern.
What the study involves? At UBC, 45 minute orientation to the study procedures and, at your home, ~10 minutes each morning for one complete menstrual cycle (21 to 35 days which means the day your period starts for one cycle and continuing with the procedures until the day your period starts for the next cycle at which time you will have completed the study!).

If you choose to be involved in this study, the procedures and visits you can expect will include the following:

1. Answering a few simple questions over the phone to determine if you are able to participate in the study (~15 minutes).

2. Study orientation at UBC during which you will meet with a member of the research team at the Human Nutrition department of UBC to be oriented to the study procedures, complete a brief questionnaire, be provided with instructions and materials for daily body temperature readings and collecting a sample of your first morning urination, have your height, weight and waist circumference measured and answer a question regarding symptoms associated your menstrual cycle (~45 minutes, at your convenience).

3. Completion of a daily temperature calendar & daily first morning urine sample at your home. Beginning the first day of your period (for a cycle you choose to be most convenient) and daily thereafter, you will measure your body temperature (orally) immediately after waking using a digital thermometer. You will record the temperature in your calendar as well as the time you woke up, whether or not flow has occurred, any sleep problems, your health status and any physical activity you performed the previous day. At UBC, we will provide you with the digital thermometer and temperature calendar and instructions to use them. You will also use a sponge vial to collect a portion of your first morning urination, label the vial and then place it immediately in your freezer for storage. On a 'label record sheet', you will record any medications or supplements taken and how the specimen was treated if it was not frozen immediately. We will contact you weekly to answer any questions you may have (~10 minutes daily for one full menstrual cycle which is 21 to 35 days).

4. Returning the frozen urine samples and temperature calendar to UBC. During your UBC orientation, we will provide you with icepacks and a Styrofoam cooler for transporting the frozen urine samples to UBC. The amount of freezer space you have available will determine the number of times you need to return the frozen vials to UBC. When you return the last of your urine samples, you will also return your completed temperature calendar and 'label record sheet'.

What's in it for me?
We will reimburse all travel expenses ($5 per trip to UBC). After completing all study procedures, you get to keep the digital thermometer and you will be provided with a $20 gift certificate and information about your menstrual cycle for yourself and to share with your doctor if you choose.
Appendix 3: QBT Validation Study Eligibility Phone Script

Hello [name]. This is Jennifer Bedford, a Ph.D. student at UBC working as a member of the research team for the study on women’s menstrual cycles. I am calling because you expressed an interest in participating. Are you still interested in participating?”

If no: “I understand. Thank you for your time and please call again if you change your mind as we are still looking for new participants”

If yes: “Great. Do you have time to speak with me now for approximately 15 minutes?”
If no: “That’s no problem. Can we set up a time that I can call you back?” Set up time and date most convenient for participant.

If yes: I would like to briefly explain the study procedures to you and then if you are interested in participating I have a few simple questions that I need to ask you in order to determine if you are able to participate in the study. The study would begin with a 45 minute meeting at UBC where I will orient you to the study procedures in detail. You will complete the study procedures each day at your home over one complete menstrual cycle which, for most women, ranges from 21 to 35 days. At the meeting, you will read and sign a consent form describing the study procedures and your rights/responsibilities as a research participant. Then we will decide which menstrual cycle is most convenient for you to complete the study procedures. You will then answer a short questionnaire with questions regarding your menstrual/reproductive history, medication use, special dietary practices, and demographic information such as employment and age. Next, I will provide you with materials and instructions for completion of a temperature calendar. This will involve recording your temperature each morning before you get out of bed using a digital thermometer which I will give you (and you can keep after the study!). This will take you ~3 minutes each day for one menstrual cycle. I will also provide you with the materials and instructions for daily collection of a sample from your first urination of the day. Each morning for one menstrual cycle (that you choose to be most convenient) you will use a sponge vial to collect a portion of your first urination which you will then store in your freezer. There is also a ‘record sheet’ where you will record any medications and supplements you took that day and how the urine specimen was treated. This will take you ~10 minutes each day for one menstrual cycle. You will return the frozen urine samples to UBC as often as required, depending on your freezer space, probably once per week. At the meeting I will provide you with icepacks and a Styrofoam cooler for you to use to return the samples as often as you require. You will receive $5 for each trip. When you return the last of the frozen samples, you will also bring back your temperature calendar and record sheet. Lastly, I will measure your height, weight and waist circumference and will ask you a question about any symptoms you experience before your menstrual period.

Your participation in the study will be completing voluntary and you can withdraw without consequence at any time. We will reimburse you for all travel, $5 per trip to UBC, and you will also receive a $20 gift certificate when all procedures are completed. At the end of the study we will provide you with information regarding your menstrual cycle as well as a copy for you to share with your doctor if you choose.”

“Would you like to continue with the questioning to see if you are eligible? This will take about 10 minutes. Your participation in this brief interview is completely voluntary and you may end the interview at any time without any consequences. Do you have time to complete this now?”

If no: “That’s no problem. Can we set up a time that I can call you back?” Set up time and date most convenient for participant.

155
If yes: “Great let’s get started! As I mentioned I will be asking you these questions in order to ensure that you are able to participate in the study. May I proceed?”

“Are you a female between 19 and 35 years of age?” [Yes - continue] [No – ineligible*]

“Are you able to read, speak and understand English?” [Yes - continue] [No – ineligible]

“Do you work at night or work shift work?” [No - continue] [Yes – ineligible]

“Would you say your sleeping patterns are consistent (i.e. wake up and go to sleep at approximately same time, most days and sleep for similar amount of time most days)?” [Yes - continue] [No – ineligible]

“Are you currently pregnant or lactating?” [No - continue] [Yes – ineligible]

“OK great. Now I am going to as
sk you some questions about your health and medication use. Because this study is examining the menstrual cycle, I need to know if you have any medical conditions or use any drugs that may affect the measurements.”

“Are you currently using the birth control pill, receiving Depo-Provera injections or using other birth control medication?”
[No - continue] [Yes – ineligible]

“Have you used either birth control pills, Depo-Provera injections or other birth control medication in the past 6 months?”
[No - continue] [Yes – ineligible]

“Do you have your period every 21 to 35 days?” [Yes continue] [No – ineligible]

“Are you in general good health and free of any chronic diseases?” [Yes - continue] [No – ineligible]

“Have you ever been treated for or diagnosed with an eating disorder?” [No - continue] [Yes – ineligible]

“Do you currently or have you ever suffered with any chronic medical conditions?”
[No - continue] If yes, “Could you please name the condition(s)?”

[Women with any of the following will be ineligible: Anorexia or bulimia nervosa, polycystic ovary syndrome, or endometriosis]

“Have you ever been sufficiently bothered by severe acne, unwanted face or body hair to consult a physician for treatment?” [No - continue] [Yes – ineligible]

“Are you currently or have you ever taken any prescription medications daily for more than 1 month?”
[No - continue] If yes, “Could you please name the medications and the duration you took them for?

[Women using any drugs that may affect ovarian function will be ineligible.]

“Are you currently or have you ever taken any herbal supplements or over-the-counter medications daily for more than 1 month?”
If yes, “Could you please name the herbal supplement and the duration you took them for?”

“OK great. The last thing I am going to ask is your height and weight so that I can make a calculation to determine your body mass index (BMI) which is an index of your weight in kilograms divided by your height in metres. We require women with BMIs >18.5 and <25 kg/m² because BMIs below and above this range have effects on the menstrual cycle.”

“What is your current body weight? ______________

What is your current height?” ______________

If BMI <18.5: “Thank you. Your BMI is XX. Unfortunately this BMI is not in the range we are looking for. Thank you for your time in answering these questions and I apologize for any inconvenience.”

If BMI >25: “Thank you. Your BMI is XX. Unfortunately this BMI is not in the range we are looking for. Thank you for your time in answering these questions and I apologize for any inconvenience.”

If BMI 18.5 to 25: “Thank you. Your BMI is XX. You are eligible to participate in the study. May we set up an appointment to meet for approximately 45 minutes at UBC?

Arrange meeting date and time.

Thank you again for your time and I look forward to meeting with you on [date] at [time]. I will provide you with $5 to defray your transportation costs at this time (you will receive another $5 each time you need to travel to UBC). As we are going to be measuring your weight and waist circumference at the meeting, please wear some light clothing (underneath your regular clothing) like a tank top or camisole and perhaps some spandex shorts or light pants.

OK so last thing, could I please get your contact information at this time? I will send you a reminder by email or phone about our meeting date/time/location

EMAIL:_________________________________________________________

Great thank you. Now the meeting is in my office at the Food & Nutrition Building is located at 2205 East Mall. Are you familiar with campus?

How will you be arriving? (Bus, parking- meter parking at bookstore).

Some landmarks: bookstore, new Michael Smith Building then us. Across from Library.

My office is on the third floor in Room 321. There will be signs posted on the walls of the building guiding you but just as a heads up when you walk into the building the stairs are on your right. Proceed up the stairs to the 3rd floor and go through the doorway then turn left and I am the first door on the left!

Please call me at 604-616-4676 if you need to re-schedule or you have any questions or need further directions to campus, the building or my office.”

*If a participant’s answer to any question determines them ineligible the following script will be used:

“Thank you. Unfortunately you are ineligible for this study because [explain based on exclusion criteria]. I appreciate your interest and thank you for your time. Have a nice day. Bye.”
Appendix 4: QBT Validation Study Questionnaires

Section completed by participant during orientation:

1. Today’s date: ____________________ (month / day / year)
2. Your birth date: __________________ (month / day / year)
3. How many years of school have you finished? (Mark the highest level completed)
   ___ I have not completed any formal schooling
   ___ Less than grade 9
   ___ Grades 9-13, without certificate, diploma, or degree
   ___ High school certificate or diploma
   ___ Trades or professional certificate or diploma
   ___ Some university without certificate or diploma
   ___ University certificate or diploma
   ___ University degree
   ___ Graduate or professional degree (MA/Sc, PhD, MBA, MD)
4. What is your current employment status? (Check all that apply)
   ___ Unemployed
   ___ Retired
   ___ Student, part time
   ___ Student, full time
   ___ Employed, part time
   ___ Employed, full time
5. With what race/ethnic group do you identify? (Check all that apply)
   ___ Caucasian
   ___ Chinese
   ___ South Asian (Indian, Pakistani, Punjabi, Sri Lankan)
   ___ Black (African, Haitian, Jamaican, Somali)
   ___ First Nations
   ___ Arab/West Asian (Armenian, Egyptian, Iranian, Lebanese)
   ___ Filipino
   ___ South East Asian (Cambodian, Indonesian, Vietnamese)
   ___ Latin American
   ___ Japanese
   ___ Korean
6. What is your current marital status?
   ___ Common-law
   ___ Divorced/separated
   ___ Married
   ___ Single
   ___ Widowed
7. Are you currently trying to lose weight? ___ Yes ___ No
8. How do you feel about your weight right now? I think I am...
   ___ Very overweight
   ___ Slightly overweight
   ___ About right
   ___ Slightly underweight
   ___ Very underweight
9. Have you ever smoked?
   ___ Yes
   ___ No (If no please proceed to question #12)

10. Do you currently smoke?
    ___ Yes
       ___ No (If no please proceed to question #12)

11. If you currently smoke, how many cigarettes per day on average do you smoke?
    ___ Less than 5 cigarettes per day
    ___ 5 to 10 cigarettes per day
    ___ 10 to 25 cigarettes per day
    ___ More than 25 cigarettes per day

12. Do you currently take any herbal supplements?
    ___ Yes
       ___ No (If no please proceed to question #14)

13. If you are currently taking herbal supplements, please list the NAME, DOSE, and BRAND of the supplement(s) and the FREQUENCY you use them (i.e. twice per day, daily, weekly, etc.):
   ________________________________________________________________
   ________________________________________________________________

14. Do you currently take any medications (including prescription, over-the-counter, homeopathic or naturopathic)?
    ___ Yes
       ___ No (If no please proceed to question #16)

15. If you are currently taking medications, please list the NAME of medication(s), what you are taking it for and the FREQUENCY you take them (i.e. twice per day, daily, weekly, etc.):
   ________________________________________________________________
   ________________________________________________________________

16. How would you describe your typical diet?
    ___ Mixed: I eat meat, dairy products, eggs, fruits and vegetables, grains
    ___ Lacto-ovo vegetarian: I DO NOT eat meat, fish or poultry, but I DO eat dairy, eggs, fruits and vegetables, grains
    ___ Vegan: I exclude ALL animal products

17. Since adulthood (18 years old), have you ever gone three or more months without a menstrual period (not including pregnancy or breastfeeding)?
    ___ Yes
       ___ No

18. Since adulthood, have your menstrual periods stopped for more than one year?
    ___ Yes
       ___ No

19. Do you or did you ever take Provera (progesterone)?
    ___ Yes
       ___ No (If no please proceed to question #20)
If yes, please answer the following questions:
a) How many months did you take Provera? __________
b) At what age(s) did you take Provera? _____________

20. Have you ever used birth control pills or oral contraceptives?
   ___ Yes
   ___ No (If no please proceed to question #21)

   If yes, please answer the following questions regarding your birth control use:
   At what age did you start? _____________
   Approximately how long did you use them? __________
   Are you still using them?
      ___ Yes
      ___ No (If no, at what age did you stop? _____________

21. How many times have you been pregnant? _____ (If none please proceed to question #24)

22. How many of these resulted in live births? ______________

23. Did you breast feed any of your children?
   ___ Yes (If yes, for how many months total counting all children
   ___ No

24. How old were you when you had your first menstrual period? ___________

25. Did you have regular periods once they began?
   ___ Yes (If yes please proceed to question #28)
   ___ No

26. If you had irregular periods, did they become regular?
   ___ Yes (If yes, at what age?)
   ___ No

27. Have your periods been made regular by medication (i.e. birth control pills)?
   ___ Yes (If yes, at what age?)
   ___ No

28. On average, how often do you have menstrual periods?
   ___ 20 days or less
   ___ 21 to 25 days
   ___ 26 to 30 days
   ___ 31 to 36 days
   ___ 37 or more days
   ___ Do not know

29. Have you ever been diagnosed with or treated for infertility or tried for more
    than 2 years and been unable to get pregnant?
   ___ Yes
   ___ No (If no please proceed to question #30)

   If yes, what was the reason?
   ___ Hormone or ovulation problem
   ___ Tubal blockage or abdominal surgery
   ___ Problem with your partner’s fertility
   ___ Other (please specify) ____________________
30. Have you ever been sufficiently bothered by severe acne, unwanted face or body hair to consult a physician for treatment?
   ___ Yes If yes, at what age?
   ___ No

Molinima question read by PhD candidate during orientation:

*Can you tell by the way you feel that your period is coming?*
   ___ Yes, every month
   ___ Yes, most months
   ___ Yes, less than half the time
   ___ Yes, once or twice a year
   ___ Never

*If yes to any of the above, what signs or symptoms indicate to you that your period is coming? (DO NOT READ SYMPTOMS BUT ALLOW PARTICIPATE TO PROVIDE)*
   ___ menstrual cramps or aching back or legs
   ___ bloating, fluid retention
   ___ increased appetite (in general or for sweet, salty or spicy foods)
   ___ moodiness (frustration, irritability, sadness)
   ___ breast tenderness in the front or nipple
   ___ breast tenderness up under the armpit
   ___ breast swelling
   ___ headaches
   ___ acne/pimples/blemishes
   ___ other, please specify ________________________________
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Temperature Calendar Instructions

Be sure to record your temperature every day. Leave your thermometer and calendar by your alarm clock or on the bedside table. If you forget, skip that day and write the date and then FORGOT across the table. Don’t try to remember later.

The following recommendations will assist you to accurately take and record your oral temperature.

1. **Day 1 is the first day of your flow (and you should start your calendar).** When you go to the bathroom in the morning, if you notice your period has started, go back and take your temperature and that is ‘Day 1’. If your period starts during the day, consider ‘Day 1’ as the following morning.

2. Take your temperature in the **morning, when you first wake**. Activity will raise your basal (resting) temperature. Although you may start your thermometer and head to the washroom, if you can, postpone this or getting out of bed until your temperature taking is finished.

3. Record the **DATE** (month/day) and the **TIME** you took your temperature (i.e. 7:20AM).

4. Record your **TEMPERATURE** as displayed on the digital thermometer (XX.X°Celsius).

5. Under ‘FLOW’ please indicate whether or not you menstruated that day (Y) or not (N).

6. Under ‘SLEEP PROBLEMS’ please rate the degree of your sleep problems using the following scale: 0=none, 1=minimal, 2=moderate, 3=moderately intense & 4=intense.

7. Under ‘HEALTH’, please record any events that may affect your morning temperature (e.g. felt like you were getting the flu, experiencing a fever, feeling unusually stressed, had a very late night, tossed and turned a lot).

8. Under ‘PHYSICAL ACTIVITY’, please record any and all activities you participated in the **PREVIOUS day** including what the activity (e.g. walking, biking, swimming, aerobics), the **intensity** of the activity (e.g. for walking, brisk vs. casual) and the **duration** (e.g. 20 mins).

Using the digital thermometer:

1. Press the **ON/OFF** button and a beep will sound (88.88 will display when used for the first time).

2. After a few seconds the display will **go blank**.

3. **Place the thermometer under your tongue at the back of your mouth.** The thermometer will begin to beep steadily for ~1 minute. If it stops, reposition the thermometer.

4. When the peak temperature is reached, the thermometer will sound **3 rapid beeps**. Record the temperature in your diary. The reading will not change while the power remains on.

5. Turn the thermometer off by pressing the purple ON/OFF button for a few seconds.

Analyzing your temperature data.

*This is optional as we will provide you with a detailed computer analysis of your menstrual cycle at the end of the study.*

If you would like to figure out whether you have ovulated and the length of your luteal phase (the time following ovulation) you can do that. First, compute the average of all the temperatures in your record, by adding them up and dividing by the number of days for which you have temperature readings. The average temperature you get can then be compared with the actual readings. If your temperature went above and stayed above that average until the day before the next flow you have ovulated. The higher temperatures should last 10-16 days. When there are between 3 and 9 days of higher temperatures, you have what is called a short luteal phase. This means that you have ovulated but the time of progesterone elevation is too short. Enjoy keeping this daily Temperature Calendar. You will learn new things about yourself!

*Please do not hesitate to call Jennifer at 604-616-4676 if you have any questions/comments/concerns or need additional calendars.*

*Thank you very much for your conscientious completion of this task!*
Appendix 6: QBT Validation Study Instructions for Daily Urine Sample Collection

**Your task:** Each morning for one menstrual cycle, you will collect a small portion of your first urination using a sponge vial which you will immediately label and store in your freezer. If you have any questions regarding this procedure, please contact anytime Jennifer at 604-616-4676.

**Materials:** If any materials are missing, call Jennifer and revisions will be sent to you.

1. **Urine collection vials:** Each morning for one menstrual cycle, you will use these sponge vials to collect a small portion of your first urination. Keep the vials in the provided Ziploc bag in your bathroom.

2. **Label Record Sheet with magnetic clip and pen:** After collecting a sample of your first urination each morning, you will use the select the appropriate label from the Record Sheet and firmly place the label on the vial. Next, fill in any supplements/medication taken and how the sample was treated.

3. **Ziploc Freezer bag:** Keep this bag in your freezer. Each morning, after collecting a sample your first urination and labelling the vial, you will place it IMMEDIATELY in this bag in your freezer.

4. **Styrofoam cooler and icepacks:** You will use these to transport your frozen urine samples to UBC. You may return your samples as many times as needed (i.e. depending on your freezer space).

**Instructions:** The accuracy of our analysis, and thus the information we provide to your regarding your menstrual cycle, will depend on the accuracy of the urine collection and storage technique. These instructions will help ensure that your urine collections are obtained correctly and will give accurate test results. Keep these instructions, the Label Record Sheet and pen on your refrigerator using the magnetic clip.

1. **When to collect the urine:** You will begin collecting a sample of your first urination the first day your menstrual flow begins for a menstrual cycle that is most convenient for you. This is also when you should begin your temperature calendar! It is OK if you urinate at night and during the early morning as normal. You only need to collect a sample from your first urination after rising for the day. If you forget to take a sample of your first urination, please take the sample later in the day when you remember. If you miss an entire day, continue the urine collection as normal the following day. Be sure to make note of any missed collection days on your Label Record Sheet. You will continue to collect a sample of your first urination EACH MORNING for one complete menstrual cycle (i.e. when flow begins for your next cycle, discontinue the collection).

2. **How to collect the urine:**
   - Before you begin your first urination in the morning, get a urine collection vial (keep the Ziploc bag of new/unused vials in the bathroom closet/cupboard.)
   - Unscrew the cap of the vial and remove the cap with attached sponge from the vial. Do not remove the sponge from the cap! The cap is designed to be a holder for the sponge.
When you sit on the toilet to urinate, hold the top of the cap with your hand, and position the sponge directly in your urine stream. You will notice the sponge expand greatly. Saturate the sponge well, until urine has “wicked” up the sponge and the sponge has expanded to fill the cap (takes 8-12 seconds). After the sponge is saturated, wait 2-3 seconds to prevent dripping.

Insert the sponge back into the collection vial and screw the cap on tightly.

When finished in the bathroom, take the vial to your freezer.

Fine the appropriate label (cycle day – will match with temperature calendar) on your Label Record Sheet and attach the label firmly around the vial. Please do not put the label on the cap portion of the vial.

Place the labelled vial into the provided Ziploc freezer bag (or other container) in your freezer.

Using the pen, make any appropriate notations on the Label Record Sheets (see next section of this document).

If the sponge falls out of the cap and into the toilet or onto the ground, please discard it. Try to get another sample (then or later in the day) with a new urine collection vial.

3. **How to complete the Label Record Sheet:** Your Label Record Sheet contains: 1) labels (which include your 3 digit study ID and cycle day) to peel off each day and affix to the vial of urine collected, and 2) a Record Sheet, where you will make any relevant notations corresponding to each day of urine collection. Each morning after you collect your urine, you will need to affix the appropriate label to the collection vial and make some minimal notations on the Label Record Sheet. Please use the provided pen (or any ballpoint pen) to enter the necessary information directly on the Record Sheet.

Please follow these step-by-step instructions:

**Select the appropriate label from the Label Record Sheet. Peel off the label and affix that label to the vial containing the morning’s urine.** If you miss a day of collecting urine, be sure to **skip the missed day’s label** when you collect the next day.

**Write down comments,** if any, in the COMMENTS Section of the Label Record Sheet. In this section we are primarily interested in anything that might 1) alter your body’s natural reproductive hormone levels, or 2) affect the quality of a urine sample.

We ask that you **please record any of the items below that apply to you.**

Record if you missed collecting urine that day (so we know that we have not misplaced one of your samples).
Record how your urine sample was treated if not immediately frozen (i.e. was kept at room temperature or unfrozen for more than a few hours, and note approximately how long it was at room temperature).

Record whether you started or stopped using any natural or prescription hormones on that day. Please indicate the name of the product, and whether you took the product on just that day, or whether you take it daily, or on some other regular basis. Examples of hormones include: any form of estrogen or progestin, Premarin, natural progesterone cream, birth control pills, Norplant (implant in upper arm), Depo Provera (3 month injection), wild yam cream, Estroven, soy supplement drinks or pills, androgens, or any other hormone that you may be taking that is not included in this list. These examples include all forms of hormones: pills, vaginal creams, vaginal rings, gels, suppositories, or patches.

Record whether you started or stopped using any prescription or non-prescription medicines or nutritional supplements. You can list these items daily, or you can list them once at the top of the record sheets so that you don’t have to make daily notations. But please remember to note any deviations from the routine, or when you stop taking an item, or start a new item. List all non-prescription substances you take, including vitamins/minerals, natural progesterone cream, wild yam cream, supplements, flaxseed, phytoestrogen supplements, herbs, and all other over-the-counter preparations. These include aspirin, Tylenol, ginseng, St. John’s Wort, soy supplements, etc.

If you have nothing to record for a day, just leave the Comments section blank!

4. How to store the urine: Your urine samples should be kept frozen or as cold as possible at all times (including when you are transporting them to UBC, see transporting section below for details). Keeping samples frozen or cold is important for preserving the reproductive hormone, progesterone, in the urine. You may store samples in your freezer in the provided Ziploc freezer bags or any other container that is convenient for you.

5. How to transport the urine to UBC: You may transport your frozen urine samples to UBC as often as you require based on your freezer space. A few days prior to wanting to return frozen samples, place two icepacks in your freezer and contact Jennifer (604-616-4676) to arrange a meeting. Just prior to leaving your home to meet Jennifer at UBC, place one of the frozen icepacks inside the Styrofoam cooler. Remove your frozen urine collection vials from your freezer, place them in one of the provided Ziploc bags and pack them on top of the first icepack in the cooler. Place the second frozen icepack on top of your frozen vials. Close the lid of the cooler securely, using tape if desired. You may place the cooler in a plastic bag for transport. Keep the cooler upright as much as possible. Proceed IMMEDIATELY to UBC. When you return the last of your frozen urine samples, remember to bring your completed Label Record Sheet and temperature calendar. Don’t worry! Jennifer will remind you of all these things when you call to arrange the meeting.

6. Trouble shooting tips!
What if I forget to take the sample in the morning? If you miss the first urination of the day, collect a sample from any urination later in the day, if you can. If this does happen, please make a note of it on your Label Record Sheet.

What if I miss a day completely? Don’t worry if you miss collecting for a day – this happens occasionally. If this does happen, please make a note of it on your Label Record Sheet. Resume as normal the next day, being sure to SKIP THE MISSED CYCLE DAYS LABEL.
What if I forget to put the collected vial in the freezer immediately?
It may occasionally happen that you leave a collection vial with a urine sample on the bathroom or kitchen counter all day (or otherwise forget to freeze it!), and only put it in the freezer later in the day. This is okay, but try to get the sample into the freezer as soon as possible. If this does happen, please make a note of it on your Label Record Sheet.

What if I have to spend a night away from home?
Since you choose the menstrual cycle to complete the procedures, try to choose a cycle where you do not have to travel. If travel comes up unexpectedly and you have not yet begun your collection, you may simply choose a different cycle! If you have to travel during the cycle and have already begun collecting your urine, you can continue to collect samples while you are away from home. If you will have access to a freezer, bring the required number of collection vials and your Label Record Sheet with you. Simply collect as normal! If you will not have access to a freezer, and will only be traveling for a few days, bring the provided Styrofoam cooler and icepacks (as well as the required number of vials and Label Record Sheet) with you. Right before you leave, place the icepacks in the cooler. Continue your collection as normal and place the collected samples in the cooler with one icepack underneath and one on top of the vials. Try to keep the samples as cool as possible (i.e. in a fridge with ice). We realize that it is sometimes hard to keep samples cold while traveling, and although it is not ideal, it happens that some urine samples end up being stored at room temperature for a day or two. Please note on your Label Record Sheet if any of your samples have been at room temperature for more than a few hours, and please let us know for approximately how long they were at room temperature.

Please do not hesitate to call Jennifer at 604-616-4676 if you have any questions. Thank you very much for your conscientious completion of this task!
**Daily Urine Sample Label Record Sheet**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

**COMMENTS**

How was the vial treated after urine was collected – frozen immediately?
Left at room temperature? If so, for how long?

Did you take any medication (over the counter or prescription) today? If yes, please list them including name, brand, amount and Drug Information Number if available.

Did you take any supplements (herbal or nutritional) today? If yes, please list them including name, brand and amount.

---

**PLEASE CONTACT JENNIFER IF YOU HAVE ANY QUESTIONS OR REQUIRE MATERIALS 604-616-4676**

ID # ____________
LABEL RECORD SHEET
Appendix 7: QBT Validation Study Ethics Approval Certificate

The University of British Columbia
Office of Research Services,
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

Certificate of Expedited Approval
Clinical Research Ethics Board Official Notification

PRINCIPAL INVESTIGATOR
Barr, S.I.

DEPARTMENT
Family & Nutr Sci

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
Other, UBC Campus

CO-INVESTIGATORS:
Bedford, Jennifer, Land & Food Systems; Keiver, Katherine, Land & Food Systems; Linden, Wolfgang, Psychology; Prior, Jerilynn, Medicine

SPONSORING AGENCIES
Canadian Institutes of Health Research

TITLE:
Determination of Ovarian Function by Least-Squares Analysis Method of Quantitative Basal Temperature: Validation Against Urinary Progesterone Metabolites

APPROVAL DATE
02 June 2006

TERM (YEARS)
1

DOCUMENTS INCLUDED IN THIS APPROVAL:
Protocol; Subject Consent Form version date May 2006; Instructions; Poster Advertisement version date April 2006; Classroom Handout/Flyer version date April 2006; Newspaper Advertisement; Letter of Initial Contact; Questionnaire Phone Script; Questionnaire

CERTIFICATION
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the Chair of the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

The CREB approval for this study expires one year from the approval date.

[Signature]

Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bellward, Chair
Dr. James McCormack, Associate Chair
Dr. John Russell, Associate Chair
Dr. Caron Strahlendorf, Associate Chair
Appendix 8: QBT Validation Study Letter of Consent

THE UNIVERSITY OF BRITISH COLUMBIA

Food, Nutrition and Health
Faculty of Land and Food Systems
2205 East Mall
Vancouver, B.C. Canada V6T 1Z4
Phone: (604) 822-2502
Fax: (604) 822-5143

Determination of ovarian function by least-squares analysis method of quantitative basal temperature: validation against urinary progesterone metabolites

Short title: Validation of a quantitative basal temperature method against urinary progesterone

Subject Information and Consent Form

Principal Investigator:
Susan Barr, PhD, RD
Professor; Food, Nutrition and Health, University of British Columbia (UBC); (604) 822-6766

Co-Investigators:
Jennifer Bedford, BSNH
PhD Candidate
Human Nutrition, UBC
(604) 616-4676
Jerilynn Prior, MD
Professor
Endocrinology & Metabolism, UBC
(604) 875-5927

Katherine Keiver, PhD
Assistant Professor
Food, Nutrition & Health
(604) 822-0421
Wolfgang Linden, PhD
Professor
Clinical Psychology, UBC
(604) 822-4156

Sponsor: Canadian Institutes of Health Research (CIHR)

Introduction: You are being invited to participate in this study after indicating your interest and meeting eligibility requirements.

Your Participation is Voluntary: Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you do not have to provide any reason for your decision not to participate. Please take the time to read this document carefully. If you wish, you may discuss it with your family, friends and doctor before you decide. Please feel free to ask any questions regarding the study procedures you may have while reading this document.

Purpose: Characterising the menstrual cycle determines whether different parts of the cycle are normal. Measuring body temperature at the time of awakening is a non-invasive way to characterise a women’s menstrual cycle. However, this method needs to be compared to other laboratory-based methods to demonstrate that it is accurate. Therefore, the purpose of this study is to access how well a computerized method of evaluating body temperature, measured upon awakening, characterises the menstrual cycle. Body temperature records will be compared to hormone levels in the urine, which is an established laboratory-based method of characterising the menstrual cycle.
Who Can Participate? You are eligible to participate in this study if you are: (1) female, (2) a resident of the Lower Mainland of British Columbia, (3) between 19 and 35 years of age, (4) regularly menstruating, (5) at a normal body weight defined as body mass index (an index of your weight in kilograms divided by your height in metres squared) between 18.5 and 25, (6) in general good health and not suffering from any chronic diseases that may affect hormone levels, (7) able to read and understand English.

Who Should Not Participate? You are not able to participate in this study if you: (1) do not meet inclusion criteria, (2) use oral contraceptives (birth control pills), receive Depo-Provera injections or other drugs that affect hormone levels, (3) have been diagnosed with or treated for an eating disorder, (4) are pregnant or lactating, (5) work at night or have an inconsistent sleep pattern.

What Does the Study Involve? This study will take place at UBC and subjects’ homes. Approximately 50 women will participate in the study. The study will occur over one complete menstrual cycle and will involve the following: orientation to the study procedures (~45 min at UBC); daily temperature records (at home); daily first morning urine sample collection (at home); and returning the temperature calendar and frozen urine specimens to UBC. Your involvement in this study will take 10-15 minutes per day over one menstrual cycle (approximately 21 to 35 days) for a total of 5 to 7 hours plus time to transport the frozen urine samples to UBC.

Specific Study Procedures: If you agree to take part in this study, the procedures and visits you can expect will include the following:

1. Eligibility assessment by phone. This involves answering a few simple questions to determine if you are able to participate in the study (see Who Can Participate). This will require approximately 15 minutes of your time. At this time, we will schedule a meeting at UBC for orientation to the study.

2. Orientation at UBC. At your convenience, you will meet with a member of the research team at the Human Nutrition department of UBC for the following: a) orientation to the study procedures; b) complete a brief questionnaire (you do not have to answer any questions that you may feel uncomfortable answering); c) be provided with instructions and materials for daily body temperature readings and daily first void urine sample collection; d) measurement of your height, weight and waist circumference while wearing light indoor clothing; and e) answering a question regarding symptoms associated with your menstrual cycle. This meeting will require approximately 45 minutes of your time.

3. Completion of a daily temperature calendar. At home, beginning the first day of your period (for your chosen cycle) and daily thereafter, you will measure your body temperature immediately after waking using a digital thermometer. You will record the temperature in your calendar as well as the time you woke up, whether or not flow has occurred, any sleep problems, your health status and any physical activity you performed the previous day. At UBC, we will provide you with the digital thermometer and temperature calendar. We will demonstrate how to use the digital thermometer and will give you written instructions to take home. We will contact you weekly to answer any questions. Completion of your calendar will take approximately 3 minutes daily for one full menstrual cycle. Most women’s menstrual cycles range from 21 to 35 days.

4. Collection of daily first void urine sample. At home, beginning the same day you start the temperature calendar, you will use a sponge vial to collect a portion of your first morning urination, label the vial and then place it immediately in your freezer for storage. On a Label
Record Sheet, you will record any medications or supplements taken and how the specimen was treated if it was not frozen immediately. You will do this each morning until your next menstrual period begins. At UBC, we will provide you with the sponge vials, Ziploc freezer bags, and Label Record Sheet with magnetic clip. We will demonstrate how to use the vials to collect your urine and will give you written instructions to take home. We will contact you weekly to answer any questions. This will take approximately 5 minutes daily for one full menstrual cycle. Most women’s menstrual cycles range from 21 to 35 days.

5. Returning the frozen urine samples and temperature calendar to UBC. As mentioned, each morning you will collect a portion of your first urination using a sponge vial which you will then store in your freezer. The amount of freezer space you have available will determine the number of times you need to return the frozen vials to UBC. During your UBC orientation, we will provide you with icepacks and a Styrofoam cooler for transporting the frozen urine samples to UBC. A few days before you need to return the frozen samples, you will contact Jennifer by phone to arrange a meeting time. Just prior to departing for UBC, you will place one frozen icepack in the Styrofoam cooler followed by the frozen samples in the Ziploc freezer bag and a second frozen icepack on top of the frozen vials. You will then bring them immediately to the Human Nutrition facilities at UBC as arranged. When you return the last of your urine samples, you will also return your completed Label Record Sheet and temperature calendar.

Risks: There are no known risks associated with participation.

Benefits: There are no known benefits associated with participation. You will receive an analysis of one menstrual cycle. We will provide a copy of the results for you to discuss with your family doctor. A summary of the research results, once completed, will be provided to you as well, if you would like to receive it.

Your Subject Rights & Responsibilities: Your participation in this study is entirely voluntary and you may refuse to participate or withdraw from the study at any time without penalty or repercussions. You may withdraw without providing any explanation of your reasons for doing so. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrollment in the study will be retained for analysis. By law, this data cannot be destroyed. Your participation in this study is not associated with any known risks of injury or illness. However, you do not waive any of your legal rights against the sponsor, investigators, or anyone else by signing this consent form. We ask that you please inform the research team if you are no longer able or willing to participate in the study. We also ask that you please inform the research team if you begin taking any medications (i.e. birth control), if you become pregnant, are trying to become pregnant or suspect you may be pregnant. Also, if you are not complying with the requirements of the study the investigators may withdraw you from the study.

Remuneration/Compensation: Upon completing all aspects of the study, you will receive a $20 gift certificate. In order to defray transportation costs to and from UBC, you will be reimbursed $5 per visit. If your travel costs exceed this amount, you may provide the receipts detailing your travel and a member of the research team will compensate your additional costs.

Confidentiality: All samples, questionnaires and other documents will be labelled with code numbers only (your name will not be associated with these) and will be kept in a locked filing cabinet in the offices of the principal investigator. After your urine samples have been analyzed, they will be disposed of. Your confidentiality will be respected, and no information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the investigator or her designate by representatives of the Canadian Institutes of
Health Research, Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. No records which identify you by name or initials will be allowed to leave the investigators’ offices.

**Contact Information:** If you have any questions or desire further information about this study before or during participation, you can contact Jennifer Bedford at (604) 616-4676 or Dr. Susan Barr at (604) 822-6766. If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services at (604) 822-8598.

**Consent to Participate:**

- I have read and understood the subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time and without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me.
- I have read this form and I freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form

<table>
<thead>
<tr>
<th>Printed Name of Subject</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Printed Name of Witness</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Printed Name of Principal Investigator or Designated Representative</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>
Appendix 9: QBT Validation Study Transportation Reimbursement Receipt

THE UNIVERSITY OF BRITISH COLUMBIA

Food, Nutrition and Health
Faculty of Land and Food Systems
2205 East Mall
Vancouver, B.C. Canada V6T 1Z4
Phone: (604) 822-2502
Fax: (604) 822-5143

Determination of ovarian function by least-squares analysis method of quantitative basal temperature: validation against urinary progesterone metabolites

TRANSPORTATION REIMBURSEMENT RECEIPT

I, ______________________________________________,

(Participant name – Please print)

acknowledge that I have received $5.00 in support of study transportation costs.

_________________________________________________________  ____________
Signature                                      Date                    Phone number (to verify receipt of the funds)

_________________________________________________________  ____________
Printed Name of Principal Investigator or Designated Representative Signature Date
Determination of ovarian function by least-squares analysis method of quantitative basal temperature: validation against urinary progesterone metabolites

GIFT CARD RECEIPT

I, ______________________________________________,

(Participant name – Please print)

acknowledge that I have received a gift certificate valued at $20.00 in support of completing the above mentioned study procedures.

____________________________________________________________________

Signature Date Phone number (to verify receipt of the funds)

Printed Name of Signature Date
Principal Investigator or
Designated Representative
Appendix 11: QBT Validation Study Individual Results

Participants were sent the following email with their individual results as an attachment as follows.

Re: Menstrual cycle study – Results

Hi [participant]. I hope this email finds you well!

Good news! I have finally completed both the study analyses as well as everyone’s individual results. I apologize that these took far too long to get to you. There were some unexpected bumps along the road, as always happens in research. The study results were presented at a conference for the Society for Menstrual Cycle Research at the beginning of June.

I have attached a file explaining the study findings as well as your personal results. Please read over them and let me know if you have any questions about anything. If you would like, we can meet to discuss them further or we could also chat over the phone.

I really appreciate your participation in my study. I believe our findings will contribute significantly to what is known about women’s menstrual health and you should feel proud you were a part of that. I hope that your participation also helped you to learn something about yourself!

Thank you again so much and please don’t hesitate to contact me by phone or email.

Jen
Determination of ovarian function by least-squares analysis method of quantitative basal temperature: validation against urinary progesterone metabolites

INDIVIDUAL SUBJECT RESULTS & STUDY FINDINGS

Principal Investigator:
Susan Barr, PhD, RD
Professor: Food, Nutrition and Health, University of British Columbia (UBC); (604) 822-6766

Co-Investigators:
Jennifer Bedford, BSNH
PhD Candidate
Human Nutrition, UBC (604) 616-4676

Jerilynn Prior, MD
Professor
Endocrinology & Metabolism, UBC (604) 875-5927

Sponsor: Canadian Institutes of Health Research (CIHR)

Thank you for your Participation. We are extremely grateful for your participation in the study and your dedication to completing the procedures! Thanks to you and our other participants, we were able to evaluate whether the body temperature record method is suitable for determining whether or not a menstrual cycle is ovulatory. This method will now be used as part of our larger project examining relationships between eating attitudes, ovarian function, stress and bone density. This document will describe the study you participated in last summer/fall including the overall study findings and your individual results. You are welcome to share your personal results with your doctor. It is important to remember that we only collected data for one menstrual cycle and, as I’m sure you know, your cycle can be quite variable.

Confidentiality. All samples, questionnaires and other documents were labelled with code numbers only (your name was not associated with these) and were kept in a locked filing cabinet. After your urine samples were analyzed, they were disposed of. No records which identify you by name or initials will be allowed to leave the investigators’ offices.

Contact Information. If you have any questions or desire further information about this study or your personal results, please contact Jennifer Bedford at (604) 616-4676 or Dr. Susan Barr at (604) 822-6766.

Background Information & Purpose of the Study. Characterising the menstrual cycle determines whether different parts of the cycle are normal. Measuring body temperature at the time of awakening is a non-invasive way to characterise a women’s menstrual cycle. However, it was important to compare this method needs to other laboratory-based methods to demonstrate that it is accurate. Therefore, this study was done to assess how well a computerized method of evaluating body temperature, measured upon awakening, characterises the menstrual cycle. Body temperature records were compared to urinary levels of an end-product of the menstrual cycle hormone progesterone, an established laboratory-based method of characterising the menstrual cycle.

A women’s menstrual cycle lasts an average of 28 days and is divided into to parts: the first half (about 14 days) is the follicular phase, then ovulation occurs (the egg is released from the ovaries), and the second half (again, about 14 days) is referred to as the luteal phase (see diagram). Levels of the reproductive hormones show a pattern over the cycle. The hormone we
are examining, progesterone, increases in the 2nd half of the cycle if ovulation has occurred. Interestingly, progesterone actually has a warming affect on the body and causes an increase of ~0.3°Celsius from the follicular phase to the luteal phase, when progesterone peaks.

**Methods & Analyses.** Fifty-three women received the study materials and instructions at UBC and 48 completed the procedures and returned all the materials. From these, 44 samples were used for the analysis. One was removed for a possible thermometer malfunction and 3 because too much data was missing. Like you, these women were between 19 and 35 years of age, had not used oral contraceptives or progesterone in the previous 6 months and self-reported regular menstrual cycles, consistent sleep patterns, and normal body weight (defined as body mass index (an index of your weight in kilograms divided by your height in metres squared) between 18.5 and 25). As you no doubt remember, each morning, for one complete menstrual cycle, you were asked to record your body temperature upon awakening and provide a portion of your first urination which was then frozen.

Your temperature calendars were analysed using a computer program called *Maximina*. This program uses what is called the least-squares analysis method of quantitative basal temperature. The program assesses whether ovulation occurred by determining whether the menstrual cycle can be divided into two phases by identifying a statistically significant difference in temperature; the day the temperature increases significantly indicates the start of the luteal phase and the end of the luteal phase is marked by the onset of menstrual flow.

Your urine samples were analyzed for a metabolite of progesterone known as pregnanediol glucuronide (PDG), and the results were analyzed to see whether levels increased during the second half of the cycle. This was done using the following procedure:
1. The levels of PDG were averaged over 5-day periods (these are shown in column B of your results sheet), and the lowest 5-day average was determined (this is shown in column C of your results sheet).
2. The level of PDG on each day of the cycle (shown in column A) was then divided by the lowest 5-day average (column C). This ratio, known as the Kassam ratio, is shown in column D of your results sheet.
3. If the Kassam ratio increased to 3.0 or above for at least 3 days in a row, the cycle was assessed as being ovulatory. The day the ratio increased to 3.0 or above indicates the start of the luteal phase and the end is marked by the onset of menstrual flow.

We then compared the results of the two methods.

**Study Findings.** The 'gold standard' PDG method, classified 40 of the 44 cycles as ovulatory. The QBT method correctly identified 39 of these. In scientific lingo, this is called true positives or sensitivity. The PDG method classified 4 of the 40 cycles as anovulatory, and QBT correctly 1 of these cycles. This is called true negatives or specificity. So relative to PDG, the QBT method has excellent sensitivity (97.5%) but poor specificity (25%). This may be due, at least in part, to the small number of anovulatory cycles in our study. For the length of the luteal phase, PDG classified 9 cycles as short and QBT correctly identified 7 of these. PDG classified 30 cycles as having normal luteal phase length and QBT correctly identified 23 of these. So for luteal phase length, the QBT method had good sensitivity (78%) and specificity (77%). In summary, while the QBT method shows promise as a non-invasive tool for assessing ovarian function, more work is needed. In particular, studies with more anovulatory cycles and cycles with short luteal phase lengths are required to further establish whether the least-squares QBT analysis method is a reliable and valid method for assessing ovarian function.
Personal Results.

**Ideal Hormone Levels and Temperature Over the Cycle**

- **FSH**
- **LH**
- **Ovary**
- **Estrogen**
- **Progesterone**
- **Basal Body Temperature**

Temperatures recorded:
- 35.5
- 36
- 36.5
- 37

Cycle Days: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23

**Urinary PDG Levels as Kassam's Ratio**

- Kassam's PDG Ratio: 0, 1000, 2000, 3000, 4000, 5000, 6000, 7000

Cycle Days: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23

**Temperature Records**

Temperature in degrees C:
- 35.5, 36, 36.5, 37
<table>
<thead>
<tr>
<th>CYCLE DAY</th>
<th>A: PDG ng/mL SG corrected</th>
<th>B: 5-D Running Average</th>
<th>C: Minimum 5-day Average</th>
<th>D: Kassam Ratio = PDG ng/mL / min 5-day average</th>
<th>Temperature (°Celsius)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**AVERAGE CYCLE TEMPERATURE:**

**FOLLICULAR PHASE AVERAGE TEMPERATURE:**

**LUTEAL PHASE AVERAGE TEMPERATURE:**

<table>
<thead>
<tr>
<th>Did Ovulation Occur?</th>
<th>Luteal Onset (Cycle Day)</th>
<th>Luteal Phase Length (&lt;10 days = short)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature Analysis Method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary PDG Analysis Method</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Interpretation.** Based on the ‘gold standard’ of the urine analysis of PDG, your cycle was [ovulatory/anovulatory] during the study period. Based on temperature records, your cycle was [ovulatory/anovulatory]. The day of luteal onset likely ranged between [XX and XX], meaning your luteal phase duration was [XX] to [XX] days. So for this cycle, your luteal phase length was considered normal/short because it was [>/<]10 days.
Appendix 12: Sensitivity and specificity of least-squares quantitative basal temperature analysis (LS-QBT) methods in determining luteal phase length (LPL) relative to Kassam’s urinary pregnanediol glucuronide (PdG) algorithm (n=35)

In addition to detecting evidence of luteal activity and estimating the day of ovulation (Chapter 2), we required a method that could detect cycles with short luteal phase duration. This is because anovulatory and short LPL cycles may have negative affects on bone in premenopausal women, which was one of the main objectives of my PhD research program (Chapter 3). Although the Kassam PdG algorithm was not designed to classify cycles by LPL, it does identify the day of luteal transition which can then be used to estimate LPL. The day of the significant temperature increase by LS-QBT occurs ~2.4 days after the serum LH surge and cycles as classified as short LPL if the luteal phase is <10 days in duration [1]. The day of sustained PdG rise occurs ~3 days following the serum LH surge [2]. We therefore examined the sensitivity and specificity of LS-QBT in classifying cycles with short versus normal LPL relative to Kassam’s PdG using <10 days (first table) and <9 days (second table) in order to account for the small discrepancy in timing relative to the serum LH surge.

<table>
<thead>
<tr>
<th>LS-QBT method</th>
<th>Short LPL by PdG&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Normal LPL by PdG&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Short LPL by LS-QBT&lt;sup&gt;b&lt;/sup&gt; (sensitivity)</th>
<th>Normal LPL by LS-QBT&lt;sup&gt;b&lt;/sup&gt; (misclassified)</th>
<th>Short LPL by LS-QBT&lt;sup&gt;b&lt;/sup&gt; (misclassified)</th>
<th>Normal LPL by LS-QBT&lt;sup&gt;b&lt;/sup&gt; (specificity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All temperatures&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.5% (7)</td>
<td>12.5% (1)</td>
<td>4% (1)</td>
<td>96% (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Royston adjusted&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62.5% (5)</td>
<td>37.5% (3)</td>
<td>15% (4)</td>
<td>85% (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-hour average wake-time&lt;sup&gt;e&lt;/sup&gt;</td>
<td>62.5% (5)</td>
<td>37.5% (3)</td>
<td>8% (2)</td>
<td>92% (23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expert reviewed&lt;sup&gt;f&lt;/sup&gt;</td>
<td>75% (5)</td>
<td>25% (2)</td>
<td>0% (0)</td>
<td>100% (22)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as proportion (%, n).

a. The Kassam PdG algorithm was not validated to assess luteal phase length. It does estimate the day of luteal onset, which occurs ~3 days following the serum LH surge. For this analyses, LPL by Kassam’s PdG algorithm is considered short if <10 days or normal if ≥10 days.

b. The day of significant temperature increase occurs ~2.4 days following the serum LH surge. Using LS-QBT, LPL is considered short if <10 days or normal if ≥10 days.

c. All recorded temperatures were included except for febrile illness (n=35).

d. All recorded temperatures were adjusted by 0.1°Celsius/hour from earliest wake-time (n=35).

e. Temperatures recorded >1 hour before or after the average wake-time were removed. Three cycles could no longer be analysed because of the number of temperature values removed (n=33).

f. Temperatures were removed based on interpretation by a reproductive endocrinologist. Six cycles could no longer be analysed because of the number of temperature values removed (n=30).
<table>
<thead>
<tr>
<th>LS-QBT method</th>
<th>Short LPL by PdG&lt;sup&gt;a&lt;/sup&gt; (sensitivity)</th>
<th>Normal LPL by PdG&lt;sup&gt;a&lt;/sup&gt; (misclassified)</th>
<th>Short LPL by PdG&lt;sup&gt;a&lt;/sup&gt; (misclassified)</th>
<th>Normal LPL by PdG&lt;sup&gt;a&lt;/sup&gt; (specificity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All temperatures&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80% (4)</td>
<td>20% (1)</td>
<td>13% (4)</td>
<td>87% (26)</td>
</tr>
<tr>
<td>Royston adjusted&lt;sup&gt;d&lt;/sup&gt;</td>
<td>60% (3)</td>
<td>40% (2)</td>
<td>20% (6)</td>
<td>80% (24)</td>
</tr>
<tr>
<td>2-hour average wake-time&lt;sup&gt;e&lt;/sup&gt;</td>
<td>40% (2)</td>
<td>60% (3)</td>
<td>12% (3)</td>
<td>88% (22)</td>
</tr>
<tr>
<td>Expert reviewed&lt;sup&gt;f&lt;/sup&gt;</td>
<td>60% (3)</td>
<td>40% (2)</td>
<td>12% (3)</td>
<td>80% (24)</td>
</tr>
</tbody>
</table>

Data are presented as proportion (%, n).

- **a.** The Kassam PdG algorithm was not validated to assess luteal phase length. It does estimate the day of luteal onset, which occurs ~3 days following the serum LH surge. For this analyses, LPL by Kassam’s PdG algorithm is considered short if <9 days or normal if ≥9 days.
- **b.** The day of significant temperature increase occurs ~2.4 days following the serum LH surge. Using LS-QBT, LPL is considered short if <10 days or normal if ≥10 days.
- **c.** All recorded temperatures were included except for febrile illness (n=35).
- **d.** All recorded temperatures were adjusted by 0.1°Celsius/hour from earliest wake-time (n=35).
- **e.** Temperatures recorded >1 hour before or after the average wake-time were removed. Three cycles could no longer be analysed because of the number of temperature values removed (n=33).
- **f.** Temperatures were removed based on interpretation by a reproductive endocrinologist. Six cycles could no longer be analysed because of the number of temperature values removed (n=30).

References


GET YOUR BONE DENSITY & DIET ANALYSED!

We are looking for healthy WOMAN aged 19-35 yrs to participate in a 2-yr research study!

Who Can Participate?
You are eligible to participate in this study if you are: (1) female, (2) a resident of the Lower Mainland of British Columbia, (3) between 19 and 35 years of age, (4) regularly menstruating, (5) at a non-obese body weight defined as body mass index (an index of your weight in kilograms divided by your height in metres squared) >18.5 and <30, (6) in general good health and not suffering from any chronic diseases that may affect bone metabolism or the endocrine or reproductive systems, (7) able to read and understand English.

Who Should Not Participate?
You are not able to participate in this study if you: (1) do not meet inclusion criteria, (2) use oral contraceptives (birth control pills), receive Depo-Provera injections or other drugs that affect menstruation, the endocrine system or bone metabolism, (3) have been diagnosed with or treated for an eating disorder, (4) are pregnant or lactating, (5) work at night or have an inconsistent sleep pattern.

What is involved?
Completed at the beginning of the study, 6 months and 2 years later:
1. A short telephone survey to determine if you are eligible to participate (~10 mins).
2. Coming to the UBC Nutrition department to receive materials & instructions and have your height, weight and waist circumference measured (~1 hour).
3. Take home questionnaire (~40 mins) and food frequency questionnaire (~1 hour) to be completed at your convenience and mailed back to us – we provide the envelope and stamp.
4. A bone density/body composition scan at Vancouver General Hospital at study entry and 2 years later – we will pay for your transportation (~30 mins).
5. 24 hour urine collection – we provide all materials and courier pick up. You pick the most convenient day to complete.
6. 12 hour blood pressure assessment (once during the two year study) – you chose the day to wear our lightweight, compact monitor which will measure your blood pressure as you go about your normal day.
7. Daily record of your body temperature - we will provide a calendar and digital thermometer (~3 mins per day for 2 years).

What’s in it for me!? We will reimburse all travel expenses and at the end of each phase of the study (entry, 6 months, 2 years) you will be provided with a $30 gift certificate (3x $30 = $90). Also after the 2 year assessment, you will be provided with information about your health including body composition, bone density, dietary intake, fertility and blood pressure. All at no cost.

For more information please call Jennifer at 604-616-4676 OR e-mail jbedford@interchange.ubc.ca
Advertisements used in University of British Columbia student newspapers

**Are you a healthy non-obese woman aged 19 to 35 who doesn’t use birth control pills? Would you like information on your bone density, body composition, dietary intake & fertility? If so we need your help for a research study!**

The study will involve the following at study entry, 6 months, & 2 years later: orientation to study procedures (at UBC); completing a questionnaire, daily temperature records, a 12-hr blood pressure assessment (once during 2 yr period), and a 24-hr urine sample collection (all at your home); measurement of your bone density/body composition at study entry and 2 years later (at VGH).

You will receive a gift certificate for each phase of the study you complete and your results at the end of 2 years. Please contact Jennifer at (604) 616-4676 or jbedford@interchange.ubc.ca for more information.

---

**Healthy, non-obese woman, 19-35 yr need for research study! You will receive information on your bone density, body composition & dietary intake!**

The study will involve the following at study entry and 6 months & 2 years later: orientation to study procedures (at UBC); completing a questionnaire, daily temperature records, a 12-hr blood pressure assessment (once during 2 yr period), and a 24-hr urine sample collection (all at your home); measurement of your bone density/body composition at study entry and 2 years later (at VGH).

You will receive a gift certificate for each phase of the study you complete and your results at the end of 2 years. Please contact (604) 616-4676 or jbedford@interchange.ubc.ca for more information.
Appendix 14: 2-year Prospective Bone Study Letter of Initial Contact (via email)

Hi [name]! Thanks for your interest in our study!

I’ve included more detailed information about the study procedures and eligibility requirements at the bottom of this email. Read the information over and let me know if you have any questions or require any additional info or clarification.

If you are still interested in participating, email me back and we can set up a time that I can call you to answer a few questions about yourself (age, health, etc) to make sure you are eligible. This takes about 10 minutes and we can do it anytime that is convenient for you - day or night. When you email me back, let me know what number I can reach you at and some convenient days/times for me to call.

If you are able to participate, we can set up a time to meet at UBC for about 45 to 60 minutes where I will orient you to the study - again at your convenience.

Let me know what you think and please contact me again if you have any questions or require further clarification and if you want to set up a time for me to call!

Thanks again for your interested and please feel free to pass the info included in this email onto any of your friends/family/co-workers who may be interested in participating :)

Jen

We are inviting regularly menstruating, non-obese women aged 19 to 35 years who are in general good health to participate in this research study being conducted by Dr. Susan Barr at the University of BC.

**Purpose:** The purpose of this study is to explore relationships between young women’s eating attitudes and behaviours, stress, ovarian function and changes in bone density over two years.

**Investigator Contact:** Jennifer Bedford (604) 616-4676, jbedford@interchange.ubc.ca

**Who Can Participate?** You are eligible to participate in this study if you are: (1) female, (2) a resident of the Lower Mainland of British Columbia, (3) between 19 and 35 years of age, (4) regularly menstruating, (5) a non-obese body weight defined as body mass index (an index of your weight in kilograms divided by your height in metres squared) between 18.5 and 30, (6) in general good health and not suffering from any chronic diseases that may affect bone metabolism or the endocrine or reproductive systems, (7) able to read and understand English.

**Who Should Not Participate?** You are not able to participate in this study if you: (1) do not meet inclusion criteria, (2) use oral contraceptives (birth control pills), receive Depo-Provera injections or other drugs that affect menstruation, the endocrine system or bone metabolism, (3) have been diagnosed with or treated for an eating disorder, (4) are pregnant or lactating, (5) work at night or have an inconsistent sleep pattern.

**What Does the Study Involve?** This study will take place at UBC, the Vancouver General Hospital and participants’ homes. Approximately 135 women will participate in the study. The 2-year study will involve the following at the beginning of the study, 6 months and 2 years later: i) eligibility assessment by phone; ii) orientation to the procedures, measurement of height, weight and waist circumference and answering a question regarding symptoms associated with menstruation (at UBC); iii) completion of a questionnaire package and Food Frequency
Questionnaire, daily temperature records, and a 24-hour urine sample (at home). At the beginning of the study and 2 years later only, a bone density/body composition scan at Vancouver General Hospital will be completed. Also, once during the study period, a 12-hour blood pressure assessment will be completed (at home). Your involvement in this 2-year study will take a total of 28.5 hours at each assessment period plus 13 hours for the blood pressure monitoring and 18 hours/year for recording your daily temperature.

**Specific Procedures:**

1. **Eligibility assessment by phone.** This involves answering a few simple questions to determine if you are able to participate or continue to participate in the study (see Who Can Participate). This will require approximately 10 minutes of your time. At this time, we will schedule a meeting at UBC for orientation to the study. This procedure will occur at study entry, 6 months and year 2.

2. **Study orientation at UBC.** At your convenience, you will meet with a member of the research team at the Human Nutrition department of UBC for the following: a) orientation to the study procedures; b) review of the questionnaire package and Food Frequency Questionnaire; c) be provided with instructions and materials for daily body temperature readings, 12-hour blood pressure monitoring (only once) and 24-hour urine collection; d) measurement of your height, weight and waist circumference while wearing light indoor clothing; e) answering a question regarding symptoms associated your menstrual cycle; and f) scheduling of a bone density/body composition scan at VGH (at study entry and year 2 only). This meeting will require approximately 1 hour of your time and will occur at study entry, 6 months and year 2. Please see details below.

3. **Completion of the Questionnaire Package & Food Frequency Questionnaire.** A member of the research team will explain how to complete the questionnaire package. You will take the questionnaire home to complete within one week and then return by mail using the addressed, stamped envelope we will provide. The questionnaire will take you 40 minutes to complete and includes questions regarding eating attitudes and behaviours, body image, stress, weight cycling, menstrual/reproductive history, lifestyle behaviours and demographics. The Food Frequency Questionnaire will take 1 hour to complete and involves questions regarding the types and amounts of food you usually consume. These will be completed at study entry, 6 months and year 2.

4. **Completion of a daily temperature calendar.** At home, beginning the first day of your period after entering the study and daily thereafter, you will measure your body temperature when you wake up using a digital thermometer. You will record the temperature in your calendar as well as the time you woke up, whether or not menstrual flow has occurred, any sleep problems, and your health status. At UBC, we will provide you with the digital thermometer and temperature calendar. We will demonstrate how to use the digital thermometer and will give you written instructions to take home. We will contact you periodically to answer any questions you may have regarding the temperature calendars. Completion of your calendar will take approximately 3 minutes daily for 2 years.

5. **Completion of 12-hour blood pressure monitoring, the day following one of your UBC visits.** While at UBC, the compact, lightweight monitor will be fitted and demonstrated. You will then have an opportunity to practice using the monitor with the aid of a research team member. You will take the monitor home with you and will begin the assessment the following morning. The arm cuff will be placed on your non-dominant arm after bathing (since the device should not get wet) and will be worn for 12 hours or after finishing dinner, whichever is later. During this time you may go about your normal day, except we ask that you refrain from heavy exercise. Each time you feel the cuff inflate (once every half hour), you will need to sit down until the cuff loosens again and then note the time and describe the activities in
which you were engaged (i.e. doing school work, watching TV, having dinner) in your provided blood pressure monitoring diary. We will arrange for courier pick up of the monitor and diary the next day. This assessment will occur once during the 2-year study.

6. **Completion of a 24-hour urine sample** within two weeks of entering the study. At UBC, we will schedule a date that is most convenient for you to complete this assessment within 2 weeks. We will explain the procedure and provide you with the necessary materials and written instructions while at UBC. The day before the set collection date, a member of the research team will contact you to review procedures and answer any questions you may have. On the chosen date, you will collect all urine for a 24-hour period but will otherwise be free to go about your normal day. The day following collection, you will complete a short questionnaire requiring approximately 15 minutes of your time and then call the research team to arrange for courier pick up of the sample. The questionnaire will be returned using a provided addressed, stamped envelope. This assessment will occur at study entry and 6 months and year 2.

7. **Measurement of bone density and body composition at VGH by dual energy x-ray absorptiometry (DEXA)** within two months of entering the study. At your UBC orientation, we will arrange your appointment on a day that is convenient for you to complete this procedure within the next two months. For this procedure, you will be asked to lie still on a padded table for approximately 20 to 30 minutes while a small x-ray detector scans over your body taking measurements of your bone density and body composition. The test is safe and painless and does not require any injections or any other discomfort. This assessment will occur at study entry and year 2.

**What’s in it for me?**
We will reimburse all travel expenses: $10 per trip to UBC and VGH (if your travel costs exceed this amount, you may provide the receipts detailing your travel and a member of the research team will compensate your additional costs). Upon completing all aspects of a study phase (study entry, 6 months and 2 years), you will receive a gift certificate valued at $30 (total of $90 if you finish entire 2 yr study). You also get to keep the digital thermometer and will be provided with copies of your assessments including bone density, body composition, dietary analysis, ovarian function analysis and 12-hr blood pressure analysis.
Appendix 15: 2-year Prospective Bone Study Eligibility Phone Script

Hello [name]. This is Jennifer Bedford a member of the research team for the study on women’s dietary attitudes and health. I am calling because you expressed an interest in participating. Are you still interested in participating?”

If no: “I understand. Thank you for your time and please call again if you change your mind as we are still looking for new participants”

If yes: “Great. Do you have time to speak with me now for approximately 15 minutes?”

If no: “That’s no problem. Can we set up a time that I can call you back?” Set up time and date most convenient for participant.

If yes: I would like to briefly explain the study procedures to you and then if you are interested in participating I have a few simple questions that I need to ask you in order to determine if you are able to participate in the study.

The study would begin with a meeting at UBC where I will orient you to the study procedures in detail. As you know this is a 2-year study so this meeting will occur once now, then again 6 months later and then again 2 years later. At the meeting, I will explain how to complete a questionnaire package (takes about 40 mins to complete) and food frequency questionnaire (takes about an hour to complete) which you will take home with you and complete them at your convenience within about a week and return them to me by mail using a stamped envelope that I will give you. Next, I will provide you with materials and instructions for completion of your temperature calendar. This will involve recording your temperature each morning before you get out of bed using a digital thermometer which I will give you (and you can keep after the study!). This will take you <3 minutes each day for the next two years. I will also provide you with the materials and instructions for a 24-hour urine collection to be completed in the next week or so. You will choose a day that is convenient to collect all your urine over a 24-hr period and when done I will arrange for a courier to pick up the urine and bring it to the Vancouver General Hospital laboratory for analysis. Also at one of the meetings (either now, 6 months or 2 years later – its up to you) I will show you how to use a compact, lightweight blood pressure monitor which you take home with you and begin wearing the following morning for a 12 hour period. You will be able to go about your normal day while wearing the monitor and will only need to sit down when it makes a measurement, every ½ hour, and write down what activities you were participating in at the time in a diary we will provide you with. Again I will arrange for courier pick up to return the equipment. Finally, I will help you set up an appointment at Vancouver General Hospital to have your body composition and bone density scan. At the end of the study I will provide you with the results of all of your scan as well as your ovulation, blood pressure and dietary analysis and you can share this information with your doctor. Lastly, I will measure your height, weight and waist circumference and will ask you a question about any symptoms you experience before your menstrual period. This will be repeated at 6 months (except bone) and 2 years later.

Your participation in the study will be completing voluntary and you can withdrawal without consequence at any time. We will reimburse you for all travel, $10 per trip to UBC and VGH and you will also receive a $30 gift certificate at study entry 6 months and 2 years. At the end of the study we will provide you with information regarding your dietary intake, fertility, blood pressure, bone density and body composition.”

“Would you like to continue with the questioning to see if you are eligible? This will take about 10 minutes. Your participation in this brief interview is completely voluntary and you may end the interview at any time without any consequences. Do you have time to complete this now?”
If no:  "That’s no problem. Can we set up a time that I can call you back?” Set up time and date most convenient for participant.

If yes:  “Great let’s get started! As I mentioned I will be asking you these questions in order to ensure that you are able to participate in the study. First are a few simple questions regarding your age and living plans. May I proceed?”

“Are you a female between 19 and 35 years of age?” [Yes - continue] [No – ineligible*]

“Are you able to read, speak and understand English?” [Yes - continue] [No – ineligible]

“Do you plan on remaining in the Vancouver area for the next two years?” [Yes - continue] [No – ineligible]

“Do you work at night or work shift work?” [No - continue] [Yes – ineligible]

“Would you say your sleeping patterns are consistent (i.e. wake up and go to sleep at approximately same time, most days and sleep for similar amount of time most days)?” [Yes - continue] [No – ineligible]

“Are you currently pregnant or lactating?” [No - continue] [Yes – ineligible]

“Do you plan on becoming pregnant in the next two years?” [No - continue] [Yes – ineligible]

“OK great. Now I am going to ask you some questions about your health and medication use. Because this study is examining bone, fertility, blood pressure and stress, I need to know if you have any medical conditions or use any drugs that may effect these measurements.”

“Are you currently using the birth control pill or receiving depo-provera injections?” [No - continue] [Yes – ineligible]

“Have you used either birth control pills or depo-provera injections in the past year?” [No - continue] [Yes – ineligible]

“Do you currently have plans to begin using birth control pills or receiving depo-provera injections within the next two years?” [No - continue] [Yes – ineligible]

“Do you have your period every 21 to 35 days?” [Yes - continue] [No – ineligible]

“Are you in general good health and free of any chronic diseases?” [No - continue] [Yes – ineligible]

“Have you ever been treated for or diagnosed with an eating disorder? [No - continue] [Yes – ineligible]

“Do you currently or have you ever suffered with any chronic medical conditions?” [No - continue] If yes, “Could you please name the condition(s)?”

[Women with any of the following will be ineligible: Anorexia or bulimia nervosa, Cushing’s syndrome, osteoporosis, renal/kidney disease, polycystic ovary syndrome, endometriosis, gastrointestinal disease (IBS, Crohn’s, ulcer, ulcerative colitis or gastric resection), seizure or convulsive disorder, thyroid disorder, primary hyperprolactinemia, multiple myeloma, chronic obstructive pulmonary disease or emphysema, hypertension]
“Have you ever been sufficiently bothered by severe acne, unwanted face or body hair to consult a physician for treatment?” [No - continue] [Yes – ineligible]

“Are you currently or have you ever taken any prescription medications daily for more than 1 month?” [No - continue]
If yes, “Could you please name the medications and the duration you took them for?

[Women who used any of the following drugs for more than six months will be ineligible: Prednisone, dexamethasone, glucocorticoids, other steroid drugs, blood pressure medication or diuretics, beta blockers (propranolol, metoprolol), thyroid hormones, anticonvulsive drugs, heparin, warfarin (Coumadin), methotrexate, cyclophosphamide, cyclosporine or other immnosuppressants, cholestyramine, gonadotropin-releasing hormones, danazol (cyclamen)]

“Are you currently or have you ever taken any herbal supplements or over-the-counter medications daily for more than 1 month?” [No - continue]
If yes, “Could you please name the herbal supplement and the duration you took them for?”

[Each herbal supplement will be researched for possible effects on menstruation, bone, cortisol, and/or blood pressure.]

“OK great. The last thing I am going to ask is your height and weight so that I can make a calculation to determine your body mass index (BMI) which is an index of your weight in kilograms divided by your height in metres. We require women with BMIs >18.5 and 25 <30 kg/m² because BMIs below and above this range have effects on bone.”

“What is your current body weight? ____________ What is your current height?”

If BMI <18.5: “Thank you. Your BMI is XX. Unfortunately this BMI is not in the range we are looking for. Thank you for your time in answering these questions and I apologize for any inconvenience.”
If BMI >30: “Thank you. Your BMI is XX. Unfortunately this BMI is not in the range we are looking for. Thank you for your time in answering these questions and I apologize for any inconvenience.”
If BMI 18.5-30: “Thank you. Your BMI is XX. You are eligible to participate in the study. May we set up an appointment to meet for approximately 1 hour at UBC? Also, would you like to complete the blood pressure assessment the day following our meeting? IF so, we need to keep in mind that you will be required to complete the 12-hr blood pressure monitoring the following day.

Arrange meeting date and time.

Thank you again for your time and I look forward to meeting with you on [date] at [time]. I will provide you with $10 to defray your transportation costs at this time (you will receive another $10 after you travel to VGH for your bone scan). As we are going to be measuring your weight and waist circumference at the meeting, please wear some light clothing (underneath your regular clothing) like a tank top or camisole and perhaps some spandex shorts or light pants. We will also be setting a date for your urine collection and bone density scan so if you have day timer or planner or scheduler you should bring that with you. Also, when we schedule your bone scan appointment I will need your care card number (MSP, OHIP) so I can either take the number down from you now or you can bring your card/number with you to the orientation.

CARE CARD: ______________________
The Food & Nutrition Building is located at 2205 East Mall. Are you familiar with campus?

How will you be arriving? (Bus, parking-meter parking at bookstore).

Some landmarks: bookstore, new Michael Smith Building then us. Across from Library.

I will meet you right inside the lobby on the day of our appointment.
Please call me at 604-616-4676 if you need to re-schedule or you have any questions.”

*If a participant’s answer to any question determines them ineligible the following script will be used:
“Thank you. Unfortunately you are ineligible for this study because [explain based on exclusion criteria]. I appreciate your interest and thank you for your time. Have a nice day. Bye.”
Appendix 16: 2-year Prospective Bone Study Letter of Consent

THE UNIVERSITY OF BRITISH COLUMBIA

Prospective Studies of Eating Attitudes in Women:
Physiological, Psychosocial & Nutritional Associations

Short title: Dietary Attitudes, Stress, Menstruation and Bone Health in Young Women

Subject Information and Consent Form

Principal Investigator:
Susan Barr, PhD, RD
Professor; Food, Nutrition and Health, University of British Columbia (UBC); (604) 822-6766

Co-Investigators:
Jennifer Bedford, BSNH
PhD Candidate
Human Nutrition, UBC
(604) 616-4676

Jerilynn Prior, MD
Professor
Endocrinology & Metabolism, UBC
(604) 875-5927

Katherine Keiver, PhD
Assistant Professor
Food, Nutrition & Health
(604) 822-0421

Wolfgang Linden, PhD
Professor
Clinical Psychology, UBC
(604) 822-4156

Sponsor: Canadian Institutes of Health Research (CIHR)

Introduction: You are being invited to participate in this study after indicating your interest and meeting eligibility requirements.

Your Participation is Voluntary: Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you do not have to provide any reason for your decision not to participate. Please take the time to read this document carefully. If you wish, you may discuss it with your family, friends and doctor before you decide. Please feel free to ask any questions regarding the study procedures you may have while reading this document.

Purpose: The purpose of this study is to explore relationships between young women’s eating attitudes and behaviours, stress, ovarian function and changes in bone density over two years.

Who Can Participate? You are eligible to participate in this study if you are: (1) female, (2) a resident of the Lower Mainland of British Columbia, (3) between 19 and 35 years of age, (4) regularly menstruating, (5) a non-obese body weight defined as body mass index (an index of your weight in kilograms divided by your height in metres squared) between 18.5 and 30, (6) in general good health and not suffering from any chronic diseases that may affect bone metabolism or the endocrine or reproductive systems, (7) able to read and understand English.
Who Should Not Participate? You are not able to participate in this study if you: (1) do not meet inclusion criteria, (2) use oral contraceptives (birth control pills), receive Depo-Provera injections or other drugs that affect menstruation, the endocrine system or bone metabolism, (3) have been diagnosed with or treated for an eating disorder, (4) are pregnant or lactating, (5) work at night or have an inconsistent sleep pattern.

What Does the Study Involve? This study will take place at UBC, the Vancouver General Hospital and participants’ homes. Approximately 135 women will participate in the study. The 2-year study will involve the following at the beginning of the study, 6 months and 2 years later: i) eligibility assessment by phone; ii) orientation to the procedures, measurement of height, weight and waist circumference and answering a question regarding symptoms associated with menstruation (at UBC); iii) completion of a questionnaire package and Food Frequency Questionnaire, daily temperature records, and a 24-hour urine sample (at home). At the beginning of the study and 2 years later only, a bone density/body composition scan at Vancouver General Hospital will be completed. Also, once during the study period, a 12-hour blood pressure assessment will be completed (at home). Your involvement in this 2-year study will take a total of 28.5 hours at each assessment period plus 13 hours for the blood pressure monitoring and 18 hours/year for recording your daily temperature.

Specific Procedures:
1. Eligibility assessment by phone. This involves answering a few simple questions to determine if you are able to participate or continue to participate in the study (see Who Can Participate). This will require approximately 15 minutes of your time. At this time, we will schedule a meeting at UBC for orientation to the study. This procedure will occur at study entry, 6 months and year 2.

2. Study orientation at UBC. At your convenience, you will meet with a member of the research team at the Human Nutrition department of UBC for the following: a) orientation to the study procedures; b) review of the questionnaire package and Food Frequency Questionnaire; c) be provided with instructions and materials for daily body temperature readings, 12-hour blood pressure monitoring (only once) and 24-hour urine collection; d) measurement of your height, weight and waist circumference while wearing light indoor clothing; e) answering a question regarding symptoms associated your menstrual cycle; and f) scheduling of a bone density/body composition scan at VGH (at study entry and year 2 only). This meeting will require approximately 1 hour of your time and will occur at study entry, 6 months and year 2. Please see details below.

3. Completion of the Questionnaire Package & Food Frequency Questionnaire. A member of the research team will explain how to complete the questionnaire package. You will take the questionnaire home to complete within one week and then return by mail using the addressed, stamped envelope we will provide. The questionnaire will take you 45 minutes to complete and includes questions regarding eating attitudes and behaviours, body image, stress, weight cycling, menstrual/reproductive history, lifestyle behaviours and demographics. The Food Frequency Questionnaire will take 1 hour to complete and involves questions regarding the types and amounts of food you usually consume. These will be completed at study entry, 6 months and year 2.

4. Completion of a daily temperature calendar. At home, beginning the first day of your period after entering the study and daily thereafter, you will measure your body temperature when you wake up using a digital thermometer. You will record the temperature in your calendar as well as the time you woke up, whether or not menstrual flow has occurred, any sleep problems, and your health status. At UBC, we will provide you with the digital thermometer and temperature calendar. We will demonstrate how to
use the digital thermometer and will give you written instructions to take home. We will contact you periodically to answer any questions you may have regarding the temperature calendars. Completion of your calendar will take approximately 3 minutes daily for 2 years.

5. Completion of 12-hour blood pressure monitoring, the day following one of your UBC visits. While at UBC, the compact, lightweight monitor will be fitted and demonstrated. You will then have an opportunity to practice using the monitor with the aid of a research team member. You will take the monitor home with you and will begin the assessment the following morning. The arm cuff will be placed on your non-dominant arm after bathing (since the device should not get wet) and will be worn for 12 hours or after finishing dinner, whichever is later. During this time you may go about your normal day, except we ask that you refrain from heavy exercise. Each time you feel the cuff inflate (once every half hour), you will need to sit down until the cuff loosens again and then note the time and describe the activities in which you were engaged (i.e. doing school work, watching TV, having dinner) in your provided blood pressure monitoring diary. We will arrange for courier pick up of the monitor and diary the next day. This assessment will occur once during the 2-year study.

6. Completion of a 24-hour urine sample within two weeks of entering the study. At UBC, we will schedule a date that is most convenient for you to complete this assessment within 2 weeks. We will explain the procedure and provide you with the necessary materials and written instructions while at UBC. The day before the set collection date, a member of the research team will contact you to review procedures and answer any questions you may have. On the chosen date, you will collect all urine for a 24-hour period but will otherwise be free to go about your normal day. The day following collection, you will complete a short questionnaire requiring approximately 15 minutes of your time and then call the research team to arrange for courier pick up of the sample. The questionnaire will be returned using a provided addressed, stamped envelope. This assessment will occur at study entry and 6 months and year 2.

7. Measurement of bone density and body composition at VGH by dual energy x-ray absorptiometry (DEXA) within two months of entering the study. At your UBC orientation, we will arrange your appointment on a day that is convenient for you to complete this procedure within the next two months. For this procedure, you will be asked to lie still on a padded table for approximately 20 to 30 minutes while a small x-ray detector scans over your body taking measurements of your bone density and body composition. The test is safe and painless and does not require any injections or any other discomfort. This assessment will occur at study entry and year 2.

**Risks:** The only risk to your involvement in this study is the exposure to a small amount of radiation through the DEXA scan. The amount of radiation to which you will be exposed is approximately equal to the amount you would receive after being outdoors for several hours.

**Benefits:** You will benefit from your participation in this study by receiving an analysis of your dietary intake and fertility, and the results of both your bone density/body composition test and blood pressure assessment at the conclusion of the study. We will also provide copies of the results for you to discuss with your family doctor. A summary of the research results, once completed, will be provided to you as well, if you would like to receive it.

**Your Participant Rights & Responsibilities:** Your participation in this study is entirely voluntary and you may refuse to participate or withdraw from the study at any time without penalty or repercussions. You may withdraw without providing any explanation of your reasons.
for doing so. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrollment in the study will be retained for analysis. By law, this data cannot be destroyed. Your participation in this study is not associated with any known risks of injury or illness. However, you do not waive any of your legal rights by signing this consent form. We ask that you please inform the research team if you are no longer able or willing to participate in the study. We also ask that you please inform the research team if you begin taking any medications (i.e. birth control), if you become pregnant, are trying to become pregnant or suspect you may be pregnant. If either of the two DEXA scans indicate your bone mass is diagnostic of osteoporosis we will inform you immediately and will provide you with a copy of the results to discuss with your family physician. At this time you will be withdrawn from the study in order to seek treatment. Also, if you are not complying with the requirements of the study the investigators may withdraw you from the study.

**Remuneration/Compensation:** Upon completing all aspects of a study phase (study entry, 6 months and 2 years), you will receive a gift certificate valued at $30. Also, in order to defray the costs of transportation you will be reimbursed $10 for each visit to either UBC or VGH. Also, if your travel costs exceed this amount, you may provide the receipts detailing your travel and a member of the research team will compensate your additional costs.

**Confidentiality:** All samples, test results, questionnaires and other documents will be labelled with code numbers only (your name will not be associated with these results or documents) and will be kept in a locked filing cabinet in the offices of the principal investigator. After your urine samples have been analyzed, they will be disposed of. Your confidentiality will be respected, and no information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the investigator or her designate by representatives of the Canadian Institutes of Health Research, Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. No records which identify you by name or initials will be allowed to leave the investigators’ offices.

**Contact Information:** If you have any questions or desire further information about this study before or during participation, you can contact Jennifer Bedford at (604) 616-4676 or Dr. Susan Barr at (604) 822-6766. If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services at (604) 822-8598.
Consent to Participate:

☐ I have read and understood the subject information and consent form.

☐ I have had sufficient time to consider the information provided and to ask for advice if necessary.

☐ I have had the opportunity to ask questions and have had satisfactory responses to my questions.

☐ I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.

☐ I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time and without changing in any way the quality of care that I receive.

☐ I understand that I am not waiving any of my legal rights as a result of signing this consent form.

☐ I understand that there is no guarantee that this study will provide any benefits to me.

☐ I have read this form and I freely consent to participate in this study.

☐ I have been told that I will receive a dated and signed copy of this form

__________________________________________________________
Printed Name of Participant ___________________________ Signature ___________________________ Date ___________________________

__________________________________________________________
Printed Name of Witness ___________________________ Signature ___________________________ Date ___________________________

__________________________________________________________
Printed Name of Principal Investigator or Designated Representative ___________________________ Signature ___________________________ Date ___________________________
Appendix 17: 2-year Prospective Bone Study Ethics Approval Certificate

The University of British Columbia
Office of Research Services,
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

Certificate of Full Board Approval
Clinical Research Ethics Board Official Notification

PRINCIPAL INVESTIGATOR
Barr, S.I.

DEPARTMENT
Family & Nutr Sci

NUMBER
C05-0257

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
Other, UBC Campus, Vancouver Coastal Health Authority

CO-INVESTIGATORS:
Bedford, Jennifer, Land & Food Systems; Keiver, Katherine, Land & Food Systems; Prior, Jerilynn, Medicine

SPONSORING AGENCIES
Canadian Institutes of Health Research

TITLE:
Prospective Exploration of Associations Among Cognitive Dietary Restraint, Cortisol Excretion, Ovarian Function and Bone Health in Premenopausal Women

APPROVAL DATE
28 June 2005

TERM (YEARS)
1

DOCUMENTS INCLUDED IN THIS APPROVAL:
Protocol; Subject Consent Form version date June 2005;
Newspaper/Newsletter Advertisement; Poster Advertisement;
Eligibility Questionnaire Phone Script; Questionnaires

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

The CREB approval for this study expires one year from the approval date.

Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bellward, Chair
Dr. James McCormack, Associate Chair
Dr. Alain Gagnon, Associate Chair
Appendix 18: 2-year Prospective Bone Study Transportation Reimbursement Receipt

THE UNIVERSITY OF BRITISH COLUMBIA

Food, Nutrition and Health
Faculty of Land and Food Systems
2205 East Mall
Vancouver, B.C. Canada V6T 1Z4
Phone: (604) 822-2502
Fax: (604) 822-5143

Prospective Studies of Eating Attitudes in Women:
Physiological, Psychosocial and Nutritional Associations

TRANSPORTATION REIMBURSEMENT RECEIPT

I, ______________________________________________,

(Participant name – Please print)

acknowledge that I have received $20.00 in support of study transportation costs.

_________________________________________  ______________________________
Signature                                                      Date                                                      Phone number (to verify receipt of the funds)

_________________________________________  ______________________________
Printed Name of                                               Signature                                                  Date
Principal Investigator or                                      
Designated Representative
Appendix 19: 2-year Prospective Bone Study Gift Card Receipt

THE UNIVERSITY OF BRITISH COLUMBIA

Food, Nutrition and Health
Faculty of Land and Food Systems
2205 East Mall
Vancouver, B.C. Canada V6T 1Z4
Phone: (604) 822-2502
Fax: (604) 822-5143

Prospective Studies of Eating Attitudes in Women:
Physiological, Psychosocial and Nutritional Associations

GIFD CARD RECEIPT

I, ________________________________________________,

(Participant name – Please print)

acknowledge that I have received a gift certificate valued at $30.00 in support of completing
the above mentioned study procedures

____________________________________________________
Signature Date Phone number (to verify receipt of the funds)

Printed Name of
Principal Investigator or
Designated Representative

____________________________________________________
Signature Date
Appendix 20: 2-year Prospective Bone Study 24-hour Urine Collection Instructions

DATE OF COMPLETION: _______________________

Reminder: put the urine collection materials in your bathroom the day before your collection date.

Your task:
You will complete one 24-hour urine collection on the day you scheduled during your orientation at UBC, listed above. If you need to change this date, please contact Jennifer. Jennifer will call you the day before your scheduled date as a reminder and to review procedures and answer any questions you may have.

Materials provided for each collection:
If any materials are missing, call Jennifer (604-616-4676) as soon as possible and another will be sent to you.

1. Two large orange collection containers: These are the containers you will use to accumulate and store all of the urine collected over the 24-hour time period. Fill one container entirely before using the 2nd. It is OK if you only require one.
2. Plastic measuring cup with handle: This will be used to collect urine with each voiding. You can hold the handle and aim to collect the urine in the cup.
3. Plastic funnel: This will make it easier to transfer the urine collected in the plastic measuring cup into the orange container.
4. Padded Addressed Envelope: When you have completed the urine collection, you will use this envelope to send the sample to the hospital laboratory via courier. The envelope contains a label to attach to the orange container when you have completed the collection, as well as the “requisition form” required by the hospital laboratory – this form is very important to include.

Instructions:
The accuracy of our analysis will depend on the accuracy of the urine collection technique. These instructions will help ensure that your 24-hour urine collection is obtained correctly and will give accurate test results. Please note that you will be collecting all of your urine during the 24-hour period.

1. How to start the collection: The urine collection will begin in the morning of your chosen day. After waking up in the morning and rising for the day, pass your urine, flush it down the toilet, and note the exact time. This is the “start time” for your 24-hour urine collection (write it down). You are now beginning the collection with an empty bladder and an empty orange collection bottle.
2. Continue with the collection for 24 hours: For the rest of that day and night (a 24-hour time period), collect all urine passed. Urine samples should be collected in the plastic measuring cup and then poured into the orange container, using the funnel to reduce the risk of spilling. Do not try to urinate directly into the orange container! You may need only one container. If you fill that container, being filling the 2nd one. Void urine prior to bowel movements in order to avoid losing urine that might normally be passed during a bowel movement. If you are away from home during your collection time, please bring the materials with you (they can be carried discretely in a bag) and continue with the collection as needed. The orange container should be kept in a cool place.
3. How to conclude the collection: The very next morning, exactly 24 hours from the start time, you should empty your bladder and add that urine to the orange collection bottle. This is your “end time” and completes the collection (write it down). Try to match your “start time” and “end time” as closely as possible (ideally, there should be no more than a 5 or 10 minute difference in these times). If you find that you must urinate an hour
or so before the end time, go ahead and do so, then drink a full glass of water so that you can urinate again at the time to end the collection.

4. **Questionnaire:** On the morning you complete your 24-hour urine collection, complete the short questionnaire (it is inside the padded envelope).

   **Note:** The questionnaire is returned in the normal paper envelope addressed to UBC, not the padded envelope containing your orange urine collection container.

5. **Daily Activity:** On the day of urine collection you should go about your normal daily activities. Any unusual physical activity should be avoided. For example, if you walk for 20 min daily it is ok to also do this on your collection day but if you complete a 60 min walk weekly this should not be chosen as the day of your collection. Unusual mental strain should also be avoided, for example, do not complete the urine collection on a day of an exam or job presentation. If you become aware of any sudden stresses on the day you have chosen for your urine collection, please contact Jennifer to reschedule.

**When you have completed the 24-hour collection...**

1. **Call Jennifer at 604-616-4676** to arrange for courier pick-up of the sample. These arrangements should be made as soon as possible after the collection is complete. If you are leaving a voicemail message, please include the address to which the courier should go. The courier should arrive at the address you request (home or office) within a few hours at most, and the sample will then be delivered to the hospital for analysis. Jennifer will call you back to confirm that the courier has been asked to pick up your package.

2. **Package the complete sample (in the orange container(s)) in the padded envelope provided.** Put the label on the orange container indicating your name and the start and end times of the collection, and ensure that the "requisition form" is included inside of the bag.

3. **Complete the mini-questionnaire.** This should be returned to Jennifer by mail in the normal stamped, addressed envelope provided.

   I started my urine collection on ____________________ (date) at _________________ (time).

   I ended my urine collection on _____________________ (date) at _________________ (time).

The completed collection samples will be sent by courier to (this should already be on the padded envelope):
Laboratory Reception
Vancouver General Hospital
Room 1302
910 West 10th Avenue
Vancouver, BC, V5Z 4E3

*Please do not hesitate to call Jennifer at 604-616-4676 if you have any questions.*

*Thank you very much for your conscientious completion of this task!*
## Appendix 21: 2-year Prospective Bone Study Temperature Calendar

<table>
<thead>
<tr>
<th>Cycle DAY</th>
<th>DATE (Month/Day)</th>
<th>TIME</th>
<th>TEMP (° Celsius)</th>
<th>FLOW (Y or N)</th>
<th>SLEEP PROBLEMS (0-4)</th>
<th>HEALTH (Feeling ill? Fever? Stressed?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reminder: Start new calendar the day period begins
Temperature Calendar Instructions

Be sure to record your temperature every day.

Leave your thermometer and calendar by your alarm clock or on the bedside table. **If you forget, skip that day and write the date and then FORGOT across the table.** Don’t try to remember later. **Use a new chart for each cycle.**

Quantitative Basal Temperature Monitoring:
The following recommendations will assist you to accurately take and record your oral temperature.

1. **Day 1 is the first day of your flow (and you should be starting on a new sheet).** When you go to the bathroom in the morning, if you notice your period has started, go back and take your temperature and that is ‘Day 1’. If your period starts during the day, consider ‘Day 1’ as the following morning.
2. Take your temperature in the morning, when you first wake. Activity will raise your basal (resting) temperature. Although you may start your thermometer and head to the washroom, if you can, postpone this or getting out of bed until your temperature taking is finished.
3. Record the DATE (month/day) and the TIME you took your temperature (i.e. 7:20AM).
4. Record your TEMPERATURE as displayed on the digital thermometer (XX.X °Celsius).
5. Under ‘FLOW’ please indicate whether or not you menstruated that day (Y) or not (N).
6. Under ‘SLEEP PROBLEMS’ please rate the degree of your sleep problems using the following scale: 0=none, 1=minimal, 2=moderate, 3=moderately intense & 4=intense.
7. Under ‘HEALTH’, please record any events that may affect your morning temperature (e.g. felt like you were getting the flu, experiencing a fever, feeling unusually stressed, had a very late night, tossed and turned a lot).

Using the digital thermometer:
1. Press the ON/OFF button and a beep will sound (88.88 will display when the thermometer is used for the first time).
2. After a few seconds the display will go blank.
3. **Place the thermometer under your tongue at the back of your mouth.** The thermometer will begin to beep steadily for ~1 minute. If it stops, reposition the thermometer.
4. When the peak temperature is reached, **the thermometer will sound 3 rapid beeps.**
   Record the temperature in your diary. The reading will not change while the power remains on.
5. Turn the thermometer off by pressing the purple ON/OFF button for a few seconds.

Analyzing your temperature data:
*This is optional as we will provide you with a detailed computer analysis of your menstrual cycle at the end of the study.*

If you would like to figure out whether you have ovulated and the length of your luteal phase (the time following ovulation) you can do that. First, compute the average of all the temperatures in your record, by adding them up and dividing by the number of days for which you have temperature readings. The average temperature you get can then be compared with the actual readings. If your temperature went above and stayed above that average until the day before the next flow you have ovulated. The higher temperatures should last 10-16 days. When there are between 3 and 9 days of higher temperatures, you have what is called a short luteal phase. This means that you have ovulated but the time of progesterone elevation is too short. Enjoy keeping this daily Temperature Calendar. You will learn new things about yourself!

**Please do not hesitate to call Jennifer at 604-616-4676 if you have any questions/comments/concerns or need additional calendars.**

Thank you very much for your conscientious completion of this task!
Appendix 22: 2-year Prospective Bone Study Bone Density Scan Instructions

Appointment Date: ______________ Day: __________ Time: __________

Location: Nuclear Medicine, Jim Pattison Pavilion South, Unit 8
          899 West 12\textsuperscript{th} Ave (Laurel & 12\textsuperscript{th}), Vancouver, BC

Directions (Please turn over for map of hospital area)

By bus:
From campus take the B-line #99. Get off at the hospital stop which is the 6\textsuperscript{th} stop at Willow Street. Walk up Heather Street from Broadway Ave to West 12\textsuperscript{th} Ave. **Turn right** (west) and walk down 12\textsuperscript{th} until you reach the under ground parking lot for the Jim Pattison Pavilion.
Alternatively, you may take any bus that gets you between Granville and Cambie near 12\textsuperscript{th} Avenue. If you require assistance finding the best bus route, please call Jennifer and she will assist you.

By car:
We encourage you to take the bus or taxi as parking at VGH can be difficult. If you chose to drive, please **leave extra time to find parking (~20 minutes)**.
There is a parkade across from the Jim Pattison Pavilion though it is often full (note the P on the map). If you are able to park here, there is a pedestrian overpass to the Pavilion.
There is 2-hour meter parking within 3-4 blocks of the hospital.
There is a small park at the corner of 18\textsuperscript{th} and Willow where parking is usually available and the 2-hour limit is rarely enforced. It takes approximately 15 minutes to walk to the Pavilion from there (north on Willow then turn left onto 12\textsuperscript{th}).
**Wherever you park, go to 12\textsuperscript{th} Ave between Heather and Laurel streets at 899 West 12\textsuperscript{th} Ave, the Jim Pattison Pavilion.**

Enter the Jim Pattison Pavilion Main Entrance at 899 West 12\textsuperscript{th} Ave.
Walk down a few steps and through the small lobby (some plants and patio furniture) until you reach the far (north) wall of the building. Following the signs to Nuclear Medicine/Bone density (Unit #8), turn down the **hallway to the left** until you reach the Nuclear Medicine unit on the left (note: it is a long hallway). **Check in with the receptionist** (give her your name and appointment time).

Once you are there, you will be asked to change into a hospital gown.
For the actual scan, you will be asked to lie still on a padded table for approximately 20 to 30 minutes while a small x-ray detector scans over your body taking measurements of your bone density and body composition. The test is safe and painless and does not require any injections or any other discomfort.
At the end of the 2-year study, we will provide you with the results of all your scans, we will explain the meaning of them and will provide a copy for you to give to your family physician if you choose.

Please call Jennifer at 604-616-4676 if you require any assistance arriving or if you need to reschedule your appointment.
Vancouver General Hospital (VGH) map for Referring Physicians
Appendix 23: 2-year Prospective Bone Study Annual Questionnaire Package

Questionnaire completed at home and returned by mail:

1. Today’s date: ____________________ (month / day / year)
2. Your birth date: ____________________ (month / day / year)

3. How many years of school have you finished? (Mark the highest level completed)
   ___ I have not completed any formal schooling
   ___ Less than grade 9
   ___ Grades 9-13, without certificate, diploma, or degree
   ___ High school certificate or diploma
   ___ Trades or professional certificate or diploma
   ___ Some university without certificate or diploma
   ___ University certificate or diploma
   ___ University degree
   ___ Graduate or professional degree (MA/Sc, PhD, MBA, MD)

4. What is your current employment status? (Check all that apply)
   ___ Unemployed
   ___ Retired
   ___ Student, part time
   ___ Student, full time
   ___ Employed, part time
   ___ Employed, full time
   ___ Other (please specify): __________________________

5. With what race/ethnic group do you identify? (Check all that apply)
   ___ Caucasian
   ___ Chinese
   ___ South Asian (Indian, Pakistani, Punjabi, Sri Lankan)
   ___ Black (African, Haitian, Jamaican, Somali)
   ___ First Nations
   ___ Arab/West Asian (Armenian, Egyptian, Iranian, Lebanese)
   ___ Filipino
   ___ South East Asian (Cambodian, Indonesian, Vietnamese)
   ___ Latin American
   ___ Japanese
   ___ Korean
   ___ Other (please specify): __________________________

6. What is your current marital status?
   ___ Common-law
   ___ Divorced/separated
   ___ Married
   ___ Single
   ___ Widowed
The following questions relate to eating behaviours. Please read each statement and circle True (T) or False (F). Please answer each question as best you can.

7. When I smell the aroma of my favourite food, I find it very difficult to keep from eating, even if I have just finished a meal. ....................................................... T  F

8. I usually eat too much at social occasions, like parties and picnics. ....................................................... T  F

9. I am usually so hungry that I eat more than three times a day. ....................................................... T  F

10. When I have eaten my quota of calories, I am usually good about not eating any more. ....................................................... T  F

11. Dieting is so hard for me because I just get too hungry. ....................................................... T  F

12. I deliberately take small helpings as a means of controlling my weight. ....................................................... T  F

13. Sometimes things just taste so good that I keep on eating even when I am no longer hungry. ....................................................... T  F

14. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat. ....................................................... T  F

15. When I feel anxious, I find myself eating. ....................................................... T  F

16. Life is too short to worry about dieting. ....................................................... T  F

17. Since my weight goes up and down, I have gone on reducing diets more than once. ....................................................... T  F

18. I often feel so hungry that I just have to eat something. ....................................................... T  F

19. When I am with someone who is overeating, I usually overeat too. ....................................................... T  F

20. I have a pretty good idea of the number of calories in common foods. ....................................................... T  F

21. Sometimes when I start eating, I just can’t seem to stop. ....................................................... T  F

22. It is not difficult for me to leave something on my plate. ....................................................... T  F

23. At certain times of the day, I get hungry because I have gotten used to eating then. ....................................................... T  F

24. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it. ....................................................... T  F

25. Being with someone who is eating often makes me hungry enough to eat also. ....................................................... T  F
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>26. When I feel blue, I often overeat.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27. I enjoy eating too much to spoil it by counting calories or watching my weight.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28. When I see a real delicacy, I often get so hungry that I have to eat right away.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. I often stop eating when I am not really full as a conscious means of limiting the amount of food that I eat.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30. I get so hungry that my stomach often seems like a bottomless pit.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31. My weight has hardly changed at all in the last two years.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33. When I feel lonely, I console myself by eating.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34. I consciously hold back at meals in order to not gain weight.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35. I sometimes get very hungry late in the evening or at night.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36. I eat anything I want, anytime I want.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37. Without even thinking about it, I take a long time to eat.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38. I count calories as a conscious means of controlling my weight.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39. I do not eat some foods because they make me fat.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40. I am always hungry enough to eat at any time.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41. I pay a great deal of attention to changes in my figure.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43. If I eat a little bit more on one day, I make up for it the next day.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44. I pay attention to my figure, but I still enjoy a variety of foods.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45. I prefer light foods that are not fattening.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46. If I eat a little bit more during one meal, I make up for it at the next meal.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47. I eat diet foods, even if they do not taste very good.</td>
<td>T</td>
<td>F</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
48. A diet would be too boring a way for me to lose weight......................... T F
49. I would rather skip a meal than stop eating in the middle of one............. T F
50. I alternate between times when I diet strictly and times when I don’t pay much attention to what and how much I eat........................................ T F
51. Sometimes I skip meals to avoid gaining weight............................... T F
52. I avoid some foods on principle even though I like them...................... T F
53. I try to stick to a plan when I lose weigh........................................... T F
54. Without a diet plan I wouldn’t know how to control my weight.............. T F
55. Quick success is most important to me during a diet........................... T F

Please answer the following questions by circling the number above the response that is most appropriate to you. Please answer all questions as best as you can.

56. How often are you dieting in a conscious effort to control your weight?

1 2 3 4
Rarely Sometimes Usually Always

57. Would a weight fluctuation of 5 lbs (~2.3 kg) affect the way you live your life?

1 2 3 4
Not at all Slightly Moderately Very much

58. How often do you feel hungry?

1 2 3 4
Only at mealtimes Sometimes Often Almost between meals between meals always

59. Do your feelings of guilt about overeating help you to control your food intake?

1 2 3 4
Never Rarely Often Always

60. How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

1 2 3 4
Easy Slightly Moderately Very difficult difficult difficult

61. How conscious are you of what you are eating?

1 2 3 4
Not at all Slightly Moderately Extremely

62. How frequently do you avoid ‘stocking up’ on tempting foods?

1 2 3 4
Almost never Seldom Usually Almost always
63. How likely are you to shop for low calorie foods?

1  2  3  4  
Unlikely Slightly unlikely Moderately likely Very likely

64. Do you eat sensibly in front of others and splurge alone?

1  2  3  4  
Never Rarely Often Always

65. How likely are you to consciously eat slowly in order to cut down on how much you eat?

1  2  3  4  
Unlikely Slightly likely Moderately likely Very likely

66. How frequently do you skip dessert because you are no longer hungry?

1  2  3  4  
Almost never Seldom At least once a week Almost everyday

67. How likely are you to consciously eat less than you want?

1  2  3  4  
Unlikely Slightly likely Moderately likely Very likely

68. Do you go on eating binges though you are not hungry?

1  2  3  4  
Never Rarely Sometimes At least once a week

69. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never ‘giving in’), what would you number yourself? Please circle the number which best applies to you most of the time.

0. Eat whatever you want, whenever you want it.
1. Usually eat whatever you want, whenever you want it.
2. Often eat whatever you want, whenever you want it.
3. Often limit food intake, rarely ‘give in’.
4. Usually limit food intake, rarely ‘give in’.
5. Constantly limiting food intake, never ‘giving in’.

70. To what extent does this statement describe your eating behaviour? ‘I start dieting in the morning, but because of any number of things during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow’.

1  2  3  4  
Not like me Little like me Pretty good Describes me description of me perfectly

71. Do you deliberately restrict your intake during meals even though you would like to eat more?

1  2  3  4  
Never Rarely Often Always
The questions in this scale relate to stress and ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don’t try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

72. In the last month, how often have you been upset because of something that happened unexpectedly?
   0 Never
   1 Almost never
   2 Sometimes
   3 Fairly often
   4 Very often

73. In the last month, how often have you felt that you were unable to control the important things in your life?
   0 Never
   1 Almost never
   2 Sometimes
   3 Fairly often
   4 Very often

74. In the last month, how often have you felt nervous and ‘stressed’?
   0 Never
   1 Almost never
   2 Sometimes
   3 Fairly often
   4 Very often

75. In the last month, how often have you dealt successfully with irritating life hassles?
   0 Never
   1 Almost never
   2 Sometimes
   3 Fairly often
   4 Very often

76. In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?
   0 Never
   1 Almost never
   2 Sometimes
   3 Fairly often
   4 Very often

77. In the last month, how often have you felt confident about your ability to handle your personal problems?
   0 Never
   1 Almost never
   2 Sometimes
   3 Fairly often
   4 Very often

78. In the last month, how often have you felt that things were going your way?
   0 Never
   1 Almost never
   2 Sometimes
   3 Fairly often
   4 Very often

211
79. In the last month, how often have you found that you could not cope with all the things that you had to do?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Almost</td>
<td>Sometimes</td>
<td>Fairly</td>
<td>Very</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
</tbody>
</table>

80. In the last month, how often have you been able to control irritations in your life?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Almost</td>
<td>Sometimes</td>
<td>Fairly</td>
<td>Very</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
</tbody>
</table>

81. In the last month, how often have you felt that you were on top of things?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Almost</td>
<td>Sometimes</td>
<td>Fairly</td>
<td>Very</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
</tbody>
</table>

82. In the last month, how often have you been angered because of things that happened that were outside of your control?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Almost</td>
<td>Sometimes</td>
<td>Fairly</td>
<td>Very</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
</tbody>
</table>

83. In the last month, how often have you found yourself thinking about things that you have to accomplish?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Almost</td>
<td>Sometimes</td>
<td>Fairly</td>
<td>Very</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
</tbody>
</table>

84. In the last month, how often have you been able to control the way you spend your time?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Almost</td>
<td>Sometimes</td>
<td>Fairly</td>
<td>Very</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
</tbody>
</table>

85. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Almost</td>
<td>Sometimes</td>
<td>Fairly</td>
<td>Very</td>
</tr>
<tr>
<td></td>
<td>never</td>
<td>often</td>
<td>often</td>
<td>often</td>
<td>often</td>
</tr>
</tbody>
</table>

We would like to know how you have been feeling about your appearance over the past month. Please read each question and circle the appropriate number. Please answer all questions the best you can.

86. Have you been so worried about your shape that you have been feeling you ought to diet?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
</table>
|   | Never | Rarely | Sometimes | Often | Very often | Always
87. Has being with thin people made you feel self-conscious about your shape?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

88. Have you ever noticed the shape of other people and felt that your own shape compared unfavourably?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

89. Has being undressed, such as when taking a bath, made you feel fat?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

90. Has eating sweets, cakes or other high calorie foods made you feel fat?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

91. Have you felt excessively large and rounded?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

92. Have you felt ashamed of your body?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

93. Has worry about your shape made you diet?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

94. Have you thought that you are the shape you are because you lack self-control?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

95. Have you worried about other people seeing rolls of fat around your waist and stomach?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

96. Have you felt that it is not fair that other people are thinner than you?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always

97. Has seeing your reflection (e.g. in a mirror or shop window) made you feel bad about your shape?

1  2  3  4  5  6
Never  Rarely Sometimes Often Very often Always
98. Have you been particularly self-conscious about your shape when in the company of other people?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Very often</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

99. Has worry about your shape made you feel you ought to exercise?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Very often</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

The following series of questions ask you to indicate the degree of agreement with the following statements about appearance. Please answer each question as best as you can by circling the appropriate number.

<table>
<thead>
<tr>
<th>The opinion others have of me is based on my appearance</th>
<th>Not at all</th>
<th>Somewhat</th>
<th>Moderately</th>
<th>A lot</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>The amount of influence I have on other people depends upon how I look</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>People will think less of me if I don't look my best</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>People would be more interested in me if I looked better</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>My relationships would improve if I looked the way I wished</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>The amount of success I have in my future job or career depends largely upon how I look</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>My appearance influences my ability to do things</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>My performance in activities (e.g. school, work, hobbies) is influenced by how I look</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>The opportunities that are available to me depend upon how I look</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>My school and work performance or opportunities would improve if I looked the way I wished</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>My value as a person depends upon how I look</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>How I feel about myself is largely based on my appearance</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I would think more highly of myself if I looked the way I wished</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Statement</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>How I look is a large part of who I am.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>It is difficult to feel good about myself when I am not looking my best.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My ability to feel happy depends upon how I look.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improving my appearance is one of the few activities that makes me feel good or like I am accomplishing something.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My life will be more exciting or rewarding if I look good.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My moods are influenced by how I look.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I would enjoy life more if I looked the way I wished.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My value as a person depends upon how I look.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How I feel about myself is largely based on my appearance.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I would think more highly of myself if I looked the way I wished.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How I look is a large part of who I am.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>It is difficult to feel good about myself when I am not looking my best.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My ability to feel happy depends upon how I look.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improving my appearance is one of the few activities that makes me feel good or like I am accomplishing something.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My life will be more exciting or rewarding if I look good.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>My moods are influenced by how I look.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I would enjoy life more if I looked the way I wished.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The following questions ask about usual physical activity. Please answer all questions choosing the most appropriate answer for each statement.

101. What is your main occupation?

______________________________________________________________.

102. At work I sit:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Always</td>
</tr>
</tbody>
</table>

103. At work I stand:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Always</td>
</tr>
</tbody>
</table>

104. At work I walk:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Always</td>
</tr>
</tbody>
</table>

105. At work I lift heavy loads:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Always</td>
</tr>
</tbody>
</table>

106. After working I am tired:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Always</td>
</tr>
</tbody>
</table>

107. At work I sweat:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Seldom</td>
<td>Sometimes</td>
<td>Often</td>
<td>Always</td>
</tr>
</tbody>
</table>

108. In comparison with others of my own age I think my work is physically:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Much</td>
<td>Lighter</td>
<td>As heavy</td>
<td>Heavier</td>
<td>Much heavier</td>
</tr>
</tbody>
</table>

109. Do you play sport (Running & biking are considered a sport)?

___ Yes
___ No (If no please skip to question 111 at the top of next page)

a. If yes, which sport do you play most frequently?

______________________________________________________________.

b. How many hours a week?

___ Less than 1 hour
___ 1 to 2 hours
___ 2 to 3 hours
___ 3 to 4 hours
___ More than 4 hours
c. How many months a year?
   — Less than 1 month
   — 1 to 3 months
   — 4 to 6 months
   — 7 to 9 months
   — More than 9 months

110. If you play a second sport: (If no please skip to question 111 at the top of next page)
   a. Which sport is it? ________________________________________________.

   b. How many hours a week?
      — Less than 1 hour
      — 1 to 2 hours
      — 2 to 3 hours
      — 3 to 4 hours
      — More than 4 hours

   c. How many months a year?
      — Less than 1 month
      — 1 to 3 months
      — 4 to 6 months
      — 7 to 9 months
      — More than 9 months

111. In comparison with others of my own age I think my physical activity during leisure time is:

   1  2  3  4  5
   Much less  Less  The same  More  Much more

112. During leisure time I sweat:

   1  2  3  4  5
   Never  Seldom  Sometimes  Often  Very often

113. During leisure time I play sport:

   1  2  3  4  5
   Never  Seldom  Sometimes  Often  Very often

114. During leisure time I watch television:

   1  2  3  4  5
   Never  Seldom  Sometimes  Often  Very often

115. During leisure time I walk:

   1  2  3  4  5
   Never  Seldom  Sometimes  Often  Very often

116. During leisure time I cycle:

   1  2  3  4  5
   Never  Seldom  Sometimes  Often  Very often
117. How many minutes do you walk and/or cycle per day to and from work, school and shopping?
   ___ Less than 5 minutes
   ___ 5 to 15 minutes
   ___ 15 to 30 minutes
   ___ 30 to 45 minutes
   ___ More than 45 minutes

The following questions are designed to assess attitudes, feelings and behaviours related to eating. Please answer all questions choosing the most appropriate answer for each statement.

118. I eat sweets and carbohydrates without feeling nervous.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

119. I think that my stomach is too big.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

120. I eat when I am upset.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

121. I stuff myself with food.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

122. I think about dieting.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

123. I think that my thighs are too large.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

124. I feel extremely guilty after overeating.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

125. I think that my stomach is just the right size.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always

126. I am terrified of gaining weight.
   1  2  3  4  5  6
   Never Rarely Sometimes Often Usually Always
<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

127. I feel satisfied with the shape of my body.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

128. I exaggerate or magnify the importance of weight.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

129. I have gone on eating binges where I felt that I could not stop.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

130. I like the shape of my buttocks.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

131. I am preoccupied with the desire to be thinner.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

132. I think about bingeing (overeating).

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

133. I think my hips are too big.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

134. I eat moderately in front of others and stuff myself when they are gone.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

135. If I gain a pound, I worry that I will keep gaining.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

136. I have the thought of trying to vomit in order to lose weight.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

137. I think that my thighs are just the right size.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

138. I think my buttocks are too large.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>
139. I eat or drink in secrecy.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
</tr>
</tbody>
</table>

140. I think that my hips are just the right size.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Often</td>
<td>Usually</td>
<td>Always</td>
</tr>
</tbody>
</table>

The following questions relate to lifestyle behaviours. Please answer all questions choosing the most appropriate answer for each statement.

141. Are you currently trying to lose weight?
   ___ Yes
   ___ No

142. How do you feel about your weight right now?
   I think I am...
   ___ Very overweight
   ___ Slightly overweight
   ___ About right
   ___ Slightly underweight
   ___ Very underweight

143. Have you ever smoked?
   ___ Yes
   ___ No (If no please proceed to question # 146)

144. Do you currently smoke?
   ___ Yes
   ___ No (If no please proceed to question # 146)

145. If you currently smoke, how many cigarettes per day on average do you smoke?
   ___ Less than 5 cigarettes per day
   ___ 5 to 10 cigarettes per day
   ___ 10 to 25 cigarettes per day
   ___ More than 25 cigarettes per day

146. Do you currently take any herbal supplements?
   ___ Yes
   ___ No (If no please proceed to question # 148)

147. If you are currently taking herbal supplements, please list the NAME, DOSE, and BRAND of the supplement(s) and the FREQUENCY you use them (i.e. twice per day, daily, weekly, etc.):

   Example: Echinacea (1000 mg), Jamieson, Daily
   ____________________________________________________________________________________

148. Do you currently take any medications (Including prescription, over-the-counter, homeopathic or naturopathic)?
   ___ Yes
   ___ No (If no please proceed to question # 150)
149. **If you are currently taking medications**, please list the NAME of medication(s), what you are taking it for and the FREQUENCY you take them (i.e. twice per day, daily, weekly, etc.):

   *Example: Midol, Menstrual cramps, ~5 days per month*

___________________________________________________________________
___________________________________________________________________

150. How would you describe your typical diet?

   ___ Mixed: I eat meat, dairy products, eggs, fruits & vegetables, grains

   ___ Lacto-ovo vegetarian: I DO NOT eat meat, fish or poultry, but I DO eat dairy, eggs, fruits & vegetables, grains

   ___ Vegan: I exclude ALL animal products

   ___ Other (please specify): ____________________________

151. Approximately how many times in your adult life have you *lost* weight in the following categories:

   ___ 5 to 9 lbs (2.3 to 4.1 kg)

   ___ 10 to 19 lbs (4.5 to 8.6 kg)

   ___ 20 to 49 lbs (9.1 to 22.2 kg)

   ___ 50 to 99 lbs (22.6 to 44.9 kg)

   ___ 100 lbs or more (45.4 kg or more)

   ___ I have never lost more than 5 lbs (2.3 kg)

152. Approximately how many times in your adult life have you *gained* weight in the following categories:

   ___ 5 to 9 lbs (2.3 to 4.1 kg)

   ___ 10 to 19 lbs (4.5 to 8.6 kg)

   ___ 20 to 49 lbs (9.1 to 22.2 kg)

   ___ 50 to 99 lbs (22.6 to 44.9 kg)

   ___ 100 lbs or more (45.4 kg or more)

   ___ I have never gained more than 5 lbs (2.3 kg)

153. Please estimate the number of times in your adult life that you have undertaken a **weight loss program** (e.g. for the purposes of losing weight only) using each of the following approaches:

   ___ Canada’s Food Guide to Healthy Eating

   ___ Exercise Program or personal trainer

   ___ Jenny Craig

   ___ Weight Watchers

   ___ L.A. Weight Loss Centre

   ___ Dr. Bernstein

   ___ Atkins, The Zone or Sugar Busters

   ___ Slim Fast or Nutrisystem

   ___ Dr. Dean Ornish

   ___ Eat Right For Your Blood Type

   ___ Herbal/health food store products

   ___ I have never used these or any other weight loss programs

   ___ Other, please specify: _______________________________________

154. Are you a ‘yo-yo’ dieter (do you lose and regain weight often)?

   

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does Not Apply</td>
<td>Disagree</td>
<td>Strongly Disagree</td>
<td>Neither disagree nor agree</td>
<td>Agree</td>
<td>Strongly Agree</td>
<td></td>
</tr>
</tbody>
</table>

221
155. If you lose weight but then begin regaining, how likely are you to feel terrible?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likelihood</td>
<td>I never</td>
<td>Not at all likely</td>
<td>Slightly likely</td>
<td>Moderately likely</td>
<td>Very likely</td>
<td>Extremely likely</td>
</tr>
</tbody>
</table>

156. If you lose weight but then begin regaining, how likely are you to go off the diet and regain?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Likelihood</td>
<td>I never</td>
<td>Not at all likely</td>
<td>Slightly likely</td>
<td>Moderately likely</td>
<td>Very likely</td>
<td>Extremely likely</td>
</tr>
</tbody>
</table>

157. If you gain back weight after dieting, do you typically gain back to a weight that is:

- _____ Much less than the weight you started at
- _____ Less than the weight you started at
- _____ The same weight you started at
- _____ More than the weight you started at
- _____ Much more than the weight you started at
- _____ I have not gained weight back after dieting
- _____ I do not diet

The following section asks questions about your reproductive history. Please answer all questions choosing the most appropriate answer for each statement.

158. Since adulthood (18 years old), have you ever gone three or more months without a menstrual period (not including pregnancy or breastfeeding)?

- ___ Yes
- ___ No

159. Since adulthood, (18 years old) have your menstrual periods stopped for more than one year?

- ___ Yes
- ___ No

160. Do you or did you ever take Provera (progesterone)?

- ___ Yes
- ___ No (If no please proceed to question #161)

If yes, please answer the following questions:

- How many months did you take Provera? __________
- At what age(s) did you take Provera? __________

161. Have you ever used birth control pills or oral contraceptives?

- ___ Yes
- ___ No (If no please proceed to question #162)

If yes, please answer the following questions regarding your birth control use:

- At what age did you start? _______ years
- Approximately how long did you use them? _____ years _____ months
- Are you still using them?
  - ___ Yes
  - ___ No (If no, at what age did you stop? _____ years)

162. How many times have you been pregnant? _____ (If none please proceed to #165)
163. How many of these resulted in live births? ________

164. Did you breastfeed any of your children?
   ___ Yes (If yes, for how many months total ____)
   ___ No

165. How old were you when you had your first menstrual period? _______ years

166. Did you have regular periods once they began?
   ___ Yes (If yes please proceed to question # 168)
   ___ No

167. If you had irregular periods, did they become regular?
   ___ Yes (If yes, at what age? __________ years)
   ___ No

168. Have your periods been made regular by medication (i.e. birth control pills)?
   ___ Yes (If yes, at what age? __________ years)
   ___ No

169. On average, how often do you have menstrual periods?
   ___ 20 days or less
   ___ 21 to 25 days
   ___ 26 to 30 days
   ___ 31 to 36 days
   ___ 37 or more days
   ___ Do not know

170. Have you ever been diagnosed with or treated for infertility or tried for more than 2 years and been unable to get pregnant?
   ___ Yes
   ___ No (If no please proceed to question # 171)
   If yes, what was the reason?
   ___ Hormone or ovulation problem
   ___ Tubal blockage or abdominal surgery
   ___ Problem with your partner’s fertility
   ___ Other (please specify) ____________________

171. Have you ever been sufficiently bothered by severe acne, unwanted face or body hair to consult a physician for treatment?
   ___ Yes (If yes, at what age? ______ years)
   ___ No

172. Have you ever fractured (broken) any bones since entering adulthood (18 years)?
   ___ Yes
   ___ No (If no please proceed to question # 173)
   If yes, please answer the following questions:
   How many adult fractures have you experienced? ______
   What was your age when the fracture(s) occurred? ______
This is the last section of the questionnaire. The questions ask about **average weekly exercise.** Please read each statement carefully.

173. Considering a 7-Day Period (a week), how many times on the average do you do the following kinds of exercise for **more than** **15 minutes** (write on each line the appropriate number).

<table>
<thead>
<tr>
<th>Exercise Type</th>
<th>Times Per Week</th>
<th>Average Mins Per Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. STRENUOUS EXERCISE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Heart beats rapidly)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i.e. running/jogging, judo, hockey, football, soccer, basketball, squash, cross country skiing, vigorous swimming or bicycling)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. MODERATE EXERCISE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Not exhausting)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i.e. fast walking, baseball, tennis, volleyball, badminton, downhill skiing, dancing, easy bicycling or swimming)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. MILD EXERCISE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Minimal effort)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i.e. yoga, archery, fishing, bowling, horseshoes, golf, easy walking)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

174. Considering a 7-Day Period (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

- ___ Often
- ___ Sometimes
- ___ Rarely
- ___ Never

**TURN OVER FOR INSTRUCTIONS TO COMPLETE.**

********************************************************************************

**To complete your questionnaire:**

- Please check that EVERY answer is complete. This is very important for our analysis.
- Ignore the ‘do not use’ columns. These will be used by the research team to ‘code’ the responses.
- Complete the Food Frequency Questionnaire. Please make sure EVERY answer is complete. We require all the information to give you an accurate analysis of your dietary intake.
- Place the **two** completed questionnaires in the provided stamped, addressed envelope and place in the mail!
- If you require another envelope or either questionnaire please call Jennifer at 604-616-4676.

You have completed the questionnaire!

*Thank you very much for your conscientious completion of this task*

********************************************************************************
Revisions at first follow-up:

Question 151 revised to: Approximately how many times in the past 6 months have you lost weight in the following categories:

- 5 to 9 lbs (2.3 to 4.1 kg)
- 10 to 19 lbs (4.5 to 8.6 kg)
- 20 to 49 lbs (9.1 to 22.2 kg)
- 50 to 99 lbs (22.6 to 44.9 kg)
- 100 lbs or more (45.4 kg or more)
- I have never lost more than 5 lbs (2.3 kg)

Question 152 revised to: Approximately how many times in the past 6 months have you gained weight in the following categories:

- 5 to 9 lbs (2.3 to 4.1 kg)
- 10 to 19 lbs (4.5 to 8.6 kg)
- 20 to 49 lbs (9.1 to 22.2 kg)
- 50 to 99 lbs (22.6 to 44.9 kg)
- 100 lbs or more (45.4 kg or more)
- I have never gained more than 5 lbs (2.3 kg)

Question 158 revised to: In the past 6 months, have you ever gone three or more months without a menstrual period (not including pregnancy or breastfeeding)?

- Yes
- No

Question 172 revised to: In the past 6 months, have you fractured (broken) any bones?

- Yes
- No

Questions removed from the questionnaire at second follow-up:

118-140, 153-157, 159-168, 170-171

Questions added to the questionnaire at second follow-up:

The following questions are designed to assess attitudes and behaviours related to eating over the previous month. Please answer all questions, choosing the most appropriate answer for each statement.

1. Have you been deliberately trying to limit the amount of food you eat to influence your shape or weight (whether or not you have succeeded)?

   0 1 2 3 4 5 6
   No days 1-5 days 6-12 days 3-15 days 16-22 days 23-27 days Every day

2. Have you gone for long periods of time (8 waking hours or more) without eating anything at all in order to influence your shape or weight?

   0 1 2 3 4 5 6
   No days 1-5 days 6-12 days 3-15 days 16-22 days 23-27 days Every day

3. Have you tried to exclude from your diet any foods that you like in order to influence your shape or weight (whether or not you have succeeded)?

   0 1 2 3 4 5 6
   No days 1-5 days 6-12 days 3-15 days 16-22 days 23-27 days Every day
4. Have you tried to follow definite rules regarding your eating (for example, a calorie limit) in order to influence your shape or weight (whether or not you have succeeded)?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

**On how many of the past 28 days...**

5. Have you had a definite desire to have an empty stomach with the aim of influencing your shape or weight?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

6. Have you had a definite desire to have a totally flat stomach?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

7. Has thinking about food, eating or calories made it very difficult to concentrate on things you are interested in (for example reading, having a conversation, or working)?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

8. Has thinking about shape or weight made it very difficult to concentrate on things you are interested in (for example reading, having a conversation, or working)?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

9. Have you had a definite fear of losing control over eating?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

10. Have you had a definite fear that you might gain weight?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

11. Have you felt fat?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

12. Have you had a secret desire to lose weight?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

For these questions, binge eating means eating what others would regard as an unusually large amount of food for the circumstances, accompanied by a sense of having lost control over eating.

13. On how many days have you eaten in secret (i.e. furtively) (do not count binge eating)?

   |   |   |   |   |   |  
---|---|---|---|---|---|  
0  | 1 | 2 | 3 | 4 | 5 | 6  
No days | 1 – 5 days | 6 – 12 days | 3 – 15 days | 16 - 22 days | 23 – 27 days | Every day  

226
14. On what proportion of the times that you have eaten have you felt guilty (felt that you’ve done wrong) because of its affect on your shape or weight (do not count binge eating)?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>None of the times</td>
<td>A few of the times</td>
<td>Less than half the time</td>
<td>Half the times</td>
<td>More than half the times</td>
<td>Most of the time</td>
<td>Every time</td>
</tr>
</tbody>
</table>

15. How concerned have you been about other people seeing you eat (Do not count binge eating)?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please fill in the appropriate number in the boxes on the right.

Over the past 28 days...

16. How many times when you have eaten what other people would regard as an unusually large amount of food (given the circumstances)?

Number of times

17. How many of these times did you have a sense of having lost control over your eating (at the time you were eating)?

18. On how many DAYS have such episodes of overeating occurred (i.e. you have eaten an unusually large amount of food and have had a sense of loss of control at the time)?

19. How many times have you made yourself sick (vomit) as a means of controlling your shape or weight?

20. How many times have you taken laxatives as a means of controlling your shape or weight?

21. How many times have you exercised in a ‘driven’ or ‘compulsive’ way as a means of controlling your weight, shape or amount of fat or to burn off calories?

22. Has your weight influenced how you think about (judge) yourself as a person?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

23. Has your shape influenced how you think about (judge) yourself as a person?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

24. How much would it upset you if you had been asked to weigh yourself once a week (no more, or less, often) for the next four weeks?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

25. How dissatisfied have you been with your weight?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

26. How dissatisfied have you been with your shape?

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
27. How uncomfortable have you felt seeing your body (for example, seeing your shape in the mirror, in a shop window reflection, while undressing or taking a bath or shower)?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

28. How uncomfortable have you felt about others seeing your shape or figure (for example, in communal changing rooms, when swimming or wearing tight clothes)?

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Markedly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

29. During the past 12 months, have you done anything to try to lose weight or to try to keep from gaining weight?

- ___ No (If no, please proceed to question #31)
- ___ Yes, I tried to lose weight
- ___ Yes, I tried to keep from gaining weight

30. **If yes**, please indicate what you did to try to lose weight or to try to keep from gaining weight. Check all that apply.

- Ate less food (amount)
- **Switched to foods with lower calories**
- Ate less fat
- Increased exercise (e.g. going to the gym, jogging, other activities that work up a sweat)
- Increased activity in daily routines (e.g. took stairs more often, walked longer distances)
- Skipped meals
- Ate ‘diet’ foods or products
- Used a liquid diet formula such as Slimfast or Optifast
- Joined a weight-loss program such as Weight Watchers, Jenny Craig or TOPS
- Followed a special diet such as South Beach, Atkins or other high protein/low carb diets
- Took diet pills described by a doctor
- Took other pills, medicines, herbs, drinks or supplements not needing a prescription
- Took laxatives or diuretics
- Vomited after eating
- Drank a lot of water
- Other(s): ______________________________________________________

31. Have you changed your typical diet in the past two years (for example, become or stopped being vegetarian or stopped eating or drinking a particular food or added a food or food group)?

- ___ No (If no, please proceed to question #32)
- ___ Yes

    **If yes**, please describe this change in detail below:

____________________________________________________________________________

____________________________________________________________________________

____________________________________________________________________________

32. Approximately how many times in the past 2 years have you lost weight in the following categories:

- ___ 5 to 9 lbs (2.3 to 4.1 kg)
- ___ 10 to 19 lbs (4.5 to 8.6 kg)
- ___ 20 to 49 lbs (9.1 to 22.2 kg)
- ___ 50 to 99 lbs (22.6 to 44.9 kg)
- ___ 100 lbs or more (45.4 kg or more)
- ___ I have not lost more than 5 lbs (2.3 kg) in the past 2 years
33. Approximately how many times in the past two years have you gained weight in the following categories:
   ____ 5 to 9 lbs (2.3 to 4.1 kg)
   ____ 10 to 19 lbs (4.5 to 8.6 kg)
   ____ 20 to 49 lbs (9.1 to 22.2 kg)
   ____ 50 to 99 lbs (22.6 to 44.9 kg)
   ____ 100 lbs or more (45.4 kg or more)
   ____ I have not gained more than 5 lbs (2.3 kg) in the past 2 years

34. Have you experienced any changes in your health status in the past 2 years (i.e. newly diagnosed medical condition)?
   ___ No (If no please proceed to question # 36)
   ___ Yes

35. If you have experienced a major health change, please describe this change in detail below including the name of any conditions/diseases:
   ______________________________________________________________________
   ______________________________________________________________________

36. Do you or did you ever take Provera (progesterone)?
   ___ No (If no please proceed to question #37)
   ___ Yes
   If yes, please answer the following questions:
   At what age(s) did you start taking Provera? ______
   Approximately how long did you take Provera? ______ years ______ months
   Did you use Provera during the study (i.e. before completing the 2nd bone scan?)
   _____ Yes (If yes, for how many months? ______)
   _____ No

37. Do you or have you ever used birth control pills/oral contraceptives?
   ___ No (If no please proceed to question #38)
   ___ Yes
   If yes, please answer the following questions regarding your birth control use:
   At what age did you start? ______ years
   Approximately how long did you use them? ______ years ______ months
   Did you use them during the study i.e. before completing the 2nd bone scan?)
   _____ Yes (If yes, for how many months? ______)
   _____ No

38. Have your periods been made regular by an intrauterine device?
   _____ No (If no, you are done)
   _____ Yes
   If yes, please specify what you used: _______________________________________
   Did you use them during the study i.e. before completing the 2nd bone scan?)
   _____ Yes (If yes, for how many months? ______)
   _____ No
The events listed below are considered ‘life stressors’. Please read each statement carefully. In the space provided, please indicate if the event has occurred to you personally over the previous 2 years by circling ‘yes’ or ‘no’.

**Did this event occur to you personally in the previous 2 years...**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HEALTH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>An injury or illness which kept you in bed a week or more or sent you to the hospital</td>
<td>Yes</td>
</tr>
<tr>
<td>2.</td>
<td>An injury or illness which was less serious than above</td>
<td>Yes</td>
</tr>
<tr>
<td>3.</td>
<td>Major dental work</td>
<td>Yes</td>
</tr>
<tr>
<td>4.</td>
<td>Major change in eating habits</td>
<td>Yes</td>
</tr>
<tr>
<td>5.</td>
<td>Major change in sleeping habits</td>
<td>Yes</td>
</tr>
<tr>
<td>6.</td>
<td>Major change in your usual type and/or amount of recreation</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<p>| WORK |   |   |
| 7. | Change to a new type of work | Yes | No |
| 8. | Change in your work hours or conditions | Yes | No |
| 9. | Change in your responsibilities at work: |   |   |
|     | More responsibilities | Yes | No |
|     | Fewer responsibilities | Yes | No |
|     | Promotion | Yes | No |
|     | Demotion | Yes | No |
|     | Transfer | Yes | No |
| 10. | Troubles at work: |   |   |
|     | With your boss | Yes | No |
|     | With coworkers | Yes | No |
|     | With persons under your supervision | Yes | No |
|     | Other work troubles | Yes | No |
| 11. | Major business adjustment | Yes | No |
| 12. | Laid off from work | Yes | No |</p>
<table>
<thead>
<tr>
<th>Event</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did this event occur to you personally in the previous 2 years...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. Fired from work</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>14. Correspondence course to help you in your work</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>HOME AND FAMILY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Major change in living conditions</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>16. Move within the same town or city</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>17. Move to a different town or city or province</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>18. Change in family get-togethers</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>19. Major change in health or behaviour of family member</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>20. Marriage</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>21. Pregnancy</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>22. Miscarriage or abortion</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>23. Birth or adoption of a child</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>24. A relative moving in with you</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>25. Spouse beginning or ending work</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>26. Child leaving home to attend college, due to marriage or other reasons</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>27. Change in arguments with spouse</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>28. In-law problems</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>29. Change in the marital status of your parents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorce</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Remarriage</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>30. Separation from spouse or partner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Due to work</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Due to marital problems</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>31. Divorce</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>32. Death of spouse</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
**Did this event occur to you personally in the previous 2 years...**

<table>
<thead>
<tr>
<th>Event</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>33. Death of other family member</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Brother/sister</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Parent</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**PERSONAL, SCHOOL, SOCIAL**

<table>
<thead>
<tr>
<th>Event</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>34. Change in personal habits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35. Beginning or ending university or college</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36. Change of university or college</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37. Being forced to withdraw from school</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38. Change in political beliefs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39. Change in religious beliefs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40. Change in social activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41. Vacation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42. New close, personal relationship</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43. Engagement to marry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44. Girlfriend or boyfriend problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45. Sexual difficulties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46. “Falling out” of a close personal relationship</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47. An accident</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48. Minor violation of the law</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49. Being held in jail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50. Death of a close friend</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51. Major decision regarding your immediate future</td>
<td></td>
<td></td>
</tr>
<tr>
<td>52. Major personal achievement</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Did this event occur to you personally in the previous 2 years... Yes No

**FINANCIAL**

53. Major change in finances........................................... Yes No
54. Loss or damage of personal property.................................. Yes No
55. Moderate purchase.......................................................... Yes No
56. Major purchase............................................................... Yes No
57. Foreclosure on a mortgage or loan........................................ Yes No

These questions are designed to assess emotional state. Please read each statement and circle a number 0, 1, 2 or 3 that indicates how much the statement applied to you over the past week. There are no right or wrong answers. Do not spend too much time on any statement.

<table>
<thead>
<tr>
<th>The rating scale is as follows: This question applies to me...</th>
<th>None of the time</th>
<th>Some of the time</th>
<th>A good part of the time</th>
<th>Most of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I found myself getting upset by quite trivial things</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. I was aware of dryness of my mouth</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. I couldn’t seem to experience any positive feeling at all</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion)</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. I just couldn’t seem to get going</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. I tended to over-react to situations</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. I had a feeling of shakiness (e.g., legs going to give way)</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. I found it difficult to relax</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. I found myself in situations that made me so anxious I was most relieved when they ended</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. I felt that I had nothing to look forward to</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. I found myself getting upset rather easily</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. I felt that I was using a lot of nervous energy</td>
<td>0 1 2 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The rating scale is as follows: This question applies to me...</td>
<td>None of the time</td>
<td>Some of the time</td>
<td>A good part of the time</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------------------------------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>13</td>
<td>I felt sad and depressed</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>I found myself getting impatient when I was delayed in any way (e.g., traffic lights, being kept waiting)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>I had a feeling of faintness</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>I felt that I had lost interest in just about everything</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>I felt I wasn't worth much as a person</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>18</td>
<td>I felt that I was rather touchy</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>I perspired noticeably (e.g., hands sweaty) in the absence of high temperatures or physical exertion</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>I felt scared without any good reason</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>I felt that life wasn't worthwhile</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>I found it hard to wind down</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>23</td>
<td>I had difficulty in swallowing</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>I couldn't seem to get any enjoyment out of the things I did</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>I was aware of the action of my heart in the absence of physical exertion (e.g., sense of heart rate increase, heart missing a beat)</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>26</td>
<td>I felt down-hearted and blue</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>27</td>
<td>I found that I was very irritable</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>I felt I was close to panic</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>29</td>
<td>I found it hard to calm down after something upset me</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>I feared that I would be &quot;thrown&quot; by some trivial but unfamiliar task</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>31</td>
<td>I was unable to become enthusiastic about anything</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>I found it difficult to tolerate interruptions to what I was doing</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>The rating scale is as follows: This question applies to me...</td>
<td>None of the time</td>
<td>Some of the time</td>
<td>A good part of the time</td>
<td>Most of the time</td>
</tr>
<tr>
<td>------------------------------------------------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>33 I was in a state of nervous tension</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>34 I felt I was pretty worthless</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>35 I was intolerant of anything that kept me from getting on with what I was doing</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>36 I felt terrified</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>37 I could see nothing in the future to be hopeful about</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>38 I felt that life was meaningless</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>39 I found myself getting agitated</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>40 I was worried about situations in which I might panic and make a fool of myself</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>41 I experienced trembling (e.g., in the hands)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>42 I found it difficult to work up the initiative to do things</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Molinima question read by PhD candidate at each data collection point

*Can you tell by the way you feel that your period is coming?*

___ Yes, every month
___ Yes, most months
___ Yes, less than half the time
___ Yes, once or twice a year
___ Never

*If yes to any of the above, what signs or symptoms indicate to you that your period is coming? (DO NOT READ SYMPTOMS BUT ALLOW PARTICIPATE TO PROVIDE)*

___ menstrual cramps or aching back or legs
___ bloating, fluid retention
___ increased appetite (in general or for sweet, salty or spicy foods)
___ moodiness (frustration, irritability, sadness)
___ breast tenderness in the front or nipple
___ breast tenderness up under the armpit
___ breast swelling
___ headaches
___ acne/pimples/blemishes
___ other, please specify ______________________________________
### Appendix 24: List of Validated Questionnaires Included in Annual Questionnaire Package

<table>
<thead>
<tr>
<th>Questionnaire</th>
<th>Questions</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three Factor Eating Questionnaire [1]</td>
<td>#7-71</td>
<td>p. 205-208</td>
</tr>
<tr>
<td>Beliefs About Appearance Scale [4]</td>
<td>#100</td>
<td>p. 212-213</td>
</tr>
<tr>
<td>Eating Disorder Inventory [6]</td>
<td>#118-140</td>
<td>p. 216-218</td>
</tr>
<tr>
<td>Life Event Scale [8]</td>
<td>#1-57</td>
<td>p. 228-231</td>
</tr>
<tr>
<td>Depression Anxiety and Stress Scale [9]</td>
<td>#1-42</td>
<td>p. 231-233</td>
</tr>
</tbody>
</table>

### References

Appendix 25: 2-year Prospective Bone Study Daily Stress Inventory

Below are listed a variety of events that may be viewed as stressful or unpleasant. Read each item carefully and decide whether or not that event occurred within the past [12/24] hours. Indicate if the event did occur (Y) or did not occur (N) in the DID EVENT OCCUR? column. If the event DID occur, indicate the amount of stress that it caused on a scale of 1 to 7 by checking the appropriate column. Please answer honestly as you can so that we may obtain accurate information.

<table>
<thead>
<tr>
<th>EVENT</th>
<th>Did Event Occur? (Y or N)</th>
<th>Amount of Stress Event Caused:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performed poorly at task</td>
<td></td>
<td>1 Not stressful</td>
</tr>
<tr>
<td>Performed poorly due to others</td>
<td></td>
<td>2 Little stress</td>
</tr>
<tr>
<td>Thought about unfinished work</td>
<td></td>
<td>3 A little stress</td>
</tr>
<tr>
<td>Hurried to meet deadline</td>
<td></td>
<td>4 Some stress</td>
</tr>
<tr>
<td>Interrupted during task/activity</td>
<td></td>
<td>5 Much stress</td>
</tr>
<tr>
<td>Someone spoiled your completed task</td>
<td></td>
<td>6 Very much stress</td>
</tr>
<tr>
<td>Did something you are unskilled at</td>
<td></td>
<td>7 Caused me to panic</td>
</tr>
<tr>
<td>Unable to complete a task</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was unorganized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Criticized or verbally attacked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ignored by others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spoke or performed in public</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dealt with rude waiter/ress/salesperson</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interrupted while talking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was forced to socialize</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Someone broke a promise/appointment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competed with someone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not hear from someone you expected to hear from</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was stared at</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experienced unwanted physical contact (crowded, pushed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was misunderstood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was embarrassed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had your sleep disturbed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVENT</td>
<td>Did Event Occur? (Y or N)</td>
<td>Amount of Stress Event Caused:</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>---------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Forgot something</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feared illness/pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experienced illness/physical discomfort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had car trouble</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Your property was damaged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unable to complete all plans for today</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ran out of food/personal article</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argued with spouse/boyfriend/girlfriend</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argued with another person</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waited longer than you wanted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interrupted while thinking/relaxing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Someone ‘cut’ ahead of you in line</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performed poorly at sport/game</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had minor accident (broke something, tore clothing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did something you did not want to do</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Someone borrowed something without your permission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had difficulty in traffic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Money problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Store lacked a desired item</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misplaced something</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad weather</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexpected expense (fines, ticket, etc.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had confrontation with an authority figure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heard some bad news</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EVENT</td>
<td>Did Event Occur? (Y or N)</td>
<td>Amount of Stress Event Caused:</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>---------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Concerned over personal appearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed to feared situation/object</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed to upsetting TV show/movie/book</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Pet peeve’ violated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failed to understand something</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worried about another’s problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experienced narrow escape from danger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had problem with kid(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Was late for work/appointment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stopped unwanted personal habit (smoking, nail biting)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other?</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other?</td>
<td>__________________________</td>
<td></td>
</tr>
</tbody>
</table>

239
Appendix 26: 2-year Prospective Bone Study 12-hour Ambulatory Blood Pressure Monitoring Instructions and Diary

DATE OF COMPLETION: ______________________

Your task: You will complete one 12-hour ambulatory blood pressure assessment on the day following your orientation at UBC. If you need to change this date, please contact Jennifer. If you have any questions or concerns at any time during your 12-hour monitoring, please contact Jennifer at 604-616-4676.

Materials provided: (If any of these materials are missing, please contact Jennifer immediately)

1. One blood pressure monitor with belt and pouch: This is the lightweight monitor you will wear for 12-hours. It is placed in a pouch and worn around your waist with your own belt or the provided belt. You are able to go about your normal day during the assessment except the monitor cannot get wet and we ask you to refrain from heavy exercise.

2. Blood pressure diary: Each time you feel the arm cuff inflate (every ½ hr); you will sit down and record the time and the activity you were engaged in (i.e. watching TV, eating dinner, etc) in this diary. Please take the diary everywhere you go during the 12-hour period.

3. Questionnaire: This short questionnaire is to be completed when you remove the monitor after your 12-hour assessment is complete. Please return the questionnaire with the other equipment. This will require only 10-15 minutes.

4. Padded Addressed Envelope: When you have completed the assessment, you will place the monitor, pouch and belt as well as this diary and the questionnaire in this envelope. The next morning, contact Jennifer to arrange for courier pick up.

Instructions: The accuracy of the analysis will depend on the accuracy of the blood pressure monitoring technique. These instructions will help ensure that your 12-hour blood pressure assessment is obtained correctly and will give accurate test results. Please call Jennifer immediately if you have any questions.

1. How to start the assessment: You will begin the assessment after you have showered. Please write down the time you begin wearing the monitor at the bottom of this form. Follow the instructions below to begin:
   - Power ON the monitor. In a few seconds, the screen will display the current time.
   - Place the monitor inside the pouch.
   - Attach the pouch to the provided belt (or your belt) on your dominant side (i.e. if you are right handed, your right side).
   - Position the cuff on your NON-DOMINANT arm (i.e. if right handed, your left arm) so that the cuff is two fingers from your elbow crease and the arrow (on the outside of the cuff) is directly over the centre of your elbow crease (closely in line with the vein you can see).
   - Tighten the cuff so that you can place two fingers between your skin and the cuff at the top and bottom.
   - Lead the hose up your arm with the cuff and place it across the back of your neck. You can place this under or over your shirt. If you have sensitive skin, place the tube over your shirt.
   - Connect the hose to the monitor.
   - The monitor is now ready to being your assessment! If you have trouble with any of these steps or are unsure if the monitor is working properly please contact Jennifer immediately.

2. Blood pressure readings & diary: The cuff will inflate once every 30 minutes during the 12 hour assessment. When you begin to feel the cuff inflate, it is important that...
you keep your arm as still as possible. We ask you to sit down for the one minute reading. If the monitor is unable to complete a successful reading (i.e. because your arm was moving), it will try again two minutes later. If the cuff becomes uncomfortable during a reading, press the STOP key on the front of the monitor. This will terminate the reading. If the cuff slips out of place, the cuff must be repositioned properly in order to obtain successful readings. If the cuff is not properly positioned, the hose is kinked or the hose has become unattached to the monitor, event codes may appear on the monitor, for example EC82. If you receive an event code or notice that the monitor is taking the second (two minute later) reading even when your arm is as still as possible, please contact Jennifer immediately and she will assist you in correcting the problem.

While sitting during the reading, we ask that you please record the following in the diary provided:

- The time of day. For example: 9 a.m.
- Your present location. For example: home, work, car, shopping mall, etc.
- The activity you were engaged in. For example: shopping, on phone, watching TV, driving car, eating, reading, making meal, etc.
- Describe your feelings at the time of the reading. For example, happy, relaxed, nervous, tired, angry, tense, etc.
- There is also space for you to add any additional comments regarding the reading including any problems that may have occurred.
- PLEASE TAKE THE DIARY WITH YOU WHEREVER YOU GO. If you miss a reading, please estimate the time, location, mood, etc and indicate in the COMMENTS section that you filled in the information later and indicate the actual time you filled out the information.

3. Care of the monitor: On the day of your blood pressure assessment you should go about your normal daily activities BUT THE MONITOR CANNOT GET WET. If you wish to exercise, you should perform the exercise in the morning, shower, and then begin your assessment. Any unusual mental strain (i.e. out of the ordinary) should be avoided. For example, do not complete the blood pressure assessment on a day of an exam. If you become aware of any sudden stresses on the day you have chosen for your blood pressure assessment, please contact Jennifer to reschedule (you will still need to courier the equipment back).

4. Finishing the assessment: Once 12 hours have passed OR you have finished your last meal of the day (whichever is the later), TURN THE MONITOR OFF. Then, please write down the time on this form. You may now carefully remove the monitor, pouch and belt. Place the equipment in the padded envelop. Then, complete the short questionnaire. Place questionnaire in the padded envelope.

5. The morning following your assessment: Prepare the padded envelope (include the monitor, pouch, belt, this diary/instructions and the questionnaire) for pick-up. When ready for shipping, please call Jennifer at 604-616-4676. If you are leaving a voicemail message, please include the address to which the courier should go. The courier should arrive at the address you request (home or office) within a few hours at the most, and the equipment will then be delivered to the research team for analysis. Jennifer will call you back to confirm that the courier has been asked to pick up your package.

I started my blood pressure assessment on _______________ (date) at _______________ (time).

I ended my blood pressure assessment on _______________ (date) at _______________ (time).
## Ambulatory Blood Pressure Monitoring Diary

*Please read instructions on following pages before completing*

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>Where are you?</th>
<th>What are you doing?</th>
<th>How do you feel?</th>
<th>Anything else to note?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Appendix 27: Correlations of Cognitive Dietary Restraint and Subclinical Ovarian Disturbances with General Stress Questionnaires

<table>
<thead>
<tr>
<th>Stress questionnaire score</th>
<th>Subclinical ovulatory disturbances&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cognitive dietary restraint score&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R value</td>
<td>P value</td>
</tr>
<tr>
<td>Life Event Scale Total&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.021</td>
<td>0.828</td>
</tr>
<tr>
<td>Health</td>
<td>0.072</td>
<td>0.451</td>
</tr>
<tr>
<td>Work</td>
<td>-0.045</td>
<td>0.638</td>
</tr>
<tr>
<td>Home and Family</td>
<td>0.115</td>
<td>0.225</td>
</tr>
<tr>
<td>Personal, School and Social</td>
<td>-0.023</td>
<td>0.809</td>
</tr>
<tr>
<td>Financial</td>
<td>-0.079</td>
<td>0.404</td>
</tr>
<tr>
<td>Depression, Anxiety, Stress Scale Total&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.069</td>
<td>0.471</td>
</tr>
<tr>
<td>Depression</td>
<td>-0.051</td>
<td>0.594</td>
</tr>
<tr>
<td>Anxiety</td>
<td>-0.008</td>
<td>0.938</td>
</tr>
<tr>
<td>Stress</td>
<td>-0.097</td>
<td>0.315</td>
</tr>
<tr>
<td>Perceived Stress Scale&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.046</td>
<td>0.631</td>
</tr>
<tr>
<td>Daily Stress Inventory - Impact&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.136</td>
<td>0.154</td>
</tr>
<tr>
<td>Daily Stress Inventory - Frequency&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.134</td>
<td>0.160</td>
</tr>
</tbody>
</table>

Data are presented as Pearson's (R) coefficients. Exact n varied by comparison as cases were excluded pairwise.

- **a.** The percentage of cycles that were anovulatory or luteal length <10 days by least squares quantitative basal temperature analysis [1].
- **b.** Restraint subscale of the Three Factor Eating Questionnaire [2] average score from baseline and first and final follow-up. Higher scores indicate an increased perception of monitoring and attempting to limit food intake in order to achieve/maintain a perceived ideal body weight.
- **c.** Life Events Scale [3] total and subscale scores based on time interval between baseline and final bone density scan. Higher scores indicate a higher occurrence of stressful life events during the study.
- **d.** Depression, Anxiety and Stress Scale [4] total and subscale scores reflecting emotional state over previous week at the final follow-up. Higher scores indicate low positive affectivity (Depression), higher physiological hyperarousal (Anxiety) and higher negative affectivity (Stress).
- **e.** Perceived Stress Scale [5] average score from baseline and first and second follow ups. Higher scores indicate higher stress perception over the previous month.
- **f.** Daily Stress Inventory [6] Impact score averaged from baseline and first and second follow during the 24-hour urine collections. Higher scores indicate perceived impact of stressful events during the 24-hour period.
- **g.** Daily Stress Inventory [6] Frequency score averaged from baseline and first and second follow during the 24-hour urine collections. Higher scores indicate a higher number of stressful events that occurred during the 24-hour period.
References


Appendix 28: Comparison of Least-squares Basal Temperature Analysis Method Relative to Other Non-invasive Methods to Detect Ovulation Regarding Cost, Participant Acceptability, Ease-of-use and Accuracy in Detecting the Day of Luteal Onset

Reviewers of the manuscript presented in Chapter 2 indicated that urine ovulation kits, the World Health Organization ‘3 over 6’ basal temperature analysis method and other computerized basal temperature analysis methods were easy to use, inexpensive and accurate methods of detecting ovulation. An edited version of our response is provided below indicating that many of these methods are too expensive for use in large epidemiological studies, have not been validated against established markers of ovulation and are not meant to be used to indicate the day of luteal onset but rather, as a means of determining the fertile period for conception.

Reviewer Comment: I am not convinced that simple urine ovulation test kits are that expensive and that they are not easy to use and acceptable to most women and that could be used in large epidemiological studies.

OUR RESPONSE: We agree that a single urine ovulation test kit is not particularly expensive. However, we are referring to studies of sample size and duration that would be powered to examine the relationship between menstrual cycle variability (progesterone) and bone and/or other health outcomes. As the reviewer mentioned it is well-established that there is considerable within-person variability in the menstrual cycle and therefore the menstrual cycle of each women would need to be tracked for a considerable period of time. In a study of only 100 women for 24 months use of the ovulation kits would represent a cost difference of almost $100,000 compared to our method of basal temperature monitoring (based on use of 1 kit ($45 CDN) per month per participant for a total per participant cost of over $1000 compared to about $20 for a digital basal temperature thermometer). While some cost savings would undoubtedly be recognized by purchasing the test kits in bulk, the cost difference is nevertheless prohibitive.

A second consideration is that women participating in epidemiological research studies are not as motivated as women seeking pregnancy or using symptothermal methods (STM) to avoid pregnancy. In our experience with the paid volunteers in our study, many participants voiced their dislike for the daily urine sampling procedure.

In addition to the expense, methods that rely on detecting the luteinizing hormone (LH) peak are not at all robust to missing or patchy data. Because the LH peak is a brief event, lasting one or two days, it is easy to miss, and so many cycles without an apparent LH peak may actually be wrongly classified as anovulatory, or will need to be censored as “undetermined”, both of which are problematic for research purposes. By contrast, luteal temperature remains high during the luteal phase, and the least-squares basal temperature analysis method (LS-QBT) method is robust to up to 1/3 missing data.
Review Comment: What about the most frequently used quantitative method of determining the temperature shift - the ‘3 over 6’ rule that is promoted by the World Health Organization and utilized in many fertility awareness methods? There are also many other authors who have developed quantitative methods for determine the temperature shift - i.e. Kippley, Roetzer, Marshall, Serena Coverline.

OUR RESPONSE: A literature search for JF Kippley reveals 16 publications of which 10 are in a Couple to Couple League (CCL) publication. According to CCL’s website [1], they are an international, Catholic, non-profit organization dedicated to promoting and teaching Natural Family Planning (NFP) to married and engaged couples. The purposes of the CCL are:
1. To meet the need for a nationwide, independent, and organized way of delivering NFP services;
2. To provide instruction in NFP that includes moral and religious values along with physiological and scientifically accurate information;
3. To train volunteer married couples to be proficient counsellors and teachers in a 99% effective method of NFP, and at no cost to those who generously undertake this training.

CCL teaches the Sympto-Thermal Method (STM) of NFP which includes 3 aspects of fertility: basal temperature, cervical mucus and physical changes in the cervix.

A literature search for J Roetzer reveals two studies which discuss the STM as well and for J Marshall reveals description of the 3 over 6 method from the 1960s.

As regards basal temperature as part of the STM, usually basal temperature is charted and analysed using the Coverline method or 3 over 6 method which are both qualitative in nature [2]. The Coverline method meets the needs of women who need to assess ovulation prospectively within a menstrual cycle in order to decide when they are infertile. The original validation paper for the LS-QBT method compared the performance of the algorithm with that of the mean temperature method of Vollman [3] and the cumulative sum method of Royston [4], and found all three methods were in agreement with the date of luteal onset by LH peak, but that the LS-QBT method was more robust to missing data (the cumulative sum method of Royston was unable to analyze 5 of 24 cycles). We use only basal temperature records as methods relating to cervical mucous are less acceptable to women who would be participating in epidemiological studies rather than for the purposes of NFP. We are not seeking to identify the fertile/infertile times during the cycle but are looking at a method to indirectly determine exposure to progesterone in the cycle. Kippley himself states: “NFP is not based on detecting ovulation but on identifying the limits of the fertile period.” As conception or avoidance of conception is not the purpose of our method, detecting the day of luteal transition is important to determine the influence of progesterone on women’s health without using hormone assays as these are costly as previously discussed.
Reviewer Comment: There are many computerized methods and online software programs that quantitatively determine the temperature shift and give a luteal phase estimate. These computerized programs have built-in algorithms. Monitors such as the Baby or LadyComp, the Rabbit, L Sophia or software that you can purchase online or chart online. Toni Weschler, author of Taking Charge of your Fertility, has an online web site for online temperature charting. This is state of the art. No mention in the article or as to what quantitative methods are used with these software programs.

OUR RESPONSE: We are aware of the many computerised programs that are currently available and discuss them in detail in the appendix to this response. These methods are designed to provide real-time feedback to women about ovulation-related events, so that they can time sexual intercourse to best achieve or avoid pregnancy. The trade-offs for these algorithms are different than in our case, where real-time analysis is not desired, and post hoc methods can use more complete data. Overall, the main issue is that these methods are not sufficiently validated against either the LH surge, progesterone or ultrasound determination of ovulation for use in research studies. The majority of studies (which are published as conference abstracts rather than peer-reviewed manuscripts in scientific journals) relate to their reliability as a means of determining fertile/infertile periods in NFP. As well, these programs do not describe the analysis method used. Many describe ‘algorithms’ but provide no details as to what these algorithms are although we can infer from the information on their websites that they use the Coverline or 3 over 6 method. Lastly, many of these programs are cost prohibitive.

Ladycomp/Babycomp

The majority of studies listed in the ‘clinical tests’ section of the Ladycomp/Babycomp website [5] are conference abstracts or are published in German (as such I cannot determine where they were published) by the same group of authors and refer to its ability to prevent pregnancy and its accuracy in defining the fertile/infertile times.

I was able to find two studies (1 full ‘report’, 1 abstract) on Ladycomp/Babycomp and hormonal indicators of ovulation. The authors discuss using an algorithm but no further details are supplied. In the abstract [6], 15 cycles were examined using LH and ultrasound. A ‘temperature shift’ was detected in all 15 cycles though estimating the day of luteal transition was less precise. In the only peer-reviewed manuscript I was able to locate [7], they discuss only reliability in preventing/achieving pregnancy rather than relative to hormonal indicators. They also mention an ‘algorithm’ but do not go into detail in the methods section. The Ladycomp/Babycomp is a considerable investment of $245 to $498 CDN for each device [8]
Cyclotest

Cyclotest® is available in Europe (no prices listed on website) and I believe it uses the 3 over 6 method as they refer to the WHO in some of their literature on the Cyclotest® method: “Symptothermal approach and temperature method, modified on the basis of the acknowledged guidelines of the WHO” [9]. I found one published abstract on Cyclotest® relative to hormonal indicators [10] which looked at 3-12 cycles from 30 experienced NFP users relative to LH and ultrasound. Of 69 ovulatory cycles, 17% did not detect a temperature shift. The only other peer-reviewed manuscript I was able to located referred to ability to properly detect the fertile/infertile times adequately [11].

Bioself

The Bioself, made by the Ladycomp/Babycomp company, is available in Canada for $245 [8]. It uses the STM. Quite a bit of work as regards progesterone, LH and contraceptive outcomes has been conducted on this method and it seems valid [12-16]. However, the cost is substantial and they do not describe which algorithm is being employed.

Taking Charge of your Fertility: Ovusoft

Ovusoft costs US$40 [17] for a potential participant to download as well as a PC for running Microsoft in order to use the program. As such, some participants may not be able to participate if this software was used. Although there is no section on clinical trials or methodology, I was able to discover that this program uses the Coverline method which is considered a qualitative method [19].

Fertility Friend

We found an additional free program called Fertility Friend and it also uses the Coverline method [20]. They even state: “The Coverline (the line drawn horizontally across your chart once ovulation is detected) is a visual tool to help you easily see your pre- and post-ovulation temperatures. It has no physiological significance whatsoever so where it is placed does not have much importance as long as it helps you to see your ovulation pattern” [21].

Sophia

The Sophia Basal Thermometer and Ovulation Prediction Computer by Ovutherm™ is a thermometer with additional input capabilities to allow women to use the STM for contraception by indicating fertile/infertile periods [22]. A PUBMED search reveals no scientific studies regarding its use. It is available for USD$41.25 per unit [22].
Rabbit Ovulation Computer

I was unable to locate information on the Rabbit software.

References


Appendix 29: Partial Correlations of 24-hour Urinary Free Cortisol (UFC) and Average Perceived Stress Scale (PSS) Scores with Questionnaire Scores

<table>
<thead>
<tr>
<th>Questionnaire score</th>
<th>UFC</th>
<th></th>
<th>PSS score</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R value</td>
<td>P value</td>
<td>R value</td>
<td>P value</td>
</tr>
<tr>
<td>Three Factor Eating Questionnaire&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive Dietary Restraint</td>
<td>0.059</td>
<td>0.517</td>
<td>-0.118</td>
<td>0.194</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>0.175</td>
<td>0.053</td>
<td>0.096</td>
<td>0.291</td>
</tr>
<tr>
<td>Hunger</td>
<td>0.096</td>
<td>0.291</td>
<td>0.234</td>
<td>0.009</td>
</tr>
<tr>
<td>Perceived Stress Scale&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.222</td>
<td>0.014</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Body Shape Questionnaire&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.102</td>
<td>0.264</td>
<td>0.110</td>
<td>0.225</td>
</tr>
<tr>
<td>Beliefs About Appearance Scale&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.066</td>
<td>0.470</td>
<td>0.307</td>
<td>0.001</td>
</tr>
<tr>
<td>Eating Disorder Exam Total&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.148</td>
<td>0.104</td>
<td>0.153</td>
<td>0.092</td>
</tr>
<tr>
<td>Restraint</td>
<td>0.137</td>
<td>0.131</td>
<td>0.034</td>
<td>0.711</td>
</tr>
<tr>
<td>Eating Concern</td>
<td>0.234</td>
<td>0.009</td>
<td>0.179</td>
<td>0.047</td>
</tr>
<tr>
<td>Weight Concern</td>
<td>0.068</td>
<td>0.457</td>
<td>0.122</td>
<td>0.179</td>
</tr>
<tr>
<td>Shape Concern</td>
<td>0.106</td>
<td>0.247</td>
<td>0.182</td>
<td>0.044</td>
</tr>
<tr>
<td>Eating Disorder Inventory subscales&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drive For Thinness</td>
<td>0.073</td>
<td>0.425</td>
<td>0.066</td>
<td>0.465</td>
</tr>
<tr>
<td>Bulimia</td>
<td>0.201</td>
<td>0.026</td>
<td>0.250</td>
<td>0.005</td>
</tr>
<tr>
<td>Body Dissatisfaction</td>
<td>0.117</td>
<td>0.201</td>
<td>0.094</td>
<td>0.302</td>
</tr>
<tr>
<td>Life Event Scale Total&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.171</td>
<td>0.060</td>
<td>0.115</td>
<td>0.207</td>
</tr>
<tr>
<td>Health</td>
<td>0.072</td>
<td>0.429</td>
<td>0.088</td>
<td>0.331</td>
</tr>
<tr>
<td>Work</td>
<td>0.076</td>
<td>0.402</td>
<td>0.194</td>
<td>0.032</td>
</tr>
<tr>
<td>Home and Family</td>
<td>0.095</td>
<td>0.296</td>
<td>0.006</td>
<td>0.947</td>
</tr>
<tr>
<td>Personal, School and Social</td>
<td>0.235</td>
<td>0.009</td>
<td>0.072</td>
<td>0.429</td>
</tr>
<tr>
<td>Financial</td>
<td>0.020</td>
<td>0.828</td>
<td>0.017</td>
<td>0.855</td>
</tr>
<tr>
<td>Depression, Anxiety, Stress Scale Total&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.203</td>
<td>0.028</td>
<td>0.608</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Depression</td>
<td>0.190</td>
<td>0.040</td>
<td>0.468</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anxiety</td>
<td>0.207</td>
<td>0.025</td>
<td>0.537</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stress</td>
<td>0.140</td>
<td>0.131</td>
<td>0.548</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daily Stress Inventory - Impact&lt;sup&gt;i&lt;/sup&gt;</td>
<td>0.185</td>
<td>0.042</td>
<td>0.341</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Daily Stress Inventory - Frequency&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.157</td>
<td>0.085</td>
<td>0.185</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Data are presented as Pearson’s partial correlation coefficients (R<sub>p</sub>) adjusted for urine volume. Exact n varied by comparison as cases were excluded pairwise. All values were
averaged from the three data collections except for the following: the Eating Disorder Examination, which was completed only at baseline; and the Eating Disorder Inventory which was completed at baseline and first follow-up only. PSS; Perceived Stress Scale; UFC, 24-hour urinary free cortisol (µg).

a. Three Factor Eating Questionnaire subscales [1]. Cognitive Dietary Restraint. higher scores indicate an increased perception of monitoring and attempting to limit food intake in order to achieve/maintain a perceived ideal body weight. Disinhibition, higher scores indicate a greater tendency to overeat when restraint is removed. Hunger, higher scores indicate an increased susceptibility to hunger

b. Perceived Stress Scale [2] for which higher scores indicate higher stress perception over the previous month.

c. Body Shape Questionnaire [3] for which higher scores indicate increased body dissatisfaction caused by feelings of being fat.

d. Beliefs About Appearance Scale [4] with higher scores indicating increased belief that appearance is important in relationships, achievement, self-view and feelings.

e. Eating Disorder Exam [5] for which higher scores suggest body attitudes that are concurrent with eating disorder pathology over the previous four weeks.

f. Eating Disorder Inventory subscales [6]. Higher scores of Drive for Thinness suggest extreme concerns with weight, dieting and the intense pursuit of thinness. Increased Bulimia scores indicate a tendency to think about and engage in uncontrolled overeating. Higher Body Dissatisfaction reflects more dissatisfaction with overall weight and specific parts of the body.

g. Life Events Scale [7] total and subscale scores based on time interval between baseline and final bone density scan. Higher scores indicate a higher occurrence of stressful life events during the study.

h. Depression, Anxiety and Stress Scale [8] total and subscale scores reflecting emotional state over previous week. Higher scores indicate low positive affectivity (Depression), higher physiological hyperarousal (Anxiety) and higher negative affectivity (Stress).

i. Daily Stress Inventory [9] Impact score averaged from baseline and first and second follow during the 24-hour urine collections. Higher scores indicate perceived impact of stressful events during the 24-hour period.

j. Daily Stress Inventory [9] Frequency score averaged from baseline and first and second follow during the 24-hour urine collections. Higher scores indicate a higher number of stressful events that occurred during the 24-hour period.

References


Appendix 30: Cognitive Dietary Restraint Score, General Stress Score, Subclinical Ovulatory Disturbances, 24-hour Urinary Free Cortisol and 2-year ΔaBMD by Ethnicity (n=123)

<table>
<thead>
<tr>
<th></th>
<th>Asian (n=77)</th>
<th>Caucasian (n=46)</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Dietary Restraintb</td>
<td>8.3 ± 0.5</td>
<td>7.3 ± 0.5</td>
<td>0.200</td>
</tr>
<tr>
<td>General stress Z-scorec</td>
<td>0.03 ± 0.1</td>
<td>-0.05 ± 0.1</td>
<td>0.621</td>
</tr>
<tr>
<td>Subclinical ovulatory disturbancesd (%)</td>
<td>40.6 ± 3.7</td>
<td>46.8 ± 4.8</td>
<td>0.331</td>
</tr>
<tr>
<td>UFC (µg/24-hour)e</td>
<td>25.6 ± 1.0</td>
<td>25.9 ± 1.4</td>
<td>0.858</td>
</tr>
<tr>
<td>Total body ΔaBMDf (%)</td>
<td>0.9 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.317</td>
</tr>
<tr>
<td>L1-4 ΔaBMDf (%)</td>
<td>1.4 ± 0.3</td>
<td>0.9 ± 0.4</td>
<td>0.311</td>
</tr>
<tr>
<td>Hip ΔaBMDf (%)</td>
<td>0.0 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>0.398</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error. UFC, 24-hour urinary free cortisol; ΔaBMD, annualised 2-year percent change in areal bone mineral density (g/cm²); L1-4, lumbar vertebrae 1-4.

a. Level of significance of difference between Asian and Caucasian participants by independent t-test or General Linear Model adjusted for covariates.
d. N=114; Adjusted for gynaecological age, body mass index (kg/m²) and the number of cycles analysed.
e. Adjusted for urine volume (L/24 hour).
f. Findings did not change with inclusion of theory-based ΔaBMD correlates: study hormone use, calcium/kcal, sport activity, or change in lean or fat mass.

References


Appendix 31: Cross-sectional Examination of Differences in 24-hour Urinary Free Cortisol by Ethnicity and Level of Cognitive Dietary Restraint (CDR) and the Ethnicity-by-CDR Interaction

<table>
<thead>
<tr>
<th></th>
<th>Higher CDR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lower CDR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Main CDR Effect P value</th>
<th>Main Ethnicity Effect P value</th>
<th>Interactive Effect P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline UFC</td>
<td>26.7 ± 1.6</td>
<td>26.7 ± 1.6</td>
<td>0.990</td>
<td>0.183</td>
<td>0.019</td>
</tr>
<tr>
<td>Asian (n=77)</td>
<td>25.6 ± 2.0</td>
<td>30.8 ± 2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (n=52)</td>
<td>27.8 ± 2.4</td>
<td>22.5 ± 2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First follow-up UFC</td>
<td>26.7 ± 1.8</td>
<td>23.0 ± 1.6</td>
<td>0.040</td>
<td>0.438</td>
<td>0.062</td>
</tr>
<tr>
<td>Asian (n=75)</td>
<td>25.3 ± 1.8</td>
<td>25.0 ± 2.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (n=44)</td>
<td>31.2 ± 2.6</td>
<td>25.0 ± 2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final follow-up UFC</td>
<td>28.2 ± 1.6</td>
<td>23.7 ± 1.5</td>
<td>0.131</td>
<td>0.453</td>
<td>0.154</td>
</tr>
<tr>
<td>Asian (n=76)</td>
<td>24.0 ± 2.1</td>
<td>23.8 ± 2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (n=47)</td>
<td>29.3 ± 2.9</td>
<td>22.2 ± 2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error. The number of participants varied for each analyses based on available of urine data at each data collection period. P values indicate differences by General Linear Modelling adjusted for corresponding 24-hour urine volume. CDR; cognitive dietary restraint; UFC, 24-hour urinary free cortisol (µg).

a. Women with Three Factor Eating Questionnaire Restraint subscale [1] scores higher than or equal to the median score. The median score was 7.0 at baseline and both follow-ups.
b. Women with Three Factor Eating Questionnaire Restraint subscale [1] scores below the median score at the corresponding data collection. The median score was 7.0 at baseline and both follow-ups.

References

Appendix 32: Pearson’s Partial Correlations of 12-hour Average Daytime Ambulatory Blood Pressure (ABP, mm Hg) and Eating and Body Attitude Questionnaire Scores at First Follow-up (n=120)

<table>
<thead>
<tr>
<th>Questionnaire Score</th>
<th>Systolic ABP</th>
<th>Diastolic ABP</th>
<th>Mean Arterial Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three Factor Eating Questionnaire(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive Dietary Restraint</td>
<td>0.033</td>
<td>0.016</td>
<td>0.041</td>
</tr>
<tr>
<td>Disinhibition</td>
<td>0.123</td>
<td>0.229(^*)</td>
<td>0.210(^*)</td>
</tr>
<tr>
<td>Hunger</td>
<td>0.098</td>
<td>0.180(^i)</td>
<td>0.145</td>
</tr>
<tr>
<td>Body Shape Questionnaire(^b)</td>
<td>0.083</td>
<td>0.243(^**)</td>
<td>0.224(^*)</td>
</tr>
<tr>
<td>Beliefs About Appearance Scale(^c)</td>
<td>0.031</td>
<td>0.099</td>
<td>0.078</td>
</tr>
<tr>
<td>Eating Disorder Inventory(^d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drive For Thinness</td>
<td>0.007</td>
<td>0.150</td>
<td>0.138</td>
</tr>
<tr>
<td>Bulimia</td>
<td>0.087</td>
<td>0.266(^**)</td>
<td>0.225(^*)</td>
</tr>
<tr>
<td>Body Dissatisfaction</td>
<td>-0.056</td>
<td>0.074</td>
<td>0.060</td>
</tr>
</tbody>
</table>

Data are presented as Pearson’s partial correlation coefficients (Rp) adjusted for age, body mass index and activity during ABP monitoring: \(^i\) \(p \leq 0.10\); \(^*\) \(p \leq 0.05\); \(^*\) \(p \leq 0.01\); \(^**\) \(p \leq 0.001\). ABP-Activity: active or sedentary during ambulatory BP measurement;

\(^a\) Three Factor Eating Questionnaire subscales [1]. Cognitive Dietary Restraint, higher scores indicate an increased perception of monitoring and attempting to limit food intake in order to achieve/maintain a perceived ideal body weight. Disinhibition, higher scores indicate a greater tendency to overeat when restraint is removed. Hunger, higher scores indicate an increased susceptibility to hunger.

\(^b\) Body Shape Questionnaire [2] for which higher scores indicate increased body dissatisfaction caused by feelings of being fat.

\(^c\) Beliefs About Appearance Scale [3] with higher scores indicating increased belief that appearance is important in relationships, achievement, self-view and feelings.

\(^d\) Eating Disorder Inventory subscales [4]. Higher scores of Drive for Thinness suggest extreme concerns with weight, dieting and the intense pursuit of thinness. Increased Bulimia scores indicate a tendency to think about and engage in uncontrolled overeating. Higher Body Dissatisfaction reflects more dissatisfaction with overall weight and specific parts of the body.

References


Appendix 33: Email Correspondence with Participants of the 2-year Prospective Bone Study

Email sent one month after study orientation:

Re: BONE STUDY – checking in :)  
Hi [participant]! Hope all is well with you!

I was just checking in to see how everything was going with the study.

Have you started taking your temperature and recording it in your calendar yet?

Have you completed the questionnaire and food frequency questionnaire yet? I do need these soon please as it is important that your answers reflect the time when you did the urine collection and bone density scan. Let me know if you need another copy. I have received your questionnaires so thank you very much!

Thanks for completing the urine collection so diligently! Also, have you completed and returned the short questionnaire about stress and put it in the envelope addressed to me along with your instruction form with the start/finish times filled in? I did receive the questionnaire you completed about stress on the day of urine collection – thanks!

Last thing! Have you received your appointment for the bone scan? When is your appointment? If you have to reschedule from the time they gave you, please let me know the new date :)  
How did the bone scan at VGH go?

Anyway thanks again so much for being such a good participant and please let me know if you have any questions at all.

Thanks again, Jen

Email reminder sent 2 days prior to urine collection procedure:

Re: Bone study - 24 hr urine collection  
Hi [participant]. Hope all is well with you!

Just checking in to see if you were up for doing the urine collection on [date]. Let me know either way please and thanks.
Also, please let me know approximately what time I can expect you to be calling me for the courier pick up on the following morning.

If you are going to complete the procedures, please take the time to read over the instructions again just to be clear on everything. The night before, you should place both the measuring cup and large orange canister in your bathroom so that you remember to start the collection in the morning. The first time you pee on the day you start, that gets flushed as normal but be sure to record the time on your forms. This is the ‘start’ time and you need to collect all your urine each and every time you pee for the next 24 hours (including in the middle of the night!). So that means,
the next morning, within 5-10 minutes of when you started, you collect your urine and that is the last sample!

Again feel free to contact me anytime by phone anytime while you are completing the procedures as I will have my cell phone with me all day just in case!

Thanks again and talk to you soon,
Jen

Email reminder sent 2 days prior to bone density scan at VGH (participants were reminded by a phone call the day before appointment):

Re: BONE SCAN @ VGH REMINDER!
Hi [participant]! Hope you are doing well :) 

I just wanted to remind you of your bone density scan scheduled for [date] at [time]. The details on how to get to VGH Nuclear Medicine Department are on the sheet I gave you with a map. It’s the same location and procedure as the first time you went.

It is VERY important that you arrive on time. Sorry to be a nag but I've had some participants showing up late or not at all and this is very costly ($225!) and also bogs down the health care system as it takes up an appointment that could have gone to someone else in need!

Anyway I'm sure this won't be the case with you (you are a very good participant and I love having you in the study!) but I just need to make sure everyone arrives from now on. Please do call me as soon as possible if you do need to reschedule the appointment.

Thanks again,
Jen

Email sent two months prior to 6-month assessment:

Re: BONE STUDY: Touching base about 6 month assessment
Hello [participant].
How are you?! I hope all is going well so far this term. Hard to believe we’re almost half way through it!

How are things going with the temperature taking? Just let me know if you had any questions about it.

I also just wanted to touch base about the 6 month assessment (I won’t bother you with the details now!) for the study. We are aiming for six months after the 24-hr urine collection was completed which for you is [date]. But since that is after the school year is over, I just wanted to make sure you would be available to come meet me at UBC. If you will not be able to come into UBC then (and complete the procedures afterwards), then we will need to get you in sooner. Which is just fine – I just need to know when I should contact you about coming in.

Does that make sense? So please let me know what your plans are and if you will be out of town during that time, when you would be available until.
If this is making no sense, just give me a call at 604-616-4676 sometime to chat about it. Sometimes I have a hard time explaining things over email!

Anyway thanks again for being such a great participant and I look forward to seeing you again and hearing how things are going.

Jen

Email sent to book 6-month assessment:

Re: BONE STUDY: 6 month meeting
Hello [participant]!

How are you?! I hope that you are doing well and enjoying 2007 thus far!

Hard to believe, but it has been almost 6 months since you enrolled in the study and so its time to start thinking about the 6-month assessment! You have been a wonderful participant in my study and I really look forward to seeing you again!

We should get together about 6 months after you completed the urine collection so that is around [date]. I know that’s not for a while but I just wanted to give you lots of ‘heads up’ time so that you could try and keep me in mind in your future plans. Let me explain what would happen at this time and then we can either set up a time to meet for about 30 minutes or I can contact you again a 2-3 weeks before and we can work something out then.

OK so like last time we will need to chat on the phone for just a few minutes so to make sure you are eligible to continue – so basically that you are still regularly menstruating, have a consistent sleep pattern, are not taking any drugs that would affect our study measurements (particularly birth control pills or injections) and that you have not become pregnant J We can do this anytime that is good for you.

Then we will need to meet in my office again at UBC for about 30 minutes. At this time you will return the temperature calendars you have completed so far, and since you would then have completed the entire baseline procedures you will get a $30 gift card! I will also give you $20 cash for transportation - $10 for your trip to VGH for the bone scan and $10 for meeting me that day at UBC.

Then we will get to the six month assessment procedures! So let me break this down ;)

1. I will provide you with more temperature calendars.
2. I will give you a questionnaire package to complete within one week and then return by mail or drop off at the office.
3. We will review the procedures for the 24-hr urine collection and I will give you the necessary new materials – by the way, do you still have an orange canister as well as the measuring cup and funnel? You will complete this as last time – on a day that is convenient for you within the 2-3 weeks following our meeting.
4. We will go over the procedures and materials for the 12-hour blood pressure monitoring. You will start this the morning following our meeting so we need to keep that in mind when you book the appointment. Unlike the urine collection however, this is easy to take along with you so you can do it on a day you have work/classes etc. In fact it is best to do it on a ‘normal’ day. Basically you will put the monitor on in the morning following our meeting, where it for 12-hours and then call me the following morning at which time I will arrange for a courier to come pick it up and bring
it back to me for the next participant to use! It’s easy (and actually really fun and interesting) and don’t worry we will go over all the details when we get together!

5. Then I will weigh you and measure you again and as you a quick question about your menstrual cycle.

6. Finally, we will talk about getting together in an additional 6 months (so one year from your first appointment) just for ~15 minutes at which time I will give you a $20 gift card for completing the six-month procedures and I will also give you another food frequency questionnaire which you will take home to complete within about a week and return by mail or drop off at the office. I will also ask you to bring in the completed temperature calendars and I will give you some new ones. Also, if you were unable to complete the blood pressure assessment at the 6-month assessment, we will take care of it then.

I know that sounds like a lot but the only new thing is the blood pressure assessment and everything else is just a repeat!

So does that all sound good?! Let me know if you have any questions and if you want to go ahead and book our 30-minute meeting now or if I should email you again a few weeks before the date I mentioned above.

Thanks again so much for being such a great participant and I look forward to seeing you again soon and hearing about how things have been going for you!

Jen

Email sent for 6-month completion:

Re: BONE STUDY – done the 6 month procedures!
Hi [participant]. Hope all is well with you!

I just wanted to say thank you so much for the time you have dedicated to the 6-month procedures for the study! I really do appreciate it very much.

So the only thing you need to do now is continue taking your temperature. I will email you again in the fall to get together for the 1-yr assessment. This will only take about 15 minutes. I will give you a gift card for the procedures you completed recently and another one of those food frequency questionnaires to take home with you.

Anyway thanks again so much for being such a good participant and please let me know if you have any questions at all.

Thanks again, Jen

Email sent to book 1-year assessment:

Re: BONE STUDY – 1 year meeting
Hey there [participant]!

Hope you that you had a great summer and are looking forward to the fall and all it brings! Can you believe it’s been one year since you started the study!? Crazy! I am so grateful for all you have done for me. I know it’s a lot of work and I appreciate it very much.
Sometime this month, we just need to touch base for about 15 minutes to take care of a few things. Its just a few questionnaires that you complete at home at your convenience so nice and easy this time around!

First I have another $30 gift card and $10 in cash for the 6-month procedures. Then we will go over the following procedures which are really easy and you take them with you to complete at home:

1. I will provide you with more temperature calendars and you will return the calendars you have completed so far.
2. I will give you another food frequency questionnaire and you will take this with you to complete at home (takes ~1 hour) and then return it by mail or drop it off at the office – just like last time!
3. Then I will weigh you and measure you again and as you a quick question about your menstrual cycle.
4. Finally, we will talk about getting together for our final meeting – approximately 2 years from when we first started!

So quick and easy this time around J

When you email me back, let me know some good days/times to meet for about 15 minutes – day, evening, weekend – whatever works best for you. And also let me know if you have any questions about anything.

Thanks again so much for being such a great participant. I really do appreciate all you have done for the study. I look forward to seeing you again soon and hearing about how things have been going for you!

Jen

Email sent after 1-year procedure completed

Re: Bone study – Happy Holidays!
Heya [participant]

I hope this email finds you well and feeling festive 😊

I just wanted to say have a GREAT holiday season. I hope that you are able to take some time off and get some relaxation!

I also just wanted to take a moment to thank you for being a part of this study - your participation will make a meaningful contribution to the area of women’s bone health. You have been such a good participant and I appreciate it very much – it’s the best holiday gift a girl good ask for.

Other than continuing to take your temperature, that’s it until the final assessment which will be 2 years from the first time we got together. I will email every once in awhile to see how things are going! Please keep me informed of any email, address or phone number changes between now and then. And of course you can contact me anytime if you have any questions or concerns.

Thanks again and happy holidays,
Jen
Email sent 6 months prior to 2-year assessment:

Re: Bone study – Touching base
Hi there [participant]!

I hope this email finds you well! It seems like the New Year just started and yet the end of term is just around the corner! I sure am looking forward to the warmer weather that is coming!

Sorry to bother you with an email but I just wanted to touch base to see how you were doing and if there had been any changes or updates in your future plans.

We only have one more data collection point left and that is approximately 2 years from our first meeting – [date].

Do you know if you will be around the Vancouver area at that time? Would you mind emailing me back and letting me know what your plans are for the fall? We can easily complete the procedures a little bit earlier or later if that's more convenient. Please just let me know so I can contact you at the appropriate time. Just as before, we will meet at UBC and then you will complete the procedures at your home within the following few weeks. If you want some more details, please just let me know when you email me back.

I will email you again to see how things are going in the summer and if you have had any changes in plans. In the meantime, please contact me anytime if you have any questions or concerns about the study. I would be really grateful if you could keep me posted on any address, email or phone number changes. And especially let me know if your plans change and you will not be around in the fall. It is no problem for you to come in earlier and I would be very grateful to have you participate in the 2-year assessment!

Thank you again so much for being so dedicated to my study. I look forward to hearing from you!

Happy spring,
Jen

Email sent 4 months prior to 2-year assessment and to return first year Temperature Calendar analysis:

Re: Bone study – Happy Summer!
Howdy [participant]!

I hope this email finds you well and that you are enjoying summer so far. I really can’t believe it’s the middle of June already!

I just wanted to touch base with all the participants to see how everyone was doing before people started going away on summer adventures and to share some your personal results! I am attaching the analysis of your menstrual cycle from the first year of the study. Please read over it and email or call me anytime if you have questions! I hope you find it interesting and also hope that it inspires you to keep taking your temperature each morning! I do appreciate it very much and the results are proving to be very important to the study. You are making a wonderful contribution as we have very little
research in the area of healthy women’s menstrual cycles over such a long period of time. So thank you for that!

The next time we are scheduled to meet is 2 years from when we started in [date]. If you know that you will not be able to come in during that time (for a quick meeting with me, then questionnaires to take home, the last 24-hr urine collection and last bone density scan @ VGH), please let me know as soon as you can and we can easily get together earlier or later. Of course you will also receive another $30 gift card and $20 in cash – wohoo!

Anyway I really hope you are having a great summer and I will email you again in the fall to arrange our final meeting. I will also have some more of your personal results then such as the blood pressure analysis.

Please email me back if you have any questions or concerns or just to let me know how things are going. Thanks again so very much for being a part of my study!

Jen

Email sent to book 2-year (final) assessment:

Hi [participant]!

How are you?! I hope that you are doing well! I can’t believe it has been 2 years since you became a part of my study! I am so grateful for all that you have done so far for the study. And now we have reached the last step – the 2 yr assessment! To get things started, we will need to meet at UBC for 30 minutes (for the last time!) to go over all the procedures and materials. You will also receive your last $30 gift card and $20 cash. I have described what needs to be done below. Once you have a chance to read it over, please email me back with some good days/times for us to meet for 30 minutes at UBC!

So at our meeting we will go over the following:

1. You will return the temperatures calendars you have completed so far and I will provide with you a couple of new ones just to get you through until the bone density scan is completed (more about that below). If you stopped taking your temperature, it’s no problem! This is the least important part of the study and I definitely want you to continue with the other aspects!

2. I will give you a questionnaire package (takes ~30 mins) as well as another food frequency questionnaire (takes ~1 hour) to complete at your leisure and then return it by mail or drop off at the office – just as before!

3. We will review the procedures and materials for another 24-hr urine collection. You will complete this on a day that is convenient for you within the 2-3 weeks following our meeting.

4. We will arrange the final bone density scan at VGH on a day that is convenient for you to complete this procedure. I am currently getting appointments for early December at the earliest. The appointment takes about 30 minutes.

5. Then I will weigh you and measure you for the last time and as you a quick question about your menstrual cycle.

I know that sounds like a lot but it is all things you have completed before and everything but the bone density scan at VGH is completed at your home!
So does that all sound good?! Let me know if you have any questions. Also, please let me know some good days/times for us to meet – day, evening, weekend – whatever works best for you over the next few weeks! Ideally we should get together by mid December but let me know if that’s not possible and we can rearrange things.

Thanks again so much for being such a great participant. I am really looking forward to seeing you again soon and hearing about the past year!

Jen

Email sent for 2-year meeting reminder (similar emails were sent for all study meetings) and to return ambulatory blood pressure analysis:

Re: BONE STUDY – friendly reminder for meeting
Hi [participant]! Hope that you are doing well!

Just a friendly reminder that we have our 2-year meeting scheduled for the Eating Attitudes and Bone Study [date] @ [time]. Please let me know as soon as possible if you need to reschedule so that another participant could come in during that time.

We will be measuring your weight, height and waist at this time so please wear some light clothing (cami under your regular shirt).

Please remember to bring your completed temperature calendars (please do not bring the one you are currently filling in). We will also set the date for the last urine collection and bone scan @ VGH so bringing your day timer would also be helpful as well as your January/February schedule as that is the earliest appts I can get over there at this time. And, if your MSP or health care card # has changed, please bring that as well or just email me the number if that’s easier.

The meeting will be at my office (same as before) which is in the Food, Nutrition and Health Building which is located at 2205 East Mall. My office is in Room 321 which is on the 3rd floor. There will be signs posted within the building to guide you there!

If you get lost or need to reschedule or anything you can call me on my cell which is 604-616-4676.

Thanks again and I look forward to hearing how things are going! Jen

PS. I have attached your blood pressure results from the 6-month assessment. I hope you find it interesting. Many more results to follow! Please do let me know if you have any questions about this or the temperature calendar analyses.

Email sent after completion of all study procedures:

RE: Bone Density Study – done!
Hi [participant]! Hope all is well with you!

I just wanted to say thank you so much for the time you have dedicated to the study over the past two years. I know I’ve said it a million times but I really do appreciate it very much. You have made a wonderful contribution to women’s health and I look forward to sharing the study findings with you and the other participants.
I have received all the materials I require from you, so you are officially finished with the study. I will of course be in touch with your personal results as we compile them. I know that you are anxious to receive them and we are doing our best to get everything out as quickly as possible. Thank you for your patience.

It has been a pleasure getting to know you over the past two years and I wish you the best of luck in all your future endeavours.

Please contact me any time if you have any questions or concerns. Or, if you just want to drop me a line to let me know how things are going, I would love to hear from you!

Thanks again, Jen 😊

Email sent before mailing bone density scan results:

Re: BONE DENSITY RESULTS!
Hey there [participant]. I hope this email finds you doing well and enjoying winter.

Some good news – I have received the results from your 2-yr bone density scan. I have 2 copies of the results (one for you and one for your doctor) as well as a letter explaining what the results actually mean to you and the health of your bones.

Unfortunately I cannot email these results as they only come to me in paper form. So I would like to mail them to you but just want to make sure I have an up to date mailing address. Please let me know where you like me to send them and I will get them off as soon as possible.

Also, please don’t hesitate to email or call me if you have any questions about the results. Thanks so much and I will keep the results from the study (both your personal findings and the findings of the whole study) coming your way!
Jen
Appendix 32: 2-year Prospective Bone Study Temperature Calendar Individual Results

THE UNIVERSITY OF BRITISH COLUMBIA

Temperature Calendar Analysis for Year 2

Principal Investigator:
Susan Barr, PhD, RD
Professor; Food, Nutrition and Health, University of British Columbia (UBC); (604) 822-6766

Co-Investigators:
Jennifer Bedford, BSNH
PhD Candidate
Human Nutrition, UBC
(604) 616-4676

Jerilynn Prior, MD
Professor
Endocrinology & Metabolism, UBC
(604) 875-5927

Sponsor: Canadian Institutes of Health Research (CIHR)

Thank you for your Participation. We are extremely grateful for your participation in the study so far and your dedication to completing the procedures. Thanks to you and our other participants, we are currently conducting the 2-year assessments. This document will give you a summary of your personal temperature calendar analysis from the second year of the study. You will learn some interesting information about your menstrual cycle from our analysis.

Contact Information. If you have any questions or desire further information about this document or anything else pertaining to the study, please contact Jennifer Bedford at (604) 616-4676 or Dr. Susan Barr at (604) 822-6766.

Background Information. Characterising the menstrual cycle determines whether different parts of the cycle are normal. Measuring body temperature at the time of awakening is a non-invasive way to characterise a woman's menstrual cycle. We will be using the data from the temperature calendars to examine the relationship between menstrual cycle characteristics and bone.

A woman's menstrual cycle lasts an average of 28 days and is divided into two parts: the first half (typically about 14 days) is the follicular phase, then ovulation occurs (the egg is released from the ovaries), and the second half (again, about 14 days) is referred to as the luteal phase. Levels of the reproductive hormones show a pattern over the cycle. The hormone we are examining, progesterone, increases in the 2nd half of the cycle if ovulation has occurred. Interestingly, progesterone actually has a warming affect on the body and causes an increase of ~0.3°Celsius from the follicular phase to the luteal phase, when progesterone peaks.

Analyses. Your temperature calendars were analysed using a computer program called Maximina. This program uses what is called the least-squares analysis method of quantitative basal temperature. The program assesses whether ovulation occurred by determining whether the menstrual cycle can be divided into two phases by identifying a statistically significant difference in temperature; the day the temperature increases significantly indicates the start of the luteal phase and the end of the luteal phase is marked by the onset of menstrual flow. We have validated this program against urinary progesterone levels and found it performs moderately well in detecting whether or not ovulation occurred.
In the table below you will find your personal temperature calendar analyses from the entire study. Each cycle is listed in the first column. The next column indicates if the Maximina program detected a significant temperature increase – indicating that ovulation likely occurred during that cycle. The program then identifies which cycle day this occurred on – the day of luteal onset. By subtracting that day from the length of that cycle, we determine luteal phase length. From a fertility perspective, a luteal phase length of more than 10 days is usually required to achieve pregnancy.

In the section below the table, you will find a summary of your analyses indicating the percentage of cycles for which ovulation did and did not (anovulatory) occur as well as the range of luteal onset and luteal phase length. It is important to remember that our menstrual cycles can be affected by many variables (stress, illness) and these results are not a medical test for fertility. If you have any questions regarding your temperature calendars, please contact me anytime. I hope that you have learned something about yourself and that you will continue your temperature recordings.

**Personal Results**

<table>
<thead>
<tr>
<th>Did Ovulation Occur?</th>
<th>Cycle Length (# of days)</th>
<th>Luteal Onset (Cycle Day)</th>
<th>Luteal Phase Length (&lt;10 days = short)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Summary.** Based on temperature records, [XX]% of your cycles during the study were ovulatory and [XX]% were anovulatory. The day of luteal onset likely ranged between [XX] and [XX], meaning your luteal phase duration was [XX] to [XX] days.
Appendix 35: 2-year Prospective Bone Study Ambulatory Blood Pressure Individual Results

THE UNIVERSITY OF BRITISH COLUMBIA

Food, Nutrition and Health
Faculty of Land and Food Systems
2205 East Mall
Vancouver, B.C. Canada V6T 1Z4
Phone: (604) 822-2502 Fax: (604) 822-5143

Ambulatory Blood Pressure Results

Principal Investigator:
Susan Barr, PhD, RD
Professor; Food, Nutrition and Health, University of British Columbia (UBC); (604) 822-6766

Co-Investigators:
Jennifer Bedford, BSNH
PhD Candidate
Human Nutrition, UBC
(604) 616-4676

Wolfgang Linden, PhD
Professor
Clinical Psychology, UBC
(604) 822-4156

Sponsor: Canadian Institutes of Health Research (CIHR)

Thank you for your Participation. We are extremely grateful for your participation in the study so far and your dedication in completing the procedures. Thanks to you and our other participants, we have completed year 1 of data collection and are looking forward to the 2-year assessments.

This document will give you a summary of your personal 12-hour ambulatory blood pressure results. Ambulatory refers to blood pressure taken while moving about rather than resting blood pressure like when you go to the doctors.

Contact Information. If you have any questions or desire further information about this document or anything else pertaining to the study, please contact Jennifer Bedford at (604) 616-4676 or Dr. Susan Barr at (604) 822-6766.

Background Information on Blood Pressure.

Blood pressure is the pressure of the blood against the walls of the arteries. Systolic blood pressure (the larger number that is presented on top), is the pressure created when the heart contracts to pump blood into the arteries and through the circulatory system. Diastolic blood pressure (the smaller number presented at the bottom), is the pressure created when the heart relaxes between beats. Blood pressure below 120/80 mm Hg (millimeters of mercury – just a unit of measurement) is considered optimal for adults. Blood pressure of 140/90 is considered high blood pressure or hypertension. Hypertension is a risk factor for heart disease and stroke. We do not expect any of the young, healthy participants in this study to have high blood pressure. Rather, we are looking at small differences in the normal range of blood pressure in relation to stress, eating attitudes and behaviours. If your blood pressure was frequently over 140/90 mmHg, it is highly recommended to see your family physician for advice.

Mean Arterial Pressure (MAP) is the average (mean) pressure within an artery over a complete cycle of one heart beat. MAP is calculated as [(2 x systolic) + diastolic/3]. Research shows that MAP is an indicator of age-related blood vessel stiffening. Normal MAP ranges from 70 to 100 mm Hg.
Pulse Pressure (PP) is another method used to assess the stiffening of the blood vessels. PP is calculated by subtracting diastolic from systolic blood pressure. Since normal blood pressure is less than 120/80, normal PP is in the range of 30 - 50 mm Hg.

Heart Rate (HR) is a measurement of your pulse – it is the number of times your heart beats in one minute. A healthy resting HR ranges between 50 and 70 beats per minute. Very physically fit individuals tend to have low heart rates and women’s heart rates are typically a beat higher than those of men.

The definitions above were adapted from the American Heart Association (www.americanheart.org)

### Personal Results

<table>
<thead>
<tr>
<th>Reading Number</th>
<th>Time Taken</th>
<th>Systolic BP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
<th>Mean Arterial Pressure (mm Hg)</th>
<th>Pulse Pressure (mm Hg)</th>
<th>Heart Rate (beats per minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 36: 2-year Prospective Bone Study Letter Accompanying Bone Density Results

The Results of your Bone Density Scan

Attached is a complete copy of the results of the two bone density scans that you had done at Vancouver General Hospital on [DATE] and [DATE].

To help you make sense of the results, we have written the following notes – please do not hesitate to contact me if something is unclear. Also, you may wish to give your doctor the extra photocopy of these results and this summary page.

Note that personal results of the bone density scan for each of the three areas measured (total body, lumbar spine, hips) can be categorized as follows:

- “normal” (bone mineral density is in the desired range)
- “below normal” (bone mineral density for the total body is lower than average for young women)
- “osteopenia” (bone mineral density at the spine or hips is lower than what is considered normal), or
- “osteoporosis” (bone mineral density at the spine or hips is low and the risk for a fracture in the future is increased).

Also, your results can be compared to the initial scan you had when you first joined the study. These changes are expressed as a percentage, with negative numbers indicating that bone density has decreased and positive numbers indicating that it has increased. It is normal for bone mineral density at the spine and hips to decrease slightly with increased age. Changes of less than 3% (either positive or negative) are not considered meaningful.

Overall the results of your bone density scan indicate the following:

<table>
<thead>
<tr>
<th>Area of body</th>
<th>Bone density category at baseline</th>
<th>Bone density category after 2 years</th>
<th>Change from initial scan*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both hips</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Changes of more than 3% are considered significant.

Please do not hesitate to contact me at (604) 616-4676 should you have any questions about any of this information. Thank you so much for participating in the study. I hope you find this information valuable and interesting.

Sincerely,

Jennifer Bedford, PhD Candidate
The first 5 pages of the attached report provide the results of the “total body” portion of the test, for your bone mineral density and for your body composition. Results are provided for the body as a whole and for different parts (e.g., left arm, left leg, etc). The following definitions may help you understand the information provided:

**BMD**: This is the abbreviation for “Bone Mineral Density” – the average concentration of bone mineral in the area of bone being measured. BMD provides an estimate of bone strength.

**T-score**: This number compares your personal result to those of young healthy women about 30 years old (who have an average T-score of zero). The T-score is used to classify BMD as “normal”, “osteopenia” or “osteoporosis”. If your T-score is between -1.0 and -2.5, you meet the criterion for osteopenia in that area; if it is less than -2.5, you meet the criterion for osteoporosis in that area.

---

The T-score is represented by standardized units as illustrated:

<table>
<thead>
<tr>
<th>“Osteoporosis”</th>
<th>“Osteopenia”</th>
<th>“Normal range”</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>-2.5</td>
<td>-1.0</td>
</tr>
<tr>
<td>-1.0</td>
<td>0</td>
<td>+1.0</td>
</tr>
</tbody>
</table>

Bone density is lower than young reference women
Similar to young women

**Z-score**: This is similar to the T-score in that it compares your personal information to others’, but in this case, the comparison is to other women the same age as you (instead of the young women about 30 years old). If your Z-score is above zero, then your bone density is relatively higher than other women of your age. If your Z-score is below zero, then your bone density is less than the average for other women your age.

**Trend**: This shows the change in your bone density since it was first measured at VGH (the baseline measurement). **BMC (g)**: This stands for “Bone Mineral Content”, and is the absolute amount of bone mineral in the section of bone being measured.

**Area (cm²)**: This indicates the size (area) of the bone being measured.

**Tissue (% Fat)**: The percentage of fat in the soft tissue (not including bone) of that area.

**Region (% Fat)**: The percentage of fat in the entire area (including bone).

**Tissue (g)**: The weight of the combined fat and lean (muscle) in that area, in grams.

---

**Pages 1 – 5: Results for your “total body” (page by page)**

**Page 1**: Here you see pictures of your skeleton. The first graph at the top of the page shows your total body bone mineral density (the white square with a black dot in it) and your T-score, and the second graph shows the % change in total body BMD from baseline. The box below the graphs shows BMD values for several regions of the body. The box at the bottom of the page shows the changes in total body BMD. Note that values for total body BMD are not generally considered when diagnosing osteoporosis or predicting fracture risk. Values at the lumbar spine and hip are used for those purposes.

Your personal result for the “total body” bone mineral density is indicated by the white square in the graph. As you can see, the T-score for your total body is [XXX], which indicates that it is [lower than/similar to] the average of healthy young women.
Page 2: This page shows additional results for your total body – the BMD, BMC, and bone area for each section of the body.

Page 3: This page shows information on your body composition (fat and lean). The first graph at the top of the page shows the percentage of your body that is fat (the numbers between 0% and 50% left hand side of the graph) and also compares your body fat to other women your age using the Z-score (negative numbers indicate that your fat percentage is lower than the average woman your age; positive numbers indicate it is higher). The second graph shows the change in your total body weight (in kg) since your first scan. The box in the middle shows the composition of major regions of your body (arms, legs, and trunk), and the box at the bottom shows changes in your body composition since your first scan.

Page 4: This page provides detailed results for the composition of different parts of your body. The “android” region is the central part of your body (your belly); the “gynoid” region includes your hips and thighs.

Page 5: This page repeats some of the information from page 3 (for example, the graph at the top of the page in the middle is the same as on page 3). The “new” information is at the bottom of the page. It shows your Body Mass Index (BMI), which is a measure of your weight relative to your height. A BMI below 18.5 is underweight, between 18.5 and 25 is normal weight, between 25 and 30 is overweight, and over 30 is considered obese. The graphic also shows what your weight would be at different BMIs.

Pages 6-7: Results for your Lumbar Spine

The next two pages (pages 6 and 7) provide the results of the bone density scan of the “lumbar spine” (i.e., the lower portion of the spine at the base of your back). This is one of the sites that is often measured for the diagnosis of osteoporosis.

Your personal result is indicated on the graph in the middle of the top of the page by the white box with the black dot in it. As you can see, your bone density falls in the “[XXXXX]” range at the lumbar spine.

Please keep in mind that the reference point here for what is considered “normal” is the bone mineral density of young healthy women about 30 years old. The T-score values compare your bone density to this reference (Again, the T-score is used for the diagnosis of osteoporosis: a T-score less than -2.5 would be classified as osteoporosis, and between -1 and -2.5 would be considered osteopenia.)

The Z-score values compare your personal results to other women at the same age. If your Z-score is a positive number (above 0) then your bone density is relatively greater than other women your age. If your Z-score is a negative number (less than 0, with a “-“ in front of it), then your bone density is less than the average woman of your age. In the table below the graph on page 6, the “Region” (“L1”, “L2”, “L1-L2” etc.) refers to the measurement for the specific vertebral body (or bodies) in your spine. You can see that the image of your own spine (on the left of the page) shows the areas filled by the four vertebral bodies measured during this part of the scan.
Finally, the box at the bottom of page 6 shows the percent changes in your BMD for your lumbar spine, since earlier measurements were taken. Changes of less than 3% are not considered meaningful.

**Page 7** provides additional results for the BMC, area, width and height of each vertebral body and various combinations.

**Pages 8-9: Results for your hips**

The next two pages (**pages 8 and 9** – the last pages of the report) provide the results of the bone density scan of your hips. This is also one of the sites often measured for the diagnosis of osteoporosis.

The presentation of these results is very similar to how the results were presented for your lumbar spine. The graph at the left hand side of the page (below the picture of your hips) shows where your personal measurements fall (you will notice there are two squares on this graph – one for your right hip and one for your left hip). The graph in the middle of the page shows the change since your first scan. And the table shows T-scores and Z-scores for the femoral neck (the narrowest area of your hip joint) and for your total hip. Again, the T-score values compare your results to those of healthy young women, and the Z-scores compare your personal results to other women of your age.

Your personal result as indicated on the graph by the white boxes shows that your bone density falls in the “[XX]” range in both hips.

**Page 9** simply presents more detailed results for a number of different areas of the hip.
Appendix 37: Pearson’s Correlations Between the Duration of Hormone Use and 2-year ΔaBMD (n=123)

<table>
<thead>
<tr>
<th></th>
<th>Study hormone use&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total hormone use&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R value</td>
<td>P value</td>
</tr>
<tr>
<td>Total body ΔaBMD&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>0.134</td>
<td>0.140</td>
</tr>
<tr>
<td>L1-4 ΔaBMD&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>-0.066</td>
<td>0.470</td>
</tr>
<tr>
<td>Hip ΔaBMD&lt;sup&gt;c&lt;/sup&gt; (%)</td>
<td>0.134</td>
<td>0.140</td>
</tr>
</tbody>
</table>

Data are presented as Pearson’s correlation coefficients (R). ΔaBMD, annualised 2-year percent change in areal bone mineral density (g/cm<sup>2</sup>); L1-4, lumbar vertebrae 1-4.

- **a.** Duration of hormone use in months (oral contraceptives, progesterone and hormonal intrauterine devices) between the first and final bone density scans. Non-users=0 months.
- **b.** Duration of hormone use in months (oral contraceptives, progesterone and hormonal intrauterine devices) between the first and final bone density scans as well as hormone use prior to study enrollment. Non-users=0 months.
- **c.** Findings did not change with inclusion of theory-based ΔaBMD correlates: calcium/kcal, sport activity, or change in lean or fat mass.
### Appendix 38: 24-hour Urinary Free Cortisol at Baseline and Follow-ups and Level of Significant Difference Between Values by Repeated Measures General Linear Model (n=116)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>First follow-up</th>
<th>Final follow-up</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour urinary free cortisol (µg/day)</td>
<td>27.2 ± 13.3</td>
<td>25.4 ± 12.3</td>
<td>24.5 ± 14.2</td>
<td>0.225</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. First follow-up was 6-12 months (average 7) after baseline, and second follow-up was 1.5-2.5 years (average 2) after baseline. Values are for the 116 participants who completed the final follow-up, and completed all three urine collections.