THE EFFECT OF MENSTRUAL CYCLE PHASE ON DIFFUSING CAPACITY OF THE LUNG

by

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ABSTRACT

Pulmonary diffusing capacity (D_L) has been observed to decrease during menses. Nonetheless, a descriptive study of alterations in this parameter with menstrual cycle phase has not been completed and the mechanism of change is not clear. Changes in resting single-breath diffusing capacity of carbon monoxide (D_{LCO}), and in its two components: pulmonary capillary blood volume (V_C), and membrane diffusing capacity (D_M) were measured in 13 normally menstruating women at points within the menstrual cycle chosen to best discriminate between the effects of oestradiol, progesterone and prostaglandins. In addition, haemoglobin concentration ([Hb]), packed cell volume (PCV) , and percent of carboxyhaemoglobin (COHb) were measured. Measurements of D_{LCO}, V_C, D_M, and [Hb] were undertaken at five testing points throughout three menstrual cycles, whilst COHb and PCV were assessed at four points within one cycle. The phase of the menstrual cycle was determined by quantitative analysis of basal body temperature recorded daily by subjects.

No changes in resting D_{LCO}, D_{LCO} divided by alveolar volume (V_A), COHb, PCV or for [Hb] corrected D_{LCO}, D_{LCO}/V_A, D_M or V_C were found using one-way repeated measures analyses of variance (ANOVAs) of the most representative ovulatory menstrual cycle for each subject. Two-way repeated measures ANOVAs of D_{LCO} and D_{LCO}/V_A; and [Hb] corrected D_{LCO} and D_{LCO}/V_A, which separated the effects of the five testing points and the ovulatory or anovulatory status of a menstrual cycle were also performed and no significant changes were observed. When the effect of the large hormonal differences between an ovulatory and an anovulatory cycle were removed, a trend towards an increase in D_L independent of the effects of [Hb] at mid-cycle and during the luteal phase compared to the early follicular phase were observed.

Notwithstanding the extreme variability of hormonal changes within the human menstrual cycle, without the benefit of hormonal analysis we have not found consistent alterations in D_{LCO} with menstrual phase in normally menstruating women. This is despite careful effort to time diffusion test with points in the cycle that should best discriminate between the hormonal effects of oestradiol, progesterone and prostaglandins.

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CHAPTER ONE: INTRODUCTION

The movement of metabolic and other gases across the alveolar membrane of the lung is a vital physiological process which takes place solely via passive diffusion. The physical properties of the alveolar membrane, the respiratory-cardiovascular interface, in particular its extreme thinness (around 1μm) and vast surface area (approximately $70m^2$) allow this exchange to take place freely in a healthy lung. It is doubtful in fact that lung diffusion limits the transfer of oxygen (O_2) from the atmosphere to metabolising tissues in healthy, untrained individuals, either in rest or during maximal exercise. Despite this, under certain circumstances lung diffusion may limit oxygen delivery, for example in diseased lungs (Crapo and Forster, 1989), at high altitude (Hastala and Berger, 1996), or in elite athletes during maximal exercise (Dempsey, 1986).

The capacity of the lungs to transfer O_2 from the lung alveoli to the red blood cell haemoglobin (Hb) in the pulmonary capillaries is called pulmonary diffusing capacity (D_L). D_L is usually measured clinically by determining the rate of disappearance of a known concentration of carbon monoxide from a single breath of a mixed gas: hence the name for the measurement: "diffusing capacity of the lungs for carbon monoxide" (D_{LCO})". For a substantial explanation of the basis of lung diffusion measurement and calculation refer to Appendix I: Lung Diffusion: Its Measurement, Components and Determinants (p48).

CYCLIC ALTERATIONS IN DIFFUSING CAPACITY

Because D_{LCO} is an important clinical measurement, often measured serially in one patient to assess changes in pulmonary function occurring as a result of pulmonary disease, it is important to be aware of other factors that may alter lung diffusion on a day to day basis as well as any cyclic variations in D_{LCO}. D_{LCO} has

traditionally been thought to undergo circadian variation, because it has been shown to decrease in individuals of both sexes by 1 to 2 percent per hour from morning to night (Cinkotai and Thompson, 1966). Nonetheless, a cyclic circadian pattern of D_{LCO} variation has not been demonstrated. Moreover, a more recent study (Frey et al., 1987) found no change in D_{LCO} at different times of the day after correcting it for COHb backpressure (an artifact of repeated diffusion testing), and for small circadian changes in haemoglobin concentration ([Hb]).

There are two ovarian phases of the human menstrual cycle The first, the follicular phase, begins on the first day of menses and lasts until ovulation. The subsequent luteal phase lasts from ovulation until the beginning of the next cycle and is characterised by vastly increased production of ovarian steroid hormones by the corpus luteum, a remnant of the ruptured ovarian follicle. For a complete review of the human menstrual cycle refer to Appendix II: The Human Menstrual Cycle.

A change in D_{LCO} with menstrual cycle phase might be expected from the recent results of Sansores et al. (1995). These researchers noted a 9.5% decrease on the third day of menses relative to a premenstrual baseline measurement. In this study, 14 healthy women (including one smoker and eight who were taking oral contraceptives), underwent six single-breath carbon-monoxide lung diffusion (D_{LCO}) measurements. A baseline measurement was obtained premenstrually (1 to 7 days before menses), then repeated measurements made on each of the first 4 days of menses, and on one occasion following menses (5 to 10 days after the onset of bleeding). In ten of the 14 women the two components of D_{LCO}, pulmonary capillary blood volume (V_C), and membrane diffusing capacity (D_M), were determined via duplicate D_{LCO} measurements at two different O_2 fractions. The authors observed no significant changes in V_C, D_M, or [Hb].

The mechanisms of this diffusion reduction are difficult to explain. D_{LCO} is a function of both pulmonary and circulatory parameters and will alter with changes

in the diffusing properties of the alveolar membrane (D_M), the pulmonary capillary blood volume (V_C), or the reaction kinetics of carbon monoxide (CO) and Hb (expressed as theta or θ). These might alter as a consequence of respiratory alterations in the luteal phase or as a direct hormonal effect.

RESPIRATORY CHANGES WITHIN THE MENSTRUAL CYCLE

The luteal phase of the menstrual cycle is associated with an increased resting and exercise minute ventilation ($\dot{V}_E$), (Schoene et al., 1981 and Jurkowski et al., 1981), raised resting hypercapnic and hypoxic drives (Schoene et al., 1981; Dombovy et al., 1987) and greater inspiratory muscle endurance (Chen and Tang, 1989) compared to the follicular phase. A potential mechanism for D_{LCO} changes is not apparent from established alterations in any of these respiratory parameters. It is possible that mild respiratory alkalosis occurring as a result of increased V_E might have a direct effect on θ. Deep breathing also has the potential to elevate D_{LCO} by raising the compliance of previously under inflated alveoli and consequently increasing the exchange surface area. Whilst large inspirations prior to a single breath might therefore raise D_{LCO} measurement, it seems unlikely that the small increase in tidal volume occurring in the luteal phase of the menstrual cycle would have a physiologically significant or even detectable effect on lung diffusion.

CIRCULATORY CHANGES WITHIN THE MENSTRUAL CYCLE

A substantial (17%) elevation of V_C (Seaton, 1972), and small (2.6%) increases in [Hb] (Jurkowski et al., 1981) have been observed in the luteal compared to the follicular phase of the menstrual cycle. The cause of these alterations is unclear but the timing of changes implicates a hormonal mechanism.

Progesterone mediated increases in Vc may have occurred during the premenstrual measurement in the Sansores et al., (1995) study. These researchers regarded hormonal changes as a possible explanation for the D_{LCO} reduction they noted during menses. Although alterations in Vc in the same study were non significant, the sample size for the assessment of Vc changes was small and a corresponding alteration in Vc might not have been detected. Progesterone levels increase around 10 to 40 fold in the luteal phase of a menstrual cycle in which normal ovulation occurs. A progesterone mediated change in D_{LCO} would therefore be expected to occur in the luteal phase of a normal ovulatory cycle, but not in an anovulatory cycle. The difference in diffusion alterations between menstrual cycles of differing ovulatory status has not been investigated.

High levels of oestrogen which occur immediately prior to ovulation in a normal menstrual cycle and following the administration of synthetic oestrogen, prevent the plasma volume drop associated with bed-rest (Fortney et al., 1988). Although unsubstantiated, a potential mechanism for this oestrogenic effect on plasma volume may involve the cardiovascular vasodilator nitric oxide (NO). Expired NO levels were shown in one study to increase almost threefold from menses until days 13 to 19 of the menstrual cycle and rapidly drop again later in the cycle (Kharitonov et al., 1994). Although ovulation was not documented in this study and the timing of reproductive hormone changes therefore unknown, the profile of NO fluctuations suggest that oestradiol, luteinising hormone or follicle stimulating hormone are the most likely hormones to initiate the response.

Mid-cycle increases in plasma volume would result in similar increases in Vc and therefore D_{LCO} by definition. Moreover, depending on the ability of the blood to restore [Hb] to normal levels, plasma volume alterations might affect the measured D_{LCO} through changes in [Hb]. Plasma volume, if it were raised too high, might also have the potential to induce pulmonary oedema via increased pressure and stress failure of pulmonary capillaries thus increasing the thickness of the

diffusion membrane and lowering D_M (and D_{LCO}). If raised oestradiol is responsible for menstrual alterations in D_{LCO}, a large increase in D_{LCO} would be expected in the late follicular phase prior to ovulation compared to moderate elevations during the luteal phase.

FURTHER EXPLANATIONS OF D_{LCO} CHANGES DURING MENSES

Alterations in the endogenous production of CO with menstrual phase have been reported in the past but not well studied (Coburn, 1970). A change in production would affect the assessment of D_{LCO} via changes in the diffusion gradient for carbon monoxide. Nonetheless, the expected magnitude of endogenous CO increase during menses as a result of red blood cell breakdown was calculated by Sansores et al. (1995), to result in only negligible declines in D_{LCO}, well short of the observed reduction.

Direct effects of steroid hormones on θ, the reaction rate of CO and haemoglobin might be considered as a potential mechanism. Reductions in D_{LCO} occurring around menstruation might also be mediated by vasoconstrictive agents (prostaglandins for example), which might alter the vasomotor tone of pulmonary capillaries thus affecting pulmonary capillary blood volume and hence D_{LCO}. Prostaglandin A_2 has a known vasoconstrictor action on pulmonary blood vessels (Patton et al., 1989) and may be involved in uterine vasoconstriction occurring just before and around the time of flow. Although its exact role in the human endometrium in vivo has not been established, Prostaglandin $F_{2\alpha}$ has also been implicated in the process of luteolysis or break-down of the corpus luteum occurring at the end of the luteal phase (Adashi et al., 1996).

LIMITATIONS OF PREVIOUS WORK

In addition to the small number of subjects who were able to undergo the diffusion partitioning procedure, the Sansores et al. (1995) study is limited in its ability to either characterise changes in D_{LCO} over the menstrual cycle or determine a likely mechanism for the observed change because it considered only the effect of a single menstrual cycle phase (menses itself) on D_{LCO} and because only one cycle for each subject was investigated. Furthermore, 8 of the 14 subjects were taking oral contraceptives and the post-menses measurement (obtained between Day 5 and Day 10 of the menstrual cycle) may have corresponded with a point in time when some of these 8 women were still taking placebo tablets, while others had resumed hormone tablets for the new cycle.

RESEARCH QUESTIONS

The main purpose of this study is to describe changes in D_{LCO} and the two components of D_{LCO} (V_C and D_M), within an ovulatory menstrual cycle of healthy regularly menstruating women. D_{LCO} expressed as a ratio to alveolar ventilation (V_A) is also analysed in an attempt to reduce error due to varying inspired volumes in the D_{LCO} procedure. Haemoglobin concentration [Hb], may also vary with menstrual cycle phase and values of D_{LCO}, D_{LCO}/V_A, V_C and D_M are reported corrected for haemoglobin. Measurement of packed cell volume (PCV or haematocrit), which reflects alterations in plasma volume, and COHb percent provide further explanatory power for significant alterations in diffusion. The following research questions provide a specific basis for the investigation.

1. Is there a change in D_{LCO} within an ovulatory menstrual cycle in regularly menstruating women?
2. Is there a change in D_{LCO}/V_A within an ovulatory menstrual cycle in regularly menstruating women?
3. Is there a change in [Hb] corrected D_{LCO} within an ovulatory menstrual cycle in regularly menstruating women?
4. Is there a change in [Hb] corrected D_{LCO}/V_A within an ovulatory menstrual cycle in regularly menstruating women?
5. Is there a change in [Hb] corrected V_C within an ovulatory menstrual cycle in regularly menstruating women?
6. Is there a change in [Hb] corrected D_M within an ovulatory menstrual cycle in regularly menstruating women?

If a significant alteration in D_{LCO} is found, the following research questions will be investigated as explanatory variables of the above.

7. Is there a change in percent COHb within an ovulatory menstrual cycle in regularly menstruating women?

8. Is there a change in PCV within an ovulatory menstrual cycle in regularly menstruating women?

The characteristics of diffusion changes between ovulatory and anovulatory menstrual cycles of individuals who display both within the study period will be compared.

9 - 12. Is the change in D_{LCO}, D_{LCO}/V_A, [Hb] corrected D_{LCO}, [Hb] corrected D_{LCO}/V_A with menstrual cycle phase different for ovulatory versus anovulatory cycles of the same regularly menstruating woman.

Finally, the reliabilities of the PCV measurement and [Hb] measurement procedures used in the study were assessed. A further comparison of the validity of the portable [Hb] analyser compared to a hospital spectometry unit was undertaken.

CHAPTER TWO: METHODOLOGY

ETHICAL APPROVAL

Ethical approval for the study was obtained from the University of British Columbia Clinical Screening Committee for Research and other Studies Involving Human Subjects. The ethical approval certificate and approved subject consent form is included (Appendix IV).

SUBJECT RECRUITMENT AND SELECTION

Volunteers were obtained through advertising on bulletin boards around the University of British Columbia (Appendix V) and through word of mouth. Initial contact with people indicating their interest in participating in the study was normally made by telephone.

Subjects included in the study had not been taking oral contraceptives for the previous 3 months, had no history of respiratory medical conditions and had not smoked regularly in the last 2 years since smoking has been shown to be associated with a decline in D_{LCO} (Frans et al., 1975). A subject who is an ex-smoker but has not smoked regularly for 2 years or more was thought to be less likely to resume smoking during the study. All subjects had also menstruated regularly for at least 5 years and a normal average cycle length of 21-36 days over the previous 12 months (Barr and Prior, 1994).

Subjects selected for the study also reported the recognition of cyclic alterations of physiological or psychological parameters during the menstrual cycle from which they believed they were able to estimate the time of ovulation and predict the occurrence of menstruation. Examples of commonly reported moliminal changes are increased viscosity of cervical mucous just prior to

ovulation and cramps just prior to menses. Subjects who had no experience with completing The Menstrual Diary (© Prior, 1996; Appendix VI) and who were not totally familiar with the timing of key events in their menstrual cycle, kept a record using this instrument for at least one month before testing.

An appointment was then made with subjects who met all selection criteria for the study. At this appointment, subjects read and signed the consent form. The timing of the testing points in the cycle was carefully explained and tentative bookings were made for the next four or five tests.

DOCUMENTATION OF MENSTRUAL DATA

The Menstrual Cycle Diary (© Prior, 1996) that allows daily recording of menstrual flow, molimina, mood fluctuations, and sub-lingual temperature was used by subjects in the study. The Diary was modified to include a measurement of supine resting heart-rate and hours of sleep (Appendix VI). Subjects were instructed to measure sub-lingual basal body temperature at approximately the same time each morning before rising using a Becton Dickenson digital thermometer. Daily basal body temperature measurements were used to provide an index of ovulation and luteal phase onset using the method of least mean squares (Prior et al., 1990b). Luteal phase onset was defined as the number of days from the first day of the quantitatively determined mean temperature rise to the day before the onset of bleeding inclusive.

Daily reports of menstrual cycle experiences (molimina), were used to increase subject interest in the project and their adherence to the study, identify the best time of testing and in the analysis to help confirm possible cycle-related changes in diffusion. Results from the menstrual charts were explained to subjects to help clarify the testing points and an attempt was made to answer any questions they had relating to their own menstrual cycle.

TIMING OF TESTING

Testing of subjects occurred at five points during the menstrual cycle (figure 1) for three cycles. Day 1 of the cycle was defined as beginning at midnight preceding the day that menstrual bleeding began irrespective of the exact time at which bleeding started. Subjects were instructed to report to the lab at the following five test points outlined below.

Test Point a. Early Menstrual

This took place on the first or second day of flow or the last day of the previous cycle. It was preferable that this Test Point occurred while menstrual cramps were were present. This time point was chosen as a time in the cycle where levels of oestrogen and progesterone are low but prostaglandins (as indicated by cramping) are present.

Test Point b. Late Menstrual

This testing point took place a few days later between Day 3 to Day 4 of the cycle (inclusive), when menstrual cramps if they had been present had subsided. At this time point levels of oestrogen, progesterone and prostaglandins are minimal.

Test Point c. Early Follicular

This took place a few days later again on Day 5 to Day 8 inclusive. At this time point follicular oestrogen levels would be expected to be moderate whilst levels of progesterone and prostaglandins are very low.

Test Point d. Midcycle

This testing point took place as close as possible to subject observed and reported thickening of cervical mucous in The Daily Menstrual Diary©. This time point would be expected to coincide with peak levels of oestrogen and low levels of

progesterone and prostaglandins that occur just prior to ovulation. Daily monitoring of cervical mucous was crucial in determining the correct time for this test point as the timing of ovulation within the cycle is quite variable (Landgren et al., 1980).

Test Point e. Mid Luteal

This took place between 3 to 7 days after a subject observed an increase in basal body temperature of around 0.35°C (Prior et al., 1990b) or between Day 17 to Day 22 of the cycle if a basal body temperature increase was not clear. An increase in temperature signifies the luteal phase onset and was quantitatively determined from basal body temperature measurements using the least mean squares analysis of Prior et al. (1990b). Analysis of the ovulatory pattern of the subject's past cycles was also used to help identify when ovulation was likely to occur and estimate the best time for mid-luteal testing for that individual.

A mid-luteal test occurring within an ovulatory cycle would be expected to coincide with moderate levels of oestrogen, high levels of progesterone and low levels of prostaglandins.

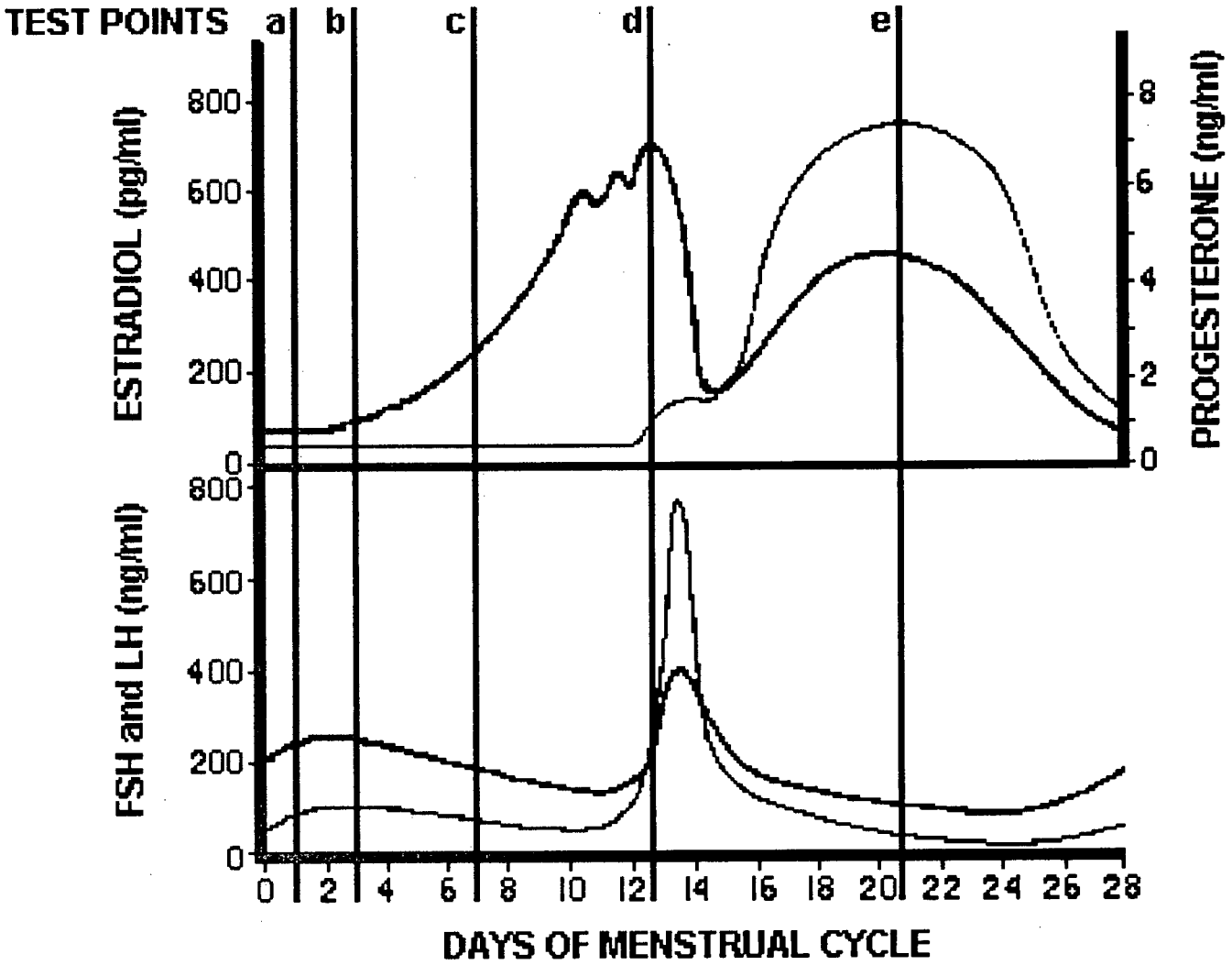

Figure 1: The Timing of Testing at Points in the Menstrual Cycle

Subjects began their first test at any of the five points depending upon the stage of their cycle they were at when they were first available for testing. For the purposes of this study, all test points were given a number and letter code. The number corresponds to the cycle number while the letter corresponds to the testing point (eg: "2a" refers to Cycle Number 2, Test Point a). Apart from a fingerprick blood sample, which was used for [Hb] measurement, and haematocrit measurement if there was enough blood, no blood or urine samples were collected at Test Point a (Early Menstrual).

At the completion of the study, the timing of visits was checked retrospectively against the subjects Menstrual Diary to ensure that the timing corresponded with the criteria. While advance warning of Test Point d (Midcycle) was provided by alterations in cervical mucous or other menstrual cycle experiences that the individual associated with ovulation (eg: mid-cycle cramps), it was possible to retrospectively compare the actual laboratory testing day with the quantitatively determined day of luteal phase onset. Ovulation and peak oestrogen would be expected to preceed the basal temperature rise by 2 to 3 days (Prior et al., 1990b) and peak progesterone levels might be expected around midway between the basal body temperature rise and the onset of the following cycle. The criteria for these two test points was clarified based on basal temperature analysis and was as follows.

Test Point d. Mid Cycle

This test point should have occurred between 5 days before basal temperature rise and the day before basal temperature rise to correspond with expected peak oestrogen.

Test Point e. Mid Luteal

This test point should have occurred between the second day of increased body temperature and three days before the onset of menses for the next cycle to correspond with expected peak progesterone.

DOCUMENTATION OF EXERCISE

Subjects used the Daily Exercise Record (Appendix VII) to record the amount of time spent engaging in either vigorous or strenuous exercise (sufficient to elevate heart-rate to greater than 150 beats per minute) or mild or moderate exercise (sufficient to elevate heart-rate to between 90 and 150 beats per minute). The primary mode of exercise each day was also recorded. Daily exercise was measured so that a reterospective assessment of exercise patterns could be used to explain aberrant D_{LCO} measurements and to help explain changes that occurred in menstrual hormonal status, particularly those changes sufficient to alter D_{LCO} or its components.

TESTING PROTOCOL

On an initial visit to the laboratory subjects completed a questionnaire detailing descriptive data including age, occupation, a brief menstrual, exercise and health history as well as a 24 hour recall of the consumption of foods high in calcium (Appendix VIII). Basic anthropometric variables (height, body mass and sum of six skinfolds) were measured and the subject's date of birth was recorded.

The timing of subsequent visits to the lab was explained to subjects and tentative dates were recorded. Where possible, each subject came into the lab at the same time for each of the 15 laboratory tests in order to minimise the effect of diurnal variation in lung diffusion (Cinkotai and Thompson, 1966). Subjects were

asked to be as consistent as possible with the timing of meals and caffeinated drinks prior to each session. Because pulmonary diffusing capacity of CO has been found to decline from 1 hour to 24 hours following maximal exercise (Sheel, 1995), subjects were also instructed not to exercise intensively 24 hours prior to their laboratory visit. The time of last vigorous exercise, last meal and last coffee or caffeinated drink was recorded.

Upon arrival in the laboratory, the subject's menstrual diary was reviewed, the date (or tentative date) and time of the next testing point were confirmed and any questions they had relating to their menstrual cycle were addressed. At Test Points b, c, d, and e for the first two cycles only blood was drawn and overnight urine collected from 11 subjects willing to undergo this procedure.

At each Test Point body weight, forced vital capacity (FVC) and the forced expired volume in 1 second (FEV1) were measured prior to assessment of diffusing capacity of the lung (D_{LCO}) and its two components D_M and V_C. At Test Point "a." when no venous blood was drawn, [Hb] was measured from a finger capillary sample.

At the final testing session, skinfolds were again measured, items of the questionnaire which might have changed over the study period were readministered and selected items from the Canadian Multicentre Osteoporosis Study (CaMOS) were administered via interview (Appendix X). Sheets for raw data entry are included in Appendix IX.

MEASUREMENT OF HEIGHT, WEIGHT AND ANTHROPOMETRIC VARIABLES

Height

The subject stood erect without shoes against a Holtain stadiometer. Subjects were instructed look straight ahead and visual inspection was used to determine if

their head was held in the Frankfort Plane: the position where an imaginary line joining the orbitale (most inferior point on the margin of the eye socket) to the tragion (notch superior to the flap of the ear at the superior aspect of the zygomatic bone) is horizontal. Upon a full inspiration, the measurement was taken as the maximum distance from the floor to the most superior point on the skull. Height was measured both on the initial and final testing session and, if the measurement differed, an average was recorded.

Weight

Body weight was determined with subjects wearing light clothing, using a Homs beam scale and measured to the nearest 0.05 kg. An average of the measurement at the initial and final testing session was recorded in the descriptive data. The subjects weight was also measured at all other test sessions to ascertain that no large fluctuations over the duration of the study occurred.

Skinfolds

Skinfold measurements were obtained following the procedure described by Ross and Marfell-Jones (1982), with the exception that all measurements, including the abdominal skinfold, were made on the right side of the body. The Triceps, Subscapular, Iliac Crest, Supraspinale, Abdominal, Anterior Thigh and Medial Calf skinfolds were each measured twice. If the two measurements varied more than 10% a third measurement was taken. The average between the two measurements varying less than 10% was recorded. If no measurement differed from the next closest by more than 10% an average of all three was used. Six skinfolds (all the above excluding Iliac Crest) were summed and recorded as the sum of six skinfolds. As skinfold assessment also took place at at the initial and final testing session, the average of the two sum of six skinfolds was reported.

MEASUREMENT OF RESTING FORCED VITAL CAPACITY (FVC) AND FORCED EXPIRED VOLUME IN ONE SECOND (FEV1)

Forced Vital Capacity (FVC) and Forced Expired Volume in 1s (FEV1) were measured at each testing session using the spirometry functions of a Collins/DS pulmonary function analyser. The subject was instructed to inspire deeply and then expire forceably: "as hard and fast as possible" until all air was "squeezed out" of their lungs. A flow versus volume loop for a whole breath cycle was completed when the subject reinspired to vital capacity. The largest FVC and FEV1 from at least two maximal tests not varying by more than 10% for either variable was recorded. The forced expired volume from 25% to 75% of the expiration (FEV_{25-75}) and the peak expired flow rate (PEFR) were recorded from the test with the greatest summed FVC and FEV1.

MEASUREMENT OF RESTING DIFFUSING CAPACITY (D_{LCO})

Resting pulmonary diffusing capacity was determined via the single-breath method first developed by Krogh (1915) and modified by Ogilvie et al. (1957) using the Collins analyser. Single breath methods as opposed to steady state measurements of diffusion are thought to better reflect the alveolar membrane and pulmonary capillary characteristics of the ventilated parts of the lungs (Forster et al., 1986). Moreover, they do not require the measurement of arterial pCO_2 for their most accurate determination. According to Forster et al. (1986) however, single-breath techniques may be less sensitive to unevenness of gas distribution and probably to non-uniformity of diffusion throughout the lung. It was thought that in healthy subjects unevenness and non-uniformity are likely to be minimal hence the use of the single-breath measurement in this investigation.

The single-breath method used to measure D_{LCO} in our laboratory consists of a rapid inspiration, a 10s breath-hold and a rapid expiration of the test gas containing

about 21% oxygen, 10% helium, 0.3% carbon monoxide and the balance nitrogen. A sample of expired gas is collected in a collection bag attached to the Collins analyser. Breath-hold during the test is timed from the beginning of inspiration to the beginning of sample collection as outlined by Ogilvie et al. (1957). While the latest American Thoracic Society (A.T.S.) recomendations (A.T.S., 1995) suggest the use of the Jones and Meade (1961) protocol which adjusts the calculation of breath hold to better reflect the CO concentration profile in the alveolar space, the results of Graham et al., (1981), suggest that in healthy subjects measures of diffusing capacity calculated via the Ogilvie method are likely to be similar to those measured via the Jones and Meade method. Both carbon dioxide (CO_2) and water (H_2O) were removed from the expired gas sample prior to analysis as the gas sample passed through a divided canister containing calcium sulphate and barium hydroxide. Concentrations of CO were measured using an infrared analyser. The concentration of expired O_2 is assumed for the purposes of calculation. In the measurement of D_{LCO}, subjects were encouraged to relax against a closed glottis and remain calm during the breath-hold to avoid performing either a Valsalva or Müller manoeuver that could under- or overestimate D_{LCO} respectively. Each diffusion measurement was also examined to ensure that the inspired volume was at least 85% of the FVC, the total time of inspiration was less than two seconds and the breath-hold time was between nine and eleven seconds as the accuracy of D_{LCO} measurements are increased in this range (Graham et al., 1981). The American Thoracic Society (A.T.S., 1995) recommends that 90% FVC be attained for each inspiration. However, subjects in this study were unable to attain this standard consistently and so the criterion was lowered to 85%. On a few occasions for 9 subjects, only one of the two tests reached 85%. If the two tests were above 80% and acceptably close to each other (below), then an average of these two tests was recorded. In line with A.T.S. recommendations (1995), D_{LCO} was determined in duplicate and repeated a third time if the initial two measurements varied from each other by more than 10% of their average. The

average of tests differing from the mean by 10% or less was reported. An interval of at least 4 minutes was allowed between tests to ensure elimination of the test gas from the lungs.

CALCULATION OF DIFFUSING CAPACITY

D_{LCO} was calculated automatically by the Collins system using equation 1 (below). The rationale for its use is outlined in Appendix I (p57).

Equation 1: Calculation of Diffusing Capacity

$$D_{LCO} = \frac{V_A \times 60}{713 \times t} \times \text{Ln}\,[(F_EHe/F_ECO) \times (F_ICO/F_IHe)] \times \text{STPD correction}$$

where D_{LCO} = diffusing capacity for CO (mlCO(STPD)/min/mmHg)

V_A = alveolar volume (ATPS in ml)

$$= V_I \times \frac{F_IHe}{F_EHe} \times 1.05$$

F_IHe = inspired He fraction

F_EHe = expired He fraction

F_ICO = inspired CO fraction

F_ECO = expired CO fraction

V_I = volume inspired

t = breath-hold time (s)

ATPS = ambient body temperature and pressure

STPD = standard temperature and pressure dry

1.05 = correction factor for 5% carbon dioxide in expired air removed prior to analysis

713 = P_B of 760mmHg - $P_{\text{water vapour at } 37^\circ C}$ of 47mmHg

CORRECTION FOR HAEMOGLOBIN CONCENTRATION

The diffusion value was adjusted for haemoglobin concentration using the formula below recommended by the American Thoracic Society in its 1995 recommendations (equation 2). Both adjusted and unadjusted diffusion measurements are reported and analysed.

Equation 2:

$$D_{LCO}\,[Hb]adjusted = D_{LCO}\,measured\,(9.38 + [Hb]) / 1.7\,[Hb]$$

where D_{LCO} [Hb]adjusted = diffusing capacity for CO corrected for [Hb] (g/dl)

(mlCO(STPD)/min/mmHg)

D_{LCO} measured = unadjusted diffusion measurement

QUANTIFICATION OF D_M AND V_C

The two components of pulmonary diffusing capacity (D_M and V_C) were measured using the single-breath method of Roughton and Forster (1957) as modified by Ogilvie et al. (1957). Two measurements of resting diffusing capacity were made using two different inspired fractions of O2 (21% and 90%). Subjects breathed for 5 minutes through a low resistance valve (Hans Rudolph, #2700B) attached to a Douglas bag filled with a gas mixture of 90% ± 5% O_2, and the balance N_2. The D_{LCO} 90% O2 test was performed in the same manner as the 21% O2. The reciprocal of D_{LCO} ($1/D_{LCO}$) or total resistance to diffusion, is the sum of two component resistances ($1/D_M$ and $1/V_C$). Mean pulmonary capillary oxygen partial pressure required for a calculation of $1/\theta$ was estimated using the alveolar gas equation. The calculation of D_M and V_C and the formula and underlying

assumptions for the use of the alveolar gas equation are outlined in Appendix I (p57).

THE RELIABILITY OF DIFFUSING CAPACITY AND ITS COMPONENTS

The reliability of the single breath procedure in measuring D_{LCO} and its components has been determined in our laboratory in a study of 9 individuals measured twice on separate days (Sheel et al., 1996). Pearson's product-moment correlation coefficients between the two measurements were r = 0.98, r = 0.84 and r = 0.92 for D_{LCO}, D_M and V_C respectively.

COLLECTION AND BATCHING OF BLOOD AND URINE SAMPLES

On the first cycle a total of approximately 16ml of blood was drawn from those subjects willing to undergo this procedure into 2, 6ml SST® vacutubes containing clot activator for later serum production, and into a 5ml vacutube (3 to 4 ml only) containing 15% K_3 EDTA for haemoglobin (Hb) and packed cell volume (PCV) analysis. On the second cycle, approximately 20ml of blood was drawn as above with an additional 3 to 4ml drawn into an airtight vacutube containing EDTA for later carboxyhaemoglobin concentration [COHb] analysis.

In addition, eight subjects who were willing collected a 45ml urine sample from their first morning excretion of the day that they were scheduled to come into the laboratory. Upon arrival at the lab, the urine sample was transferred to a standard household freezer in the laboratory. At the end of the week urine samples were transferred to -70°C.

MEASUREMENT OF PACKED CELL VOLUME AND HAEMOGLOBIN CONCENTRATION

Haemoglobin was measured at each testing session. For sessions when venous blood was not drawn, a pin-prick capillary blood sample was used. When venous samples were already taken, the whole, unclotted blood was slowly drawn into a 3ml syringe from the gently rotated vacutube and a drop from the syringe was allowed to saturate the measuring cuvette. Total [Hb] was then analysed using a HemoCue A13 portable β-hemoglobin photometer in the laboratory. In addition, [COHb] and total [Hb] were measured for one cycle (four time-points) in each subject at Vancouver General Hospital using an OSM3 hemoximeter. Samples were taken from the refrigerated storage in the laboratory and transported on ice-packs for this analysis. Both same day and backdated samples were checked to ascertain that a delay did not affect percent COHb determination.

A sample from the remaining whole unclotted blood was injected into two microcapillary tubes and spun in an International Electric Company (IEC) microcapillary centrifuge model M8 centrifuge for 3 minutes. PCV was determined from the spun samples using a Sherwood Micro-Hematocrit Tube Reader and the average from the two microcapillary tubes recorded.

DATA ANALYSIS

In order to answer the research questions, the data analysis was divided into three parts. Firstly to investigate changes in lung diffusion and diffusion-related variables, a "best" ovulatory cycle was selected for each subject and a difference over the five test points investigated.

Secondly, the different profiles of diffusion change between ovulatory and anovulatory cycles in the same subject were investigated by selecting the best ovulatory and best anovulatory cycle.

Finally, analyses of reliability of the [Hb] and PCV measurements were made in two ways. Firstly, intraclass correlation coefficients for four repeated measurements (consecutive where possible), from the Test Points at which blood was drawn were determined. Secondly, at a random Test Point for each subject, duplicate measurements were analysed. Thus the stability (or reliability of the measurements over time), controlled for changes in [Hb] and PCV with menstrual cycle phase, were compared to a same day reliability of the measurement procedures. The validity of the [Hb] measurements made at our laboratory was determined from cross-measurements of venous blood total [Hb] on our laboratory portable HemoCue [Hb] analyser and the total [Hb] measurement made on the OSM hemoximeter located at Vancouver General Hospital. One Test in which both measurements were made will be selected at random for each subject. Although any spectrometry measurement uses only a very small sample of whole blood (cf. blood chemistry analysis), this OSM hemoximeter was deemed as a suitable criterion for the determination of validity of our equipment. The manufacturer reports the total standard error for measurements of total [Hb] and [HbCO] made on the equipment to be 0.4 g/dl and 0.6% respectively for a 13.8 g/dl sample of oxygenated blood. No comprehensive study of Hemo-Cue reliability is reported in the manufacturer's manual although the stated accuracy of the apparatus is ±0.3 g/dl.

CRITERIA FOR THE SELECTION OF THE "BEST" CYCLE FOR ANALYSIS

One cycle was chosen for each subject for investigation of diffusion alterations. This procedure prevented the overrepresentation of one subject in the investigation whilst providing a solution to effectively deal with missing test points and anovulatory menstrual cycles.

The criteria for selecting the best cycle were as follows.

1. Diffusion tests met the criteria outlined previously (p18-20).

2. Cycle data was complete. No more than 33% of temperature readings (or any 3 at mid-cycle) were missing or affected by reported illness or taking at the wrong time (Prior et al., 1990b).

3. The cycle was ovulatory as confirmed by basal body temperature methods.

4. The menstrual period was between 3 and 6 days in length and the cycle (defined by the luteal phase cycle point (point "e") was of normal length: between 21 and 36 days (Prior, 1996).

The cycle chosen normally began at the same cycle point that the subject originally started participating in the laboratory sessions, unless it was impossible to select five sequential testing points meeting the criteria above starting at this test point. Ovulation was assessed using The Menstrual Diary© which begins each cycle with the onset of menstruation. Whether a cycle for analysis was to be classed as ovulatory or not depended on the basal body temperature analysis of Test Points d and e. (Mid Cycle and Mid-Luteal Test Points) irrespective of where the cycle began. If the cycle began at Test Point e, both the preceding and proceeding cycle needed to be ovulatory.

STATISTICAL ANALYSIS

Descriptive statistics of subject characteristics including age, height, weight and lung function parameters were calculated using Microsoft™ Excel 5.0.

The first analysis of changes in diffusion parameters during a normal ovulatory cycle was made via a One-Way Repeated Measures Analysis of Variance (RM-ANOVA) over the five Test Points. Significant trends across the Test Points were also investigated using trend analysis. All pair-wise Tukey's post-hoc tests were chosen as the statistic used to investigate the nature of any significant changes in the overall F statistics that were observed. The second analysis of ovulatory and anovulatory cycles was investigated using a Two-Way (Ovulatory Status (2 levels) x

Test Point (5 levels)) RM-ANOVA and testing for a significant interaction between the groups. The significance of the regression equation determined was also tested. Finally for the reliability and validity studies, Intraclass Correlation Coefficients were calculated for the repeated measurements using Statistical Package for the Social Sciences, Version x (SPSSx) for Windows. The Intraclass Correlation Coefficient is a more appropriate measurement of reliability than Pearson's (interclass) Correlation Coefficient (Vincent, 1995, p178). Refer to Appendix III for the formula and an explanation. For all analyses α was set at 0.05.

CHAPTER THREE: RESULTS

RECRUITMENT OF SUBJECTS

Subjects were largely self-selected because the criteria for entry were outlined on the advertisement, however three individuals who were ineligible to participate contacted by telephone. A total of 19 subjects agreed to enter the study. Four withdrew from the study before the first laboratory visit due to other time commitments. Two subjects withdrew after their first laboratory visit, one because of other commitments and one for feelings of discomfort with the laboratory procedure. One subject agreed to participate but was not willing to have blood drawn and no blood was drawn from another subject after medical staff experienced undue difficulty in drawing blood from her. One subject (subject 21) was only able to participate for two rather than three menstrual cycles and. Thus, a total of 13 subjects (11 from whom blood was drawn) are included in the analysis.

GENERAL SUBJECT CHARACTERISTICS

All the subjects were associated with the university either as staff (n=3), students (n=9) or in one case, a recently graduated student. They were 168.8±4.2cm in height and weighed 67.1±21.2kg. In accordance with the selection criteria, no subjects smoked regularly, none had a respiratory or endocrine medical condition and none had taken oral contraceptives in the last year. All subjects had a history of regular menstruation and all reported that they were normally able to predict the onset of menses. They reported a cycle length of 29.6±2.0 days and a 4.9±1.3 day duration of menses. Their average age at menarche was 12.5±1.3 years. None of the subjects had been pregnant in the past. Summary data of subject characteristics is included in Appendix XII (p59).

SUBJECT ACTIVITY

All subjects were healthy and active. Three were competitive athletes at the time of the study. Analysis of the open question for subjects to list the vigorous activity they performed regularly revealed that they performed 6.0±3.1 hours per week of vigorous activity ranging from 0 to 10.1 hours per week at the time of the study. All had spent more than one hour per week vigorously active in the past for an average of 9.3±4.2 years. The question from the Canada Multicentre Study of Osteoporosis (CaMOS), which divides activity into strenuous sport, vigorous work and moderate activity elicited values of 6.5±4.9, 1.5±1.3, and 6.1±3.9 hours per week respectively. Hours of sitting per week ranged from 1.5 to 13.5 hours per week (7.2±3.3 hours/week).

LUNG FUNCTION CHARACTERISTICS

Since all subjects were active and had no respiratory conditions, all lung function variables were within the normal range (Table 1). Blood haemoglobin concentration (recorded as an average between the initial and final laboratory reading was 13.1±1.0 mg/dl. The packed cell volume was 39.0±2.9%.

Ten of the thirteen subjects experienced difficulty in consistently attaining an inspired volume of 90% of forced vital capacity (FVC) in all four diffusion tests during each test session. Ten subjects were able to attain 85% in at least one of the two tests at both O_2 fractions at every session. For the three subjects who could not, care was taken in the selection of the "best" cycle for analysis to include test sessions that met this criterion.

TABLE ONE: Lung Function Characteristics of Subjects
showing forced vital capacity (FVC), forced expired volume in 1s (FEV1), forced expired volume between 25% and 75% of expiratory time (FEV25-75), and peak expiratory flow rate (PEFR)

SUBJECT	FVC (l)	FEV1 (l/s)	FEV 25-75 (l)	PEFR (l/s)
00	4.39	3.67	3.58	9.29
01	4.14	3.40	3.11	7.69
03	3.76	3.43	4.17	7.04
04	5.77	4.91	5.18	9.59
08	3.95	3.25	3.22	6.58
12	4.47	3.92	4.35	9.20
13	3.76	3.46	4.59	7.02
16	4.08	3.74	4.47	6.99
18	3.35	2.72	2.55	5.30
19	3.89	3.50	4.40	9.52
20	3.96	3.50	4.07	8.36
21	4.46	3.85	4.48	9.93
22	4.56	3.82	3.76	7.72
MEAN	4.19	3.63	3.99	8.02
S.D.	0.59	0.49	0.72	1.42

ANALYSIS OF CYCLES

The 13 subjects completed testing over a total of 38 full menstrual cycles (two for Subject 21 and three for all the rest). The cycle characteristics for each subject are shown in Table 2. Because subjects began attending the lab at different times throughout their cycle, but kept their Menstrual Diary for the period of time leading up to and following their initial and final laboratory visit, there are more than 3 cycles listed for some subjects. In addition one subject, (Subject 12), was not able to attend laboratory sessions for one month, but kept her Menstrual Diary during the intervening time. One subject (Subject 19), missed a menstrual period. This is represented in the table below as a particularly long cycle. Basal body temperature analysis confirmed that 6 (16%) of the 37 menstrual cycles with sufficient data recorded were anovulatory.

ANALYSIS OF TESTING POINTS

A timetable which shows the dates of diffusion testing for each subject, and corresponding cycle day for the diffusion measurements is included (Appendix XI). The timetable also shows the cycle length (CL) for the cycle beginning at Point "a" and the day of luteal phase onset as determined by the basal body temperature rise. The Test points chosen for the "best" ovulatory cycle and the anovulatory cycle are also shown in Appendix XI on a separate timetable.

When the best ovulatory cycles were chosen for each subject, data from four Test Points (in three subjects) were missing because the subject was unable to attend at that time. For the anovulatory cycles recorded by six subjects, there were two missing Test Points. Missing values were replaced in the statistical analysis with the grand cell mean corrected for the subject mean and for the test point mean. In two cycles chosen as the best ovulatory cycle, the length of menses was more than 6 days (7 days for Subject 13 and 11 days for Subject 19). Subject 04, whose best ovulatory

TABLE TWO: Summary of Menstrual Cycles

showing cycle number in study, cycle length (days), length of menses (days), and length of the luteal phase*[1].

SUBJECT	CYCLE NO.	CYCLE LENGTH	MENSES LENGTH	LUTEAL LENGTH (t value)
00	1	26	5	11 (2.07)
	2	23	6	0
	3	30	5	9 (7.94)
01	1	27	6	14 (5.75)
	2	26	5	11 (6.57)
	3	28	5	13 (11.67)
03	1	38	6	9 (3.86)
	2	27	6	9 (5.26)
	3	41	7	0
		31	6	8 (4.64)
04	1	26	5	6 (2.49)
	2	27	5	11 (2.29)
	3	28	7	I.D.*[2]
	4	26	7	I.D.
08	1	28	4	6 (2.53)
	2	28	3	10 (5.99)
	3	34	5	9 (3.78)
12	1	29	4	15 (2.14)
	2	26	I.D.	0
	3	27	I.D.	I.D.
	4	33	I.D.	I.D.
	5	I.D.	I.D.	I.D.
13	1	27	7	10 (3.49)
	2	29	5	7 (4.76)
	3	25	7	0
16	1	29	7	14 (2.07)
	2	27	5	11 (4.96)
	3	28	5	14 (3.44)
	4	27	5	12
18	1	32	7	13 (8.06)
	2	32	6	14 (9.30)
	3	34	8	0
	4	31	5	17
19	1	29	11	6 (3.75)
	2	59	10	0
	3	I.D.	I.D.	I.D.
20	1	38	6	14 (7.92)
	2	31	6	15 (8.27)
	3	35	5	13 (6.31)
	4	I.D.	I.D.	I.D.
21	1	30	I.D.	10 (6.16)
	2	34	5	9 (4.56)
	3	33	4	I.D.
22	1	28	I.D.	I.D.
	2	29	I.D.	9 (6.45)
	3	27	4	14 (12.25)
	4	26	4	13

*[1] Luteal length was measured from the day of onset of basal body temperature rise (inclusive) to the day of onset of menses (exclusive). A luteal length of 0 means that the menstrual cycle was anovulatory. The t value is for the difference in basal temperatures between the follicular and luteal phase (greater than 2.0 is considered acceptable).

*[2] Insufficient data to determine this value

cycle was 26 days in length, had recorded only 15 basal temperatures for that cycle and therefore fell just short of the criteria for sufficient data. Despite there being inadequate recordings, the mean difference between temperatures in the follicular and luteal phase for that subject was allowable (t = 2.49) and was included as an ovulatory cycle. The anovulatory cycles appeared to be relatively more disturbed in terms of cycle length and length of bleeding. Two of the six anovulatory cycles were longer than 36 days and four exhibited menstrual periods greater than 6 days. One anovulatory cycle with only 16 (out of 26) recorded temperatures was also used.

The proportion of cycle Testing Days that occurred outside the strict timing criteria previously outlined (p11-13) is shown below (Table 3). The details of this information may be examined by comparing Diffusion Testing Timetable (Appendix XI) with Table 2. A total of 10 tests for ovulatory cycles did not occur at the correct time. Nine were only one day outside the criteria and one (Subject 20, Test Point d) occurred two days late. Three tests originally scheduled as Test Point e met the criteria for Test Points d (2) and a (1) and were thus analysed as such. All tests occurred within the correct time frame for the anovulatory cycles. Test Points "d" and "e" took place between Day 15 and 19, and between Day 19 and 23 of the menstrual cycle respectively.

BLOOD SAMPLE ANALYSIS TESTING DATES

Two timetables showing the date of testing, and corresponding cycle day for collection of blood samples for Hb and COHb assessment are included (Appendix XI). In addition to the smaller number of samples, they differ from the diffusion time-table in minor details when blood testing took place on the previous or subsequent day.

TABLE THREE: An Analysis of Test Points

BEST OVULATORY CYCLE	a	b	c	d	e
Number of Missing Test Points	1	1	0	0	2
Timing of Tests Outside Criteria	2	2	0	5	1
Total Test Points	13	13	13	13	13

ANOVULATORY CYCLE	a	b	c	d	e
Number of Missing Test Points	1	0	0	0	1
Timing of Tests Outside Criteria	0	0	0	0	0
Total Test Points	6	6	6	6	6

BEST CYCLE ANALYSIS

No significant change across the five test points was noted in D_{LCO} or D_{LCO}/V_A (Figure 2 and 3). Adjustment for [Hb] alterations made no difference to the results for D_{LCO}, and D_{LCO}/V_A (Figures 4 and 5), although a significant difference in [Hb] between Test Points of an ovulatory cycle was observed (Figure 6). Tukey's post-hoc test for all pairwise comparisons at the $\alpha=0.05$ significance level showed that only the largest difference from Test Point a to Test Point e was significant. The change in [Hb] was characterised by an average 5% increase from the early follicular to the mid luteal phase.

No changes were found in [Hb] adjusted or unadjusted V_C or D_M over 5 Test Points. For these parameters however, 16 missing values (25%), were replaced with mean corrected values.

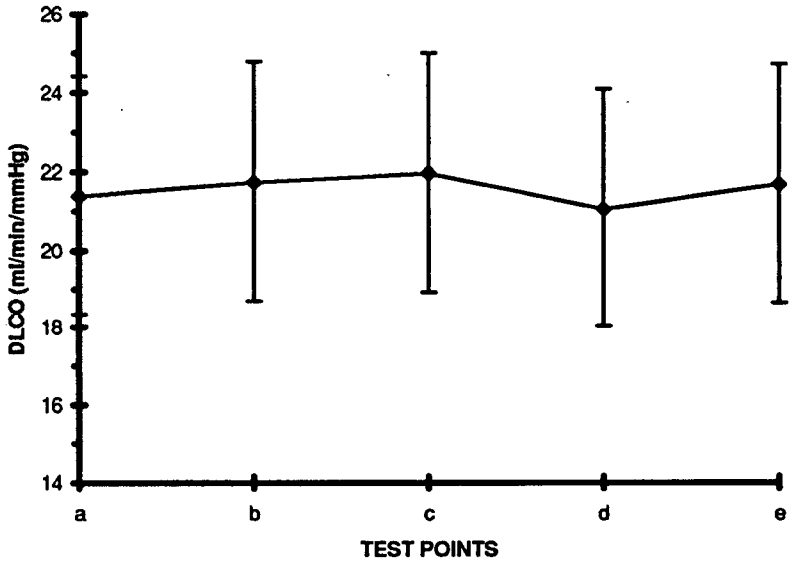

Figure 2: Diffusing Capacity Measured at Five Test Points Within Ovulatory Menstrual Cycles of 13 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

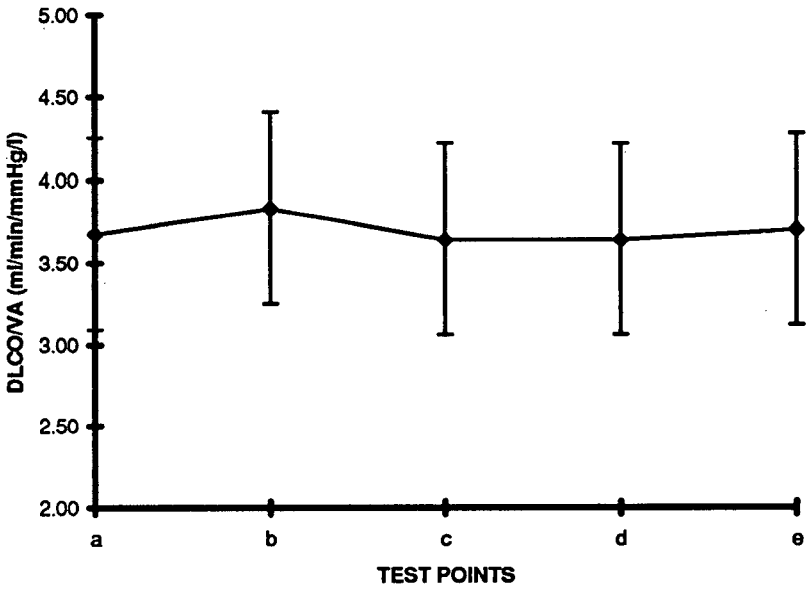

Figure 3: Diffusing Capacity/Alveolar Ventilation Ratio Measured at Five Test Points Within Ovulatory Menstrual Cycles of 13 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

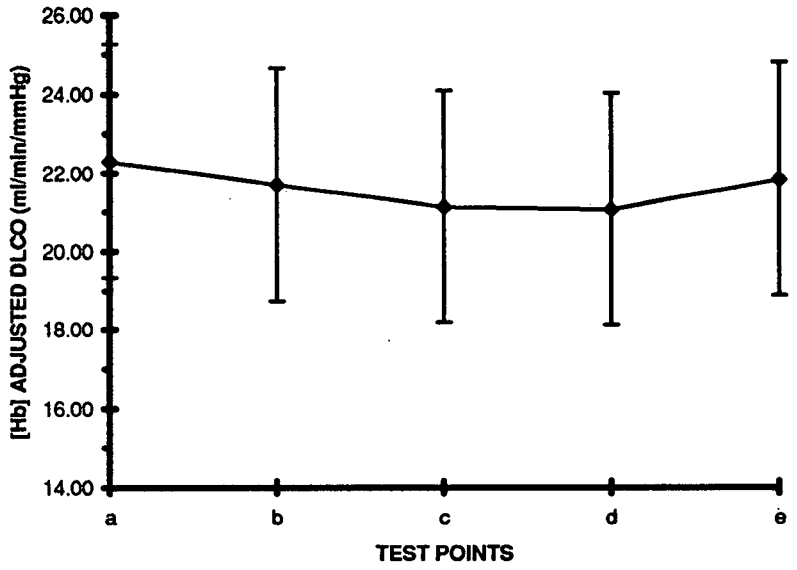

Figure 4: Haemoglobin Corrected Diffusing Capacity Measured at Five Test Points Within Ovulatory Menstrual Cycles of 13 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

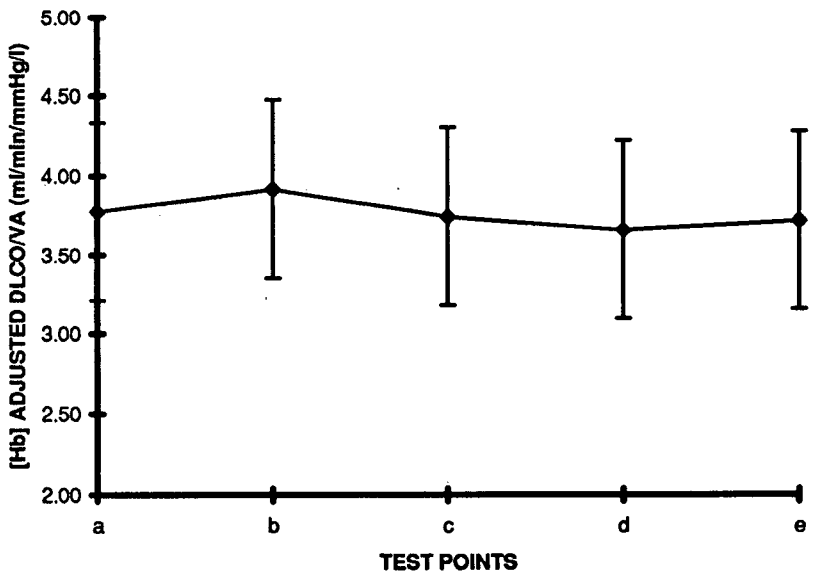

Figure 5: Haemoglobin Corrected Diffusing Capacity/Alveolar Ventilation Ratio Measured at Five Test Points Within Ovulatory Menstrual Cycles of 13 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

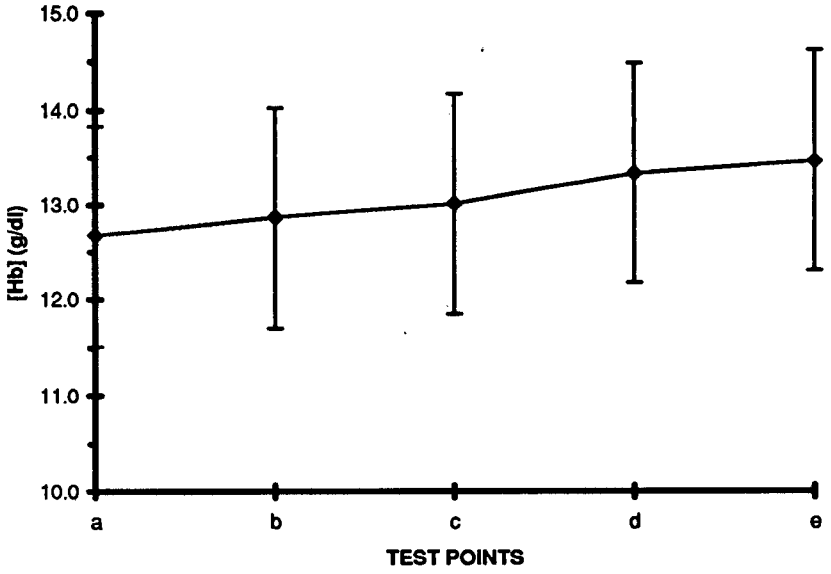

Figure 6: Haemoglobin Changes Measured at Five Test Points Within Ovulatory Menstrual Cycles of 13 Women.
Bars represent standard deviations from the mean. Test Point e is significantly different from Test Point a.

OVULATORY VERSUS ANOVULATORY CYCLES

When diffusion measurements of the 6 subjects who showed a variation in menstrual cycle status over the 3 months were analysed, [Hb] adjusted D_{LCO} changes over the 5 test points approached significance ($p=0.066$). There was also a slight trend towards a difference in D_{LCO}/V_A and D_{LCO}/V_A adjusted for [Hb]. All three measurements tended to fall from the late menstrual measurement to mid-cycle. [Hb] adjusted D_{LCO} tended to undergo steady decline from Test Point a to d and then a small rise at Test Point e. Figures 7 to 10 show trends in the changes of these variables in ovulatory and anovulatory cycles of subjects who recorded both during the study.

There was no change in non [Hb] adjusted D_{LCO}, nor in adjusted or unadjusted D_M or V_C over the 5 test points. No changes were observed in any of the diffusion variables measured from the ovulatory to the anovulatory cycle. Additionally, there were no significant interactions between menstrual status and Test Point for any of the diffusion variables.

CHANGES IN CARBOXYHAEMOGLOBIN WITH MENSTRUAL CYCLE PHASE

There was no change in COHb percentage measured at 4 Test Points for all 11 subjects. Seven of the subjects exhibited ovulatory cycles for this analysis. Separating out the potential differences in COHb changes in ovulatory subjects from the three subjects who did not ovulate during that cycle by using a Mixed Method Repeated Measures ANOVA, revealed no change in COHb over the test points and no interaction between menstrual status and Test Point (Figure 11).

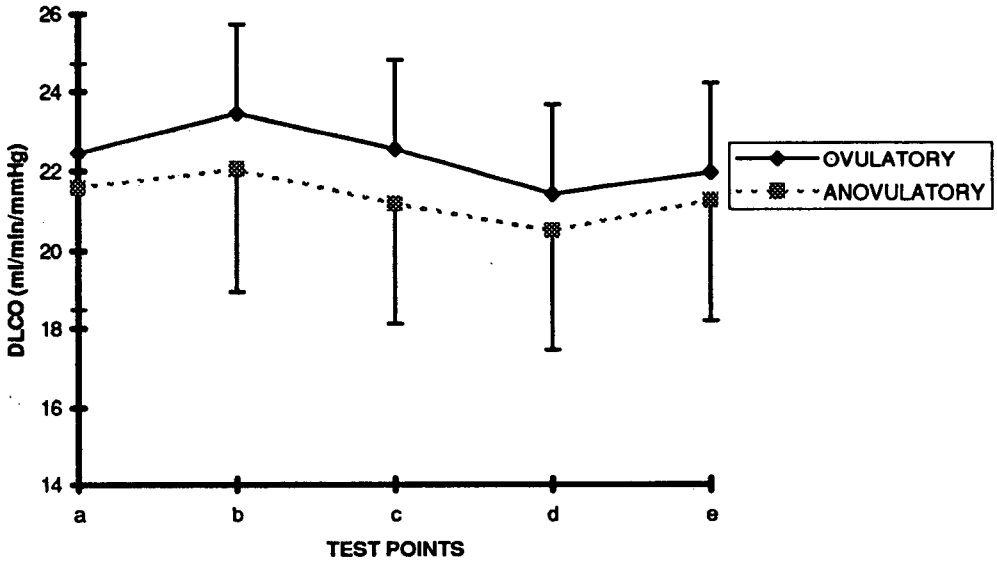

Figure 7: Diffusing Capacity Measured at Five Test Points Within Ovulatory and Anovulatory Menstrual Cycles of 6 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

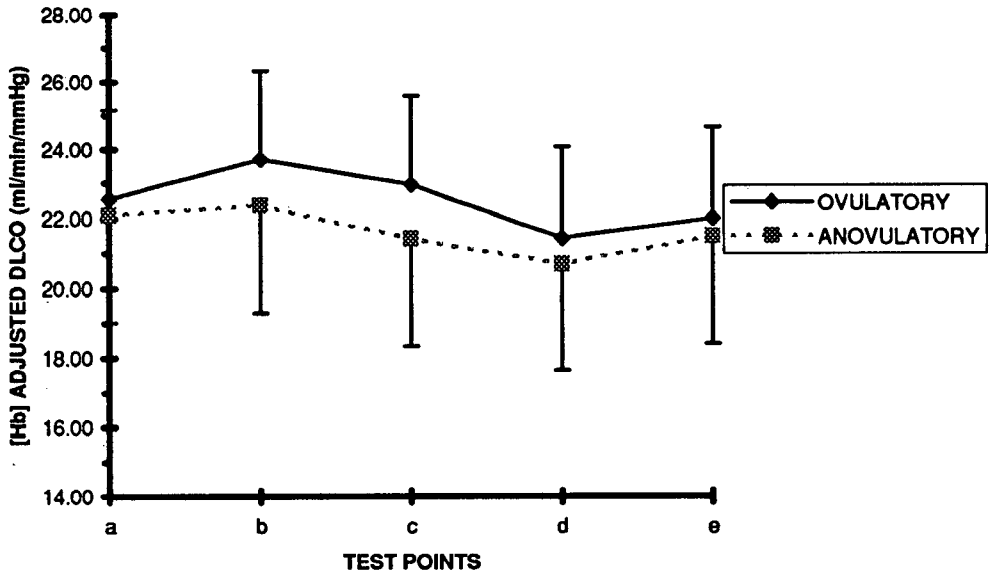

Figure 8: Haemoglobin Corrected Diffusing Capacity Measured at Five Test Points Within Ovulatory and Anovulatory Menstrual Cycles of 6 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

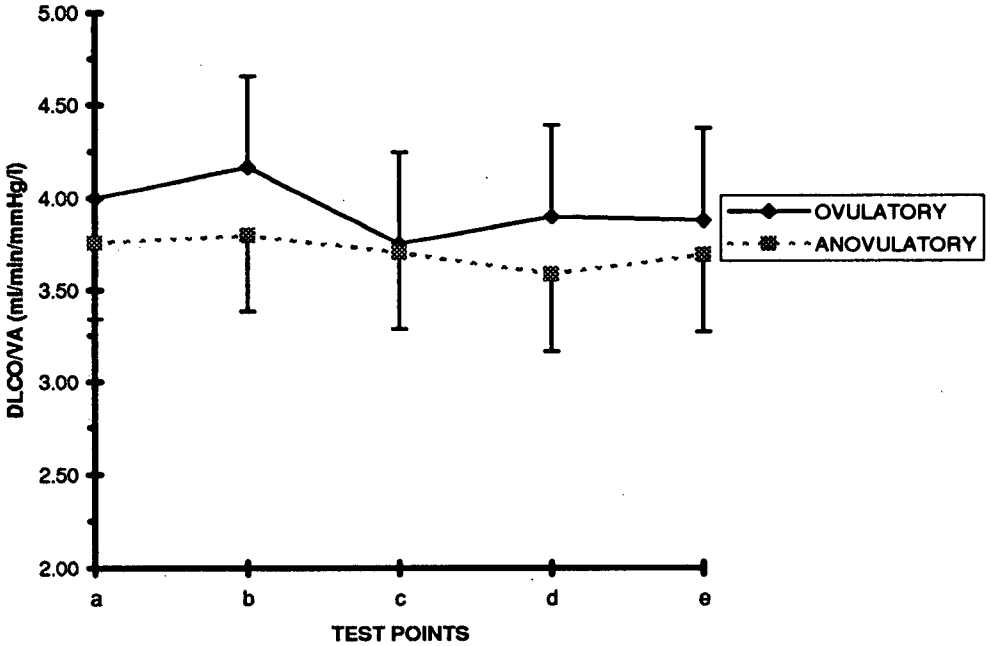

Figure 9: Diffusing Capacity/Alveolar Ventilation Ratio Measured at Five Test Points Within Ovulatory and Anovulatory Menstrual Cycles of 6 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

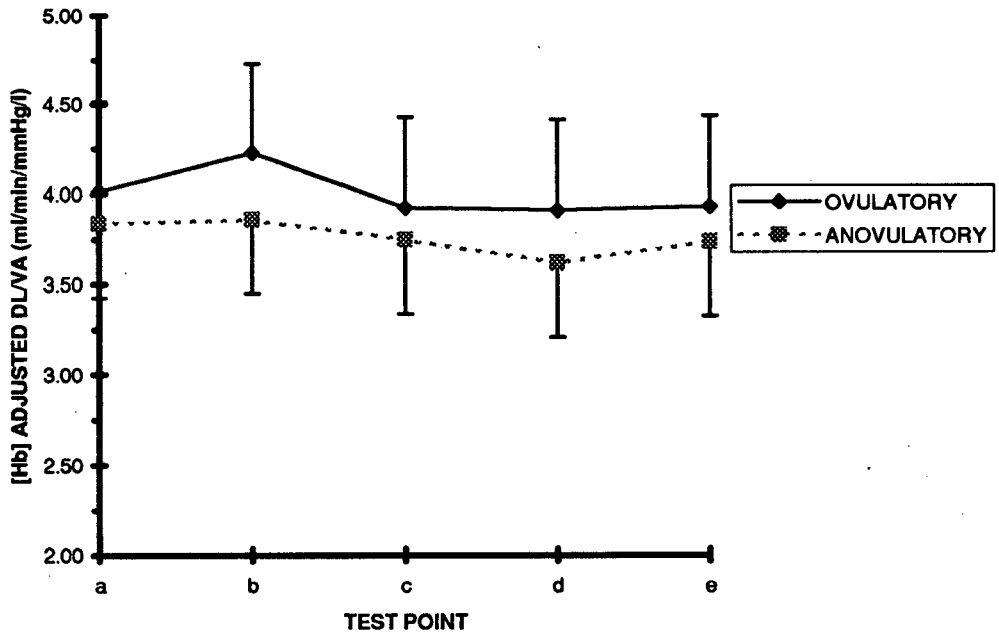

Figure 10: Haemoglobin Corrected Diffusing Capacity/Alveolar Ventilation Ratio Measured at Five Test Points Within Ovulatory and Anovulatory Menstrual Cycles of 6 Women.
Bars represent standard deviations from the mean. No significant alterations were observed.

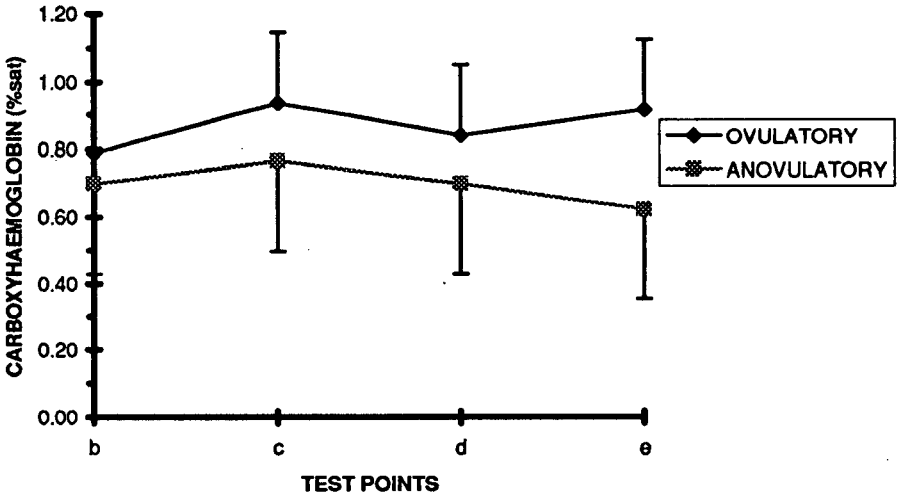

Figure 11: Percent Carboxyhaemoglobin Measured at Five Test Points Within the Menstrual Cycles of 7 Ovulating and 3 Non-Ovulating Women.
Bars represent standard deviations from the mean. No significant changes between test points, differences between the two groups or interactions found.

CHANGES IN PCV WITH MENSTRUAL PHASE

No alteration in PCV over 4 Test Points was observed when measurements from all 11 subjects were analysed. Data from nine subjects who exhibited an ovulatory cycle during the phase of PCV measurement were also analysed and no changes over the menstrual cycle were found.

RELIABILITY OF THE PACKED CELL VOLUME MEASUREMENTS

The test-retest reliability of two measurements of PCV, drawn from the same whole blood sample and spun at the same time in our laboratory was very high and significant (r intraclass = 0.99; $r^2 = 0.98$; $p<0.01$). Over four tests measured at different phases in the menstrual cycle and after the separation of any consistent change across the Test Points, the reliability of PCV measurement in one subject was much lower and non-significant (r intraclass = 0.25; $r^2 = 0.06$).

RELIABILITY AND VALIDITY OF THE HAEMOGLOBIN CONCENTRATION MEASUREMENTS

Test retest reliability for the measurement of [Hb] in our laboratory was also very high ($r = 0.98$; $r^2 = 0.96$; $p<0.01$). The reliability of this measurement procedure undertaken at four points in the menstrual cycle after removing consistent changes with menstrual phase was still high ($r = 0.96$; $r^2 = 0.92$; $p<0.01$).

The Intraclass Correlation Coefficient for the relationship between our measurement procedure and hospital based spectroscopy was ($r = 0.89$; $r^2 = 0.85$; $p<0.01$). There was a significant trial effect ($p<0.01$) and our laboratory procedure overestimated [Hb] compared to the OSM unit by an average of 0.63 g/100ml or 5%.

CHAPTER FOUR: DISCUSSION

RECRUITMENT AND CYCLE CHARACTERISTICS OF SUBJECTS

Of the subjects who were initially recruited for the study, only 68% completed the entire project. As only two of subjects who later withdrew attended the first laboratory visit, there is little information available to assess a difference in characteristics between those who completed the study and those who did not. The subjects who withdrew were of a similar educational and occupational background to the subjects who remained in the study and no obvious selection bias is apparent. Moreover, since the initial sample was non-random, the consequences of a selection bias as a result of subject withdrawal is likely to be relatively unimportant.

Only 72% (81 out of 113) carefully selected women met the further screening criterion of two consecutive ovulatory cycles in the study of Prior et al. (1990a). Moreover, of the 81 women who remained in their 12 month study, only 66 (81%) completed it and only 13 (20%) of these women had consistently ovulatory menstrual cycles throughout the year. Screening for the current study was not as rigid as for the Prior et al. study. In the current study, subjects were not required to demonstrate two consecutive ovulatory cycles before entering the study. Subjects in the current study did however report a good knowledge of the timing and nature of events within their cycle and it was assumed that this was likely to be related to hormonal consistency. It was anticipated that at least 35% of subjects recruited for the study would either withdraw or not exhibit consistent ovulatory cycles for the three cycles. In fact, 32% of recruited subjects withdrew from the study and of the 13 who remained only six ovulated consistently for the time they were in the study. Two subjects recorded insufficient data to determine if they ovulated consistently or not.

DIFFUSION CHANGES WITHIN THE "BEST" OVULATORY MENSTRUAL CYCLE

Neither D_{LCO} nor its components was found to alter during ovulatory menstrual cycles in this group of women. While a change in [Hb] was observed, correction of the diffusion measurements for [Hb] did not affect the result. Differences in V_A from test to test or over the three month period could not explain the lack of significance in D_{LCO} either because there was no change in D_{LCO} expressed as a ratio of V_A. In addition, V_A was very consistent. The mean intra-subject standard deviation of V_A was 0.23 litres, less than 4% of the mean.

For a One Way Repeated Measures ANOVA, power is correctly determined using the noncentrality parameter lambda (λ) (Winer et al., 1991). λ is essentially an effect size measure, analogous to Cohen's "d", whose variance term in the denominator is equivalent to the mean squared error (MS_{error}) from the ANOVA table. In turn, MS_{error} can be estimated from the mean intratrial variance and the mean correlation coefficient of all pairwise trials as shown in Equation 3.

Equation 3:

$$\lambda = \frac{n \sum (\mu_i - \mu)^2}{\sigma^2_e (1 - \bar{\rho})}$$

where λ (lambda) = the noncentrality coefficient

n = number of subjects

$\sum (\mu_i - \mu)^2$ = the sum of the squared differences between each trial mean and the overall mean

σ^2_e = the common within cell variance (estimated from the average variance among scores of the dependent variable within a group)

$\bar{\rho}$ (rho) = the average of the correlation coefficients between all pairs of trials

In the current study, the power to detect a 10% difference in D_{LCO} from the highest to the lowest Test Point, assuming an even spread of D_{LCO} at other Test Points, was greater than 99.9%. The power to detect the same magnitude difference was even greater for [Hb] adjusted D_{LCO} and [Hb] adjusted D_{LCO}/V_A, but was lower (75.5%) for [Hb] adjusted V_C. The power to detect a 5% change in D_{LCO}, [Hb] adjusted D_{LCO} and [Hb] adjusted D_{LCO}/V_A was still high: 59%, 74% and 70% respectively. More than the required number of subjects to detect with confidence the 9.5% difference in D_{LCO} noted by Sansores et al (1995) were recruited for this study in order to ensure enough consistently ovulating women.

HAEMOGLOBIN CONCENTRATION CHANGES WITHIN THE "BEST" OVULATORY MENSTRUAL CYCLE

The luteal phase increase in [Hb] is in agreement with the findings of Jurkowski et al. (1981). While these researchers noted a 3% increase in the luteal phase of healthy subjects in whom ovulation was hormonally confirmed, in the current study a 5% alteration was observed. Past studies of [Hb] changes throughout the menstrual cycle have produced spurious results. While Vellar (1994) and Dombovy et al. (1987) noted luteal phase decreases in [Hb] neither of these studies confirmed the timing or existence of a luteal phase using basal body temperature or hormonal analysis. Lebrun et al. (1993), in a study quantitatively confirming ovulation, observed no change in [Hb] from the follicular to luteal phase. In the past, cyclic changes in [Hb] have been thought to occur as a result of increases in plasma volume and lowered PCV. Both progesterone and oestradiol may mediate fluid retention probably through stimulation of the renin-angiotensin system and increased production or activity of anti-diuretic hormone. Exogenous oestrogen also prevents plasma volume reduction with bed rest and the positive influence of endogenous oestradiol on plasma volume appears to be greater around the time of

ovulation when progesterone levels are low (Fortney et al., 1988). Luteal increases in plasma volume and concomitant reduction in PCV would result in a lowered [Hb] and cannot explain the luteal increases in [Hb] noted in this study. Further evidence that [Hb] changes occur independently of hormonally mediated haemostatic alterations in this study are the lack of change noted in PCV and the lack of difference in [Hb] changes over the Test Points between ovulatory and anovulatory cycles.

The [Hb] rise from menses to luteal phase in this study may simply reflect the gradual restoration of red blood cells lost during menstruation. A 16% increase in caloric consumption in the luteal phase compared to the follicular phase has been previously noted (Barr et al., 1995). Although this study did not analyse differences in micronutrient intake, it is reasonable to suggest that iron intake is also higher in this phase and may accelerate this restoration process. Menstrual phase alterations in iron intake would be likely to have an important effect in the [Hb] of individuals with a suboptimal iron status.

DIFFUSION CHANGES IN OVULATORY VERSUS ANOVULATORY CYCLES

While no alterations in any of the diffusion measurements were noted with this smaller group of subjects, a non-significant tendency for [Hb] corrected D_{LCO} to change existed, as did weak trends in both corrected and uncorrected D_{LCO}/V_A. At Test Point c in the ovulatory cycle, one subject (00), recorded a value much higher than normal. Removal of this subject from the analysis and correction of this data point to an average value, resulted in a significant change in [Hb] corrected D_{LCO} with menstrual cycle phase. However, no reason for this discrepancy could be determined from the diffusion test results. The subject had not exercised intensely in the previous day and had not consumed a large meal or recently drunk coffee. The test was completed at the same time of day as usual. The elevated record could

be due in part to the unusually high V_A but correction for V_A fluctuations in all the subjects did not produce significant results.

The pattern of these trends in D_L fluctuation was towards a small (5%) increase from early in menses until later in menses followed by a drop later in the follicular and luteal phases. Seaton (1972) noted a substantial decline (14.8%) in V_C from 7 to 10 days following menstruation compared to 2 to 4 days prior to menstruation. Although there was on average a corresponding 4.4% drop in D_{LCO}, this difference was not statistically significant. The author suggests that progesterone mediated premenstrual distension of the pulmonary capillary bed, possibly secondary to respiratory arteriole or venous distension is the most likely explanation of his findings. The results of this study which carefully documented rises in basal body temperature, a secondary effect of progesterone, and found no corresponding change in D_{LCO} do not support this rationalisation. An alternative interpretation of Seaton's results would be that peaking oestradiol (around day 7 to 10 of the menstrual cycle) caused the alteration in V_C. This would be in agreement with the trend of a midcycle rise in D_{LCO} observed in the current study, although subjects in this study tended to ovulate much later than Day 7 to 10.

Sansores et al. (1995), in contrast, noted a decline in diffusion during menses. These researchers investigated D_{LCO} change during menses itself and recorded daily changes during this time of the cycle thus investigating more closely a specific phase of the menstrual cycle, menses itself. Mean intra-individual coefficients of variation for the parameter D_{LCO} in the Sansores et al. (1995) study was lower than in the current study: 4.8% as opposed to 8.8%. This is not surprising given that in the Sansores et al. (1995) study, the trials were conducted over a few days compared to the current study which analysed trials conducted over a monthly cycle.

The current study has carefully controlled for the effects of hormonal changes by selecting testing points to coincide with peak oestradiol and progesterone levels and assessing menstrual cycles for the occurrence of ovulation. Accordingly, the

lack of significant change in diffusion would appear to make the possibility of hormonally induced diffusion changes unlikely.

FURTHER MECHANISMS OF DIFFUSION CHANGE

Percentage COHb changes with menstrual cycle phase might be at least partially responsible for the D_{LCO} changes during menstruation observed by Sansores et al. (1995). These researchers reject COHb changes as an explanation of their observed D_{LCO} decline as the magnitude of COHb increase required to lower D_{LCO} by 10% was too great to be accounted for by red cell break-down. Coburn et al. (1970) report a doubling of CO production in the luteal phase of the menstrual cycle. Excess endogenous CO production may be a result of the breakdown of haemoglobin from senescent blood cells but may also reflect increased degradation of other haem compounds particularly of hemoproteins in the liver and other organs (Coburn, 1970). This excess production is unlikely to be reflected to any great extent in the blood as it is cleared from the body via the lungs and is masked by uptake from the environment. No change in whole blood COHb percent with menstrual cycle phase was noted in the current study.

Repeated measurements of D_{LCO} will raise blood [COHb] and thus lower successive determination of D_{LCO}. We have found that percentage COHb increases around 1.5% following a partitioned diffusion test in our laboratory. Following the test or repeated tests it declines at a rate of approximately 1% per hour (Stewart et al., 1997). Complete clearance of raised [COHb] may therefore take some hours and depends upon alveolar ventilation, pulmonary capillary PO_2 and upon D_{LCO} itself. It seems very unlikely that any of the decrease in D_{LCO} noted by Sansores et al. (1995) was explained by [COHb] accumulation with repeated testing, given that tests were completed 24 hours apart.

Prior to this investigation we felt that levels of prostaglandins associated with menstrual cycle cramps might have been responsible for the observed changes in D_{LCO}, perhaps acting via pulmonary arteriole vasoconstriction or alveolar membrane fluid retention. The incidence of menstrual cramps although it was assessed in The Menstrual Diary© was very unpredictable in both its occurrence for a particular cycle and in its timing. The few testing points that coincided with subjects reported cramping seemed to elicit D_{LCO} measurements within the subject's normal range. Moreover, neither changes in Vc nor DM with menstrual cycle phase (including the early and late menses testing points) were significant and this does not provide support for the prostaglandin initiated mechanisms described.

Hormone independent alterations in NO are a possible alternative explanation for D_{LCO} changes during menses. If this were the case however, it is hard to explain why hormone dependent changes in NO production (Kharitonov et al., 1994) were not observed.

RELIABILITY AND VALIDITY OF PACKED CELL VOLUME AND HAEMOGLOBIN CONCENTRATION MEASUREMENTS

Both PCV and [Hb] measurements obtained in our laboratory were highly reproducible. Approximately 98% and 96% of the variation in one measurement of PCV and [Hb] respectively could be explained by the variation in a measurement just preceding it. In addition, 92% of the variation in [Hb] was consistent over time after the effect of systematic alterations over the menstrual cycle were removed. Over time PCV determination was much less reliable reflecting changes in PCV over time that were not consistent between subjects and were not related to menstrual cycle changes since this was controlled. Only 6% of the variation in a PCV measurement was retained in repeated test over a month. It seems therefore

that PCV undergoes fluctuation with time that is not systematic between individuals.

The portable Hemocue analyser used in our lab for [Hb] determination appears to overestimate [Hb] compared to hospital based spectometry. The overestimation for a subject with a [Hb] of approximately 12 g/dl would be in the order of about 0.6 g/dl. This is outside the stated accuracy of both pieces of equipment (0.4 and 0.3 g/dl for the OSM and Hemocue respectively). In assessing the validity of the Hemocue, there is obviously a question of faith regarding the authenticity of manufacturer accuracy reports. The OSM spectometer in Vancouver Hospital outlined comprehensive laboratory trials in the determination of its reliability and accuracy compared to blood chemistry analysis. It therefore seems to be a more trustworthy standard and it could be assumed that the Hemocue in our laboratory did indeed consistently overestimate [Hb] by at least 0.2 g/dl.

CHAPTER FOUR: CONCLUSION

This study has resulted in the following findings.

1. There is no change in D_{LCO} or D_{LCO}/V_A either corrected or uncorrected for [Hb] with menstrual cycle phase of an ovulatory cycle in regularly menstruating healthy women.
2. There is no change in [Hb] corrected V_C or D_M; or in [COHb] or PCV with menstrual cycle phase of an ovulatory cycle in regularly menstruating healthy women.
3. There is a small (5%) increase in [Hb] from the early follicular to mid luteal phase of an ovulatory menstrual cycle in regularly menstruating healthy women.
4. There is no difference in menstrual cycle related D_{LCO} or D_{LCO}/V_A changes between ovulatory and anovulatory cycles in the same subject.
5. There is no difference in menstrual cycle related [COHb] changes between ovulatory and anovulatory cycles in different subjects.

Given that the timing of diffusion testing was designed to maximise reproductive hormone changes and that no difference in diffusing capacity exists between ovulatory and anovulatory cycles, a hormonally mediated alteration in D_L over the menstrual cycle is unlikely. The avoidance of female subjects in studies related to pulmonary diffusion because of supposed phase related changes appears to be unfounded.

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Abbreviations

CO Carbon Monoxide

COHb Carboxyhaemoglobin
[COHb] Carboxyhaemoglobin Concentration

D_{LCO} Diffusing Capacity of Carbon Monoxide
Units: ml CO (STPD) per mmHg Partial Pressure Change

D_M Diffusing Capacity of the Alveolar Membrane

Hb Haemoglobin
[Hb] Haemoglobin Concentration

NO Nitric Oxide

O_2 Molecular Oxygen

P_ACO Partial Pressure of Alveolar Carbon Monoxide

P_cCO Partial Pressure of Pulmonary Capillary Carbon Monoxide

pCO_2 Partial Pressure of Carbon Dioxide

PCV Packed Cell Volume (haematocrit)

pO_2 Partial Pressure of Molecular Oxygen

V_A Alveolar Volume
Units: ml (BTPS)

V_C Pulmonary Capillary Blood Volume

$\dot{V}CO$ Rate of Carbon Monoxide Production

$\dot{V}CO_2$ Rate of Carbon Dioxide Production

$\dot{V}O_2$ Rate of Oxygen Production

Appendix I: Lung Diffusion: Its Measurement, Components and Determinants.

DIFFUSING CAPACITY OF THE LUNG

Diffusing capacity of the lung entails the transfer of O_2 from the atmospheric side of the alveolar epithelium to its binding with erythrocytic haemoglobin. While both steady-state and a number of variations of single-breath techniques are available for the measurement of D_{LCO}, lung diffusion is most commonly assessed clinically using a single-breath method originally developed by Krogh (1915). This method quantifies the rate of disappearance of CO from the alveolar space within the lung following the inspiration and breath hold (usually 10s) of a known concentration of CO and assumes that the diffusion gradient for CO across the exchange surface does not limit its transport. This assumption is valid because carbon monoxide has a very high affinity to haemoglobin and at low concentrations is effectively removed from the blood plasma upon its diffusion across the membrane. This ensures that a large CO concentration driving force is maintained between the epithelial layer of the alveolar membrane (on the atmospheric side) and the pulmonary capillary blood plasma even when pulmonary capillary blood perfusion is relatively low.

In general, D_{LCO} is the volume of carbon monoxide diffusing across the alveolar membrane per mmHg partial pressure change from mean alveolar air to mixed pulmonary capillary blood (equation 1).

Equation 1: Basic Equation for Diffusing Capacity

$$D_{LCO} = \frac{\dot{V}CO}{P_ACO - P_cCO}$$

where D_{LCO} = diffusing capacity of CO

$\dot{V}CO$ = volume (ml) of CO transferred per minute

and $P_ACO - P_cCO$ = difference between mean alveolar and capillary CO partial pressure

In the single breath procedure, the lungs may be considered as a closed bag (of volume V_A: the alveolar volume) from which CO is removed at an exponential rate proportional to its concentration gradient. Under normal circumstances, the pulmonary capillary CO partial pressure (P_cCO) is negligible and ignored. D_{LCO} at breath-hold time (t) is a function of the initial and final alveolar fraction of CO and the alveolar volume (V_A) (equation 2).

Equation 2: The Krogh Equation for Single Breath Diffusing Capacity

$$D_{LCO} = \frac{V_A \times 60}{713 \times t} \times \text{Ln}\,[F_ACO_0/F_ACO_t] \times \text{STPD correction}$$

where D_{LCO} (mlCO/min/mmHg) STPD

V_A = alveolar volume (ATPS in ml)

t = breath-hold time (60 in the numerator converts seconds to minutes)

713 constant reflecting CO transfer

Ln = natural logarithm

F_ACO_0 = initial alveolar CO concentration

F_ACO_t = alveolar CO concentration at the end of breath-hold

STPD = standard temperature and pressure dry

1.05 = correction factor for 5% carbon dioxide in expired air removed prior to analysis

713 = the correction factor for conversion from concentration gradient to partial pressure difference

= P_B of 760mmHg - $P_{water\ vapour\ at\ 37^\circ C}$ of 47mmHg

In the single breath procedure, an inert gas that will not diffuse across the alveolar membrane (usually helium (He)) is present in the inspired mixture and has two purposes: to assess the initial alveolar CO fraction from the inspired fraction; and to determine the V_A via its dilution in the total lung volume.

Because helium does not diffuse to any great extent, the ratio of inspired He fraction to alveolar He fraction (assumed to be equivalent to the expired He fraction), will equal the ratio of inspired CO fraction to alveolar CO fraction after inspiration but before any diffusion has occurred. Thus, the initial fraction of CO

(F_{ACO_0}) may be determined from the change in helium fraction from inspired to alveolar air multiplied by the inspired CO fraction (equation 3).

Equation 3: Calculation of the Initial Alveolar CO Fraction.

$$F_{ACO_0} = F_{ICO} \times \frac{F_{EHe}}{F_{IHe}}$$

where F_{ICO} = inspired CO fraction

F_{EHe} = expired helium fraction

F_{IHe} = inspired helium fraction

If both sides of the equation are divided by the expired CO fraction (assumed equivalent to the alveolar CO concentration at the end of breath-hold time ($F_{ECO} = F_{ACO_t}$), the following equation is obtained (equation 4).

Equation 4: Calculation of the Initial to Final Alveolar CO Fraction Ratio.

$$\frac{F_{ACO_0}}{F_{ACO_t}} = \frac{F_{ICO} \times \frac{F_{EHe}}{F_{IHe}}}{F_{ECO}}$$

$$= \frac{F_{EHe} \times F_{ICO}}{F_{ECO} \times F_{IHe}}$$

where F_{ECO} = expired CO fraction

As long as the change in the FCO:FHe ratio from inspired to expired gas remains proportional to changes in either CO or He fraction, the relationship is independent of the actual quantites of inspired CO or He. It is convenient to assume

that F_ICO is equal to F_IHe cancelling these variables out of the equation. The ratio [F_ACO_0/F_ACO_t] may thus be regarded as equal to [(F_EHe/F_ECO) x (F_ICO/F_IHe)] and is used in the calculation of DL_{CO} in this study (Equation 2, p20).

V_A may also be determined via He dilution in the total lung volume (which includes the inspired volume and the residual volume) with a correction made for the dead-space of the diffusion instrument and for anatomical dead-space (equation 5).

Equation 5: Calculation of the Alveolar Volume.

$$V_A = (V_I - V_D) \times \frac{F_IHe}{F_EHe} \times 1.05$$

where V_A = alveolar volume

V_I = inspired volume

V_D = dead space (anatomical and instrument)

F_IHe = inspired He fraction

F_EHe = expired CO fraction

1.05 = dilution factor for the CO2 fraction which is normally chemically removed from the sample

DL_{CO} as a ratio of V_A may be reported to correct for variations in inspired ventilation and lung size (equation 6).

Equation 6: Calculation of Diffusing Capacity to Alveolar Volume Ratio

$$DL_{CO}/V_A = DL_{CO}/V_A \text{ (l BTPS)}$$

where BTPS = Body Temperature and Pressure Saturated

COMPONENTS OF LUNG DIFFUSION

Because diffusing capacity of the lung may be considered as a conductance of the lung for CO, its inverse, the total resistance of the lung ($1/D_{LCO}$), can be partitioned into two component resistances as shown (equation 7).

Equation 7: Partitioning of Diffusing Capacity into its Membrane Component and Pulmonary Capillary Volume Component

$$\frac{1}{D_{LCO}} = \frac{1}{D_M} + \frac{1}{\theta \times V_C}$$

where D_{LCO} = diffusing capacity of the lung for carbon monoxide

D_M = diffusion across the alveolar membrane

θ = the reaction rate of carbon monoxide with haemoglobin

and V_C = volume of the pulmonary capillaries

In essence, $1/\theta V_C$ may be considered as the component of resistance that varies with changes in P_AO_2 and $1/D_M$ as all other resistance (Crapo and Forster, 1989). These authors describe a useful anatomical model for the partitioning which though not strictly correct is helpful in understanding the factors affecting diffusion. Using this model, the first resistance ($1/D_M$), can be thought of as the resistance due to the movement of CO across the alveolar membrane to the surface of a red blood cell. This movement requires the crossing of 3 cell layers (the alveolar epithelium, the alveolar basement membrane and the capillary endothelium), the interstitial fluid within the alveolar membrane and a layer of blood plasma. Aveolar membrane diffusion (D_M) therefore depends on the thickness and composition of

the involved pathways, which may be altered in disease (as for example in pulmonary fibrosis); and the surface areas of air-tissue and tissue-blood interfaces, which may in turn be affected by changes in the pulmonary capillary blood supply or alveolar ventillation. D_M may also be decreased by changes in the back pressure of CO caused by CO bound haemoglobin already circulating in pulmonary arterial blood.

The second resistance ($1/\theta V_C$), is a product of two resistances: one due to the reaction of CO with the red blood cell haemoglobin ($1/\theta$), and the other to the pulmonary capillary blood volume ($1/V_C$). The combined resistance, depends upon the surface area of the erythrocytes, diffusion across the erythrocyte membrane and within the blood cell as well as the chemical reaction of CO with haemoglobin. The chemical reaction term (θ) refers to a predetermined rate that 1ml of normal [Hb] can pick up CO per 1mmHg concentration gradient. Consequently, changes in the concentration of erythrocytic haemoglobin and factors which affect the saturation kinetics of the reaction (namely the partial pressures of O_2 and CO_2 (pO_2 and pCO_2), pH, and temperature) all affect the combined resistance. Because θ varies in a standard way with alterations in pO_2, both $1/V_C$ and $1/D_M$ may be calculated from the slope and y-intercept respectively of a linear line of $1/D_{LCO}$ plotted against $1/\theta$. Specifically, the slope of the line estimates $1/V_C$, while the Y-intercept represents $1/D_M$. Each value of $1/\theta$ is normally calculated as described by Forster et al. (1986). It is the mean capillary O_2 tension ($P\bar{c}O_2$) which determines θ and this is very difficult to quantify. While the alveolar partial pressure of oxygen (P_AO_2) is typically the same as the end capillary partial pressure of oxygen ($P_{ec}CO_2$), $P\bar{c}O_2$ is assumed to be 15mmHg lower. Note that θ is also a function of [Hb], as its uptake depends on the number of red cells present (equation 8).

Equation 8: Determination of Theta.

$$\frac{1}{\theta} = \frac{0.34 + [0.006 \times P\bar{c}O_2]}{\frac{[Hb]}{15}}$$

where θ (theta) = the reaction rate of CO and red blood cell haemoglobin

$P\bar{c}O_2$ = mean capillary partial pressure of oxygen

$= P_AO_2 - 15$

[Hb] = haemoglobin concentration

P_AO_2 can be estimated by using the alveolar gas equation (equation 9), assuming a respiratory exchange ratio (RER) of 0.8 and that the arterial pressure of carbon dioxide (PaCO2) is equal to an alveolar P_ACO_2 of 40 mmHg.

Equation 9. Alveolar Gas Equation

$$P_AO2 = [F_IO2 \times (P_B - 47)] - P_ACO2 \left[F_IO2 + \frac{(1 - F_IO2)}{RER}\right]$$

where P_AO_2 = partial pressure of alveolar oxygen

F_IO_2 = fraction of inspired oxygen

P_B = barometric pressure

P_ACO_2 = partial pressure of alveolar carbon monoxide

F_IO_2 = fraction of inspired oxygen

RER = respiratory exchange ratio

FACTORS AFFECTING LUNG DIFFUSION

An individuals pulmonary diffusing capacity is largely determined by anthropometric factors. Larger D_{LCO} is correlated with greater body dimensions including weight, height, surface area and lung volume (Crapo and Forster, 1989). Reduction in D_{LCO} results from both obstructive and fibrotic lung disorders, marijuana and cigarette smoking, pulmonary oedema, and anaemia. Increases may occur in association with asthma, pulmonary haemorrhage, and left to right circulatory shunts (Crapo and Forster, 1989). In healthy individuals, D_{LCO} also depends on body position, increasing 15% from the upright to supine or prone position (Chang et al., 1992).

In addition, D_{LCO} increases during exercise. Turcotte et al. (1992), noted 42% and 65% increases in diffusing capacity during moderate and intense exercise respectively. Paradoxically, a number of researchers have noted reductions in D_{LCO} following maximal exercise (reviewed by Sheel, 1995). The magnitude of the post-exercise decrease reported in the literature varies from 2% to 19% depending on intensity and duration of the exercise bout and the time following exercise that diffusion capacity is measured. Miles et al. (1983) observed 2% reductions in D_{LCO}, while Manier et al. (1991), found 10% reductions in D_{LCO}, 24 hours and 28 minutes respectively after the completion of a marathon. Sheel (1995), in a study designed to follow the timecourse of post-exercise alterations in D_{LCO}, observed a peak (13%), reduction in pulmonary diffusion six hours following maximal exercise which was still 6% below baseline 24 hours after the exercise bout.

Although exercise training induced changes in D_{LCO} have not been reported in the literature, physically active people generally have a higher D_{LCO} value (Crapo and Forster, 1989). An increase in D_{LCO} is likely to occur in trained athletes as a result of increased total blood volume and hence pulmonary capillary blood volume. Sheel (1995), found small (7.5%) but statistically non-significant differences

between the D_{LCO} of a group of highly trained and a group of moderately trained males.

Appendix II: The Human Menstrual Cycle

THE HUMAN MENSTRUAL CYCLE

The menstrual cycle of humans and old world primates is characterised by circa-lunar fluctuations in the levels of hormones of the hypothalamic-pituitary-ovarian (HPO) axis, early-cycle bleeding (menstruation), and mid-cycle release of a mature ovum (ovulation) (figure 2). The phases of the menstrual cycle may be categorised by ovarian hormone changes or changes in the uterine lining. During the early part of the ovarian "follicular" phase, reproductive hormone levels are low and menstruation occurs. Increasing levels of oestrogen cause the cessation of menstruation and the onset of development of an encapsulated ovum (the follicle). At this time, the uterine lining (endometrium) undergoes proliferation: it begins to thicken and develop a new blood supply. A late follicular shift from the negative feedback regulatory action of oestrogens on the hypothalamus to a positive feedback mechanism results in a rapid but short-lived increase in oestrogen levels and a slightly delayed gonadtrophin level rise. This results in the rupture of the mature follicle and exocytosis of the ovum from the ovary marking ovulation. The remainder of the follicle becomes a steroid hormone producing body known as the corpus luteum. During the ovarian "luteal" phase, the corpus luteum produces large amounts of both progesterone and oestradiol which act to maintain the uterine lining. In the latter part of the luteal phase corpus luteal function declines. Lowered steroid hormonal levels, mediated by the action of locally produced prostaglandins, initiate transient vasoconstriction of arteries leading to the endometrium. Necrotic erosion of the superficial layer of the ishaemic endometrium by proteolytic enzymes released following leukocyte and macrophage invasion, results in the shedding of outer cell layers of the endometrium and rupture of blood vessels causing the onset of menstruation.

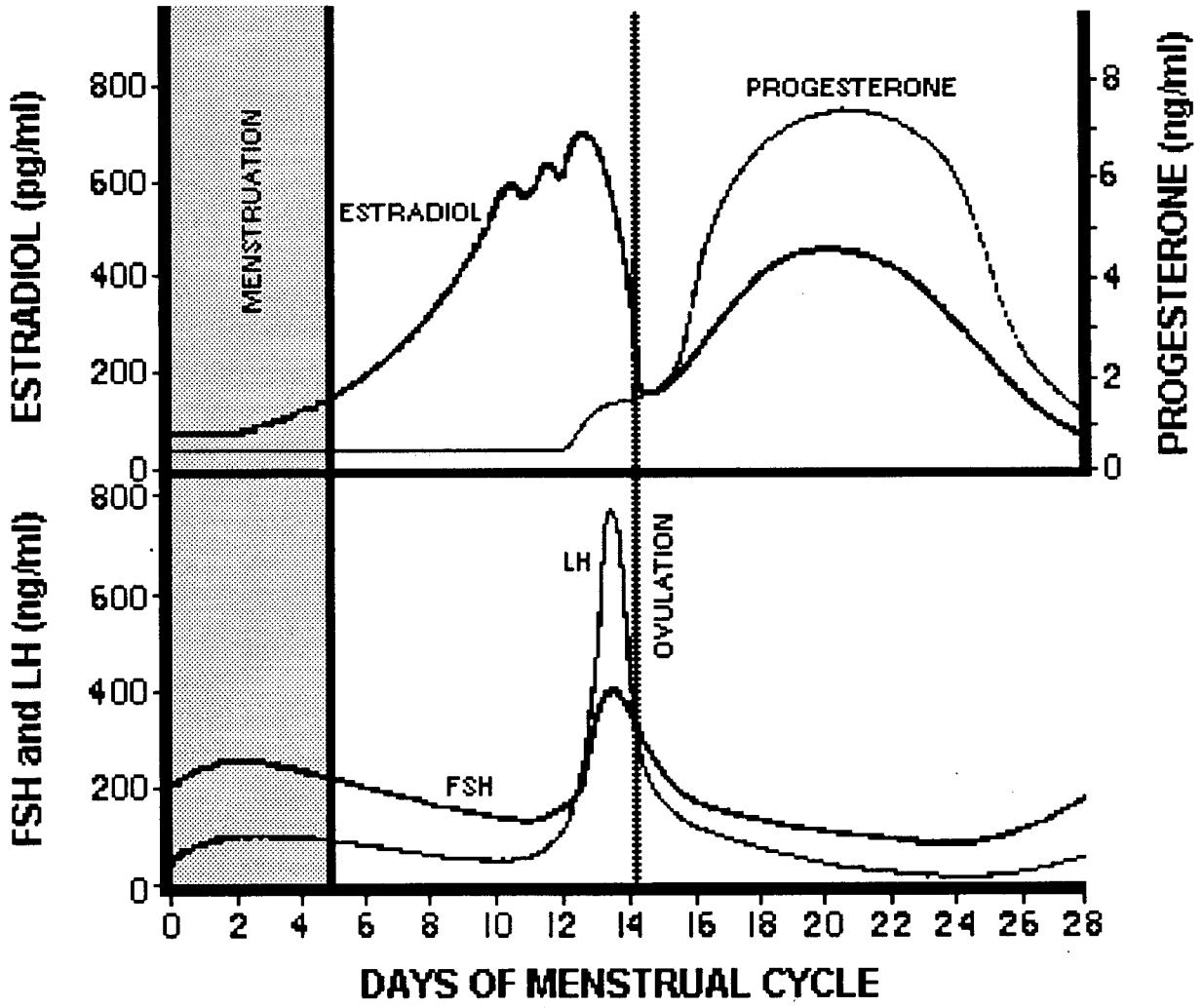

Figure 12: The Human Menstrual Cycle (Adapted from Guyton, 1996)

CHANGES IN RESPIRATORY FUNCTION OVER THE HUMAN MENSTRUAL CYCLE

Menstrual cycle phase is already known to influence respiratory function. Both resting (Shoene et al., 1981) and maximal exercise (Jurkowski et al., 1981) minute ventilation ($\dot{V}E$) increases in the luteal phase of the menstrual cycle compared to the follicular phase. Resting hypercapnic and hypoxic drives are also higher in the luteal phase (Schoene et al., 1981; Dombovy et al., 1987). Menstrual phase dependent alterations in ventilatory drive are thought to be a result of progesterone mediated stimulation of central respiratory centres. Firstly, the synthetic progestin medroxyprogesterone acetate (MPA), which increases ventilatory drive, has been previously identified in the cerebrospinal fluid (Scoggin et al., 1978). Secondly, the changes in oral occlusion pressure found by Schoene and coworkers (1981) to coincide with changes in ventilatory drive are generally thought to reflect total neural drive. Peripheral factors may also be involved in ventilatory changes over the menstrual cycle; Chen and Tang (1989) have noted a higher inspiratory muscle endurance in the mid to late luteal phase compared to the mid follicular phase.

Appendix III: Calculation of Intraclass Correlation Coefficient

The Intraclass Correlation Coefficient may be calculated from a Repeated Measures ANOVA using the following formula (Equation 1, from Vincent, 1995, pg 179).

Equation 1: Intraclass Correlation Coefficient

$$r_{intraclass} = \frac{MS_{subject} - MS_{trial+error}}{MS_{subject}}$$

where $r_{intraclass}$ = Intraclass Correlation Coefficient

$MS_{trial+error}$ = Mean Square of Trial Effect and Error

$MS_{subject}$ = Between Subjects Mean Square

Because both PCV and [Hb] repeated measurements were made on separate days at different points of the menstrual cycle, day to day variation and/or menstrual cycle effects will cause changes in the means of the separate trials. In order to assess only the measurement error (or reliability of the measurement procedure), the trial effect should be ignored. Vincent (1995) recommends the use of the following modified formula in this situation (Equation 2).

Equation 2: Modified Intraclass Correlation Coefficient

$$r_{2intraclass} = \frac{MS_{subject} - MS_{error}}{MS_{subject}}$$

where $r_{2intraclass}$ = Modified Intraclass Correlation Coefficient

MS_{error} = Error Mean Square

$MS_{subject}$ = Between Subjects Mean Square

Appendix IV: Ethical Approval Certificate and Consent Form

Appendix VI: Menstrual Cycle Diary

Menstrual Cycle Diary

Name: _____ Month: _____ Year: _____

Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Date																															
Tampons/pads/day																															

Record 0 = none, 1 = minimal, 2 = moderate, 3 = moderately intense, 4 = very intense

Amount Flow																															
Cramps																															
Breast Sore: Front																															
Breast Sore: Side																															
Fluid Retention																															
Mucous Secretion																															
Constipation																															
Headache																															
Sleep Problems																															
Feeling Frustrated																															
Feeling Depressed																															
Feeling Anxious																															

Record M = much less, L = a little less, U = usual, Y = a little increased, Z = much increased

Appetite																															
Breast Size																															
Interest in sex																															
Feeling of energy																															
Feeling of self-worth																															
Outside stresses																															
Basal Temperature SUPINE HEART RATE																															
Comments (temperature taken late, feeling sick, poor sleep, etc) HOURS OF SLEEP																															

Appendix VII: Daily Exercise Record

DAILY EXERCISE RECORD

Name: _____ Month: _____ Year: _____

Date	**Men Day**	Vigorous/ Strenuous Exercise (HR 10s >25) (hrs:mins)	Mild/ Moderate Exercise (HR 10s 15-25) (hrs:mins)	**Mode** (eg: running, walking, swimming, resistance training)	**Comments**
	1	:	:		
	2	:	:		
	3	:	:		
	4	:	:		
	5	:	:		
	6	:	:		
	7	:	:		
	8	:	:		
	9	:	:		
	10	:	:		
	11	:	:		
	12	:	:		
	13	:	:		
	14	:	:		
	15	:	:		
	16	:	:		
	17	:	:		
	18	:	:		
	19	:	:		
	20	:	:		
	21	:	:		
	22	:	:		
	23	:	:		
	24	:	:		
	25	:	:		
	26	:	:		
	27	:	:		
	28	:	:		
	29	:	:		
	30	:	:		
	31	:	:		

Appendix VIII: Initial Questionnaire

INITIAL VERSION

GENERAL AND HEALTH QUESTIONNAIRE

Code Number_____ Date/Time_____

Birthday _____
day / month / year

1. How would you describe your work?

_____

2. What's your partnership status?

partnered ☐ single ☐ separated ☐ divorced ☐

3. Who is at home with you?

no-one ☐ family ☐ partner ☐ roommate(s) ☐

4. How many years of schooling do you have?

less than 8 ☐ 8 - 12 ☐ 13 - 16 ☐ more than 16 ☐

5. Are you on any hormones (including the pill) or other medications? Yes ☐ No ☐

If yes, please list them _____

_____

_____

6. Do you have asthma, other lung problems or significant illness? Yes ☐ No ☐

7. Is there anyone related to you who has/had a broken bone (without a fall or accident) or who is getting/became shorter and developing/developed a hunched back?
Yes ☐ No ☐

8. Do you currently smoke? Yes ☐ No ☐

9. Are you a past smoker? Yes ☐ No ☐

If yes, how long since you stopped? _____
months/years

10. Do you currently drink alcohol? Yes ☐ No ☐

If yes, how many glasses of wine or beer do you drink per week?

1 or 2 ☐ 3 - 7 ☐ 8 - 12 ☐ more than 12 ☐

If yes, how many glasses of spirits do you drink per week?

1 or 2 ☐ 3 - 7 ☐ 8 - 12 ☐ more than 12 ☐

11. Do you currently drink caffeine containing drinks (non-herbal tea, coffee, or coke)?

If yes, how many glasses do you drink per week?

1 or 2 ☐ 3 - 7 ☐ 8 - 12 ☐ more than 12 ☐

12. Are you on a diet for a health concern or to lose weight? Yes ☐ No ☐

If yes, please explain?

_____

_____

_____

_____

_____

13. Has your weight changed in the past 5 years? Yes ☐ No ☐

If yes, has it? increased ☐ decreased ☐ fluctuated ☐

How much has it changed/fluctuated? _____ kgs _____ lbs

14. How much do you feel you should weigh? _____ kgs _____ lbs

15. What was your highest non-pregnant weight? _____ kgs _____ lbs

16. What was your lowest non-pregnant weight? _____ kgs _____ lbs

NUTRITION

17. Are you taking any multiple vitamins? Yes ☐ No ☐

18. Are you taking any calcium supplements? Yes ☐ No ☐

If yes: How many mg are there in each pill? _____

How many pills do you take each day? _____

How long have you been taking them? _____

19. Have you had any calcium containing foods (milk, yoghurt, cheese, creamed cheese, canned salmon, etc) over the last 24 hours?

Food	Amount
Breakfast	
Lunch	
Dinner	

EXERCISE

20. Do you do an hour or more of vigorous exercise per week? Yes ☐ No ☐

If yes, please indicate the type of activity, the average frequency and duration.

Type of Activity	Frequency (no sessions per week)	Duration (time per session)

21. How many months over the last year have you exercised a similar amount?

_____ months

22. How many years **(over the last 5)** have you exercised a similar amount?

_____ years

23. Have you done more than 1 hour of aerobic exercise per week in the past? Yes ☐ No ☐

If yes, how many years did you do this for? _____ years

MENSTRUAL CYCLE

24. Are you having regular periods? Yes ☐ No ☐

25. How long is your cycle length? _____ days

26. How many days long is your flow? _____ days

27. Can you usually tell, by the way you feel, that your period is coming? Yes ☐ No ☐

28. Do you usually experience the following symptoms?
If yes, please indicate the time in your cycle that you notice them (respond "intermittently" if this seems the most appropriate answer).

			Time in cycle
Breast Tenderness	Yes ☐	No ☐	
Appetite Changes	Yes ☐	No ☐	
Mood Changes	Yes ☐	No ☐	
Fluid Retention	Yes ☐	No ☐	
Stretchy Vaginal Mucous	Yes ☐	No ☐	

29. Are there any changes in your symptoms after your flow starts? Yes ☐ No ☐

If yes, please explain?

30. How many times did you menstruate in the last year?

none ☐ 1-2 ☐ 3-6 ☐ 7-9 ☐ 10-17 ☐ more than 17 ☐

31. Have you menstruated in the last 6 months? Yes ☐ No ☐

32. How many periods have you missed in the last 5 years? _____ periods
(normal = 10-14/year)

33. Have you been pregnant in the last 5 years (full = 10 months)? Yes ☐ No ☐

If yes: How many months after delivery until your period started? _____ periods

34. Have you recently had PMS? Yes ☐ No ☐

If yes, please describe your symptoms and their timing in relation to flow?

35. Are you currently taking oral contraceptives? Yes ☐ No ☐

36. Have you taken them in the past? Yes ☐ No ☐

If yes: How long did you use them for? _____ months/years

How long ago since you used them last? _____ months/years

37. Did you have regular periods when you stopped the pills? Yes ☐ No ☐

If no: How long were they irregular for? _____ months/years

Appendix IX: Data Sheets

INITIAL SUBJECT DATA SHEET **Code** _____

Date___/___/___ **Height (m)** _____ **DOB** ___/___/___(___)
day month year

Triceps _____ _____ _____ _____ **Subscap** _____ _____ _____ _____

SupSpi _____ _____ _____ _____ **Abdom** _____ _____ _____ _____

AntThi _____ _____ _____ _____ **MedCalf**_____ _____ _____ _____

SupIliac (IliacCrest) _____ _____ _____ _____ **S of Six** _____

Any Respiratory or Endocrine Medical Conditions Y/N

Current Smoking Y/N

Past Smoking Y/N **Length ago** _____ **No per day** _____

Oral Contraceptives Y/N

Past Oral Contraceptives Y/N **Length ago** _____ **Type** _____

Menstruate Regularly Y/N **Able to predict onset of bleeding Y/N**

No of times menstruated in the last year **None** **1-3** **4-7** **8-10** **10-17** **>17**

Training/Sport _____

Hrs/Week (average training or intensive exercise) _____ **Months/Year** _____

SUBJECT DATA SHEET Code _____

Date___/___/___ Time _____ Weight (kg) _____
day month year

P_B (mmHg)_____ T_{ROOM} (°C)_____ Humidity (%)_____

Test Time 1 2 3 4 5 Day in menstrual cycle _____

Time at: Last Vig Ex _____ Last Meal _____ Last Coffee _____

FVC (ml) _____ FEV1 (ml) _____%___ FEV25-75 _____ PEFR _____

Hb (mg/100dl) _____ PCV (haematocrit) _____ COHb_____

Oes _____ Prog _____ FiO2 (between) _____

21% Tests I and II

FiO2 _____VinBTPS _____ He _____ CO _____ Hold_____

21% DLCO (ave) _____ VA (ave) _____ D/VA (ave) _____

90% Tests III and IV

FiO2 _____VinBTPS _____ He _____ CO _____ Hold_____

90% DLCO (ave) _____ VA (ave) _____ D/VA (ave) _____

DM _____ VC _____

FINAL SUBJECT DATA SHEET

Code _____

Date___/___/___ Height (m) _____ DOB ___/___/___(___)
day month year

Triceps _____ _____ _____ _____ Subscap _____ _____ _____ _____

SupSpi _____ _____ _____ _____ Abdom _____ _____ _____ _____

AntThi _____ _____ _____ _____ MedCalf_____ _____ _____ _____

SupIliac (IliacCrest) _____ _____ _____ _____ S of Six _____

Any Respiratory or Endocrine Medical Conditions Y/N

Current Smoking Y/N

Oral Contraceptives Y/N

Menstruation During Study (circle)

Did not Menstruate Irregular Cycles Regular Cycles

Training/Sport _____

Hrs/Week (average training or intensive exercise) _____ Months/Year _____

Appendix X: Final Questionnaire

FINAL VERSION

GENERAL AND HEALTH QUESTIONNAIRE

Code Number_____ Date/Time_____

1. Are you on any hormones (including the pill) or other medications? Yes ☐ No ☐

   If yes, please list them _____

   _____

   _____

2. Do you have asthma, other lung problems or significant illness? Yes ☐ No ☐

3. Do you currently smoke? Yes ☐ No ☐

4. Do you currently drink alcohol? Yes ☐ No ☐

   If yes, how many glasses of wine or beer do you drink per week?

   1 or 2 ☐ 3 - 7 ☐ 8 - 12 ☐ more than 12 ☐

   If yes, how many glasses of spirits do you drink per week?

   1 or 2 ☐ 3 - 7 ☐ 8 - 12 ☐ more than 12 ☐

5. Do you currently drink caffeine containing drinks (non-herbal tea, coffee, or coke)?

   If yes, how many glasses do you drink per week?

   1 or 2 ☐ 3 - 7 ☐ 8 - 12 ☐ more than 12 ☐

NUTRITION

6. Are you taking any multiple vitamins? Yes ☐ No ☐

7. Are you taking any calcium supplements? Yes ☐ No ☐

If yes: How many mg are there in each pill? _____

How many pills do you take each day? _____

How long have you been taking them? _____

8. Have you had any calcium containing foods (milk, yoghurt, cheese, creamed cheese, canned salmon, etc) over the last 24 hours?

Food	Amount
Breakfast	
Lunch	
Dinner	

9. Are you on a diet for a health concern or to lose weight? Yes ☐ No ☐

If yes, please explain?

EXERCISE

10. Do you do an hour or more of vigorous exercise per week? Yes ☐ No ☐

If yes, please indicate the type of activity, the average frequency and duration.

Type of Activity	Frequency (no sessions per week)	Duration (time per session)

11. How many months over the last year have you exercised a similar amount?

_____ months

12. How many years **(over the last 5)** have you exercised a similar amount?

If yes, how many years did you do this for? _____ years

13. Have you done more than 1 hour of aerobic exercise per week in the past? Yes ☐ No ☐

_____ years

MENSTRUAL CYCLE

14. Are you having regular periods? Yes ☐ No ☐

15. How long is your cycle length? _____ days

16. How many days long is your flow? _____ days

17. Can you usually tell, by the way you feel, that your period is coming? Yes ☐ No ☐

18. Do you usually experience the following symptoms?
If yes, please indicate the time in your cycle that you notice them (respond "intermittently" if this seems the most appropriate answer).

			Time in cycle
Breast Tenderness	Yes ☐	No ☐	_____
Appetite Changes	Yes ☐	No ☐	_____
Mood Changes	Yes ☐	No ☐	_____
Fluid Retention	Yes ☐	No ☐	_____
Stretchy Vaginal Mucous	Yes ☐	No ☐	_____

19. Are there any changes in your symptoms after your flow starts? Yes ☐ No ☐

If yes, please explain?

_____

_____

_____

_____

20. Over the study have you?

not menstruated at all ☐ menstruated abnormally ☐ menstruated normally ☐

21. Over the study have you taken oral contraceptives? Yes ☐ No ☐

Respondent I.D. # _____

In this section I would like to ask you questions that will help us understand how women's hormones relate to bone structure. We ask everyone these questions.

5. REPRODUCTIVE HISTORY (FEMALES)

5.1 * Before menopause, have you ever gone 3 months or more without a menstrual period?
(not including pregnancy or during breastfeeding)

☐ Yes ☐ No → Go to 5.2

What was the longest single period of time without a menstrual flow? _____ months

If you count all the periods you have missed throughout your menstruating years, how many months would that be? _____ months
(this question asks for the cumulative time)

5.2 * Have your menstrual periods stopped for more than one year?
(No period one year or more after last menstruation)

☐ Yes ☐ No

→ At what age? _____ years

5.12 How old were you when you had your first menstrual period? _____ years

5.3 Have you had your uterus removed (*hysterectomy*)?

☐ Yes ☐ No

→ At what age? _____ years

5.4* Have you ever had one or both ovaries removed?

☐ Yes, one ovary removed at what age? _____
☐ Yes, both ovaries removed at what age? _____
(if ovaries were removed on separate occasions, write the age at which the second ovary was removed)
☐ Yes, do not know how many at what age? _____
☐ No

* See notes in manual

I'm going to ask you a few questions on your eating habits.

8.7 a) I am going to read two sentences for you. Please answer True (T) or False (F) for each statement as it pertains to you.

I enjoy eating too much to spoil it by counting calories or watching my weight. T ☐ F ☐

I consciously hold back at meals in order not to gain weight. T ☐ F ☐

b) Which of these best describes you?

On a scale of 0 to 5, where 0 means no restraint in eating (*eating whatever you want, whenever you want it*) and 5 means total restraint (*constantly limiting food intake and never "giving in"*), what number would you give yourself?

0 Eat whatever you want, whenever you want it
1 Usually eat whatever you want, whenever you want it
2 Often eat whatever you want, whenever you want it
3 Often limit food intake, but often "give in"
4 Usually limit food intake, rarely "give in"
5 Constantly limiting food intake, never "giving in"

Now the questions I will ask will relate to the use of tobacco.

9. TOBACCO

9.1 Have you ever used any of the following tobacco products daily for at least 6 months?

Cigarettes	☐ Yes	☐ No	
Pipes	☐ Yes	☐ No	
Cigars	☐ Yes	☐ No	
Chewing tobacco	☐ Yes	☐ No	→ If NO to all: go to 9.3

Now I will ask you in detail about the foods you eat

10. FOOD INTAKE

10.1 * How often (on the average) have you eaten the following items?

	During the last 12 months?					In your 30's (If subject 40 years or over)				In your teens?				As a child?			
Fold	Never	servings per month	servings per week	servings per day	Serving Size	Never	Less	Same	More	Never	Less	Same	More	Never	Less	Same	More
Milk to drink incl. choc. milk & hot cocoa w/milk					☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup) ☐ 375 ml (1.5 cup)												
Milk on cereal					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup)												
Milk/cream in tea/coffee					☐ 15 ml (1 tbsp) ☐ 30 ml (2 tbsp) ☐ 60 ml (4 tbsp)												
Milk desserts (tapioca, rice pudding)					☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup)												
Hard cheese (to eat, in sandwich or mixed dish)					☐ 15 g (0.5 oz) ☐ 30 g (1 oz) ☐ 60 g (2 oz)												
Yogurt					☐ 125 ml (0.5 cup) ☐ 175 ml (single) ☐ 250 ml (1 cup)												
Ice-cream, ice milk or frozen yogurt					☐ 125 ml (0.5 cup) ☐ 250 ml (1.0 cup) ☐ 375 ml (1.5 cup)												
Cream soups made with milk					☐ 125 ml (0.5 cup) ☐ 160 ml (.67 cup) ☐ 250 ml (1.0 cup)												

* See notes in manual

Respondent I.D. # _____

	During the last 12 months?					In your 30's (If subject 40 years or over)				In your teens?				As a child?			
Food	Never	servings per month	servings per week	servings per day	Serving Size	Never	Less	Same	More	Never	Less	Same	More	Never	Less	Same	More
Canned salmon or sardines with bones					☐ 30 g (1 oz) ☐ 60 g (2 oz) ☐ 90 g (3 oz)												
Broccoli					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Dark leafy greens (bok choy, kale, gailan (Chinese broccoli), collards, dandelion greens)					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Dried peas or beans (navy, pinto, kidney)					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Whole wheat buns, bread, rolls, bagels					☐ 1 serving = 1 slice ½ bagel ½ pita												
White bread, buns, rolls, bagels, etc.					☐ 1 serving = 1 slice ½ bagel ½ pita												
Tofu					☐ 60 ml (.25 cup) ☐ 125 ml (0.5 cup) ☐ 250 ml (1 cup)												
Multivitamin, Vit. D or cod liver oil					☐ 1 supplement												
Calcium suppl. or "TUMS"					☐ 200 mg ☐ 300 mg ☐ 500 mg												

Respondent I.D. # _____

Now some questions about the liquids/fluids you might choose to drink.

BEVERAGES *

10.2 How many of the following drinks did you consume?

In these questions, one serving of alcoholic beverage is:
- 1 bottle or can of beer or a glass of draft (12 oz):
- 1 glass of wine or a wine cooler (4-5 oz)
- 1 straight or mixed drink with (1-1½ oz) hard liquor

- 1 serving of tea or coffee is 6 oz
- 1 serving of cola is 12 oz - 1 can (355 ml)

100 Beverages		During the past 12 months?				In your 30's (If subject is 40 years or over)				When in your teens?			
		None	Serving /month	Serving /week	Serving /day	None	Less	Same	More	None	Less	Same	More
Coffee	caffeinated												
	decaffeinated												
Tea	caffeinated												
	decaffeinated												
Colas	caffeinated												
	decaffeinated												
Alcoholic beverages													

* See notes in manual

Respondent I.D. # _____

Respondent I.D. # _____

In this section I will ask you about your physical activities and exercise.

11. PHYSICAL ACTIVITY

11.1 During a typical week in the past 6 months, how much time did you usually spend walking to work or school or while doing errands?

- ☐ None
- ☐ Less than 1 hour
- ☐ Between 1-5 hour
- ☐ Between 6-10 hours
- ☐ Between 11-20 hours
- ☐ More than 20 hours

11.2 Which of the following describes the paid work you usually do or what you consider your job? Or if retired or unemployed, which best describes your (*past or longest*) job?

- ☐ I am usually sitting during the day and do not walk around very much
- ☐ I stand or walk quite a lot during the day but I do not have to lift or carry heavy things
- ☐ I usually lift or carry light loads or I often have to climb stairs or hills
- ☐ I do heavy work or have to carry loads

11.3 Do you currently participate in any regular activity or programme (*either on your own or in a formal class*)?

☐ Yes ☐ No

→ How many times a week? _____

→ How long per session ? _____ minutes

11.4* On the average, during the last year, how many hours <u>in a week</u> did you spend in the following activities?

	Never	1/2-1 hr	2-3 hrs	4-6 hrs	7-10 hrs	11-20 hrs	21-30 hrs	31 hrs +
STRENUOUS SPORTS (such as jogging, bicycling on hills, tennis, racquetball, swimming laps, aerobics)								
VIGOROUS WORK (such as moving heavy furniture, loading or unloading trucks, shovelling, weight lifting, or equivalent manual labour)								
MODERATE ACTIVITY (such as housework, brisk walking, golfing, bowling, bicycling on level ground, gardening)								

11.5 * On the average, during the last year, how many hours <u>in a day</u> did you spend in the following sitting activities?

	Never	Less than 1 hr	1 to 2 hrs	3 to 4 hrs	5 to 6 hrs	7 to 10 hrs	11 hrs or more
Sitting in car or bus							
Sitting at work							
Watching TV							
Sitting at meals							
Other sitting activities (such as reading, playing cards, sewing)							

11.6 On the average, during the last year, how many hours <u>in a day</u> did you sleep (*include naps*)?

☐ 5 hours or less ☐ 7 hours ☐ 9 hours
☐ 6 hours ☐ 8 hours ☐ 10 hours or more

* See notes in manual

11.7 * Rate your overall level of physical activity compared to your peers during certain times in your past life.

	When you were about 50 if subject 60 y. and over	When you were about 30 if subject 40 y. and over	Teenager	Child
A lot less active				
Somewhat less active				
About the same				
Somewhat more active				
A lot more active				

Now I want to ask you questions about being in the sunlight

12. SUNLIGHT EXPOSURE

12.1 * Did you ever expose a considerable part of your body to direct sunlight?

A. During the past 12 months?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

If 60 years old or more.

B. When you were about 50 years old?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

If 40 years old or more.

C. When you were about 30 years old?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

For all.

D. When you were a child or teenager?
- ☐ never
- ☐ seldom
- ☐ regularly
- ☐ often

* See notes in manual

Appendix XI: Timetables of Testing Dates

DATES OF DIFFUSION TESTING

	Day 1	Day 3	Day 6	Day 17	Day 21	Day 1	Day 3	Day 6	Day 19	Day 23	Day -1	Day 3	Day 7	Day 17	Day 24
CBMS00	22/03 1a	24/03 1b	27/03 1c	07/04 1d	11/04 1e	17/04 2a	19/04 2b	22/04 2c	05/05 2d	miss 2e	09/05 3a	12/05 3b	16/05 3c	26/05 3d	02/06 3e
	CL = 26					CL = 23					CL = 30				
		Day 1	Day 3	Day 7	Day 14	Day 20	Day 2	Day 4	Day 8	Day 17	Day 21	Day 2	Day 4	Day 7	Day 17
CBMS01		26/02 1a	28/02 1b	04/03 1c	11/03 1d	17/03 2e	26/03 2a	28/03 2b	01/04 2c	10/04 2d	14/04 3e	21/04 3a	23/04 3b	26/04 3c	06/05 3d
	CL = 30	CL = 27					CL = 26					CL = 28			
	Day 23														
	12/05 4e														
	Day 24	Day 2	Day 5	Day 8	Day 15	Day 23	Day 2	Day 4	Day 7	Day 17	Day 23	Day 3	Day 4	Day 7	Day 18
CBMS03	26/02 1e	14/03 1a	17/03 1b	20/03 1c	27/03 1d	04/04 2e	10/04 2a	12/04 2b	15/04 2c	25/04 2d	01/05 3e	22/05 3a	23/05 3b	26/05 3c	06/06 3d
	CL = 38	CL = 27					CL = 41					CL = 31			
	Day 13	Day 23		Day 3	Day 6		Day 20			Day 7	Day 14	Day 20	Day 1	Day 4	Day 6
CBMS04	14/04 1d	24/04 1e	miss 1a	28/04 1b	01/05 1c	miss 2d	15/05 2e	miss 2a	miss 2b	29/05 2c	05/06 3d	11/06 3e	miss 3a	23/06 3b	25/03 3c
	CL = 26		CL = 27					CL = 28					CL = 26		
	Day 2	Day 4	Day 7	Day 14	Day 21		Day 3	Day 5	Day 18	Day 21	Day 2	Day 4	Day 7	Day 18	Day 21
CBMS08	01/03 1a	03/03 1b	06/03 1c	13/03 1d	20/03 1e	miss 2a	30/03 2b	01/04 2c	14/04 2d	17/04 2e	26/04 3a	28/04 3b	01/05 3c	12/05 3d	15/05 3e
	CL = 28					CL = 28					CL = 34				
	Day 2		Day 7	Day 15			Day 3	Day 7	Day 15	Day 22					Day21
CBMS12	13/02 1a	miss 1b	18/02 1c	28/02 1d	miss 1e	miss 2a	15/03 2b	19/03 2c	27/03 2d	03/04 2e	miss 3a	miss 3b	miss 3c	miss 3d	28/04 3e
	CL = 29					CL = 26					CL = 27				
	Day 2	Day 4	Day 8	Day 16	Day 22	Day 3									
	06/05 4a	08/05 4b	12/05 4c	20/05 4d	26/05 4e	09/06 5a									
	CL = 33					CL = ?									

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF DIFFUSION TESTING

	Day -1	Day 3	Day 5	Day 15	Day 20	Day 1	Day 4	Day 6	Day 15	Day 21	Day -1	Day 3	Day 6	Day 15	Day 19
CBMS13	28/02 1a	03/03 1b	05/03 1c	15/03 1d	20/03 1e	28/03 2a	31/03 2b	02/04 2c	11/04 2d	17/04 2e	25/04 3a	28/04 3b	01/05 3c	10/05 3d	14/05 3e
	CL = 27					CL = 29					CL = 25				
	Day 9	Day 18	Day 23		Day 3	Day 7	Day 17	Day 25	Day 2		Day 11	Day 17	Day 27	Day 2	
CBMS16	12/03 1c	21/03 1d	26/03 1e	miss 1a	04/04 1b	08/04 2c	18/04 2d	26/04 2e	30/04 2a	miss 2b	09/05 2c	15/05 3d	25/05 3e	28/05 3a	miss 3b
	CL = 29			CL = 27					CL = 28					CL = 27	
	Day 10														
	05/06 3c														
	Day 22	Day 1	Day 3	Day 8	Day 15	Day 22	Day 1	Day 4	Day 8	Day 15	Day 22	Day -1	Day 2	Day 6	Day 15
CBMS18	03/04 1e	14/04 1a	16/04 1b	21/04 1c	28/04 1d	05/05 2e	16/05 2a	19/05 2b	23/05 2c	30/05 2d	06/06 3e	18/06 3a	20/06 3b	24/06 3c	04/07 3d
	CL = 32	CL = 32					CL = 34					CL = 31			
	Day 5	Day 8	Day 16	Day 22	Day 2		Day 7	Day 16	Day 22	Day 2	Day 4	Day 13	Day 21	Day 25	Day 1
CBMS19	21/03 1b	24/03 1c	01/04 1d	07/04 1e	16/04 1a	miss 2b	21/04 2c	30/04 2d	06/05 2e	14/06 2a	16/06 3b	20/06 3c	03/07 3d	07/07 3e	miss 3a
	CL = 29				CL = 59					CL = ?					
	Day 8	Day 17	Day 22	Day 3	Day 5	Day 7	Day 19	Day 23		Day 4	Day 8	Day 21	Day 24	Day -4	Day 1
CBMS20	26/03 1c	04/04 1d	09/04 1e	28/04 1a	30/04 1b	02/05 2c	14/05 2d	19/05 2e	miss 2a	30/05 2b	03/06 3c	16/06 3d	19/06 3e	27/06 3a	miss 3b
	CL = 38			CL = 31					CL = 35						
	Day 22	Day -2	Day 2	Day 7	Day 19	Day 25		Day 3	Day 7	Day 17					
CBMS21	04/04 1e	11/04 1a	14/04 1b	19/04 1c	01/05 1d	07/05 2e	miss 2a	19/05 2b	23/05 2c	02/06 2d					
	CL = 30	CL = 34					CL = 33								
	Day 24	Day 2	Day 4	Day 8	Day 13	Day 22	Day 1	Day 3	Day 8	Day 13	Day 20	Day 2	Day 4	Day 8	Day 21
CBMS22	04/04 1e	10/04 1a	12/04 1b	16/04 1c	22/04 1d	30/04 2e	08/05 2a	10/05 2b	15/05 2c	20/05 2d	27/05 3e	05/06 3a	07/06 3b	11/06 3c	24/063e
	CL = 28	CL = 29					CL = 27					CL = 26			

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF DIFFUSION TESTING SELECTED AS BEST OVULATORY CYCLE

											Day -1	Day 3	Day 7	Day 17	Day 24
CBMS00											09/05 3a	12/05 3b	16/05 3c	26/05 3d	02/06 3e
											CL = 30				
												Day 2	Day 4	Day 7	Day 17
CBMS01												21/04 3a	23/04 3b	26/04 3c	06/05 3d
												CL = 28			
	Day 23														
	12/05 4e														
		Day 2	Day 5	Day 8	Day 15	Day 23									
CBMS03		14/03 1a	17/03 1b	20/03 1c	27/03 1d	04/04 2e									
		CL = 27													
	Day 13	Day 23		Day 3	Day 6										
CBMS04	14/04 1d	24/04 1e	miss 1a	28/04 1b	01/05 1c										
	CL = 26		CL = 27												
									Day 18	Day 21	Day 2	Day 4	Day 7		
CBMS08									14/04 2d	17/04 2e	26/04 3a	28/04 3b	01/05 3c		
											CL = 34				
	Day 2		Day 7	Day 15											
CBMS12	13/02 1a	miss 1b	18/02 1c	28/02 1d	miss 1e										
	CL = 29														

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF DIFFUSION TESTING SELECTED AS BEST OVULATORY CYCLE

	Day -1	Day 3	Day 5	Day 15	Day 20										
CBMS13	28/02 1a	03/03 1b	05/03 1c	15/03 1d	20/03 1e										
	CL = 27														
					Day 3	Day 7	Day 17	Day 25	Day 2						
CBMS16					04/04 1b	08/04 2c	18/04 2d	26/04 2e	30/04 2a						
									CL = 28						
	Day 22	Day 1	Day 3	Day 8	Day 15										
CBMS18	03/04 1e	14/04 1a	16/04 1b	21/04 1c	28/04 1d										
	CL = 32	CL = 32													
	Day 5	Day 8	Day 16	Day 22	Day 2										
CBMS19	21/03 1b	24/03 1c	01/04 1d	07/04 1e	16/04 1a										
	CL = 29				CL = 59										
				Day 3	Day 5	Day 7	Day 19	Day 23							
CBMS20				28/04 1a	30/04 1b	02/05 2c	14/05 2d	19/05 2e							
				CL = 31											
	Day 22	Day -2	Day 2	Day 7	Day 25										
CBMS21	04/04 1e	11/04 1a	14/04 1b	19/04 1c	07/05 1d										
	CL = 30	CL = 34													
						Day 22	Day 1	Day 3	Day 8	Day 13					
CBMS22						30/04 2e	08/05 2a	10/05 2b	15/05 2c	20/05 2d					
							CL = 27								

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF DIFFUSION TESTING SELECTED AS BEST ANOVULATORY CYCLE

						Day 1	Day 3	Day 6	Day 19	Day 23					
CBMS00						17/04 2a	19/04 2b	22/04 2c	05/05 2d	miss 2e					
						CL = 23									
							Day 2	Day 4	Day 7	Day 17	Day 23				
CBMS03							10/04 2a	12/04 2b	15/04 2c	25/04 2d	01/05 3e				
							CL = 41								
							Day 3	Day 7	Day 15	Day 22					
CBMS12						miss 2a	15/03 2b	19/03 2c	27/03 2d	03/04 2e					
						CL = 26									
											Day -1	Day 3	Day 6	Day 15	Day 19
CBMS13											25/04 3a	28/04 3b	01/05 3c	10/05 3d	14/05 3e
											CL = 25				
							Day 1	Day 4	Day 8	Day 15	Day 22				
CBMS18							16/05 2a	19/05 2b	23/05 2c	30/05 2d	06/06 3e				
							CL = 34								
							Day 7	Day 16	Day 22	Day 2	Day 4				
CBMS19							21/04 2c	30/04 2d	06/05 2e	14/06 2a	16/06 3b				
										CL = ?					

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF SERUM SAMPLING

		Day 3	Day 6	Day 17	Day 21		Day -1	Day 6	Day 19						Day 24
CBMS00		24/03 1b	27/03 1c	07/04 1d	11/04 1e		19/04 2b	22/04 2c	05/05 2d	miss 2e					02/06 3e
	CL = 26					CL = 23					CL = 30				
	Day 18		Day 3	Day 7	Day 14	Day 20		Day 4	Day 8	Day 17	Day 21				
CBMS01	13/02 1e		28/02 1b	04/03 1c	11/03 1d	17/03 2e		28/03 2b	01/04 2c	10/04 2d	14/04 3e				
	CL = 30	CL = 27					CL = 26					CL = 28			
	Day 23														
	12/05 4e														
	Day 23		Day 5	Day 8	Day 15	Day 23		Day 4	Day 7	Day 17	Day 23				Day 25
CBMS03	25/02 1e		17/03 1b	20/03 1c	27/03 1d	04/04 2e		12/04 2b	15/04 2c	25/04 2d	01/05 3e				13/05 4e
	CL = 38	CL = 27					CL = 41					CL = 31			
	Day 13	Day 23		Day 3	Day 6		Day 20			Day 7	Day 14	Day 20			
CBMS04	14/04 1d	24/04 1e		28/04 1b	01/05 1c	miss 2d	15/05 2e		miss 2b	29/05 2c	05/06 3d	11/06 3e			
	CL = 26		CL = 27					CL = 28					CL = 26		
		Day 4	Day 7	Day 14	Day 21		Day 3		Day 18	Day 21					Day 21
CBMS08		03/03 1b	06/03 1c	13/03 1d	20/03 1e		30/03 2b	miss 2c	14/04 2d	17/04 2e					15/05 3e
	CL = 28					CL = 28					CL = 34				
		Day 2					Day 3	Day 7	Day 15	Day 22					Day21
CBMS12		13/02 1b					15/03 2b	19/03 2c	27/03 2d	03/04 2e					28/04 3e
	CL = 29					CL = 26					CL = 27				
		Day 4	Day 8	Day 16	Day 22										
		08/05 4b	12/05 4c	20/05 4d	26/05 4e										
	CL = 33					CL = ?									

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF SERUM SAMPLING

		Day 3													
CBMS13		03/03 1b													
	CL = 27					CL = 29					CL = 25				
	Day 9	Day 18	Day 23			Day 7	Day 17	Day 25			Day 11	Day 18	Day 27		
CBMS16	12/03 1c	21/03 1d	26/03 1e		miss 1b	08/04 2c	18/04 2d	26/04 2e		miss 2b	09/05 2c	16/05 3d	25/05 3e		
	CL = 29			CL = 27					CL = 28					CL = 27	
	05/06 3c														
	Day 22		Day 3	Day 8	Day 15	Day 22		Day 4	Day 8	Day 15					Day 15
CBMS18	03/04 1e		16/04 1b	21/04 1c	28/04 1d	05/05 2e		19/05 2b	23/05 2c	30/05 2d					04/07 3d
	CL = 32	CL = 32					CL = 34					CL = 31			
	Day 5	Day 8	Day 16	Day 22			Day 7	Day 16	Day 22		Day 4			Day 25	
CBMS19	21/03 1b	24/03 1c	01/04 1d	07/04 1e			21/04 2c	30/04 2d	06/05 2e		16/06 3b			07/07 3e	
	CL = 29		miss E2		CL = 59					CL = ?					
	Day 8	Day 17	Day 22		Day 5	Day 7	Day 19	Day 23							
CBMS20	26/03 1c	04/04 1d	09/04 1e		30/04 1b	02/05 2c	14/05 2d	19/05 2e							
	CL = 38			CL = 31					CL = 35						
	Day 22		Day 2	Day 7	Day 19	Day 25			Day 7						
CBMS21	04/04 1e		14/04 1b	19/04 1c	01/05 1d	07/05 2e			23/05 2c	miss 2d					
	CL = 30	CL = 34					CL = 33								
CBMS22															
	CL = 28	CL = 29					CL = 27					CL = 26			

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF CARBOXYHAEMOGLOBIN ANALYSIS

						Day 1		Day 6	Day 19	Day 23					
CBMS00						17/04 2a		22/04 2c	05/05 2d	miss 2e					
	CL = 26					CL = 23					CL = 30				
								Day 4	Day 8	Day 17	Day 21				
CBMS01								28/03 2b	01/04 2c	10/04 2d	14/04 3e				
	CL = 30	CL = 27					CL = 26					CL = 28			
	Day 23														
	12/05 4e														
						Day 23		Day 4	Day 7	Day 17	Day 23				
CBMS03						04/04 2e		12/04 2b	15/04 2c	25/04 2d	01/05 3e				
	CL = 38	CL = 27					CL = 41					CL = 31			
	Day 13	Day 23		Day 3	Day 6										
CBMS04	14/04 1d	24/04 1e		28/04 1b	01/05 1c										
	CL = 26		CL = 27					CL = 28					CL = 26		
								Day 5	Day 18	Day 21					Day 6
CBMS08								01/04 2c	14/04 2d	17/04 2e					03/06 4b
	CL = 28					CL = 28					CL = 34				
									Day 15	Day 22					
CBMS12									27/03 2d	03/04 2e					
		Day 4	Day 8												
		08/05 4b	12/05 4c												
	CL = 33					CL = ?									

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))

DATES OF PACKED CELL VOLUME ANALYSIS

		*Day 3	*Day 6	*Day 17	*Day 21	Day 1		Day 6	Day 19						Day 24
CBMS00		24/03 1b	27/03 1c	07/04 1d	11/04 1e	17/04 2a		22/04 2c	05/05 2d						02/06 3e
	CL = 26					CL = 23					CL = 30				
					Day 14	Day 20		*Day 4	*Day 8	*Day 17	*Day 21				
CBMS01					11/03 1d	17/03 2e		28/03 2b	01/04 2c	10/04 2d	14/04 3e				
	CL = 30	CL = 27					CL = 26					CL = 28			
	Day 23														
	12/05 4e														
			*Day 5	*Day 8	*Day 15	*Day 23		Day 4	Day 7	Day 17	Day 23				
CBMS03			17/03 1b	20/03 1c	27/03 1d	04/04 2e		12/04 2b	15/04 2c	25/04 2d	01/05 3e				
	CL = 38	CL = 27					CL = 41					CL = 31			
	*Day 13	*Day 23		*Day 3	*Day 6		Day 20				Day 14	Day 20			
CBMS04	14/04 1d	24/04 1e		28/04 1b	01/05 1c		15/05 2e				05/06 3d	11/06 3e			
	CL = 26		CL = 27					CL = 28					CL = 26		
		*Day 4	*Day 7	*Day 21					Day 18	*Day 21				Day 21	Day 6
CBMS08		03/03 1b	06/03 1c	20/03 1d					14/04 2d	17/04 2e				15/05 3e	03/06 4b
	CL = 28					CL = 28					CL = 34				
									Day 15	Day 22					Day21
CBMS12									27/03 2d	03/04 2e					28/04 3e
	CL = 29					CL = 26					CL = 27				
		*Day 4	*Day 8	*Day 16	*Day 22										
		08/05 4b	12/05 4c	20/05 4d	26/05 4e										
	CL = 33					CL = ?									

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))
* Dates chosen for cycle analysis

DATES OF PACKED CELL VOLUME ANALYSIS

CBMS13															
	CL = 27					CL = 29					CL = 25				
	Day 9	Day 18	Day 23			Day 7		Day 25			Day 11	Day 17	Day 27		
CBMS16	12/03 1c	21/03 1d	26/03 1e			08/04 2c		26/04 2e			09/05 2c	15/05 3d	25/05 3e		
	CL = 29			CL = 27					CL = 28					CL = 27	
	Day 10														
	05/06 3c														
	Day 22		*Day 3	*Day 8	*Day 15	Day 22		Day 4	Day 8	Day 15					
CBMS18	03/04 1e		16/04 1b	21/04 1c	28/04 1d	05/05 2e		19/05 2b	23/05 2c	30/05 2d					
	CL = 32	CL = 32					CL = 34					CL = 31			
				Day 22			*Day 7	*Day 16	*Day 22		*Day 4				
CBMS19				07/04 1e			21/04 2c	30/04 2d	06/05 2e		16/06 2b				
	CL = 29				CL = 59					CL = ?					
	Day 8	Day 17	Day 22			*Day 7	*Day 19	*Day 23		Day 4	Day 8	Day 21			
CBMS20	26/03 1c	04/04 1d	09/04 1e			02/05 2c	14/05 2d	19/05 2e		30/05 2b	03/06 3c	16/06 3d			
	CL = 38			CL = 31					CL = 35						
	*Day 22		*Day 2		*Day 25			Day 3	*Day 7	Day 17					
CBMS21	04/04 1e		14/04 1b		07/05 1d		miss 2a	19/05 2b	23/05 2c	02/06 2d					
	CL = 30	CL = 34					CL = 33								
CBMS22															
	CL = 28	CL = 29					CL = 27					CL = 26			

Day (refers to day of menstrual cycle)
1a (refers to Cycle Number and Test Point)
CL (cycle length (days))
* Dates chosen for cycle analysis

Appendix XII: Raw Data Summary

DIFFUSION CAPACITY OF CARBON MONOXIDE MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						**ANOVULATORY**		**CYCLE**	**ANALYSIS**		
	DLCO CHANGES OVER TEST POINTS						DLCO CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	23.72	22.64	27.02	22.35	23.03	23.75	23.86	24.95	24.03	24.00	miss	24.21
CBMS01	19.36	18.30	17.90	18.73	18.62	18.58						
CBMS03	27.06	25.60	21.79	23.03	20.70	23.64	21.29	21.28	21.18	19.07	20.04	20.57
CBMS04	miss	21.50	21.49	19.74	21.74	21.12						
CBMS08	18.05	16.37	16.84	16.61	17.04	16.98						
CBMS12	22.62	miss	22.93	21.29	miss	22.28	miss	25.74	25.70	25.27	26.16	25.72
CBMS13	23.27	21.81	19.43	22.13	20.41	21.41	17.26	17.68	17.68	17.01	18.81	17.69
CBMS16	17.25	20.92	24.71	19.54	20.09	20.50						
CBMS18	19.62	20.85	18.88	19.00	22.38	20.15	21.74	22.33	20.03	18.98	18.77	20.37
CBMS19	18.51	27.38	25.27	20.70	miss	22.97	19.90	20.25	18.47	18.67	19.90	19.44
CBMS20	16.88	18.08	22.46	18.42	22.46	19.66						
CBMS21	24.76	23.64	20.94	21.90	24.77	23.20						
CBMS22	25.80	23.10	25.82	30.26	25.39	26.07						
MEAN	21.41	21.68	21.96	21.05	21.51	**21.52**	20.81	22.04	21.18	20.50	20.74	**21.07**
STADEV	3.54	3.14	3.16	3.33	2.49		2.44	3.00	3.15	3.31	3.09	

Missing values have been replaced by overall means corrected for subject mean and test point (a-e) mean in the statistical analysis.
Bolded values represent overall means.

DIFFUSION CAPACITY OF CARBON MONOXIDE TO ALVEOLAR VOLUME RATIO MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						ANOVULATORY CYCLE ANALYSIS					
	DLCO/VA CHANGES OVER TEST POINTS						DLCO/VA CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	3.76	3.44	3.81	3.48	3.62	3.62	3.74	3.86	3.79	3.73	miss	3.78
CBMS01	3.41	3.17	3.14	3.37	3.26	3.27						
CBMS03	4.86	4.28	3.62	4.31	3.67	4.15	3.63	3.67	3.65	3.18	3.59	3.54
CBMS04	miss	2.84	2.77	2.58	2.79	2.75						
CBMS08	3.33	3.06	3.14	3.12	3.23	3.18						
CBMS12	3.53	miss	3.43	3.43	miss	3.46	miss	4.19	3.94	4.06	3.90	4.02
CBMS13	4.17	3.81	3.60	4.38	3.86	3.96	3.27	3.22	3.13	3.09	3.34	3.21
CBMS16	3.34	4.86	4.48	4.12	4.39	4.24						
CBMS18	4.23	4.86	4.26	4.35	4.77	4.49	4.42	4.39	4.48	4.12	4.20	4.32
CBMS19	3.46	5.02	3.81	3.46	miss	3.94	3.48	3.48	3.25	3.33	3.36	3.38
CBMS20	2.97	3.14	3.90	3.01	3.20	3.24						
CBMS21	3.70	3.95	3.34	3.48	3.78	3.65						
CBMS22	4.20	3.76	4.03	4.23	4.18	4.08						
MEAN	3.75	3.85	3.64	3.64	3.70	**3.71**	3.71	3.80	3.71	3.59	3.68	**3.70**
STADEV	0.53	0.76	0.48	0.58	0.58		0.44	0.44	0.49	0.45	0.37	

Missing values have been replaced by overall menas corrected for subject mean and test pojnt (a-e) mena in the statistical analysis.
Bolded values represent overall means.

DIFFUSION CAPACITY OF THE ALVEOLAR MEMBRANE MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						ANOVULATORY	CYCLE	ANALYSIS			
	DM CHANGES OVER TEST POINTS						DM CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	33.88	33.76	39.99	34.06	36.82	35.70	35.34	35.89	34.06	36.18	miss	35.37
CBMS01	26.56	25.36	26.42	26.45	24.72	25.90						
CBMS03	36.37	33.70	27.87	miss	miss	32.65	miss	miss	miss	28.46	28.45	28.45
CBMS04	miss	32.85	32.62	miss	31.96	32.48						
CBMS08	26.69	23.06	25.88	miss	23.90	24.88						
CBMS12	29.19	miss	31.54	27.96	miss	29.57	miss	32.87	34.36	miss	miss	33.61
CBMS13	32.22	29.74	26.41	27.84	25.92	28.42	22.45	22.63	24.90	22.43	24.85	23.45
CBMS16	24.24	miss	miss	26.06	25.95	25.42						
CBMS18	miss	29.10	26.56	26.12	miss	27.26	miss	30.43	29.57	27.93	28.29	29.06
CBMS19	26.19	37.83	34.59	miss	miss	32.87	32.75	31.20	28.52	28.41	32.75	30.73
CBMS20	23.81	26.72	31.85	26.58	31.85	28.16						
CBMS21	miss	miss	30.45	33.57	miss	32.01						
CBMS22	38.08	32.27	35.37	42.49	35.65	36.77						
MEAN	29.72	30.44	30.80	30.13	29.60	**30.18**	30.18	30.60	30.28	28.68	28.59	**29.67**
STADEV	5.10	4.49	4.41	5.58	5.11		6.82	4.92	3.98	4.90	3.24	

Missing values have been replaced by overall means corrected for subject mean and test point (a-e) mena in the statistical analysis.
Bolded values represent overall means.

PULMONARY CAPILLARY BLOOD VOLUME MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						**ANOVULATORY**		**CYCLE**	**ANALYSIS**		
	VC CHANGES OVER TEST POINTS						VC CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	49.20	43.19	54.00	38.79	35.00	44.04	44.86	44.58	48.03	44.83	miss	45.58
CBMS01	46.03	38.74	34.03	40.88	46.59	41.26						
CBMS03	69.79	72.77	73.62	miss	miss	72.06	miss	miss	miss	43.12	53.43	48.28
CBMS04	miss	45.68	43.82	miss	47.51	45.67						
CBMS08	32.80	31.68	29.17	miss	35.43	32.27						
CBMS12	67.66	miss	50.65	50.70	miss	56.34	miss	69.07	59.81	miss	miss	64.44
CBMS13	46.87	45.88	42.26	56.23	56.95	49.64	45.73	46.26	36.96	41.82	45.26	43.20
CBMS16	38.12	miss	miss	53.93	59.31	50.45						
CBMS18	miss	44.06	38.29	40.41	miss	40.92	miss	50.71	37.35	33.95	32.16	38.54
CBMS19	39.26	62.45	63.86	miss	miss	55.19	33.77	39.54	32.01	36.46	33.77	35.11
CBMS20	43.21	42.07	46.91	37.70	46.91	43.36						
CBMS21	miss	miss	46.23	41.54	miss	43.89						
CBMS22	52.76	54.09	miss	64.19	50.04	55.27						
MEAN	48.57	48.06	47.53	47.15	47.22	**47.74**	41.45	50.03	42.83	40.04	41.16	**43.34**
STADEV	12.09	12.00	12.83	9.40	8.76		6.67	11.37	11.15	4.62	10.05	

Missing values have been replaced by overall menas corrected for subject mean and test point (a-e) mean in the statistical programme.
Bolded values represent overall means.

HAEMOGLOBIN ADJUSTED DIFFUSION CAPACITY OF CARBON MONOXIDE MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						ANOVULATORY	CYCLE	ANALYSIS			
	ADJUSTED DLCO CHANGES OVER TEST POINTS						ADJUSTED DLCO CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	23.72	22.85	27.54	22.02	22.25	23.68	23.75	23.92	23.64	24.19	miss	23.87
CBMS01	19.73	18.08	17.96	18.97	18.65	18.68						
CBMS03	27.86	27.13	23.75	24.60	22.20	25.11	23.98	24.03	23.70	20.78	22.42	22.98
CBMS04	miss	23.43	22.73	21.47	23.00	22.66						
CBMS08	17.78	15.82	16.69	16.14	16.81	16.65						
CBMS12	23.37	miss	22.66	20.74	miss	22.26	miss	25.51	25.36	24.73	25.81	25.35
CBMS13	22.54	21.13	18.93	20.95	20.17	20.74	17.21	17.22	17.52	16.62	18.43	17.40
CBMS16	23.25	20.86	23.94	20.71	20.94	21.94						
CBMS18	19.11	20.72	18.47	18.56	21.86	19.74	21.30	22.33	19.91	18.44	18.18	20.03
CBMS19	18.68	27.64	26.39	21.77	miss	23.62	20.92	21.18	18.39	19.50	20.60	20.12
CBMS20	18.47	19.96	20.62	18.59	22.56	20.04						
CBMS21	25.76	25.05	22.28	22.36	25.08	24.11						
CBMS22	26.47	23.70	*25.74	30.26	24.73	26.18						
MEAN	22.23	22.20	22.13	21.32	21.66	**21.90**	21.43	22.36	21.42	20.71	21.09	**21.41**
STADEV	3.43	3.49	3.42	3.43	2.44		2.74	2.93	3.23	3.22	3.16	

Missing values are replaced by overall means corrected for subject and test point mean.
* [Hb] value missing. Adjusted DLCO value was calculated from mean [Hb] corrected for cycle number and test point.
Bolded values represent overall means.

HAEMOGLOBIN ADJUSTED DIFFUSION CAPACITY TO ALVEOLAR VOLUME RATIO MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						**ANOVULATORY**		**CYCLE**	**ANALYSIS**		
	ADJUSTED DLCO/VA CHANGES OVER TEST POINTS						ADJUSTED DLCO/VA CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	3.77	3.47	3.88	3.42	3.50	3.61	3.72	3.70	3.72	3.77	miss	3.73
CBMS01	3.47	3.13	3.15	3.41	3.26	3.29						
CBMS03	5.01	4.54	3.94	4.61	3.94	4.41	4.09	4.14	4.09	3.47	4.02	3.96
CBMS04	miss	3.10	2.93	2.81	2.95	2.95						
CBMS08	3.28	2.96	3.11	3.03	3.18	3.11						
CBMS12	3.65	miss	3.61	3.26	miss	3.51	miss	4.15	3.89	3.98	3.85	3.97
CBMS13	4.04	3.69	3.50	4.14	3.82	3.84	3.27	3.14	3.10	3.02	3.27	3.16
CBMS16	3.63	4.83	4.45	4.00	4.23	4.23						
CBMS18	4.13	4.83	4.17	4.25	4.66	4.41	4.33	4.39	4.45	4.00	4.07	4.25
CBMS19	3.49	5.07	4.43	3.80	miss	4.20	3.59	3.65	3.24	3.48	3.48	3.49
CBMS20	3.25	3.47	3.88	3.04	3.22	3.37						
CBMS21	3.91	4.19	3.56	3.55	3.82	3.81						
CBMS22	4.30	3.86	*4.02	4.23	4.07	4.10						
MEAN	3.83	3.93	3.74	3.66	3.70	**3.77**	3.80	3.86	3.75	3.62	3.74	**3.75**
STADEV	0.50	0.75	0.49	0.56	0.52		0.42	0.46	0.51	0.37	0.35	

Missing values are replaced by overall means corrected for subject and test point mean.
* [Hb] value missing. Adjusted DLCO value was calculated from mean [Hb] corrected for cycle number and test point.
Bolded values represent overall means.

HAEMOGLOBIN ADJUSTED DIFFUSION CAPACITY OF THE ALVEOLAR MEMBRANE MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						**ANOVULATORY**	**CYCLE**	**ANALYSIS**			
	ADJUSTED DM CHANGES OVER TEST POINTS						ADJUSTED DM CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	33.88	34.08	40.76	33.56	35.57	35.57	35.18	34.40	33.51	36.46	miss	34.89
CBMS01	27.07	25.06	26.50	26.79	24.75	26.03						
CBMS03	37.44	35.72	30.37	miss	miss	34.51	miss	miss	miss	31.02	31.83	31.42
CBMS04	miss	35.80	34.51	miss	33.81	34.71						
CBMS08	26.29	22.28	25.64	miss	23.58	24.45						
CBMS12	30.16	miss	31.17	27.23	miss	29.52	miss	32.57	33.90	miss	miss	33.24
CBMS13	31.21	28.81	25.72	26.35	25.61	27.54	22.39	22.05	24.68	21.91	24.34	23.07
CBMS16	32.92	miss	miss	27.62	27.05	29.19						
CBMS18	miss	28.92	25.98	25.51	miss	26.80	miss	30.43	29.39	27.13	27.41	28.59
CBMS19	26.44	38.18	36.12	miss	miss	33.58	33.75	32.64	28.39	29.67	33.90	31.67
CBMS20	26.05	29.50	25.96	26.83	32.00	28.07						
CBMS21	miss	miss	32.40	34.27	miss	33.34						
CBMS22	39.07	33.11	*35.26	42.49	34.73	36.93						
MEAN	31.05	31.15	30.87	30.07	29.64	**30.62**	30.44	30.42	29.98	29.24	29.37	**29.86**
STADEV	4.74	5.09	5.06	5.64	4.89		7.01	4.89	3.84	5.33	4.31	

Missing values are replaced by overall means corrected for subject and test point mean.

* [Hb] value missing. Adjusted DLCO value was calculated from mean [Hb] corrected for cycle number and test point.

Bolded values represent overall means.

HAEMOGLOBIN ADJUSTED PULMONARY CAPILLARY BLOOD VOLUME MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						**ANOVULATORY**		**CYCLE**	**ANALYSIS**		
	ADJUSTED VC CHANGES OVER TEST POINTS						ADJUSTED VC CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	49.20	43.60	55.04	38.21	33.82	43.97	44.66	42.73	47.25	45.18	miss	44.96
CBMS01	46.92	38.28	34.14	41.40	46.66	41.48						
CBMS03	71.86	77.13	80.24	miss	miss	76.41	miss	miss	miss	47.00	59.78	53.39
CBMS04	miss	49.78	46.35	miss	50.26	48.80						
CBMS08	32.32	30.60	28.91	miss	34.95	31.70						
CBMS12	69.91	miss	50.05	49.39	miss	56.45	miss	68.45	59.01	miss	miss	63.73
CBMS13	45.41	44.44	41.16	53.23	56.27	48.10	45.59	45.06	36.63	40.85	44.34	42.49
CBMS16	57.07	miss	miss	57.16	61.82	58.68						
CBMS18	miss	43.79	37.46	39.47	miss	40.24	miss	50.71	37.12	32.98	31.16	37.99
CBMS19	39.63	63.04	66.69	miss	miss	56.45	37.65	41.36	31.87	38.08	34.95	36.78
CBMS20	47.29	46.44	60.76	38.05	47.13	47.93						
CBMS21	miss	miss	49.19	42.42	miss	45.80						
CBMS22	54.14	55.51	*58.48	64.19	48.74	56.21						
MEAN	51.37	49.26	50.71	47.06	47.46	**49.35**	42.63	49.66	42.38	40.82	42.56	**43.74**
STADEV	12.39	13.20	14.60	9.42	9.54		4.34	11.09	10.86	5.62	12.75	

Missing values are replaced by overall means corrected for subject and test point mean.
* [Hb] value missing. Adjusted DLCO value was calculated from mean [Hb] corrected for cycle number and test point.
Bolded values represent overall means.

HAEMOGLOBIN CONCENTRATION CHANGES MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS				(Hb)		ANOVULATORY		CYCLE	ANALYSIS		(Hb)
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	13.40	13.10	12.80	13.90	14.60	13.56	13.55	14.90	13.95	13.15	miss	13.89
CBMS01	12.80	13.80	13.30	13.00	13.35	13.25						
CBMS03	12.50	11.70	11.00	11.50	11.40	11.62	10.25	10.20	10.40	11.00	10.40	10.45
CBMS04	miss	11.00	11.75	11.05	11.75	11.39						
CBMS08	13.90	14.60	13.70	14.40	13.85	14.09						
CBMS12	12.40	miss	13.80	14.30	miss	13.50	miss	13.70	13.85	14.13	13.85	13.88
CBMS13	14.50	14.50	14.30	15.40	13.80	14.50	13.50	14.30	13.70	14.20	14.10	13.96
CBMS16	11.10	13.60	13.60	14.40	14.70	13.48						
CBMS18	14.30	13.60	14.15	14.20	14.20	14.09	14.10	13.40	13.60	14.40	14.50	14.00
CBMS19	13.10	13.10	12.10	11.90	miss	12.55	12.40	12.05	13.55	12.10	12.35	12.49
CBMS20	10.90	10.70	13.55	13.10	13.25	12.30						
CBMS21	12.20	11.70	11.60	12.75	13.00	12.25						
CBMS22	12.60	12.60	13.50	13.40	14.30	13.28						
MEAN	12.81	12.83	13.01	13.33	13.47	**13.09**	12.76	13.09	13.18	13.16	13.04	**13.06**
STADEV	1.13	1.30	1.06	1.28	1.09		1.53	1.71	1.37	1.37	1.69	

Missing values have been replaced by overall menas corrected for subject mean and test point (a-e) mean in the statistical analysis.
Bolded values represent overall means.

CARBOXYHAEMOGLOBIN CONCENTRATION MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

		COHB	SUMMARY			
	b	c	d	e	MEAN	status
CBMS00	0.75	0.70	0.55	0.65	0.66	AN
CBMS01	0.80	1.20	miss	0.90	0.97	OV
CBMS03	0.85	1.10	1.00	miss	0.98	OV/AN
CBMS04	0.75	0.90	0.85	1.00	0.88	OV
CBMS08	0.90	0.90	1.00	1.50	1.08	OV
CBMS12	0.75	0.85	0.80	0.75	0.79	ID
CBMS16	miss	0.75	0.50	0.40	0.55	OV
CBMS18	1.05	1.15	0.90	0.90	1.00	OV
CBMS19	0.50	0.50	0.55	0.25	0.45	AN
CBMS20	1.00	0.65	0.90	0.95	0.88	OV
CBMS21	0.50	1.00	0.80	miss	0.77	OV
MEAN	0.79	0.88	0.79	0.81	**0.82**	
ST DEV	0.18	0.22	0.19	0.36		

Missing values are replaced by overall means corrected for subject and test point mean.
Bolded value represents overall mean.

PACKED CELL VOLUME CHANGES OVER THE MENSTRUAL CYCLE OF ALL CYCLES AND OVULATORY CYCLES

PCV SUMMARY	ALL CYCLES					OVULATORY CYCLES				
	b	c	d	e	MEAN	b	c	d	e	MEAN
CBMS00	41.25	39.00	41.25	40.00	40.38	41.25	39.00	41.25	40.00	40.38
CBMS01	37.50	39.25	40.00	42.25	39.75	37.50	39.25	40.00	42.25	39.75
CBMS03	34.00	35.25	42.75	32.75	36.19	34.00	35.25	42.75	32.75	36.19
CBMS04	35.00	37.25	34.00	37.00	35.81	35.00	37.25	34.00	37.00	35.81
CBMS08	36.25	39.50	36.50	41.50	38.44	36.25	39.50	36.50	41.50	38.44
CBMS12	37.50	38.50	41.50	41.50	39.75					
CBMS16	miss	37.75	35.25	33.25	35.42	miss	37.75	35.25	33.25	35.42
CBMS18	39.00	41.00	41.00	42.00	40.75					
CBMS19	38.00	41.25	37.50	39.50	39.06	39.00	41.00	41.00	42.00	40.75
CBMS20	miss	42.00	40.50	40.00	40.83	miss	42.00	40.50	40.00	40.83
CBMS21	36.50	miss	39.25	31.25	35.67	36.50	miss	39.25	31.25	35.67
MEAN	37.22	39.08	39.05	38.27	**38.45**	37.07	38.88	38.94	37.78	**38.21**
ST DEV	1.54	2.03	2.74	4.02		1.64	2.13	2.87	4.26	

Missing values are replaced by overall means corrected for subject and test point mean in the statistical analysis.
Bolded values represent overall means.

RELIABILITY AND VALIDITY OF HAEMOGLOBIN CONCENTRATION AND PACKED CELL VOLUME MEASUREMENT

SUBJECT	Hb1	Hb2	Hb3	Hb4		Hb1	Hb2		Hblab	Hbvgh
CBMS00	13.55	13.95	13.15	13.65		13.10	13.20		13.95	13.00
CBMS01	13.50	14.85	14.10	14.40		14.00	13.00		14.10	13.18
CBMS03	10.20	10.40	11.00	11.40		10.20	10.20		10.20	10.40
CBMS04	11.00	11.75	11.05	11.75		11.20	10.80		11.00	10.50
CBMS08	14.30	14.00	14.40	13.85		14.30	13.70		14.30	13.85
CBMS12	13.00	13.15	14.10	13.85		13.90	14.30		13.00	11.05
CBMS16	12.85	14.05	13.35	12.15		13.20	13.50		12.85	12.35
CBMS18	13.60	14.15	14.20	14.70		14.20	14.10		14.15	13.95
CBMS19	12.05	13.55	12.10	12.35		12.10	12.00		12.05	11.65
CBMS20	12.85	13.55	13.10	13.25		12.40	12.50		12.85	12.05
CBMS21	11.70	12.95	12.90	12.75		12.40	12.50		12.95	12.45
SUBJECT	PCV1	PCV2	PCV3	PCV4		PCV1	PCV2			
CBMS00	41.25	39.00	41.25	40.00		40.00	40.00			
CBMS01	42.75	36.25	37.50	38.50		38.50	38.50			
CBMS03	34.00	35.25	42.75	32.75		34.00	34.00			
CBMS04	34.00	37.00	35.00	37.25		34.00	34.00			
CBMS08	36.25	39.50	37.25	36.50		36.50	36.50			
CBMS12	36.00	39.00	37.50	38.50		38.50	38.50			
CBMS16	37.75	35.25	33.25	41.25		37.50	38.00			
CBMS18	39.00	41.00	41.00	42.00		41.50	40.50			
CBMS19	41.25	37.50	39.50	38.00		38.00	37.00			
CBMS20	41.75	37.25	33.00	42.00		33.00	33.00			
CBMS21	36.50	39.50	31.25	39.25		36.50	36.50			

ALVEOLAR VOLUME MEASURED AT FIVE TEST POINTS WITHIN AN OVULATORY AND AN ANOVULATORY CYCLE

	BEST OVULATORY CYCLE ANALYSIS						ANOVULATORY	CYCLE	ANALYSIS			
	VA CHANGES OVER TEST POINTS						VA CHANGES OVER TEST POINTS					
	a	b	c	d	e	MEAN	a	b	c	d	e	MEAN
CBMS00	6.30	6.59	7.09	6.43	6.35	6.55	6.38	6.47	6.35	6.42	miss	6.41
CBMS01	5.68	5.77	5.70	5.56	5.72	5.69						
CBMS03	5.56	5.98	6.02	5.34	5.63	5.71	5.87	5.80	5.80	5.99	5.58	5.87
CBMS04	miss	7.56	7.75	7.64	7.79	7.69						
CBMS08	5.42	5.35	5.36	5.32	5.28	5.35						
CBMS12	6.40	miss	6.36	6.36	miss	6.37	miss	6.15	6.52	6.22	6.70	6.40
CBMS13	5.58	5.73	5.40	5.06	5.28	5.41	5.27	5.49	5.35	5.51	5.64	5.45
CBMS16	6.41	6.59	6.77	6.47	6.25	6.50						
CBMS18	4.63	4.29	4.43	4.37	4.69	4.48	4.92	5.09	4.47	4.61	4.55	4.73
CBMS19	5.35	5.45	5.71	5.35	miss	5.47	5.82	5.81	5.68	5.60	5.92	5.77
CBMS20	5.68	5.76	5.31	6.12	7.01	5.98						
CBMS21	6.59	5.98	6.26	6.30	6.56	6.34						
CBMS22	6.15	6.14	6.40	7.15	6.07	6.38						
MEAN	5.81	5.93	6.04	5.96	6.06	**5.96**	5.65	5.80	5.70	5.73	5.68	**5.71**
STADEV	0.57	0.79	0.87	0.90	0.88		0.57	0.48	0.74	0.65	0.77	

Change in alveolar volume independent of DLCO was not analysed statistically.
Bolded values represent overall means.