BODY COMPOSITION AND BODY FAT DISTRIBUTION CHANGES AFTER SHORT-TERM WEIGHT GAIN IN ANOREXIA NERVOSA PATIENTS

by

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ABSTRACT

The most commonly described psychological abnormality in Anorexia Nervosa (AN) is a distorted perception of body weight and shape. Anorexia nervosa patients typically fear that weight gain is accompanied by preferential fat deposition in the abdomen, hips and thighs; and this fear may contribute to their resistance in gaining weight even when this is of medical necessity. One objective of this study was to investigate body composition and body fat distribution changes that accompany short-term weight gain in AN patients. Another objective was to assess the level of agreement in the measurement of change in percentage body fat in patients with AN pre- and post-weight gain, as determined by two body composition assessment methods: bioelectrical impedance analysis (BIA) and dual energy X-ray absorptiometry (DEXA).

Twenty six female subjects, 28±7 years of age (mean ± SD), initial BMI 17±2 kg/m^2, who met the diagnostic criteria for AN completed the study. Subjects were recruited from the inpatient and outpatient Eating Disorders Clinics, St. Paul's Hospital, Vancouver, BC. Body composition and body fat distribution changes were assessed by skinfold (SKF), circumference (CIRC), and DEXA methods. Bioelectrical impedance analysis was used to measure the change in percentage body fat pre- and post-weight gain, and this change was compared to that obtained by DEXA. Skinfold and CIRC measurements were performed at 9 body sites; DEXA was used to quantify body fat mass in the subscapular, waist and thigh regions. Measurements by all methods were performed at baseline, and at the point of maximum weight gain.

Results of body composition changes included a highly significant weight gain of 6.7±5.3 kg ($p < .001$). This weight gain was achieved by significant gains in body fat ($p < .001$), lean body mass ($p < .05$), and bone mineral content ($p < .01$). Total body fat was, however, the component which increased to the greatest extent. Analysis of absolute and relative changes pre- and post-weight gain as assessed by SKF and CIRC indicated a greater

fat deposition in the central regions (chest, abdomen, hip and thigh) than in the extremities (arm and calf). However, comparison of body fat mass change (kg) in the subscapular, waist and thigh regions as measured by DEXA indicated no significant differences among these 3 central regions (subscapular: 1.7$\pm$1.2, waist: 1.8$\pm$1.3, thigh: 1.5$\pm$1.0; p = .10). Comparison of measurement of change in percentage body fat upon weight gain between BIA and DEXA indicated poor agreement between the two methods. It appears that single-frequency BIA may not be sensitive enough to reliably quantify changes in body composition in AN patients.

Overall, the preliminary findings of this study suggest that although weight gain in AN patients is accompanied by greater fat deposition in the central regions than in the extremities, there is no preferential fat accumulation in any of the central regions. Therefore, the gynoid fat distribution pattern in these patients is preserved despite renourishment and subsequent weight gain. This also implies that significant weight gain does not predispose these patients to the health risks associated with central body fat distribution.

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CHAPTER ONE

INTRODUCTION

A) Rationale

In recent years, there has been an increasing interest among the scientific community in the area of regional fat distribution, particularly in relation to obesity. It is now well-established that many of the health risks of human obesity are due to the distribution of body fat rather than the absolute amount of body fat (Wadden et al., 1988; Vansant, Den-Besten, Westrate, & Deurenberg, 1988). Android (upper body or central) fat distribution which is most commonly seen in men, has been strongly associated with certain metabolic aberrations such as diabetes mellitus, hypertriglyceridemia and hypertension (Lapidus et al., 1984; Ohlson, Larsson, & Svardsudd, 1985). In contrast, these metabolic disturbances have been found to be less common in gynoid (lower body or peripheral) fat distribution most commonly found in women, whose fat predominates in the gluteal-femoral region (Vansant et al., 1988).

In recent decades, there has been a dramatic increase in the incidence of the Eating Disorders (ED) Anorexia Nervosa (AN) and Bulimia Nervosa (BN). These disorders are of complex etiology and are characterized by a relentless pursuit of thinness manifested by strict dieting, obsessive exercising, vomiting or laxative abuse which subsequently leads to significant weight loss, often to the point of emaciation (Davis, 1988; King, 1991; Garfinkel & Garner, 1982; Woodside & Garfinkel, 1989). The majority of AN patients are typically dissatisfied with their body weight and shape regardless of their actual weight. Their dissatisfaction may focus on specific areas of the body such as the abdomen, hips, and thighs, or it may encompass contempt against the entire body. They also view their bodies in a distorted way, and can identify areas where they are convinced there is too much fat accumulation, even though objective evidence does not indicate this to be the case (Davis, 1988). Unpublished observations (Drs Birmingham & Goldner, Eating Disorders Clinic, St. Paul's Hospital) have further substantiated that fear of weight gain by AN patients is enhanced by their belief that this gain is accompanied by preferential fat distribution in the abdominal area. As a consequence, they are reluctant to accept appropriate nourishment, and they avoid gaining weight even when this is of medical necessity.

Despite the fact that this fear of "fatness" is a central feature in AN, there has been surprisingly little investigation and only minimal understanding of the biology of body composition in patients with this condition (Mitchell & Truswell, 1987). As well, to date there has been no study conducted to examine fat distribution before and after weight gain in AN patients. Thus, no empirical data are available to substantiate or refute these patients' claim that upon refeeding body fat accumulation occurs preferentially in specific areas.

The goal of this research was to better understand the nature of body composition and fat distribution changes that accompany short-term weight gain (up to 6 months) in AN patients. It was anticipated that the results of this study would provide relevant information for use in the development of future nutrition intervention strategies for the treatment of this patient population.

B) <u>Null Hypotheses Tested</u>

1. There will be no difference in the composition of body weight (fat vs lean tissue) in patients with AN pre- and post-weight gain.

2. There will be no difference in the distribution of body fat in patients with AN pre- and post-weight gain.

3. There will be no difference in the measurement of change in percentage body fat in patients with AN pre- and post-weight gain, as determined by bioelectrical impedance analysis (BIA) and dual energy X-ray absorptiometry (DEXA).

C) <u>Study Objectives</u>

1. To determine whether there are changes in body composition pre- and post-weight gain in patients with AN, as determined by dual energy X-ray absorptiometry (DEXA).

2. To determine whether there are changes in body fat distribution pre- and post-weight gain in patients with AN, as determined by skinfolds (SKF), circumferences (CIRC), and dual energy X-ray absorptiometry (DEXA).

3. To determine whether there are differences in the measurement of change in percentage body fat in patients with AN pre- and post-weight gain, as determined by bioelectrical impedance analysis (BIA) and dual energy X-ray absorptiometry (DEXA).

CHAPTER TWO

LITERATURE REVIEW

A review of the literature was done to gain an understanding of body composition patterns that characterize AN, the changes in body composition and body fat distribution that occur upon weight gain in these patients, and the methodologies used to assess such changes.

This chapter first provides an overview of the physiology of starvation. This is followed by a description of body composition patterns in normal-weight individuals, and definitions of body composition terminology are provided. The body composition characteristics of AN are then discussed, followed by a review of the body composition and body fat distribution changes that accompany weight gain in AN. Lastly, measurement techniques for body composition and body fat distribution assessment are outlined.

A) Physiology of Starvation

During starvation, the human body undergoes a series of hormonal and metabolic changes which lead to modifications in body composition. The supply of calories is selectively drawn from adipose tissue, in an attempt to spare the breakdown of vital proteins (Mitchell & Truswell, 1987). Each gram of either protein or glycogen is associated with 3-4 grams of water which are excreted during energy utilization. In contrast, fat is not water-bound (Mitchell, & Truswell 1987).

Fuel is required by the body primarily to supply energy for vital functions. A primary fuel is glucose, two-thirds of which (100-145 g) is required by the brain, and one-third by muscles and red blood cells. Glucose is mainly stored as glycogen in the liver. This reserve amounts to approximately 100 g of glucose, which would be enough to sustain the needs of the brain for only 12-16 hours (Cahill, 1970). As a result, gluconeogenesis occurs and protein in skeletal muscle is broken down for the production of glucose by the liver. At the same time, triglycerides in adipose tissue are broken down, to provide muscles with energy (Mitchell & Truswell, 1987).

During the early stages of total starvation, protein breakdown occurs at a high rate. Since 1 g of protein is bound to 3-4 g of water, this protein loss accompanied by water loss causes the initial weight reduction to be rapid (Mitchell & Truswell, 1987). As the starvation process continues, more of the weight loss is accounted for by body fat mobilization. Since fat is a richer energy source than protein, each unit of body fat consumed provides relatively more energy, therefore there is a slowing down in the rate of weight loss as starvation continues. At this time, metabolic rate is also reduced as a biological adaptation in preserving energy sources and reducing energy needs (Keys, Brozek, Henschel, Mickelsen, & Taylor, 1950).

After 3-4 days, the process of ketosis commences, in which fatty acids released from fat depots are oxidized in the liver to acetoacetic acid and its derivatives. During this time, the brain and other tissues become accustomed to using ketones as energy substrates instead of glucose (Mitchell & Truswell, 1987). However, even after this period, the breakdown of body protein is not completely eliminated in fasted subjects. In fact, the body continues to require some glucose from protein breakdown. Nitrogen, in the form of urea and ammonia continues to be excreted in the urine (Young & Scrimshaw, 1971).

The physiology of semistarvation which closely resembles that of the anorexic state, has been studied less intensively than total starvation. One important physiological distinction between total and semistarved states is that there is no evidence of ketonuria in semistarvation (Keys et al., 1950). Similarly ketonuria is uncommon in patients with AN, although increased plasma concentrations of ketoacids are often present (Garfinkel & Garner, 1982). Elevated levels of low density lipoprotein (LDL) cholesterol are commonly seen in AN (Blendis & Crisp, 1968), possibly as a consequence of the enhanced mobilization of adipose tissue (Russell & Beumont, 1987).

In summary, starvation and semistarvation appear to progressively select fat as a body fuel. In total starvation, ketogenesis protects against the excess utilization of proteins; however, negative nitrogen balance and protein loss still occur. In contrast, during semistarvation the carbohydrate intake usually provides sufficient glucose so that ketonuria does not develop. Some protein loss persists, which appears to decrease as semistarvation continues (Mitchell & Truswell, 1987).

B) Normal Body Composition

To explore body composition changes that accompany starvation in general, and AN in particular, one needs to have a basic understanding of body composition in normal healthy individuals.

The study of human body composition has a relatively recent history, spanning mainly the last one hundred years (Wang, Pierson, & Heymsfield, 1992). Body composition analysis can provide a tool for characterizing populations; a reference basis for nutritional counselling; and an aid in identifying patterns characteristic of various disease entities (Buskirk, 1987). Thus, body composition measurements can have wide applicability in the fields of clinical nutrition, medicine, and physical education (Buskirk, 1987).

Despite the advances that have been made in the acquisition of knowledge in body composition, there are two principal problems that make the study of body composition difficult: First, terminology used to define body composition components/compartments is often unclear. An example of this is the frequent interchangeable use of terms like fat-free mass and lean body mass when in fact these represent different body compartments. This lack of clear definitions can result in errors in published body composition equations and models (Wang et al., 1992). Second, the only method that allows direct measurement of body composition is cadaveric analysis, and to date, very few cadaveric studies have been undertaken (Brodie, 1988). Thus, the majority of the body composition methods commonly used are indirect in nature. As such, a number of assumptions are made that may not necessarily be valid (Martin, Ross, Drinkwater, & Clarys, 1985). Taking into consideration these inherent shortcomings of body composition measurement, it became imperative to define the key body compartments that were relevant to the present research study.

Fat is the term used to describe the chemical substance which consists of glyceryl esters of fatty acids. Fat is one component of adipose tissue (Gurr & Harwood, 1991). Lipid is not synonymous with fat. Lipid refers to a group of chemical compounds that are insoluble in water but very soluble in organic solvents. There are fifty different lipids recognized in humans. Glyceryl esters of fatty acids which are referred to as fat are only a subcategory of total lipid (Gurr & Harwood, 1991). Adipose tissue (AT) describes the anatomical tissue which is comprised of fat cells (adipocytes), as well as the cellular matrix made up of protein, water and minerals. Adipose tissue can be either subcutaneous (beneath the skin), or visceral (surrounding body organs) (Snyder et al., 1984).

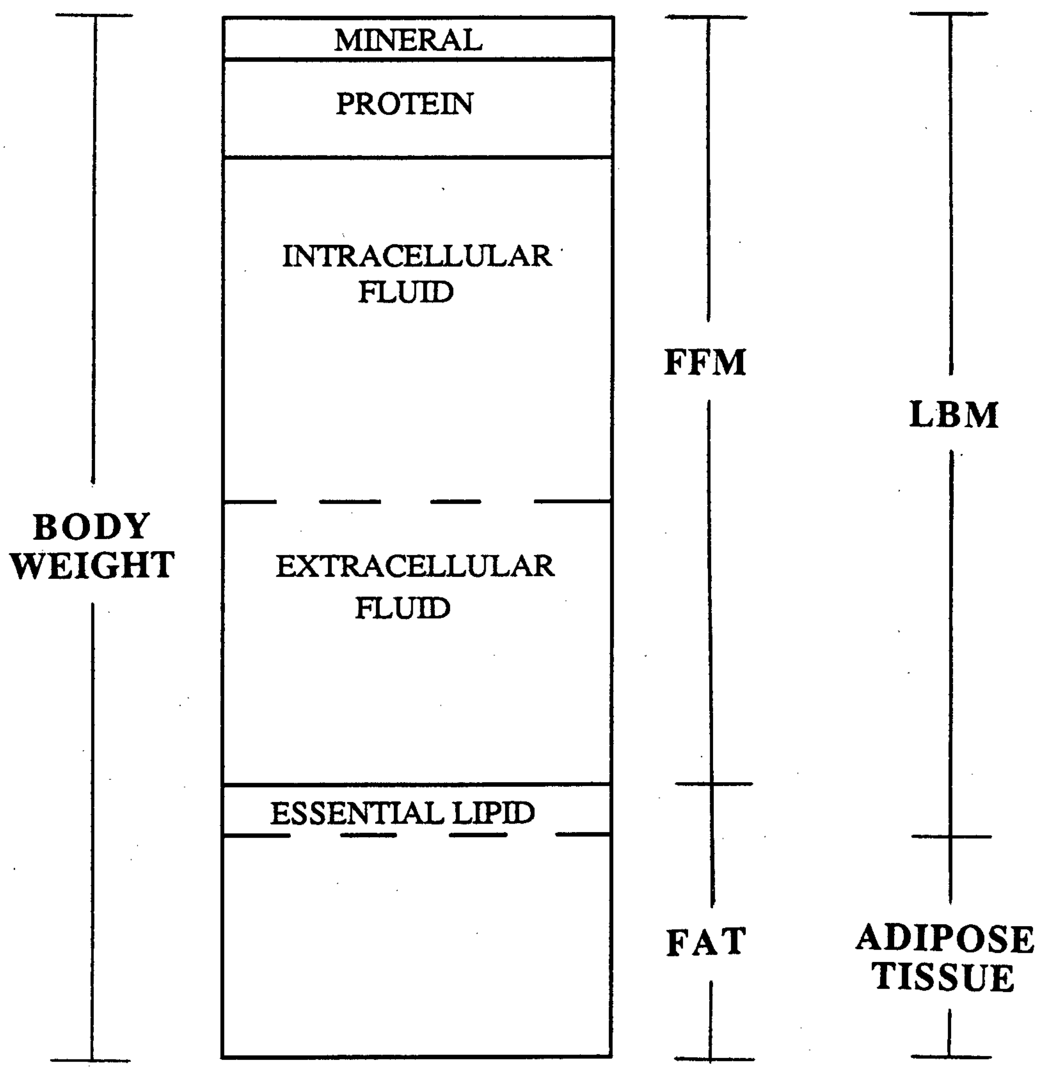
Lean body mass (LBM) is the sum of all tissues in the body excluding AT. In addition to water, protein, and mineral, LBM also consists of "essential" lipid; lipid which is found in the marrow of bones, in cell membranes, within the central nervous system and in internal organs (Behnke, Osserman, & Welham, 1953; McArdle, Katch, & Katch, 1991). Lean body mass can be expressed as total mass minus adipose tissue. Fat-free mass (FFM) is not synonymous with LBM. Fat-free mass excludes all lipid, that is, all chemically extractable lipid from a corpse or animal carcass (Mitchell & Truswell, 1987). Thus, unlike LBM, FFM does not include the "essential" lipid component (Mitchell & Truswell, 1987). Fat-free mass can be expressed as LBM minus essential lipid.

Total body water (TBW) constitutes the largest body compartment. In an average person, it can comprise from 50 to 65% of the total body weight (Moore, 1963). Total body water consists of two fractional components: a) intracellular fluid (ICF), fluid found inside the cell, and b) extracellular fluid (ECF), the non-metabolizing fluid surrounding cells which provides a medium for gaseous exchange, nutrient transfer, and excretion of metabolic end products (Wang et al., 1992). Extracellular fluid is in turn distributed into two main compartments: i) plasma, which represents the fluid portion of blood, and ii) interstitial fluid (ISF), fluid surrounding body tissues.

A simplified form of the major body composition compartments is depicted in Figure 1.

Figure 1:

Major body composition compartments



C) Body Composition Characteristics of Anorexia Nervosa

AN has the following effects on body compartments:

1) Body Fat

The few studies that have quantified percentage body fat in the wasted anorexic patient have utilized a variety of body composition methods. In a study by Russell et al. (1983), 6 AN patients, with a mean age of 23.8 years, and with clinically severe malnutrition of four year duration, were found to have 14% body fat. To determine percentage body fat, anthropometric evaluation was done by measuring triceps and iliac SKF thicknesses. In a group of 15 patients with AN, with a mean age of 24 years, Mayo-Smith et al. (1989) measured triceps, biceps, subscapular, and suprailiac SKF, and calculated percentage body fat using the Durnin and Womersley equation (1974). They obtained a value of 13% body fat, in comparison to 29% for their 39 healthy female volunteers. In the same study, computed tomography (CT) was used to assess subcutaneous and visceral fat. In the AN patients subcutaneous fat was 20% and visceral fat was 50% that of normal-weight volunteers. Mazess, Barden & Ohlrich (1990b) used dual-photon absorptiometry (DPA) to measure body fat in 11 AN patients, with a mean age of 21 years, with more than a one year history of AN, and a history of amenorrhea ranging from 1 to 8 years. They obtained a value of 7.8% body fat, in contrast to a value of 26% seen in the 22 control subjects. Although the above researchers utilized different methodologies, they all observed significantly lower body fat values in malnourished AN patients compared to a range of 25-35% body fat commonly seen in normal-weight females (Mitchell & Truswell, 1987).

There has been significant interest in the relationship of body fat to menstrual status and endocrine factors in AN. From studies of adolescent women with primary and secondary amenorrhea, Frisch and MacArthur (1974) proposed that the onset and maintenance of regular menstrual function are both dependent upon maintenance of a minimum weight for height. This hypothesis, however, has been rejected. A more widely accepted theory is that menstruation is determined by multiple factors related to the processes of growth and development which are regulated by central nervous system maturation, rather than specifically to body fat percentage (Mitchell & Truswell, 1987). Nevertheless, this hypothesis has provided a framework for exploring the relationship between gonadotrophin secretion and changes in body fat in AN patients (Jeuniewic, Brown, Garfinkel, & Moldofsky, 1978).

2) Fluid and Electrolyte Status

Edema has been recognized as a common consequence of severe starvation (Mitchell & Truswell 1987). The cause of this "famine edema" is still not well understood. Under normal conditions, the body has the ability to closely regulate the amount of total body fluid (Reiff & Reiff, 1992). However, this is not the case in AN patients, who often experience rebound fluid retention, manifested by thirst, decreased urinary output, or puffiness in the fingers, ankles and face. This phenomenon may occur for a variety of reasons including low albumin levels, excessive periods of time spent standing, bingeing on large quantities of salty foods (Kaplan, 1990), self-induced vomiting, or laxative and diuretic abuse (Reiff & Reiff, 1992). The body's adaptive response to these phenomena is to retain more total fluids than normal. It is thus not uncommon for AN patients to experience 2-5 kg weight gain following rehydration. Since these patients find weight gain so frightening, they are unable to distinguish between long-term and temporary water weight shifts, and tend to view all weight increases as changes in body fat related to caloric intake. They respond by further restricting, exercising, bingeing and purgeing, creating a vicious cycle that is resistant to treatment. With respect to the specific impact of edema on body composition, studies in undernourished populations and AN patients have found that ECF space accounts for an increased proportion of total body weight (Moore, McMurray, Parker, & Magnus, 1956; Dempsey et al., 1984; Vaisman, Corey, Rossi, Goldberg, & Pencharz, 1988b). As well, ICF as measured by the difference of TBW and ECF has been shown to decrease, reflecting the decrease in cellular tissue (McCance & Widdowson, 1951; Moore et al., 1956).

3) Skeletal System

There has been increasing concern over the long-term effects of AN on bone structure. In fact, a number of reports have found significant osteoporosis and pathologic bone fractures in AN patients (Biller, Saxe, & Herzog, 1989; Rigotti, Neer, Skates, Herzog, & Nussbaum, 1991). The pathophysiology of osteoporosis is multifactorial and not well understood. Several metabolic consequences of AN could adversely affect skeletal mass, including estrogen deficiency, secondary hyperparathyroidism due to low calcium intake or vitamin D deficiency, cortisol excess and protein-energy malnutrition (Rigotti et al., 1991; Salisbury & Mitchell, 1991; Seeman, Szmukler, Formica, Tsalamandris, & Mestrovic, 1992).

Mazess et al., (1990b), assessed bone mineral content (BMC) and bone mineral density (BMD) of the total skeleton, BMD of the lumbar spine and the proximal femur, and total body soft-tissue composition using DPA in 11 female patients with AN. Their findings confirmed those of previous studies that AN is accompanied by decreased percentage body fat, a reduction in BMC by 10%, and preferential spinal osteopenia.

The first longitudinal study on changes in bone mass in AN was reported by Rigotti et al. (1991). This study investigated whether any therapeutic intervention, particularly weight gain would lead to increased bone mass in 27 women with AN, who were followed for an average period of 27 months. No significant change in cortical bone density of the radius as assessed by single-photon absorptiometry was observed during the follow-up period, even though most of the patients had gained some weight, exercised regularly, resumed menses, and increased their calcium intake. As well, no difference was observed between those subjects who had recovered to within 15-20% of their ideal body weight and those who had not. These results were unexpected and contrasted with studies in which estrogen-deficient women had increased cortical mass following treatment and resumption of menses (Klibanski & Greenspan, 1986). Although the results of the Rigotti et al. study are preliminary, there is concern that bone mass remains low despite recovery from AN. It further suggests that the occurrence of AN in young individuals may adversely affect skeletal integrity during adulthood resulting in a predisposition to osteoporotic fractures (Rigotti et al., 1991). To date, no controlled trials of estrogen replacement or calcium supplementation in AN have been reported, thus the proper treatment for decreased bone mineral density in these patients remains unknown. Until further research is undertaken in this area, the most important interventions appear to be medical stabilization and weight gain (Salisbury & Mitchell, 1991).

4) Skin Thickness

One of the assumptions underlying the use of SKF is constancy of skin thickness. However, results of a cross-sectional study by Savvas et al. (1989) who investigated the effects of AN on skin thickness do not support this assumption. Thirty six women with AN were compared with a control group of 33 female volunteers of comparable age. The median forearm skin thickness, assessed by a radiological technique developed by one of the authors, was significantly higher (0.88 mm) in the control group compared to the AN patients (0.70 mm). This finding implies that although the contribution of skin to total SKF thickness is generally not large, it may lead to overestimation of body fat in underweight AN patients..

D) Body Composition and Body Fat Distribution Changes after Weight Gain in Anorexia Nervosa

While numerous studies have examined the psychological and neuroendocrine aspects of AN, few have looked at body composition. Some of these studies have utilized techniques which have questionable validity in starved populations. As well, the majority of research has focused on changes that occur in total body composition with refeeding. As a result, when SKF measurements are performed, only sums of SKF thicknesses are reported as indicators of total body fat. It thus becomes impossible to evaluate the differential site responses and the distribution patterns of adipose tissue that may accompany refeeding (Himes, 1988).

A well-known study which examined body composition changes during nutritional rehabilitation (Keys et al., 1950), focused on refeeding after prolonged experimental semistarvation. When starvation is used as a model for AN, caution must be used as there are clinical differences between starved and anorexic populations. In order to lose weight, AN patients frequently engage in excessive exercise, and may also resort to purgeing and diuretic abuse. In contrast, the experimentally starved subjects voluntarily reduce their exercise levels during the period of weight loss (Mitchell & Truswell, 1987). As well, semistarvation studies normally attempt to replicate the food intake of populations in famine areas by using male subjects whose average daily intake is approximately 1,600 kcal (Keys et al., 1950). This is discrepant from the intake of AN patients which is usually between 700-800 kcal per day (Beumont, Chambers, Rouse, & Abraham, 1981). These figures clearly demonstrate that in terms of caloric intake the starvation of AN patients can be more severe than semistarvation reported in the experimental groups. Furthermore, semistarvation studies have primarily used male subjects, whereas anorexia studies typically use female subjects. Gender differences may affect body composition in ways that make comparisons difficult. Despite these differences, studies of semistarved populations are a useful analogue for AN.

The classic study of semistarvation by Keys et al. (1950) was undertaken at the University of Minnesota towards the end of the Second World War. The 32 male subjects participated in a program of 3 months controlled diet, 6 months of semistarvation, and 3 months of nutritional rehabilitation. During the semistarvation period, the male subjects displayed emotional and personality changes also seen in AN patients. They exhibited intense preoccupation with food, they spent prolonged periods eating their meals, and some had episodes of binge eating. Their mood became irritable, their concentration was poor, their sleep was disturbed, and they became socially withdrawn. At the end of the first 3 months of rehabilitation, subjects had regained 86% of their control weight. During the first 2 weeks of ad libitum eating, most subjects achieved food intakes as high as 7,000-10,000 kcal/day; however, eventually intakes levelled off to 3,200-4,500 kcal/day. By 5 months of rehabilitation when weight had been regained to equal previous control levels, subjects were approaching normal food intakes.

In the Minnesota study a number of body composition measurements were performed. Body density was used to measure body fat, and extracellular fluid volume was determined by thiocyanate dilution. The fluid findings along with assumed constants for the density of the edema fluid and constants for the maintenance of bone minerals determined by bone density, were used to correct the final determination of body density and thus body fat. "Active tissue" mass (mainly skeletal muscle, organs and red cells) was calculated from the difference between body weight, and the sum of fat, thiocyanate space and bone minerals. Lastly, body protein was measured by nitrogen balance and muscle mass was determined by creatinine excretion.

During refeeding, a more rapid recovery of adipose tissue compared to that of muscle was observed. At the stage of complete weight regain, the fat content was approximately 120% that of the control values. However, the active tissue mass was still 8% below the control. In terms of body fat distribution changes during nutritional rehabilitation, the male subjects regained their body CIRC to levels that equalled or exceeded the prestarvation basal values. At the end of the rehabilitation period, abdominal CIRC had the greatest rate of increase, followed by the thigh, arm and calf CIRC.

Results from the Minnesota study indicate that nutritional rehabilitation of severely undernourished adults is accompanied by a more rapid increase in AT compared to muscle. As well, CIRC measurements revealed preferential deposition of fat in the abdominal and thigh regions rather than the extremities. Although these observations are significant, the anthropometric measurements were performed at a limited number of body sites. Also, as previously mentioned, fat deposition patterns during refeeding in males cannot be extrapolated to anorexic women, as fat distribution patterns during weight gain may be different between the sexes.

Russell & Mezey (1962) investigated the changes in body composition upon refeeding in AN patients. Their objectives were to determine the composition of the tissues synthesized when AN patients were placed on a high-calorie diet, and the energy intake required to yield a given weight gain. Four AN patients, 18-22 years of age, with an initial weight range of 30-44 kg were treated for 5-6 weeks in a Metabolic Unit, where they were very closely supervised. During this time, the patients were provided with a liquid diet which was gradually increased to intakes of 3,500-5,500 kcal daily. Energy balance was measured by keeping a daily record of the patients' activities, the metabolic cost of which was estimated twice weekly by indirect calorimetry. Nitrogen balance was also measured daily. As well, SKF thicknesses were measured at 5 sites (triceps, scapula, midaxilla, abdomen and upper thigh), to determine the distribution of subcutaneous fat deposits. At the end of 6 weeks, weight gain in the range of 6-12 kg was achieved. An average energy requirement of 7,500 calories for a 1 kg weight gain was found. The results of the energy and nitrogen balance studies indicated that 1 kg of deposited tissue contained 77% fat, 7% protein and 16% water in chemical form. In other words, a weight gain of 1 kg was due to an increase of 770 g of fatty tissue and 230 g of cellular matter, the latter including an expansion of the intracellular space by 160 g (assuming that 70% of cellular matter is water). According to the authors, the extracellular space remained unaltered. It is unclear, however, why they assumed that the 16% water gain was entirely intracellular fluid. When changes in SKF thickness were expressed either in absolute or relative terms, no preferential fat deposition patterns were observed. However, when mean gains in SKF thickness (mm of subcutaneous fat) were expressed per kg of weight gained, the following results were obtained: abdomen (0.44 mm/kg), thigh (0.39 mm/kg), axilla (0.28 mm/kg), triceps (0.07 mm/kg), and scapula (0.05 mm/kg). These indicate a greater deposition of subcutaneous fat in the abdomen and thigh regions. Results from the Russell & Mezey study are thus in agreement with the Minnesota study in terms of the preferential deposition of subcutaneous fat that appears to occur in the abdomen and thigh regions upon refeeding.

Russell et al. (1983) examined changes in muscle function in relation to body composition changes, during an 8-week refeeding period, in 1 male and 5 female severely depleted AN patients. Anthropometric evaluation included weight, height, midarm muscle CIRC, wrist diameter, forearm diameter, iliac SKF (in females), pectoral SKF (in males), and triceps SKF. From these parameters, LBM and percentage body fat were calculated. Patients received an average of 2,100 kcal per day during the 8-week period. A weight gain of approximately 7.5 kg was achieved, with LBM and body fat accounting for 68% and 32% of the weight gain respectively.

Forbes et al. (1984) investigated the effect on body composition of two dietary regimes consisting of different protein content, during a 40-day rehabilitation of 2 male and 10 female AN patients. Patients were fed a hypercaloric diet providing 20% of energy from protein (n=5), or 10% of energy from protein (n=7). The initial caloric intake ranged from 1,600-1,800 kcal/day; this was increased by 400 kcal increments as needed to ensure a weight gain of at least 150 g/day. Nitrogen balance tests and potassium-40 assays were implemented. Body composition measurements included SKF thickness at the biceps, triceps and subscapula, and CIRC at the abdomen, buttocks, mid-arm and forearm. The investigators found no advantage in using high protein diets in the rehabilitation of AN patients. In terms of anthropometric changes during recovery, both groups had the greatest increase in the abdomen and buttocks CIRC with minimal changes in the arm CIRC, or in the biceps, triceps and subscapula SKF. Lean body mass was estimated by the potassium-40 method which assumes that FFM contains 68.1 mEq of potassium/kg in males, and 64.2 mEq/kg in females (Forbes, Gallup & Hursh, 1961). It was found that 70% of the 5 kg weight gain achieved was due to LBM in the high protein group and 61% in the low protein group. Due to the variability among subjects, this difference was not statistically significant.

Pirke et al. (1986) examined the body composition changes upon weight gain in 16 female patients with AN, as compared to 11 female normal-weight controls. The potassium-40 method was used to determine body cell mass (defined as the mass of organs, muscles, and ICF). Refeeding of the AN patients was accompanied by a weight gain of 7.9 kg; 55% of the regained weight was fat, and 45% was LBM. The authors noted that body cell mass was reduced in underweight anorexics when compared to normal-weight controls, but that it was normalized rapidly during weight gain, even before ideal body weight was achieved. However, despite significant gains in body weight and body fat during refeeding, body fat mass in the AN patients was still significantly lower than that of controls.

Vaisman et al., (1988b), investigated the changes in body composition before and during 2 months of refeeding in 13 adolescent girls with AN. Body fat mass and FFM were derived from the sum of triceps, biceps, subscapula and suprailiac SKF. Intracellular fluid was assessed by the potassium-40 assay, and ECF was measured as the bromide space after oral bromide administration. The authors noted a gradual increase in weight, body fat mass, FFM and total body potassium (TBK) during refeeding. Despite the fact that none of the subjects had clinically detectable edema, ECF was expanded on admission and increased in all patients during the first 5 weeks of treatment, however it later subsided. Overall, refeeding was accompanied by a weight gain of approximately 6 kg, and percentage body fat increased from 16% to 21%. In terms of the composition of the regained weight, 50% was fat and 43% was LBM; approximately 7% of the regained weight was unaccounted for in terms of its composition. The authors concluded that most of the changes in FFM over the first weeks of refeeding could be accounted for by an expansion in ECF.

Melchior, Rigaud, Rozen, Malon, & Apfelbaum (1989), followed 11 female AN patients for a period of 5 months. Body composition measurements included SKF at 4 body sites. A weight gain of approximately 8 kg was observed, 52% of which was comprised of fat mass and 48% of LBM.

Lastly Russell et al. (1994) examined the effects of weight restoration on body composition in AN patients, using anthropometry and in vivo neutron-capture analysis (IVNCA). Thirty two females with AN and 29 matched control subjects were recruited for this study. Total body protein was obtained from measurements of total body nitrogen by IVNCA; total body fat and LBM were derived from the sum of 4 SKF. The initial mean weight of the AN patients was 73% of that of control subjects, increasing to 90% of mean weight of control subjects after refeeding. Overall, a 10 kg weight gain was achieved in the AN subjects. Compared with the control group, AN patients' nitrogen was initially depleted by 25%, increased by 18%, but remained 11% below control values. Body fat was depleted by 58%, increased by 90%, but remained 22% below control values. Therefore, despite a greater initial depletion and a subsequently greater net gain, body fat remained relatively more depleted after refeeding, than did nitrogen and protein. It appears that protein stores are less affected during depletion, and thus remain closer to control values at the end of rehabilitation.

Table 1 summarizes the findings of these studies.

Table 1:

Summary of studies investigating the composition of regained weight in AN patients[a]

Authors	# of Subjects	Weight Gain (kg)	Method	Body Fat (%)[b]	LBM (%)[b]
Russell & Mezey (1962)	4 Females	8.0	SKF[c]	77.0	23.0
Russell et al. (1983)	5 Females & 1 Male	7.5	SKF	32.0	68.0
Forbes et al. (1984)	10 Females & 2 Males	5.0	SKF TBK[d]	61.0[e] 70.0[f]	39.0[e] 30.0[f]
Pirke et al. (1986)	16 Females	7.9	TBK	55.0	45.0
Vaisman et al. (1988b)	13 Females	5.4	SKF TBK	50.0 --	-- 43.0
Melchior et al. (1989)	11 Females	8.4	SKF	52.0	48.0
Russell et al. (1994)	32 Females	9.9	SKF	57.6	42.4

[a] adapted from Russell, J. D., Mira, M., Allen, B. J., Stewart, P. M., Vizzard, J., Arthur, B., & Beumont, P. J. V. (1994). Protein repletion and treatment in anorexia nervosa. *American Journal of Clinical Nutrition*, 59, 98-102

[b] % of regained weight

[c] skinfold thickness

[d] total body potassium

[e] value for low protein-fed group

[f] value for high protein-fed group

This review of the literature indicates that the results reported by various studies on the composition of tissue deposited during weight gain are discrepant. These discrepancies may be due to differences in sample size, initial state of nutrition, sex, rate of weight gain, composition of the diet, degree of physical activity, presence of edema, and the varying body composition methods used, some of which may give false interpretations when used in starved subjects (Mitchell & Truswell, 1987). For example, there are problems with the use of the potassium-40 assay in assessing body composition in AN patients. Hannan, Cowen, Freeman, & Shapiro (1990b) demonstrated that the relationship between TBK and FFM is not linear in patients at low weight. Use of the assumed constants for the relationship between TBK and FFM would underestimate the latter at low body weights, leading to overestimation of the difference before and after refeeding and an erroneously higher proportion of FFM in the regained tissue. Also, a number of investigators have made the incorrect assumption that LBM contains a constant amount of potassium, when in fact it is FFM which is related to TBK. As has been previously mentioned, LBM and FFM are not synonymous, thus interchangeable use of these terms can lead to errors.

In terms of the body fat distribution component of these studies, there is general agreement that preferential AT deposition in the abdomen and thighs may occur during the refeeding of AN patients, as assessed by SKF and CIRC. However, it is important to note that the number of subjects used in these studies was small, thus limiting statistical power. Furthermore, the selection of body sites for the measurement of anthropometric parameters was limited. As well, only SKF and CIRC were used; and to date, none of the more reliable technologies including dual energy X-ray absorptiometry (DEXA) have been utilized to explore fat distribution changes in refed AN patients. For the above reasons more carefully designed studies are warranted to further explore this area.

E) Measurement Techniques of Body Composition and Body Fat Distribution

This century has seen the advent of a number of techniques for measuring body compositon and AT distribution. Traditionally, hydrodensitometry was one of the most commonly utilized research techniques for measurement of body composition. In fact, it was and it is still considered by some to be the gold standard in body composition despite its limitations as a quantitative index of fat content (Lohman, 1981). Hydrodensitometry uses underwater weighing (UWW) to measure whole-body density. Percentage body fat is then mathematically derived based on the assumption that the body can be divided into 2 compartments: fat mass which has a density of 0.9 g/cm^3 and FFM which has a density of 1.1 g/cm^3 (Siri, 1956). This method, however, does not take into account differences in the densities and proportions of FFM constituents. This can lead to fat-free densities different from 1.1g/cm^3 and thus to potentially large errors. Bone mineral, although a relatively small compartment, has the highest density, and the percentage contribution of bone to LBM can vary with age, exercise habits, and various disease states (eg. osteoporosis and AN) (Wang et al., 1992b; Johansson et al., 1993). Further disadvantages associated with this method are that UWW requires specialized equipment and experienced personnel; it is dependent on the cooperation of the subject (full underwater submersion is required which may be difficult for some subjects); and it is affected by the air content of the lungs (pulmonary residual volume) and the gas content of the gastrointestinal tract (Craig & Ware, 1967; Bedell, Marshall, Dubois, & Harris, 1956).

Skinfold and CIRC measurements have also been used routinely. The combination of SKF and CIRC measurements provide valuable information on the relationship between adipose and lean tissues (Sjostrom, 1988). The utility of SKF has been twofold: first, they provide a relatively simple and non-invasive method of estimating general fatness, and second, they can be used in the characterization of the distribution of subcutaneous AT (Harrison et al., 1988). Similarly, CIRC and selected CIRC ratios have been used as indices of the patterning of subcutaneous AT (Callaway et al., 1988).

In subsequent years, TBK and TBW methods were developed for the determination of LBM, and TBN for the measurement of body protein (Mitchell & Truswell, 1987). Total body potassium is used as an indication of FFM because more than 90% of potassium is found within non-fat cells (Brodie, 1988). Total body potassium can be estimated by measuring ^{40}K (the radioactive form of potassium which is naturally present in the body in a constant proportion of the TBK) in individuals placed in a suitable shielded whole body counter. Thus, FFM is estimated on the assumption that each kg of FFM consists of 63 mmol of potassium (Fidanza, 1980). However, there has been increased controversy over the value of the ratio of body potassium to FFM. In fact, it has been demonstrated that the relationship between TBK and FFM is not linear in various disease states including AN (Hannan et al., 1990b). Total body water can be determined with an isotope dilution technique using deuterium or tritium (Gibson, 1990). This method is based on the assumption that 73.2% of FFM consists of water. The major limitation of this technique is the assumption made that the FFM of an adult contains a constant percentage of water (Fidanza, 1980; Gibson, 1990). Total body nitrogen is used to assess protein status, and it is based on the principle that nitrogen bears a fixed ratio to the mass of protein (1 g N : 6.25 g protein). However, muscularity, body hydration, and the protein and mineral content of the skeleton can affect the relationship of TBN to LBM, thus compromising the validity of this method (Brodie, 1988).

Since the beginning of the 1940s, several investigators have used radiological techniques to measure subcutaneous AT thickness. During the 1980's and 1990's, computed tomography (CT) has been used for measurements of AT. Computed tomography is currently considered to be the most reproducible body fat technique available, and it is the new gold standard of body composition (Sjostrom, Kvist, Cederblad, & Tylen, 1986; Grauer, Moss, Cann, & Goldberg, 1984; Armellini et al., 1993). Although CT has the distinctive advantage of measuring both subcutaneous and visceral fat, the amount of irradiation involved and the high cost of utilization, limit its use in field surveys and routine clinical assessments (Ferland et al., 1989; Kuczmarski, Fanelli, & Koch, 1987). The usefulness of ultrasonography (US) for measurement of body composition is also currently being investigated. This technique allows sonographic imaging of the internal body. The operation of US is based on the pulse-echo principle: a beam of high frequency sound waves is emitted, which penetrates the skin surface, passes through the AT and is then reflected off the fat-muscle interface. The time taken for the echo to return to the US receiver is converted to a distance score, and in this manner subcutaneous AT thickness can be measured (van der Koy

& Seidell, 1993). Ultrasound overcomes some of the limitations of CT in that it is portable, it does not emit radiation, and it is less expensive (Kuczmarski et al., 1987). However, several sources of measurement error are possible with this technique. These include the inevitable compression created as good contact between the skin and the transducer is required to form an image, as well as the difficulty in identifying which of the reflecting surfaces represent the boundaries of subcutaneous fat (Orphanidou, McCargar, Birmingham, Mathieson, & Goldner, 1994).

The development of dual energy X-ray absorptiometry (DEXA) has added further insights into total and regional body fat composition, and skeletal physiology (Lohman, 1992). Finally, bioelectrical impedance analysis (BIA) is increasingly being used in total body fat, TBW and LBM determinations (Brylowski, 1992).

In the remainder of this section, the underlying principles of SKF, CIRC, DEXA, and BIA will be outlined, and the usefulness of each in assessing body composition and fat distribution in AN patients will be discussed.

1) Skinfolds

One of the most practical and most widely used approaches to the assessment of body fat has been the use of SKF (Lohman, 1992). The fact that 50-70% of body fat is located subcutaneously, has given SKF a preeminent position in the quantification of body fatness (Garn, Sullivan, & Hawthorne, 1987; Lohman, 1992). Skinfolds provide a fast, inexpensive, and convenient way of measuring subcutaneous fat in clinical and field settings (Gibson, 1991).

Skinfold calipers are used to measure the thickness of a double layer of skin and subcutaneous AT. Various combinations of sites for SKF measurements have been suggested as the basis for equations for predicting total body fat content. The most widely used combination is that of four SKF, utilizing the sum of biceps, triceps, subscapula and suprailiac, proposed by Durnin & Womersley (1974). The authors produced linear regression equations for the estimation of body density (and therefore body fat) from the logarithm of the total of the four SKF thicknesses, for males and females, seventeen to seventy two years of age. The major limitation of this choice of SKF sites is that it encompasses only the upper body. There was subsequent development of other body fat prediction equations, (Jackson, Pollock & Ward, 1980; Jackson & Pollock, 1978), which utilize up to seven SKF sites, with inclusion of both the upper and lower body.

There are two controversies regarding the use of SKF equations (Lohman, 1992). One is the concept that SKF equations are often population specific, and are thus not applicable to other populations or even other samples of the same population. A second controversy relates to the accurate measurement of SKF, and the various technical errors associated with it. This procedure requires training and skill, since choice of an incorrect site can lead to significantly false results (Lohman, 1981). For skilled observers performing SKF in individuals with normal body composition, the standard error of estimation is approximately 3-4% of the body weight (Lohman, 1981; Durnin & Womersley, 1974). In unskilled investigators, however, the error is expected to be much greater.

A number of assumptions underlying the use of SKF have also been questioned, particularly when examined in terms of direct evidence from cadaver studies (Martin et al., 1985). These authors specifically challenged five of these assumptions. First, they argue that constant compressibility cannot be assumed, as there is a decline in caliper reading over the first minute of caliper application to the SKF. The cause of this variation may be a varying proportion of interstitial water in the AT (Brans, Summers, Dweck, & Cassidy, 1974). As well, similar thicknesses of subcutaneous AT may yield different caliper readings due to different degrees of tissue compressibility. Second, constancy of skin thickness cannot be assumed, as there is skin thickness variability in different body sites. Although it is recognized that the contribution of skin to total SKF thickness is generally not large, it may lead to significant errors in lean subjects, including underweight AN patients. Third, the patterning of subcutaneous AT exhibits very wide inter-individual variation. This warrants the selection of body sites from both the upper and lower body, and emphasizes the potential hazards of using fat prediction formulas that do not contain lower limb sites. Fourth, it has been shown that as the level of adiposity increases, its water content decreases, whereas fat content increases. Thus two identical thicknesses of AT may contain different fat concentrations. This implies that a constant fraction of fat in the AT cannot be assumed. And finally, SKF calipers can only measure subcutaneous AT. Thus, in order to estimate total body fat, assumptions must be made about the relationship between internal and subcutaneous fat.

Most studies have employed SKF thicknesses in investigations of total body composition. Their use as indicators of fat distribution has been reported in obese subjects during weight reduction (Gray et al., 1990; Sjostrom et al., 1986; Wadden et al., 1988). Their use in studies involving AN patients has been minimal, and has primarily concentrated on total body composition (Forbes et al., 1984; Vaisman et al., 1988a). An exception is the study by Russell & Mezey (1962), in which the authors examined body fat distribution after weight gain in patients with AN. One shortcoming of these anorexia nervosa studies has been that SKF measurements were performed only at a limited number of body sites, primarily in the upper body.

Body composition changes that commonly occur in AN may affect the accuracy of measurement by SKF. For example, edema in skin and subcutaneous tissues which occurs secondary to the relative increase in the ECF space in these patients, may mask the full extent of the subcutaneous tissue lost. However, the decreased amount of subcutaneous fat observed in AN patients allows for easier anatomical identification and access to body sites. Despite these inherent shortcomings, SKF measurements have the potential to provide valuable fat distribution information, if they are performed by experienced observers, and a wide selection of body sites is utilized.

2) Circumferences

Circumferences are useful in measuring the size of cross-sectional and circumferential dimensions of the body (Callaway et al., 1988). Ratios between selected CIRC of the trunk and limbs can provide indices of the patterning of subcutaneous AT (Callaway et al., 1988). Waist CIRC is an index of deep AT. When used in a ratio with the thigh or hip CIRC, waist CIRC is an indicator of the degree of android distribution of AT; the higher the waist:hip (WHR) or the waist:thigh ratio (WTR), the greater the degree of upper body obesity. A WHR greater than 0.95 in males and greater than 0.80 in females is considered to represent android body fat distribution (Ross, Leger, Marliss, Morris, & Gougeon, 1991). In contast, hip and thigh CIRC are associated with gynoid, or lower body obesity (Lohman, 1992).

The relationship between WHR and various diseases has been widely investigated. In a large prospective study of middle-aged men (Larsson et al., 1984), a significant association was observed between coronary heart disease and WHR (waist measured at the level of the umbilicus, and hip measured at the level of the iliac crest). This association was not as significant, however, when body mass index (BMI), SKF or CIRC were examined individually. In another prospective study, Lapidus et al. (1984) found the WHR measured in women (waist measured midway between the lower rib margin and iliac crest, and hip measured at the widest point between hip and buttock) to be a stronger predictor of cardiovascular disease than BMI or subscapular and triceps SKF.

A limitation in the use of CIRC measurements is that they do not allow evaluation of the potential contribution of visceral or subcutaneous fat, or LBM (Ross et al., 1991). An additional challenge is that various studies have used different anatomical definitions for the waist CIRC. Some authors consider the waist to be the narrowest part of the torso, others consider it to be at the point of the umbilicus (Ross et al., 1991). These discrepancies imply that CIRC location as well as fat depots are confounded in CIRC ratios (Lohman, 1992).

Only one study (Forbes, 1990) has utilized WHR in the investigation of a spectrum of diseases. This included 2 males and 30 females with AN. Although the actual CIRC data obtained were not included, the author stated that these patients had WHR which were normally distributed despite the marked loss in weight.

Overall, the use of CIRC ratios can be very helpful in observing changes in fat distribution patterns in AN patients, provided that sites from both the upper and lower body are used, and various CIRC ratios are measured.

3) Dual Energy X-ray Absorptiometry

Dual photon absorptiometry and more recently, DEXA were the first techniques available to study variation in the BMC of the body (Lohman, 1992). Recently, the application of DEXA has been expanded to estimate the fat and lean contents of the soft tissue of the body (Lohman, 1992).

In 1987, the first commercially available dual energy X-ray absorptiometer was designed to estimate spine BMC with greater precision and less radiation exposure than the dual photon absorptiometer. Comparisons between DPA and the new X-ray technique have shown good agreement; however, DEXA has shorter scanning times and increased precision (Shephard, 1991).

The fundamental principle on which DEXA is based, involves the differential attenuation by tissues of transmitted X-rays (Mazess, Barden, Bisek, & Hanson, 1990a). A high stability X-ray generator produces photons over a broad spectrum of energy levels. This photon output is filtered to produce two distinct peaks which distinguish bone from soft tissue (XR-Series Bone Densitometer Operator's Guide, 1992), with soft tissue being comprised of fat and non-skeletal fat-free mass (Heymsfield, Wang, Heshka, Kehayias, & Pierson, 1989). Total body scans with DEXA take only 14-20 minutes. Also the radiation dose is extremely low: 0.02 - 0.05 mRem is needed to scan the entire body, in contrast to a typical chest X-ray which emits 40 mRem (Lohman, 1992).

Dual energy X-ray absorptiometry is based on a three-compartment body composition model, and is thus able to quantify BMC (regional or whole body), total body fat content and lean tissue mass directly (Hart, Wilkie, Edwards, & Cunningham, 1992). This implies that DEXA measurement, unlike UWW and SKF which employ the two-compartment model for estimating total body fat and FFM, does not rely on the assumptions that BMC represents a fixed fraction of LBM (Hart et al., 1992). In a review of techniques and assumptions in measuring body fat, Martin & Drinkwater (1991) indicated that variation in bone density may influence the accuracy of fat values estimated from UWW. These authors proposed that alterations in bone density may underestimate the FFM component by 34% or overestimate it by up to 168%. Although bone density is affected by age, gender, menopausal status, and certain disease states, UWW cannot account for the variation in BMC, as it uses a constant value for the density of FFM (Pritchard et al., 1993). This points to the potential usefulness of DEXA in making improved estimates of FFM due to its capability of measuring BMC more precisely.

The short-term precision of DEXA has been described by Mazess et al. (1990a), for whole-body and regional estimates of lean tissue, bone, mineral, and fat based on 10 repeated measurements in each of 12 subjects (2 measurements per day) over a five to seven-day period. The authors reported the coefficients of variation for BMC and BMD of the total body to be 1.5% and 0.6% respectively. For percentage body fat in the soft tissue, the precision of measurement (standard deviation) was 1.2%. When bone mineral mass (g) was expressed as a percentage of fat-free soft tissue mass, a mean of 5.8% was found with a precision of 0.13% (coefficient of variation = 2.3%). This small amount of variation offers a degree of precision in estimating BMC never before available (Lohman, 1992).

Heymsfield et al. (1989), correlated percentage body fat of 13 subjects measured with DPA with the mean percentage body fat estimated by four criterion methods (UWW, TBW, TBK, and neutron activation analysis). The correlation coefficient was 0.95 and the standard error of estimation was equal to 2.5%. This is one of the lowest prediction errors found between two methods.

Going et al. (1993) studied the ability of DEXA to detect small changes (<2 kg) in body composition in 17 men and women during a dehydration-rehydration protocol, and compared this to UWW and deuterium dilution methods. Since water is found solely in lean tissue, the resulting changes in body weight were expected to reflect equal changes in LBM with estimates of bone and fat remaining unchanged. As expected, bone mass and fat estimates were unaffected by changes in hydration, however DEXA had a somewhat limited capacity to resolve small changes in soft-tissue mass secondary to fluctuations in hydration in LBM. In comparison to DEXA, UWW was found to be more affected by changes in FFM. Despite the somewhat limited ability of DEXA to resolve small changes in body weight into its lean and fat components, the changes induced in some subjects were not much greater than the reported limits of precision for this technique. On average, DEXA correctly attributed 98% of the change in body weight with dehydration to a change in FFM, whereas the change in FFM by UWW was only 65% of the change in body weight. As well, the change in FFM by DEXA was significantly correlated with the change in body weight ($r=0.67$), in contrast to a non-significant correlation of $r=0.28$ in UWW. The authors concluded that DEXA has potential for providing accurate estimates of changes in body composition in studies of longer duration in which greater changes in body mass occur.

DEXA may also be useful in assessing body fat distribution and regional body composition. For the estimation of trunk composition and abdominal fat, this method may offer a practical way to validate anthropometric dimensions as indices of abdominal fat (Lohman, 1992). Ley et al. (1992) used DEXA to investigate the effects of gender and menopausal status on body fat distribution and body composition in nonobese, healthy male and female volunteers. Dual energy X-ray absorptiometry was used to estimate total fat and total lean tissue. Also, default software lines were positioned to divide body measurements into the following four regions or "boxes," all of which had the same height: a) android waist region located between the upper part of the dorsal vertebra #12 and the iliac crest, b) android subscapular region which had its lower border at the level of the upper part of the dorsal vertebrae #12, c) gynoid hip region which had its upper aspect through the most superior point of the inner pelvis, and d) gynoid thigh region with its superior border at the level of the inferior border of the gynoid hip boxed region. One important region that was omitted, however, was the abdominal area surrounding the umbilicus. The researchers observed that their boxed regional measurements provided the opportunity to examine specific regions of fat distribution. Their findings, which corresponded to what was expected (in terms of the regional differences between men and women, and between pre- and post-menopausal women) supported the potential usefulness of DEXA in fat distribution investigations.

Because DEXA is two dimensional, it cannot differentiate between visceral and subcutaneous AT, in contrast to CT which permits such differentiation. However, CT has the major draw-backs of being expensive, time-consuming, and requiring a relatively high radiation dose. Svendsen, Hassager, Bergmann, & Christiansen (1992) used CT to develop a model which could predict visceral AT in postmenopausal women, as measured by DEXA combined with anthropometry. The percentage body fat determined by DEXA and the mean attenuation of soft tissue by CT were found to be highly correlated, with an error of estimation of 3%. The authors concluded that DEXA measurements of abdominal fat appear to be valid, referring to the potential that DEXA has in estimating regional body composition and abdominal fat.

In summary, the precision of DEXA in its primary role of measuring bone is excellent (Mazess et al., 1990a). In addition, measurements done with DEXA are both safe and quick, requiring minimal cooperation from the subject, and the equipment is much less expensive than for other techniques, especially CT. Such ease means that populations of great interest, such as the very young, the very old, and the sick can often be studied by DEXA when other methods cannot be applied (Roubenoff, Kehayias, Dawson-Hughes, & Heymsfield, 1993).

Despite these capabilities of DEXA, there are limitations associated with it. Although DEXA is based on a three-compartment model, it is still not free of the assumption of uniform hydration (Roubenoff et al., 1993). Abnormal hydration which is often seen in AN patients, can alter an individual's ratio of mass attenuation coefficient for lean tissue mass, which in turn can lead to errors in the estimation of lean tissue. Because lean and fat mass are summed to give total body weight, an error in lean mass is propagated to the fat compartment, where it may be proportionally much larger (Roubenoff & Kehayias, 1991). Also, DEXA measurements of bone are sensitive to the anteroposterior thickness of the body, so that results may be systematically different between thin and obese individuals (Roubenoff et al., 1993). Another consideration with DEXA technology is its precision in regional measurements of soft tissue body composition. It has been indicated, for example, that DEXA cannot distinguish clearly between soft tissue and bone in the thorax, because the arrangement of the ribs and spine prevents the X-ray beam from finding much bone-free soft tissue mass. This can subsequently lead to imprecise estimates of thoracic composition (Roubenoff et al., 1993). In view of these observations, the precision of DEXA in the determination of regional body composition needs to be further explored.

The above account describes both the capabilities and limitations of DEXA as a soft-tissue measurement of body composition. Nevertheless, due to its ability to provide direct measurement of bone tissue and skeletal mass, it is ideally suited for measuring, among other parameters, the bone mass in AN patients, which has been shown, in various studies, to be reduced (Rigotti et al., 1991; Biller et al., 1989; Salisbury & Mitchell, 1991). Dual energy X-ray absorptiometry also has potential as both a body composition and a regional fat distribution measurement technique. A unique feature of the present study is that it is the first one reported to utilize DEXA in examining both whole body composition and body fat distribution upon refeeding in AN patients.

4) Bioelectrical Impedance Analysis

Bioelectrical Impedance Analysis (BIA) is a relatively new body composition method, which is becoming increasingly popular in the laboratory, clinical and field assessment of human body composition, as it is safe, non-invasive, rapid, portable, inexpensive and easy to operate (Lukaski, 1990; Kushner, 1992). The underlying principle of BIA is that the impedance (Z) of a geometrically isotropic conductor is related to its length (L) and configuration, its cross-sectional area (A), and applied signal frequency (Kushner, 1992). In the body, lean tissues represent a low resistance, high conductance electrical pathway, because they contain large amounts of water and conducting electrolytes. In contrast, fat and bone which contain small amounts of fluid and conducting electrolytes are low conductance, high resistance electrical pathways. Impedance is a function of resistance (R) and reactance (X) across biological tissues (Twyman, & Liedtke, 1987). Resistance is equal to the pure opposition to flow of an alternating current. Reactance is the opposition to flow of an electrical current caused by capacitance produced by tissue interfaces and cell membranes. By definition, a capacitor consists of two or more conducting plates separated from one another by an insulating non-conductive material used to store the electrical charge. In the body, cell membranes theoretically act as capacitors with a bilayer of polar proteins and phospholipids separated by a core of non-conductive lipid (Kushner, 1992).

As stated above, the underlying principle of BIA is that the body is an isotropic conductor with a uniform length and cross-sectional area. However, this assumption is not entirely true. The geometrical shape of the human body more closely approximates a series of five cylinders (two arms, two legs and trunk) excluding the head. Since R is inversely proportional to the cross-sectional area, the upper and lower extremities (which have the smallest cross-sectional area) will have the most influence on whole-body R measurements. Conversely, the trunk which contains approximately 50% of the body mass, will contribute <5-12% of total body R (Patterson, 1989).

Bioelectrical Impedance Analysis assumes that FFM is linearly related to body height^2/body resistance or body height^2/impedance (Van Loan, 1990), and that FFM consists of a constant proportion of water (ie. TBW=0.732 FFM). However, in conditions of altered fluid and electrolyte status, the TBW:FFM ratio, and the intracellular to extracellular fluid volume may be altered (Lukaski, 1990). Thus, although it might be reasonable to speculate that BIA may be useful in estimating TBW, extrapolating this to FFM, probably yields spurious results (Cohn, 1985).

The most frequently used BIA analyzers are of single frequency, which measure impedance at a fixed 50 kHz frequency with a constant 800 μA alternating current (Chumlea & Guo, 1994). However, these analyzers do not allow the fractionation and quantification of TBW into ICF and ECF components (Chumlea & Guo, 1994).

Current applications of the tetrapolar BIA method have focused primarily on healthy individuals for the development and validation of prediction models (Lukaski, 1990). A critical and unresolved question is the validity of BIA in estimating body composition among patients with altered hydration state, including AN patients. To date, very few studies have explored the potential of BIA as a measure of body composition in AN. Hannan et al. (1990b) evaluated BIA in 38 anorexic women with a wide range of BMIs. Fat-free mass was obtained from the mean of three methods: TBW, TBK and prompt neutron activation of nitrogen and was then regressed against impedance to establish the prediction equation with the smallest standard error. This prediction equation used the body habitus parameters of weight, height^2/R and shoulder width. They also derived a BIA prediction equation for FFM estimation. Their results indicated that BIA compared favourably with the other methods even in patients with very low BMI. One major shortcoming of the prediction equation derived was, however, that it was based on three methods that also make body composition assumptions which may not be valid in AN patients. In a more recent study by Hannan and co-investigators (1993a), the ability of BIA and anthropometry to measure percentage body fat and its changes in patients with ED, was assessed by determining their agreement with

DEXA which was used as the reference method. The authors concluded that in these patients, BIA offers no significant improvement over equations based on anthropometry alone, for the prediction of either percentage body fat or its changes.

It appears that the marked shifts in fluid balance observed in AN may limit the usefulness of BIA which relies on the estimation of total body water as a predictor of lean body mass (LBM) (Krahn, Rock, Dechert, Nairn, & Hasse, 1993; Rock & Curran-Celentano, 1994). Calculation of LBM from TBW may in fact overestimate LBM in this patient population (Hannan, Cowen, Freeman, Mackie & Shapiro, 1990a). More long-term studies are required to further explore the clinical application of BIA in AN, possibly through the utilization of segmental and multifrequency BIA, which have the added advantage of assessing ICF and ECF compartments.

This review of the literature suggests that despite the fact that loss of body weight and concern about fatness are essential features of AN, there has been little investigation and minimal understanding of the biology of body composition in this condition. Only a few studies have examined the body composition changes which occur upon weight gain in AN patients. Furthermore, the results of these studies have been discrepant, primarily due to the utilization of varying body composition methodologies which may give false interpretations when used in starved subjects. As well, to date there has been no formal examination of changes in fat distribution during weight gain in these patients. No empirical data exist to substantiate or refute the patients' claim that body fat accumulation occurs preferentially in specific body regions upon refeeding. Thus the objective of the present investigation was to address unanswered questions regarding body composition and body fat distribution changes which occur during refeeding in AN, through the utilization of traditional as well as modern body composition methods.

CHAPTER THREE

METHODOLOGY

A) Pilot Study

Prior to the commencement of the present study a pilot study was undertaken to determine an appropriate method for the measurement of subcutaneous body fat (please see Appendix 1 for publication). Computed tomography can differentiate between subcutaneous and visceral fat, and is thus considered to be the gold standard for quantifying body fat depots particularly in the abdominal region (Ashwell, Cole & Dixon, 1985, van der Koy & Seidell, 1993). However, the dose of radiation and the high cost associated with its use, limit its utilization in routine body composition studies. Ultrasonography, which involves a more sophisticated technology than SKF, overcomes some of the limitations of CT in that it is portable, it does not emit radiation, and it is less expensive to operate (Kuczmarski et al., 1987).

The purpose of this pilot study was to compare US and SKF measurement of subcutaneous fat at 3 abdominal sites with CT which was considered to be the gold standard. If a good correlation was obtained between US and CT measurements, then US would subsequently be used to assess body fat gain in AN patients.

This was a cross-sectional study. Twenty two subjects (13 men and 9 women), ages 24-81 volunteered to participate in the study. All subjects were recruited from a group of patients who had been scheduled for an abdominal or pelvic scan by CT for diagnostic purposes in the Department of Radiology, St. Paul's Hospital, Vancouver, BC.

Body composition assessment included subcutaneous fat measurement by CT, US, and SKF at 3 distinct abdominal sites located 5 cm to the left of midline at 6 cm (site 1), 9 cm (site 2), and 12 cm (site 3) caudal to the inferior tip of the xiphoid process. The relationships among the 3 methods were analyzed by determining Pearson correlation coefficients. A graphical method described by Bland & Altman (1986) was also used to assess agreement among the 3 methods.

Significant correlation coefficients were observed between SKF and CT at all 3 abdominal sites (site 1, r = .60, p = .003; site 2, r = .70, p = .0001; site 3, r = .73, p = .0001). Ultrasound and CT methods only showed a significant correlation at site 3 (r = .54, p = .009). As well, the graphical method revealed that the variation in the US measurements was much greater than that of SKF when compared to CT values.

The results of this study indicated that relative agreement in the measurement of subcutaneous body fat between SKF and CT was superior to that exhibited between US and CT. As a result of this pilot study, US was not used to investigate body fat distribution changes following weight gain in patients with AN. Instead, DEXA was used to assess body composition and body fat distribution changes in this patient population.

B) <u>Experimental Design</u>

The present study followed an observational cohort research design. Twenty six malnourished, underweight females, ages 18-45, who met the diagnostic criteria for AN completed the study. Subjects were either inpatients or outpatients of the Eating Disorders Clinic, St. Paul's Hospital, Vancouver, BC. They all followed an individualized weight gain regime, and at the time of their recruitment to the study, they were receiving intensive psychiatric and psychological treatment.

Patients were followed for a period of 6 months, or until maximum weight was achieved, whichever occurred first. Assessments of dietary intake and measurements of body composition and body fat distribution were performed at baseline and at the point of maximum weight. Final measurements were performed once the weight agreed upon by the Clinic's treatment team and the patient was achieved and maintained for two consecutive weeks (see Section E of this chapter for details). Monthly follow-up visits were scheduled with the patients, as a means of maintaining contact, developing rapport, and monitoring their progress. Food intake was assessed by 4-day food records. Body composition measurements included: weight (wt), height (ht), elbow and wrist breadths (for frame size determination), SKF and CIRC at 9 sites, DEXA and BIA. All measurements and monthly follow-ups took place at St. Paul's Hospital.

This study received ethics approval by the University of British Columbia and St. Paul's Hospital Clinical Screening Committees for Research and Other Studies Involving Human Subjects (Appendix 2). Figure 2 outlines the experimental protocol.

C) Subject Recruitment

All subjects recruited for the study were under the medical care of the attending internist of the Eating Disorders Clinic at St. Paul's Hospital (Dr. C.L. Birmingham). Patients were eligible for enrollment in the study if they met the diagnostic criteria for AN as outlined in the Diagnostic and Statistical Manual of Mental Disorders, 3rd. ed. revised (American Psychiatric Association, 1987) (Appendix 3). Only patients who appeared motivated to follow the treatment protocol and gain weight were recruited.

Preliminary information regarding the objectives of the study was provided to potential participants in the form of a handout (Appendix 4) by Dr. Birmingham. Once they indicated a willingness to participate in the study, they received a detailed explanation of the study and were asked to sign a consent form (Appendix 5). Once written consent was obtained, the patient was enrolled in the study and measurements commenced. A target of 30 patients was established for recruitment.

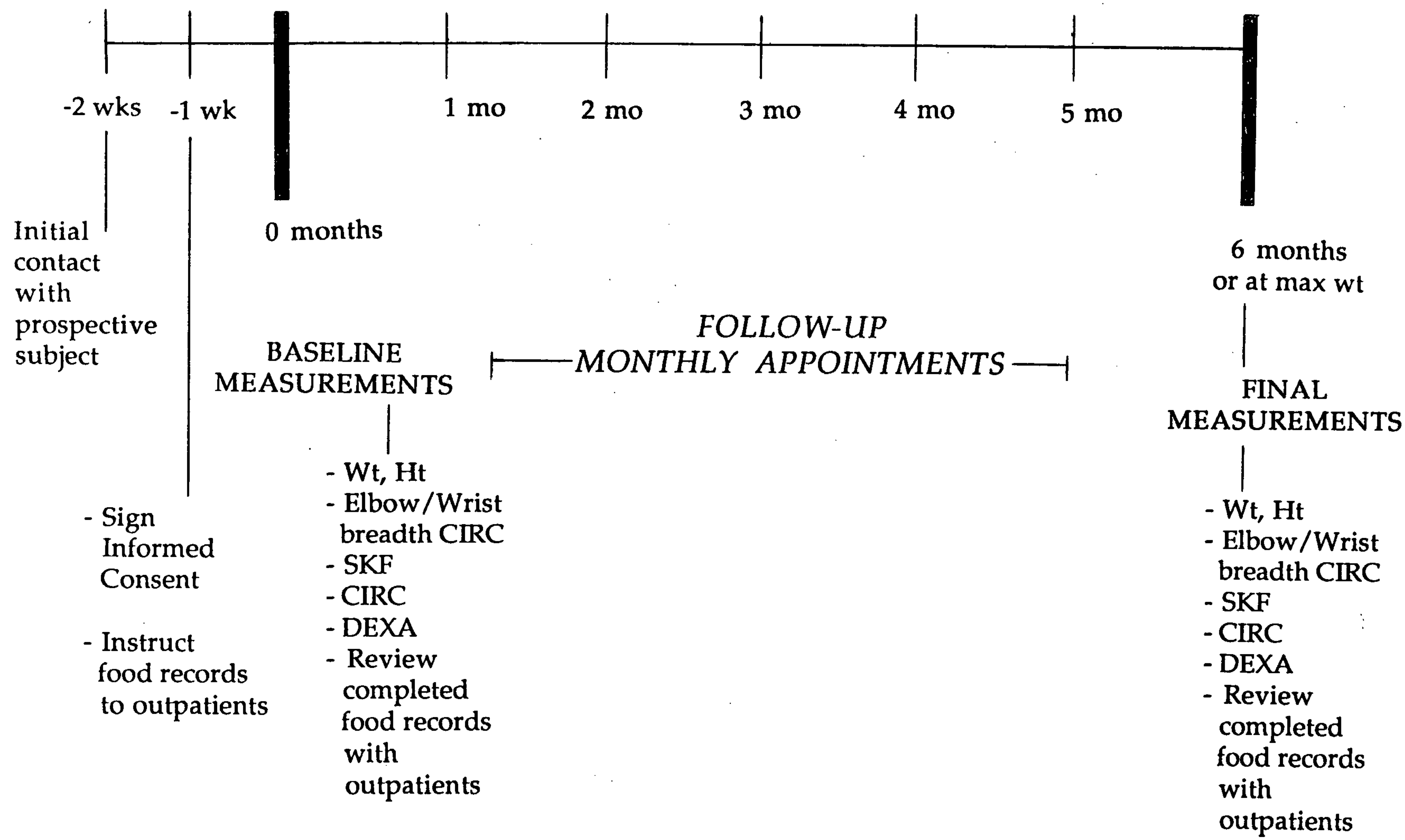


Figure 2: Experimental Protocol

D) Measurements of Body Composition and Body Fat Distribution

1) Anthropometry

A weight history is crucial in the initial evaluation of patients with AN (Kaplan, 1990). Baseline measurements included the adult premorbid weight which is the weight prior to any attempt by the patient to alter body weight or shape. It is commonly used as a crude approximation of a patient's individual set point, the weight which is genetically determined for physiological homeostasis (Kaplan, 1990). For patients who develop AN in early adolescence, prior to the closing of skeletal epiphyses there is no reliable way of establishing a premorbid adult weight (Kaplan, 1990). In such instances, an approximation of this weight was obtained by considering the average weight for age and height. The weight history also included the menstrual weight threshold (the weight at which amenorrhea or oligomenorrhea occurs) and the patient's desired weight. The discrepancy between the patient's desired weight and the premorbid weight was used as a rough measure of the patient's drive for thinness and degree of body image distortion.

All anthropometric measurements were performed by the same experienced investigator, to eliminate inter-observer variation. Patients with AN are unreliable in their self-estimation of body weight (Kaplan, 1990), thus baseline and final weights were established by objective measurement rather than by self-report. The patients' weight was obtained using DEXA. Height measurements were determined to the nearest cm and were taken while subjects stood erect without shoes. Body mass index was then calculated as weight in kg/height in m^2.

Frame size was determined by measuring elbow breadth (the distance between the epicondyles of the humerus) and wrist breadth (the distance between the ulnar and the radial styloid processes), using a sliding caliper. The procedure oulined in the Anthropometric Standardization Reference Manual (Wilmore et al., 1988) was followed. Measurements were taken to the nearest 0.1 mm and results were compared to standards adapted from Frisancho & Flegel (1983).

Edema status was assessed by the internist physician, by applying thumb pressure on the anterior tibial plateau. Edema was graded on a scale from 0 to 3 (0 = trace edema, 1 = definite edema, 2 = moderate edema, and 3 = marked edema). An edema of 3 was used as an exclusion criterion, as marked edema has been shown to confound the accuracy of SKF (Martin et al., 1985; Rock & Curran-Celentano, 1994) and BIA measurements (Hannan et al., 1990b; Kushner, 1992; Krahn et al., 1993).

Skinfold measurements were performed using Harpenden calipers (Creative Health Products, Plymouth, MI). These precision calipers are designed to measure the compressed double fold of subcutaneous fat and skin. They exert a defined and constant pressure of 10 g/mm^2 throughout the range of measured SKF and have a standard pinch area of 20-40 mm^2 (Brozek, & Henschel, 1963). For all the SKF measurements, the subjects stood erect with feet together and arms at the side. Caliper readings were read 3 seconds after the calipers were applied to the selected body site (Gibson, 1991). Three caliper measurements were taken at each site, and the median value was recorded to the nearest 0.1 mm. One measurement was done at all body sites, and this pattern was repeated for two more times to obtain a total of three measurements. This technique was employed in order to allow the tissue at each site to return to its original form after being compressed by the caliper.

Skinfold measurements were taken on the right side of the body at 9 sites: triceps, chest, subscapula, axilla, abdomen a (5 cm to the left of the umbilicus), and abdomen b (3 cm lateral to the midpoint of the umbilicus and 1 cm inferior to it), iliac, thigh and calf. A diversity of sites located in both upper and lower body was chosen to enable a more comprehensive examination of fat distribution changes with time. Skinfolds were measured at two abdomen sites, since a central aspect of this study was to determine whether preferential deposition of fat occurs in the abdomen region. The prodecures outlined in the Anthropometric Standardization Reference Manual (Harrison et al., 1988), were used as guidelines for the anatomical identification of body sites, and for the appropriate SKF measurement techniques. Appendix 6 includes diagramatic representations of the various SKF sites utilized in the study.

In the present study DEXA was the principal method used to determine body composition changes which occurred upon weight gain, in contrast to other studies which have used SKF to quantify these changes. Thus, in order to make direct comparisons with previous studies, body composition changes were also assessed by SKF. The equation for predicting body density in women developed by Jackson et al. (1980) was used. This equation utilizes 7 body sites: triceps, chest, subscapula, axilla, abdomen, iliac and thigh. The value obtained for body density was then used to determine percentage body fat by the Siri formula (Siri, 1961).

Circumferences were measured using a flexible, nonstretch tape. Measurements were done in triplicate, and the median was recorded to the nearest 0.1 cm. Circumferences were measured at 9 sites: arm, forearm, chest, waist a (at the level of the smallest circumference to the torso), waist b (at the level of the umbilicus), hip, thigh a (mid-thigh), thigh b (proximal thigh), and calf. All circumference procedures were performed according to the Anthropometric Standardization Reference Manual (Callaway et al., 1988). A detailed depiction of CIRC measurements is included in Appendix 7.

Ratios between selected CIRC of the trunk and limbs were used to provide indices of body fat distribution. The following ratios were calculated: waist a:hip, waist b:hip, waist a:thigh a, waist a:thigh b, waist b:thigh a, and waist b:thigh b.

2) Dual Energy X-ray Absorptiometry

All DEXA measurements were performed by an experienced technologist at the Department of Nuclear Medicine, St. Paul's Hospital, at baseline and at the end of the study.

An XR-26 MARK II/HS (high speed) DEXA scanner (Norland Corporation, Fort Atkinson, WI), was used.

During the DEXA measurement, patients were dressed in hospital gowns with all metal objects removed. The patient lay in a supine position, with the scanner placed directly above the top of the centre of the patient's head. The entire body was scanned from head to toes within 14 minutes, and an image was generated on an adjacent computer screen (Appendix 8a shows a whole body image computer print-out). This body image is divided into regions pre-determined by the XR-Series Control/Analysis computer software program (Norland Corporation, Fort Atkinson, WI) (Appendix 8b). One of the limitations of this program is that the pre-determined regions cannot be removed. However, a maximum of three new regions can be created provided that they are given names different from the existing ones. Appendix 8c, depicts the three regions that were introduced in an attempt to determine the fat distribution changes that occurred upon refeeding in the upper and lower body. These three regions were named as follows: a) subscapular region: located between the anterior axillary fold and the lowest point of the rib cage, b) waist region: between the lowest point of the rib cage and the uppermost point of the trochanter, c) thigh region: between the uppermost point of the trochanter and the mid-thigh. The DEXA data utilized were changes observed with time in: i) total body weight (kg), total body fat (kg), total percentage body fat, and whole body LBM (kg), and ii) body fat (kg) in each of the three regions. Changes in bone mineral content (kg) observed upon refeeding were also measured.

It is important to note that in the literature, android (upper body or central) fat distribution is defined as excess fat in the abdomen (McArdle et al., 1991). In contrast, gynoid (lower body or peripheral) fat distribution is associated with fat located in the hips and thighs (McArdle et al., 1991). However, for the purposes of the present study, the chest, abdomen, hips and thighs were all defined as central regions, whereas the arm and calf were classified as extremities.

3) Bioelectrical Impedance Analysis

A tetrapolar impedance plethysmograph (RJL Systems, Detroit, MI) with a 800 uA current at a frequency of 50 kHz, was used to determine body resistance (R) and body reactance (Xc) (Lukaski, Johnson, Bolonchuk, & Lykken, 1985). Measurements were made within 30 minutes of voiding, with the subject lying on a bed in a supine position, with socks and shoes removed. Spot electrodes were placed on the right hand dorsal surface at the distal metacarpal and on the right foot at the distal metatarsal. Electrodes were also placed between the distal prominence of the radius and ulna, and between the medial and lateral malleoli at the ankle (Lukaski, Bolonchuk, Hall, & Siders, 1986). A diagrammatic depiction of electrode placement is included in Appendix 9. Three measurements of R and Xc respectively were taken, and the median of each was recorded. The RJL BIA 101 Version 1.0 software (RJL Systems, Detroit, MI) was used to calculate percentage body fat. The above software package has been designed to estimate body fat percentage in individuals with normal body composition, and thus its applicability in AN patients is unknown. In an attempt to make more accurate estimations of percentage body fat in this study sample, a more updated BIA software package called Fluid Analysis 1.0a (RJL Systems, Detroit, MI) was also utilized. According to the manufacturer, this software version is more sensitive to hydration changes. Thus, it was anticipated that this analysis could provide further understanding of hydration changes in AN patients who experience considerable edema upon refeeding.

E) Meal Support and Renourishment Protocol

The critical factor for recovery in AN is the restoration of a healthy weight (Casper, 1982). Various approaches to weight restoration are used in clinics world-wide. Most advocate the promotion of normal eating in AN patients, without the use of liquid supplements, enteral tube feeds, or parenteral nutrition (Williams, Touyz & Beumont, 1985). In fact, some clinicians consider these artificial methods of nourishment as intrusive and anti-therapeutic (Garner, 1985). In contrast, there are those who propose that the use of liquid supplements and tube feeding allow easier weight recovery under certain conditions (Kennedy & Abbas, 1993; Williams, 1958). Anorexic individuals who require a large caloric intake to achieve weight restoration sometimes find it acceptable to eat a normal amount of food at meals and snacks and to increase their caloric intake by drinking liquid supplements. Supplementation may be considered a medicine that can be discontinued when the individual's goal weight has been achieved (Fairburn & Cooper, 1989). Occasionally, anorexic patients prefer exclusive nasogastric tube feeding for weight restoration. Tube feeding may allow these individuals to distance themselves from their ambivalence over actively eating and gaining weight. However, the resumption of normal eating behaviours should be established as early as possible during the course of treatment.

To achieve weight restoration in AN patients, the Eating Disorders Clinic at St. Paul's Hospital has implemented a meal support and renourishment protocol, which allows the assessment and documentation of patient eating behaviour and dietary intake. Upon hospital admission, low-weight patients are expected to gain 1 kg/week, and 1% body fat. This is accomplished by an appropriate meal selection plan developed and agreed upon by the inpatient dietitian and the patient. The patient's caloric intake is gradually increased from 1,200 kcal to a level that is more appropriate for age and height (usually in the range of 2,400-3,500 kcal). Meals are supervised by nursing staff, in order to provide patients with encouragement and support, to monitor the amount of food consumed, and to prevent hiding or disposal of food by patients. Food that is not eaten by the patient during each meal, is replaced in the form of a liquid supplement, which the patient is required to consume after the meal. Patients are required to spend 1-2 hours on supervised bed rest after their main meals (lunch and supper) to prevent overexercising or self-induced vomiting. If a patient has not made progress in gaining weight and increasing body fat, the possibility of tube feeding is discussed with the patient, and a combination of oral and nasogastric tube feeding is implemented. As medical and nutritional stability impoves, patients are encouraged to become more physically active. Once the agreed weight and percentage body fat are achieved (usually between 22-25% body fat), a maintenance diet is instituted. Patients, however, are advised to stay 1-2% above the maintenance percentage body fat as a safety margin. Following discharge, patients are followed as oupatients once a week, through the Outpatient Nutrition Clinic conducted by the attending internist and a dietitian.

The aim of the present study was not the provision of nutritional intervention during the rehabilitation of AN patients. Instead, the objective was to monitor the changes in body composition and body fat distribution which accompanied the weight gain accomplished as a result of the established refeeding protocol followed by the clinic.

F) Assessment of Dietary Intake and Exercise Behaviour

1) Dietary Intake Assessment

The dietary intake of subjects who received outpatient treatment was assessed by two four-day food records (Appendix 10) completed at baseline and at the end of the study. Intake of inpatients was monitored by calorie counts performed by the attending inpatient dietitian. One of the major issues in assessing nutritional status in individuals with chronic diseases including AN, is how to accurately measure usual intake of nutrients (Potosky, Block, & Hartman, 1990; Schoeller, 1990). Despite the importance of measuring dietary intake, the existing dietary assessment methods have never been fully validated (Block, 1982).

The validity of a dietary intake assessment method describes the degree to which it measures what it is intended to measure (Block, 1982). It is difficult to validate food records because the "truth" about dietary intake is never known with absolute certainty (Gibson, 1991). Even if actual food intake, monitored by unobstrusive weighed observations compares favorably with results obtained from food records maintained during the same period, there is no guarantee that the records represent the usual food intake (Block, 1982).

Precision of a dietary intake method in terms of reliability and reproducibility is another important consideration. A dietary assessment method is considered precise if it gives very similar results when used repeatedly in the same situation (Gibson, 1991). A seven-day food record has been found to be appropriate for estimating the average intake of individuals. However, its use can place a considerable burden on respondents and problems with compliance may arise (Barrett-Connor, 1991; Gibson, 1991). In one study (Gersovitz, Madden, & Smiciklas-Wright, 1978) it was reported that food records completed at the early stages of record-keeping (days 1 and 2) were more valid than those from days 5, 6 and 7. As well, a 4-day food record has been shown to estimate usual protein intake within 10%, with 95% confidence for a small group of individuals (Basiotis, Welsh, Cronin, Kelsay, & Mertz, 1987). For the above reasons, the use of 4-day food records seemed appropriate.

Patients were asked to record the time of day, location and amounts of all foods and beverages that they consumed for a period of four days. Three consecutive weekdays and one weekend day were included to account for potential day-of-the-week-effect on food and nutrient intake (Gibson, 1991). Patients were provided with written guidelines on how to complete the food records. They were instructed on how to describe cooking methods, foods eaten and how to estimate portion sizes. Those patients who exhibited purging behaviour were asked to record the foods that they had purged. Once the completed food records had been reviewed with the patient, the energy content (kcal) of the food intake reported, was determined using the Food Processor II Software Package (ESHA Research, Salem, OR). The food intake record form was adapted from the food intake form used by the Dietary Department, University Hospital, UBC site, and that developed for a previous clinical nutrition research study (Reid, 1990).

2) Exercise Behaviour

Excessive physical activity is a common feature of AN (Kron, Katz, Gorzynski, & Weiner, 1978; Falk, Halmi, & Tryon, 1985). Although many authors have commented on hyperactivity in their AN patients, it is only recently that the importance of this behaviour in the symptomatology of AN has been recognized. Epling, Pierce & Stefan (1983), and Epling & Pierce (1991) have suggested that the association of increasing activity and decreasing food intake is crucial for the pathogenesis of a subset of AN known as "activity anorexia." It has long been known that exercise is accompanied by a paradoxical decrease in appetite in AN patients (Beumont, Arthur, Russell, & Touyz, 1994). In fact, it is common among severely emaciated patients to engage in increased activity as their weight decreases, until they become unable to perform basic tasks of daily living (Beumont et al., 1994). In contrast to most healthy individuals who exercise for the purposes of fitness, health, enjoyment, and recreation, AN patients view exercise as a means of losing weight, disposing calories and gaining feelings of self-worth. Their exercising regimes are rigid, inflexible, and addictive, and the frequent admission among patients of feelings of chronic fatigue, further confirm that their level of activity is excessive (Beumont et al., 1994). Typical exercise patterns in AN include continously standing or being in motion, and spending a considerable part of the day pacing, jogging, powerwalking, or doing calisthenics (Kaye, Gwirtsman, Obarzanek, & George, 1988). This is compounded by restless hyperactivity, characterized by a patient's difficulty in keeping still even for short time periods, becoming distressed if constrained to do so, and performing tasks in an anxious and hurried manner (Beumont et al. 1994).

Since the current diagnostic criteria for AN (American Psychiatric Association, 1987) make no reference to activity anorexia, the Eating Disorders Clinic, St. Paul's Hospital uses the following common features encountered in overexercising AN patients (Beumont et al., 1994; Thompson & Trattner-Sherman, 1993) as criteria: a) insistence on exercising daily, as if life depends on it; b) experiencing "withdrawal" symptoms such as irritability, anxiety, and depression when restrained from exercising and c) continuing to exercise even when medically contraindicated. Presence of all of the above features is used as an indication of overexercising behaviour. In the present study, information on the exercise behaviour of patients was based on these criteria.

G) Statistical Analysis of the Data

The Systat PC statistical program (Systat Inc, Evanston, Il) was used to perform all statistical tests. The acceptable level of significance was set at $p < .05$. The difference between initial and final body weight was analyzed using a one-factor repeated measures ANOVA. Differences between pre- and post-weight gain body composition parameters (ie. body fat, percentage body fat, LBM, and bone mineral content) were assessed using a repeated measures ANCOVA design, with initial body weight as the covariate.

Body fat distribution changes which accompanied weight gain were assessed using a repeated measures ANCOVA with initial body weight as the covariate. Both the absolute and the relative change which occurred at each body site (for SKF, CIRC), or body region (for DEXA) following weight gain, were analyzed. Post-hoc contrasts on the absolute and relative change among means for the various body sites and regions were conducted by the Scheffe method for complex contrasts.

A graphical method described by Bland & Altman (1986) was used to assess agreement between BIA and DEXA in the measurement of change in percentage body fat. These authors propose that this method is useful when the true values of certain parameters (eg. percentage body fat) remain unknown. Under such circumstances a new method has to be evaluated by comparison with an established technique, rather than with the true quantity. Thus when two methods are compared, neither provides an unequivocally correct measurement, however, it is still possible to assess the degree of agreement between them. According to these authors, the use of the Pearson correlation coefficient is inappropriate when measurement techniques are compared. In other words, a high level of correlation does not necessarily signify that two methods produce similar results. One assumption made when this graphical method was used to assess agreement between DEXA and BIA was that DEXA was the more accurate, and thus the more established technique for the measurement of change in percentage body fat.

CHAPTER FOUR

RESULTS

A) Study Participants

Twenty six of the 30 patients enrolled in the study completed both baseline and final measurements. Of the 4 who did not complete the study, one patient died of cardiac failure and the remaining 3 patients chose not to participate in the final measurements. Seventeen of the 26 patients had both sets of measurements performed while they were inpatients. Three of the patients had one set of measurements done as inpatients, and the other set as outpatients. The remaining 6 subjects completed all measurements while followed on an outpatient basis, and they did not receive inpatient treatment during the study. The 20 subjects who received inpatient treatment had an average of 1.7 hospitalizations during the study, with a mean length of hospitalization of 11 weeks. These patients were enrolled in the study for an average of 20 weeks. In contrast, outpatients were followed for an average of 48 weeks to provide them with a longer time interval during which they could achieve weight gain, as they did not receive the vigorous refeeding protocol that the inpatients received. Table 2 provides a detailed hospitalization profile and time period of enrollment in the study of the 26 subjects.

The data analyzed were based on the 26 patients who completed both baseline and final measurements. In addition, the data of inpatients only were analyzed and these results are included in Appendix 11. No differences were observed in the results of the overall data as compared to the inpatient data, with the exception of one relative circumference and one circumference ratio. These differences are discussed in pertinent sections in Chapter 5.

Table 2:

Hospitalization profile and length of study enrollment of AN patients

Subject #	# of Hospitalizations During Study	Total Length of Hospitalizations (weeks)	Total Length of Time Enrolled in Study (weeks)
Inpatients			
1	1	12	12
2	2	12	12
3	4	20	32
4	1	8	8
5	1	6	36
6	2	6	40
7	2	11	12
8	1	5	5
9	1	12	12
10	2	3	36
11	2	12	28
12	2	15	24
13	2	16	16
14	1	10	10
15	1	8	8
16	1	12	12
17	2	12	12
18	2	12	12
19	1	12	32
20	2	23	44
Mean	*1.7*	*11*	*20*
Outpatients			
21	--	--	48
22	--	--	48
23	--	--	48
24	--	--	48
25	--	--	48
26	--	--	48

B) Baseline Data

Only the data of the 26 patients who completed the study were included in the analysis. All data are presented as mean $\pm$ SD. Baseline subject characteristics are shown in Table 3. The low baseline values in weight and BMI, and the long history of AN in these patients reflect the chronicity and severity of their malnourished state.

Table 3:

Baseline subject characteristics of patients with AN[a]

Characteristic	Baseline
Patients (n)	26
Sex	F
Age (yr)	27.6 ± 6.6
Disease duration (yr)	10.6 ± 6.4
Weight[b] (kg)	43.6 ± 5.4
Height (cm)	162.3 ± 5.7
BMI[c] (kg/m^2)	16.5 ± 1.9

[a] mean $\pm$ SD
[b] weight (kg) obtained by DEXA
[c] BMI = body mass index (weight [kg]/height [m^2])

C) Dietary Intake and Exercise Behaviour

Table 4 depicts the estimated post-weight gain maximum energy intake (kcal/day) for the 26 subjects during the course of their participation in the study. Subjects 1-20 received inpatient hospitalization, and calorie counts for these patients were performed by the attending inpatient dietitian. In 16 of the 20 inpatients, refeeding was achieved by a combination of oral intake and tube feeding; the remaining 4 inpatients were renourished solely by means of oral intake. Subjects 21-26 were outpatients, and they were asked to complete 4-day food records, which were analyzed using the Food Processor II software package (ESHA Research, Salem, OR). Overall, the post-weight gain maximum energy intake of hospitalized patients was 3,450 kcal, which was greater than that of 1,260 kcal seen in the outpatients. This difference in caloric intake is due to the rigorous refeeding protocol implemented by the inpatient clinic. Seventeen of the 26 subjects engaged in purging behaviours, whereas the remaining 9 patients restricted their food intake without purging. Table 4 also provides information on the exercise behaviour exhibited; 23 of the 26 subjects were overexercisers.

Table 4:

Estimated post-weight gain energy intake and purging and exercise behaviours of patients with AN

Subject #	Mode of feeding	Estimated Post-Weight Gain Energy Intake (kcal/day)	Purging Behaviour	Overexerciser
Inpatients[a]				
1	oral	2000	no	yes
2	oral + tube-feed	4500	no	yes
3	oral + tube-feed	4650	yes	yes
4	oral + tube-feed	3750	no	yes
5	oral + tube-feed	4100	yes	yes
6	oral + tube-feed	3910	yes	no
7	oral + tube-feed	3300	yes	no
8	oral	3500	yes	yes
9	oral + tube-feed	4700	yes	yes
10	oral + tube-feed	3500	yes	yes
11	oral + tube-feed	4200	no	yes
12	oral + tube-feed	3300	yes	yes
13	oral + tube-feed	2700	no	yes
14	oral	2000	no	yes
15	oral	3190	no	yes
16	oral + tube-feed	3500	yes	yes
17	oral + tube-feed	3850	yes	yes
18	oral + tube-feed	2600	yes	yes
19	oral + tube-feed	2640	yes	yes
20	oral + tube-feed	3140	yes	yes
Outpatients[b]				
21	oral	2000	yes	yes
22	oral	1000	yes	yes
23	oral	1040	yes	yes
24	oral	1330	no	yes
25	oral	2000	no	yes
26	oral	200	yes	no

a for inpatients, energy intake estimated by attending dietitian
b for outpatients, energy intake estimated by 4-day food records completed by the subjects

D) Body Composition Changes

The mean values for body composition parameters pre- and post-weight gain are shown in Table 5. The weight gain of 6.7±5.3 kg achieved by the subjects was found to be highly significant (p = .000), as well as the increase in BMI of 2.5±2.0 kg/m^2 (p = .000). An increase of 8.2±5.4 in percentage body fat and an increase of 5.3±3.5 kg in total body fat were both found to be highly significant (p = .000). Significant increases in total LBM (1.4±2.9 kg; p = .03) and total bone mineral content (0.057±0.089 kg; p = .005) were also observed.

Measurement of percentage body fat using SKF indicated pre- and post-weight gain percentage body fat values of 10.0±2.2 and 14.4±2.6 respectively. An increase of 4.5±2.2 in percentage body fat as determined by SKF was found to be highly significant (p = .000). It can be seen that the pre- and post-weight gain values of percentage body fat as determined by SKF are discrepant from those obtained by DEXA.

Table 5:

Comparison of body composition parameters pre- and post-weight gain in patients with AN

Characteristic	Pre	Post
Weight[b] (kg)	43.6 ± 5.4[a]	50.3 ± 5.9***
Height (cm)	162.3 ± 5.7	--
BMI[c] (kg/m^2)	16.5 ± 1.9	19.0 ± 1.7***
Percentage body fat[b]	18.8 ± 6.2	27.0 ± 5.1***
Total body fat[b] (kg)	8.3 ± 3.2	13.6 ± 3.3***
Total lean body mass[b] (kg)	33.1 ± 4.3	34.5 ± 5.0*
Total bone mineral content[b] (kg)	2.1 ± 0.2	2.2 ± 0.2**

a mean ± SD
b values obtained by DEXA
c BMI = body mass index (weight [kg]/height [m^2])
* $p < .05$ pre vs post weight gain
** $p < .01$ pre vs post weight gain
*** $p < .001$ pre vs post weight gain

E) Body Fat Distribution Changes

Body fat distribution changes that accompanied weight gain in the 26 patients were analyzed separately for each of the 3 methods (ie. SKF, CIRC and DEXA). Both the absolute and the relative change which occurred at each body site (for SKF, CIRC) or body region (for DEXA) following weight gain, were analyzed. In the post-hoc statistical analysis for contrasts, all possible comparisons between individual body sites or combinations of these were analyzed. However, only those contrasts which had body fat distribution relevance, and which were found to be statistically significant are presented.

1) Skinfolds

Tables 6a and 6b describe the results obtained from the analysis of the absolute SKF change. The dependent variable, Absolute SKF Change was measured as the amount of change in mm of subcutaneous body fat, subtracting the initial from the final measurement. The greatest mean absolute SKF change (mm) was observed at the thigh followed by abdomen b, abdomen a, iliac, triceps, calf, axilla, subscapula, and chest (see Table 6a).

A repeated measures ANCOVA was performed on the dependent variable Absolute SKF Change. The within-subjects independent variable was body site, with 9 body locations. The covariate was initial body weight. There was a significant effect of body site, $F(8,200) = 18.272, p < .05$. The full table of results from the ANCOVA are shown in Table 6b.

Post-hoc contrasts on Absolute Skinfold Change among selected subsets of means for the various body sites were conducted by the Scheffe method for complex contrasts. All contrasts were judged against a Scheffe F'_{crit} of 15.8 for $\alpha = .05$ and 200 degrees of freedom.

Four contrasts were found to be significant. The first significant contrast was between the composite of the mean absolute SKF changes in the central body sites by contrast to the composite of the mean absolute SKF changes in the extremities. For the purpose of SKF measurements in the present study, the subscapula, axilla, abdomen a, abdomen b, iliac, and thigh were defined as central body sites, while triceps, chest, and calf were considered as extremity sites. The chest SKF was classified as an extremity site due to its location slightly below the shoulder (see Appendix 6). The composite mean absolute SKF change in the central body sites (4.3 mm) was significantly higher than the mean absolute SKF change in the extremities (2.4 mm), $F(1,200) = 55.9, p < .05$.

The second significant contrast was between the composite of the mean absolute SKF changes in the subscapula and axilla, by contrast to the composite of the mean absolute SKF changes in abdomen a and abdomen b. The composite mean absolute SKF change in the abdomen a and abdomen b (5.3 mm) was significantly higher than that of the composite of the subscapula and axilla (2.5 mm), F (1,200) = 59.5, $p < .05$.

The third significant contrast was between the composite of the mean absolute SKF changes in the subscapula and axilla, by contrast to the mean absolute SKF change at the iliac site. The mean absolute SKF change at the iliac site (4.5 mm) was significantly higher than that of the composite of the subscapula and axilla (2.5 mm), F (1,200) = 20.5, $p < .05$.

Lastly, the fourth significant contrast was between the composite of the mean absolute SKF changes in the subscapula and axilla, by contrast to the mean absolute SKF change at the thigh site. The mean absolute SKF change at the thigh (5.7 mm) was significantly higher than that of the composite of the subscapula and axilla (2.5 mm), F (1,200) = 50.2, $p < .05$.

Table 6a:

Measurement of subcutaneous fat at 9 sites in AN patients - Pre, post, and absolute change

Body site	Pre	Post	Absolute Change (mm)
Triceps	7.0 ± 2.9[a]	10.4 ± 2.8	3.4 ± 2.1
Chest	2.8 ± 0.9	4.0 ± 1.2	1.2 ± 0.9
Subscapula	5.6 ± 1.5	8.1 ± 2.2	2.5 ± 1.4
Axilla	4.2 ± 1.2	6.7 ± 2.3	2.5 ± 2.1
Abdomen a	5.2 ± 1.6	10.5 ± 3.8	5.3 ± 3.4
Abdomen b	5.2 ± 1.5	10.5 ± 3.8	5.3 ± 3.5
Iliac	4.5 ± 1.8	9.0 ± 4.0	4.5 ± 3.1
Thigh	9.4 ± 4.1	15.1 ± 3.9	5.7 ± 3.2
Calf	6.3 ± 3.2	9.0 ± 3.2	2.7 ± 1.5

[a] mean ± SD

Table 6b:

ANCOVA fully repeated measures of absolute subcutaneous body fat change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	498.143	8	62.268	18.272	0.000
ERROR	681.560	200	3.408		

Tables 7a and 7b describe the results obtained from the analysis of the relative SKF change (expressed as percentage change). The dependent variable, Relative SKF Change was measured as the amount of change in mm of subcutaneous body fat, subtracting the initial from the final measurement, dividing by the initial value, and multiplying by 100. The greatest relative SKF change was observed at abdomen b, followed by abdomen a, iliac, thigh, axilla, triceps, calf, subscapula, and chest (see Table 7a).

A repeated measures ANCOVA was performed on the dependent variable Relative SKF Change, with the within-subjects independent variable being body site with 9 body locations, and initial body weight used as the covariate. A significant effect of body site, F $(8,200) = 9.129$, $p < .05$ was found. The full table of results from the ANCOVA are shown in Table 7b. Post-hoc contrast analysis by the Scheffe method, with F'_{crit} of 15.8 for $\alpha = .05$ and 200 degrees of freedom indicated 3 contrasts of statistical significance.

The first significant contrast was between the composite of the mean relative SKF changes in the central body sites by contrast to the composite of the mean relative SKF changes in the extremities. The central body sites were the subscapula, axilla, abdomen a, abdomen b, iliac, and thigh, and the extremities were triceps, chest, and calf. The composite mean relative SKF change in the central body sites (88.2%) was significantly higher than the mean relative SKF change in the extremities (55.1%), F $(1,200) = 29.2$, $p < .05$.

The second significant contrast was between the composite of the mean relative SKF changes in the subscapula and axilla, by contrast to the composite of the mean relative SKF changes in the abdomen a and abdomen b. The composite mean relative SKF change in the abdomen a and abdomen b (111.9%) was significantly higher than that of the composite of the subscapula and axilla (57.7%), F (1,200) = 39.2, $p < .05$.

The third significant contrast was between the composite of the mean relative SKF changes in the subscapula and axilla, by contrast to the mean relative SKF change at the iliac site. The mean relative SKF change at the iliac site (109.2%) was significantly higher than that of the composite of the subscapula and axilla (57.7%), F (1,200) = 23.6, $p < .05$.

Overall, the contrasts found to be significant with absolute SKF changes were the same as those when relative SKF changes were analyzed, with the exception of the comparison between the composite of the subscapula and axilla, and the thigh; this contrast was not found to be significant with relative SKF change.

Table 7a:

Relative subcutaneous fat change at 9 sites in AN patients

Body site	Relative Change (%)
Triceps	65.8 $\pm$ 62.6[a]
Chest	45.8 $\pm$ 38.4
Subscapula	48.1 $\pm$ 28.4
Axilla	67.3 $\pm$ 57.6
Abdomen a	111.5 $\pm$ 93.8
Abdomen b	112.3 $\pm$ 86.9
Iliac	109.2 $\pm$ 75.7
Thigh	80.8 $\pm$ 65.6
Calf	53.8 $\pm$ 45.1

[a] mean $\pm$ SD

Table 7b:

ANCOVA fully repeated measures of relative subcutaneous body fat change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	14.272	8	1.784	9.129	0.000
ERROR	39.084	200	0.195		

2) Circumferences

The same statistical procedures used for SKF changes were utilized in the analysis of the CIRC data. The only difference in the CIRC analysis was that the **chest CIRC** was considered to be a central body site, due to its anatomical location. This is in contrast to the **chest SKF** which, due to its anotomical location, was defined as an extremity. Tables 8a and 8b indicate the results obtained from the analysis of the absolute CIRC change. The dependent variable, Absolute CIRC Change was measured as the amount of circumference change in cm, subtracting the initial from the final measurement. The greatest mean absolute CIRC change (cm) was observed at waist a followed by hip, waist b, thigh b, thigh a, chest, arm, calf, and forearm (see Table 8a).

A repeated measures ANCOVA was performed on the dependent variable Absolute CIRC Change, with the within-subjects independent variable being CIRC body site with 9 body locations, and initial body weight used as the covariate. A significant effect of body site, F (8,200) = 26.059, $p < .05$ was found. The full table of results from the ANCOVA are shown in Table 8b. Post-hoc contrast analysis by the Scheffe method, with F'_{crit} of 15.8 for $\alpha = .05$ and 200 degrees of freedom indicated 3 contrasts of statistical significance.

The first significant contrast was between the composite of the mean absolute CIRC changes in the central body sites by contrast to the composite of the mean CIRC changes in the extremities. The central body sites were the chest, waist a, waist b, hip, thigh a and thigh b and the extremities included the arm, forearm, and calf. The composite mean absolute

CIRC change in the central body sites (5.9 cm) was significantly higher than the mean absolute CIRC change in the extremities (2.0 cm), $F\ (1,200) = 179.3, p < .05$.

The second significant contrast was between the mean absolute CIRC change at the chest, by contrast to the composite of the mean absolute CIRC change at waist a and waist b. The composite mean CIRC change at waist a and waist b (6.7 cm) was significantly higher than that at the chest (4.2 cm), $F\ (1,200) = 25.2, p < .05$.

The third significant contrast was between the mean absolute CIRC change at the chest, by contrast to the mean absolute CIRC change at the hip. The mean absolute CIRC change at the hip (6.6 cm) was significantly higher than that at the chest (4.2 cm), $F\ (1,200) = 16.8, p < .05$.

Table 8a:

Measurement of circumference change at 9 sites in AN patients - Pre, post, and absolute change

Body site	Pre	Post	Absolute Change (cm)
Arm	19.6 $\pm$ 2.1[a]	22.3 $\pm$ 1.7	2.7 $\pm$ 1.7
Forearm	20.3 $\pm$ 1.2	21.6 $\pm$ 0.9	1.3 $\pm$ 1.0
Chest	74.4 $\pm$ 4.2	78.6 $\pm$ 4.2	4.2 $\pm$ 2.8
Waist a	59.3 $\pm$ 4.2	66.2 $\pm$ 5.0	6.9 $\pm$ 4.2
Waist b	63.9 $\pm$ 5.1	70.5 $\pm$ 6.3	6.5 $\pm$ 4.6
Hip	79.0 $\pm$ 4.9	85.5 $\pm$ 5.1	6.6 $\pm$ 4.3
Thigh a	39.2 $\pm$ 3.6	43.9 $\pm$ 2.6	4.8 $\pm$ 3.4
Thigh b	42.5 $\pm$ 4.8	49.1 $\pm$ 3.4	6.5 $\pm$ 4.5
Calf	30.5 $\pm$ 1.5	32.5 $\pm$ 1.4	2.0 $\pm$ 1.4

[a] mean $\pm$ SD

Table 8b:

ANCOVA fully repeated measures of absolute circumference change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	929.135	8	116.142	26.059	0.000
ERROR	891.391	200	4.457		

Tables 9a and 9b indicate the results obtained from the analysis of the relative CIRC change (expressed as percentage change). The dependent variable, Relative CIRC Change was measured as the amount of circumference change in cm, subtracting the initial from the final measurement, dividing by the initial value, and multiplying by 100. The greatest mean relative CIRC change was observed at thigh b, followed by the arm, thigh a, waist a, waist b, hip, forearm, calf, and chest (see Table 9a).

A repeated measures ANCOVA was performed on the dependent variable Relative CIRC Change, with the within-subjects independent variable being CIRC body site with 9 body locations, and initial body weight used as the covariate. A significant effect of body site, F (8,200) = 12.619, $p < .05$ was found. The full table of results from the ANCOVA are shown in Table 9b. Post-hoc contrast analysis by the Scheffe method, with F_{crit} of 15.8 for α=.05 and 200 degrees of freedom indicated 3 contrasts of statistical significance.

The first significant contrast was between the mean relative CIRC change at the chest, by contrast to the composite of the mean relative CIRC change at waist a and waist b. The composite mean relative CIRC change at waist a and waist b (11.0%) was significantly higher than that at the chest (5.0%), F (1,200) = 20.7, $p < .05$.

The second significant contrast was between the mean relative CIRC change at the chest, by contrast to the composite of the mean relative CIRC change at thigh a and thigh b. The composite mean relative CIRC change at thigh a and thigh b (15.0%) was significantly higher than that at the chest (5.0%), F (1,200) = 57.7, $p < .05$.

The third significant contrast was between the mean relative CIRC change at the hip, by contrast to the composite of the mean relative CIRC change at thigh a and thigh b. The composite mean relative CIRC change at thigh a and thigh b (15.0%) was significantly higher than that at the hip (8.8%), $F\ (1{,}200) = 22.0, p < .05$.

Overall, the contrasts found to be significant with absolute CIRC changes were different from those when relative CIRC changes were analyzed, with the exception of the comparison between the chest and the composite of waist a and waist b. This contrast was significant when analyzed both in absolute and relative terms.

Table 9a:

Relative circumference change at 9 sites in AN patients

Body site	Relative Change (%)
Arm	14.2 ± 10.4[a]
Forearm	6.5 ± 5.7
Chest	5.0 ± 3.9
Waist a	11.5 ± 7.6
Waist b	10.4 ± 7.7
Hip	8.8 ± 5.9
Thigh a	13.1 ± 10.2
Thigh b	16.9 ± 12.8
Calf	6.2 ± 5.1

[a] mean ± SD

Table 9b:

ANCOVA fully repeated measures of relative circumference change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	0.298	8	0.037	12.619	0.000
ERROR	0.591	200	0.003		

3) Circumference Ratios

In addition to individual CIRC, certain CIRC ratios were measured and then comparisons of these were made pre- and post-weight gain in order to determine whether changes in body fat distribution had occurred. Table 10 depicts these results. An initial mean waist a:hip ratio of 0.75, reflects a gynoid fat distribution at baseline. Although the waist a:hip ratio significantly increased with weight gain ($p < .001$), a post waist a:hip ratio of 0.77 indicates preservation of the gynoid body shape. Significant were also, the pre- and post-weight gain changes in the waist b:hip and the waist b:thigh b ratios ($p < .05$). The changes in the remaining ratios were not statistically significant.

Table 10:

Selected circumference ratios pre- and post-weight gain in patients with AN

Circumference Ratio[a]	Pre	Post
Waist a:Hip	0.75 ± 0.04[b]	0.77 ± 0.04***
Waist b:Hip	0.81 ± 0.05	0.82 ± 0.05*
Waist a:Thigh a	1.52 ± 0.13	1.51 ± 0.11
Waist b:Thigh a	1.64 ± 0.19	1.61 ± 0.14
Waist a:Thigh b	1.41 ± 0.13	1.35 ± 0.11
Waist b:Thigh b	1.52 ± 0.18	1.44 ± 0.14*

a circumferences measured in cm
b mean ± SD
* $p < .05$ pre vs post weight gain
*** $p < .001$ pre vs post weight gain

4) Dual Energy X-ray Absorptiometry Analysis

Tables 11a - 11c describe the results obtained from analysis of the data measured by DEXA. Data of only 24 of the 26 subjects were utilized in this body composition analysis.

For two subjects the baseline amount of body fat in one of the three regions was too low to be quantified by the DEXA computer software. Table 11a depicts the results obtained from the analysis of absolute and relative body fat mass change as measured by DEXA. The greatest mean absolute body fat mass change (kg) was observed at the waist region, followed by the subscapular and thigh regions. The greatest mean relative body fat mass change (expressed as percentage change) was obtained at the subscapular region, followed by the waist, and thigh regions. The relative body fat mass change observed in the 3 regions was very large, particularly that obtained in the subscapular region (1375.4%). An explanation for this is that in some of the patients, initial values of body fat were very low, leading to very large relative changes.

A repeated measures ANCOVA was performed on the dependent variables Absolute Body Fat Change and Relative Body Fat Change respectively. The within-subjects independent variable was body region, with 3 body locations, and the covariate was initial body weight. No significant effect of body region was found either in terms of absolute change, $F\ (2,46) = 2.377, p > .05$ (see Table 11b) or relative change, $F\ (2,46) = 2.131, p > .05$ (see Table 11c). For this reason subsequent statistical analysis by post-hoc contrasts was not executed.

Table 11a:

DEXA measurement of fat mass at 3 regions in AN patients - Pre, post, absolute change, and relative change

Body region	Pre	Post	Absolute Change (kg)	Relative Change (%)
Subscapular	0.822 ± 0.760[a]	2.532 ± 1.194	1.710 ± 1.159	1375.4 ± 3359.1
Waist	1.286 ± 0.806	3.106 ± 1.183	1.820 ± 1.264	617.1 ± 1211.7
Thigh	2.212 ± 1.041	3.685 ± 1.083	1.473 ± 0.963	114.8 ± 156.6

[a] mean $\pm$ SD

Table 11b:

ANCOVA fully repeated measures of absolute fat mass change as measured by DEXA with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY REGION	1.396	2	0.698	2.377	0.104
ERROR	13.505	46	0.294		

Table 11c:

ANCOVA fully repeated measures of relative fat mass change as measured by DEXA with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY REGION	1430.772	2	715.386	2.131	0.130
ERROR	15444.550	46	335.751		

F) Estimation of Change in Percentage Body Fat: Comparison Between Bioelectrical Impedance Analysis and Dual Energy X-ray Absorptiometry

The standard RJL BIA 101 Version 1.0 software (RJL Systems, Detroit, MI) generated percentage body fat values for both baseline and final measurements in 14 of the 26 patients. In the remaining 12 patients, the baseline value for percentage body fat was too low to be detected by the BIA method. Thus, only data of the 14 patients are presented. The change in percentage body fat determined by BIA was calculated by subtracting the initial from the final value generated by the BIA software. This change was compared to the change in percentage body fat determined by DEXA, to assess agreement between the two methods.

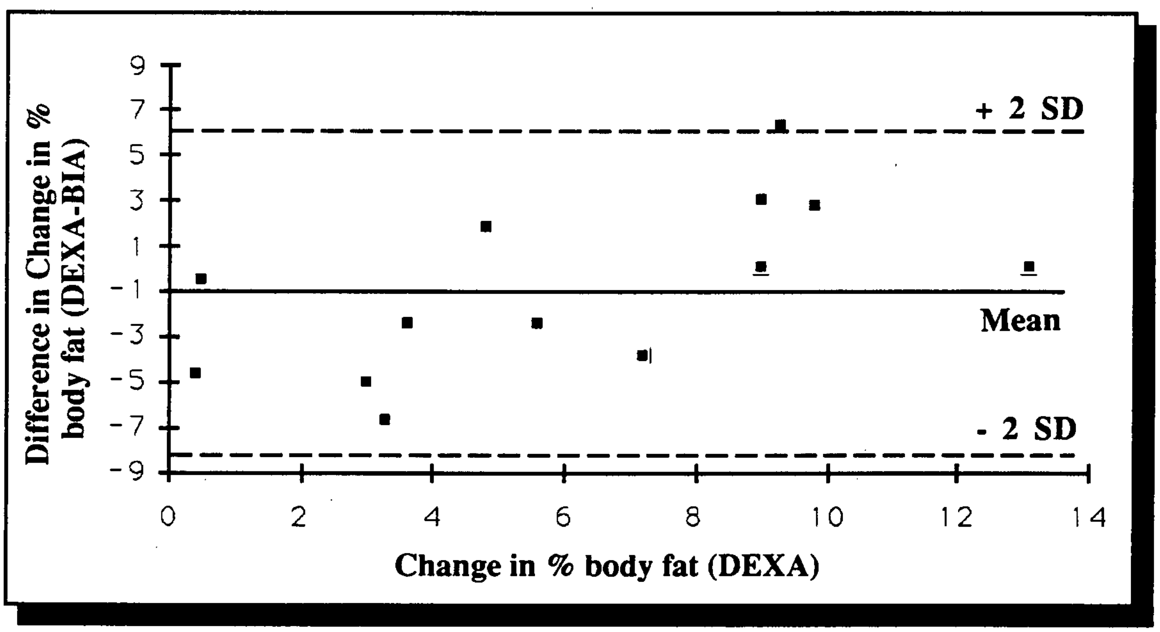
Figure 3 shows the difference in the measurement of change in percentage body fat between DEXA and BIA plotted against the change in percentage body fat as determined by

DEXA. The mean±2 SD of the differences are presented. A mean difference of -1±7.2 in the measurement of change in percentage body fat was found between the two methods. Therefore the change in percentage body fat varied by approximately 3.5% between the two methods, a value that is significant.

In addition to the standard BIA software program, a more updated BIA software package called Fluid Analysis 1.0a (RJL Systems, Detroit, MI) was used in the analysis, which according to the manufacturer, is more sensitive to hydration changes. However, this program was able to provide both baseline and final values of percentage body fat in one patient only. In the remaining patients, a value of 0% body fat was generated, labelling these patients as edematous. Thus, data obtained from Fluid Analysis 1.0a were not used.

Figure 3:

Differences in the measurement of change in percentage body fat between DEXA and BIA



CHAPTER 5

DISCUSSION

A) Major Findings

Anorexia Nervosa is a complex disease entity, characterized by alteration and impairment of mental and somatic functions. The most perplexing, yet most commonly described perceptual abnormality in AN, is the patient's inability to assess body size accurately. Some patients exhibit an extraordinary loathing for all parts of their body; others view selected areas such as the stomach, hip or thighs as disproportionate to the rest of the body. Thus, these patients become convinced that dieting and excessive physical activity are essential in order to eliminate these "protruding" areas, and they refuse nourishment even when this is medically necessary.

In recent years, the association of upper body and abdominal fat with increased risk of several chronic diseases has resulted in a renewed interest in body fat distribution. Although to date, numerous investigators have examined the fat distribution changes which accompany weight loss, very few studies have been done to assess the patterns of body fat following weight gain. Thus there are no data available to support or refute the claims made by AN patients that renourishment leads to preferential fat deposition in specific areas of the body.

The objectives of this study were three-fold: to determine the body composition changes which accompany weight gain in AN patients; to assess the changes in the distribution of body fat upon weight gain in patients with AN; and to compare measurements of change in percentage body fat pre- and post-weight gain in AN patients between DEXA and BIA techniques.

The main findings of this study are as follows. The rigorous refeeding regime that characterized the hospitalization of AN subjects led to the achievement of a significant increase in body weight. This weight gain included statistically significant gains in total body fat, total LBM, and total BMC. Total body fat was, however, the component which increased the most. In terms of the fat distribution changes which accompanied weight gain, there was general agreement among the results obtained by SKF, CIRC, and DEXA methods. As assessed by SKF and CIRC, there was a significantly greater deposition of body fat in the central regions (ie. chest, abdomen, hip and thigh), than in the extremities (ie. arm and calf). However, when body fat deposition changes pre- and post-weight gain in the abdomen, hip and thigh were compared using the 3 methods, no significant differences were observed among the regions. That is, there was no preferential deposition of body fat in any one of these 3 central regions. Body fat was deposited proportionately in these regions, with no change observed in fat distribution patterns. This finding was confirmed by the analysis of data obtained from CIRC ratios. Although there was a significant increase in the WHR pre- and post-weight gain, the gynoid body fat distribution pattern was preserved. Lastly, the agreement obtained in the measurement of change in percentage body fat upon renourishment between BIA and DEXA was poor. It appears that single-frequency BIA may not be sensitive enough to adequately quantify changes in body composition parameters in patients with AN.

B) Body Composition Changes

Recently, there has been increasing emphasis on the use of body composition analysis in the nutritional assessment of malnourished patients. One objective of this study was to determine the changes in body composition parameters upon normalization of AN patients' clinical and nutritional status following refeeding.

A highly significant weight gain as measured by DEXA (6.7$\pm$5.3 kg), and a highly significant increase in BMI (2.5 kg/m^2) was observed in the 26 AN patients who completed the study. This weight gain was the outcome of the rigorous refeeding protocol followed by the inpatient Eating Disorders Clinic, which consisted primarily of a combination of oral and nasogastric tube feeding. The composition of the weight gain as assessed by DEXA was 78% fat mass, 21% LBM, and 1% bone mineral. Compared to other studies, the amount of fat mass expressed as a percentage of weight gained in the present study is the highest ever reported.

There could be a number of reasons for the above discrepancy. First, the degree of initial malnutrition and the amount of weight gain achieved by patients are variable among studies. Second, various body compartments are given different definitions by different investigators. For example some use the term LBM synonymously with FFM, while others view LBM as being comprised of an additional component known as "essential" lipid. To further complicate this issue, LBM measured by DEXA refers to bone mineral-free fat-free mass. And third, the body composition measurement methods used in these studies (eg. SKF and TBK) are indirect in nature, and as such a number of assumptions are made which may not be valid, especially when body composition changes in AN are being examined.

The body composition results obtained in the present study are in close agreement with those of Russell & Mezey (1962). The initial weight, and the amount of weight gain achieved in 4 AN patients, was similar to that obtained in the present study. Using energy and nitrogen balance studies, these investigators found that 1 kg of deposited tissue contained 77% fat, 7% protein, and 16% water. They also indicated that the 16% water gain was entirely ICF, but it is unclear how they made this assumption. The small sample size used is a definite limitation in generalizing their results.

Some studies have examined body composition changes in refed AN patients using the TBK technique (Forbes et al., 1984; Pirke et al., 1986; & Vaisman et al., 1988b). The composition of regained weight as determined in the above studies was 55-70% fat mass, and 30-45% LBM. Thus, the amount of LBM regained upon refeeding in the present study was less than that observed in the literature. This discrepancy can be partly explained by the variability in the amount and type of physical activity that AN patients were permitted to engage in during treatment. It appears that in most of the above studies, patients were allowed to participate in supervised aerobic and nonaerobic activities, contingent on a satisfactory rate of weight gain and reasonable compliance. In contrast, patients in this study were more constrained in terms of their physical activity, and they were encouraged to become more physically active only as their medical and nutritional stability improved. As well, the limitations associated with TBK, which was the method used in these studies must be recognized. Hannan et al. (1990b) demonstrated that the relationship between TBK and FFM is not linear in patients at low weight. Use of the assumed constants for the relationship between TBK and FFM would underestimate the latter at low body weights, leading to overestimation of the difference before and after refeeding and an erroneously higher proportion of FFM in the regained tissue.

A number of studies have examined body composition changes following weight gain in AN patients using SKF (Russell et al., 1983; Forbes et al., 1984; Vaisman et al., 1988b; Melchior et al.; 1989; Russell et al., 1994). According to these studies, the composition of regained weight ranged from 32-70% fat mass and 30-68% LBM. Thus, there appears to be a significant discrepancy between the DEXA results of the present study and those from the SKF studies.

In order to determine whether this discrepancy was due to methodological differences between DEXA and SKF, or the result of differences in refeeding protocols among studies, body composition parameters of the present study were also estimated using SKF measurements. The equation for predicting body density in women developed by Jackson et al. (1980), which is based on 7 SKF measurements was used. Measurement of percentage body fat using SKF indicated pre- and post-weight gain percentage body fat values of 10% and 14.4% respectively (versus pre- and post-weight gain values of 18.8% and 27.0% estimated by DEXA). Although an increase of 4.5 in percentage body fat as determined by SKF was highly significant, it was only one-half of the increase estimated by DEXA. The composition of the weight gain as assessed by SKF was 43% fat mass and 57% LBM, in contrast to the DEXA values of 78% and 21% respectively. Overall, these results are discrepant from the DEXA data, however, they are in agreement with previous SKF studies. Thus these findings imply that the discrepancies in body composition observed between the present study and the literature are primarily due to methodological differences between DEXA and SKF.

The validity of using SKF equations to predict percentage body fat in various disease entities including AN is questionable (Lohman, 1992). Skinfold equations, including the one proposed by Jackson et al. (1980) are population specific, and cannot be extrapolated to other populations since the relationship between density and percentage body fat changes (Lohman, 1992). As well, SKF are based on the two-compartment model for estimating total body fat and FFM, and rely on the assumption that BMC represents a fixed fraction of LBM (Hart et al., 1992). However, constancy of BMC in AN cannot be assumed, since significant osteoporosis and pathologic bone fractures have been well documented in these patients (Biller et al., 1989; Rigotti et al., 1991; Salisbury & Mitchell, 1991; Seeman et al., 1992). Dual energy X-ray absorptiometry, on the other hand, is based on a three-compartment body composition model, and is able to quantify BMC directly. Thus despite its shortcomings, DEXA appears to be a more valid method than SKF in quantifying body composition changes and weight gain constitution in refed AN patients.

In a more recent study, Russell et al. (1994) investigated the effects of weight restoration on body composition in AN patients, using anthropometry and IVNCA. Thirty two females with AN and 29 matched control subjects were recruited for this study. Total body protein was obtained from measurements of total body nitrogen by IVNCA. Total body fat and LBM were derived from the sum of 4 SKF. Overall, a 10 kg weight gain was achieved in the AN subjects which, according to SKF measurements, was comprised of 58% fat and 42% LBM. These findings are in agreement with those of previous SKF studies. However, the authors proceeded to compare the results from the AN patients with those of the control group, an analysis that yielded different results. Compared with the control group, AN patients' nitrogen was initially depleted by 25%, increased by 18%, but remained 11% below control values. Body fat was depleted by 58%, increased by 90%, but remained 22% below control values. Therefore, despite a greater initial depletion and a subsequently greater net gain, body fat remained relatively more depleted after refeeding, than did nitrogen and protein. No comparisons could be made with the present study as there was no control group, an acknowledged limitation of the present study (see Chapter 6, Section B).

These authors emphasized the usefulness of IVNCA in measuring TBN, which in their opinion permits direct estimation of the protein component of the body. In contrast to these authors' claims, it has been indicated that IVNCA has certain limitations (Brodie, 1988; Ryde, Laskey, Morgan, & Compston, 1993). These include the utilization of the factor of 6.25 to relate nitrogen to protein in all body tissues. As well, body hydration, and the protein and mineral content of the skeleton, factors affected by the anorectic state, may influence the relationship of TBN to LBM thus leading to erroneous results.

An important question is how the body composition of renourished AN patients compares with that of healthy, normal-weight females of similar age. To address this issue, the findings of studies which investigated body composition in normal-weight premenopausal females using DEXA, were compared with the results of this study. Only 2 pertinent studies were found in the literature, as the majority of research has focused on body composition in groups of individuals with heterogeneous age and weight ranges. Hansen et al. (1993) compared 4 methods for predicting body composition in 100 premenopausal females, ages 28-39. One of the 4 methods used was DEXA. Exclusion criteria pertaining to subject recruitment included participation in any regular exercise program for 2 years prior to the study; experiencing less than 10 normal menses in the previous year; and the use of oral contraceptives or other drugs or medications known to affect bone metabolism. The subjects had a BMI of 22.3 kg/m^2. Body composition measurements as assessed by DEXA indicated that fat comprised an average of 30% of body weight, and bone mineral content totalled 2.6 kg (or 4% of body weight). No numerical values were provided for LBM; however, it appears reasonable to assume that LBM was the difference between body weight and the sum of body fat and bone mineral content. This gives a LBM value of 40 kg (or 66% of body weight). In our study, the renourished AN patients had a BMI of 19 kg/m^2. Twenty seven percent of body weight was comprised of body fat, LBM accounted for 34.5 kg (or 69% of body weight), and bone mineral content was 2.2 kg (or 4% of body weight). These results suggest that the body composition of renourished AN patients is comparable to that of normal-weight premenopausal women.

Rico, Revilla, Villa, Alvarez del Buergo, & Ruiz-Conteras (1994) investigated the principal determinants of bone mass in 50 postpubertal women, ages 14-18 yrs using DEXA. Exclusion criteria included a history of menstrual disorders or findings suggestive of gynecologic problems. In addition to determinations of bone mineral content, the investigators examined fat mass and LBM. Subjects had a BMI of 21.7 kg/m^2. Body composition measurements using DEXA indicated that body weight consisted of 27% fat, 68% LBM and 5% bone mineral content. As with the Hansen et al. study, comparison of these results with the present study suggest that the body composition of renourished AN patients is similar to that of postpubertal females.

A final comment is related to the measurement of percentage body fat using DEXA. There are 2 ways in which percentage body fat measured by DEXA can be calculated. First, by knowing the mass of the fat, lean and bone compartments, fat mass can be calculated as a percentage of the total body weight. The second method involves the use of the physical densities of the 3 compartments to calculate the average tissue density. Siri (Siri, 1961) or Brozek equations (Brozek, Grande, Anderson, & Keys, 1963) can then be utilized to compute percentage body fat. The first method avoids the assumption made by the Siri and Brozek equations that bone mass is a fixed fraction of total nonfat mass. However, this straightforward DEXA calculation does not give percentage body fat values which agree with UWW. In the present study, a percentage body fat value of 27% was obtained in the renourished AN patients using method 1, in contrast to 20.5% using method 2 - a discrepancy which is significant. In view of this disagreement, it is necessary to determine which of the techniques contains the error and why. It appears that although the percentage body fat values obtained by DEXA and UWW differ considerably, the 2 methods agree very closely in the measurement of body tissue density (Nord & Payne, 1994). It has been suggested that this discrepancy may be due to the currently used Brozek and Siri equations (Nord & Payne, 1994). Thus, the principal assumptions underlying the derivation of these equations, such as density of lean tissue and fraction of bone mineral in the lean compartment, need to be continuously challenged.

C) Body Fat Distribution Changes

Body fat distribution after weight gain in AN patients was investigated for two reasons. First, an upper body fat distribution has been shown to be a significant predictor of cardiovascular disease mortality in both men (Larsson et al., 1984); and women (Lapidus et al., 1984). As well, increased amounts of abdominal fat have been associated with elevated triglyceride and LDL subfraction levels, reduced HDL cholesterol levels (Terry, Stefanick, Haskell, & Wood, 1991; Despres et al., 1989) and impaired glucose metabolism in premenopausal females (Peiris, Mueller, Struve, Smith & Kissebah, 1987; Evans, Hoffman, Kalkhoff, & Kissebah, 1984). One of the aims of this study was to determine whether aggressive refeeding in AN patients results in preferential deposition of body fat in the abdomen area which, over time, could potentially lead to atherogenic metabolic profiles for these women. Second, AN patients typically refuse to accept appropriate nourishment during treatment, as they believe that this will lead to excessive fat accumulation in certain body areas - specifically the abdomen, hips and thighs. One objective of this study was to provide empirical data to either substantiate or refute these patients' beliefs; and to provide a basis for developing strategies to help patients cope with the body changes which accompany weight gain.

1) Skinfolds

Results obtained from SKF measurements indicated that in terms of absolute change, there was a significantly greater deposition of body fat in the central regions (chest, abdomen, hip and thigh) than in the extremities (arm and calf). This finding comes as no surprise. Preferential deposition of body fat in the central regions rather than the extremities under conditions of weight gain constitutes the norm (Lapidus et al., 1984; Ohlson et al., 1985). The greatest absolute SKF change was observed at the thigh, followed by the 2 abdomen sites, the iliac, triceps, calf, axilla, subscapula, and lastly the chest. Individual or combinations of body sites were then compared to determine specific patterns of body fat distribution. One important finding of this analysis was that when the absolute SKF changes in the abdomen, iliac and thigh sites were compared, no significant differences among these 3 sites were found. This suggests that there was no preferential deposition of body fat in any of these 3 central regions; rather, body fat appears to be deposited proportionately in the 3 regions. Significant differences were obtained, however, when the composite of the subscapula and axilla sites both located in the thoracic region, were compared with each of the abdomen, iliac and thigh sites. This finding indicates that during renourishment of AN patients, less body fat is deposited in the upper central sites (subscapula and axilla) in comparison to the lower central sites (abdomen, iliac and thigh).

Similar results were obtained when SKF data was analyzed in relative terms. A significantly greater deposition of body fat was observed in the central regions than in the extremities. The greatest relative change was observed at the 2 abdomen sites, followed by the iliac, thigh, axilla, triceps, calf, subscapula and chest. Although the sequence of sites in terms of the relative SKF increase achieved varies from that of the absolute change, this variation is slight, and the results essentially lead to the same conclusion - that there is greater deposition of body fat in the abdomen, iliac and thigh compared to the remaining sites. As well, when the relative SKF changes in the abdomen, iliac and thigh sites were compared, no significant differences among these 3 sites were found. When the composite of the subscapula and axilla sites were compared with each of the abdomen, iliac and thigh sites, significant differences were obtained with the abdomen and iliac, but not with the thigh. Overall, results from the absolute and relative SKF change analysis were in agreement.

There is only one study in the literature that the above results can be compared to, and this study was conducted by Russell & Mezey (1962). These investigators performed SKF measurements at 5 body sites to determine the distribution of subcutaneous fat in 4 AN patients undergoing refeeding. These patients were 18-22 years of age, and received intensive treatment for 5 weeks, during which they achieved an average weight gain of 8 kg. In terms of absolute SKF gains, the greatest increase was observed at the thigh, followed by the abdomen, subscapula, axilla, and upper arm. The relative SKF gains occurred, in descending order, at the abdomen, thigh, axilla, subscapula and upper arm. These findings are in close agreement with the results obtained in this study. It is important to note, however, that there were only 4 patients in the Russell & Mezey study, thus generalizability of their results must be viewed with caution.

2) Circumferences

CIRC measurements were also performed in order to obtain further information on the fat distribution changes that occur upon weight gain in AN patients (although it is recognized that CIRC measurements do not permit evaluation of the individual contributions of visceral fat, subcutaneous fat and LBM). As with SKF results, a significantly greater absolute CIRC increase was observed in the central regions (chest, waist, hip and thigh) compared to the extremities (arm, forearm and calf). The greatest absolute CIRC gain was observed at waist a, followed by the hip, waist b, thigh b, thigh a, chest, arm, calf and forearm. Again, no significant differences were obtained when the waist, hip and thigh CIRC were compared with each other, indicating that no preferential deposition of body fat had occurred in any of these 3 central sites. Comparisons between the chest CIRC and each of the waist, hip and thigh CIRC, revealed a significant difference between the chest and waist, and between the chest and hip, but not with the thigh CIRC. This finding supports the observations made with absolute SKF results, namely that during the refeeding of AN patients, less body fat is deposited in the upper central region (chest) compared to lower central ones (waist and hip). A possible explanation as to why no significant difference was obtained between the chest and thigh, was because the change in the chest CIRC was compared to the composite of the change in thigh a and thigh b CIRC. The change that was observed in thigh a was approximately 1.8 cm less than that observed in thigh b, thus reducing the combined mean for both the thigh CIRC, and subsequently leading to a non-significant difference when compared to the change in the chest CIRC.

The results obtained from the analysis of relative CIRC change are more variable, and thus more difficult to interpret. The greatest relative CIRC change was observed at thigh b, followed by the arm, thigh a, waist a, waist b, hip, forearm, calf and chest. An unexpected finding was the relative increase in the arm CIRC, which ranked second in magnitude following the change in thigh b. Being an extremity site, it would have been expected that less change would have been obtained at the arm in comparison to the central sites. A possible explanation for this discrepancy is the fact that pre-weight gain, the arm CIRC was small in magnitude and any amount of post-weight change achieved would result in a magnified relative change (since division by a small initial value leads to a large number). The same explanation can be given in terms of the greater relative CIRC change observed at the forearm and calf compared to the chest. For this reason, no significant differences in relative CIRC increase were observed in the central regions (chest, waist, hip and thigh) compared to the extremities (arm, forearm and calf).

When data of inpatients were analyzed separately (see Appendix 11), results were similar to those of the overall data. However, one additional comparison was found significant, and that was between the relative CIRC change of the composite of waist a and b and that of the composite of thigh a and b. In inpatients then, it appears that in terms of relative change, there was a significantly greater body fat deposition in the thigh regions than the waist.

Selected CIRC ratios were also determined pre- and post-weight gain, and these were used as indices of body fat distribution change. A significant change in WHR from 0.75 to 0.77 was observed. This increase in the WHR is expected, as increased levels of body fatness invariably lead to an increased WHR. It is important to note that the post-weight gain WHR value of 0.77 is below 0.8 - the cut-off point that characterizes upper body fat distribution. This indicates that despite a significant weight gain, these patients were able to retain a lower-body fat distribution shape. Additionally, a decrease was observed between pre- and post-weight gain in all the measured WTR; however, only the change in the waist b to thigh b ratio was statistically significant. This general trend of a decrease in the WTR suggests that there was a greater increase in the thigh CIRC in comparison to the waist CIRC. This confirms the observation that the gynoid body fat distribution in the AN patients was maintained post-weight gain.

An additional statistically significant decrease was observed in inpatients in the waist a to thigh b ratio pre- and post-weight gain. This finding substantiates the general trends observed in the overall data described above.

There are no studies cited in the literature examining the effect of weight gain on CIRC change in AN patients; thus no comparisons could be made with the results obtained in this study.

3) Dual Energy X-ray Absorptiometry Analysis

The overall results obtained by DEXA were in agreement with those by SKF and CIRC. Examination of the absolute change in fat mass in the 3 DEXA regions revealed no significant differences. Despite the lack of statistical significance, the greatest absolute change was observed at the waist region, followed by the subscapular, and thigh. It should be noted that there was a large degree of variability (presented in terms of standard deviation) in the amount of fat mass gained after refeeding. Noteworthy is the lack of significance obtained when the subscapular region was compared to the waist and thigh regions respectively. This finding was not in agreement with observations made with SKF and CIRC, both of which indicated significantly less deposition of body fat in the thorax in comparison to the waist and thigh. A possible explanation for this discrepancy is that the chest SKF and chest CIRC are located in the upper end of the thoracic region, whereas the DEXA subscapular region spans the entire thoracic region. It appears that the chest SKF and chest CIRC are located closer to extremity sites rather than central sites, and this could possibly explain these differences.

Similarly, no significant differences were obtained when the relative changes in fat mass in the 3 DEXA regions were compared. This again can be attributed to the large amount of variability in the results; in fact the relative changes in fat mass for all 3 DEXA regions had standard deviations which exceeded the mean value. The greatest relative change was observed in the subscapula, followed by the waist and thigh. The relative change in the subscapular region was 1375%, a value which is strikingly high. This can be explained by the fact that 15 of the 24 subjects had baseline subscapular fat mass values which were less than 1 kg - with one patient having a value of only 13 g. When the difference between initial and final fat mass is divided by a very small baseline value to determine the relative change, the result obtained is very large, and this explains the magnified value obtained with the subscapular region. Thus, although the absolute change in fat mass in the 3 regions was not large, the low initial values in some patients led to very large relative changes. It is also important to note that the DEXA data of 24 of the 26 patients were utilized, because in 2 subjects the baseline amount of body fat in the subscapular region was too low to be quantified by the DEXA computer software. It has been indicated that DEXA cannot distinguish clearly between soft tissue and bone in the thorax, because the arrangement of the ribs and spine prevents the x-ray beam from finding bone-free soft tissue mass, so that estimates of thoracic composition tend to be imprecise (Roubenoff et al., 1993). This problem would become more pronounced in malnourished AN patients, who, as has been shown, have very little fat mass in the thorax. It is thus unclear as to whether the results obtained for the subscapular region are accurate, and no information is available in the literature to make any comparisons.

Overall, the results obtained by all 3 methods are in general agreement. Although direct comparisons among the 3 methods cannot be made as each evaluates a different body composition component (ie. subcutaneous fat in SKF, subcutaneous fat and muscle in CIRC, fat mass in DEXA), all 3 methods are suggestive of the same observation. It appears that there is no preferential deposition of body fat in any one of the 3 central regions during the refeeding of malnourished AN patients. Instead, body fat appears to be deposited proportionately in the 3 regions, thus causing no alterations in fat distribution patterns. This was confirmed by the data from CIRC ratios. It thus appears that the gynoid body fat distribution was preserved in the refed AN patients. This is desirable because this type of body fat distribution is reported to be protective against atherogenic risk. These preliminary findings also substantiate the claims of AN patients that weight gain leads to greater deposition of body fat in the abdomen, hips and thighs, compared to other parts of the body.

One interesting question that remains to be answered is how the body fat distribution of renourished AN patients compares to that of healthy, premenopausal females. Such comparisons are difficult to make, as the number of studies done in this area are limited, and the methodologies used are variable. Only 2 pertinent studies were found in the literature and the findings of these are discussed below. Ley et al. (1992) used DEXA to investigate the effects of gender and menstrual status on body fat distribution in nonobese, healthy male and female volunteers. In addition to male and post-menopausal female subjects, the study included 61 women, ages 19-51, who were premenopausal, as judged by a regular menstrual cycle and no menopausal symptoms. None of these subjects were obese (mean BMI was 22 kg/m^2), or receiving sex-steroid therapy, and they were all in good health. To assess body fat distribution, the investigators used DEXA default software lines and divided body measurements into the following four regions or "boxes," all of which had the same height: android subscapular region; android waist region; gynoid hip region; and gynoid thigh region. One important region that was omitted, however, was the abdominal area surrounding the umbilicus. The amount of fat mass (kg) in each of the 4 regions was: 2.1 subscapular fat, 2.3 waist fat, 3.6 hip fat, and 3.3 thigh fat. Comparison of these results with those of the present study is difficult because the DEXA compartments chosen were different. It appears, however, that these subjects had significantly greater fat mass in the hip and thigh regions than the renourished AN patients. Whether the fat distribution seen in these 61 females is reflective of the fat distribution patterns in the general premenopausal female population is unknown. Thus, comparisons must be made with caution.

Williams et al. (1993) examined the relationship between fat distribution and dehydroepiandrosterone sulfate (an abundant steroid in humans thought to have an antiobesity effect) in premenopausal females. Ninety six healthy females, ages 28-39 completed the study. Regional fat distribution was assessed by circumference ratios. As well, DEXA was used to measure fat mass in the trunk and leg regions. A mean WHR of 0.73 and WTR of 1.49 were obtained respectively. In the renourished AN patients of our study, a value of 0.77 for WHR and 1.35 for WTR were obtained respectively. Although the WHR results obtained in the 2 studies appear to be in agreement, the WTR values are discrepant. The authors do not indicate whether they utilized mid- or proximal thigh in their measurements; the location of measurements may thus be a possible explanation for this discrepancy.

D) Estimation of Change in Percentage Body Fat upon Refeeding: Comparison between Bioelectrical Impedance Analysis and Dual Energy X-ray Absorptiometry

The results of this study indicate poor agreement between DEXA and BIA for the measurement of change in percentage body fat upon refeeding in AN patients. This is reflected by the fact that the change in percentage body fat varied by approximately 3.5% between the two methods, a value that is significant.

The refeeding of AN patients is accompanied by significant body composition changes (Vaisman et al., 1988b). For example, TBW has been reported to increase, with an expansion of ECF at the expense of the ICF compartment. The marked shifts in fluid balance that accompany refeeding in AN may limit the usefulness of BIA which relies on the estimation of TBW as a predictor of LBM (Krahn et al., 1993; Rock & Curran-Celentano, 1994). Calculation of LBM from TBW has been shown to overestimate LBM in this patient population (Hannan et al., 1990a). Hannan et al. (1990b), compared FFM in anorexic subjects as determined by BIA, TBW, TBK, and nitrogen neutron activation. As well, they derived a BIA prediction equation for FFM estimation using weight, height, resistance, and shoulder width. Their results indicated that BIA compared favourably with the other methods even in patients with very low BMI. However, all the methods used in the study are indirect in nature, and make assumptions regarding body composition that may not be valid in AN.

In a more recent study, Hannan and co-investigators (1993b) evaluated the use of DEXA for the assessment of body composition in 37 anorexic females. They obtained a high correlation between FFM measured by DEXA and that measured by prompt neutron activation analysis and TBW. They concluded that DEXA was a suitable method of assessing FFM and that the reproducibility of the results obtained was sufficiently good to allow changes in body composition to be followed during the course of the patients' treatment. Hannan, Cowen, Freeman, Wrate & Barton (1993a) assessed the ability of BIA and anthropometry to measure percentage body fat and its changes in 24 refed AN patients, by determining their agreement with DEXA which was used as the reference method. The authors concluded that when compared to DEXA, BIA offers no significant improvement over equations based on anthropometry alone, for the prediction of either percentage body fat or its changes in AN patients.

Various investigators have explored the clinical application of BIA in other disease entities which are characterized by malnutrition and altered hydration state including acquired immunodeficiency syndrome (AIDS) (Wang et al., 1992b); Crohn's disease (Royall et al., 1994); chronic hemodialysis (Rammohan & Aplasca, 1992); and intensive care patients (Robert et al., 1993). The findings of these studies vary in terms of the validity and reliability of BIA, due to differences in methodology, and the use of different prediction equations. Bioelectrical impedance analysis body composition equations are derived by regression analysis and thus tend to be population specific. This implies that body composition estimates are adjusted toward the group mean, leading to under- or overestimation of body fatness in patients whose body composition differs from the mean. It also appears that the assumption made by whole-body BIA that the body is an isotropic conductor with uniform length and cross-sectional area may not be valid (Kushner, 1992). As well, the use of a 50-kHz current in single-frequency bioelectrical impedance analyzers does not allow the fractionation of TBW into intra- and extracellular components (Chumlea & Guo, 1994).

In the present study, use of the standard RJL BIA 101 Version 1.0 software (RJL Systems, Detroit, MI) was able to generate percentage body fat values for both baseline and final measurements in only 14 of the 26 patients. Thus an attempt was made to estimate percentage body fat more accurately by using, in addition to the manufacturer's BIA formula, a more updated BIA software package - Fluid Analysis 1.0a (RJL Systems, Detroit, MI), which according to the manufacturer, is more sensitive to hydration changes. However, this program was able to provide both baseline and final values of percentage body fat in one patient only. In the remaining 25 patients, the program produced a value of 0% body fat, labelling these patients as edematous.

These results show that BIA has limited ability to assess changes in percentage body fat, following refeeding in patients with AN. Also as indicated by a graphical method, BIA compares poorly to DEXA in the measurement of changes in percentage body fat. More long-term studies are warranted to further explore the clinical application of BIA in AN, possibly through the use of segmental and multifrequency BIA.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

A) Conclusions

One of the fundamental clinical features of AN is an extreme concern for body weight and shape. Anorexia nervosa patients are dissatisfied with their bodies, and they fear that if they gain weight, that body fat will be deposited preferentially in the abdomen, hips and thighs. Subsequently this fear may contribute to their resistance in gaining weight even when they are obviously emaciated. The primary objective of this study was to address the patients' concerns by investigating body composition and body fat distribution changes that characterize short-term weight gain in AN.

Results of body composition changes included a highly significant weight gain which consisted of significant gains in body fat , LBM, and BMC. Total body fat was found to be the component which increased the most. The discrepancies in body composition changes observed between the present study and the literature are primarily due to methodological differences.

Analysis of absolute and relative changes in body fat distribution by SKF and CIRC revealed a greater fat deposition in the central regions than in the extremities. Comparison of fat mass change among the 3 central regions as measured by DEXA, however, indicated no significant differences. These results were also supported by CIRC ratios. It appears that the gynoid fat distribution pattern (believed to be protective against the health risks associated with android body fat distribution) is preserved in these patients, despite the significant weight gain achieved during refeeding. It is recognized, however, that promoting an increase in body fat improves morbidity and mortality, and overides the potential risk of cardiovascular disease in these patients over the long term.

Lastly, a poor agreement was obtained between BIA and DEXA for the measurement of change in percentage body fat. Single-frequency BIA may thus not be sensitive enough to quantify changes in body composition in patients with AN.

More studies are needed to further validate the use of sophisticated body composition methods in AN. In the meantime, the findings of the present study can constitute an important component of nutrition education strategies for the treatment of AN.

B) <u>Limitations to the Study</u>

Twenty six of 30 subjects successfully completed both baseline and final measurements. However, only 20 of these, who received at least one inpatient treatment achieved significant gains in body weight and body fat. Six of the subjects who were recruited as outpatients either refused inpatient treatment or were medically stable, and thus did not require urgent hospitalization. Whether or not outpatients would consent to inpatient treatment was beyond the investigators' control, but the fact that they did not receive aggressive inpatient refeeding and were subsequently unable to make significant gains in weight was a limitation to the study.

This was an observational cohort study following a group of AN patients and determining body composition and body fat distribution changes upon weight gain. It would have been beneficial if age- and height-matched female controls were also recruited, in order to determine how body composition parameters in AN compare with those in a normal-weight female population.

The limitations of all body composition methods used in this study, including DEXA, are recognized as these methods are indirect in nature, and thus make assumptions that may not be valid in AN. To date, there is no information available in the literature regarding the optimal selection of regions for body fat distribution measurements using DEXA. Thus it is uncertain whether the selection of DEXA regions in this study was fully appropriate. In addition, regional tissue variability might have had an effect on the body fat distribution results obtained. Hydration status was not uniform during the various stages of the study. Patients were typically dehydrated prior to renourishment, but were euhydrated once they had reached a maintenance weight. Since fluid gains have an effect on LBM, the big fluid shifts that accompany refeeding in AN could have introduced error in the measurement of LBM. As well, inter-observer reliability for DEXA was not tested in this study, thus the reliability of DEXA in measuring body composition and body fat distribution was assumed without being directly assessed. Lastly, the pre-morbid body fat distribution in these patients is unknown. It is possible that following weight gain, body fat distribution changed to its pre-morbid form, rather than being the direct outcome of nutrition intervention.

C) Future Research

The focus of the present study was to determine the body composition and body fat distribution changes that occur upon maximum weight gain after renourishment in AN patients. A question which remains unanswered is whether body composition and/or body fat distribution are altered as AN patients continue to maintain an increased body weight. Currently, the Eating Disorders Clinic at St. Paul's Hospital operates two day programs designed to provide outpatient therapy to those AN patients who have received inpatient treatment and then proceed to a weight-maintenance nutritional regime. These patients would be ideal for recruitment in such a study.

As emphasized previously, there are a number of challenges associated with the field of body composition assessment, the primary one being the indirect nature of the currently used body composition methods. Similar challenges exist in the area of body fat distribution. Since various investigators have used different indices to assess fat distribution, no concensus has yet been reached on the best approach to assess fat distribution in relation to health and disease. It is imperative that future research directions in the area of fat distribution include the validation of DEXA in various disease states, including AN. Such a development would thus clarify the association of fat distribution versus total body fatness in relation to disease risk.

D) Recommendations for Practice

Body image distortion and a morbid fear of becoming fat are two of the most common features of AN. In fact, weight and shape concerns have a central role in governing the anorexic patient's evaluation of her sense of self-esteem. Some patients express dissatisfaction towards their entire body, while others view selected body sites such as the stomach, hips and thighs as "protruding" and disproportionate to the rest of the body. Thus a central aspect of treatment is, through various strategies including nutrition intervention, to facilitate the patient in overcoming these misconceptions. The preliminary findings of the present research study could provide a framework for educating AN patients about the body composition and body fat distribution changes that accompany weight gain, and could help in alleviating the patients' concerns regarding these changes. For example, it would be beneficial for the anorexic individual to be forewarned that during the initial stages of weight restoration, the abdomen appears to be disproportionately large due to intestinal distension, but that this subsides as physical health improves. As well, patients could be reassured that despite weight gain the gynoid body fat distribution pattern is preserved, which in turn provides protection against the health risks associated with an android body shape. It could also be emphasized that the body composition patterns achieved following renourishment resemble those of a normal-weight female, and that this constitutes a foundation for recovery. Lastly, exploring the essential physiological functions of a gynoid body shape during various stages of the lifecycle, could help the anorexic patient to face her fears of gaining weight and encourage her to aspire towards a healthier body and an improved sense of self-worth.

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APPENDICES

APPENDIX 1

PILOT STUDY PUBLICATION

Reprinted from JOURNAL OF THE AMERICAN DIETETIC ASSOCIATION, Vol. 94, No 8, August 1994

Accuracy of subcutaneous fat measurement: Comparison of skinfold calipers, ultrasound, and computed tomography

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ABSTRACT

Objective The purpose of this study was to compare skinfold caliper and ultrasound measurement of subcutaneous body fat at three abdominal sites with computed tomography, which is considered to be the gold standard.
Design This was a cross-sectional study in which computed-tomography, ultrasound, and skinfold caliper measurements were made at three distinct abdominal sites. All body composition and anthropometric measurements were performed on each subject on one occasion.
Subjects Twenty-two subjects were recruited (13 men and 9 women). Mean ages (±standard deviation) were 43±4 years for the women and 51±18 years for the men. All subjects had been previously scheduled for an abdominal or pelvic computed-tomography scan at the Department of Radiology, St Paul's Hospital, Vancouver, British Columbia, Canada, and participated in the study on a volunteer basis.
Main outcome measures A better agreement was found between the skinfold calipers and computed-tomography methods than between the ultrasound and computed-tomography method for the measurement of subcutaneous body fat. This was observed when the data were analyzed for both correlational agreement and for graphical interpretation.
Statistical analyses performed The relationships among skinfold, ultrasound, and computed-tomography measurements were analyzed by determining Pearson correlation coefficients. A graphical method described by Bland and Altman was also used to assess agreement among the three methods.
Results Significant correlation coefficients were observed between skinfold calipers and computed tomography at all three abdominal sites (site 1, r=.60, P=.003; site 2, r=.70, P=.0001; site 3, r=.73, P=.0001). Ultrasound and computed-tomography methods only showed a significant correlation at site 3 (r=.54; P=.009). The graphical method revealed that the variation in the ultrasound measurements was much greater than that of the skinfold measurements when compared to computed-tomography values.
Applications/conclusions The results of this study indicated that relative agreement in the measurement of subcutaneous body fat between skinfold and computed-tomography measurements was superior to that exhibited between ultrasound and computed-tomography measurements. This finding enhances the potential use of skinfold calipers in the clinical setting, particularly in view of the fact that measurement of subcutaneous body fat at different body sites is becoming increasingly important for the characterization of risk of certain disease states. *J Am Diet Assoc. 1994;94:855-858.*

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Measurement of subcutaneous fat thickness at distinct body sites is an important component in the assessment of regional fat distribution. Investigators generally agree that disproportionate fat distribution plays a major role in the development of certain metabolic disorders and can ultimately influence morbidity and mortality (1,2). More specifically, android (upper body) fat distribution, most commonly observed in men, has been strongly associated with diabetes mellitus, hypertriglyceridemia, and hypertension (3-5). In contrast, these metabolic aberrations have shown little association with gynoid (lower body) fat distribution, which is more common in women (6).

Table
Characteristics of study subjects

Subjects	Age (y)	Weight (kg)	Height (cm)	BMI[a]	WHR[b]
	←		mean ± SD[c]		→
Men (n = 13)	51 ± 18	73.4 ± 9.9	174.0 ± 6.1	24.3 ± 3.8	0.96 ± 0.06
Women (n = 9)	43 ± 14	59.9 ± 10.2	164.2 ± 5.7	22.1 ± 2.6	0.82 ± 0.06

[a]BMI = body mass index (weight [kg]/height [m^2]).
[b]WHR = waist to hip ratio (cm/cm). Waist circumference was measured at the level of the umbilicus. Hip circumference was measured at the level of the maximum posterior extension of the buttocks.
[c]SD = standard deviation.

Traditionally, skinfold calipers have been the most widely used tool for evaluating body fat (7). They provide a fast, inexpensive, and convenient way of measuring subcutaneous fat in field situations (8). Some of the problems associated with this technique include selecting an appropriate site, accurately defining the area to be measured, and having sufficient experience to obtain reproducible and reliable results (7). Furthermore, a number of assumptions have been questioned regarding the use of skinfolds, including the constancy of skinfold compressibility and skin thickness and adipose tissue fat content (9).

Recently, there has been increased interest in the use of radiologic techniques for assessing body composition. Adaptation of contemporary instrumentation was deemed necessary to overcome some of the limitations of skinfold calipers. Computed tomography, which has the unique feature of differentiating between subcutaneous and visceral fat, is considered to be the reference method for quantifying body-fat depots, particularly in the abdominal area (10-12). However, the dose of radiation and the high cost limit the use of computed tomography in field surveys and routine clinical assessments (13,14). The usefulness of ultrasonography for measurement of body composition has also been investigated (8,12,14-19). Ultrasound overcomes some of the limitations of computed tomography in that it is portable, it does not emit radiation, and it is less expensive (14).

The purpose of this investigation was to compare skinfold caliper and ultrasound measurements of subcutaneous body fat at three abdominal sites with computed-tomography measurements. We assumed that computed tomography represented the "gold standard."

METHODS

Subjects

Twenty-two subjects (13 men and 9 women), 24 to 81 years of age, volunteered to participate in the study. All subjects were recruited from a group of patients who had been scheduled for an abdominal or pelvic scan by computed tomography for diagnostic purposes in the Department of Radiology, St Paul's Hospital, Vancouver, British Columbia, Canada. The study was approved by the human ethics committees of St Paul's Hospital and the University of British Columbia. Informed consent was obtained from all subjects before their participation.

Procedures

Body composition and anthropometric measurements were taken for each subject in the following sequence: fat measurement by computed tomography, ultrasound, and skinfold calipers; weight; height; and waist to hip ratio. At the time of measurement, all subjects were dressed in hospital gowns. All measurements for each individual were done at the same time, on the same day, within a 1-hour period. The calibration of all equipment was checked on a regular basis.

The abdominal site is difficult to measure by ultrasound; nevertheless, the location was of specific interest. This was a pilot study to determine whether measurement with skinfold calipers and/or ultrasound was a reliable technique to use in a subsequent study to investigate body-fat deposition during weight gain in patients with anorexia nervosa.

Measurement sites were marked with an indelible marker on the patient's skin, just before the computed-tomography scan was performed. A small, radiopaque grid marker was affixed to the skin to allow the sites to be identified. All measurement sites chosen were 5 cm to the left of midline, at 6 cm (site 1), 9 cm (site 2), and 12 cm (site 3) caudal to the inferior tip of the xiphoid process. All the computed-tomography scans were performed on a modern high-resolution scanner (spatial resolution range=0.1 mm) (GE Highlight Advantage, GE Medical Systems, Milwaukee, Wis) and were made directly from the operator's console by one of the authors, a radiologist (J.M.).

Skinfold calipers seem to be the desirable method for measuring subcutaneous fat in clinical settings; the method is accurate, quick, easy to perform, noninvasive, and inexpensive

The patients were then transferred to a separate room where ultrasound measurements were taken at the identical sites by one of two trained ultrasound technicians. All the measurements were made with a commercially available, high-resolution, real-time ultrasound unit (Aloka 650, Aloka Overseas Corporation, Tokyo, Japan) using a 5-MHz probe. The transducers were applied to the skin and then gradually withdrawn, and the ultrasound measurements were made with the least compression possible. Care was taken so that the transducer was perpendicular to the skin surface to avoid off-axis measurement errors. Both ultrasound and computed-tomography measurements were rounded off to the nearest millimeter.

Skinfold measurements were obtained by means of Harpenden calipers (Creative Health Products, Plymouth, Mich). Three measurements were taken at each site while the subject was standing. Half of the double fold of skin and subcutaneous fat was recorded. The mean value of the two measurements with the smallest difference was then calculated. All measurements were made by the same experienced investigator (C.O.).

Weight, height, and waist to hip ratio were measured to further describe the population. The waist circumference was measured at the level of the umbilicus, and the hip circumference was measured at the level of the maximum posterior extension of the buttocks (20).

Statistical Analysis

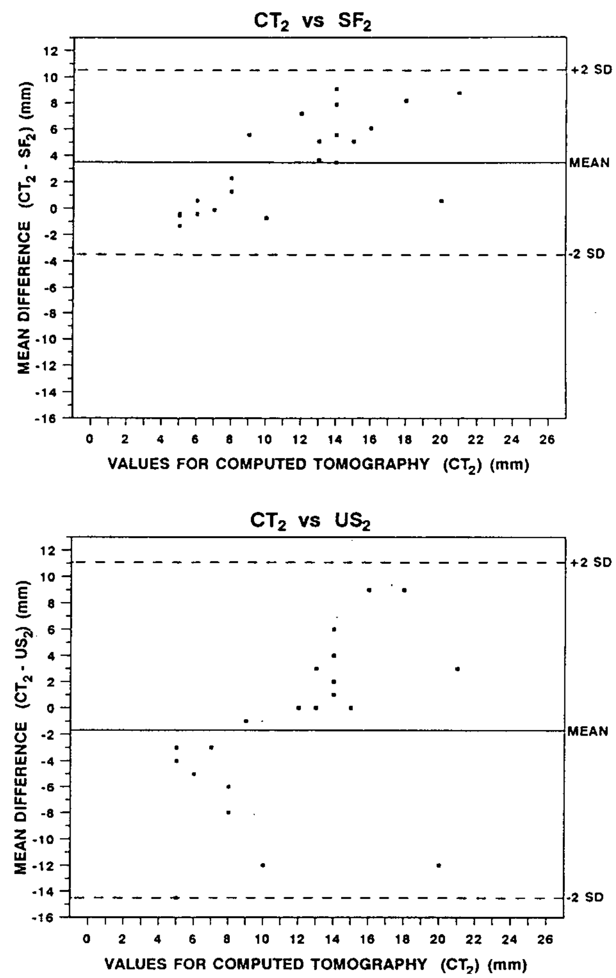
A sample-size calculation revealed that a sample of 22 would have suitable statistical power. The relationships among skinfold, ultrasound, and computed-tomography measurements were analyzed by determining Pearson correlation coefficients (Systat statistical software, version 4.1, 1989, Systat Corporation, Evanston, Ill). In addition, a graphical method described by Bland and Altman (21) was used to assess agreement between different methods.

RESULTS

Measurements of subcutaneous fat using the three different methods were completed on 22 subjects (13 men and 9 women). Subject characteristics are shown in the Table. Comparison of the methods by Pearson correlation coefficients showed significant correlations at site 1 for skinfold 1 vs ultrasound 1 (r=.59; P=.004) and skinfold 1 vs computed tomography 1 (r=.60; P=.003), but not for ultrasound 1 vs computed tomography 1. Significant correlations at site 2 were observed for skinfold 2 vs ultrasound 2 (r=.66; P=.001) and skinfold 2 vs computed tomography 2 (r=.70; P=.0001), but not for ultrasound 2 vs computed tomography 2. At site 3, significant correlations were observed for all measurements: skinfold 3 vs ultrasound 3 (r=.64; P=.001), skinfold 3 vs computed tomography 3 (r=.73; P=.0001), and ultrasound 3 vs computed tomography 3 (r=.54; P=.009).

When the data for men and women were analyzed separately, of the nine possible comparisons (three measurements at three sites), eight of nine comparisons showed strong significant correlations for the men. In the women, only three of nine comparisons showed significant correlations (P<.05) (skinfold vs computed tomography at all three sites).

The Figure shows the mean differences at site 2 between computed-tomography and skinfold measurements and between computed-tomography and ultrasound measurements plotted against the absolute values for computed tomography. The Figure shows ±2 standard deviations of the mean differences. The variation in the ultrasound measurements is much greater than that of the skinfold measurements compared with computed-tomography values.



Mean differences in subcutaneous fat measurement at abdominal site 2. CT=computed tomography; SF=skinfold calipers; US=ultrasound; SD=standard deviation.

DISCUSSION

In this study, for the measurement of subcutaneous body fat skinfold measurements showed better agreement with the results from computed tomography than did ultrasound. This was the case whether the data were analyzed for correlational agreement or graphical interpretation (21).

Because of the limitations of using skinfold calipers (measurement error, compressibility) or computed tomography (radiation, expense), we anticipated that ultrasound might be a suitable alternative measurement tool to avoid these problems. Kuczmarski et al (14) found that body fat can be estimated with nearly the same degree of accuracy using either ultrasound or skinfold calipers. Other studies (13,18) also suggest that ultrasound is a reliable method for measurement of fat depots. This conclusion, however, is not supported by our study.

Several sources of measurement error are possible with ultrasound. The variability of ultrasound measurement of subcutaneous fat is primarily attributable to direct compression of the subcutaneous fat by the pressure of the overlying transducer. Even when care is taken to minimize the amount of pressure on the abdominal wall, good contact between the skin and the transducer is required to form an image; the result is a minimum, but inevitable, amount of compression. Additionally, in some cases it is difficult to identify which of the reflecting surfaces represent the boundaries of subcutaneous fat. Furthermore, axial measurements in ultrasound depend on the speed of transmission of sound through tissue. Most ultrasound machines are calibrated to assume that the sound wave is passing through uniform soft tissue, but there are slight differences in the speed of sound wave propagation through fat compared with muscle or hepatic parenchyma. Finally, if the transducer is not held perpendicular to the skin surface, an off-axis artifact is introduced, thereby causing overestimation of the actual distance (18). The disagreement between studies may be due, in part, to differences in equipment used, technical procedures, and sites measured (8).

Our study was conducted based on the assumption that computed tomography is the gold standard for fat measurement. Although studies have been done using cadavers (10), which provide a direct measure of body fat, in most cases this research option is not possible. Researchers generally agree that computed tomography provides the greatest precision of all indirect methods currently available (10,11,13).

In clinical settings, accuracy of the skinfold caliper method can be enhanced by setting standards for site selection and measurement techniques and by using one observer

An unexpected result was the weak correlation observed between methods when only the data from women were analyzed. This result could not be explained by differences in absolute measures between men and women. The data from men alone showed significant correlations in eight of nine possible relationships. Our findings suggest not only gender differences but also characteristics inherent to abdominal fat in women that make it difficult to measure. The structure and function of the fat tissue may be highly variable because of differences in fat-cell size, number, membrane structure, or mobilizability. We acknowledge, however, that this finding may be an artifact of the small sample size.

As a result of this pilot study, ultrasound was not used in a subsequent study to investigate body-fat gain in patients with anorexia nervosa.

APPLICATIONS

The results of our study indicate that relative agreement in measurement of subcutaneous body fat between skinfold calipers and computed tomography was superior to that exhibited between ultrasound and computed tomography. This finding enhances the potential use of skinfold calipers in the clinical setting.

A practical comparison among the three methods shows that skinfold calipers, like ultrasound, are noninvasive. Computed tomography is invasive, as it uses x-rays. In terms of cost, computed tomography is three times as expensive as ultrasound; both methods require about the same amount of time. Measurements by skinfold calipers are the easiest and quickest to perform. The only cost is associated with the initial purchase of the calipers.

Site selection and measurement technique account for substantial differences between observers. With a single observer, however, skinfold measurements can yield extremely reproducible results (22). Although training is required, a standardized measurement routine can be readily established (22). ■

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APPENDIX 2

ETHICS APPROVAL CERTIFICATES

APPENDIX 3

SUBJECT CRITERIA

Based on:

American Psychiatric Association. (1987).
Diagnostic and Statistical Manual of Mental Disorders (3rd. ed. revised).
Washington, DC: Author.

Diagnostic Criteria for Anorexia Nervosa

A. Refusal to maintain body weight over a minimal normal weight for age and height, eg. weight loss leading to maintenance of body weight 15% below that expected; or failure to make expected weight gain during period of growth, leading to body weight 15% below that expected.

B. Intense fear of gaining weight or becoming fat, even though underweight.

C. Disturbance in the way in which one's body weight, size, or shape is experienced, eg. the person claims to "feel fat" even when emaciated, believes that one area of the body is "too fat" even when obviously underweight.

D. Absence of at least three consecutive menstrual cycles when otherwise expected to occur (primary of secondary amenorrhea).

APPENDIX 4

SUBJECT RECRUITMENT ADVERTISEMENT

APPENDIX 5

INFORMED WRITTEN CONSENT

3. Bioelectrical Impedance Assessment (BIA)
   This method will be done to measure body composition, It is a non-invasive procedure that can be done while you are lying down. Small electrodes are attached to one wrist and one ankle, and measurement of resistance of a small electrical current is used for the calculation of body composition.

4. Dual Energy X-ray Absorptiometry (DEXA)
   This is also a non-invasive method for measuring body composition. It involves laying on a bed for approximately 10 minutes. There is a low level of radiation exposure which is less than 1/10th that of a standard chest x-ray. This is a low level of radiation and it is not associated with any known side effects.

5. Dietary Intake Records
   You will be asked to record everything that you eat and drink for four days.

6. Physical Activity Assessment
   You will be asked to complete a questionnaire to estimate the frequency, duration and intensity of any physical activity that you have participated in, for four days.

** Each appointment will take 1 hour, so your entire participation in the study will require no more than 2 hours.

Risks and Significant Side Effects

There are no risks associated with skinfold, bioelectrical impedance, or dual energy x-ray absorptiometry measurements.

Potential Benefits

Although the question as to what areas of the body gain fat during weight gain has been addressed, it has not been previously investigated. The results of this study will shed some light into the fat redistribution patterns that occur during weight gain. It will also aid in the nutritional assessment and intervention for patients with eating disorders.

Monetary Compensation

Unfortunately, we will be unable to provide monetary compensation for your participation in the study.

Confidentiality

Only the investigators listed on page one, and the study co-ordinator Ms. Orphanidou will have access to confidential data which identifies you by name or initials. All possible precautions will be taken to assure your anonymity and the confidentiality of the data gathered in the study.

All of the data obtained will be coded by number, and no names will ever be used in any reports of the study. Only combined group data will be presented.

If you have any questions or concerns at any time during the study, you may contact Dr. Birmingham, Dr. McCargar, or Ms. Orphanidou at the phone numbers listed above. You will be informed of any significant new information pertaining to your safety.

❄ ❄ ❄ ❄ ❄ ❄ ❄ ❄

I have read the above information and I have had the opportunity to ask questions to help me understand what my participation would involve. I freely consent to participate in the study and acknowledge receipt of a copy of the consent form.

________________________________ _______________

Signature of Participant Date

____

Signature of Guardian (if applicable)

____

Signature of Witness

APPENDIX 6

ANTHROPOMETRIC MEASUREMENT STANDARDS

SKINFOLDS

Adopted from:

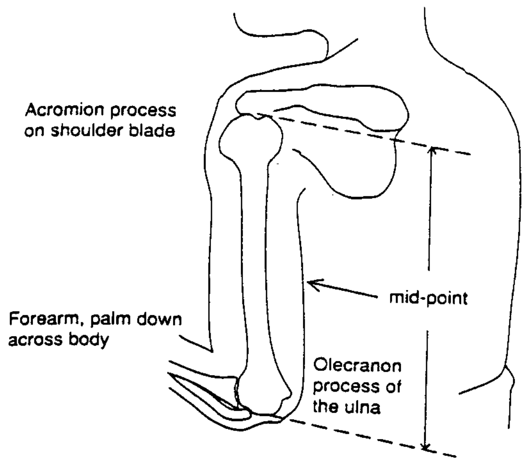
1. **Gibson, R. S. (1991).** ***Principles of Nutritional Assessment,*** **New York: Oxford University Press.**

2. **T. G. Lohman, A. F. Roche, R. Martorell, (Eds.),** ***Anthropometric Standardization Reference Manual.*** **Illinois: Human Kinetics Books.**

Triceps Skinfold

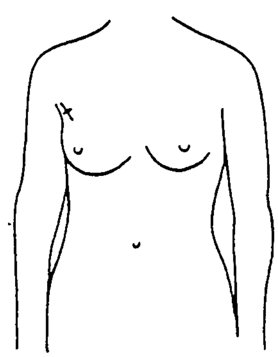
The triceps SKF is taken on the back of the right arm at the mid-point between the tip of the acromion and the tip of the olecranon, with the elbow flexed to 90°.

The triceps SKF is picked up with the left thumb and index finger approximately 1 cm proximal to the marked level, and the tips of the calipers are applied to the SKF at the marked level.



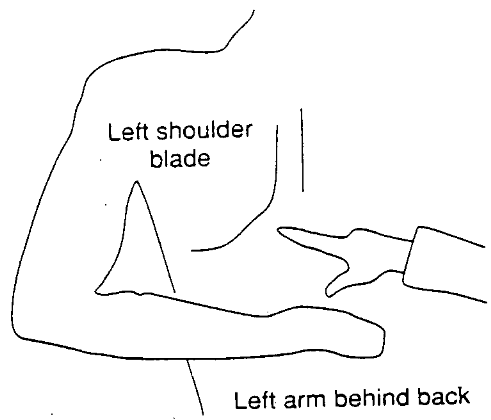
Chest Skinfold

Chest SKF thickness is measured using a skinfold with its long axis directed to the nipple. The SKF is picked up as high as possible. The thickness is measured 1 cm inferior to this.



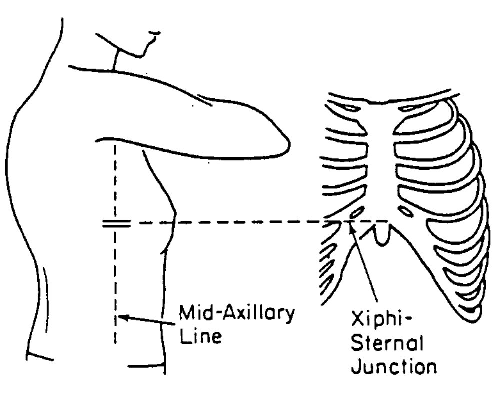
Subscapula Skinfold

The participant stands with the shoulders relaxed and the arms by the sides. The SKF is raised so it can be measured on a diagonal line coming from the vertebral border of the scapula to a point 1 cm beneath the inferior angle. The SKF runs downward and outward at an angle approximately 45° to the spine.



Axilla Skinfold

Measured at the level of the xiphi-sternal junction, in the mid-axillary line, with the SKF horizontal.



Abdomen Skinfolds

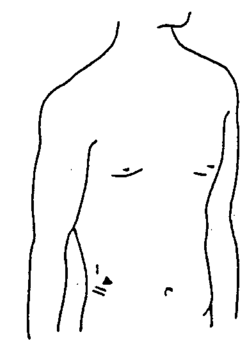
Abdomen SKF measurements were performed at 2 sites:

Abdomen a: Vertical SKF raised 3 cm lateral to the mid-point of the umbilicus and 1 cm inferior to it.

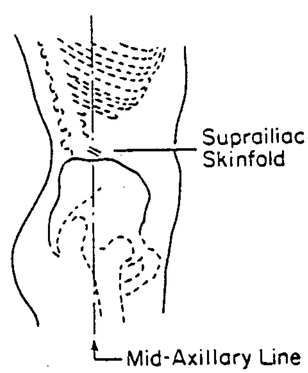
Abdomen b: Vertical SKF raised 5 cm lateral to and at the level of the umbilicus.

Iliac Skinfold

Measured in the mid-axillary line immediately superior to the iliac crest. An oblique SKF is grasped just posterior to the mid-axillary line following the natural cleavage lines of the skin. It is aligned infero-medially at 45^{o} to the horizontal. The caliper jaws are applied about 1 cm from the fingers holding the SKF.

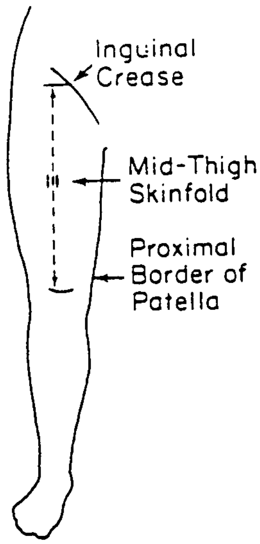


1. Standardized Suprailiac Site



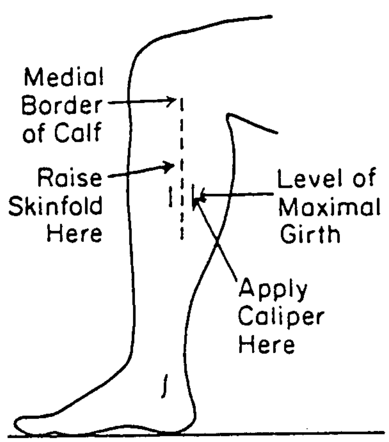
Thigh Skinfold

A vertical fold is measured at the midline of the anterior aspect of the thigh, midway between the inguinal crease and the proximal border of the patella.



Calf Skinfold

The knee and hip are flexed to approximately 90°. The level of the maximum calf circumference is marked on the medial aspect of the calf. From a position in front of the subject, a SKF is raised parallel to the long axis of the calf on its medial aspect.



Medial Aspect

APPENDIX 7

ANTHROPOMETRIC MEASUREMENT STANDARDS

CIRCUMFERENCES

Adopted from:

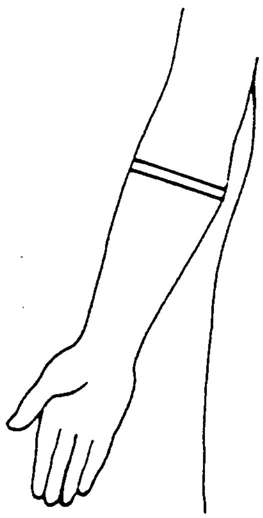
T. G. Lohman, A. F. Roche, R. Martorell, (Eds.),
Anthropometric Standardization Reference Manual.
Illinois: Human Kinetics Books.

Arm Circumference

Measured at the mid-point between the tip of the acromion and the tip of the olecranon.

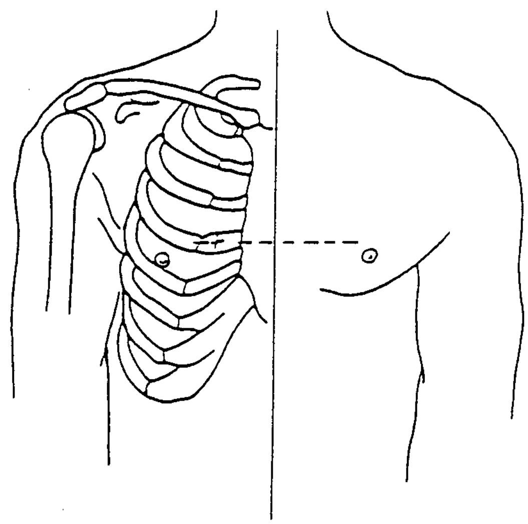
Forearm Circumference

Measured with the arm hanging downward and palms facing anteriorly. The tape is placed loosely around the proximal part of the forearm, perpendicular to its long axis, and moved up and down until the level of the maximum CIRC is located.



Chest Circumference

Measured at the level of the fourth costo-sternal joints. The tape is held in the right hand while the free end of the tape is passed in front of the subject and retrieved by the measurer's left hand as it passes around the subject's back. The measurement is made in a horizontal plane at the end of a normal expiration.



Waist Circumferences

Waist CIRC measurements were performed at 2 sites:

Waist a: measured at the level of the natural waist which is the narrowest part of the torso, as seen from the anterior aspect.

Waist b: measured at the level of the umbilicus.

Hip Circumference

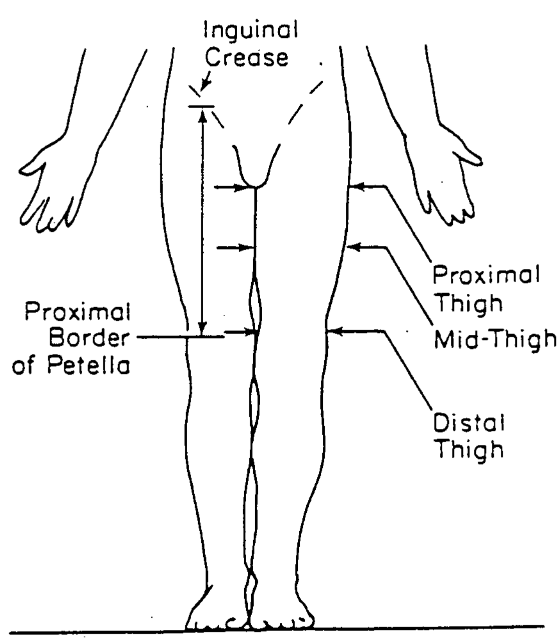
The measurer stands at the site of the subject so that the level of maximum extension of the buttocks can be seen. The tape is placed around the buttocks in a horizontal plane at this level.

Thigh Circumferences

Thigh CIRC measurements were performed at 2 sites:

Thigh a (mid-thigh): The tape is placed horizontally around the thigh at the level of the thigh SKF measurement, ie. midway between the mid-point of the inguinal crease and the proximal border of the patella.

Thigh b (proximal thigh): The tape is passed horizontally around the thigh immediately distal to the gluteal furrow.



Calf Circumference

The tape measure is positioned horizontally around the calf and moved up and down to locate the maximum CIRC in a plane perpendicular to the long axis of the calf.

APPENDIX 8a

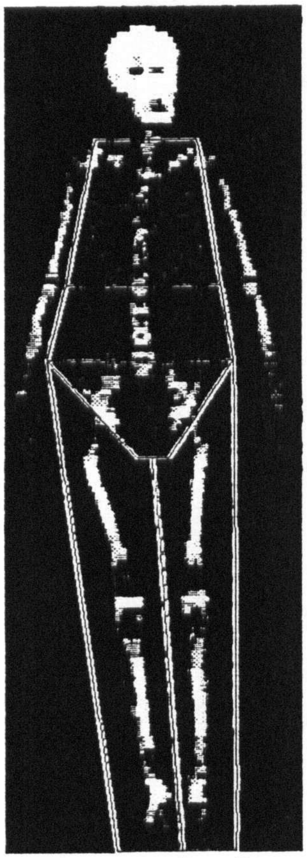
DEXA WHOLE BODY IMAGE

COMPUTER PRINT-OUT

St. Paul's Hospital Nuclear Medicine Dept.
1081 Burrard St. Vancouver, B.C. Ph. 631-5008

Name		*Ethnic*	CAUCASIAN
ID	J539	*Height*	170
Age	29 *Sex* Female	*Weight*	47.7

L H Body 08/20/93 Sequence 1



Bone image not for diagnosis

MD

Total BMD (g/cm) :	0.821
Total BMC (g) :	2033
Total Lean Mass(g):	34811
Total Fat Mass (g):	11041
Total Fat % :	23.1
Siri UWE Fat % :	17.7
Brozek UWE Fat % :	17.6
Soft Tissue Fat % :	24.1
% TBMC/FFM :	5.5

STD CV for Total BMD: 1.0 See Guide for other CVs.
6.5 x13.0 mm, 180 mm/s, 61.75 cm Rev. 2.5.0 / 1.3.0 Calib. 08/20/93

COMMENTS

St. Paul's Hospital Nuclear Medicine Dept.
1081 Burrard St. Vancouver, B.C. Ph. 631-5008

Name		*ID*	J539
Address		*Ethnic*	CAUCASIAN
		Age	29
		Menoage	
Telephone	*days* *eves*	*Sex*	Female
History		*Height*	170
		Weight	47.7
Treatment		*Armspan*	
Medications			
Comments			

SCAN INFORMATION

Type	Body		
Scan Date-Seq	08/20/93 1	*Resolution*	6.5 x 13.0 mm
Analysis Date	08/20/93	*Speed*	180 mm/s
Calibration Date	08/20/93	*Width*	61.75 cm
Technician	RF	*Host/Scanner*	2.5.0 / 1.3.0
Physician	DR. BIRMINGHAM	*Analysis Revision*	2.5.0

DETAILED RESULTS

	BMD	BMC	AREA	LENGTH	WIDTH	LEAN MASS	FAT MASS
	g/cm^2	g	cm^2	cm	cm	g	g
Head	1.466	436.4	297.7			3394	747.8
Trunk	0.727	529.6	728.0			16911	3833
Abdomen	0.705	234.7	333.1			9961	1566
Arms	0.562	322.1	573.3			2841	1640
Legs	0.850	744.7	876.3			11665	4821
Total	**0.821**	**2033**	**2475**			**34811**	**11041**

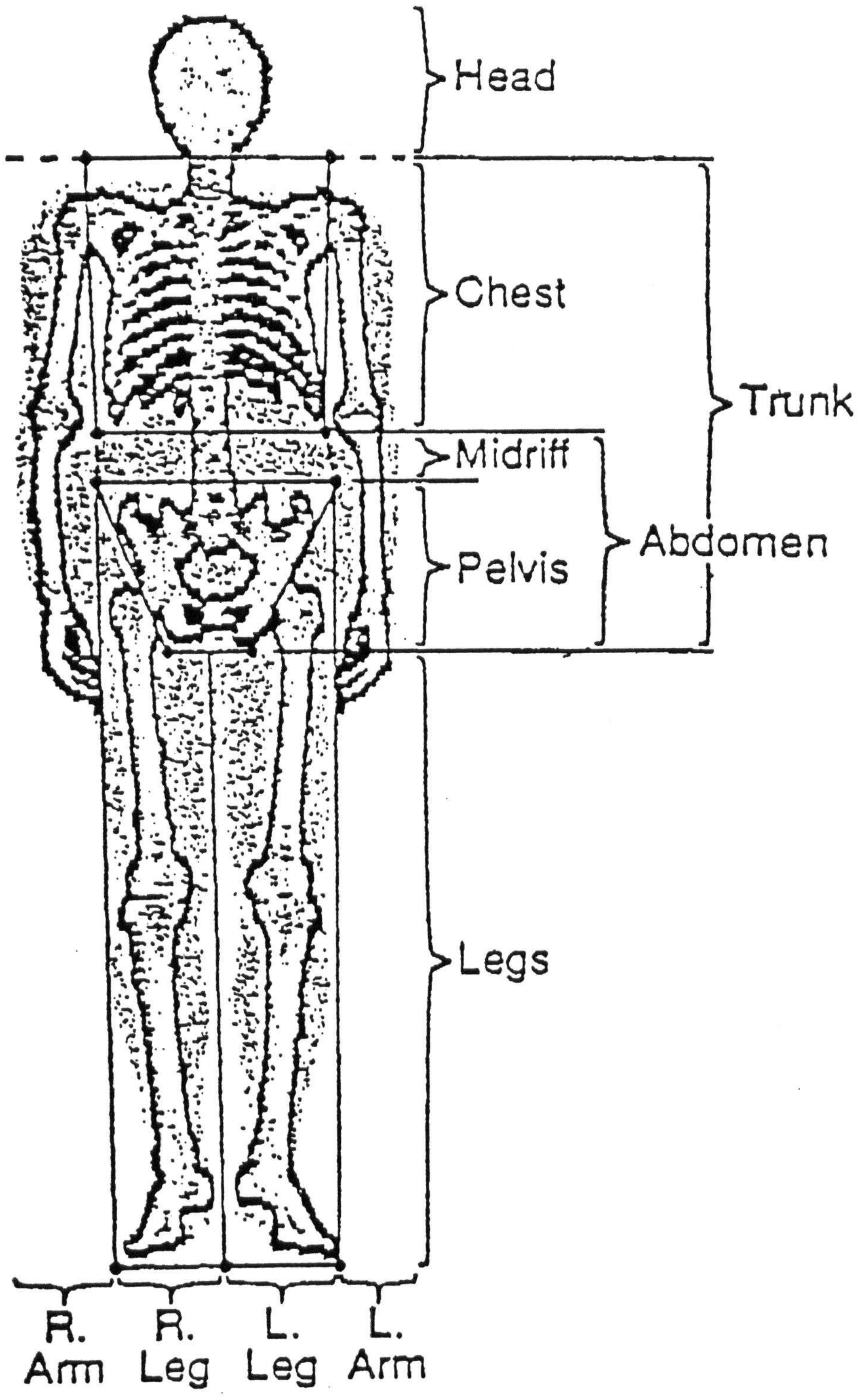
QUANTITIES MEASURED

Whole body calculations are performed for the following quantities:

AREA	=	Cursor region area, in cm^2
BMC	=	Bone mineral content, in g
BMD	=	Bone mineral density, in g/cm^2
STM	=	Soft tissue mass, in g
TBM	=	Total body mass, in g
TBMC	=	Total BMC
TSTM	=	Total STM

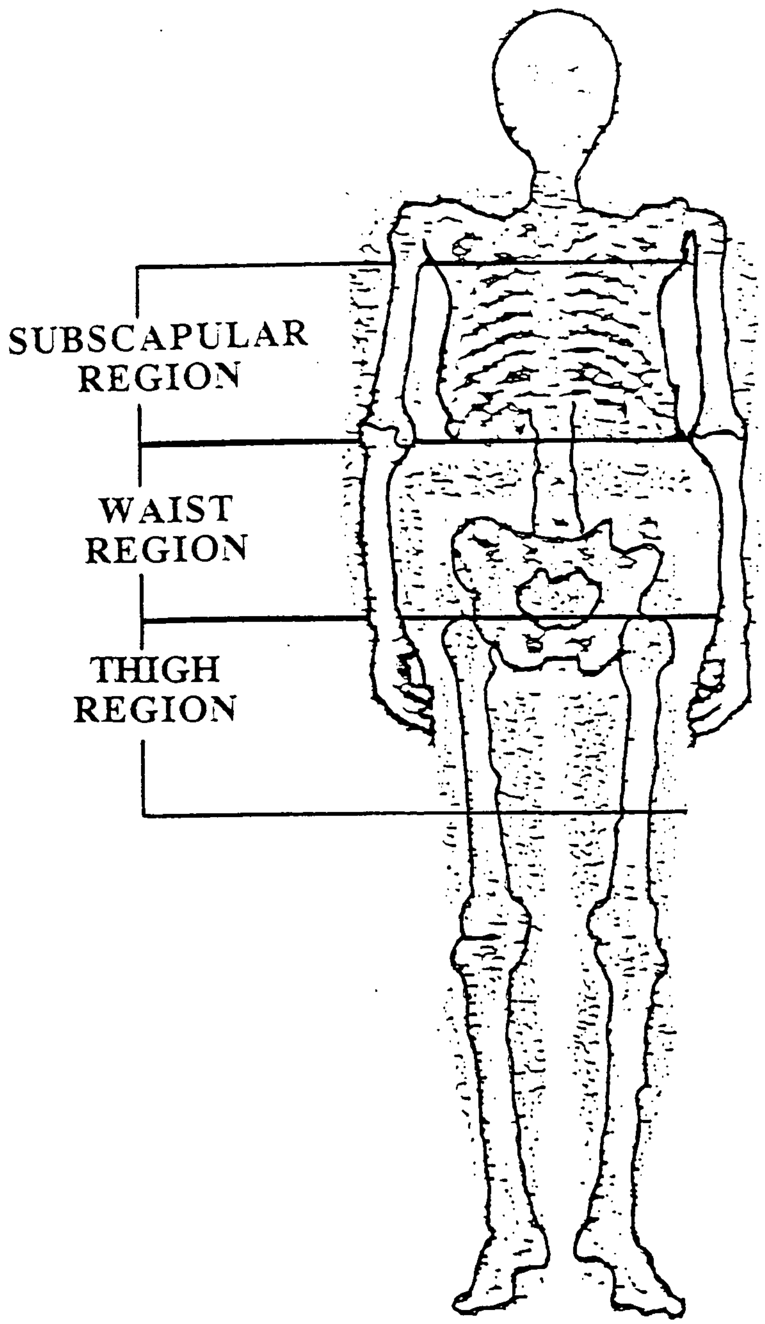
APPENDIX 8b

PRE-DETERMINED DEXA REGIONS


Head
Chest
Trunk
Midriff
Abdomen
Pelvis
Legs
R.
Arm
R.
Leg
L.
Leg
L.
Arm

APPENDIX 8c

DEXA REGIONS USED IN STUDY

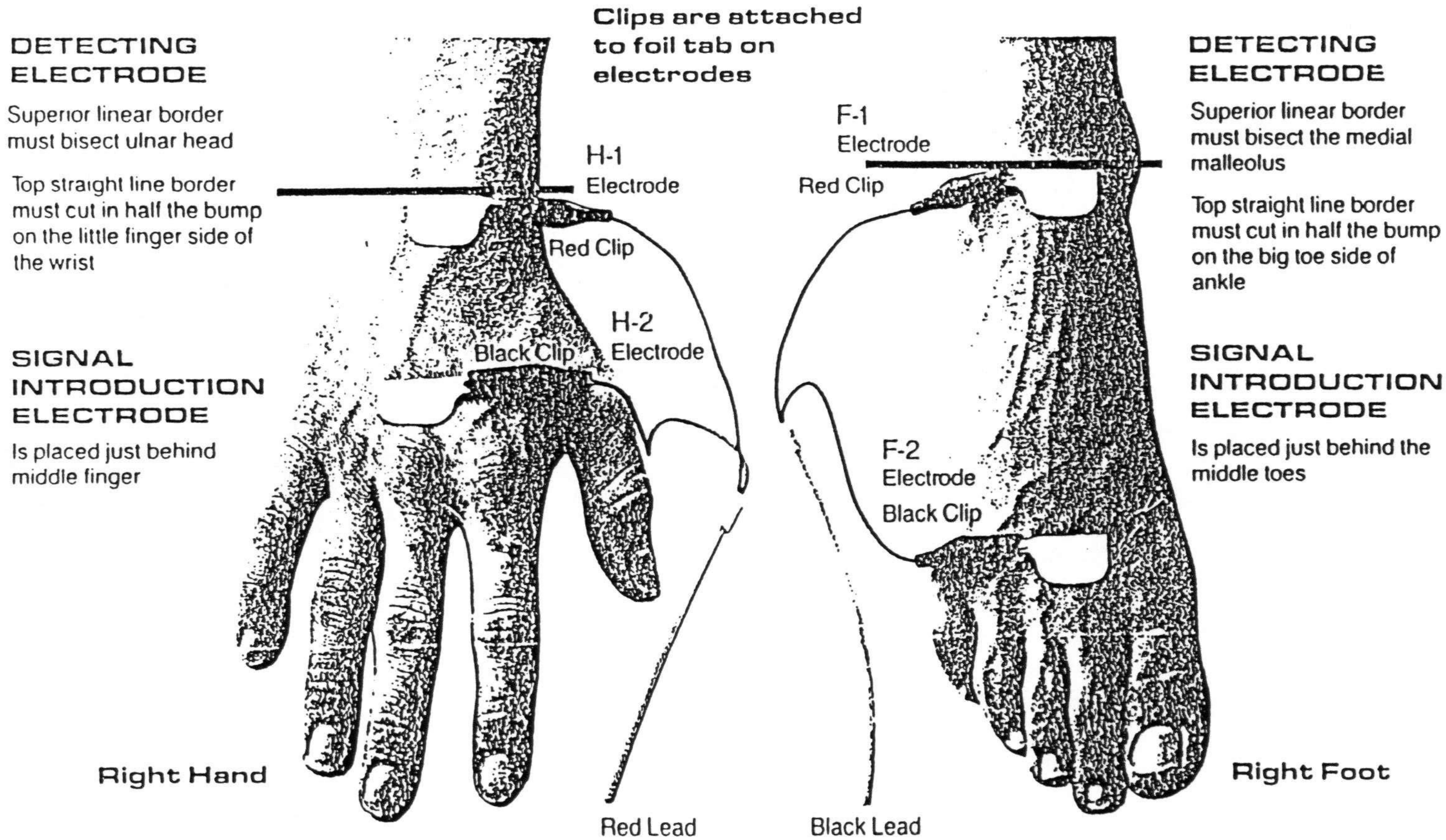


DEXA REGIONS

APPENDIX 9

BIA ELECTRODE PLACEMENT

ILLUSTRATION OF ELECTRODE PLACEMENT IN BIA



SOURCE: *Bioelectrical Impedance User's Manual. A review of Body Composition Techniques.* RJL Systems, Inc., Detroit, USA, p. 33, 1989.

APPENDIX 10

FOOD INTAKE RECORD FORM

GUIDELINES FOR KEEPING A FOOD RECORD

A FOOD RECORD IS A DETAILED DESCRIPTION OF EACH FOOD OR BEVERAGE ITEM TAKEN OVER 24 HRS DURING A DAY. AN ACCURATELY COMPLETED FOOD RECORD CAN PROVIDE VALUABLE INFORMATION ABOUT THE NUTRITIONAL CONTENT OF AN INDIVIDUAL'S USUAL DIET.

TO ASSESS YOUR DIET RECORD CORRECTLY, WE MUST BE ABLE TO CLEARLY PICTURE THE FOODS AND BEVERAGES THAT YOU HAVE RECORDED. THE GUIDELINES BELOW DESCRIBE THE INFORMATION THAT IS IMPORTANT FOR YOU TO RECORD. **PLEASE READ THESE GUIDELINES BEFORE YOU START FILLING IN YOUR FOOD RECORDS.**

PLEASE KEEP A RECORD OF EVERYTHING THAT YOU EAT OR DRINK ON THE ATTACHED FORMS, FOR A PERIOD OF 4 DAYS (3 CONSECUTIVE WEEKDAYS AND 1 WEEKEND DAY).

1. **THE PORTION SIZE (QUANTITY)** NEEDS TO BE ACCURATELY RECORDED

   You can discribe portion sizes in as many ways as you like.

   For example, you might record:

Volume

1 cup *or* 8 oz *or* 250 ml of 2%milk
1 tablespoon *or* 15 ml of peanut butter
1 teaspoon *or* 5 ml of sugar

Size

1 medium egg, poached
1 small apple
1 medium blueberry muffin

Weight

2 oz *or* 60 grams of hamburger meat *or* chicken *or* fish

2. DETAILED DESCRIPTION OF FOOD ITEMS IS ESSENTIAL

Tell us as much as you can about the foods that you eat.

BE SPECIFIC ABOUT THE TYPE OF FOOD, BRAND NAME IF APPLICABLE, AND THE CONTENT OF MIXED DISHES.

For example:

If you eat cookies, please tell us what type (eg. chocolate chip), what brand name (eg. Dare's or homemade), as well as the size (eg. 2"). If you have milk, tell us if it is canned or fresh, and whether it is whole, 2% or skim, as well as the amount (eg. 1 cup).

DESCRIBE MIXED FOODS AS IF YOU WERE WRITING A RECIPE

Everyone has their own way of making everyday foods - please tell us how you do it!

For example:

If you made a cheese sandwich:
What type of bread and cheese did you use?
Did you add margarine of butter?
Did you use mayonnaise or miracle whip?
Did you add lettuce or tomato slices?
How much of each item did you use?
If you did not make the food yourself, describe the contents as best as you can.

For example:

If you had 1 cup of tuna casserole, let us know that it was about 1/2 macaroni, 1/4 tuna, and 1/4 peas and celery.

3. RECORD IMMEDIATELY AFTER EACH MEAL AND SNACK

Take your food record with you if you go out to eat.

Please keep track throughout the day - it is easy to forget exactly what you have eaten!

HERE IS AN EXAMPLE FOR YOU TO USE AS A GUIDE:

Last Name: Smith First Name: Mary

Diet Record Day #: 1 Day of Week: Tuesday Date: March 24/93

TIME & PLACE	DESCRIPTION OF FOOD & BEVERAGE ITEMS	QUANTITY CONSUMED Please specify amounts eg. oz, g, tsp, cups etc.
Lunch 12:30 At home	• Macaroni and cheese	
	- cooked macaroni noodles	- 1 cup
	- homemade cheese sauce (made with flour, butter, cheddar cheese, 2% milk)	- 1/2 cup
	• orange Juice	- 1 cup
	• whole wheat dinner roll	- 3" in diameter
	• Margarine	- 2 teaspoons.

FOOD RECORD

Last Name: ______________________________ First Name: ______________

Diet Record Day #: _____ Day of Week: __________________ Date:______________

TIME & PLACE	DESCRIPTION OF FOOD & BEVERAGE ITEMS	QUANTITY CONSUMED Please specify amounts eg. oz, g, tsp, cups etc.

Did you take a vitamin/mineral supplement on this day? (Y/N) _______
If yes, please state the type, brand name, and the number of pills you took. ________________

Was this a fairly typical day for you? (Y/N) ________
If not, please give reason(s): ____

APPENDIX 11

DATA ANALYSIS OF INPATIENTS

BODY COMPOSITION CHANGES

Table 1:

Comparison of body composition parameters pre- and post-weight gain in 20 AN inpatients

Characteristic	Pre	Post
Weight[b] (kg)	43.0 ± 5.9^{a}	51.3 ± 6.1***
Height (cm)	163.0 ± 6.0	--
BMI[c] (kg/m^2)	15.4 ± 1.6	18.9 ± 1.3***
Percentage body fat[b]	16.8 ± 5.3	26.4 ± 5.2***
Total body fat[b] (kg)	7.4 ± 2.9	13.6 ± 3.4***
Total lean body mass[b] (kg)	33.6 ± 4.6	35.6 ± 5.1**
Total bone mineral content[b] (kg)	2.1 ± 0.2	2.2 ± 0.2**

a mean $\pm$ SD
b values obtained by DEXA
c BMI = body mass index (weight [kg]/height [m^2])
** $p < .01$ pre vs post weight gain
*** $p < .001$ pre vs post weight gain

CIRCUMFERENCE RATIOS

Table 2:

Selected circumference ratios pre- and post-weight gain in 20 AN inpatients

Circumference Ratio[a]	Pre	Post
Waist a:Hip	0.75 ± 0.04[b]	0.78 ± 0.04***
Waist b:Hip	0.82 ± 0.05	0.84 ± 0.05*
Waist a:Thigh a	1.56 ± 0.13	1.54 ± 0.11
Waist b:Thigh a	1.67 ± 0.19	1.65 ± 0.13
Waist a:Thigh b	1.44 ± 0.13	1.37 ± 0.12*
Waist b:Thigh b	1.57 ± 0.18	1.47 ± 0.14**

a circumferences measured in cm
b mean ± SD
* $p < .05$ pre vs post weight gain
** $p < .01$ pre vs post weight gain
*** $p < .001$ pre vs post weight gain

ABSOLUTE SKINFOLD CHANGE

Table 3a:

Absolute change of subcutaneous fat at 9 sites in 20 AN inpatients

Body Site	Absolute Change (mm)
Triceps	3.6 ± 1.9[a]
Chest	1.4 ± 0.9
Subscapula	2.8 ± 1.3
Axilla	3.1 ± 2.0
Abdomen a	6.2 ± 3.0
Abdomen b	6.4 ± 3.1
Iliac	5.3 ± 2.9
Thigh	6.8 ± 2.7
Calf	3.1 ± 1.3

[a] mean ± SD

Table 3b:

ANCOVA fully repeated measures of absolute subcutaneous body fat change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	572.189	8	71.524	20.589	0.000
ERROR	528.022	152	3.474		

RELATIVE SKINFOLD CHANGE

Table 4a:

Relative change of subcutaneous fat at 9 sites in 20 AN inpatients

Body Site	Relative Change (%)
Triceps	77.5 ± 66.3[a]
Chest	52.4 ± 39.1
Subscapula	55.9 ± 26.2
Axilla	80.9 ± 57.5
Abdomen a	134.1 ± 94.5
Abdomen b	137.3 ± 82.5
Iliac	130.2 ± 70.5
Thigh	100.1 ± 62.4
Calf	66.3 ± 33.9

[a] mean ± SD

Table 4b:

ANCOVA fully repeated measures of relative subcutaneous body fat change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	17.229	8	2.154	9.751	0.000
ERROR	33.571	152	0.221		

ABSOLUTE CIRCUMFERENCE CHANGE

Table 5a:

Absolute circumference change at 9 sites in 20 AN inpatients

Body Site	Absolute Change (cm)
Arm	3.1 ± 1.6[a]
Forearm	1.5 ± 1.1
Chest	4.6 ± 2.8
Waist a	8.1 ± 4.0
Waist b	7.8 ± 4.3
Hip	7.7 ± 4.0
Thigh a	5.6 ± 3.2
Thigh b	7.9 ± 4.1
Calf	2.4 ± 1.4

[a] mean ± SD

Table 5b:

ANCOVA fully repeated measures of absolute circumference change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	1019.448	8	127.431	27.359	0.000
ERROR	707.981	152	4.658		

RELATIVE CIRCUMFERENCE CHANGE

Table 6a:

Relative circumference change at 9 sites in 20 AN inpatients

Body Site	Relative Change (%)
Arm	17.0 ± 9.9[a]
Forearm	7.7 ± 6.0
Chest	6.3 ± 3.8
Waist a	13.8 ± 7.3
Waist b	12.4 ± 7.6
Hip	10.0 ± 5.6
Thigh a	15.3 ± 10.1
Thigh b	20.1 ± 12.3
Calf	7.9 ± 4.9

[a] mean ± SD

Table 6b:

ANCOVA fully repeated measures of relative circumference change with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY SITE	0.301	8	0.038	15.051	0.000
ERROR	0.380	152	0.002		

ABSOLUTE BODY FAT MASS CHANGE AS MEASURED BY DEXA

Table 7a:

Absolute body fat mass change at 3 body regions as measured by DEXA in 18 AN inpatients

Body region	Absolute Change (kg)
Subscapular	1.842 ± 1.164[a]
Waist	2.184 ± 1.135
Thigh	1.767 ± 0.806

[a] mean ± SD

Table 7b:

ANCOVA fully repeated measures of absolute fat mass change as measured by DEXA with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY REGION	1.666	2	0.833	3.243	0.051
ERROR	8.731	34	0.257		

RELATIVE BODY FAT MASS CHANGE AS MEASURED BY DEXA

Table 8a:

Relative body fat mass change at 3 body regions as measured by DEXA in 18 AN inpatients

Body region	Relative Change (%)
Subscapular	1628.5 ± 3815.6[a]
Waist	811.3 ± 1351.3
Thigh	147.0 ± 169.0

[a] mean ± SD

Table 8b:

ANCOVA fully repeated measures of relative fat mass change as measured by DEXA with initial body weight as the covariate

SOURCE	SS	DF	MS	F	P
BODY REGION	1294.148	2	647.074	1.473	0.244
ERROR	14940.743	34	439.434		