

The effects of fluctuations in oestrogen and progesterone during the menstrual cycle on glucose homeostasis, energy balance, exercise and premenstrual syndrome

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Note if anything has been removed from thesis: pages 272 & 280

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**The Effects of Fluctuations in Oestrogen and Progesterone
during the Menstrual Cycle on Glucose Homeostasis, Energy
Balance, Exercise and Premenstrual Syndrome**

Sarah Elizabeth Hillier

**A thesis submitted in partial fulfilment of the requirements of
Oxford Brookes University for the award of
Doctor of Philosophy**

January 2014

The following has been
excluded at the request of
the university

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Abstract

It is widely accepted that the loss of sex hormones after the menopause is strongly linked with cancer, insulin resistance and obesity, with variations in sex hormone concentrations being responsible in part, for the differences reported in energy metabolism and glycaemic control between genders. As such a greater understanding of the effects that oestrogen and progesterone may have on women's health and their potential long term consequences is required. This thesis reports evidence of three studies investigating the effects of oestrogen and progesterone during the menstrual cycle on glucose and insulin response, energy expenditure, substrate oxidation and premenstrual syndrome (PMS).

The aim of the first study was to investigate glucose and insulin responses to a 75g glucose load during different phases of the menstrual cycle. Venous blood samples for baseline measurements of oestradiol and progesterone were collected every other week day from eighteen regularly menstruating women for one complete menstrual cycle. An oral glucose tolerance test (OGTT, 75g glucose) was performed on three separate days during the next complete menstrual cycle. The results report an increase in glucose area under the curve (AUC) and insulin AUC during the luteal phase ($P<0.05$) compared to the menstrual and follicular phase, but no significant differences in insulin sensitivity between phases. The findings indicate that both oestrogen and progesterone during the luteal phase may affect glycaemic response and this potentially has significant implications for the development of type 2 diabetes over prolonged periods of time.

The second study investigated energy expenditure and substrate oxidation at rest and during a 30 minute moderate intensity walking exercise within the three phases of the menstrual cycle. Sex hormone concentrations were collected in the same manner as study 1. Nineteen women undertook resting measures and ten for exercise. The study reports a decrease in carbohydrate (CHO) oxidation ($P<0.05$) and a marginal increase in fat oxidation ($P=0.06$) during the follicular phase at rest, with no difference in energy expenditure at rest. No difference in energy expenditure or substrate oxidation between phases was reported during exercise. The results indicate high oestrogen concentrations during the follicular phase only may mediate the changes in substrate oxidation response reported at rest.

The final study investigated the effects of a 12 week moderate intensity exercise intervention on symptoms of PMS, quality of life and dietary intake and determined whether any such effect was associated with changes in oestrogen and progesterone concentrations in a randomised control trial. Twenty-five sedentary women, identified as suffering from PMS symptoms, were recruited to the trial spanning four menstrual cycles, the first serving as a baseline followed by three cycles of intervention. Participants were randomly assigned to either an exercise (EX) group (n=13), which involved three supervised 30-minute moderate-intensity (70-80% HR max) treadmill walking exercise sessions per week, or to a control (CON) group (n=12), which involved attending a 90-minute, one-to-one meeting once per week with the investigator.

The results report a reduction in PMS symptoms following the exercise intervention ($P < 0.05$), with no differences in the control (CON) group. Averaged dietary intake over the three intervention cycles reports a decrease in CHO intake (% total energy intake, TEI) during the luteal phase, compared to the menstrual (45.5% vs. 50.1%) and follicular (45.5 vs. 49.9%) phases in the EX group, with no difference in the CON group. No significant difference was reported in overall energy intake in either group. In addition, no significant difference was reported in plasma oestrogen and progesterone concentrations over the four cycles in either group (EX and CON). The findings indicate that the exercise regime reduces PMS symptoms and CHO intake (%TEI), but that these are not mediated in response to changes in oestrogen and progesterone hormone concentrations. As such exercise may therefore be an effective symptom management tool for women suffering with PMS. Further studies are essential to determine the exact duration and intensity required for the most effective symptom relief.

The results reported in this thesis provide evidence that the circulating reproductive hormones oestrogen and progesterone during the menstrual cycle have significant implications for energy regulation, glycaemic control and women suffering from PMS. As such, both hormones need to be investigated further in larger research studies to determine their potential long-term adverse effects on health and well-being in women.

Achievements

Prizes Awarded

Pfizer Prize: Received for an oral communication entitled 'Glycaemic response and insulin sensitivity during the menstrual cycle in women'. *Physiology 2011, The Physiological Society*

Nigel Groome Award: Received for best poster presentation entitled 'Exercise, symptoms of premenstrual syndrome and quality of life: a proposal'. *Postgraduate Symposium 2012, Oxford Brookes University*

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To end, for anyone inspired enough to embark on completing a PhD, my tip is to choose a topic that you are happy to talk about to a stranger. During my journey, I've lost count of the amount of times someone at a party/bar/sports event/conference has asked me about the topic I was researching. Normally there was one of two responses when I said the menstrual cycle and nutrition.

1. Oh right. (turns and walks away swiftly)
2. REALLY! Are you going to prove why I need chocolate when I'm on my period??

It may have seemed a strange topic to study for many, but you can't help where you get your nerdy kicks from.

Enjoy.

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Abbreviations

ACC	Acetyl-CoA Carboxylase
ACTH	Adrenocorticotrophic Hormone
Akt	Protein Kinase B
AMPK	Adenosine Monophosphate-Activated Protein Kinase
AUC	Area under the curve
ArKO	Androgen Receptor Knock Out
ATP	Adenosine Triphosphate
AUC	Area under the curve
BBT	Basal Body Temperature
B-HAD	B -Hydroxyacyl-CoA Dehydrogenase
BMR	Basal Metabolic Rate
CGMS	Continuous Glucose Monitoring System
CHO	Carbohydrate
CM	Carbohydrate Metabolism
CO₂	Carbon Dioxide
CPT I & II	Carnitine Palmityltransferase 1 And 2
CV%	Percentage Covariance
Db/Db	Leptin Receptor Deficient Diabetic Mice
E1G	Esterone Glucoronide
E₂	Oestradiol / Oestrogen
EE	Energy Expenditure
EDL	Extensor Digtorum Longus
EGH1	Euglycemic Hyperinsulinemic Clamps
ERα	Oestrogen Receptor Alpha
ERβ	Oestrogen Receptor Beta
FA	Fatty Acid
FABPpm	Fatty Acid Transport Protein
FAT/CD36	Fatty Acid Translocase
FATP	Fatty Acid Binding Protein
FBG	Fasting Blood Glucose

FP	Follicular Phase
FPI	Fasting Plasma Insulin
FSH	Follicle Stimulating Hormone
G1P	Glucose 1-Phosphate
G6P	Glucose - 6 - Phosphate
GLP-1	Glucagon-Like Peptide-1
GLUT2	Glucose Transport Protein 2
GLUT4	Glucose Transport Protein 4
GnRH	Gonadotrophin Releasing Hormone
GR	Glucose Response
GSH-R	Growth Hormone Secretagogue Receptors
GT	Glucose Tolerance
H⁺	Hydrogen
HGC	Hyperglycaemic Clamp
HK	Hexokinase
HOMA-IR	Homeostasis Model of Insulin Resistance
HSL	Hormone Sensitive Lipase
Ire	Insulin Receptor
IRS	Insulin Receptor Substrate
IS	Insulin Sensitivity
ITT	Insulin Tolerance Test
IVGTT	Intravenous Glucose Tolerance Test
KO	Knock Out
LCFA	Long Chain Fatty Acid
LH	Luteinising Hormone
LP	Luteal Phase
LPL	Lipoprotein Lipase
MCAD	Medium Chain Fat Oxidative Dehydrogenases
MDQ	Menstrual Distress Questionnaire
MP	Menstrual Phase
mRNA	Messenger Ribonucleic Acid
NS	Non-Significant

O₂	Oxygen
OGTT	Oral Glucose Tolerance Test
<i>Ob/Ob</i>	Obese mice
P	Progesterone
P13-K	Phosphatidylinositide 3-Kinases
PDH	Pyruvate Dehydrogenase
Pdiol-3g	Pregnanediol Glucoronide
PDK1	Pyruvate Dehydrogenase Kinase
PDK4	Pyruvate Dehydrogenase Kinase 4
PFK	Phosphofructokinase
PIP3	Phospholipid Phosphatidylinositol 4, 5 Bisphosphate
PMS	Premenstrual Syndrome
POMC	Proopiomelanocortin
PPARα	Peroxisome Proliferation Activator Receptor α
PPARδ	Peroxisome Proliferation Activator Receptor δ
PSST	Premenstrual Syndrome Screening Test
QoL	Quality of Life
Ra	Glucose Rate of Appearance
RAS	Renin-Angiotensin System
Rd	Glucose Rate of Disappearance
RER	Respiratory Exchange Ratio
RMR	Resting Metabolic Rate
SGLT-1	Sodium Glucose Transport Protein
SMR	Sleeping Metabolic Rate
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TCA Cycle	Tricarboxylic Acid Cycle
TEI%	Percentage Total Energy Intake
TFPα	Trifunctional Protein
VLDL-TG	Very Low Density Triglycerides
Y	Years

Chapter 1: Introduction and Literature Review

1: Introduction

Effectively regulating energy balance is widely accepted to be of paramount importance to reduce the risk of developing metabolic diseases such as type 2 diabetes, cardiovascular disease, obesity and cancer. With the increasing number of women diagnosed as overweight or obese in the UK, understanding the potential cause is essential (Department of Health, 2004). Current research has reported strong differences in energy metabolism and glycaemic control which are linked to effective energy balance, between genders. The differences between men and women may be as a result of variations in reproductive sex hormone concentrations, in particular oestrogen and progesterone (Lovejoy *et al.*, 2009; Tarnopolsky and Ruby, 2001).

It has been reported that premenopausal women have an increased insulin sensitivity compared to aged-matched men (Carr, 2003). With significant increases in type 2 diabetes development, body weight and cancer prevalence also reported in postmenopausal women compared to premenopausal women, potentially as a result of the sharp reduction in sex hormones following menopause (Carr, 2003). In addition, the development of gestational diabetes is often reported during pregnancy when both oestrogen and progesterone are present in high concentrations (Kalkhoff *et al.*, 1970; Kirwan *et al.*, 2002; Nagira *et al.*, 2006). This indicates that women may be more sensitive to changes in sex hormone concentrations compared to men, with the greatest variation reported during extreme concentrations of oestrogen and progesterone (high or low) such as those during pregnancy or menopause. However, there is currently very little research conducted on the possible effects of changes in both oestrogen and progesterone concentrations that are experienced during the most reproductive phase of a women's life cycle (the menstrual cycle).

In addition, both oestrogen and progesterone have also been identified as playing a significant role in female only disorders such as premenstrual syndrome (PMS), which appears only during the luteal phase of the menstrual cycle when both sex hormones are elevated. PMS is reported to affect between 75-85% of women of reproductive age (Halbreich, 2004) and the occurrence of PMS symptoms is

linked to an altered sensitivity to circulating oestrogen and progesterone during the luteal phase (American College of Obstetricians & Gynecologists, 2000). As a consequence, fluctuating concentrations of these hormones may exacerbate other health complications and result in adverse quality of life.

As such, by understanding the potential role of oestrogen and progesterone during different phases of the menstrual cycle in energy regulation, glycaemic control and PMS pathways, the apparent differences reported between genders and the potential consequences for women who suffer from PMS may be better explained. This may enable better weight and symptom management and reduce potential adverse effects on energy balance, health and well-being in women.

This thesis aims to investigate the potential effects of oestrogen and progesterone during the menstrual cycle in regularly menstruating women on energy balance, health and well-being. Its focus is on the effects of these two hormones on energy metabolism, glycaemic response and premenstrual syndrome (PMS).

1.1: Literature Review

The following chapter details the basic physiology of the menstrual cycle and reviews the available literature investigating the variation in cycle duration and phase duration between women. It also discusses the significant implications this variation has on research conducted on women during the menstrual cycle. In addition, the available literature investigating the potential role of oestrogen and progesterone in energy regulation, including; glucose homeostasis, energy expenditure and substrate oxidation as well as dietary intake and the effects of exercise on PMS is also reviewed. Furthermore, literature describing the potential mechanisms by which these two hormones during the menstrual cycle may affect these specific areas and the potential consequences these have is also reviewed.

1.1.1: The Menstrual Cycle: Basic Physiology

The menstrual cycle is a co-ordination of sequential interactions that occur between the hypothalamus, anterior pituitary, uterus and the ovaries and its sole purpose is for reproduction of human life. Menarche (age approx. 9-15 y) marks the start of cyclic ovarian function, beginning during puberty and spanning until menopause (age approx. 45-55 y) (Silberstein and Merriam, 2000). The

hypothalamic-pituitary-ovarian axis coordinates the normal ovarian functioning in order to bring about a successful menstrual cycle (Figure 1.1).

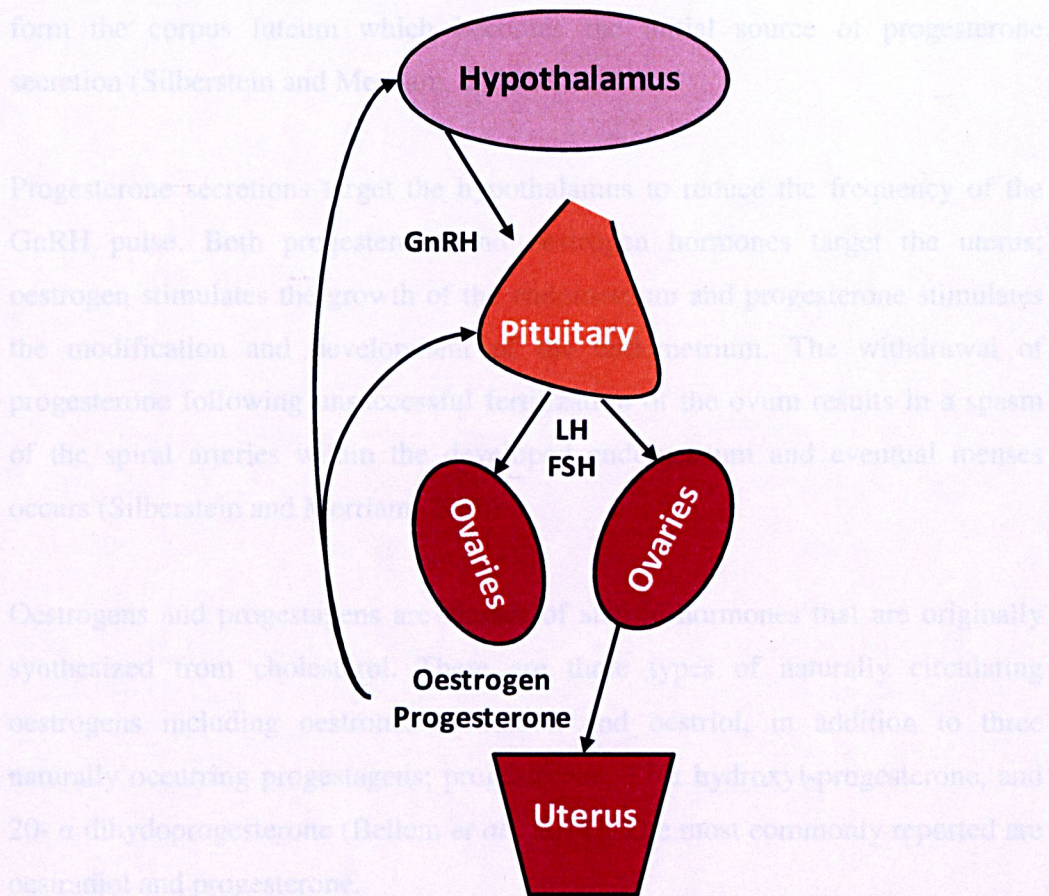


Figure 1.1: Schematic example of the hypothalamic-pituitary-ovarian axis. Gonadotrophin releasing hormone (GnRH), luteinizing hormone (LH) and follicle stimulating hormone (FSH)

Gonadotrophin releasing hormone (GnRH) is secreted by the hypothalamus in a pulsatile manner, approximately one pulse per hour and stimulates the pituitary gland, where both luteinizing hormone (LH) and follicle stimulating hormone (FSH) are secreted (Gonzalez-Ortiz *et al.*, 1998; Silberstein and Merriam, 2000). FSH and LH stimulate follicular growth in the ovaries and secretions of the sex hormones, oestrogen and progesterone (The American College of Obstetricians and Gynecologists, 1998). Oestrogen exerts a negative feedback on the pituitary gland which modulates the secretions of FSH as the follicle grows. During the middle of the menstrual cycle when the follicle has matured, oestrogen concentrations reach a threshold and the inhibition is reversed to create a surge in

LH concentrations. The developing follicle releases an oocyte into the fallopian tube known as ovulation and this oocyte is now known as an ovum. Following the release of the ovum, the granulosa cells of the ovulated follicle are modified to form the corpus luteum which becomes the initial source of progesterone secretion (Silberstein and Merriam, 2000).

Progesterone secretions target the hypothalamus to reduce the frequency of the GnRH pulse. Both progesterone and oestrogen hormones target the uterus; oestrogen stimulates the growth of the endometrium and progesterone stimulates the modification and development of the endometrium. The withdrawal of progesterone following unsuccessful fertilization of the ovum results in a spasm of the spiral arteries within the developed endometrium and eventual menses occurs (Silberstein and Merriam, 2000).

Oestrogens and progestagens are classes of steroid hormones that are originally synthesized from cholesterol. There are three types of naturally circulating oestrogens including oestrone, oestradiol, and oestriol, in addition to three naturally occurring progestagens; progesterone, 17- α hydroxyl-progesterone, and 20- α dihydropregesterone (Bellem *et al.*, 2011). The most commonly reported are oestradiol and progesterone.

1.1.2: Hormonal changes during the menstrual cycle

There are four hormones that fluctuate throughout the menstrual cycle; these include FSH, LH, oestrogen (E2 – oestradiol) and progesterone (P) (Figure 1.2). During a standard 28 day cycle, FSH concentrations rise at the end of the follicular phase during ovulation and are elevated for the last two days of the luteal phase, continuing until day 4 of menstruation (days 27, 28 and 1-4). Concentrations in LH surge up to 36 hours prior to ovulation.

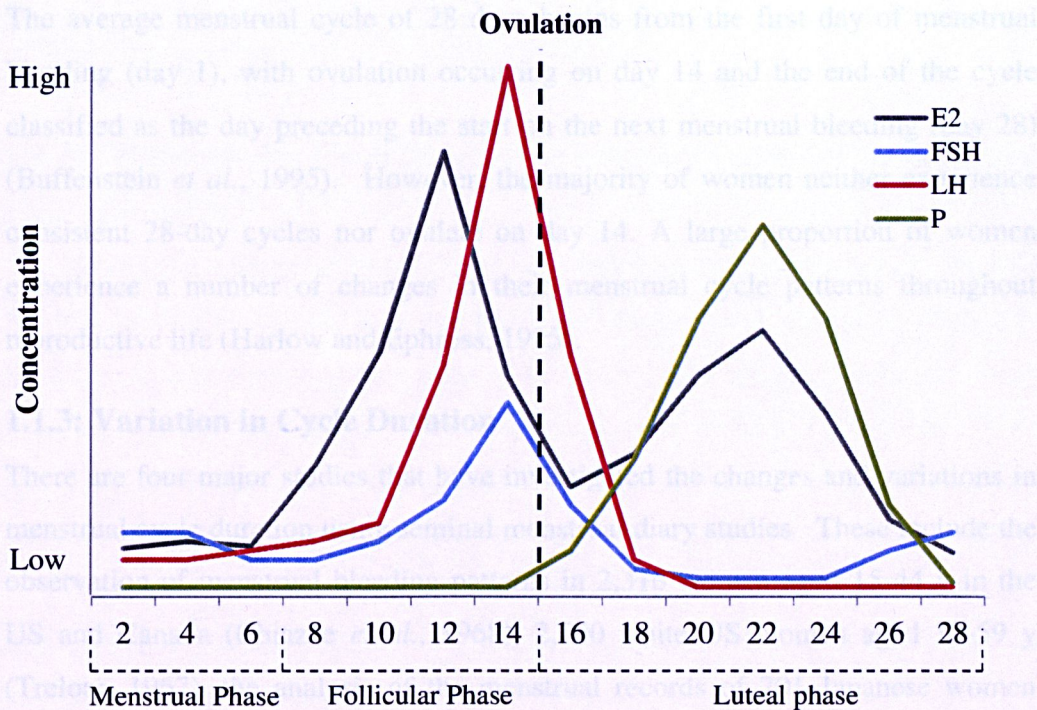


Figure 1.2: Schematic changes in the plasma concentrations of FSH, LH, E2 and P during a ‘standard’ menstrual cycle. Follicle Stimulating Hormone (FSH, mIU/ml), Luteinizing Hormone (LH, mIU/ml), Oestrogen (E2, μg/ml) and Progesterone (P, ng/ml)

Oestrogen concentrations remain low during the menstrual phase but begin to increase approximately one week prior to ovulation (days 5-11) (Table 1.1). Oestrogen concentrations peak before ovulation, sharply decline and rise again following the formation of the corpus luteum during the luteal phase. The rise in oestrogen during the luteal phase is not to the same elevated concentration as during the follicular phase (Table 1.1). Progesterone concentrations remain low throughout the menstrual and follicular phases, increasing after ovulation. This coincides with the formation of the corpus luteum which is the initial source of the high concentrations of progesterone (days 16-28).

Table 1.1: Standard hormone concentrations for oestrogen and progesterone during ovulatory menstrual cycle phases (Larsen *et al.*, 2003)

	Menstrual	Follicular	Luteal
Oestrogen / Oestradiol (pg/ml)	20-60	>200	<200
Progesterone (ng/ml)	<2	<2	2-20

The average menstrual cycle of 28 days begins from the first day of menstrual bleeding (day 1), with ovulation occurring on day 14 and the end of the cycle classified as the day preceding the start on the next menstrual bleeding (day 28) (Buffenstein *et al.*, 1995). However, the majority of women neither experience consistent 28-day cycles nor ovulate on day 14. A large proportion of women experience a number of changes in their menstrual cycle patterns throughout reproductive life (Harlow and Ephross, 1995).

1.1.3: Variation in Cycle Duration

There are four major studies that have investigated the changes and variations in menstrual cycle duration using seminal menstrual diary studies. These include the observation of menstrual bleeding patterns in 2,316 women aged 15-44 y in the US and Canada (Chiazze *et al.*, 1968), 2,700 white US women aged 10-59 y (Treloar, 1967), the analysis of the menstrual records of 701 Japanese women aged 13-52 y (Matsumoto, 1962) and observations of 691 Swiss women aged 11-58 y (Vollman, 1977). These large studies detail the distribution of cycle duration by age using a cross-section of mean population cycle durations and variance in complete menstrual life (Harlow and Ephross, 1995).

The menstrual cycle has been reported in 95% of all cycles as having a duration of between 15-45 days with an average of 28.1 days; however only 77% of women average a 25-31 day cycle (Chiazze *et al.*, 1968). The data demonstrate that the degree of irregularity of a sample size of 2,316 women is such that only 13% of the cycles observed had a difference between the shortest and longest cycle of less than 6 days (Chiazze *et al.*, 1968). Investigations have shown that there is large variation in cycle duration for women during the first few years post menarche and women who are entering menopausal transition or menopause (Harlow and Ephross, 1995). This variation is categorized by a pattern of short and long intervals between menstrual bleeding (Treloar, 1967). The greatest average cycle duration is found in women aged 15-19 y, with a steady decline until women reach 40-44 y (Chiazze *et al.*, 1968). Between the ages of 20-40 y (mid-menstrual life), cycle duration becomes the most regular with the least variation between menstrual bleeding intervals (Treloar, 1967). These results indicate that there is a

large degree of variation in cycle duration and a 28 day menstrual cycle is not standard for all women at all ages.

1.1.4: Variation in Ovulation, Menstruation, Follicular and Luteal Phase Duration

The duration of menstrual bleeding has been reported to range from between 2 to 12 days, with 80% of bleeds lasting between 3 to 6 days (Matsumoto, 1962). It has been demonstrated that women aged 35 y plus bleed approximately half a day less than women aged 20-24 y and experience fewer menstrual bleeds of longer than 7 days (Matsumoto, 1962). Menstrual bleeds of longer than 8 days duration are more commonly seen in cycles that are anovulatory (Matsumoto, 1962). Anovulatory or anovulation is a term given when the successful development of a follicle and the stimulation of its release from the ovary into the fallopian tube as an ovum does not occur.

Ovulation plays an important role in the regulation of progesterone hormone; however ovulation does not occur in all menstrual cycles. The frequency of anovulatory cycles is associated with menstrual cycle duration. Short (16-20 days) and long (>45 days) cycles are up to 30% more likely to be anovulatory than cycles that are of 25-35 days in duration (Harlow and Ephross, 1995). Therefore the frequency of anovulation is greatest in women immediately post menarche (aged 12-17 y) and premenopausal (aged approximately >45 y). This has been demonstrated by a number of studies that have tested the frequency of ovulation in menstrual cycles through measurements of basal body temperature (BBT) and LH concentrations in urine (Matsumoto, 1962; Metcalf, 1983; Vollman, 1977). Furthermore, women aged 25-39 y, experience anovulation in approximately 2-7% of their cycles compared with 34% of cycles in women older than 50 y of age (Harlow and Ephross, 1995). This suggests that there is a direct association with variability in menstrual cycle duration, ovulation and age.

Follicular and luteal phase duration is estimated by identifying the day of ovulation and is measured through a number of different techniques including changes in BBT, cervical mucus and measurements of plasma LH (Buffenstein *et al.*, 1995). The duration of the follicular phase has been shown to range between

10 to 23 days (14 ± 1 day) with the luteal phase ranging from 8 to 17 days (14 ± 1 day) for women with normal menstrual cycle duration (Donaldson *et al.*, 2009). Women with longer follicular phase duration (mean = 17-18 days) frequently demonstrate longer total menstrual cycle duration, (Vollman, 1977). Mean follicular phase duration corresponds with mean cycle duration and age, as the standard mean of 14 days for women aged 18-24 y reduces to 10.5 days for women over the age of 45 y, correlating to a decline in total menstrual cycle duration (Bartoshuk, 1978).

1.1.5: Variation in Plasma Hormone Concentrations

Variations within plasma concentrations of both oestrogen and progesterone hormones are greatest within young women (below 20 y) and those over 40 y (Treloar, 1967). Observations of hormone concentrations have shown an association with women who have high oestrogen concentrations during the first 6 cycle days with a shorter menstruation and a subsequent follicular phase (Landgren *et al.*, 1980). The study also shows high LH concentrations before the LH peak are associated with both longer follicular phase and consequently longer overall menstrual cycle duration (Landgren *et al.*, 1980). The data suggests that if there are variations of high or low concentrations of both oestrogen and LH within a given menstrual cycle, these can affect cycle duration.

Progesterone plasma concentrations appear to be dependent on whether the cycle is ovulatory or anovulatory. A greater number of anovulatory cycles occur in women <20 y old, resulting in progesterone concentrations being comparatively lower than during an ovulatory cycle (Metcalf, 1983). Similarly, women aged 40-55 y are also found to experience a greater number of anovulatory cycles, reporting lower urine pregnanediol concentrations (an inactive breakdown product of progesterone) in comparison to cycles that were ovulatory (Harlow and Ephross, 1995; Metcalf and Livesey, 1985).

1.1.6: Summary

The current literature reports cycle duration and individual phase duration vary considerably between women, but indicate that the least variability is experienced in women between the ages of 20 – 40 y. As the typical '28 day cycle' may not occur in all reproductively aged women, this may result in studies reporting

flawed data if menstrual cycle phase is not assigned correctly and assumptions are made. This therefore could have significant implications for any research study investigating the effects of sex hormone concentrations during different phases of the menstrual cycle.

As such for the purposes of this thesis, the menstrual cycle is divided into three distinct phases: menstrual, follicular and luteal. The menstrual phase is defined as the first day of menstruation to the last day of menstrual bleeding. The follicular phase is defined as the days from the end of menstruation until ovulation. The day after ovulation until the onset of a new menstrual bleed defines the luteal phase (Buffenstein *et al.*, 1995). Phases will be defined through the measurement of both plasma oestrogen and progesterone and as a result will determine ovulatory cycles through the presence of elevated progesterone concentrations.

1.2: Glucose Homeostasis and Insulin Sensitivity

The prevalence of metabolic diseases such as obesity, dyslipidaemia and insulin resistance is steadily rising (Magliano *et al.*, 2008; Zimmet *et al.*, 2005) and these often lead to an increased risk in developing heart disease and type 2 diabetes (Grundy, 2008). The control of blood glucose homeostasis plays an essential role in type 2 diabetes and it is well established that insulin plays a pivotal role in its development and progression.

Blood glucose homeostasis is a carefully controlled interplay between a number of tissues including; brain, skeletal muscle, liver, adipose tissue and pancreas. Blood glucose concentrations are regulated by insulin and glucagon secretions, which target key tissues to maintain glucose homeostasis. During periods of increased plasma glucose following the consumption of a meal, insulin is secreted by the pancreas. This hormone regulates glucose uptake within the skeletal muscle and adipose tissue (Eckel *et al.*, 2005; Fritsche *et al.*, 2008), increases liver glycogen synthesis and inhibits gluconeogenesis and glycogenolysis (Lin and Sun, 2010). The majority of insulin stimulated glucose uptake (75%) occurs within the skeletal muscle tissues, with 10-20% occurring in adipose tissue (Kahn, 1996). Blood glucose concentrations rise (hyperglycaemia) following the consumption of a meal, causing pancreatic β -cell insulin secretions to increase insulin stimulated glucose uptake. Insulin resistance occurs when insulin secreted by the pancreas is unable to effectively facilitate insulin stimulated glucose uptake, resulting in a reduction in glucose uptake (Vranic, 1992). Pancreatic β -cell dysfunction can also be a cause of insulin resistance, as β -cell dysfunction reduces the quantity of insulin produced eventually causing inefficient insulin stimulated glucose uptake (Livingstone and Collison, 2002).

1.2.1: Insulin Stimulated Glucose Uptake Pathway

The process of glucose uptake into adipocytes, skeletal muscle cells or liver storage is mediated by insulin. Skeletal muscle insulin stimulated glucose uptake begins with insulin binding to its receptor on the cell membrane. The receptor has two extracellular α -subunits and two trans membrane β -subunits with tyrosine kinase activity. When insulin binds to the α -subunit, a trans phosphorylation of the β -subunits occurs. The activated receptor phosphorylates tyrosine residues on

a number of the intracellular signalling proteins, including insulin receptor substrate (IRS) 1-4. IRS proteins interact with the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3-K) enzyme leading to its full activation. This results in phospholipid phosphatidylinositol 4, 5 bisphosphate (PIP3) production which activates and localizes protein kinases such as pyruvate dehydrogenase kinase (PDK1) and subsequently initiates the activation of Protein Kinase B (Akt) (Chang *et al.*, 2004). The activation of PI3-K and Akt via the IRS signalling pathway is essential for insulin-stimulated glucose transporter (GLUT4) translocation (Saltiel and Kahn, 2001). This activation leads to the translocation of the GLUT 4, from the intracellular vesicles to the cell membrane. Once GLUT4 is present on the cell membrane, glucose can be transported into the cell by facilitated diffusion (Saltiel and Kahn, 2001) (Figure 1.3).

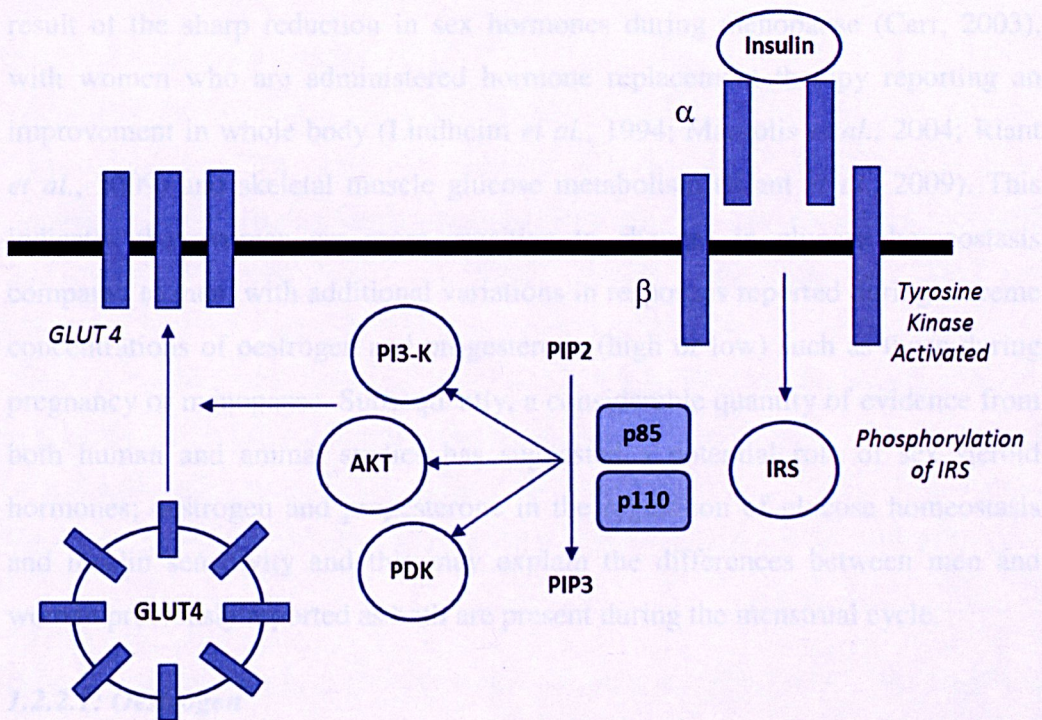


Figure 1.3: Insulin signalling pathway leading to glucose uptake in skeletal muscle. GLUT4: Glucose transporter, IRS: Insulin receptor substrate, PIP2/3: phospholipid phosphatidylinositol 4, 5 bisphosphate, PI3-K: Phosphatidylinositide 3-kinases, AKT: Protein Kinase B, PDK: Pyruvate dehydrogenase kinase

GLUT4 is predominantly expressed in skeletal muscle and adipose tissue and therefore represents a rate-limiting step in insulin-stimulated glucose uptake (Bjornholm and Zierath, 2005). In addition, over or under expression of this

protein can lead to insulin resistance and consequently impaired glucose homeostasis (Takeda *et al.*, 2003).

1.2.2: Glucose Homeostasis, Insulin Sensitivity and Sex Hormones

A number of recent clinical investigations have shown strong correlations between sex hormones and aspects of the metabolic syndrome, in particular, type 2 diabetes (Salpeter *et al.*, 2006). It has been reported that premenopausal women have an increased insulin sensitivity compared to aged-matched men (Carr, 2003). In addition, gestational diabetes development is often reported during human pregnancy when both oestrogen and progesterone are present in high concentrations (Kalkhoff *et al.*, 1970; Kirwan *et al.*, 2002; Nagira *et al.*, 2006). Significant increases in type 2 diabetes development are also reported in postmenopausal women compared to premenopausal women, potentially as a result of the sharp reduction in sex hormones during menopause (Carr, 2003), with women who are administered hormone replacement therapy reporting an improvement in whole body (Lindheim *et al.*, 1994; Margolis *et al.*, 2004; Riant *et al.*, 2009) and skeletal muscle glucose metabolism (Riant *et al.*, 2009). This indicates that women are more sensitive to changes in glucose homeostasis compared to men, with additional variations in responses reported during extreme concentrations of oestrogen and progesterone (high or low) such as those during pregnancy or menopause. Subsequently, a considerable quantity of evidence from both human and animal studies has suggested a potential role of sex steroid hormones; oestrogen and progesterone in the regulation of glucose homeostasis and insulin sensitivity and this may explain the differences between men and women previously reported as both are present during the menstrual cycle.

1.2.2.1: Oestrogen

There have been a number of studies that have investigated the potential role of oestrogen in glucose homeostasis and insulin sensitivity. Some of the clearest human evidence is reported in research that has implemented artificial concentrations of sex steroids. Postmenopausal women that are administered oestrogen replacement therapy have shown an improved glucose homeostasis (Lindheim *et al.*, 1994). Similarly, in rodent models, insulin sensitivity and glucose metabolism are significantly impaired following ovariectomy, with

oestrogen treatment reported to reverse the decrease in insulin sensitivity (Kumagai *et al.*, 1993).

However, the differences reported between studies may be due to the varying concentrations of oestrogens administered. Using a euglycemic hyperinsulinemic clamp technique it has been shown that oestrogen demonstrates a dose-dependent relationship to insulin sensitivity measures in rat. At low concentrations (0.003 - 0.006nM for 9 days), oestrogen displays no significant impairment of glucose or insulin, but that high concentrations of oestrogen (0.3 – 0.6 nM for 9 days) were associated with insulin resistance (Gonzalez *et al.*, 2002). It was hypothesised that this may be a result of a down-regulation of IRS-1 expression (Gonzalez *et al.*, 2003) and a decrease in the quantity of insulin receptors present in skeletal muscle tissues (Gonzalez *et al.*, 2002).

1.2.2.2: Oestrogen Receptors (ER)

Although the evidence to date suggests insulin sensitivity and glucose homeostasis are influenced by oestrogen is plentiful, the molecular basis is unclear, with very little information available. Oestrogen has two known receptors that regulate its physiological signalling; oestrogen receptor alpha (ER α) and oestrogen receptor beta (ER β). It has been suggested that both receptors play a significant role in the translocation of GLUT4 following insulin stimulation. ER are highly expressed in insulin-sensitive tissues (Baltgalvis *et al.*, 2010), located within white adipose tissue and skeletal muscle (Barros *et al.*, 2009). However the ER receptors exhibit differing roles in the regulation of glucose homeostasis.

1.2.2.3: ER α

ER α appears to display a positive effect on glucose homeostasis. ER α knockout (KO) mice have been associated with obesity and insulin resistance (Bryzgalova *et al.*, 2006; Heine *et al.*, 2000), with evidence reporting a decrease in glucose tolerance primarily caused by an impaired insulin action in skeletal muscle (Ribas *et al.*, 2010). It has been demonstrated that in the absence of ER α there is a significantly reduced glucose uptake in skeletal muscle (Bryzgalova *et al.*, 2006). In addition, *ob/ob* mice (obese mice) that were administered the ER α specific agonist propylpyrazoletriyl (Pazol *et al.*, 2004), demonstrated an improved glucose tolerance and insulin sensitivity (Lundholm *et al.*, 2008), by increasing

GLUT4 translocation to the cell membrane (Galluzzo *et al.*, 2009). In skeletal muscle cell culture models, ER α activation also displays an increase in glucose uptake within skeletal muscle as a result of insulin stimulation (Nagira *et al.*, 2006). An increase in ER α activation through an increase in oestrogen concentrations is reported to increase GLUT4 translocation and expression within both skeletal muscle and adipose tissue (Ropero *et al.*, 2008), with a decrease in GLUT4 expression reported in ER α -KO mice (Barros *et al.*, 2006b; Ribas *et al.*, 2010).

Similarly, ER α activation has also been reported to increase AMPK in skeletal muscle cells (D'eon *et al.*, 2008). These findings therefore suggest that ER α may act as a positive modulator of both AMPK activation and insulin signalling in skeletal muscle (Spangenburg *et al.*, 2012). AMPK is an essential regulator of malonyl-CoA formation as it inhibits the enzyme glycerol-3-phosphate acetyltransferase, which initiates acetyl-CoA carboxylase (ACC) (Steinberg *et al.*, 2006). AMPK also increases the activity of malonyl-CoA decarboxylase, which breaks down malonyl-CoA to acetyl-CoA (Steinberg *et al.*, 2006). In addition, the ability of ER α to regulate glucose uptake via GLUT4 may also be dependent on the specific fibre type, particularly as GLUT4 transcription has been reported to increase in extensor digitorum longus (EDL, fast-twitch) muscle fibres but not in the soleus (slow-twitch) (Gorres *et al.*, 2011).

1.2.2.4: ER β

In contrast, ER β displays a more suppressive role in GLUT4 gene expression (Barros *et al.*, 2006b). The suppressive role of ER β is supported in oestrogen deficient aromatase knockout (ArKO) mice that are treated with an ER β agonist, resulting in the decrease in GLUT4 gene expression within the muscle cells (Barros *et al.*, 2006a). However, its exact role in glucose homeostasis remains uncertain and controversial, particularly as ER β -KO mice at 3 months old display regular glucose homeostasis (Bryzgalova *et al.*, 2006), but at 8 month old ER β -KO mice present with hypoglycaemia and increased GLUT4 concentrations in skeletal muscle (Barros *et al.*, 2009).

1.2.2.5: *ER α and ER β*

It is clear from the evidence that both ER α and ER β demonstrate a complex interrelated relationship that varies between target tissues. ER β has also been shown to be a competitive inhibitor of ER α action, resulting in a decrease in glucose metabolism (Barros *et al.*, 2006b). Consequently the net effect of oestrogen may depend on the ratio or balance between these two receptors (Barros *et al.*, 2006b; Dieudonne *et al.*, 2004; Pedersen *et al.*, 2001).

There is currently little evidence investigating the ratio of both ERs between menstrual cycle phases, however high concentrations of oestrogen are reported to increase ER expression and as ER α is the most dominant ER in premenopausal women (Sakaguchi *et al.*, 2003), this receptor may be expressed in greater quantities during periods of high oestrogen concentrations such as the follicular phase. However there has been some research that reports no significant difference in ER α mRNA or ER β mRNA concentrations in adipose tissue between pre and postmenopausal women (Shin *et al.*, 2007). This suggests that systemic oestrogen concentrations may not be a significant determinant of ER ratio in adipose tissue (Shin *et al.*, 2007) and subsequently could possibly remain stable regardless of oestrogen fluctuations during the menstrual cycle.

However, ER β concentrations have been recorded to be significantly higher in women compared to men (Dieudonne *et al.*, 2004) and it has been noted those women with a greater ER β : ER α ratios also display the greatest degree of adiposity (Shin *et al.*, 2007). Therefore the distribution of adipose tissue may be of greater importance rather than the absolute oestrogen concentration to determine ER ratio. However the pathway regulating ER ratio in both adipose and skeletal muscle tissue is currently unknown and requires further investigation.

Overall, the evidence suggests oestrogen displays a positive effect on glucose response and insulin sensitivity, however hypo- or hyper oestrogen is associated with increased insulin resistance (Livingstone and Collison, 2002). In addition, the evidence suggests that the ability of oestrogens to mediate glucose uptake and insulin sensitivity may depend on the expression and activation patterns of both ERs (Spangenburg *et al.*, 2012).

1.2.2.6: Progesterone

The metabolic effects of progesterone are most clearly demonstrated during pregnancy, when amongst other hormones, it is secreted in high concentrations to maintain the gestational environment. It is well reported that during pregnancy, insulin sensitivity decreases and gestational diabetes can develop (Kirwan *et al.*, 2002; Nagira *et al.*, 2006), which would suggest that progesterone may play an important role in glucose homeostasis. Progesterone has also been implicated as a key modulating hormone in glucose homeostasis in women that are administered oral contraceptives. Exogenous progestin have been reported to reduce insulin sensitivity in female participants, whilst studies using combined hormonal treatments attribute progesterone as the dominant hormone responsible for the decreased insulin sensitivity (Diamond *et al.*, 1989; Spellacy, 1982; Spellacy *et al.*, 1990). Progesterone's main function is predominantly to maintain pregnancy through blocking myometrial muscle contractions. The withdrawal of progesterone initiates labour by transforming the myometrial muscle to the labour state (Mesiano *et al.*, 2010).

1.2.2.7: Progesterone Receptors

Progesterone receptors are expressed as two isoforms, PR-A and PR-B, which arise from a single gene (Kastner *et al.*, 1990). As a consequence of developmental (Shyamala *et al.*, 1990) and reproductive status (Duffy *et al.*, 1997) the ratio of both receptors varies in different reproductive tissues. PR-A and PR-B differ by the additional sequence of amino acids at the amino terminus of PR-B (Wen *et al.*, 1994).

Progesterone receptors regulate a diverse number of physiological functions, including cellular differentiation, embryonic development and physiological homeostasis (Mesiano *et al.*, 2010). Both PR have the ability to interact with a variety of co-activator, co-repressor proteins and co-regulators, this illustrates the role of these receptors in mediating different tissue-specific response of progesterone receptors to steroid ligands (Conneely *et al.*, 2002).

PR-A has been reported to repress the activity of PR-B, which can extend to other steroid receptors including ER α (Mcdonnell *et al.*, 1994). PR-A displays the ability to inhibit the transcriptional responses of both PR-B and ER α , which could result in a reduced response to progesterone in certain tissues. This contributes to the anti-oestrogenic activity of progesterone previously reported; however which tissue types are affected remains uncertain (Conneely *et al.*, 2002). Research has reported an increase in the concentration of progesterone receptors in endometrial tissue during the late follicular phase just before ovulation and a gradual fall in concentration by the late luteal phase just before menstruation (Ingamells *et al.*, 1996).

A small number of studies have attempted to investigate the potential role of progesterone in glucose homeostasis and to determine some its potential mechanisms. Wada *et al.* (2010) investigated the molecular mechanisms by which progesterone may affect insulin signalling pathways by measuring glucose uptake in adipocytes (3T3-L1) using a cell culture model (progesterone 10^{-8} – 10^{-4} mg/ml). The research indicated that progesterone inhibits GLUT4 translocation which reduces insulin-stimulated glucose uptake (Wada *et al.*, 2010). The authors suggest progesterone suppresses the PI3-kinase mediated pathway, by decreasing insulin-induced tyrosine phosphorylation of IRS-1 and therefore increasing IRS-1 degradation. These findings are of significant importance as IRS-1 degradation is one of the main mechanisms that can cause insulin resistance (Ricort *et al.*, 1995; Sasaoka *et al.*, 2005). In addition, IRS-1 association with PI3-K subunit p85 α was significantly reduced following progesterone administration, with insulin induced phosphorylation of Akt1 and Ak2 also reduced, causing a decrease in insulin-stimulated glucose uptake downstream of Akt. However, the results in this study were only reported with progesterone concentrations of 10^{-4} mg/ml, which is a concentration more likely to be reported during late pregnancy (Wada *et al.*, 2010), whereas concentrations during the luteal phase are approximately 10^{-7} mg/ml (Tulchins *et al.*, 1972). This suggests that progesterone may only affect insulin stimulated glucose uptake at high concentrations and may not cause any changes during lower progesterone concentration states such as those reported during different phases of the menstrual cycle.

However, Picard *et al.* (2002) also reports elevated progesterone concentrations in normal and diabetic *db/db* mice (leptin receptor deficient) are associated with hyperglycaemia and increased susceptibility to developing diabetes. The evidence also reported stimulation of pancreatic β -cell hyperplasia and suggests a significant role of progesterone in the cellular adaptations to hyperglycaemia (Picard *et al.*, 2002). The authors also explored the response in progesterone receptor knock-out (PR-KO) mice and reported blocking the PR resulted in an improved glucose tolerance due to an increase in insulin secretions. It is believed that the enhanced pancreatic function reported in these mice, may have been due to the number of β -cells present within the pancreatic islets, as islet cells in PR-KO mice are reported to have a twofold greater β -cell count (Picard *et al.*, 2002).

1.2.2.8: Oestrogen and Progesterone

Considering the research in human and animal studies for both oestrogen and progesterone, the findings suggest that rather than one individual hormone creating a significant impact on glucose homeostasis and insulin sensitivity, it may be more likely that both hormones play a significant role. Oestrogen appears to maintain insulin sensitivity at physiological concentrations, whereas at higher concentrations or in combination with progesterone, insulin sensitivity appears to be reduced, potentially as a result of a manipulation in insulin stimulated glucose uptake (Livingstone and Collison, 2002).

1.2.2.9: Rodent Studies

Research using ovariectomized mice has observed a decrease in glucose uptake, with oestrogen administration reversing this therefore resulting in a two fold increase in glucose uptake. However, when progesterone is administered in combination, this action is antagonized. The study also reports no significant differences in GLUT4 quantities at low oestrogen concentrations, but in supraphysiological doses, GLUT4 mRNA was significantly reduced in adipose tissue (Picard *et al.*, 2002). In similar studies, low concentrations of combined oestrogen and progesterone resulted in a decrease in GLUT4 mRNA in adipose tissue cells. However, the response was not significant in skeletal muscle and therefore the potential effects on overall insulin sensitivity and glucose homeostasis remain uncertain (Sugaya *et al.*, 2000).

1.2.2.10: Human Studies

Specific human studies investigating oestrogen and progesterone have included those administered with exogenous hormone concentrations. Studies administering combined hormonal contraceptives (50-500µg progesterone: 30-40 µg oestrogen) have reported significant decreases in insulin sensitivity (Godsland *et al.*, 1992), suggesting a combination of oestrogen and progesterone may result in significant disturbances in glucose homeostasis. The combined hormone therapies also significantly reduced sensitivity by 30-40%, regardless of the concentration administered (Godsland *et al.*, 1992). In addition, the assessment of glucose metabolism and insulin sensitivity during different phases of the menstrual cycle at the receptor and post-receptor level within adipose tissue has also been assessed (Marsden *et al.*, 1996). Subcutaneous adipose tissue biopsies were taken in female patients in either the follicular or luteal phase of the menstrual cycle. The study reports a significant reduction in insulin receptor binding in the luteal phase, however the results suggest a preservation of overall insulin action between the two phases (Marsden *et al.*, 1996). This suggests that both sex hormones (oestrogen and progesterone) present within the luteal phase may be responsible for changes in glucose homeostasis and insulin sensitivity, although the exact overall contribution is unclear as the effects in skeletal muscle were not assessed (Marsden *et al.*, 1996).

The current evidence appears to be uncertain as to which sex hormone may affect glucose homeostasis and insulin sensitivity. However, the evidence does suggest women may experience changes in insulin-stimulated glucose uptake as a result of the variations in sex hormone concentrations. These changes therefore could be experienced during the monthly variations in both oestrogen and progesterone concentrations reported during the menstrual cycle.

1.2.3: Glucose Homeostasis and Insulin Sensitivity during the Menstrual Cycle

Whilst there is good evidence to indicate that oestrogen and progesterone may affect glucose response (GR) and insulin sensitivity (IS) there is conflicting evidence on their effects during different phases of the menstrual cycle on GR and IS. Some studies suggest GR and IS decrease during the luteal phase of the

menstrual cycle (Diamond *et al.*, 1989; Escalante Pulido and Alpizar Salazar, 1999; Ezenwaka *et al.*, 1993; Goldner *et al.*, 2004; Gonzalez-Ortiz *et al.*, 1998; Jarrett and Graver, 1968; Marsden *et al.*, 1996; Peppler *et al.*, 1978; Pulido and Salazar, 1999; Trout *et al.*, 2007; Valdes and Elkind-Hirsch, 1991; Yeung *et al.*, 2010) (Table 1.2), with others reporting no significant difference across the menstrual cycle (Bingley *et al.*, 2008; Bonora *et al.*, 1987; Cudworth and Walker, 1975; Diamond *et al.*, 1993; Goldman and Eckerlin, 1970; Goldman *et al.*, 1968; Lundman *et al.*, 1994; Okey, 1925; Reid *et al.*, 1986; Reinke *et al.*, 1972; Spellacy, 1967; Spellacy *et al.*, 1990; Toth *et al.*, 1987; Ykijarvinen, 1984) (Table 1.3).

Valdes and Elkind-Hirsch (1991) measured IS in 8 women during the menstrual, follicular and midluteal phases of the menstrual cycle using the tolbutamide-modified intravenous glucose tolerance test (IVGTT). The results suggest a significant decrease in IS during the mid-luteal phases. Using the same method of IS analysis, Pulido and Salazar (1999) measured IS in both follicular (day 7-9) and luteal phase (day 22-24) in 12 Caucasian women. The results show significantly lower IS during the luteal phase compared to the follicular. This is also supported by a study conducted in 14 non-Caucasian women (Ezenwaka *et al.*, 1993). Similarly, a study conducted by Diamond *et al.* (1989) using a hyperglycaemic clamp test measured glucose uptake as an indication of IS during the menstrual cycle in both follicular (day 3-10) and luteal (day 20-25) phases. The results indicated a significant increase in glucose uptake during the follicular phase compared to the luteal. These conclusions are supported by Peppler *et al.* (1978) and Jarrett and Graver (1968) who both performed oral glucose tolerance tests (OGTT) during follicular and luteal phases of the menstrual cycle, using simple non-specific testing days to identify cycle phase. The studies report elevated fasting glucose concentrations and impaired glucose tolerance during the luteal phase. All of these studies determined menstrual cycle phase by approximating calendar day and using day 14 as a standard day to differentiate between cycle phases (follicular and luteal). It is therefore uncertain if these women were allocated the correct phase or tested during changes in hormone concentrations which may have had significant implications in the results reported.

Table 1.2: Glucose response and insulin sensitivity during the menstrual cycle in women - Significant reports

Study	Sample size	Days measured	Method	BBT	Hormones	Results
DePirro <i>et al.</i> (1978)	8	1 day weekly	¹²⁵ I-Insulin binding technique	N	N	Ire decreased during LP *
Diamond <i>et al.</i> (1989)	8	2 days; 3-10 & 20-25	2 HGC - CM	N	Y	Glucose uptake increased in FP *
Ezenwaka <i>et al.</i> (1993)	14	1 day FP / LP	1 IVGTT - IS	Y	N	IS decreased during LP *
Goldner <i>et al.</i> (2004)	4 T1D	Daily x 3 cycles	CGMS & 6 daily finger pricks. - CM	N	Y	Hyperglycaemia during LP * Large inter-cycle variability
Gonzalez-Ortiz <i>et al.</i> (1998)	6 6 T2D	2 days; 3-8 & 20-25	2 ITT	Y	Y	IS decreased in control * NS in T2D parent group
Jarrett (1968)	10	1 day weekly	3-4 OGTT - GT	Y	N	GT increased during FP *
Marsden <i>et al.</i> (1996)	23	1 day FP = 10 women 1 day LP = 14 women	Adipose tissue biopsy	N	Y	Ire decreased during LP * IS NS
Peppler <i>et al.</i> (1978)	192	1 day FP / LP	2 OGTT - GT	N	N	Glucose increased during LP *
Pulido and Salazar (1999)	12	2 days; 7-9 & 22-24	2 IVGTT - IS	N	Y	IS decreased in LP *
Trout <i>et al.</i> (2007)	5 T2D	2 days; 6-8 & 21-23	2 IVGTT - IS	N	Y	Fasting glucose increased during LP IS decreased during LP in 3/5 women
Valdes and Elkind-Hirsch (1991)	8	3; MP, FP, LP	3 IVGTT - IS	Y	Y	IS decreased in LP *
Yeung <i>et al.</i> (2010)	257	8 per cycle	FBG, FPI, HOMA-IR	N	Y	HOMA-IR increased in LP *

BBT - Basal Body Temperature, T1D - Type 1 Diabetic, T2D - Type 2 Diabetic, MP - Menstrual Phase, FP - Follicular Phase, LP - Luteal Phase, IVGTT - Intravenous Glucose Tolerance Test, OGTT - Oral Glucose Tolerance Test, HGC - Hyperglycaemic Clamp, CGMS - Continuous Glucose Monitoring System, ITT - Insulin Tolerance Test, Ire - Insulin Receptor, IS - Insulin Sensitivity, HOMA-IR - Homeostasis Measurement Of Insulin Resistance, CM - Carbohydrate Metabolism, GT - Glucose Tolerance, P - Progesterone, * - Significant Difference P<0.05, NS - Not Significant.

Table 1.3: Glucose response and insulin sensitivity during the menstrual cycle in women - Non significant reports

Study	Sample size	Days measured	Method	BBT	Hormones	Results
Bingley <i>et al.</i> (2008)	13	1 day FP / LP 1 repeat in 2 nd cycle	3 IVGTT - IS	N	P only	NS between phase and duplicate phase
Bonora <i>et al.</i> (1987)	110	1 day FP = 55 women 1 day LP = 55 women	1 OGTT - CM	N	N	NS in either fasting state or after OGTT between groups
Cudworth and Walker (1975)	20	3 days; 8-10, 17-19 & 26-28	3 OGTT - CM	N	N	NS Large inter- and intra-individual variation
Diamond <i>et al.</i> (1993)	5	1 day FP / LP	2 EGHI - IS	N	Y	NS
Goldman <i>et al.</i> (1968)	80	Daily	3 IVGTT	Y	N	NS
Goldman and Eckertlin (1970)	15	1 day FP / LP	2 IVGTT	Y	N	NS
Lundman <i>et al.</i> (1994)	20 20 T1D	Daily x 2 cycles	FPG / FPI	N	N	NS
Okey (1925)	26	1 day weekly	FBG	N	N	NS
Reid <i>et al.</i> (1986)	5 6 PMS	2 days FP / LP	4 OGTT	N	Y	NS
Reinke <i>et al.</i> (1972)	10	5 days; 7-9, 13,14,15 & 23-25	5 FBG	N	Y	NS
Spellacy (1967)	19	1 day FP / LP	2 IVGTT - CM	Y	N	NS

BBT - Basal Body Temperature, T1D - Type 1 Diabetic, T2D - Type 2 Diabetic, MP - Menstrual Phase, FP - Follicular Phase, LP - Luteal Phase, PMS - Premenstrual Syndrome, IVGTT - Intravenous Glucose Tolerance Test, OGTT - Oral Glucose Tolerance Test, HGC - Hyperglycaemic Clamp, EGHI - Euglycaemic Hyperinsulinaemic Clamps, IS - Insulin Sensitivity, FBG - Fasting Blood Glucose, FPI - Fasting Plasma Insulin, CM - Carbohydrate Metabolism, P - Progesterone, NS - Not Significant.

Table 1.3 continued: Glucose response and insulin sensitivity during the menstrual cycle in women - Non significant reports

Study	Sample size	Days measured	Method	BBT	Hormones	Results
Spellacy <i>et al.</i> (1990)	9 17 PMS	1 day FP / LP	2 OGTT - CM	Y	Y	NS within cycles in either group
Toth <i>et al.</i> (1987)	6	3 days: 1-6, 9-14 & 20-26 x 3 cycles	3 OGTT, IRe and EGHI GM & IS	N	N	NS CM or IS IRe decreased during luteal but NS
Yki-Jarvinen (1984)	7	1 day FP / LP	2 EGHI - IS	N	Y	NS

BBT - Basal Body Temperature, FP - Follicular Phase, LP - Luteal Phase, PMS - Premenstrual Syndrome, OGTT - Oral Glucose Tolerance Test, EGHI - Euglycaemic Hyperinsulinaemic Clamps, IRe - Insulin Receptor, IS - Insulin Sensitivity, CM - Carbohydrate Metabolism, GM - Glucose Metabolism, P - Progesterone, NS - Not Significant.

Fasting plasma glucose concentrations in addition to fasting insulin concentrations have also been measured to determine insulin resistance using HOMA-IR equations. A recent investigation was conducted in 257 women at 8 different time points throughout the menstrual cycle, with plasma oestradiol and progesterone measurements taken to confirm cycle phase. The results concluded HOMA-IR was significantly elevated during the luteal phase compared to the follicular. In addition, HOMA-IR and insulin were positively associated with both oestradiol and progesterone hormone concentrations (Yeung *et al.*, 2010), indicating a possible interaction between both sex hormones and glucose homeostasis. In contrast, measurements of fasting glucose concentrations have been reported as not significantly different between menstrual cycle phases in daily (Lundman *et al.*, 1994) and weekly sessions (Okey, 1925; Reinke *et al.*, 1972).

In addition, studies investigating the effects of IS and GR in women with diagnosed type 2 diabetes have also shown variations during different menstrual cycle phases. Participants were analysed using a continuous glucose monitoring system (CGMS) or frequently sampled IVGTT with measurements of oestrogen and progesterone concentrations taken in both phases. Both studies report hyperglycaemia and decreased IS during the luteal phase of the menstrual cycle (Goldner *et al.*, 2004; Trout *et al.*, 2007). This suggests that both oestrogen and progesterone may continue to impair insulin-stimulated glucose uptake even when insulin resistance has been diagnosed.

In contrast, several studies have also reported no significant differences in either GR or IS during the menstrual cycle. Yki-Jarvinen (1984) performed euglycemic hyperinsulinemic clamp tests during the follicular and luteal phases of the menstrual cycle in 7 women. The results show no significant difference in insulin mediated glucose metabolism between the two phases. Oestrogen and progesterone concentrations were recorded for testing sessions completed during the two menstrual cycle phases, but the results report no significant relationship between oestrogen or progesterone and glucose metabolism. Using the same testing method (clamp) Diamond *et al.* (1993) measured IS in 5 women during both follicular and luteal phases of the menstrual cycle. However endogenous sex hormones were not reported in this study, with phase definition occurring by

counting cycle days. The results indicate no significant difference in IS across the different phases of the menstrual cycle. Similar results were reported by Toth *et al.* (1987) in 6 women measured three times during the menstrual cycle for three complete cycles. No significant difference was found in IS and glucose metabolism during the menstrual cycle using both euglycemic clamp technique and an OGTT in this study.

In addition, Bonora *et al.* (1987), Reid *et al.* (1986) and Cudworth and Walker (1975) previously reported no significant difference in OGTT response during either phase of the menstrual cycle, with phase definition determined from the start of menstruation (day 1). The results also indicate a large inter and intra individual variation in GR and IS (Cudworth and Walker, 1975). A recent study by Bingley *et al.* (2008) performed IVGTT and reported progesterone concentrations during the follicular and luteal phases, with a repeat measurement during one phase in a second cycle. The results indicated no significant difference in IS between phases or duplicate phase in the 13 women analysed. These conclusions support earlier research studies using IVGTT method to determine IS during the menstrual cycle (Goldman and Eckerlin, 1970; Goldman *et al.*, 1968; Spellacy, 1967; Spellacy *et al.*, 1990). Spellacy *et al.* (1967) recorded BBT to distinguish menstrual cycle phases for IVGTT to be completed in 19 women. Blood glucose and insulin were measured and found to have no significant differences between phases. Goldman *et al.* (1968 and 1970) conducted monthly testing in 80 and 15 women respectively using control and progesterone therapy groups to determine the effects on IS and GR. Basal body temperature (BBT) was measured to determine ovulation and distinguish menstrual cycle phases. The results reported no significant difference between menstrual cycle phases in control or progesterone therapy testing groups. This suggests that neither oestrogen nor progesterone modulate glucose homeostasis and insulin sensitivity during the menstrual cycle as previously reported. However many of these studies differentiate menstrual cycle phases using BBT rather than measuring concentrations of sex hormones and may have resulted in women being assigned to the incorrect phase. This also prevents the results from establishing whether either or both sex hormones may affect glucose homeostasis and insulin sensitivity as they were not measured directly.

1.2.4: Summary

The current evidence on the potential role of oestrogen and progesterone in glucose homeostasis and insulin sensitivity during the menstrual cycle is inconsistent. There have been a number of proposed mechanistic pathways that have implicated both hormones may affect the control of glucose homeostasis and insulin sensitivity. Oestrogen has been reported to increase insulin sensitivity but this may be dependent on which receptor is stimulated ($ER\alpha$ or $ER\beta$) and the exact concentration of oestrogen present in the plasma. Research also suggests progesterone may act as the modulating hormone by significantly reducing the insulin signalling cascade and therefore preventing effective insulin-stimulated glucose uptake (Wada *et al.*, 2010). This could account for the significant changes in glucose homeostasis and insulin sensitivity previously reported during the luteal phase, as both hormones are present in high concentrations during this phase.

1.3: Energy Expenditure and Substrate Oxidation

Changes in energy expenditure, carbohydrate (CHO) and fat oxidation have significant health implications for women and in particular for the development of insulin resistance and type 2 diabetes (Cefalu, 2001). As the prevalence of metabolic diseases such as obesity and insulin resistance is steadily rising (Magliano *et al.*, 2008; Zimmet *et al.*, 2005) it is essential to determine the effects that sex hormones may have on these pathways.

1.3.1: Energy Expenditure

Energy expenditure comprises three main components: diet-induced thermogenesis (DIT), physical activity (PA) and the largest component (70%) basal metabolic rate (BMR): with BMR defined as the minimal activity of tissue cells under steady state conditions (Schofield, 1985). The main determinant of BMR is body weight and body composition and in general, men have a higher BMR than women as they tend to have a greater muscle mass.

BMR can be used to calculate total energy expenditure (TEE), through the Harris-Benedict principle which predicts TEE by multiplying BMR by physical activity level. However the validity of this method is based on the assumption that BMR remains largely stable (CV 2-4%) over time (Henry *et al.*, 1989). In addition, these observations have been largely made in men, with very little consideration for potential gender variations and no consideration for the potential influence of fluctuating sex hormones during the menstrual cycle.

1.3.2: Substrate Oxidation

The metabolism of CHO and fats as metabolic fuels during rest and aerobic exercise plays an important role in calculating energy expenditure. More specifically, changes in substrate oxidation (CHO and fat) can have significant health implications in the development of obesity and type 2 diabetes (Cefalu, 2001). By measuring oxygen consumption and carbon dioxide production through indirect calorimetry at rest (RMR) and during aerobic exercise, the ratio of CHO and fat oxidation can be calculated using a number of validated predictive equations (Jeukendrup and Wallis, 2005). CHO and fat are the main utilized sources of energy that enable the maintenance of a constant glucose supply to the brain during rest and enable efficient muscle contractions during exercise. The

requirement of these two energy sources significantly increases during exercise compared to resting states. The overall contribution of CHO and fat metabolism to total energy expenditure is tightly hormonally regulated, with the entire system promoting a glucose supply to the brain with a secondary response to supply the contracting muscles (D'eon *et al.*, 2002; Isacco *et al.*, 2012).

1.3.2.1: Carbohydrate Metabolism

CHO metabolism is initiated by the digestion and absorption of CHO within the small intestine. Following the breakdown of CHO to glucose, absorption of glucose occurs via an active transport mechanism. The sodium glucose transport protein (SGLT-1) actively transports two sodium ions and glucose molecules into the intestinal endothelium. Glucose then enters the blood circulation via the glucose transport protein GLUT 2 to complete absorption; GLUT 2 also regulates transport into, and out of, the liver. The process of glucose uptake into adipocytes, skeletal muscle cells or the liver is mediated by insulin (Figure 1.4).

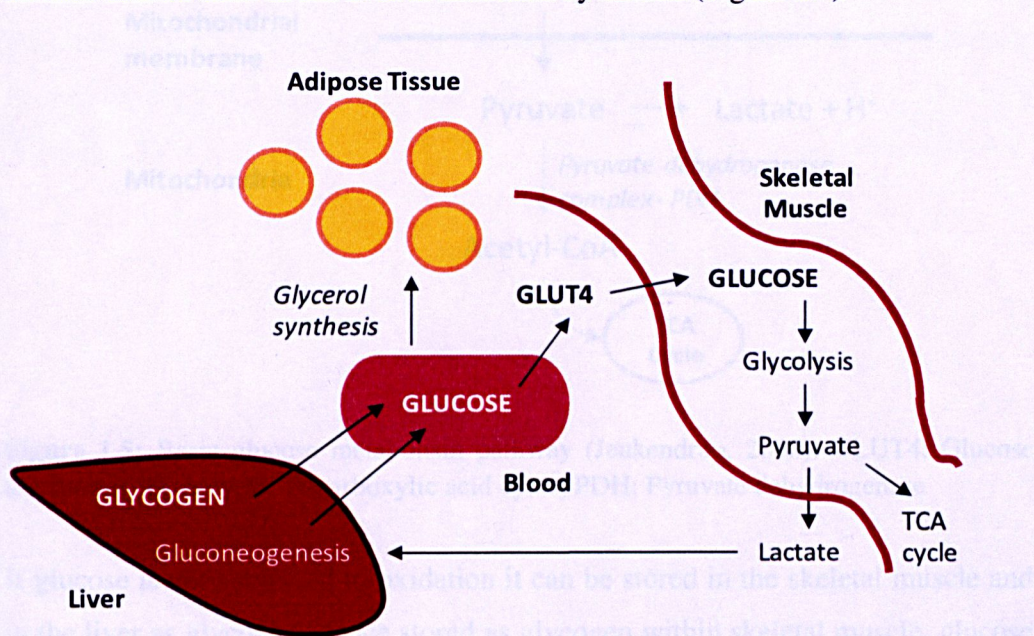


Figure 1.4: Schematic overview of the major carbohydrate metabolism pathway

Insulin stimulated glucose uptake in skeletal muscle begins with insulin binding to its receptor on the cell membrane. Specific details of this process are given in section 1.2. Approximately 75% of insulin stimulated glucose uptake occurs within skeletal muscle (Kahn, 1996) and therefore this will be discussed in the greatest detail. Once glucose enters the muscle cell it may be subject to further

breakdown within the cytosol via glycolysis, resulting in the formation of pyruvate (Figure 1.5). Pyruvate then enters the mitochondrion where it is then further broken down by pyruvate dehydrogenase (PDH) to form acetyl-CoA; this then enters the tricarboxylic acid cycle (TCA) for further breakdown and adenosine triphosphate (ATP) formation to provide energy.

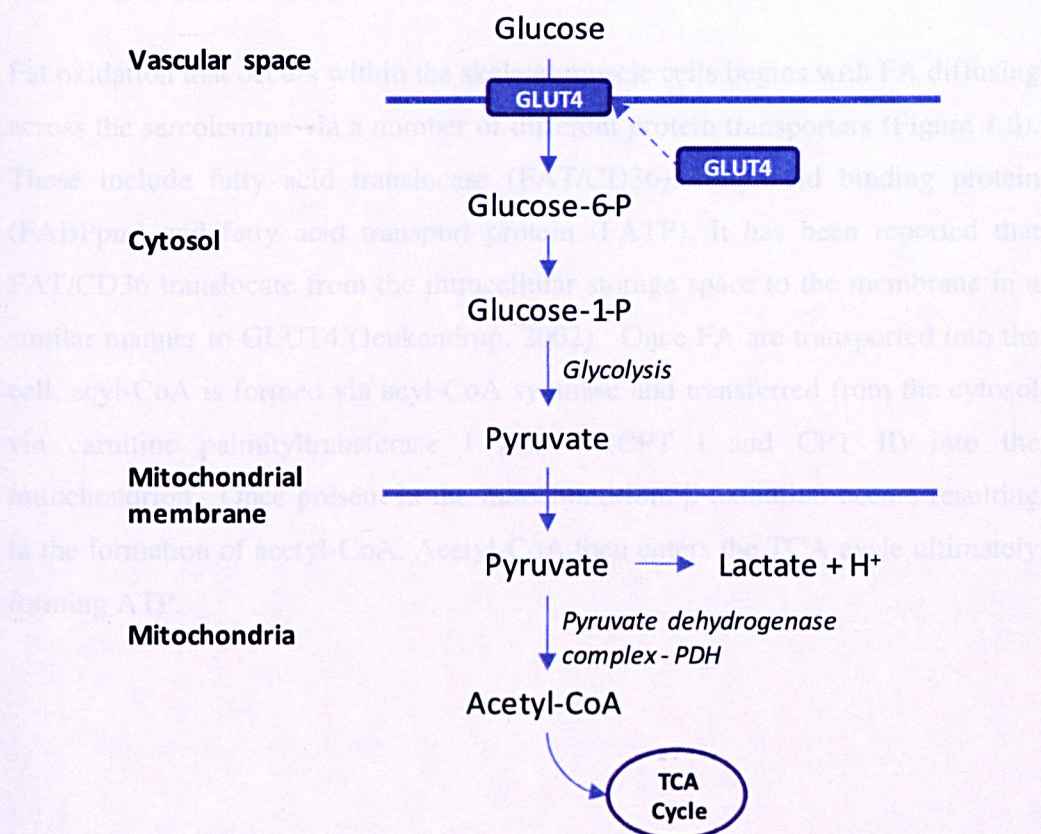


Figure 1.5: Basic glucose metabolism pathway (Jeukendrup, 2002). GLUT4: Glucose transporter, TCA cycle: Tricarboxylic acid cycle, PDH: Pyruvate dehydrogenase

If glucose is not subjected to oxidation it can be stored in the skeletal muscle and in the liver as glycogen. Once stored as glycogen within skeletal muscle, glucose can be utilized through the hydrolysis of glycogen and can be partially oxidized to pyruvate, which can subsequently enter the TCA cycle where it is fully oxidised or can be reduced to lactate. When lactate is formed, it may leave the skeletal muscle and can be either converted back to glucose via gluconeogenesis in the liver, be stored as glycogen, be used in lipogenesis or it may enter the mitochondrion and enter the TCA cycle. Stored glycogen within the liver can also be hydrolysed to provide a glucose supply to the brain and exercising muscles.

1.3.2.2: Fat Metabolism

Fat metabolism is initiated following the ingestion of fat molecules (lipids) in the stomach and small intestine. These are then absorbed into the bloodstream and form lipoproteins. Lipolysis is initiated when triglycerides are hydrolysed by lipoprotein lipase (LPL) and release fatty acids (FA) and glycerol; the FA can then be utilized in the skeletal muscle or stored once re-esterified.

Fat oxidation that occurs within the skeletal muscle cells begins with FA diffusing across the sarcolemma via a number of different protein transporters (Figure 1.6). These include fatty acid translocase (FAT/CD36), fatty acid binding protein (FABPpm) and fatty acid transport protein (FATP). It has been reported that FAT/CD36 translocate from the intracellular storage space to the membrane in a similar manner to GLUT4 (Jeukendrup, 2002). Once FA are transported into the cell, acyl-CoA is formed via acyl-CoA synthase and transferred from the cytosol via carnitine palmitoyltransferase 1 and 2 (CPT I and CPT II) into the mitochondrion. Once present in the mitochondrion, β -oxidation occurs resulting in the formation of acetyl-CoA. Acetyl-CoA then enters the TCA cycle ultimately forming ATP.

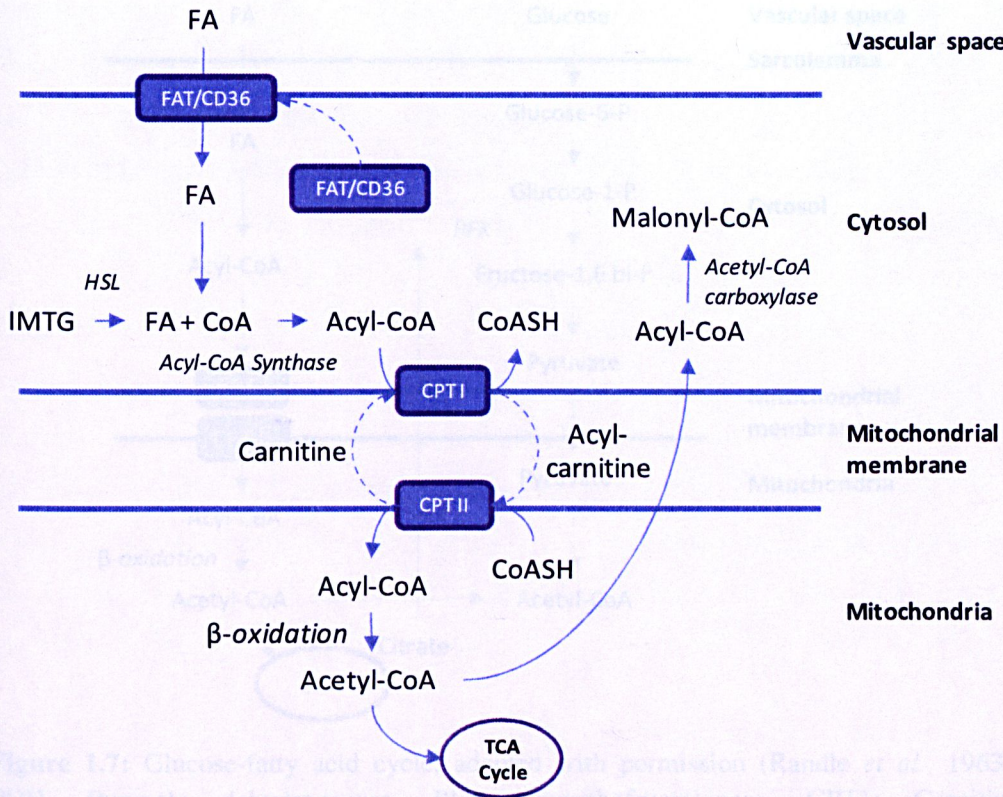


Figure 1.6: Basic fatty acid uptake pathway (Jeukendrup, 2002). FA: Fatty acids, FAT/CD36: Fatty Acid Translocase, IMTG: intramuscular triacylglycerol, HSL: Hormone sensitive lipase, CoA: Coenzyme A, CPT1: Carnitine Palmitoyltransferase I, TCA cycle: tricarboxylic acid cycle

1.3.3: Interaction between CHO and Fat Oxidation

1.3.3.1: Glucose-fatty acid cycle

The interaction between CHO and fat oxidation at rest has been previously partially explained by the glucose-fatty acid (GFA) cycle proposed by Randle *et al.* (1963). The theory states that an increase in FA plasma concentration results in an increase in FA uptake. This increase in FA uptake would result in an increase in β-oxidation in the mitochondria. Subsequently an accumulation of acetyl-CoA and citrate formation in the TCA cycle would occur. An increase in acetyl-CoA concentrations inhibits the breakdown of pyruvate via pyruvate dehydrogenase (PDH) inhibition. In addition, an increase in citrate could inhibit phosphofructokinase (PFK), which is a rate limiting step in glycolysis. This in turn increases glucose-6-phosphate accumulation which inhibits hexokinase (HK) and leads to a decrease in skeletal muscle glucose uptake (Figure 1.7).

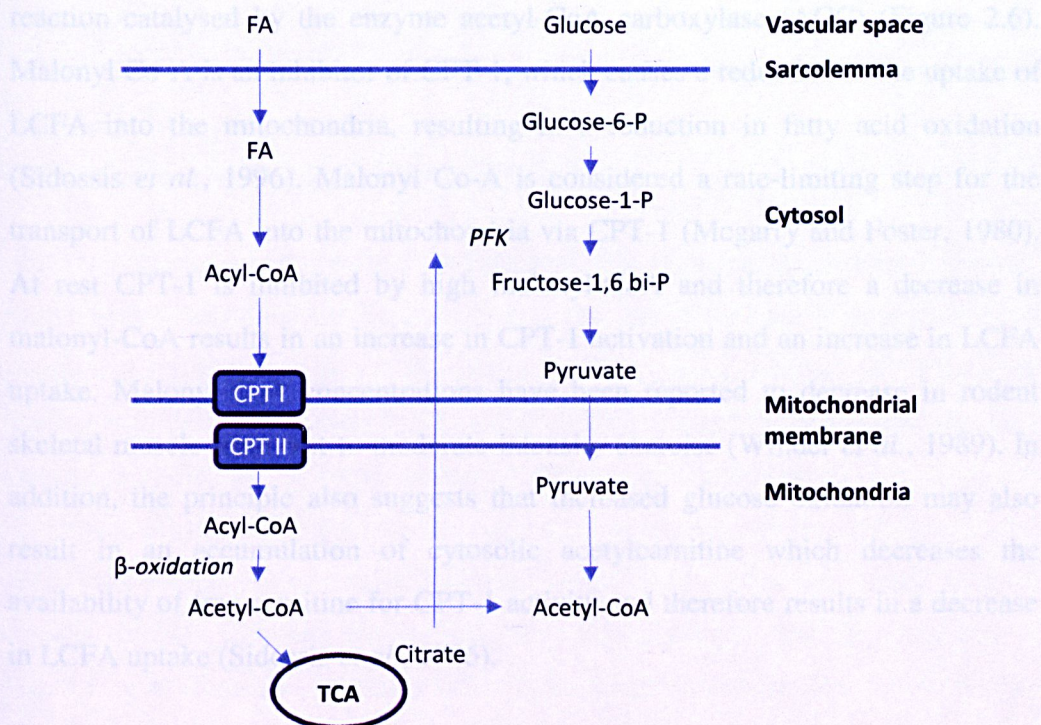


Figure 1.7: Glucose-fatty acid cycle, adapted with permission (Randle *et al.*, 1963). PDH: Pyruvate dehydrogenase, PFK: Phosphofructokinase, CPT1: Carnitine Palmitoyltransferase I, FA: Fatty acids, TCA cycle: Tricarboxylic acid cycle

However there is no evidence to suggest that this cycle occurs during exercise in skeletal muscle (Jeukendrup, 2002). There are a number of potential regulators of the interaction between CHO and fat oxidation and these include the transport of FAs from the vascular space across the sarcolemma into the cytosol via specific carrier proteins including FAT/CD36, FAMPpm and FATP. A second includes the hydrolysis of FAs from the intramuscular triacylglycerol (IMTG) by hormone sensitive lipase (HSL). IMTG stores have been located to the mitochondria as lipid droplets and are reported to reduce in size during exercise (Hoppeler *et al.*, 1985).

1.3.3.2: Reverse glucose-fatty acid cycle

More recently and universally accepted is the reverse GFA cycle (Sidossis *et al.*, 1996; Wolfe, 1998). As the name implies, this principle suggests that glucose and insulin regulate fatty acid oxidation and this occurs via a reduction in mitochondrial LCFA uptake (Sidossis *et al.*, 1996). An increase in glucose uptake, causes an increase in pyruvate availability, which results in the formation of excess malonyl Co-A. Malonyl-CoA is formed from acetyl-CoA which is a

reaction catalysed by the enzyme acetyl-CoA carboxylase (ACC) (Figure 2.6). Malonyl Co-A is an inhibitor of CPT-1, which causes a reduction in the uptake of LCFA into the mitochondria, resulting in a reduction in fatty acid oxidation (Sidossis *et al.*, 1996). Malonyl Co-A is considered a rate-limiting step for the transport of LCFA into the mitochondria via CPT-1 (Mcgarry and Foster, 1980). At rest CPT-1 is inhibited by high malonyl-CoA and therefore a decrease in malonyl-CoA results in an increase in CPT-1 activation and an increase in LCFA uptake. Malonyl-CoA concentrations have been reported to decrease in rodent skeletal muscle from rest to moderate-intensity exercise (Winder *et al.*, 1989). In addition, the principle also suggests that increased glucose oxidation may also result in an accumulation of cytosolic acetylcarnitine which decreases the availability of free carnitine for CPT-1 activity and therefore results in a decrease in LCFA uptake (Sidossis *et al.*, 1996).

However, the majority of the work to form this principle has been done in rodent studies or in vitro. The very limited human studies state that malonyl-CoA may not regulate fat oxidation at different exercise intensities (Odland *et al.*, 1996; Odland *et al.*, 1998) and that the availability of CHO may be more of an important factor determining the utilization of FA (Saha *et al.*, 1995).

1.3.4: Energy Expenditure, CHO Oxidation, Fat Oxidation and Sex

Hormones

A number of different factors have been previously reported to influence energy expenditure and substrate oxidation including; age, ethnicity, body composition (Arciero *et al.*, 1993; Henry and Rees, 1991; Poehlman, 1993; Poehlman *et al.*, 1993), exercise intensity (Goedecke *et al.*, 2000; Venables *et al.*, 2005), training status (Jeukendrup *et al.*, 1997), diet (Goedecke *et al.*, 2000) and hormonal status (Devries *et al.*, 2006). In particular, previous evidence has reported significant differences in energy expenditure and substrate oxidation between genders (Tarnopolsky, 2008). During exercise of the same relative intensity, men appear to increase CHO oxidation whereas women increase fat oxidation (Tarnopolsky *et al.*, 1990). Similar results have been reported in those performing moderate intensity exercise (65% $\text{VO}_{2\text{max}}$) and during high intensity exercise (80% $\text{VO}_{2\text{max}}$) (Froberg and Pedersen, 1984). In addition, men receiving oestrogen treatment

report an increase in fat and a decrease in CHO oxidation during exercise (Hamadeh *et al.*, 2005). Subsequently, it has been proposed that sex hormones, oestrogen and progesterone may play a significant role in determining the differences between genders (Tarnopolsky, 2008).

Rodent studies have supported the potential link between differences in energy expenditure, substrate oxidation, gender and sex hormones. Early research conducted by Hatta *et al.* (1988), on rats following an ovariectomy who were treated with daily oestrogen (2 µg), oestrogen (2 µg) and progesterone (2 mg) or a control vehicle for 8 days, indicated significant increases in fat oxidation following 60 minutes of exercise in the oestrogen only treated rats. Similarly, male rats treated with oestrogen display an increase in FA and triacylglycerol muscle content, showing lipid availability and glycogen sparing compared to control rats (Rooney *et al.*, 1993). Additional rodent studies have further supported the results and suggest that oestrogen only treatment in male rats causes an increase in fat and a decrease in CHO oxidation (Ellis *et al.*, 1994).

However, although sex hormones have been proposed to be responsible for the potential differences between genders, very little focus has been given to the possible effects of fluctuating oestrogen and progesterone concentrations during the menstrual cycle. Understanding the role that oestrogen and progesterone may have in manipulating the oxidation of both substrates is essential in order to determine the effects on total energy expenditure and the potential health consequences this may lead to in women.

There are a number of potential pathways which could lead to subsequent changes in energy expenditure as a result of fluctuations in oestrogen and progesterone concentrations during the menstrual cycle. As energy expenditure is by definition a representation of the amount of energy metabolised by the body, then the source of that utilized energy (CHO and fat) needs to also be investigated.

1.3.4.1: Fat Oxidation - Oestrogen

It has been recently proposed that fat oxidation is promoted through the presence of oestrogen receptor alpha (ER α) (Foryst-Ludwig and Kintscher, 2010). Stimulation of ERe has been reported to stimulate lipolysis, increase fat oxidation

and improve glucose tolerance and insulin sensitivity in both men and women (Foryst-Ludwig and Kintscher, 2010).

It is been reported that oestrogen via ER α may stimulate fat oxidation through a number of different pathways. Oestrogen administration has been reported to increase AMP-activated kinase (AMPK) activation 5-fold in ovariectomized mice compared to placebo controlled (D'eon *et al.*, 2005). AMPK is an essential regulator of malonyl-CoA; it inhibits the enzyme glycerol-3-phosphate acyltransferase, which initiates ACC (Steinberg *et al.*, 2006). Malonyl-CoA is an inhibitor of CPT-1 and therefore reduces the ability for LCFA to enter the mitochondrion. AMPK also increases the activity of malonyl-CoA decarboxylase, which breaks down malonyl-CoA to acetyl-CoA (Steinberg *et al.*, 2006). Furthermore, a decrease in Carnitine Palmityltransferase 1 (CPT 1) and β - Hydroxyacyl-CoA Dehydrogenase (β -HAD) activity has been reported in female rats following ovariectomy (Campbell *et al.*, 2001). The evidence reports a restoration of CPT-1 and β -HAD enzyme activity following oestrogen only treatment which was not replicated during the progesterone only treatment. As a result this suggests oestrogen promotes LCFA uptake into the skeletal muscle resulting in an increase in β -oxidation and the formation of acetyl-CoA, which could lead to a decrease in glucose uptake (Figure 1.8).

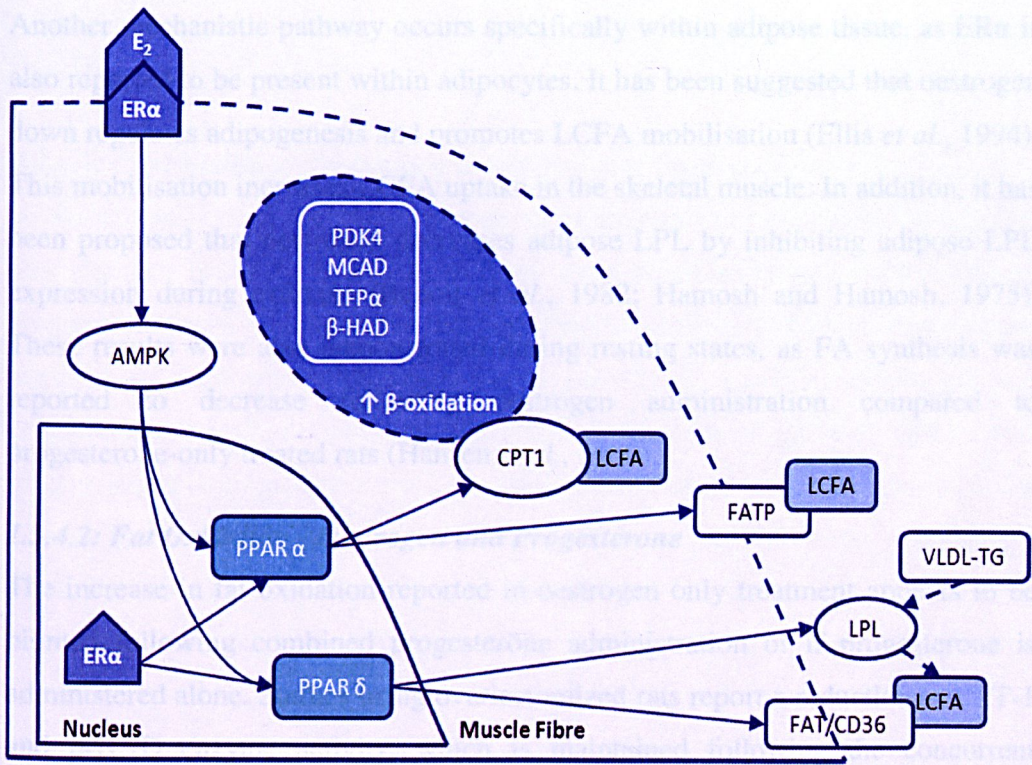


Figure 1.8: Proposed metabolic pathway of oestrogen to increase fat oxidation (Oosthuysen and Bosch, 2012). PPAR: peroxisome proliferation activator receptor, ER: Oestrogen Receptor, E₂: Oestrogen, FAT/CD36: Fatty Acid Translocase, FATP: Fatty Acid Transport Protein, LPL: Lipoprotein Lipase, LCFA: Long Chain Fatty Acid, VLDL-TG: Very Low Density Lipoprotein Triacylglycerol, AMPK: AMP-Activated Kinase, PDK4: Pyruvate Dehydrogenase Kinase 4, MCAD: Medium Chain Fat Oxidative Dehydrogenases, TFPα: Trifunctional Protein, β-HAD: β -Hydroxyacyl-CoA Dehydrogenase, CPT 1: Carnitine Palmitoyltransferase 1.

In addition, oestrogen has also been reported to up regulate peroxisome proliferation activator receptors α and δ (PPAR) (D'eon *et al.*, 2005) (Figure 1.8). This group of nuclear hormone receptors stimulates gene expression of the transport protein and enzymes (FAT/CD36, FABPpm and FATP) that are essential for long-chain fatty acid (LCFA) uptake. PPAR α and δ receptors directly increase the expression of a number of key β -oxidation enzymes (medium chain fat oxidative dehydrogenases MCAD; trifunctional protein TFP α and β -HAD) and are inhibitors of pyruvate dehydrogenase kinase 4 (PDK4), which leads to an increase in fat oxidation.

Another mechanistic pathway occurs specifically within adipose tissue, as ER α is also reported to be present within adipocytes. It has been suggested that oestrogen down regulates adipogenesis and promotes LCFA mobilisation (Ellis *et al.*, 1994). This mobilisation increases LCFA uptake in the skeletal muscle. In addition, it has been proposed that oestrogen decreases adipose LPL by inhibiting adipose LPL expression during exercise (Benoit *et al.*, 1982; Hamosh and Hamosh, 1975). These results were also demonstrated during resting states, as FA synthesis was reported to decrease following oestrogen administration compared to progesterone-only treated rats (Hansen *et al.*, 1980).

1.3.4.2: Fat Oxidation - Oestrogen and Progesterone

The increase in fat oxidation reported in oestrogen only treatment appears to be blunted following combined progesterone administration or if progesterone is administered alone. Studies using ovariectomized rats report a reduction in CPT-1 and β -HAD enzyme activity, which is maintained following the concurrent administration of both oestrogen and progesterone (Campbell and Febbraio, 2001). However, oestrogen only administration restores effective CPT-1 and β -HAD enzyme activity. This demonstrates that oestrogen alone has a potentially positive effect on fat oxidation whereas in combination with progesterone it may exhibit an inhibitory effect (D'eon *et al.*, 2005; Hatta *et al.*, 1988).

In addition, oestrogen only administration has been reported to increase PPAR α and δ in skeletal muscle (Campbell *et al.*, 2003). Rodent studies observing ovariectomized rats infused with oestrogen, oestrogen and progesterone and progesterone only hormone concentrations reports an increase in PPAR expression in oestrogen only rats, with both progesterone only and oestrogen and progesterone infused rats displaying a blunted PPAR expression (Campbell *et al.*, 2003).

1.3.4.3: Fat Oxidation - Summary

Overall, the present evidence suggests oestrogen alone promotes fat oxidation through a potential increase in AMPK activation and PPAR α and δ up regulation. The evidence also suggests that this activation is not reported in progesterone only or oestrogen and progesterone treatment. This suggests that an increase in fat

oxidation may only be observed in oestrogen only situations such as during the follicular phase.

1.3.4.4: Carbohydrate Oxidation – Oestrogen and Progesterone

In addition to its link with fat oxidation, there has been some evidence to suggest that changes in CHO oxidation may be as a result of more specific steroid mediated changes in glucose uptake and insulin sensitivity. Changes in glucose homeostasis have been reported to be influenced by both oestrogen and progesterone hormones (section 1.2), with a decrease in glucose uptake and insulin sensitivity reported during the luteal phase when both sex hormones are present (Elkind-Hirsch *et al.*, 1986; Ezenwaka *et al.*, 1993; Jarrett and Graver, 1968; Peppler *et al.*, 1978; Pulido and Salazar, 1999; Valdes and Elkind-Hirsch, 1991). In particular, oestrogen has been promoted as displaying a beneficial effect on glucose homeostasis but with an increased risk of insulin resistance development following hypo- or hyper oestrogen concentrations (Livingstone and Collison, 2002). Progesterone on the other hand has been reported to decrease glucose homeostasis, with further studies suggesting that progesterone can cause a reduction in glucose homeostasis via a decrease in GLUT4 mRNA expression in adipose tissue (Sugaya *et al.*, 2000).

As a result, a decrease in CHO oxidation may be due to subsequent changes in glucose availability that have been reported as a result of changes in sex hormone concentrations. A reduction in glucose uptake in muscle and adipose tissue could result in decrease in glucose availability for oxidation and subsequently would increase the requirement for alternative energy sources. As changes in glucose homeostasis have been reported during the luteal phase it is possible that a combination of oestrogen and progesterone hormones could cause a significant change in substrate oxidation and potentially overall energy expenditure.

1.3.5: Current Literature Investigating Energy Expenditure and Substrate Oxidation at Rest

Studies measuring energy expenditure at rest during the menstrual cycle are often measures of basal metabolic rate (BMR) or resting metabolic rate (RMR) where either one representing the largest component of total energy expenditure. Early research examined the variability in energy expenditure during the menstrual

cycle with little appreciation for changes that may occur at times other than during the menstrual phase. Research studies conducted in the 1920s (Blunt, 1921; Snell, 1920; Wakeham, 1923; Wiltshire, 1921) reveal the controversy over whether measurements of basal metabolism vary periodically in conjunction with the menstrual cycle (Table 1.4). Investigations did report differences in RMR and BMR between different stages of the menstrual cycle (Hitchcock, 1929; Snell, 1920; Wakeham, 1923), with a significant rise in metabolism found 7-10 days before menstruation (luteal phase) and a decrease during the early follicular phase (Snell, 1920; Wakeham, 1923). This cyclic pattern was also displayed with a 5-10% rise in BMR being recorded in the week preceding menstrual flow (luteal phase) (Hitchcock, 1929). Research also conducted in this decade demonstrated that inter-menstrual values vary considerably between subjects, but variations between phases of the menstrual cycle were often too small to be considered significant (Blunt, 1921; Wiltshire, 1921).

One of the most comprehensive studies since the 1920s investigating changes in BMR during the menstrual cycle was by Solomon *et al.* (1982). Dietary intake and physical activity were maintained during a single cycle and BBT was measured to indicate time of ovulation to distinguish menstrual phases. The results demonstrated a decrease in BMR during menstruation, reaching its lowest point during the follicular phase, followed by a rise just before the beginning of the next menstrual cycle (luteal phase). It was concluded that BMR varied significantly within the menstrual cycle in five of the six women tested.

Table 1.4: Energy expenditure and substrate oxidation during the menstrual cycle at rest

Study	Sample size	Days measured	Method	BBT	Hormones	Results
Blunt (1921)	14	MP, FP, LP	Benedict portable respiration apparatus	N	N	1.6% lower BMR during MP - NS
Snell (1920)	10	1	Respiratory gas collection	N	N	BMR decreased during FP BMR increased during LP
Wiltshire (1921)	5	MP, FP, OP, LP	Respiratory gas collection	N	N	NS in BMR
Wakeham (1923)	24	1 x weekly; 6 weeks	Benedict portable respiration apparatus	N	N	BMR decreased during FP BMR increased during LP
Hitchcock (1929)	20	2/3 x weekly; 3 cycles	Benedict portable respiration apparatus	Y	N	BMR increased during LP (5-10%)
Solomon <i>et al.</i> (1982)	6	92 days; daily x 1 week, every other day there after	Open circuit respiration chamber. Dietary intake manipulated	Y	N	BMR decreased during FP BMR increased during LP
Webb (1986)	10 1 Control	1 x 9-14 day; 9 weeks	36hr direct calorimetry Respiratory gas collection Dietary intake standardised	N	P only	24hr EE - decreased during FP, increased during LP (9%)
Bisdee <i>et al.</i> (1989b) (1989a)	8	1x weekly; 6 weeks; 36hr per visit	Metabolic-ward, whole body indirect calorimetry. Dietary intake standardised	Y	LH only	24hr EE - decreased during FP, increased during LP (2.5%) SMR - decreased during FP, increased during LP (6.1%) CHO oxidation decreased during FP
Meijer <i>et al.</i> (1992)	16	1 day FP / LP	Respiration calorimetry chamber	N	N	SMR increased during LP (7.7%)

BBT - Basal body temperature, BMR - Basal metabolic rate, 24hr EE - Twenty-four hour energy expenditure, SMR - Sleeping metabolic rate, RMR - Resting metabolic rate, MP - Menstrual Phase, FP - Follicular phase, LP - Luteal phase, P - Progesterone, * - Significant difference P<0.05, NS - Not significant.

Table 1.4 continued: Energy expenditure and substrate oxidation during the menstrual cycle at rest

Study	Sample size	Days measured	Method	BBT	Hormones	Results
Howe <i>et al.</i> (1993)	14 (REE) 12 (24hr EE)	MP, FP, LP	REE – Respiratory gas collection 24hr EE – Respiration calorimetry chamber	N	Y	REE – NS; 24hr EE - decreased during FP; SMR - decreased during FP, increased during LP
Westrate (1993)	23	2 days FP / LP	Respiratory gas collection	N	N	NS in RMR or substrate oxidation
Piers <i>et al.</i> (1995)	13	2 days FP / LP	Respiratory gas collection Dietary intake standardised	N	Y	NS in RMR or substrate oxidation Protein oxidation decreased in FP
Toth <i>et al.</i> (1999)	59	45 women FP 14 women LP	Respiratory gas collection Dietary intake standardised	N	Y	NS in RMR or substrate oxidation No sex hormone correlations
Matsuo <i>et al.</i> (1998)	9	1 day FP / LP	Respiratory gas collection	N	Y	RMR increased during LP Fat oxidation increased during LP
Curtis <i>et al.</i> (1996)	12	Daily; 5 weeks	Respiratory gas collection	N	N	BMR decreased during FP Intra-individual variation (up to 12%)
Matsuo <i>et al.</i> (1999)	7	1 day FP / LP	Respiratory gas collection	Y	N	RMR increased during LP Fat oxidation increased during LP
Horton <i>et al.</i> (2002)	13	MP, FP, LP	RMR Respiratory gas collection	Y	N	RMR increased during LP (NS)
Henry <i>et al.</i> (2003)	19	3 x weekly	Respiratory gas collection	N	N	NS in RMR Intra-individual variation in 9/19 (5-10%)
Vaiksaar <i>et al.</i> (2011)	11	1 day FP / LP	RMR Respiratory gas collection	N	Y	NS in substrate oxidation at rest or exercise

BBT – Basal body temperature, BMR – Basal metabolic rate, 24hr EE – Twenty-four hour energy expenditure, SMR – Sleeping metabolic rate, RMR – Resting metabolic rate, MP – Menstrual Phase, FP – Follicular Phase, LP – Luteal phase, * – Significant difference P<0.05, NS – Not significant.

Significant increases in metabolic rate during the luteal phase are also reported from studies examining BMR and SMR (sleeping metabolic rate). Bisdee *et al.* (1989b) investigated 24 hour energy expenditure using SMR and BMR measurements in 8 women. Ovulation was predicted from measurements of BBT with women pre-tested for 9-13 weeks to establish average cycle duration. The results for 24 hour energy expenditure indicated a decrease during the late follicular phase (just before ovulation) followed by a rise during the late luteal phase (Bisdee *et al.*, 1989b). A follow on study by the same author indicated that in addition to a decrease in energy expenditure during the follicular phase, CHO oxidation was also lower during this phase (Bisdee *et al.*, 1989a). These results are supported by research conducted on 16 women measuring SMR by indirect calorimetry (Meijer *et al.*, 1992). Participants had SMR measurements taken continuously between 3am and 6am whilst sleeping in a respiratory chamber. The results confirm SMR was higher during the luteal phase compared to the follicular phase (Meijer *et al.*, 1992).

These significant results were also confirmed by Webb (1986) who observed a 9% increase in 24 hour energy expenditure during the luteal phase of the menstrual cycle but with no details reported for substrate oxidation, however strict methodological conditions were applied to this study which may have amplified the observed results (Webb, 1986). In addition, Curtis *et al.* (1996) examined BMR variation daily in 12 women for 5 weeks. The results indicated BMR was lower during the follicular phase compared to the luteal phase, displaying a biphasic pattern similarly reported in the previous research. However the intra-individual variation measured by coefficient of variance (CV) exhibited a greater variation in six of the twelve women tested (<12%) with a CV in the other six women similar to that found in men (2-4%). This suggests that the biological constant of BMR is not necessarily true for all women as it is in men and the authors elucidated that this may be attributed to the fluctuations in sex hormones oestrogen and progesterone experienced during the menstrual cycle (Curtis *et al.*, 1996).

In contrast, further literature indicates no significant difference in metabolic rate and energy expenditure during the menstrual cycle phases (Howe *et al.*, 1993;

Piers *et al.*, 1995; Weststrate, 1993). RMR measurements were examined on three separate occasions during the menstrual cycle in 14 regularly menstruating women. BBT was measured to estimate ovulation, with testing days adjusted for total cycle duration. Oestrogen and progesterone concentrations were also measured during each testing session to confirm cycle phase. The results indicate no significant difference in RMR during the menstrual cycle (Howe *et al.*, 1993). These results are supported by research using 14 non-Caucasian female participants measuring RMR twice during both follicular (day 6-10) and luteal (day 21-25) phases of the menstrual cycle, with measurements of oestrogen and progesterone concentrations also reported (Piers *et al.*, 1995). The results suggest no significant difference in RMR or substrate oxidation rates between the two phases (Piers *et al.*, 1995). These findings also confirm previous research conducted on 23 women investigating RMR and DIT changes during the menstrual cycle (Weststrate, 1993). Testing days were defined from estimated cycle phase duration, reporting measurements made pre and post predicted ovulation. The results indicate no significant difference in either RMR or DIT during the menstrual cycle, with no significant changes in CHO or fat oxidation (Weststrate, 1993).

Furthermore, a recent study conducted by Henry *et al.* (2003) recruited 19 women, measuring ovulation to define menstrual cycle phases. The results found no biphasic pattern in RMR during the menstrual cycle. However, the results do report large intra-individual variation in RMR measurements, with 9 participants displaying a CV greater than 5% and 10 women exhibiting similar variations to that found in men (2-4%). This intra-individual variation repeats previous data reported (Curtis *et al.*, 1996), suggesting individual variation may have an important influence on individual energy requirements that are based on BMR values. This may explain why there is some controversy between the results as changes in energy expenditure may occur in some women but not in others.

The current evidence investigating energy expenditure and substrate oxidation during different phases of the menstrual cycle reports inconsistent findings. A significant proportion of the evidence suggests RMR increases during the luteal phase when both oestrogen and progesterone concentrations are elevated with a

reduction in CHO oxidation reported. However there is also a proportion of the evidence that suggests there is no significant difference between menstrual cycle phases. The inconsistent findings at rest may be due to the changes in energy expenditure and substrate oxidation being too small to be detected using small participant numbers and therefore more consistent data may be reported with a larger sample size or during exercise, as the specific pathways controlling energy expenditure and substrate oxidation may be up regulated.

1.3.6: Current Literature Investigating Energy Expenditure and Substrate Oxidation during Exercise

Previous evidence has reported a significant difference in energy expenditure and substrate oxidation between genders during exercise (Tarnopolsky, 2008). In particular, men display an increase in CHO oxidation, whereas females display an increase in fat oxidation when relative exercise intensity is matched (Tarnopolsky *et al.*, 1990), indicating sex hormones may play a significant role. Rodent studies support this link, with evidence suggesting that oestrogen only treatment results in an increase in fat and a decrease CHO oxidation during exercise (Hatta *et al.*, 1988).

Previous studies investigating the effects of sex hormones during the menstrual cycle on exercising women have reported conflicting evidence when comparing menstrual cycle phases (Table 1.5). Hackney *et al.* (1994) reported significantly lower CHO oxidation during the luteal phase following exercise at 35% and 60% VO_2 max. Similarly, Went *et al.* (1997) report significant decreases in CHO oxidation during the luteal phase at 30% and 50% VO_2 max, with significant increases in fat oxidation also reported at 65% and 70% VO_2 max (Hackney, 1999; Hackney *et al.*, 2000). High oestrogen concentrations have also been reported to increase glycogen sparing compared to low oestrogen concentrations (Hackney, 1990). Similarly, two studies have reported a decrease in blood glucose rate appearance (Ra) and disappearance (Rd) during the luteal phase following moderate-intensity (50% VO_2 max) exercise (Campbell *et al.*, 2001; Zderic *et al.*, 2001). This corresponds to a decrease in CHO oxidation indicating that both hormones may affect substrate oxidation.

However, there are a number of other studies that report no significant differences in fat or CHO oxidation following exercise during different phases of the menstrual cycle (Braun *et al.*, 2000; Desouza *et al.*, 1990; Galliven *et al.*, 1997; Horton *et al.*, 2002; Mclay *et al.*, 2007; Suh *et al.*, 2002, 2003; Vaiksaar *et al.*, 2011). A study conducted in 13 women, reports no significant differences in CHO or fat oxidation rates during any phase of the menstrual cycle following a 90-minute exercise test at an intensity of 50% VO_2 max (Horton *et al.*, 2002). This is supported by a recent study conducted in 9 women who performed an intermittent cycling test followed by a 16km time-trial during the follicular and luteal phases of the menstrual cycle (Mclay *et al.*, 2007). The tests were preceded by either a 3 day normal diet (5.2g/kg/day) or a 3 day CHO loading diet (8.4g/kg/day). The results indicated no change in time-trial performance and no significant difference in substrate oxidation during either menstrual cycle phase (Mclay *et al.*, 2007). Similarly at increased exercise intensities (70% VO_2 max), 11 female rowers performed rowing ergometer exercise during follicular and luteal phases. The study reports no significant differences in substrate oxidation, resting respiratory exchange ratio (RER) or RER after exercise during either menstrual cycle phase (Vaiksaar *et al.*, 2011). However, at these exercise intensities, fat oxidation is minimal, with CHO oxidation being the main energy source. Therefore the contribution of fat oxidation at high exercise intensities would always be reduced due to the exercise intensity rather than any potential hormonal or phase specific changes.

In summary, the majority of studies indicate that fat oxidation increases and CHO oxidation decreases during the luteal phase of the menstrual cycle following exercise at various intensities. As both oestrogen and progesterone are present in this phase it could indicate that both hormones may be responsible for some of the changes reported. However the current evidence is inconsistent and subject to methodological inconsistencies particularly when exercising at high intensities, which could account for some of the variation reported and therefore requires further investigation

Table 1.5: Energy expenditure and substrate oxidation during exercise within different phases of the menstrual cycle

Study	Sample size	Days measured	Method	BBT	Hormones	Results
Desouza <i>et al.</i> (1990)	8 amenorrhea 8 eumenorrhea	FP, LP and amenorrhea	80% VO ₂ max and maximal run	N	LH only	NS differences in substrate oxidation based on RER CHO oxidation decreased during LP at 30% and 60% Fat oxidation increased during LP at 30% and 60%
Hackney <i>et al.</i> (1994)	9	1 day FP / LP	35%, 60% and 75% VO ₂ max: treadmill	-	-	NS in RER at 75%, NS at 70%: Significant changes at 30% and 50% Increased Fat oxidation during LP Decreased CHO oxidation during LP
Wenz <i>et al.</i> (1997)	9	1 day FP / LP	30%, 50%, 70% VO ₂ max: cycling 3 day low CHO and high CHO diet	-	-	Increased FA concentrations Decreased Ra and Rd with E2 NS in substrate oxidation
Ruby <i>et al.</i> (1997)	6 amenorrhea	MP, FP, LP	65% VO ₂ max and maximal run: treadmill	N	Y	NS in substrate oxidation
Galliven <i>et al.</i> (1997)	8	FP, OP, LP	70% VO ₂ max	N	Y	NS in substrate oxidation
Hackney <i>et al.</i> (1999)	8	1 day FP / LP	70% VO ₂ max: cycling Muscle biopsy	Y	Y	Increased muscle glycogen during LP Increased Fat oxidation during LP
Matsuo <i>et al.</i> (1999)	7	1 day FP / LP	60% VO ₂ max: cycling	Y	N	Fat oxidation increased during LP

MP – Menstrual Phase, FP – Follicular Phase, OP – Ovulatory Phase, LP – Luteal Phase, LH – Luteinising Hormone, Ra – Glucose Appearance Rate, Rd – Glucose Disappearance Rate, RER – Respiratory Exchange Ratio, CHO – Carbohydrate, FA – Fatty Acid, E2 – Oestradiol, * – Significant Difference
P<0.05, NS – Not Significant

Table 1.5 continued: Energy expenditure and substrate oxidation in exercise during the menstrual cycle

Study	Sample size	Days measured	Method	BBT	Hormones	Results
Braun <i>et al.</i> (2000)	16	1 day FP / LP	50% VO ₂ max	N	Y	NS in glucose Ra and Rd NS in substrate oxidation
Hackney <i>et al.</i> (2000)	8 4 anovulatory	1 day MP / LP	65% VO ₂ max: treadmill	N	Y	NS in EE Increased Fat oxidation during LP Decreased CHO oxidation during LP
Zderic <i>et al.</i> (2001)	6	1 day FP / LP	70% and 90% Lactate threshold: cycle	Y	Y	90%: Decreased Ra and Ra during LP Increased Fat oxidation during LP Decreased CHO oxidation during LP
Horton <i>et al.</i> (2002)	13	MP, FP, LP	50% VO ₂ max: cycle	Y	N	NS in substrate oxidation NS in Ra and Rd
Suh <i>et al.</i> (2002)	8	1 day FP / LP	45% and 65% VO ₂ max: cycle	N	Y	NS in Ra and Rd Decreased Ra and Rd during LP
Suh <i>et al.</i> (2003)	8 OC	1 day FP / LP	45% and 65% VO ₂ max: cycle	N	Y	NS in substrate oxidation during exercise
Devries <i>et al.</i> (2006)	13 6 OC	1 day FP / LP	65% VO ₂ max: cycle Muscle biopsy	N	Y	Decreased Ra and Rd during LP

MP – Menstrual Phase, FP – Follicular Phase, LP – Luteal Phase, OC – Oral Contraceptive, Ra – Glucose Appearance Rate, Rd – Glucose Disappearance Rate, RER – Respiratory Exchange Ratio, CHO – Carbohydrate, FA – Fatty Acid, E2 – Oestradiol, EE – Energy Expenditure, * – Significant Difference
P<0.05, NS – Not Significant

Table 1.5 continued: Energy expenditure and substrate oxidation in exercise during the menstrual cycle

Study	Sample size	Days measured	Method	BBT	Hormones	Results
Mclay <i>et al.</i> (2007)	9	1 day FP / LP	45% to 75% VO ₂ max: cycle 16km Time-Trial 3 day CHO loading or normal diet	Y	Y	NS in Time Trial performance NS in substrate oxidation
Fu <i>et al.</i> (2009)	12	1 day FP / LP	65% VO ₂ max: cycling Muscle biopsy	N	Y	Increased mRNA for Fat oxidation genes in LP
Vaiksaar <i>et al.</i> (2011)	11	1 day FP / LP	70% VO ₂ max: rowing	N	Y	NS in substrate oxidation

FP – Follicular Phase, LP – Luteal Phase, CHO – Carbohydrate, mRNA – Messenger Ribonucleic Acid, * – Significant Difference P<0.05, NS – Not Significant

1.3.7: Summary

Previous research investigating energy expenditure and substrate oxidation during rest and exercise is conflicting and inconsistent. Studies investigating resting measures report an increase in RMR during the luteal phase, with some evidence indicating a decrease in CHO oxidation. During exercise, current evidence reports a decrease in CHO oxidation and an increase in fat oxidation during the luteal phase when oestrogen and progesterone concentrations are elevated.

Mechanistic evidence suggests that this may be due to oestrogen receptors (ER β) located in the skeletal muscle and adipose tissue which directly affect glucose uptake by reducing GLUT4 expression and translocation. With oestrogen also reported to promote fat oxidation through a potential increase in AMPK activation and PPAR α and δ up regulation. In addition, the reported interaction between both fat and CHO oxidation has provided evidence for an increased FA availability and uptake which results in a decrease in glucose uptake as a result of oestrogen stimulation. However the limited evidence also suggests that progesterone may blunt or antagonise the effects of oestrogen but the exact mechanisms or pathways remain unclear.

The differences reported between resting and exercising measures may be due to the up regulation of CHO and fat oxidation pathways as a result of performing exercise and therefore the potential changes may be detected more efficiently and possibly reduce any potential experimental noise. In addition, the number of oestrogen receptors ER α and ER β present in skeletal muscle has been reported to increase with the level of endurance training (Wiik *et al.*, 2005); subsequently a greater oestrogen stimulated AMPK response during exercise may explain the differences reported between rest and exercise.

Overall, the evidence suggests that sex hormones may elicit changes in energy expenditure and substrate oxidation during rest and exercise throughout different phases of the menstrual cycle. However the exact mechanistic pathways involved remain unclear and require further investigation.

1.4: Dietary Intake during the Menstrual Cycle with Special Reference to Premenstrual Syndrome

Whilst the previous sections suggest there may be an effect of the sex hormones on energy expenditure and thus potentially weight during the cycle, there are other factors that need to be considered. The regulation of human appetite plays a pivotal role in the complex nature of human energy balance and body weight stability. With the current national statistics reporting 57% of women in England are now classified as overweight or obese (Department of Health, 2004), understanding the exact reasons for this is now more essential than ever.

1.4.1: Energy Regulation

Human energy regulation is centrally controlled by the hypothalamus with a balanced interaction between two sets of neurons occurring within the arcuate nucleus of the hypothalamus. The activation of neuropeptide Y (NPY) and Agouti related protein (AgRP) neurons promotes food intake, whereas pro-opiomelanocortin (POMC) displays an anorexigenic effect (Pelletier *et al.*, 2007; Sainsbury *et al.*, 2002). The major peripheral organs include the stomach, intestines, liver, pancreas, skeletal muscle and adipose tissue (Figure 1.9). These organs secrete a variety of hormones in response to short term dietary intake, including many gastrointestinal hormones (e.g. ghrelin, cholecystikinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY), insulin and leptin).

Following a reduction in blood glucose concentration, the stomach and duodenum secrete the hormone ghrelin known as the 'hunger hormone' to facilitate an eating episode. After the consumption of a meal (following liquid or solid food ingestion), food is broken down into its basic components. The presence of nutrients within the small intestine results in the release of a number of gastrointestinal hormones responsible for appetite regulation in order to terminate the eating episode and stimulate satiety (Feinle *et al.*, 2003). These include GLP-1 which is derived from pre-proglucagon and secreted upon food ingestion by the proximal gastrointestinal tract. PYY 3-36 and CCK are also secreted by the gastrointestinal tract and display an anorexigenic effect, inducing satiety. Ghrelin is secreted from the stomach and duodenum and also decreases after feeding stops.

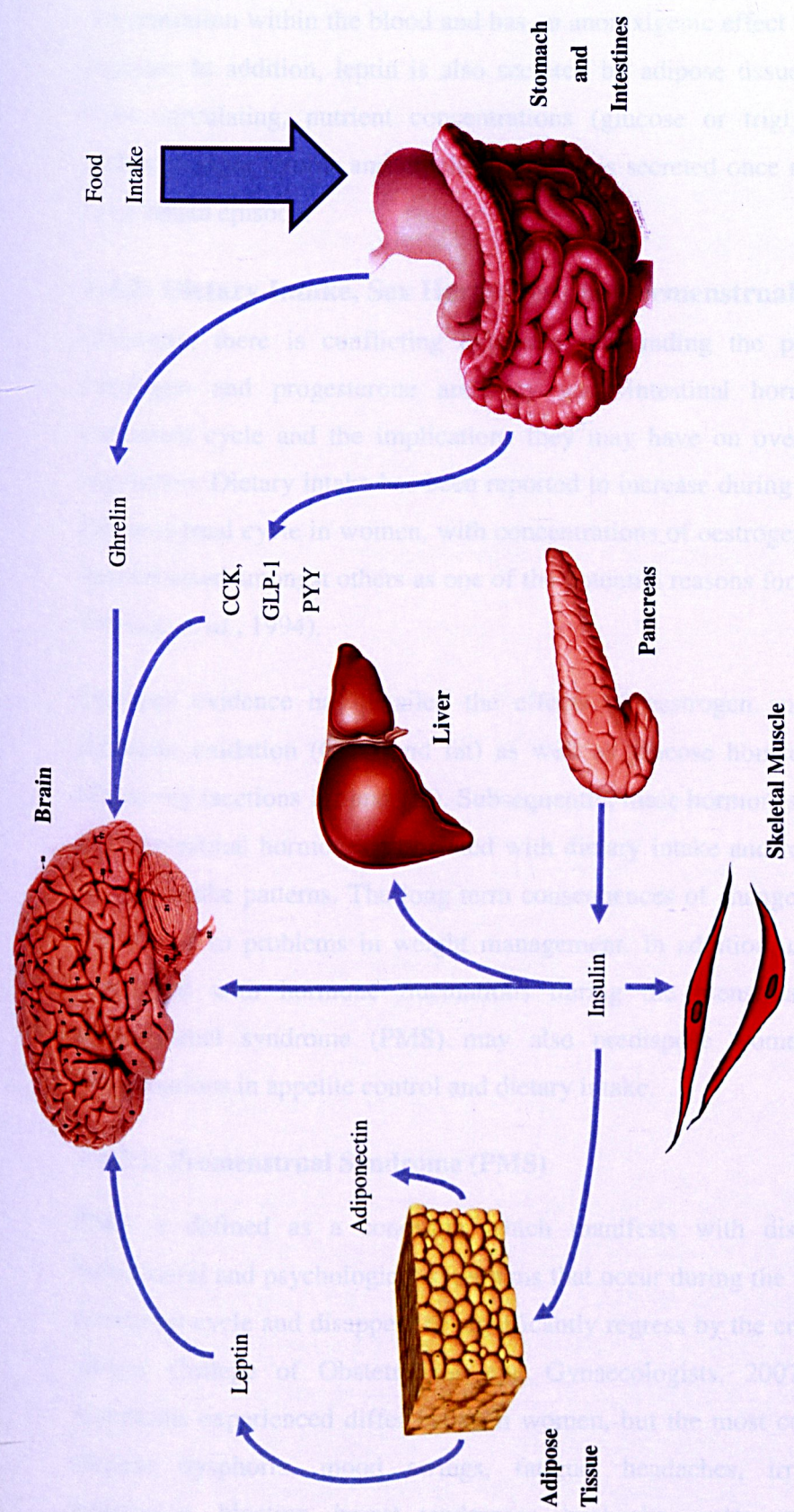


Figure 1.9: Schematic diagram of the energy regulation pathways and main organs and hormones involved. CCK: Cholecystokinin, GLP-1: Glucagon-like peptide-1, PYY: peptide YY.

Insulin is secreted by the pancreas in response to an increased glucose concentration within the blood and has an anorexigenic effect through the arcuate nucleus. In addition, leptin is also secreted by adipose tissue to induce satiety. Once circulating, nutrient concentrations (glucose or triglycerides) begin to reduce, hunger returns and ghrelin hormone is secreted once more to stimulate a food intake episode.

1.4.2: Dietary Intake, Sex Hormones and Premenstrual Syndrome

Currently, there is conflicting evidence surrounding the potential effects of oestrogen and progesterone and key gastrointestinal hormones during the menstrual cycle and the implications they may have on overall human energy regulation. Dietary intake has been reported to increase during the luteal phase of the menstrual cycle in women, with concentrations of oestrogen and progesterone hypothesised amongst others as one of the potential reasons for this (Dalvit, 1981; Johnson *et al.*, 1994).

Previous evidence has detailed the effects of oestrogen and progesterone in substrate oxidation (CHO and fat) as well as glucose homeostasis and insulin sensitivity (sections 1.2 and 1.3). Subsequently, these hormones may also alter the gastrointestinal hormones associated with dietary intake and result in changes in dietary intake patterns. The long term consequences of changes in dietary intake could lead to problems in weight management. In addition, conditions that are associated with hormone fluctuations during the menstrual cycle, such as premenstrual syndrome (PMS) may also predispose women to changes or exacerbations in appetite control and dietary intake.

1.4.2.1: Premenstrual Syndrome (PMS)

PMS is defined as a condition which manifests with distressing physical, behavioural and psychological symptoms that occur during the luteal phase of the menstrual cycle and disappear or significantly regress by the end of menstruation (Royal College of Obstetricians and Gynaecologists, 2007). The types of symptoms experienced differ between women, but the most common symptoms include dysphoria, mood swings, fatigue, headaches, irritability, tension, aggression, bloating, breast tenderness, backache, water retention and food

cravings (American College of Obstetricians & Gynecologists, 2000). A diagnosis of PMS is given to women who suffer from at least 5 symptoms during the luteal phase that subsides by the end of menstruation (Freeman, 2003; Royal College of Obstetricians and Gynaecologists, 2007).

The prevalence of PMS ranges between 75-85% of women of reproductive age (Halbreich *et al.*, 2007) and is more prevalent in women who are obese, exercise little and are of lower socioeconomic status (Royal College of Obstetricians and Gynaecologists, 2007). The symptoms have high adverse effects on quality of life (QoL) (Yonkers *et al.*, 2008). The UK Royal College of Obstetricians and Gynecologists (2007) has defined the different types of PMS based on the symptom severity experienced and their effects on daily activity (Table 1.6).

Table 1.6: UK Royal College of Obstetricians and Gynecologists (RCOG) definitions of PMS

Type	Definition
PMS	PMS symptoms experienced before menstruation and are completely relieved by the end of menstruation
Mild	Does not interfere with personal/social and professional life
Moderate	Interferes with personal/social and professional life but still able to function and interact
Severe	Unable to interact personally/socially/professionally – withdraws from social and professional activities

PMS symptoms occur during the luteal phase of the menstrual cycle when increased oestrogen and progesterone concentrations are present and when progesterone peaks in concentration (Stoddard *et al.*, 2007). However the exact aetiology of PMS remains uncertain and multifactorial, with much of the evidence suggesting women with PMS display a sensitivity to normal fluctuations in oestrogen and progesterone during the menstrual cycle (American College of Obstetricians & Gynecologists, 2000). This is particularly evidenced by women who use hormonal contraception or experience anovulatory cycles as they report fewer PMS symptoms (Freeman, 1997; Shangold *et al.*, 1990). The cyclical fluctuations in sex hormone concentrations may additionally alter brain

neurotransmitters that affect the hypothalamus. This has been reported to cause abnormal serotonin function which is associated with depressive symptoms (Frackiewicz and Shiovitz, 2001; Meltzer, 1989; Rapkin *et al.*, 1987).

As changes in dietary intake and food cravings are implicated in the aetiology and symptoms of PMS (American College of Obstetricians & Gynecologists, 2000), there is evidence to suggest that dietary manipulations may assist as potential treatments to relieve symptoms, which in turn could impact on the health and well-being of women (Johnson *et al.*, 1995). Given the high prevalence of women suffering from PMS symptoms (Halbreich *et al.*, 2007) it is likely that studies investigating dietary intake during the menstrual cycle did not screen for PMS symptoms and therefore may have included women that suffer from the syndrome. Consequently, it is essential to understand the potential influence oestrogen and progesterone may have on dietary intake, and to take into account the potential effect of conditions such as PMS.

One of the most common PMS symptom reported is food cravings or changes in dietary intake and this is potentially in response to the increased sensitivity to the effects of oestrogen and progesterone during the luteal phase on insulin stimulated glucose uptake and metabolism. However there is contradictory evidence regarding the differences in quantitative hormonal concentrations between PMS and non-PMS women. Some evidence reports a significant increase in both oestrogen and progesterone concentrations in PMS women compared to those who do not suffer with PMS (Reid and Yen, 1981). However, other previous evidence indicates that the absolute concentrations of sex hormones may not differ and that women who suffer from PMS may be more susceptible to changes in hormone concentrations and their proposed metabolic effects (Schmidt *et al.*, 1988). It would therefore suggest that both oestrogen and progesterone may play a pivotal role in energy intake during different phases of the menstrual cycle in both PMS and non-PMS suffering women.

1.4.3: The Effects of Sex Hormones on Energy Regulation

There are a number of potential pathways by which oestrogen and progesterone may act to influence dietary intake; in particular, there is evidence to suggest sex

hormone receptors are located within areas of the brain highly activated during dietary intake (McEwen *et al.*, 1997). Evidence has also suggested a possible interaction between gastrointestinal hormones secreted during energy regulation and sex hormones which may have significant implications in energy intake.

1.4.3.1: Hypothalamus

NPY and AgRP mRNA expressed in the hypothalamus are heavily implicated in the development of obesity. It has been suggested that the reduction in oestrogen concentrations as a result of ovariectomy could be responsible for the increased expression of NPY and AgRP mRNA within the hypothalamus previously reported (Clegg *et al.*, 2007; Pelletier *et al.*, 2007), which has resulted in an increase in food intake and a decrease in physical activity (Sainsbury *et al.*, 2002). Similar results in menopausal women report a significant reduction in 24 hour energy expenditure and physical activity following the reduction in oestrogen hormone concentrations after menopause (Lovejoy *et al.*, 2008). This indicates that a reduction in oestrogen concentrations may result in an increased food intake, which over prolonged periods of time could lead to weight gain.

1.4.3.2: Leptin

Leptin, resistin and adiponectin are among several adipokines that are produced within adipose tissue and are known to play a role in the control of dietary intake (Lago *et al.*, 2007; Meier and Gressner, 2004; Mitchell *et al.*, 2005). Leptin is an appetite-suppressing hormone secreted by adipose tissue and has been reported as displaying an anorexigenic (fasting) effect on energy intake, but leptin is unable to prevent overconsumption in the short-term (Mcneil and Doucet, 2012). The concentration of leptin present in plasma is proportional to the quantity of body fat mass (Murphy and Bloom, 2004). It has been reported that women of reproductive age have higher concentrations of leptin compared to men, even after adjusting for body fat (Rosenbaum *et al.*, 1996). Leptin plays a pivotal role in the onset of menses during puberty and is required to maintain effective reproductive function throughout life (Goumenou *et al.*, 2003). It has been suggested that plasma concentrations of leptin between 1.2ng/ml - 1.85ng/ml are required to stimulate FSH and LH production and therefore stimulate effective ovulatory cycles (Welt *et al.*, 2004).

In addition to its requirement for effective ovulatory cycles, there have been a number of studies that report variation in plasma leptin concentrations during different phases of the menstrual cycle. A comprehensive study by Riad-Gabriel *et al.* (1998) was conducted in 9 women. The results demonstrated fasting leptin concentrations were significantly elevated during the luteal phase of the menstrual cycle (14.9 ng/ml menstrual phase vs. 20.4 ng/ml luteal phase). This evidence is supported by similar research conducted in 18 women in whom fasting blood samples were taken on days 3, 10, 17 and 24 of the menstrual cycle. The study reports a significant increase in leptin (35%) during the luteal phase compared with the menstrual (11.2 ng/ml vs. 15.12 ng/ml respectively) (Mannucci *et al.*, 1998). Additionally, some studies have reported between 35-60% increases in leptin concentrations during the luteal phase compared to the early follicular phase (Al-Harithy *et al.*, 2006; Hardie *et al.*, 1997; Thong *et al.*, 2000).

However, there are a number of other studies that report no significant difference in plasma leptin concentrations across different menstrual cycle phases (Capobianco *et al.*, 2010; Mills *et al.*, 1998; Teirmaa *et al.*, 1998). It has been suggested that the number of discrepancies within the data could be due to the frequency in which leptin measures are taken during studies (Mcneil and Doucet, 2012), with a significant difference in leptin concentrations reported between phases in studies taking 4 or more samples compared to non-significant data in studies recording leptin concentrations less frequently.

It has been reported that the expression of leptin receptors (*leprb*) present within the ARC area of the brain is colocalized with $ER\alpha$ (Diano *et al.*, 1998), with the presence of oestrogens within the ARC causing a down-regulation of *leprb* mRNA expression (Bennett *et al.*, 1999). This indicates a closely coupled interaction between these two receptors. In addition, rodent models report heightened central leptin sensitivity during increased oestrogen concentrations (Ainslie *et al.*, 2001; Clegg *et al.*, 2006; Clegg *et al.*, 2003), with increased leptin concentrations also reported following oestrogen hormone therapy (Dedeoglu *et al.*, 2009). However, others have reported no significant changes in leptin concentrations following hormone therapy (Castracane *et al.*, 1998). This indicates that during periods of elevated oestrogen (such as the follicular phase);

leprb may be more sensitive even if this does not affect overall plasma leptin concentrations. This suggests that leptin may play an important role in the regulation of gonadotropin and ovarian steroidogenesis during the menstrual cycle as well as potentially causing changes in dietary intake (Asimakopoulos *et al.*, 2009).

1.4.3.3: Resistin and Adiponectin

A recent study has investigated the role of two other known adipokines, resistin and adiponectin, which may also have a significant role in dietary intake changes during the menstrual cycle. Sixteen healthy women were recruited to determine fasting plasma concentrations of leptin, resistin and adiponectin during one menstrual cycle. The results demonstrated leptin concentrations were significantly elevated during the luteal phase supporting previous research conducted (Mcneil and Doucet, 2012). Resistin concentrations were also significantly elevated during the luteal phase compared to the follicular phase. However, adiponectin concentrations remained stable over the entire menstrual cycle, displaying no differences between phases (Asimakopoulos *et al.*, 2009). This data supports a previous smaller study reporting no significant differences in adiponectin concentrations during the menstrual cycle (Kleiblova *et al.*, 2006).

1.4.3.4: Ghrelin

Ghrelin is a 28 amino acid hormone that is often known as the 'hunger hormone' as it is released by the hypothalamus via the stomach and duodenum to stimulate an episode of eating. It is secreted in high concentrations before a meal during a fasting state (Toshinai *et al.*, 2001) and reduces 1-2 hours post food consumption (Cummings *et al.*, 2001). The hormone circulates in the blood in acylated and unacylated forms, with octanoylation at serine-3 (acylated ghrelin) required for it to bind to growth hormone secretagogue receptors (GSH-R) (Van Der Lely *et al.*, 2004).

There is evidence to suggest that there is an interaction between ghrelin, oestrogen and progesterone concentrations. Ovariectomy in rats is associated with an increase in food intake and a decrease in physical activity which leads to weight gain (Sainsbury *et al.*, 2002). However, in ovariectomized ghrelin knock-out (KO) mice, no increase in food intake or body weight is reported (Clegg *et al.*, 2007).

This indicates oestrogen may be responsible for potentially inhibiting ghrelin signalling (Lovejoy *et al.*, 2009). However, there is evidence to suggest that oestrogen administration does not alter ghrelin concentrations (Dafopoulos *et al.*, 2010). As a result, it is unlikely that ghrelin is secreted in large enough concentrations to result in changes in oestrogen or progesterone concentrations during different phases of the menstrual cycle and that the changes are more apparent during extreme hormone concentrations such as after menopause. However it may explain the differences in dietary intake recorded between anovulatory and ovulatory cycles, amenorrheic and eumenorrheic cycles as the hormone concentrations vary considerable during these cycles.

Conversely, there is evidence to suggest that ghrelin concentrations may also interact with normal oestrogen and progesterone concentrations. A number of studies have reported a possible link between ghrelin and LH pulse frequency which regulates oestrogen and progesterone concentrations. A study conducted in 6 women mapped nocturnal secretions of LH and FSH following 4 injections of ghrelin. The results indicate a reduction in LH and FSH secretions following the ghrelin injections (Kluge *et al.*, 2012). It has been suggested that ghrelin potentially inhibits GnRH (Kluge, 2009), which inhibits the LH pulse as this is controlled by the hypothalamus (Krey *et al.*, 1975). The hypothalamus also expresses a large number of growth hormone secretagogue (GHS) receptors (Gnanapavan *et al.*, 2002), which are required along with octanoylation at serine-3 (acylated ghrelin) to bind ghrelin (Van Der Lely *et al.*, 2004). It has been suggested that ghrelin may influence GnRH concentration release which has a significant knock on effect to the production of oestrogen and progesterone. This therefore may explain the reduction in sex hormone concentrations previously reported following ghrelin administration (Fang *et al.*, 2012).

1.4.3.5: Gastric Emptying

There is increasing evidence to suggest that the rate of gastric emptying plays a pivotal role in short-term energy intake (Sepple and Read, 1989). Gastric emptying is the process by which food leaves the stomach and enters the duodenum, the rate at which this process occurs can influence the gastrointestinal hormones involved in dietary intake regulation. Gastric emptying is recognized as

a contributor to postprandial blood glucose homeostasis, as a slow rate of gastric emptying can lower the initial and overall glycaemic response (Jones *et al.*, 1996). Additionally, glucose intake has been reported to stimulate the release of GLP-1 hormone, but is highly dependent on the rate of gastric emptying into the small intestine (O'donovan *et al.*, 2004). Secretions of GLP-1 account for approximately 50% of the increase in plasma insulin following oral glucose consumption, which may have profound effects on dietary intake.

Previous studies have reported inconsistent results when assessing gastric emptying during different phases of the menstrual cycle (Brennan *et al.*, 2009; Caballero-Plasencia *et al.*, 1999; Gill *et al.*, 1987; Horowitz *et al.*, 1985; Mones *et al.*, 1993). A recent comprehensive study has investigated the rate of gastric emptying, gastrointestinal hormones, glycaemic response, insulin response and food intake during the follicular and luteal phases of the menstrual cycle following a 90 minute oral glucose test (50g glucose) (Brennan *et al.*, 2009). The results indicate a reduction in gastric emptying during the follicular phase, corresponding to a reduction in GLP-1 secretions and subsequent glycaemic and insulinemic responses. Food intake during a buffet-style meal following the conclusion of the 90 minute glucose test also recorded a reduction in overall energy intake during the follicular phase testing sessions (Brennan *et al.*, 2009). It is therefore suggested that a reduction in food intake during the follicular phase compared to the luteal phase may be as a result of a reduction in gastric emptying and a subsequent reduction in gastrointestinal hormone secretions associated with appetite regulation. As gastric emptying appears not to change during the luteal phase, this may explain the differences in dietary intake recorded between menstrual cycle phases. However there have also been several studies to date that suggest gastric emptying does not change during different phases of the menstrual cycle (Caballero-Plasencia *et al.*, 1999; Horowitz *et al.*, 1985).

As gastrointestinal hormones have been reported to have mixed roles during the menstrual cycle and appear to not differentiate between PMS and non-PMS women, the variation in dietary intake during the menstrual cycle may be as a result of other hormonal and neural interactions. An example of this neural influence might include serotonin, which is heavily implicated in the aetiology of

PMS and strongly links mood with dietary intake (American College of Obstetricians & Gynecologists, 2000).

1.4.3.6: Serotonin

Obesity in some populations has been previously described as a disease of mood in which appetite control is sacrificed to appease depression or an anxiety state (Wurtman, 1993). As such it has been suggested that there is a strong link between mood and dietary intake, which is clearly demonstrated by certain conditions, including seasonal affective disorder, depression and PMS. Individuals that are affected by these conditions eat 'normally' until they experience the depression. This is evident by a temporary relief in symptoms of depression or anxiety following an increase in high CHO foods (Wurtman, 1993).

It has been well documented that the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT or its metabolite 5-hydroxyindoleacetic acid, 5-HIAA) plays an important role in eating behaviour (Blundell, 1977) and in particular has been implicated as the mediating factor in the relationship between mood and appetite (Wurtman, 1993). In addition, plasma serotonin concentrations are at their lowest during the luteal phase of the menstrual cycle (Tam *et al.*, 1985; Taylor *et al.*, 1984), with concentrations in PMS sufferers also reported to be lowest during this phase (Rapkin *et al.*, 1987). Subsequently it has been suggested that changes in energy intake and macronutrient intake (in particular CHO intake) are as a result of a reduction in serotonin concentrations.

It has been demonstrated that increases in tryptophan may stimulate an increase in serotonin synthesis (Fernstrum and Wurtman, 1971) and it is believed that this may be an adaptive mechanism, designed to compensate for the reduction in serotonin during the luteal phase. As a result this could explain the increase in energy intake previously reported during this phase (Dye and Blundell, 1997; Rapkin *et al.*, 1987). Increases in energy intake may therefore be a compensatory response to a decrease in serotonin concentrations rather than any direct oestrogen or progesterone concentration change.

1.4.3.7: Serotonin and Insulin

A potential feedback loop has been hypothesised between CHO intake, insulin secretion, serotonin production and its precursor tryptophan (Fernstrum and Wurtman, 1971). Insulin administration is known to decrease the concentration of glucose within plasma; however insulin secretions concomitantly increase tryptophan concentrations when given as an injection or as part of a CHO rich diet. One specific rodent study reports an increase in serotonin concentrations of 25-36% from baseline following a CHO diet (5 g/hr. for 3 hrs.). In addition, an increase in CHO rich foods has been found to increase the availability of tryptophan within the brain, relative to other large neutral amino acids (Blundell, 1992). As tryptophan competes with amino acids for receptor mediated transport across the blood brain barrier, a reduction in amino acids therefore increases the ability to synthesise serotonin (Wurtman *et al.*, 1989). However this work has been challenged as a potential mechanism in humans, as many studies report not only increases in CHO intake but also cravings for foods that are high in fat and energy density during the luteal phase. This increase in fat intake is potentially due to its association with pleasure, which may relieve some PMS symptoms. As such the diet may contain too much fat and protein to facilitate the increase in tryptophan concentrations to stimulate serotonin, as previously hypothesised (Cross *et al.*, 2001).

The variation in success rate of the proposed CHO/serotonin theories could be due to the reporting of an increase in CHO intake in some women but not all. This variation may depend on their underlying susceptibility to increases in CHO intake in some women and therefore may be modulated via different unknown mechanisms other than the ones proposed. As insulin has already been reported to increase during the luteal phase of the menstrual cycle potentially due to the presence of oestrogen and progesterone hormone receptors within the insulin stimulated glucose uptake pathway (section 1.2), the interaction between sex hormones oestrogen and progesterone and these pathways may similarly influence the production of serotonin.

1.4.4: Current Literature on Changes in Dietary Intake during different Menstrual Cycle Phases

Recent literature has reported inconsistent results in potential variations in dietary intake during different phases of the menstrual cycle in both PMS and non-PMS suffering women. Studies to date have demonstrated that energy intake may increase between 12-38% during the luteal phase as compared to the follicular phase (Dalvit, 1981). However, the exact macronutrient content of this intake varies, with increased CHO consumption (Dalvit, 1981), increased CHO and decreased fat consumption (Brzezinski *et al.*, 1990; Dalvit-Mcphillips, 1983), increased fat consumption (Johnson *et al.*, 1994; Tarasuk and Beaton, 1991) and increased fat and protein consumption (Gallant *et al.*, 1987) all reported during the luteal phase (Table 1.7).

Conversely, other dietary intake studies have reported no specific variation in macronutrient intake despite increases in overall energy intake (Lyons *et al.*, 1989) (Table 1.8). The most notable non-significant studies include those investigating women taking oral contraceptives, suffering from PMS or experiencing anovulatory cycles (Barr *et al.*, 1995; Gallant *et al.*, 1987; Piers *et al.*, 1995; Schweiger *et al.*, 1992; Wurtman *et al.*, 1989). Hormonal contraception and anovulatory cycles both cause changes or modifications in sex hormone concentrations during the menstrual cycle. Anovulatory cycles in particular are associated with a reduced progesterone concentration and this may account for the non-significant differences in dietary intake reported in some studies. However, there is some controversy as to whether changes in dietary intake are a result of luteal phase hormone concentrations that are typical of all women or if they are exacerbated in women that suffer from PMS (Dye and Blundell, 1997). Several studies do not specify if the participants were suffering from PMS related symptoms or not when investigating dietary intake during the menstrual cycle. There have been a select few that have attempted to make comparisons between women with PMS and non-PMS control groups (Bryant *et al.*, 2006; Brzezinski *et al.*, 1990; Cross *et al.*, 2001; Wurtman *et al.*, 1989) with some authors investigating dietary intake in PMS women only (Johnson *et al.*, 1995).

Table 1.7: Current literature investigating energy intake during different phases of the menstrual cycle in women

Study	Sample size	Days measured	BBT	Hormones	Luteal (LP)	Energy Intake (MJ)			LP vs. FP
						Menstrual (MP)	Follicular (FP)	Ovulatory (OP)	
Abraham <i>et al.</i> (1981)	23	Daily, 1 cycle	N	N	Nr	Nr	Nr	Nr	LP>FP*
Dalvit (1981)	8	10 days FP / LP	N	N	8.12		6.02		LP>FP*
Dalvit-McPhillips (1983)	8	10 days FP / LP	N	N	7.22		5.22		LP>FP*
Pliner and Fleming (1983)	34	1 day FP / LP	N	N	8.42		7.49		LP>FP*
Giannini <i>et al.</i> (1985)	20	10 days FP / LP	-	-	Nr	Nr	Nr	Nr	NS
Manocha <i>et al.</i> (1986)	11	10 days FP / LP	N	N	6.74		5.44		LP>FP*
Gallant <i>et al.</i> (1987)	9 10 PMS	3 days FP / LP	N	N	8.09		6.27		LP>FP*
					6.56		5.71		NS
Oram (1987)	6	10 days FP / LP	N	N	11.56		8.74		LP>FP*
Sophos <i>et al.</i> (1987)	9	7 days FP / LP	Y	N	7.79		7.44		NS
Lissner <i>et al.</i> (1988)	23	10 days FP / LP	N	N	9.77		9.41		LP>FP*
McCoy <i>et al.</i> (1988)	12	8 days FP / LP	Y	N	8.95		8.27		LP>FP*
Gong <i>et al.</i> (1989)	7	Daily, 1 cycle	Y	N	8.54	7.89	7.67	7.39	LP>FP*

Nr - Measure But Actual Intake Not Recorded, MP - Menstrual Phase, FP - Follicular Phase, OP - Ovulatory Phase, LP - Luteal Phase, NS - Non Significant, * - Significant Difference P<0.05, PMS - Premenstrual Syndrome, LH Only - Luteinizing Hormone, BBT - Basal Body Temperature, Hormones - Oestrogen And Progesterone, MJ - Mega Joules

Table 1.7 continued: Current literature investigating energy intake during different phases of the menstrual cycle in women

Study	Sample size	Days measured	BBT	Hormones	Luteal (LP)	Energy Intake (MJ)			LP vs. FP
						Menstrual (MP)	Follicular (FP)	Ovulatory (OP)	
Hrboticky <i>et al.</i> (1989)	8	1 day FP / LP	Y	N	7.72		7.52		NS
Lyons <i>et al.</i> (1989)	25	Daily, 35 days	N	LH only	9.13	9.05	8.45	7.87	LP>FP*
Wurtman <i>et al.</i> (1989)	9	1 day FP / LP	Y	N	8.46		8.72		NS
	19 PMS				10.02		7.92		LP>FP*
Brzezinski <i>et al.</i> (1990)	17 PMS	1 day FP / LP	-	-	7.28		6.04		LP>FP*
Tarasuk and Beaton (1991)	14	10 days FP / LP	N	N	8.00		7.62		LP>FP*
Schweiger <i>et al.</i> (1992)	13	12 days FP / LP	N	Y	9.42		9.08		LP>FP*
Fong and Kretch (1993)	9	Daily	Y	N	10.46	10.31	9.87	9.39	NS
Netter <i>et al.</i> (1993)	40	9 days FP / LP	-	-	Nr		Nr		LP>FP*
Johnson <i>et al.</i> (1994)	26	Daily	Y	Y	7.84	7.6	7.15	7.15	LP>FP*
Lariviere <i>et al.</i> (1994)	8	1 day FP / LP	N	N	8.29		7.39		LP>FP*
Martini <i>et al.</i> (1994)	18	3 days FP / LP	Y	Y	7.98		7.32		LP>FP*
Rogers and Jas (1994)	42	25 days FP	-	-	3.35		1.46		LP>FP*
		17 days LP							
Barr <i>et al.</i> (1995)	29 ovulatory	3 days; MP, FP, LP	Y	N	3.27		8.01	Nr	LP>FP*
	13 anovulatory				7.91		8.21	Nr	NS

MP – Menstrual Phase, FP – Follicular Phase, OP – Ovulatory Phase, LP – Luteal Phase, NS – Non Significant, * – Significant Difference P<0.05, PMS – Premenstrual Syndrome, LH Only – Luteinizing Hormone, BBT – Basal Body Temperature, Hormones – Oestrogen and Progesterone, MJ – Mega Joules

Table 1.7 continued: Current literature investigating energy intake during different phases of the menstrual cycle in women

Study	Sample size	Days measured	BBT	Hormones	Luteal (LP)	Energy Intake (MJ)			LP vs. FP
						Menstrual (MP)	Follicular (FP)	Ovulatory (OP)	
Piers <i>et al.</i> (1995)	13	2 days FP / LP	N	Y	7.12		7.12		NS
Li <i>et al.</i> (1999)	20	3 days FP / LP	N	LH only	6.98		6.09		LP>FP*
Cross <i>et al.</i> (2001)	40	4 days FP / LP	N	N	7.89		7.43		LP>FP*
	82 PMS				8.65		6.05		LP>FP*
Reimer <i>et al.</i> (2005)	9	3 days FP / LP	N	Y	8.74		7.33		LP>FP*
Bryant <i>et al.</i> (2006)	27	3 days FP / LP	N	N	8.17		8.29		NS
	31 PMS				8.38		8.58		
Ismail <i>et al.</i> (2009)	43	3; MP, FP, LP	N	N	5.25	4.71	5.70		LP>FP*

MP – Menstrual Phase, FP – Follicular Phase, OP – Ovulatory Phase, LP – Luteal Phase, NS – Non Significant, * – Significant Difference $P<0.05$, PMS – Premenstrual Syndrome, LH Only – Luteinizing Hormone, BBT – Basal Body Temperature, Hormones – Oestrogen and Progesterone, MJ – Mega Joules

Table 1.8: Current literature investigating macronutrient intake (% total energy intake, TEI) during different phases of the menstrual cycle

Study	Sample size	Days measured	BBT	Hormones	Carbohydrate			Fat			Protein		
					FP	LP	Nr*	FP	LP	Nr	FP	LP	Nr*
Abraham <i>et al.</i> (1981)	23	Daily	N	N	Nr	Nr*	Nr	Nr	Nr	Nr	Nr	Nr	Nr*
Dalvit-McPhillips (1983)	8	10 days FP / LP	N	N	39.9	55.8*	44.4	32.6	15.7	11.6			
Gallant <i>et al.</i> (1987)	9	3 days FP / LP	N	N	43.1	40.9	36.6	37.7*	13.9	15.7			
	10 PMS				34.6	40.0	48.8	43.6	15.0	16.0			
Oram (1987)	6	10 days FP / LP	N	N	46.5	42.2	37.1	38.1	14.5	16.4			
Sophos <i>et al.</i> (1987)	9	7 days FP / LP	Y	N	47.3	46.1	36.4	33.7	15.2	15.1			
Lyons <i>et al.</i> (1989)	25	Daily, 35 days	N	LH only	47.0	45.9	35.8	36.8	13.5	13.4			
Hrboticky <i>et al.</i> (1989)	8	1 day FP / LP	Y	N	54.0	54.0	28.0	28.3	13.4	13.4			
Brzezinski <i>et al.</i> (1990)	17 PMS	1 day FP / LP	-	-	28.6	32.5*	48.6	48.1*	21.1	17.0			
Tarasuk and Beaton (1991)	14	10 days FP / LP	N	N	43.8	43.1	36.0	37.6*	14.6	14.1			
Schweiger <i>et al.</i> (1992)	13	12 days FP / LP	N	Y	42.0	43.0	41.0	40.0	13.0	14.0			
Fong and Kretch (1993)	9	Daily	Y	N	44.2	44.5	41.9	41.7	12.9	13.0			

Nr - Measure But Actual Intake Not Recorded, FP - Follicular Phase, LP - Luteal Phase, * - Significant difference compared to FP P<0.05, PMS - Premenstrual Syndrome, LH Only - Luteinizing Hormone, BBT - Basal Body Temperature, Hormones - Oestrogen and Progesterone.

Table 1.8 continued: Current literature investigating macronutrient intake (% total energy intake, TEI) during different phases of the menstrual cycle

Study	Sample size	Days measured	BBT	Hormones	Carbohydrate		Fat		Protein	
					FP	LP	FP	LP	FP	LP
Johnson <i>et al.</i> (1994)	26	Daily	Y	Y	47.5	47.9	35.4	37.4*	15.4	14.7
Martini <i>et al.</i> (1994)	18	3 days FP / LP	Y	Y	51.3	50.1	33.1	34.2	15.6	15.6
Barr <i>et al.</i> (1995)	29	3 days; FP, OP &	Y	N	57.5	56.1	28.9	31.3	12.7	12.4
	13 anovulatory	LP			56.3	54.1	30.5	32.9	12.5	13.1
Li <i>et al.</i> (1999)	20	3 days FP / LP	N	LH only	51.0	52.0*	19.0	18.0	29.0	31.0*
Cross <i>et al.</i> (2001)	40	4 days FP / LP	N	N	44.0	45.0	35.0	33.0	16.0	17.0
	82 PMS				46.0	45.0	35.0	33.0	16.0	19.0
Reimer <i>et al.</i> (2005)	9	3 days FP / LP	N	Y	53.0	48.0	29.0	34.0	14.0	15.0
Bryant <i>et al.</i> (2006)	27	3 days FP / LP	N	N	48.0	50.0	36.0	34.0	15.0	14.0
	31 PMS				46.0	47.0	38.0	37.0	15.0	15.0
Ismail <i>et al.</i> (2009)	43	3; MP, FP, LP	N	N	51.9	50.9	32.4	33.6	15.7	15.4

FP – Follicular Phase, OP – Ovulatory Phase, LP – Luteal Phase, * – Significant difference compared to FP P<0.05, PMS – Premenstrual Syndrome, LH Only – Luteinizing Hormone, BBT – Basal Body Temperature, Hormones – Oestrogen and Progesterone

Wurtman *et al.* (1989) examined dietary intake and subjective assessments of appetite and food cravings in 37 women during the follicular and luteal phases of the menstrual cycle. The study was divided into two groups; 19 women who had reported severe PMS symptoms during the previous 12 months were compared to a control group (n=9) with no history of PMS. Dietary intake was recorded for 1 day during the early follicular (days 4-9) and late luteal (days 25-28) phases of their menstrual cycles. During the two testing sessions, participants were given a choice of six isocaloric and isofat meals with food weighed before and after consumption. PMS symptoms were assessed using a modified Abraham Premenstrual Symptomatology Questionnaire which retrospectively assessed the previous 5 days. The control group reported similar symptoms during both phases, with the PMS group reporting a significant rise in symptom severity during the luteal phase of the menstrual cycle. Energy intake within the PMS group increased during the luteal phase compared to the follicular (1892 ± 104 kcal/day; 2395 ± 93 kcal/day), with a significant proportion of this due to an increase in CHO intake. The control group reported no significant differences in energy intake or CHO consumption during the menstrual cycle. In addition, protein intake did not significantly differ between cycle phases in either control or PMS group. These results suggest that PMS is associated with an increase in energy intake and an increase in CHO intake during the luteal phase (Wurtman *et al.*, 1989).

A follow on clinical trial was then conducted by the same group (Brzezinski *et al.*, 1990), using the same experimental protocol in 17 PMS women. Baseline energy intake was reported to be significantly higher during the luteal phase compared to the follicular, with a significant increase in CHO and fat intake also reported. Protein consumption was not significantly different during either phase of the menstrual cycle. Despite this study having similar methodological weaknesses to the previous investigation (Wurtman *et al.*, 1989), the findings do confirm that women suffering from PMS symptoms demonstrate an increase in total energy intake and more specifically an increase in CHO and fat consumption during the luteal phase of the menstrual cycle (Brzezinski *et al.*, 1990).

Johnson *et al.* (1995) also investigated dietary intake during different phases of the menstrual cycle in 26 women with PMS. BBT was recorded daily to identify ovulation, with a blood sample drawn once a week for 4 weeks to identify menstrual cycle phases using FSH, LH, oestrogen and progesterone. PMS related symptoms were recorded using the Menstrual Distress Questionnaire (MDQ) (Moos, 1968). Following dietary analysis the results demonstrated an increase in energy intake during the luteal phase (by 163.9 kcal/day). CHO and fat intake also significantly increased during the luteal phase but not protein intake. CHO and protein, expressed as a proportion of total energy intake (%TEI), was consistent between the two phases; however fat consumption (%TEI) did increase during the luteal phase (by 2%). The results support the findings of the previous studies (Brzezinski *et al.*, 1990; Wurtman *et al.*, 1989) indicating a significant increase in energy intake during the luteal phase and report an increase in both CHO and fat intake (%TEI). Johnson *et al.* (1995) also reported concentrations of progesterone and LH to identify anovulatory cycles, which many of the previous studies failed to do. Anovulatory cycles have been reported to be a confounding variable that may influence energy intake (Barr *et al.*, 1994; Gallant *et al.*, 1987; Piers *et al.*, 1995; Schweiger *et al.*, 1992; Wurtman *et al.*, 1989), particularly in women with PMS, as the presence of ovulation and the peak in progesterone is required by definition for the condition to exist. As a result this therefore could account for some of the non-significant results reported.

In addition, the previous evidence has predominantly reported dietary intake in normal weight women only (with and without PMS). However a recent study was conducted in 144 women who were overweight (BMI 24-37 kg/m²), where 88 were suffering from PMS and 56 were not (Cross *et al.*, 2001). Dietary intake was assessed in both groups using 4-day diet diaries in the follicular and luteal phases over two menstrual cycles. Energy and macronutrient intake was also calculated according to food categories. Energy intake was significantly increased during the luteal phase compared to the follicular phase within the PMS group. Of the extra energy consumed, a significant increase in CHO, simple sugars and fat was recorded in the PMS group. The largest food group to increase was recorded in the 'cakes and desserts' and 'high sugar foods' categories during the luteal phase which may explain the increase in CHO and fats reported. The non-PMS group

also reported an increase in energy and fat intake during the luteal phase. However when intake fat was adjusted for its proportion of total energy (%TEI) there was no significant difference. The results support previous findings (Wurtman *et al.*, 1989) and indicate that women with PMS who are overweight also demonstrate an increase in dietary intake during the luteal phase.

Conversely, Bryant *et al.* (2006) conducted a similar study investigating dietary intake in women with PMS (n=31) and a non-PMS control group (n=27). PMS symptoms were measured using a Daily Symptom Rating questionnaire and dietary data were collected using 3 day food diaries during the follicular (days 4-6) and luteal (days -6, -5 and -4) phases. No significant difference was reported in energy or macronutrient intake in the PMS group during either phase. A significant increase was reported in the consumption of alcohol and non-milk extrinsic sugars (NMES) in the PMS group during the luteal phase. NMES are sugars that are not naturally incorporated into the cellular structure of the food, but have been added to potentially enhance the flavour (Health Education Authority, 1999). These added sugars are present in high quantities in confectionary, biscuits, cakes and fruit juices. Additionally, these nutrients were positively correlated with the severity of symptoms recorded within the PMS group. In comparison, energy intake increased during the luteal phase of the control group, supporting previous literature (Dalvit-Mcphillips, 1983; Reimer *et al.*, 2005). The variation between groups may be due to participant recruitment numbers in this study; however changes in NMES within the PMS group were reported and may represent the CHO and fat increases in the previous studies.

1.4.5: Methodological Limitations within Dietary Intake Studies

Researchers investigating dietary intake in female participants are confronted with a number of methodological problems that may account for some of the inconsistent results reported. Many studies informed participants of the nature of the research before collecting data which has been shown to influence reporting (Ruble, 1977). In addition, there are a wide variety of methods that can be used to record dietary intake all of which experience varying levels of participant bias. A large proportion of research studies perform self-reported intake through a food diary, whereas others are asked to complete weighed records either at home or

within a laboratory setting. The method implemented has a significant impact on the amount of under reporting that occurs, with self-reported dietary intake biased towards underestimation (Black *et al.*, 1991). In addition, the rate of under reporting varies depending on the subgroup of population observed, with the overweight and obese recording the greatest levels of under reporting (approx. 30%) (Plankey *et al.*, 1997; Rennie *et al.*, 2007; Westerterpplantenga *et al.*, 1991).

Furthermore, the method used to analyse dietary intake can also have a significant impact on overall intake. An example of this is supported by Johnson *et al.* (1994) who concluded that CHO and fat intake increased during the luteal phase of the menstrual cycle in 26 women. However when expressed as a proportion of total energy intake (%TEI), significant increases in fat intake during the luteal phase were reported, but CHO and protein consumption displayed little variation across both phases (Johnson *et al.*, 1994). Absolute increases in macronutrient intake have similarly been reported as increasing between phases, with the percentage of total energy intake failing to differ (Barr *et al.*, 1995). Reporting macronutrient intake as a proportion of total energy intake (%TEI) allows the relative change to total intake to be recorded. In addition, the data represent true variation in macronutrient intake rather than in response to one or two specific food items and is a much better representation of any possible variation over time.

1.4.6: Summary

There is considerable amount of evidence to suggest that a change in energy intake occurs during different phases of the menstrual cycle, with the majority of research reporting a significant increase during the luteal phase with potential increase in specific macronutrient selection (CHO and fat). In addition, evidence of energy intake changes in PMS sufferers suggests a similar increase in energy intake and CHO intake during the luteal phase of ovulatory cycles. This is accounting for the large range of methodological practices that are implemented.

The exact mechanistic pathway by which sex hormones may influence the regulation of dietary intake is currently unclear. Both oestrogen and progesterone and in conjunction with other gastrointestinal hormones have been implicated as possible sources of explanation. The effect of oestrogen and progesterone on substrate oxidation and glucose homeostasis has also been proposed as a possible

mechanistic pathway. Serotonin synthesis via tryptophan metabolism has been strongly implicated as a potential mechanism in both PMS and non-PMS suffering women. However due to the complexity of energy regulation, a combination of all of these pathways may elucidate to the potential mechanisms involved.

Regardless of the mechanistic uncertainty, the current body of evidence strongly indicates that dietary intake does significantly increase during different phases of the menstrual cycle, particularly within the luteal phase. This has significant consequences for energy regulation and energy balance in women, which has long term health implications including the development of obesity.

1.5: Exercise and Symptoms of Premenstrual Syndrome (PMS)

As previously discussed, PMS symptoms occur during the luteal phase when both oestrogen and progesterone concentrations are present (section 1.4). However due to the uncertainty in its aetiology, treatments for PMS vary greatly. The UK Royal College of Obstetricians and Gynecologists (2007) currently recommends general advice about exercise, diet and stress reduction before starting hormonal treatment as the first line of symptom management. The second line of treatment focuses purely on hormonal treatments, with a combination of oestrogen and progesterone therapies and higher dose selective serotonin reuptake inhibitors (SSRIs) recommended. The third and fourth line of treatment encompass more extreme hormonal therapy which may include GnRH analogues, total abdominal hysterectomy and hormone-replacement therapy (Royal College of Obstetricians and Gynaecologists, 2007).

1.5.1: Exercise and PMS

Despite lifestyle interventions, such as exercise, being recommended before more complex and potentially distressing hormonal treatment is initiated to treat PMS symptoms; very little research has been conducted to determine its success rate. Physical activity is recommended for general populations to prevent and manage chronic conditions including coronary heart disease, type 2 diabetes, obesity and cancer (World Health Organisation, 2010). Exercise participation has also been reported to play a pivotal role in preventing and improving mental health, psychological well-being and quality of life (QoL) (Department of Health, 2004; Hassmen *et al.*, 2000; Stoddard *et al.*, 2007). Specifically, physical activity has been reported to enhance psychological well-being by improving self-perception, self-esteem, mood and sleep quality, as well as reducing anxiety and fatigue (World Health Organisation, 2010). Similarly, in some clinical conditions (such as gastroenteritis), exercise can improve physical symptoms such as constipation and bloating (Daley *et al.*, 2008). With this in mind exercise participation for women who suffer with PMS may produce positive outcomes for symptom relief.

Previous observational research suggests physically active women report fewer PMS symptoms when compared to those who are sedentary (Aganoff and Boyle, 1994; Choi and Salmon, 1995). In addition, female athletes who train vigorously

also report fewer menstrual cycle related symptoms (Case and Reid, 1998). The possible pathways by which exercise may alleviate PMS symptoms are currently unclear; however, women who perform regular high-intensity physical activity experience a greater frequency of anovulatory cycles as a result of lower sex hormone concentrations (Shangold *et al.*, 1990). This may account for some of the reduced symptoms previously reported in physically active women (Case and Reid, 1998). In addition, concentrations of β -endorphins also increase during exercise and are implicated in areas of the brain associated with pain, emotion and behaviour (Dalayeun *et al.*, 1993).

1.5.1.1: Sex Hormone Concentrations

Previous evidence in female athletes has suggested that vigorous exercise often causes amenorrhea or secondary amenorrhea (Speed, 2007) and subsequently may lower the frequency and severity of PMS symptoms reported (Case and Reid, 1998). In addition, some intense exercise training regimes can also lower body fat which can lead to a reductions in oestrogen production (Reilly, 2000). This reduces sufficient oestrogen feedback to the pituitary and may result in an increase in reported anovulatory cycles (Reilly, 2000).

In studies employing moderate exercise regimes the evidence is more inconsistent. Some studies have reported a reduction in sex steroid concentrations (Bullen *et al.*, 1984; Keizer *et al.*, 1987; Morris *et al.*, 1999), with others reporting no significant differences (Prior *et al.*, 1987; Ronkainen *et al.*, 1985). A more recent investigation reports a significant decrease in oestrogen and progesterone concentrations following a moderate intensity exercise regime in women with PMS (Stoddard *et al.*, 2007). However, the hormone concentrations were measured in urine samples and had been left for up to 4 days before processing in some circumstances. The time-lag between excretion and sample processing may have reduced the detectable hormone content and therefore the resulting concentration.

1.5.1.2: β -endorphins

Performing exercise has been reported to increase secretions of the endogenous opioid β -endorphin from the pituitary gland into the blood. β -endorphins are neuropeptides, 31-amino acids long which are cleaved from proopiomelanocortin

(POMC) along with adrenocorticotrophic hormone (ACTH) (Hirsch and Millington, 1991). β -endorphins are secreted by the pituitary gland and the receptors are found in the hypothalamus and limbic systems of the brain; both areas are associated with pain, emotion and behaviour (Dalayeun *et al.*, 1993). The β -endorphin system is responsible for the regulation of a number of mechanisms and pathways including blood pressure, pain perception and body temperature. β -endorphins secreted in response to pain, reduce the symptoms by inhibiting the electrical responses to sensory nerves (Dalayeun *et al.*, 1993).

It has been hypothesised that increased β -endorphin concentrations during exercise interact with the hypothalamic pituitary adrenal axis (HPA) by decreasing the release of FSH (Gonzalez-Ortiz *et al.*, 1998) and LH (Chuong *et al.*, 1985). This subsequently reduces successful follicular development and results in a decrease in oestrogen and progesterone secretions (Drinkwater *et al.*, 1986; Shangold *et al.*, 1990). This indicates that β -endorphins may inhibit the LH surge as a result of a down-regulation of the HPA axis and in turn increases the frequency of anovulatory cycles.

The role of β -endorphins in the pathophysiology of PMS was first suggested in the 1980 (Reid and Yen, 1981). Evidence suggests women with PMS have lower β -endorphin concentrations compared to those without such a diagnosis (Chuong *et al.*, 1985). This is supported in a recent study measuring plasma β -endorphins, ACTH and cortisol in women with PMS ($n=27$) and non-PMS women ($n=27$) during the menstrual cycle following an ischemic pain test. The results report significantly lower resting β -endorphins and cortisol in the PMS group during the luteal phase compared to the control group. Pain unpleasantness was reported as worse during the luteal compared to the follicular phase, corresponding to a simultaneously lower β -endorphin concentration during the luteal phase (Straneva *et al.*, 2002). Subsequently, it could be suggested that the naturally lower concentrations of β -endorphins reported in women who suffer from PMS could account for some of the symptoms associated with this syndrome, particularly as β -endorphin receptors are present in parts of the brain associated with pain, emotion and behaviour (Dalayeun *et al.*, 1993).

It is well documented that performing exercise increases the release of β -endorphin from the pituitary gland into the blood. Aerobic exercise at intensities of $>70\%$ of $\text{VO}_{2\text{max}}$ has been reported to increase plasma β -endorphin concentrations (Goldfarb *et al.*, 1990; Kraemer *et al.*, 1989). Similar results were reported following 8 weeks of moderate training (30 min of treadmill running at anaerobic threshold, 3 times per week) in young women (Heitkamp *et al.*, 1998). As exercise is associated with an increase in β -endorphin concentrations (Bortz *et al.*, 1981; Farrell *et al.*, 1982), and PMS women display a naturally lower β -endorphin concentration compared to non-PMS women, there is the potential that exercise participation in PMS women could lead to an improved β -endorphin concentration. This may lead to a reduction in some PMS symptoms experienced and improve feelings of well-being.

1.5.2: Current Literature Investigating Exercise Interventions on PMS Symptoms

The two earliest studies (Table 1.9) investigating the effects of exercise on PMS symptoms reported significant improvements in PMS symptoms following a 3 and 6 month exercise programmes (Prior *et al.*, 1986; Prior *et al.*, 1987). The first 3 month study recruited 14 women (8 exercise and 6 control) that were diagnosed with type 1 diabetes (Prior *et al.*, 1986). The study reported significant decreases in some PMS symptoms including breast tenderness and water retention. However the symptoms were recorded retrospectively and therefore could reflect participant bias and ineffective recall (Prior *et al.*, 1986). The second 6 month study recruited a total of 21 women, 8 sedentary, 7 marathon training runners and 6 normally active controls (Prior *et al.*, 1987). These women reported symptoms prospectively and displayed a similar significant decrease in PMS following the longer exercise programme, with no differences in symptoms reported in the control group (Prior *et al.*, 1987).

Another study (Steege and Blumenthal, 1993) investigated the effects of aerobic compared to strength training during a 12 week exercise programme (Table 1.9). Twenty three women were recruited, 11 assigned to the strength training and 12 to the aerobic training. The participants were instructed to complete menstrual symptom questionnaires before the exercise programme began and then again

after the programme finished. The study reports improvements in symptoms recorded in both exercise groups, but with greater symptom reductions (18/23 symptoms vs. 3/23 symptoms) in the aerobic exercise training group (Steege and Blumenthal, 1993). This suggests that the type of physical activity may also determine the success of the reduction in PMS symptoms recorded.

The most recent intervention investigating the effects of exercise on PMS symptoms was conducted in 14 sedentary women (Table 1.9) (Stoddard *et al.*, 2007). The women were enrolled on a 24 week exercise-training programme (60-85% maximum heart rate) following a Baseline menstrual cycle. PMS symptom scores were collected daily using the menstrual distress questionnaire (MDQ) (Moos, 1968) during Baseline and between week 22 and 26 of the exercise programme. Urine concentrations of oestrone glucuronide (E1G) and pregnanediol glucuronide (Pdiol-3g) were also measured. The moderate intensity exercise programme caused a decrease in some PMS symptoms in particular water retention and pain between baseline and post-intervention (Stoddard *et al.*, 2007). The study also reports a significant reduction in mean and peak E1G and peak Pdiol-3g during the luteal phase. The study indicates that a 24 week exercise programme in previously sedentary women who suffer from PMS results in a decrease in some PMS symptoms and a reduction in sex hormones during the luteal phase.

Table 1.9: Current literature investigating the effects of an exercise intervention on symptoms of PMS

Study	Sample Size	Hormones or BBT	Study Design	Results
Prior <i>et al.</i> (1986)	8 exercise (ExG) 6 control with T1D (CG)	BBT - Daily Plasma E ₂ and P taken d18-26 only	Non-randomized controlled intervention trial (3m) 2-4 running sessions weekly, 10-15 minutes (3m) Symptom diary recorded retrospectively	Decrease in some PMS symptoms (breast tenderness & fluid retention) and global scores CG had lower baseline scores at 0m compared to the ExG
Prior <i>et al.</i> (1987)	8 sedentary (ST), 7 marathon (MT), 6 control (C-NT)	BBT - Daily Plasma E ₂ and Prog taken d18-26 only	Non-randomized controlled intervention trial (6m) 2-4 x running sessions weekly, alternated intense to less intense sessions (ST) Prospective daily symptom diaries recorded – (MCQ or DSD)	NS change in mean BMI in all groups Decrease in breast tenderness, fluid retention and global scores at 6m in ST and MT NS change in symptom scores in C-NT group NS change E2 and Prog levels in ST group
Steege and Blumenthal, (1993)	11 strength (ST) 12 aerobic (Soeters <i>et al.</i>)	BBT – N Hormones - N	Randomized intervention trial (3m) AER- 3 x 60mins for 3m (70-85% VO ₂ max) ST – circuit training (<50% VO ₂ max)	Increase VO ₂ max after 3m in AER group Decrease in global symptom scores after 3m in AER AER: 18/23 symptoms improved after 3m ST: 3/23 symptoms improved after 3m
Stoddard <i>et al.</i> (2007)	14 sedentary (ST)	BBT – N Urine E1G and Pdiol-3g taken d11 onwards	Intervention trial (24w) 3 x weekly exercise sessions (60-85% HR max) Post-test data was collected week 22-26 Prospective daily symptom diaries recorded – (MCQ or DSD)	Decrease some PMS symptoms (fluid retention & pain) and global scores after 3m NS impaired concentration Decrease mean and peak E1G and peak Pdiol-3g after 3m

P - Progesterone, Pdiol-3g – Urinary Pregnanediol-3-Glucuronide, E₂ - Oestradiol, E1G – Urinary Oestrone-Glucuronide, BBT - Basal Body Temperature, T1D – Type 1 Diabetes, M – Months, MCQ – Menstrual Cycle Questionnaire, DSD – Daily Symptom Diary, LP – Luteal Phase, NS – Not Significant. D-Day

1.5.3: Methodological Limitations

All four studies investigating the effects of an exercise intervention on PMS symptoms reported a decrease in symptoms between pre and post exercise, regardless of intervention duration or type and despite the relatively small sample sizes in all studies. However, none of the identified studies employed a specific non-exercising control arm within their methodology, which would minimise the effects of external variables and increase the reliability of the results. Although one study did collect data from a sedentary control group (Prior *et al.*, 1986), these women were diagnosed with type 1 diabetes and were not randomly assigned to both groups. It is unclear whether women with type 1 diabetes would respond differently to an exercise intervention to reduce their PMS symptoms. However, there is strong evidence to suggest that oestrogen and progesterone play a role in glucose uptake and insulin sensitivity which is pivotal in maintaining glucose homeostasis in type 1 diabetic women and therefore may cause a different symptom response compared to non-diabetic women due to the potential role of insulin in serotonin production (Fernstrum and Wurtman, 1971). As both groups were not homogenous it is uncertain if the differences in symptoms reported between groups were due to other variables and not just variations in exercise participation.

The methodologies employed in these trials made little attempt to control for factors known to affect the prevalence and severity of PMS. The studies were unable to provide an assessment of ovulation status, an important factor as ovulatory cycles have a greater effect on PMS symptoms compared to anovulatory cycles (Shangold *et al.*, 1990). In addition, neither menstrual cycle duration nor sex hormone concentrations before and after interventions were explicitly reported within any of the studies. Although urinary progesterone and oestrogen concentrations were measured during one study (Stoddard *et al.*, 2007), the samples were left for up to 4 days before processing which could have had a significant impact on the reliability of the results reported. Current guidelines recommend urine samples be processed or frozen within 48 hours of voiding to reduce potential metabolite degradation. The inclusion of more consistent and reliable sex hormone concentration data within study trials would allow any significant effects of exercise on PMS symptoms and QoL to be attributed appropriately and may provide potential mechanistic details.

1.5.4: Summary

There is growing evidence to suggest that physical activity prevents a number of chronic and mental health conditions and improves psychological well-being and QoL (Department of Health, 2011). The current limited research investigating the effects of exercise on PMS symptoms reports a decrease in symptoms following an exercise intervention programme, however the research to date has been performed using small participant numbers and is of low methodological quality.

The potential mechanisms by which exercise may reduce some PMS symptoms may occur directly by reducing oestrogen and progesterone hormone concentrations, increasing anovulatory cycles and indirectly by increasing β -endorphins concentrations. However there is currently no clear mechanistic pathway by which the reductions in PMS symptoms following exercise may occur and therefore requires further investigation. A greater number of studies are needed to determine the effectiveness of different exercise regimes on reducing PMS symptoms and in particular require randomized control trials in order to reduce participant expectation and possible placebo effect that can occur in this population.

1.6: Literature Review Summary

The literature review here covers four general areas: energy expenditure, substrate oxidation; glucose homeostasis and insulin sensitivity, dietary intake with special reference to PMS: and the effects of exercise on PMS symptoms. Specifically, previous literature has been reviewed investigating the potential role oestrogen and progesterone has in the individual mechanistic pathways, alongside details of previous studies investigating the effects of the two hormones during different menstrual cycle phases.

As discussed in the literature review, there is evidence throughout the four areas to suggest a lack of consistent methodology to determine oestrogen and progesterone concentrations to accurately identify menstrual cycle phase. The most common methods implemented include BBT, as an indicator of ovulation, or standard cycle duration of 28 days, with day 14 as a guide mid-point. For many women simply counting from the first day of menstrual bleeding may accurately determine the different phases; however there has been extensive research to suggest that there is inter-individual variation in total cycle duration, cycle phase duration and peak hormone concentrations of both sex hormones, with cycle duration ranging between 15 and 45 days (Chiazze *et al.*, 1968; Vollman, 1977). Equally there is also variation between follicular and luteal phase duration in young (less than 20 y) and mature (older than 40 y) women (Chiazze *et al.*, 1968), with the luteal phase duration significantly reduced in anovulatory cycles as new cycles are stimulated by a lack of progesterone secretions from the corpus luteum. By analysing sex hormone concentrations during the menstrual cycle, a clearer understanding of which hormone or combination of hormones may be driving the changes between cycle phases will be developed. These would enable a greater understanding of the potential significant implications on overall health and well-being in women and the long-term consequences this may cause.

1.7: Aims and Objectives

This thesis aims to determine the effects of fluctuating sex hormones, oestrogen and progesterone during the menstrual cycle on energy regulation, glucose and insulin response, dietary intake and in mediating the effects of exercise on PMS.

To fulfil this aim, the thesis objectives were:

1. To measure oestrogen and progesterone concentrations in plasma within different phases of the menstrual cycle
2. Investigate glycaemic response and insulin sensitivity during the individual phases of the menstrual cycle
3. Investigate energy expenditure and substrate oxidation at rest and whilst exercising during individual phases of the menstrual cycle
4. Investigate the effects of an exercise intervention on symptoms of PMS, QoL and dietary intake

It is hypothesised that changes in energy regulation, glucose and insulin response, dietary intake and PMS symptoms will be reported during the menstrual cycle, due to the changes in sex hormone concentrations of oestrogen and progesterone, reported within the three menstrual cycle phases.

The results of these investigations will determine if any of these processes are mediated by either oestrogen or progesterone or both and potentially shed light on the differences in metabolic responses between genders. In order to pursue the aims of this thesis, a series of human investigations were undertaken to determine the effects of fluctuating oestrogen and progesterone during the menstrual cycle, including two observation studies and one randomised control trial.

Chapter 2: Methodology and Common Methods

In order to minimise the range of other variables that may influence the proposed aims, careful consideration has been taken to determine the most effective methodological approaches to fulfil each objective. The following chapter discusses a range of methodologies currently available, justifies the inclusion of those selected in the current series of studies and describes the common methods implemented during all studies in this thesis (chapter 3, 4 and 5). Permissions to use the enclosed pictures are given in appendix 1.

2.1: Methodology

2.1.1: Menstrual Cycle Phase Definition

2.1.1.1: Objective of assessment

To determine each individual menstrual cycle phase (menstrual, follicular and luteal) in all study participants.

2.1.1.2: Possible methods

There are a number of methodologies that have been implemented in order to define menstrual cycle phases (Table 2.1). The most obvious and well implemented method is by counting menstrual cycle days. This method assumes that cycle duration remains averaged at 28 days. However regular menstrual cycles can range between 25-31 days (Chiazze *et al.*, 1968) and the follicular phase duration can vary considerably (Donaldson *et al.*, 2009). As such, counting forward from the start of menstrual bleeding (day 0) and assuming oestrogen peaks at day 14, with peak progesterone at day 22, is likely to be considerably inaccurate. Furthermore, the counting method does not account for menstrual cycles that are anovulatory and therefore are likely to result in significantly lower progesterone concentrations (Harlow and Ephross, 1995).

Table 2.1: Advantages and disadvantages of the varying methods used to determine menstrual cycle phases

Method	BBT		LH surge		Sex hormones		Cervical Mucus	
	Collection method	Self-collected	Self-collected	Self-collected	Self-collected or trained personnel	Self-collected or trained personnel	Self-collected or trained personnel	Self-collected or trained personnel
Invasiveness		Non-invasive – oral temperature	Non-invasive – in urine		Invasive, depending on collection method		Dependent on collection method	
Sample characteristic		Represents increase in body temperature associated with ovulation	Represents LH surge associated with ovulation		Concentrations represent values over whole menstrual cycle		Consistency and quantity changes during different phases of the whole cycle	
Advantages		Easy to measure and record Daily sampling possible	Daily sampling possible Easy to store Not a biohazard (unless blood contamination)		Accurate phase definition Hormone concentrations measured Various methods of collection (blood, saliva, urine)		Easy to measure and record Daily sampling possible	
Disadvantages		Accuracy subject to other increase in temperature such as fever/infection Only details ovulation, not all phases No known hormone concentrations	Urine sticks can be expensive over time Assays for urine collection – time consuming Only details ovulation, not all phases		Daily samples may be difficult to achieve Hormone assays require specialised equipment Biohazard		Social acceptability in some countries is very low	

BBT: Basal body temperature, LH: Luteinising hormone

More direct methods of determining menstrual cycle phases include measurements of oestrogen and progesterone concentrations within plasma and cervical mucus. Each of these methods has advantages and disadvantages associated with them (Table 2.1).

2.1.1.3: Justification for use

The most accurate method to define menstrual cycle phases is by measuring hormone concentrations directly. The varying methods by which these data can be collected are also an advantage. This also enables the reported variation in cycle and phase duration (Matsumoto, 1962; Vollman, 1977) to be considered in each individual participant.

2.1.2: Oestrogen and Progesterone

2.1.2.1: Objective of assessment

To determine oestrogen (oestradiol) and progesterone hormone concentrations during the menstrual cycle, to enable clear menstrual cycle phase definition based on individual participant hormone profiles.

2.1.2.2: Possible methods

There are a number of different methodologies that have been previously implemented to estimate or measure oestrogen and progesterone concentrations during the menstrual cycle. These include acquiring bodily fluids (urine, saliva or blood) to identify hormone concentrations. Each bodily fluid has both advantages and disadvantages associated with it (Table 2.2); however consideration has to be given to the convenience and practicality of the method for the participants and research team conducting the sample collections.

Table 2.2: Advantages and disadvantages of the varying methods used to measure oestrogens and progestagens

	Plasma / Serum	Urine	Saliva
Collection method	Trained personnel	Self-collected	Self-collected
Invasiveness	Invasive	Non-invasive	Non-invasive
Sample characteristic	Concentration represents a single point in time	Concentration represents a pooled value over time (6-8 hours)	Concentration represents a value over a time
Advantages	Represents active form at one specific time point	Daily sampling possible Less sensitive hormone assay required Easy to store Not a biohazard (unless blood contamination)	Represents the bio available portion of hormone Daily sampling possible Easy to store Not a biohazard (unless blood contamination)
Disadvantages	Collection method requires specific training Sample requires spinning before storage to comply with the HTA. Daily sampling can be difficult Considered a biohazard	Collections have variable water content – requires measurement of specific gravity or creatinine for correction	Very low concentrations present in sample Uses highly sensitive hormone assay. Sample requires spinning before storage to comply with the HTA.

2.1.2.3: Justification for use

The most direct and commonly used method to measure hormone concentrations is in plasma or serum via venous blood, as this represents the most active form of the hormone at one specific time point (Bellem *et al.*, 2011). The method does however require specialist trained individuals to take the sample and is considered a biohazard, therefore storage and disposal guidelines must be adhered to. However the hormones measured are of high concentration and can be replicated following adequate sample collection using venous blood. As a result, the current series of investigations will collect venous blood samples in order to determine plasma concentrations of oestrogen and progesterone. The method and principles used to measure the hormone concentrations will be discussed in section 2.2.14.

2.1.3: Energy Expenditure

2.1.3.1: Objective of assessment

To determine energy expenditure at rest and whilst exercising, during the three phases of the menstrual cycle

2.1.3.2: Possible methods

Energy expenditure can be measured by either the assessment of heat production (direct calorimetry) or by oxygen (O₂) consumption and carbon dioxide (CO₂) production (indirect calorimetry). Direct calorimetry was first described by Lavoisier in the late 18th century and this technique of directly measuring heat production allows energy expenditure to directly be measured; however the technique is highly demanding and as such is now infrequently used.

Indirect calorimetry measures energy production via respiratory gas analysis of O₂ consumption and CO₂ production through the oxidation of protein, carbohydrate (CHO), fat and alcohol. Respiratory gasses can be collected easily at rest and during exercise, and the determination of energy expenditure can be calculated using a validated equation (Jeukendrup and Wallis, 2005).

$$\text{Energy Expenditure (kcal)} = (3.87 \times VO_2) + (1.2 \times VCO_2)$$

In addition, the double-labelled water technique can be used to ascertain an accurate measurement of total energy expenditure in habitual human conditions. The technique requires isotopically labelled water to be consumed, to enable water to be tracked through the body. Based on a number of assumptions, the rate of CO₂ production and energy expenditure can be calculated based on the rates of isotope loss from the body. The advantage of this method is its accuracy and ability to determine expenditure in habitual conditions; however the technique is very expensive and therefore is unable to be used in many studies.

2.1.3.3: Justification for use

In this study indirect calorimetry measurements were made in both resting and exercise protocols to ensure consistency. Respiratory gasses using indirect calorimetry can be collected over short periods of time, with minimal intrusion for

the participant and can be conducted for limited cost which is ideal for the current population studied.

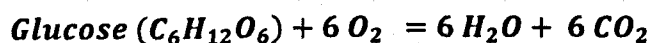
2.1.4: Substrate Oxidation Equations

2.1.4.1: Objective of assessment

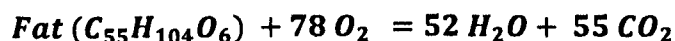
To determine substrate oxidation rates at rest and whilst exercising, during the three phases of the menstrual cycle

2.1.4.2: Explanation of the method

During indirect calorimetry measurements (at rest and exercising) O_2 consumption (VO_2) and CO_2 production (VCO_2) is recorded, enabling the type and rate of fuel oxidation within the body to be calculated (Frayn, 1983). The calculation of substrate oxidation under normal circumstances includes the following equations.



6 mol of O_2 are consumed and 6 mol of CO_2 are produced for each mol of glucose (180g) oxidized, therefore the respiratory quotient ($RQ = VCO_2 / VO_2$) is 1.00.



78 mol of O_2 are consumed and 55 mol of CO_2 produced for each mol of fat (861g) oxidized, therefore the respiratory quotient ($RQ = VCO_2 / VO_2$) is ~0.7.

Protein oxidation can be estimated from urinary nitrogen excretion and most urinary nitrogen is in the form of urea. Therefore, 1g urinary nitrogen arises from approximately 6.25g protein. As such, in a participant oxidising c gram of CHO and f grams of fat per minute and excreting n grams of urinary nitrogen per minute, the total O_2 consumption would be:

$$VO_2 \text{ (l/min)} = 0.746c + 2.03 f + 6.04 n$$

$$VCO_2 \text{ (l/min)} = 0.743c + 1.43 f + 4.89 n$$

To determine the unknown variables of c and f, average CHO and fat oxidation rates can be calculated using VO_2 and VCO_2 . In the current series of investigations it was

assumed that protein oxidation was negligible and therefore n was zero (Jeukendrup and Wallis, 2005). The following equations were used to calculate average CHO and fat oxidation (Jeukendrup and Wallis, 2005).

$$\text{Carbohydrate Oxidation: CHO (g/min)} = (4.21 \times VCO_2) - (2.96 \times VO_2)$$

$$\text{Fat Oxidation: FAT (g/min)} = (1.695 \times VO_2) - (1.70 \times VCO_2)$$

$$\text{Fat Oxidation (\%EE)} = \frac{(FAT \times 9.75)}{EE} \times 100$$

$$\text{Carbohydrate Oxidation (\%EE)} = 100 - \text{FAT (\%EE)}$$

During the exercise protocol implemented in study 3, respiratory gases were collected using a Douglas bag for two minutes every ten minutes whilst participants were exercising (70-80% HR max). A sample of collected expired air was taken for gas analysis (Servomex Gas Analyser, 1440C, Servomex Group Ltd, UK) and total volume expired, VO_2 , l/min and VCO_2 , l/min were calculated. Further details are given in section 2.2.11.

It is assumed that during indirect calorimetry all O_2 consumed and CO_2 produced is derived from oxidative processes. To obtain fat and CHO oxidation concentrations, both O_2 consumption and CO_2 production are therefore required. Protein oxidation is estimated from urinary nitrogen excretion; however it is assumed that the contribution of protein oxidation during rest and exercise to total energy expenditure is negligible (Jeukendrup and Wallis, 2005). Before each testing session the Servomex (Servomex Gas Analyser, 1440C, Servomex Group Ltd, UK) was calibrated for gas concentration analysis with nitrogen (0% O_2 and 0% CO_2 , British Oxygen Company Gases, London UK) and a gas of known concentration (21% O_2 and 8% CO_2 , British Oxygen Company Gases, London UK).

2.1.4.3: Justification for use

The equations (Jeukendrup and Wallis, 2005) used to calculate substrate oxidation in both rest and exercising study protocols remained the same and are the most accurate for the current population. These did not require adjustment as the participants

recruited were of good health and therefore were not subjected to disturbances in metabolism which can be reported in some clinical populations (Frayn, 1983).

2.1.5: Glucose Response and Insulin Sensitivity

2.1.5.1: Objective of assessment

To determine glucose response and insulin sensitivity during the menstrual, follicular and luteal phases of the menstrual cycle

2.1.5.2: Possible methods

2.1.5.2.1: Hyperinsulinemic Euglycemic Glucose Clamp

The most direct method of measuring glucose response and insulin sensitivity is the glucose clamp technique (Table 2.3). This is classified as the 'gold standard' method and directly measures the effects of insulin on glucose utilization under steady state controlled conditions (DeFronzo *et al.*, 1979). There are a number of clamp protocols that can be utilized: the most frequently used being the Hyperinsulinemic Euglycemic Clamp. However, as insulin can be set at different concentrations, this method may stimulate the body to respond outside its normal physiological range to determine the efficiency of either glucose uptake or insulin sensitivity. The validity of a clamp technique depends on the achievement of steady-state conditions and as such requires medical cover and a highly trained individual to perform the procedure.

2.1.5.2.2: Intravenous Glucose Tolerance Test (IVGTT)

This method provides indirect measurements of insulin sensitivity using a minimal model with data obtained via the completion of a frequently sampled IVGTT (Bergman *et al.*, 1979). The minimal model approach uses dynamic data from the IVGTT and assumes that the glucose disappearance is in response to glucose or insulin and occurs at a monoexponential rate. Despite the IVGTT being easier to complete than a clamp, the procedure still requires trained individuals and data collection ranges between as little as 10 minutes post glucose infusion to 300 minutes with the complete procedure including set up being much more time consuming.

2.1.5.3: Oral glucose tolerance test (OGTT)

As the glucose clamp technique can represent values outside of the normal physiological range, OGTT is often the simplest, most easily accessible and physiologically relevant method compared to both glucose clamp and IVGTT (World Health Organization, 2006). The OGTT method allows both the cephalic and oral stages of eating to be included in the methods, which allows the gastrointestinal peptide pathways (including ghrelin) to be stimulated. These are of importance as they are implicated within glucose kinetics and the release of insulin (Broglia *et al.*, 2003; Erdmann *et al.*, 2004; Murphy *et al.*, 2006). In addition, this method does not elevate insulin above its normal physiological range, which potentially allows more physiologically relevant data to be collected. However, unlike the clamp and IVGTT methods, the OGTT does not report insulin sensitivity (IS) *per se*, as glucose tolerance and IS are not equivalent concepts. Glucose tolerance determines how effectively glucose is cleared from the body whereas insulin sensitivity is an indication of how sensitive the body is to the effects of insulin. However the data collected from OGTT can be easily used by surrogate indices to determine IS.

2.1.5.4: Validity of OGTT

The reproducibility of OGTT method can be subject to variable glucose absorption and splanchnic glucose uptake. However, poor reliability and reproducibility of repeat measure in OGTT can be largely attributed to poor pre-test control. Replicating dietary intake and reducing physical activity are imperative to ensure successful duplicate measurements, particularly as these factors can vary energy expenditure and glycogen store depletion (Holloszy, 2005).

2.1.5.5: Justification for use

Given the time/money constraints associated with measuring glucose response and insulin sensitivity using the clamp and IVGTT techniques, OGTT were performed to provide measurements of glucose tolerance and data that could be used to establish IS. As IS cannot be measured directly by performing OGTT a number of surrogate indexes were performed using the data gathered and these are discussed in table 2.3.

Table 2.3: Direct and indirect methods of assessing glucose response and insulin sensitivity

	Hyperinsulinemic Euglycemic Glucose Clamp	Intravenous Glucose Tolerance Test (IVGTT)	Oral Glucose Tolerance Test (OGTT)
General method	Reference or 'gold' standard Constant infusion of insulin to produce hyperinsulinemic state Glucose analysed every 5-10 minutes. 20% dextrose infused to clamp blood glucose concentrations in the normal range (euglycemic)	Bolus of glucose is infused over 2 minutes starting at time 0 Blood samples taken for glucose and insulin concentrations Data are subjected to minimal model analysis	75g glucose bolus orally consumed Blood samples taken regularly for glucose and insulin concentrations for 120 minutes
Sample collection	Intravenous	Intravenous	Intravenous or finger prick
Advantages	Directly measures whole body glucose disposal at a given level of insulinemia under steady-state conditions	IVGTT is easier to use than the clamp Insulin Sensitivity, glucose effectiveness and β -cell function can be derived from a single dynamic test	OGTT reflects the efficiency of the body to dispose of glucose after an OG load. Mimics the glucose and insulin dynamics of physiological conditions more closely than conditions in the clamp or IVGTT
Disadvantages	Time consuming Labour intensive Expensive Requires experienced operator Cost/benefit ratio to be considered Clamp utilizes steady-state insulin concentrations that may supraphysiological	Multiple blood sampling over 3 hour period Model over simplifies the physiology of glucose homeostasis Labour intensive Requires experienced operator	Multiple blood sampling over 3 hour period Glucose tolerance and Insulin Sensitivity are not equivalent concepts - OGTT does not give Insulin Sensitivity per se but the data can be used by surrogate indexes

2.1.6: Sample Surrogate Indexes for Insulin Sensitivity

2.1.6.1: Objective of assessment

To determine insulin sensitivity following an OGTT during the menstrual, follicular and luteal phases of the menstrual cycle

2.1.6.2: Possible methods

2.1.6.2.1: Homeostasis model assessment (HOMA)

This assessment is a model of the interaction between glucose and insulin that is used to predict fasting steady-state glucose and insulin concentrations for a wide range of insulin resistance (IR) and β -cell functions (Matthews *et al.*, 1985). It is widely used and most studies use the simple equation to determine a surrogate index of insulin resistance

$$\frac{\text{Fasting Insulin } \left(\mu \frac{U}{mL} \right) \times \text{Fasting Glucose } \left(\frac{mmol}{L} \right)}{22.5}$$

The model assumes a feedback loop between the liver and β -cell (Matthews *et al.*, 1985). HOMA-IR is closely correlated with estimates of insulin resistance assessed by euglycemic clamp ($R=0.88$), fasting insulin ($R=0.81$) and hyperglycaemic clamp tests ($R=0.69$) (Matthews *et al.*, 1985). However HOMA-IR does report low correlations in patients with advanced type 2 diabetes (Kanauchi, 2002).

2.1.6.2.2: Whole body insulin sensitivity index (ISI)

This index reflects a composite estimate of hepatic and muscle insulin sensitivity determined from OGTT data (Matsuda and DeFronzo, 1999).

$$\frac{10,000}{\sqrt{(FPG \times FPI) \times (Mean OGTT In) \times (Mean OGTT Gl)}}$$

FPG is the fasting plasma glucose concentration; FPI is the fasting plasma insulin concentration; Mean OGTT In is the mean oral glucose tolerance test insulin concentration; Mean OGTT Gl is the mean oral glucose tolerance test glucose concentration. 10,000 represents a constant that allows numbers ranging between 1 and 12 to be obtained and the square root conversion is used to correct the nonlinear distribution of values.

This model has been validated as a tool to measure IS against the hyperinsulinemic euglycemic clamp by using data collected from OGTT ($R=0.73$, $P<0.01$) (Matsuda and DeFronzo, 1999). In addition, this method has been validated in cohorts who display a mix of IS states including normal glycaemia, impaired glycaemia and diabetes (Matsuda and DeFronzo, 1999).

2.1.6.2.3: Insulinogenic Index (IGI)

Early phase insulin secretions can also be calculated as a ratio of the increment of plasma insulin (at 30 minutes) to glucose concentrations (at 30 minutes) using the following equation (Seltzer *et al.*, 1967):

$$\frac{IRI\ 30 - IRI\ 0}{PG\ 30 - PG\ 0} \times n$$

IRI 30 is the plasma insulin concentration at 30 minutes; IRI 0 is the plasma insulin concentration at 0 minutes baseline; PG 30 is the plasma glucose concentration at 30 minutes; PG 0 is the plasma glucose concentration at 0 minutes baseline. n represent the number of observations within the current sample within an individual.

This model has been validated as a tool to measure insulin secretions, comparing OGTT and intravenous glucose tolerance test (IVGTT) using participants who display a variety of IS states (Seltzer *et al.*, 1967). A strong correlation has been reported between first phase insulin response measured using the IGI equation and IVGTT ($r = 0.88$) (Kosaka *et al.*, 1996).

2.1.6.2.4: Disposition Index (DI)

The relationship between IS and insulin secretions can be measured using the following equation (Bergman *et al.*, 1981):

$$IGI \times ISI$$

IGI is the total score representing the Insulinogenic index; ISI is the total score representing insulin sensitivity index.

The index is a measure of tolerance which includes the contributions of the individual factors that are related to insulin. It has been suggested that the relationship between insulin sensitivity and insulin secretions is approximately

hyperbolic and therefore the product of the two variables is constant in individuals with the same degree of glucose tolerance (Kahn *et al.*, 1993).

2.1.6.3: Justification of use

HOMA-IR is the most widely used and simplest equation to determine a surrogate index of insulin resistance. In addition, whole body insulin sensitivity index (ISI) is also validated as a tool to measure insulin sensitivity from OGTT data, with insulinogenic index (IGI) also validated by OGTT for early phase insulin secretions. A combination of these validated indices was used following the completion of OGTT data collection, to ascertain possible IS and insulin resistance changes during different phases of the cycle.

2.1.7: Premenstrual Syndrome (PMS) assessment

2.1.7.1: Objective of assessment

To determine PMS symptoms, establish correct diagnosis and detect any changes in symptoms experienced.

2.1.7.2: Possible methods and critique

There is currently great concern surrounding whether the PMS phenomenon actually exists, moreover what the exact definitions should be, for example how to measure mood and well-being and how to differentiate between distress and normal states.

The initial assessment tools in the 1930's were based on correlations of daily psychoanalytic sessions based on dream analysis with physiological assessments of the menstrual cycle (Benedek and Rubenstein, 1939a; Benedek and Rubenstein, 1939b). However this more systematic investigation was rare and the majority of studies based their diagnosis on distress at clinical impression. However this approach was criticised based on its purely clinical perspective (Coppin and Kessel, 1963).

The definition of PMS combines a number of different symptoms and as a result a clear diagnosis can be difficult to achieve. Currently there is no biological test to assist in the diagnosis of PMS; therefore the diagnosis is based purely on accurately reported symptoms. There are over 300 possible symptoms that have been associated

with the luteal phase of the menstrual cycle; however there are only a small number that are regularly present within PMS assessment tools (Halbreich, 2003). Current recommendations include daily or alternate day symptom ratings (Moos, 1968; Royal College of Obstetricians and Gynaecologists, 2007) as this enables the essential component of determining the severity of symptoms during the luteal phase and the extent of the reduction during menstruation to be reported.

As a rule, any assessment tool used should be relevant to the population who experience the condition to be measured. A reliable assessment technique should assess the direct experience of menstrual cycle symptoms rather than any possible learned responses and it is critical that the tool distinguishes women who experience PMS from other disorders that include gynaecological and affective disorders. Additionally it should differentiate the severity of symptoms experienced by women according to the recognized diagnostic criteria.

The current criterion that is universally implemented is derived from the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) or newly released DSM V guidelines. These guidelines suggest daily symptoms ratings over two months are required to obtain a diagnosis of PMS or PMDD (premenstrual dysphoric disorder) (American Psychiatric Association, 2013). The definition of PMS requires women to experience at least one affective symptom during the final week before the onset of menstruation, which start to improve within a few days of the onset of menstruation (American Psychiatric Association, 2013). However the definitions in DSM have been criticised as focussing on affective experiences rather than somatic and excluding women with physical symptoms only (O'Brien, 1993). As such it has been suggested that the DSM definition of PMDD is only one example of a broader range of PMS experiences, which still remains open for interpretation and variation between research groups and clinicians (Gallant *et al.*, 1992a, b). However, as with any questionnaire a negative emphasis can be created and the possibility of the creation of expectations and self-fulfilling prophecies is abundant.

A number of other different PMS symptom tools have been discussed, detailing positive and negative aspects of each tool.

2.1.7.2.1: Retrospective Questionnaires

2.1.7.2.1.1: Menstrual Distress Questionnaire (MDQ)

The first attempt at a PMS symptom questionnaire using psychometric principles was made by Rudolf Moos in the 1960's and is known as the Menstrual Distress Questionnaires (MDQ) (Moos, 1968). The MDQ is one of the most widely used in menstrual cycle research and has had 15 other questionnaires developed as a result, but is also one of the most widely criticised (Richardson, 1990). The questionnaire is divided into two forms (Form C and T), with 46 individual symptoms on each form, divided into 8 key groups. These include pain, water retention, autonomic reactions, negative effect, impaired concentration, behaviour change, arousal and control. Form C is used as a screening tool to enable women to report retrospective symptoms experienced during each of the three phases of the most recent menstrual cycle (premenstrual/luteal, intermenstrual/follicular and menstrual). Form C is designed to identify the potentially significant symptoms experienced (Moos, 1968). The internal consistencies and correlations have also been reported in the design of this questionnaire and are moderate to high. Together with Form T, this questionnaire provides both retrospective and prospective symptom analysis.

2.1.7.2.1.2: Premenstrual Assessment Form (PAF)

This questionnaire is the most comprehensively developed to assess premenstrual symptoms and includes 95 items which cover changes in mood, behaviour and physical condition (Halbreich *et al.*, 1982). These items were selected from a pool of over 200 symptoms derived from previous literature; the items were reduced in order to reduce overlap. The final 95 items were clustered into 18 groups, with the construct validity and concurrent validity being confirmed (Beere, 1990). Construct validity is the validity by which the measurement tool (questionnaire) actually represents the construct that is being investigated, whereas concurrent validity is an indication that the test correlates well with a similar measure that has been previously validated. A previous investigation gave women who were previously diagnosed with PMS ($n = 30$) and controls ($n=16$) the PAF to complete. The results reported high sensitivity but low specificity which allowed differentiation between groups but failed to provide enough information to diagnose PMS (Pires and Calil,

2000). To complete the PAF, 30 minutes is required and therefore may be impractical in some research settings (York *et al.*, 1989).

2.1.7.2.1.3: Premenstrual Symptom Screening Tool (PSST)

This tool is quick and easy to use and is designed as a screening assessment method to identify PMS sufferers, measuring both the severity and impact of symptoms (Steiner *et al.*, 2003). The tool was developed as a less time consuming and potentially more practical assessment than the 1-2 cycle prospective charting often recommended. The PSST reflects and 'translates' the complex categorical DSM-IV criteria, with measures of symptom severity (Steiner *et al.*, 2003). The validity of this screening tool has been established in the vigorous evaluation of 519 women (Steiner *et al.*, 2003). The results suggest good correlation between its findings and those previously using prospective diagnosis methods (Gehlert and Hartlage, 1997; Sternfeld *et al.*, 2002). There are a number of limitations that are highlighted whilst using the screening tool, predominantly the inability to differentiate between non PMS and mild PMS categories in some cases (Steiner *et al.*, 2003). However the tool is simple to use and provides a quicker and easier diagnosis method to identify PMS individuals compared to the PAF.

2.1.7.2.2: Prospective Questionnaires

2.1.7.2.2.1 : Daily Symptom Report (DSR)

The 17-item DSR lists a number of key items required for PMS diagnosis. It is designed to be an easy to use brief tool that can be administered daily over extended periods of time. There have been a number of studies using the DSR method that report significant changes in symptom scores after treatments (Freeman *et al.*, 1996). As a result a validity study has been conducted comparing a PMS group (n=170) to a non-PMS group (n=54) to examine the reliability of the DSR. The study reports high internal consistency of the DSR (17-items), indicating that the measure is a reliable index to diagnose PMS. The study also suggests that PMS has a moderate overlap with a number of conditions that are also associated with the menstrual cycle. As a result the DSR is able to measure the broader collection of PMS symptoms (Freeman *et al.*, 1996).

2.1.7.2.2.2: Menstrual Symptomatology Questionnaire (MSQ)

This instrument was developed from the Menstrual Distress Questionnaire (Moos, 1968), with the author removing 25 items and replacing them with 5 theoretical constructs collectively named Premenstrual Tension Syndromes (PMTS). The author postulated that premenstrual distress was caused by small but not exclusive nutritional imbalances and that these imbalances would result in 5 specific groups of symptoms (Abraham, 1980). Individual items were rated out of three (1 – mild; 2 – moderate, 3 – severe; Table 2.4).

Table 2.4: Classification of Premenstrual Tension Syndromes using the Menstrual Symptomatology Questionnaire (MSQ) developed by Abraham (1980).

PMT – A	PMT – H	PMT – C	PMT – D	PMT – P
Anxiety	Weight gain	Increased appetite	Depression	Pain
Irritability	Abdominal	Sweet food cravings	Withdrawal	
Nervous	bloating	Fainting	Suicidal	
tension	Breast congestion	Fatigue	ideation	
	Mastalgia	Palpitation	Lethargy	
	Oedema (face and	Headache	Confusion	
	extremities)	Tension	Incoherence	

Diagnosis of PMTS requires at least one subgroup of symptoms to be scored as moderate to severe the week before menstruation, with the same subgroup reported as mild or no symptoms after menstruation. This criteria does allow for the variation in symptoms experienced to be recorded; however this may lead to the over inclusion of participants compared to the adopted American College of Obstetricians and Gynaecologist (ACOG) definitions.

Previous reports consider the validity of the MSQ to be accurate considering the robust theoretical formulation Abraham presents to define PMTS subtypes (Beere, 1990). However without external validation this does not represent a useful instrument to measure PMS symptoms, particularly as there is little evidence to support the specifics of Abraham's theories

2.1.7.2.2.3: *Menstrual Distress Questionnaire (MDQ)*

Form T of the MDQ developed by Moos (Moos, 1968) enables symptoms to be reported on a specific day and is used to ascertain the exact changes and prevalence of PMS symptoms during the cycle phases. In general, a 30% change in symptoms between the luteal/menstrual and follicular phases is required to be diagnosed with PMS. Form T is often used to indicate the beginning of treatment and it has been reported that the use of self-report instruments encourages women to observe the symptoms more accurately. As a result this enables the identification of experiences and techniques that may alleviate symptoms (Moos, 1968).

2.1.7.2.3: *Validity of MDQ*

The reliability and validity of the MDQ questionnaire has been previously confirmed by reports of significant correlations between original symptom scores and the second menstrual cycle scores ($n=15$). The internal consistencies and correlations have also been reported in the design of this questionnaire and are moderate to high. The subscales range from 0.92 – 0.64, with high internal consistencies reported in other investigations (Monagle *et al.*, 1993; Rose *et al.*, 2008; Sigmon *et al.*, 2000a; Sigmon *et al.*, 2000b).

There are some limitations to the MDQ assessment tool, predominantly based around the sample population used in order to assess the validity of the questionnaire. The final questionnaire was based on the responses of 839 wives of graduates at a large Western university. As such the MDQ is heavily criticised and from a psychometric perspective has inadequate scale construction and factor analysis for the subscales (Richardson, 1990). The large sample used to determine the questionnaires validity (839 housewives) was very homogenous in age (25.2 ± 3.9 y) and years of education (15.2 ± 1.7 y). Indeed, when validated against the responses of an older population, the scores and factor structure of the questionnaire changes (Morse and Dennerstein, 1988). As such the data were reported from a very limited source and may not represent all women that are likely to experience PMS (Siegel *et al.*, 1987).

In addition, one researcher obtained the responses of men and women to determine the validity of the MDQ, asking both sets of participants to rate each item according

to their perception of what women experience. The results indicate a similar correlation between the women in both studies, however Parlee (1974) suggests that a stereotype of menstrual experiences may be influencing both men and women's accounts of symptoms and suggests that the questionnaire focuses primarily on the negative experiences of PMS, which may mislead the participant (Parlee, 1974).

Further criticism is given concerning the number of experiences listed on the MDQ questionnaire (47 experiences). Steiner *et al* (1980) suggests that the MDQ is too long to effectively measure different changes in PMS symptoms, whereas Halbriech *et al* (1982) indicates the MDQ is too short and therefore is unable to increase the sensitivity for differentiating among different types of changes. As such, Halbriech *et al* (1982) devised the Premenstrual Assessment Form (PAF) to provide greater variety of items and different types and subtypes of PMS to be diagnosed.

It has been suggested that PMS is experienced throughout women's reproductive years regardless of background or ethnicity, increasing in prevalence with age and number of natural cycles (Warner and Bancroft, 1990). As a result the MDQ may only be reliable using the demographic population that it was developed with (Sveindottir and Backstrom, 2000).

2.1.7.2.4: Justification for use

As the current investigation studied women of reproductive age (18-40 y) to establish the effects of an exercise intervention of PMS symptoms, the use of MDQ to determine symptoms is believed to be an effective and validated tool. The questionnaire is also well used within the scientific community and covers symptoms reported to be present under the new DSM 5 criterion.

2.1.7.2.5: Component structure of questionnaires

The use of the prospective symptom questionnaires is widely accepted in the literature as the most appropriate method of investigating PMS, with item-specific scales highly recommended due to their ease of use and sensitivity to treatment effects (Bryant and Dye, 2004; Steiner *et al.*, 1999). Many questionnaire developers utilize a Principal Component Analysis (PCA) to reduce the extensive data collected by the questionnaires into a more manageable size and increase the structure of the

symptoms recorded (Allen *et al.*, 1991; Choi and Salmon, 1995; Freeman *et al.*, 1996; Moos, 1968).

The DSR (Freeman *et al.*, 1996) divides its 17 symptoms into 4 components, describing mood, behavioural, pain and physical symptoms. Its use as a diagnostic tool is widely adopted (Bryant *et al.*, 2005) and has been used to assess the effect of different treatments (Freeman *et al.*, 1994; Freeman *et al.*, 1995; Freeman *et al.*, 1993). However criticism has surrounded this questionnaire as components should be made up of more than three items (Zwick and Velicer, 1986) and the DSR has only two items (symptoms) within its physical component (Canning *et al.*, 2012). This method of component extraction is criticised for severely overestimating component numbers (Fabrigar *et al.*, 1999; McWilliams *et al.*, 2001), which could result in over emphasising the minor components.

A recent study by Canning *et al* (2012) suggests these approaches would benefit from reducing the number of components to two, physical and psychological. The authors modified the DSR questionnaire to include 20 symptoms and divided them into two components, instead of the original 17 symptoms and 4 components. The study suggests that the use of the two component model provides a more practical and clinically relevant means to investigate and identify PMS (Canning *et al.*, 2012). The study reports psychological symptoms were more important when determining if women suffered with PMS and not and indicated that the severity of both physical and psychological symptoms was greatest in the PMS group compared to control demonstrating that the two components could aid in the determination of PMS sufferers (Canning *et al.*, 2012).

The MDQ designed by Moos (1968) categories its 46 symptoms into 8 components (pain, water retention, autonomic reaction, negative affect, impaired concentration, behaviour change, arousal and control). This large number of components could potentially lead to an exaggeration of symptoms changes similar to Freeman *et al* (1996) DSR approach. If a two component approach was implemented using the MDQ symptoms, then potentially categories pain, water retention, autonomic reaction and impaired concentration would fall into the physical component with

negative affect, behaviour change and arousal categorised in the psychological component.

As the current investigation implemented an exercise intervention it is possible that a two component approach would be useful in determining if the changes in symptoms recorded in response to the exercise were specifically physical or psychological rather than arbitrary categories that may be difficult for the general population to understand.

2.1.7.2.6: Prospective vs. Retrospective symptom analysis

As with all research that is conducted in women that suffer from PMS, the method by which the diagnosed symptoms are assessed can be a source of unreliability and misclassification. Many studies implement either a retrospective or prospective assessment of symptoms using a number of different questionnaires. Current literature extensively promotes the use of prospective symptom analysis compared to retrospective analysis (Royal College of Obstetricians and Gynaecologists, 2007). This is generally administered in the form of a questionnaire which includes daily or alternate day symptoms ratings (Moos, 1968; Royal College of Obstetricians and Gynaecologists, 2007) as this enables the essential component of determining the severity of symptoms during the luteal phase to be reported. Previous reports suggest prospective analysis regularly demonstrates a significant cyclic fluctuation in somatic and psychological symptoms (Moos, 1968). With similar research suggesting that women may recall symptoms in retrospective analysis by relating them to their previous beliefs about PMS symptoms (Mcfarland *et al.*, 1989).

However, as many women may only experience symptoms for one or two days during the luteal phases the most intense symptoms are most likely to be reported in retrospective analysis as it relies on recall (Woods *et al.*, 1982). As a result, daily symptom ratings in prospective assessment may average the severity of symptoms experienced as a result of the number of symptom free days also present within that phase. Consequently, this may lead to an artificially lower symptom level (Sampson and Prescott, 1981). Nevertheless, retrospective analysis may also lead to an over exaggeration of symptoms experienced in different phases.

2.1.7.2.7: Justification for use

There have been some suggestions that results from both prospective and retrospective methods are similar in symptom analysis (Schilling, 1981; Taylor, 1979), with two studies reporting high consistency of symptoms using both methods (Abplanalp, 1983; Haskett *et al.*, 1980). It therefore may be more accurate to use both prospective and retrospective analysis in recording PMS symptoms.

However the retrospective nature of some questionnaires is subject to a large response bias as a result of stereotypes, expectation, and poor memory or inters cycle variability (Parlee, 1974; Richardson, 1990). As such the work of Parlee (1974) alongside Ruble (1977) has convinced the medical and clinical research community that prospective symptom assessment is the most effective method of recording PMS symptoms. As a result, both prospective (MDQ Form T) and retrospective (PSST and MDQ Form C) symptom questionnaires will be administered (chapter 5).

2.1.7.2.8: Timing of PMS symptom analysis

The definition and accuracy of PMS symptoms experienced relies heavily on the exact timing of symptom measurement during the menstrual cycle. Much uncertainty currently surrounds the exact timing of symptoms during the menstrual cycle and which should be included under the diagnosis criteria of PMS. The ACOG definition currently states symptoms occurring 5 days before menses and reducing within 4 days of menstruation are regarded as PMS (American College of Obstetricians & Gynecologists, 2000). However, it has been suggested that this definition is too narrow and may exclude some women (Bancroft, 1993). There are variations of this definition and these include 7 days before menstruation with up to 7 days after menstruation, representing the luteal and follicular phases respectively (Thys-Jacobs *et al.*, 2007; Walker *et al.*, 1998). However it is important to ensure that recorded symptoms are not an exacerbation or worsening of other physical or mental disorders, particularly during menstruation.

Further research has reported the variability in the number and range of days PMS symptoms are experienced, making its diagnosis criteria further complicated. Research has identified symptoms to be substantially worse 6 days before the onset of menstruation with a peak at 2 days before menstruation (Angst *et al.*, 2001;

Pearlstein *et al.*, 2005). The results also identified that 34-46% of women studied (n=279) also reported moderate to severe symptoms on day 1 of menstruation. The most common symptoms reported included anger/anxiety (76%), anxiety/tension (71%), tired/lethargic (58%) and mood swings (58%) (Pearlstein *et al.*, 2005), all of which are commonly assessed in all PMS symptom questionnaires. However the results did indicate that peak day varied by up to 4 days in 45% of women studied (Pearlstein *et al.*, 2005). This suggest that a range of 7-8 days before the onset of menstruation and at least one menstruation day should be included in premenstrual or luteal phase symptom analysis (Pearlstein *et al.*, 2005).

The most recent guidelines have proposed the assessment phase be extended up to 14 days prior to menstruation including up to 5 days during menstruation, with symptoms occurring for between 2 and 14 days (Halbreich *et al.*, 2007). The symptoms should also be asymptomatic (non-existent to mild) during the follicular phase (approximately day 6 to 10) (Halbreich *et al.*, 2007). The symptoms must also be associated with impairment in daily functioning causing suffering, emotional or physical distress.

2.1.7.2.9: Justification for use

As a result, the current investigation required PMS symptoms to be at least 30% greater during the luteal phase compared to the follicular phase. Symptoms during the luteal phase were defined as up to 10 days prior to menstruation including the first day of menstruation. The follicular phase began from the end of menstruation and was defined by at least 5-7 asymptomatic days before the luteal phase. These definitions were flexible in order to account for variations in phase and total cycle duration between participants.

2.1.8: Quality of Life (QoL) assessment

2.1.8.1: Objective for assessment

The objective of this assessment was to determine the effects of an exercise intervention on overall quality of life (QoL). QoL is a term that describes an overall sense of well-being, including aspects of happiness and satisfaction with life as a

whole. As such, it is a broad and subjective measure as opposed to specific and objective (Centers for Disease Control and Prevention, 2000).

2.1.8.2: Possible methods

World Health Organisation Quality of Life (WHOQOL-100)

This questionnaire is one of the most extensive and well used QoL questionnaires to date. The original WHOQOL-100 questionnaire was developed from a genuine need for an international measure of QoL and to incorporate a promotion of a holistic approach to health not simply medical. The questionnaire consists of 100 items which included four items of each of the 24 facets of quality of life, and four items relating to the overall quality of life and general health facet. These were then grouped into six domains.

World Health Organisation Quality of Life (WHOQOL-BREF)

Following the administration of the WHOQOL-100 to the scientific community it was noted that the WHOQOL-100 was too lengthy for practical use, therefore the WHOQOL-BREF was developed to provide a short form of QoL assessment. The WHOQOL-BREF contains 26 questions, with one item from each of the original (WHOQOL-100) 24 facets and an additional two items from the overall quality of life and general health facet. These items were grouped into four domains known as physical health, psychological, social relationships and environment.

However both questionnaires have strict usage and guidelines attached to ensure they are used as an appropriate QoL assessment tool. The WHOQOL guidelines state that a minimum of 300 participants are required to ensure the instrument is piloted correctly. For these reasons it was not feasible to use either of these two questionnaires within the current research investigations.

Health related quality of life (HRQoL)

The health related quality of life (HRQoL) questionnaire was administered at the end of each completed menstrual cycle to retrospectively analyse the effects of the exercise intervention of QoL (appendix 2). To determine changes in QoL, the number of unhealthy days (mental and physical) were added together and subtracted

from 30 (approximate days in one month). Similarly, the number of specific healthy days ranging from tension, sleeplessness, physical pain was also recorded.

2.1.8.3: Justification for use

Due to the strict usage guidelines imposed with the WHOQOL questionnaires, it was deemed inappropriate to use them in the current investigation. The validity of the HRQoL questionnaire has been extensively reported for use in both general and clinical populations, assessing its construct, criterion, concurrent and predictive validity (Newschaffer, 1998) and as such makes it an ideal tool for the current population.

2.1.9: Dietary Assessment

2.1.9.1: Objectives for assessment

To determine dietary intake during different phases of the menstrual cycle in women suffering from PMS

2.1.9.2: Possible methods

There are several methods that can be implemented to assess dietary intake in humans including carefully controlled laboratory settings which have been previously described in varying forms including an assessment of their limitations (Benelam, 2009; Hill *et al.*, 1995). Alternative methods of recording dietary intake include the use of food diaries or records and can be in a variety of forms. These include weighed food diaries (2, 3, 5, 7 days in duration), food frequency questionnaires (FFQ) and 24 hour dietary recalls; each possesses a number of strengths and limitations associated with their use (Table 2.5).

2.1.9.3: The 24 hour dietary recall interview

The method most suited to the current population demographic and the objectives is the 24 hour dietary recall (Nelson, 2003). This method of dietary assessment allows quantified and detailed intake to be assessed with increased precision. Due to its retrospective nature, the technique also requires minimal literacy and burden to each participant and does not cause dietary behaviour modifications that often occur using other dietary assessment methods (Buzzard *et al.*, 1996). However, its main

limitation includes the reliance upon knowledge and accurate memory of the participant.

The 24 hour dietary recall interview involves a three multi-pass method whereby the participant is asked to recall their intake from the beginning of the day, working through each item. The multi-pass method is able to stimulate participant's cognitive recall in greater detail by placing the dietary intake within context rather than over-load in specific detail more commonly used in the single-pass method. As a result the number of items missed is greatly reduced and therefore increases the accuracy of the dietary intake recall (Jonnalagadda *et al.*, 2000).

The method uses at least three passes during the interview, each with a different emphasis (Conway *et al.*, 2003). The first pass involves the use of a 'quick list' and involves minimal input from the interviewer. The list identifies the main foods consumed and the location at which they were consumed. This forms the basis of a basic structure to the previous day and allows any obvious gaps in meals to be identified.

The second pass allows much more detail to be added concerning the exact food description, specific brands consumed, cooking methods implemented or portion sizes. The interviewer uses open questions in order to gain greater detail, with food portion photographs used to prompt recall for common food items. In addition, familiar standard portion sizes can also be used as prompts to estimate similar items, such as a can of cola. More specific questions can also be asked by the interviewer during this pass in order to ascertain greater detail, including 'did you add milk/sugar in your tea?' or 'was the bacon fried or grilled?' The final pass reviews the more detailed list by reading the entire list of items back to the participants to determine any inconsistencies and stimulate any additional recall.

2.1.9.4: Use of food portion photographs

The use of food photographs, memory aids or food models has been demonstrated to increase the accuracy of 24 hour dietary recall data (Chambers *et al.*, 2000). The current investigation used comprehensive reference food photographs (Nelson, 2002).

Table 2.5: Dietary assessment methodology strengths and limitations

Dietary Assessment	Strengths	Limitations
Food Diaries (varying durations)	Variety of application approaches with frames <24 hours. Can be pre-coded or open weighed Quantified (if weighed) and detailed intake; open ended format Relatively accurate information on individual meal, eating frequency and cooking practices	Requires literacy High burden on participant and researcher Multiple days required. Possible decrease in completion quality over time Recording alters usual eating habits High underreporting with differences between normal and obese individuals
Food Frequency Questionnaire (FFQ)	Low administration cost Low participant burden Attempts normal intake in one assessment Source of information on true non-consumers	Requires literacy Can produce incomplete data sets High measurement error (small food lists, no quantification, imprecise estimation of portion size) Used for population data rather than individual data
Food Recall (24 hour)	Variety of application approaches Paper vs. computer, in-person vs. telephone Portion size models vs. measurement aids Literacy not required and Low participant burden Quantified and detailed intake Information on individual meal, eating frequency and cooking practices Does not affect eating behaviour	Relies on knowledge and accurate memory Multiple days required to estimate typical intake Likely to omit infrequently consumed foods Possible interviewer bias

2.1.9.5: Validity of multiple-pass 24 hour dietary recall

No self-reported dietary instrument can measure dietary intake completely error free (Illner *et al.*, 2012). Nevertheless research has reported 24 hour dietary recall displays good validity and measurement property compared to food diaries and FFQ (Carroll *et al.*, 2012; Prentice *et al.*, 2011; Schatzkin *et al.*, 2003). In addition, when comparing 24 hour recalls to weighed food records previous studies have reported similar observed intakes in adults (Gersovitz *et al.*, 1978; Harrison *et al.*, 2000). However previous national dietary surveys using multiple-pass methods, suggests that underreporting may affect up to 15% of all 24 hour dietary recalls (Krebs-Smith *et al.*, 2000).

There are a number of factors that increase misreporting in dietary assessment including BMI, age, sex and physical activity (Ballardbarbash *et al.*, 1996). The greatest effect being BMI, as overweight and obese participants are the most likely to underreport and underweight participants are the most likely to over-report dietary intake (Rennie *et al.*, 2007).

When comparing different dietary intake methods, previous evidence has demonstrated the least under-reporting recorded as a result of the multiple-pass 24 hour dietary recall methodology compared to the FFQ; 7-day food record; 1-day self-administered and single-pass 24 hour recall (Sawaya *et al.*, 1996). However the results maintained a high degree of variation as experienced in all dietary intake assessment techniques, with some under-reporting and some over-reporting recorded.

2.1.9.6: Justification for use

24 hour dietary recall interviews demonstrate the least under-reporting of all the dietary assessment techniques. As a result this method was conducted by trained personnel, once weekly during each phase of the menstrual cycle throughout the duration of study 3 (appendix 3). The 24 hour Triple Pass interview technique was implemented with food photographs to aid in the participant recall process (Nelson, 2002) (appendix 4).

2.2: Common Methods

2.2.1: *Ethical approval*

Ethical approval for all studies was obtained from Oxford Brookes University Research Ethics Committee (UREC) in accordance to the guidelines laid down by the Declaration of Helsinki prior to study initiation (UREC registration number: 100470 and 110571). UREC ethical approval letters and participant consent forms are shown in appendix 5 and 6. The approval covered all aspects of the study protocols, information sheets and recruitment procedures.

All participants gave written informed consent before taking part in any of the studies and were free to discontinue the study at any point. On completing all elements of the study protocol, participants were debriefed and given compensation for their time.

2.2.2: *Participant recruitment*

Participants for each of the studies were recruited using a number of different strategies. These include advertisements via posters, social-networking sites, tailored-websites and personal communications (appendix 7). Participants were aged 18-40 y and members of the public from the Oxford area.

2.2.3: *Inclusion criteria*

- **Female, aged 18-40 y;** Women in this age group experience the least variability in their menstrual cycle and therefore are likely to provide the best results when measuring oestrogen and progesterone concentrations (Chiazze *et al.*, 1968; Vollman, 1977).
- **BMI: 18.5 – 30 kg/m²;** Women in this BMI range are less likely to experience adverse health problems and the least variability in their menstrual cycle.
- **Fasting blood glucose: <6.0 mmol/L;** This is the standard glucose concentration cut-off implemented to indicate a lack of pre-diabetes (Brouns *et al.*, 2005).

Study 3 only

- **Suffer from premenstrual syndrome (PMS);** Women were required to experience a 30% increase in PMS symptoms during the follicular and luteal phase of the menstrual cycle as assessed by the MDQ and PSST questionnaire (further details below).
- **Exercise <1hour per week;** To determine the effects of the exercise intervention on PMS symptoms, participants were required to be sedentary to limit the effect of external exercise regimes on the intervention and results.

2.2.4: Exclusion criteria

- **Taking any prescription medication, no genetic or metabolic diseases;** This exclusion criteria reduces the number of external variables that may influence the expected study outcome as well any potential effect on oestrogen and progesterone concentrations.
- **Taking any form of hormonal contraception, given birth <2y, were lactating or breastfeeding, pregnant, or presented with <3 previous regular menstrual cycles;** These factors potentially result in abnormal menstrual cycle patterns. Women were required to be regularly menstruating and therefore experience the 'normal' fluctuations of both oestrogen and progesterone.

2.2.5: Study Protocols

A standard protocol was implemented during each study methodology to ensure consistency between study designs (Figure 2.1 and 2.2).

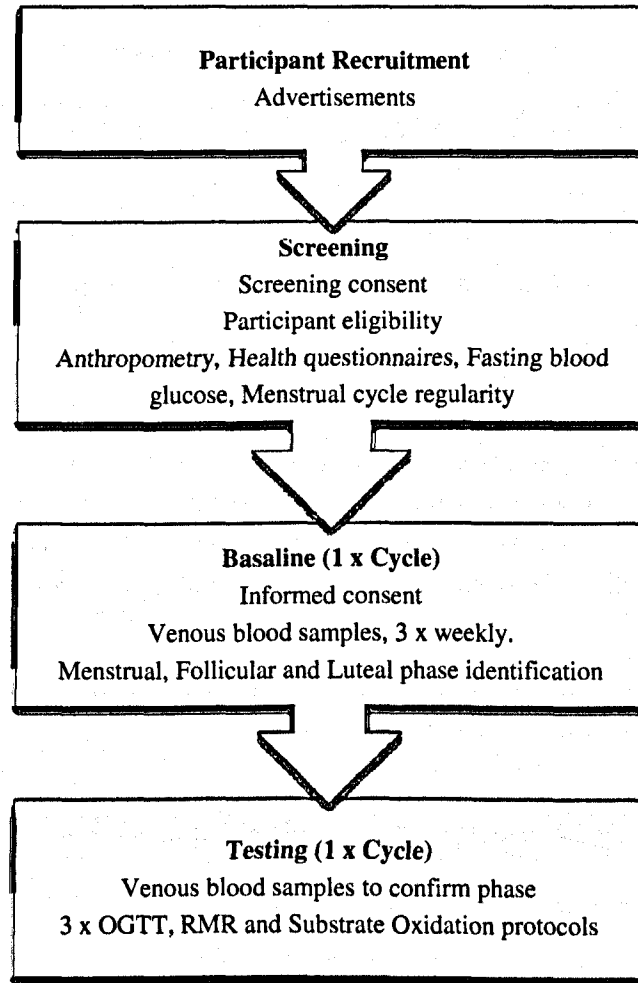


Figure 2.1: Study protocol implemented during study 1 and 2 including stages of recruitment and data collection

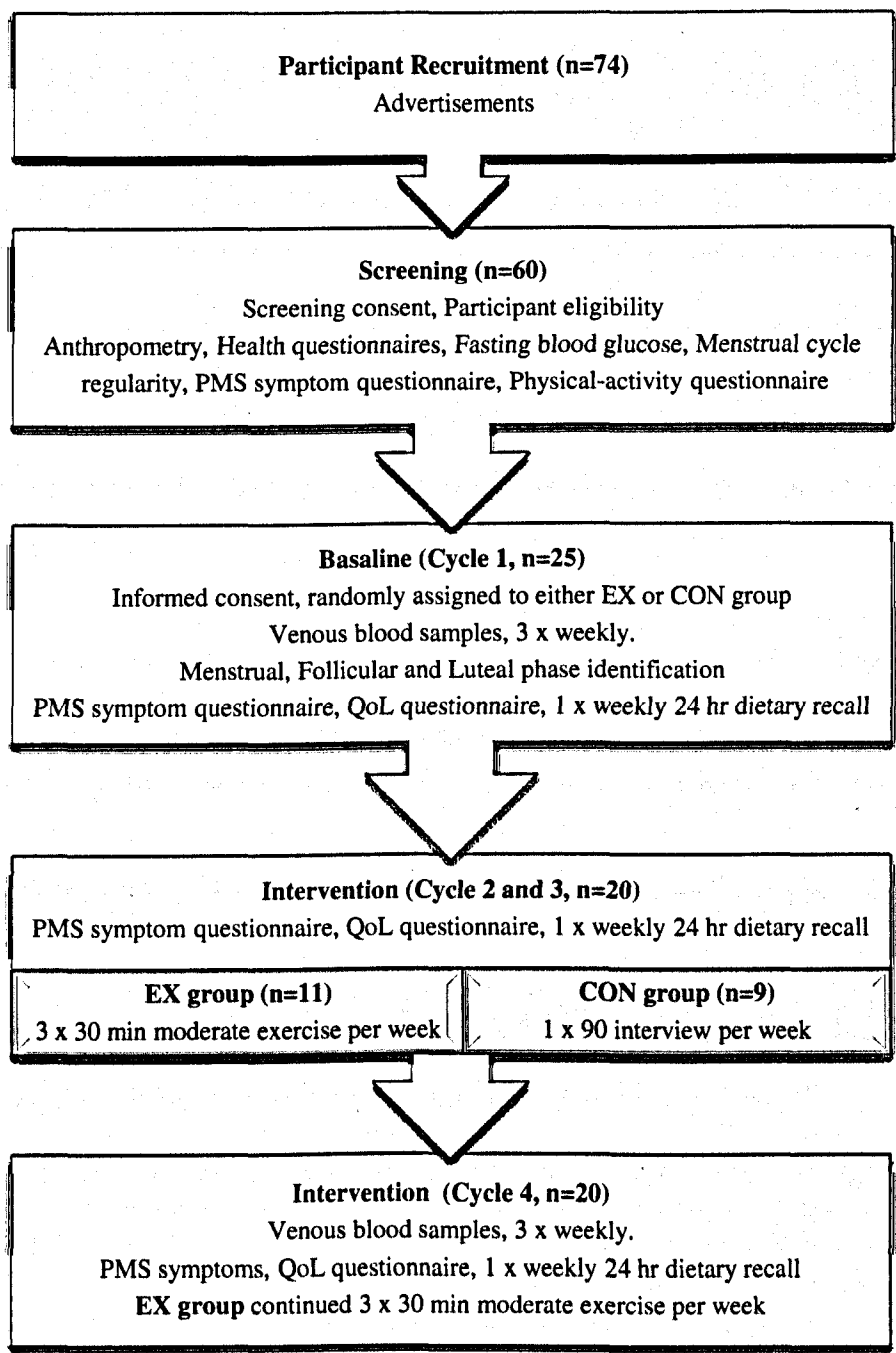


Figure 2.2: Study protocol implemented during study 3 including stages of recruitment and data collection.

2.2.6: Power Calculations and Randomisation

Power analysis for each investigation was achieved by G*Power software (Ver. 3.0.10, Universitat Kiel, Kiel, Germany), using data from the existing literature to determine sample size required. A 10% dropout rate was added to each study to ensure the power calculation was met.

For the first study, investigating the glucose and insulin response during the three phases of the menstrual cycle the power analysis calculations were based on results from a previous study completed by Jarrett and Graver (1968). In order to have the power (0.8) to detect a significant effect (0.772) in glucose response a sample size of 16 participants were required. Taking into account the ~10% drop out rate associated with longitudinal studies a total sample size of 18 participants were recruited.

To investigate energy expenditure and substrate oxidation during the menstrual cycle, the power analysis calculations were based on data from a previous study conducted by Howe *et al.* (1993). In order to have the power (0.8) to detect a moderate significant effect (0.724) in energy expenditure, 18 participants were required. Taking into account the ~10% drop out rate, 20 participants should have been recruited however only 19 participants were recruited.

To investigate the effects of an exercise intervention on PMS symptom scores, the power analysis calculations were based on the results from a previous study conducted by Steege and Blumenthal (1993). In order to have the power (0.8) to detect a significant effect (0.449) in PMS symptom scores, 41 participants were required. Taking into account the ~10% drop out rate associated with longitudinal training studies a total sample size of 46 were required. However, due to time and budget restrictions a total of 25 participants were recruited to this study.

Participant randomisation occurred in study 3 to determine which participants would be assigned to the exercise intervention group or control group. Participant numbers (1-46) were randomised using an online randomisation calculator which generated two groups of numbers (n=23 each), representing the exercise and control group (www.randomizer.org).

2.2.7: Screening

Following the expression of interest through the implemented participant recruitment techniques, individuals were sent further study details via email and invited to a screening visit. During the screening visit, the individuals were asked to complete a self-reported health questionnaire, were subjected to an anthropometric assessment and signed an informed consent form (appendix 10).

Once the individual completed the screening visit, eligibility to the study protocol was determined. If eligibility was confirmed arrangements for Baseline measures to begin were scheduled and individuals were then given a participant number. If the individual was not eligible for the study, the exact reasons were explained clearly and sensitively to them.

2.2.8: Baseline

2.2.8.1: Blood collection and storage

Venous blood samples (6ml) were collected three times per week (Monday, Wednesday and Friday) in the current series of investigations via the venepuncture technique, using one-use holders, needles and ethylenediaminetetraacetic acid (EDTA)-containing tubes (K2E 10.8mg, Becton Dickinson, UK) (Figure 2.3).



Figure 2.3: Venous blood samples collected in EDTA-containing tubes (K2E 10.8mg, Becton Dickinson, UK).

Blood collected into the EDTA-containing tubes was centrifuged at 4°C, 4000 rpm for 10 minutes (MC-6; Sarstedt Ltd., Leicester, UK) and aliquots of plasma were stored at -80°C for future hormone analysis. Exact hormone analysis methodology is described in section 2.2.14.

Hormone concentrations of oestrogen and progesterone were used to determine menstrual cycle phases in each individual participant in all investigations. The menstrual phase was defined as low oestradiol and progesterone concentrations (20-60 pg/ml and <2ng / ml respectively) (Larsen *et al.*, 2003). The follicular phase was defined by oestradiol concentrations of >200 pg/ml with progesterone remaining low (<2ng/ml) (Larsen *et al.*, 2003). The luteal phase was defined as peak progesterone between 2-20 ng/ml (Larsen *et al.*, 2003). Due to the reported variations in total cycle duration, phase duration and sex hormone concentrations reported between reproductive women, ovulation was confirmed by identifying progesterone concentrations of <2 ng/ml during the luteal phase.

2.2.9: Data Collection

2.2.9.1: Pre-test controls

During study 1 and 2, participants were instructed to maintain similar pre-test controls the day and evening before testing. These included participants being advised to restrict their intake of alcohol and caffeine-containing drinks and to avoid physical activity on the day prior to each test. The participants were also instructed to consume the same evening meal before each testing day, with water allowed *ad libitum*. The participants were instructed to be 10-12 hours fasted before each test session. These pre-test controls were implemented in order to reduce variability between repeated measurements and individual methodologies (Brouns *et al.*, 2005; Compher *et al.*, 2006). On the day of testing, all participants were asked to provide details of dietary intake and physical activity for the previous evening to encourage and check for compliance.

2.2.10: Blood Glucose and Plasma Insulin

2.2.10.1: Oral Glucose Tolerance Test (OGTT)

To determine glucose response and insulin sensitivity during different phases of the menstrual cycle, a standard 75g oral glucose tolerance test (OGTT) was performed.

The protocol implemented to measure blood glucose response was adopted from that described in Brouns *et al.* (2005) and is in line with the current recommendations by the FAO/WHO (1998). Participants arrived at the laboratory following a 12 hour overnight fast before 10.00 am; capillary blood samples were taken by finger-prick using a single-use lancing system (Unistik 3, Owen Munford, Woodstock, UK) (Figure 3.4). To minimise plasma dilution fingertips were not squeezed but gently massaged to extract the blood and 5 μ l was used immediately to measure blood glucose using an automatic blood glucose analyser (Glucose 201+, HemoCue AB, Sweden). In addition, 300 μ l of blood was collected into Microvette CB 300 capillary blood collection tubes treated with EDTA (Sarstedt, Nümbrecht, Germany). The blood tubes were centrifuged at 4000 rpm for 10 minutes (MC-6; Sarstedt Ltd., Leicester, UK) and aliquots of plasma were stored at -80°C .



Figure 2.4: The HemoCue 201+ Glucose analyser and the Unistik 2 single-use lancing device.

Two fasting samples were taken at -5 and 0 minutes, with participants then ingesting a glucose load consisting of 75g glucose (Lloyds Pharmacy Ltd, UK) made up with water to a volume of 250 ml. Further finger-prick blood samples were collected post glucose load consumption (Figure 2.5).

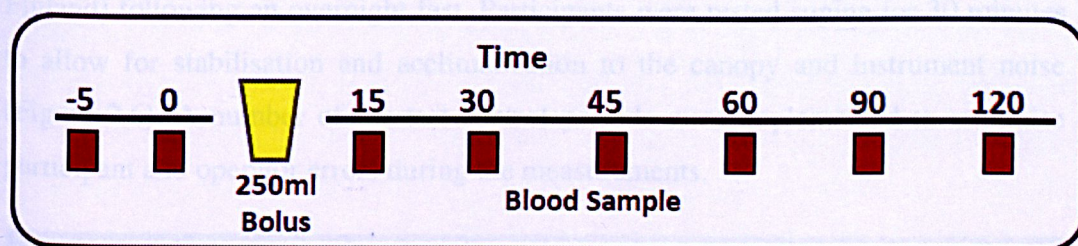


Figure 2.5: Methodology implemented during OGTT.

Blood glucose response was plotted for each subject at each time point, using change-from-baseline values. The glucose response was expressed as incremental area under the curve (IAUC) ignoring the area below Baseline and calculated geometrically (FAO/WHO, 1998).

The HemoCue instruments used in the study were calibrated against an automated glucose analyser (YSI 2300 stat, YSI Inc., Yellow Springs, Ohio, USA) which is a pre-validated instrument for the determination of blood glucose concentrations (Wolever *et al.*, 2006). The HemoCue reports within-day CV of 3.5% and between – day of 2.7% (Glucose 201+, HemoCue AB, Sweden). The HemoCue utilizes a chemical micro method in order to determine glucose concentrations in whole blood. The method is split into two chemical reaction phases: the first known as haemolysis involves disintegration of the erythrocyte membranes using saponin. The second is a modified glucose dehydrogenase method where by tetrazolium salt is used to obtain glucose quantification in visible light and is known as the glucose reaction. The glucose dehydrogenase within the glucose reaction phase acts as a catalyst for the oxidation of β -D-glucose, forming nicotinamide adenine dinucleotide (NADH) which, in the presence of diaphoresis, produces a coloured formazan with MTT which is a form of tetrazolium salt. The formazan is quantified photometrically using two wavelength photometric methods at 660 nm and 840 nm, resulting in a glucose concentration being detected in the whole blood sample (Glucose 201+, HemoCue AB, Sweden).

2.2.11: Energy Expenditure

2.2.11.1: Resting Metabolic Rate (RMR)

To determine resting metabolic rate (RMR), indirect calorimetry was performed before 10.00 am using the ventilated hood system (Deltatrac Datex, Helsinki, Finland) following an overnight fast. Participants were rested supine for 30 minutes to allow for stabilisation and acclimatization to the canopy and instrument noise (Figure 2.6). A number of pre-test control periods were implemented to minimise participant and operator errors during the measurements.



Figure 2.6: Deltatrac Datex hood and canopy used to perform RMR indirect calorimetry measures

Respiratory gas exchange was then measured for 30 minutes with breath by breath gas analysis (VO_2 and VCO_2 , l/min). This analysis allowed calculations of average energy expenditure (resting metabolic rate, RMR) and respiratory quotient to be made using validated equations as detailed in section 2.13 (Jeukendrup and Wallis, 2005). The methodology implemented was chosen to ensure the most accurate measurements of O_2 consumption and CO_2 production were achieved during a resting state to accurately determine RMR (Compher *et al.*, 2006).

2.2.11.2: Exercise

All exercise sessions were completed using a treadmill and set at an intensity rate of 70-80% predicted age related heart rate maximum (HR max):

220 – Participant Age

Heart rate was measured during all exercise sessions using a Polar FS1 HR Monitor (Polar Electroy, Finland). The supervised exercise regime was conducted on a treadmill either within the Human Performance Laboratory or in the Centre for Sport at Oxford Brookes University. The walking regime included a 5 minute warm-up (50% HR max), 30 minutes (70-80% HR max) and a 2-minute cool down (50% HR max). The exercise intensity averaged 5.8 ± 0.4 km at 5.9 ± 1.0 incline to allow for a comfortable brisk walk for 30 minutes.

During exercise sessions of study 1, indirect calorimetry was performed and respiratory gases were collected using a Douglas bag for two minutes every ten minutes whilst participants were exercising (70-80% HR max). Whilst exercising, participants wore a nose clip and breathed through a mouthpiece, with respiratory gases collected using a Douglas bag (Figure 2.7).

A sample of expired air was analysed (Servomex Gas Analyser, 1440C, Servomex Group Ltd, UK) for O₂ and CO₂ concentrations and total volume expired. Gas concentrations were then used to calculate oxygen (VO₂, l/min) uptake and carbon dioxide (VCO₂, l/min) production allowing carbohydrate (CHO) and fat oxidation (g/min) to be calculated using validated equations as detailed in section 2.13 (Jeukendrup and Wallis, 2005).

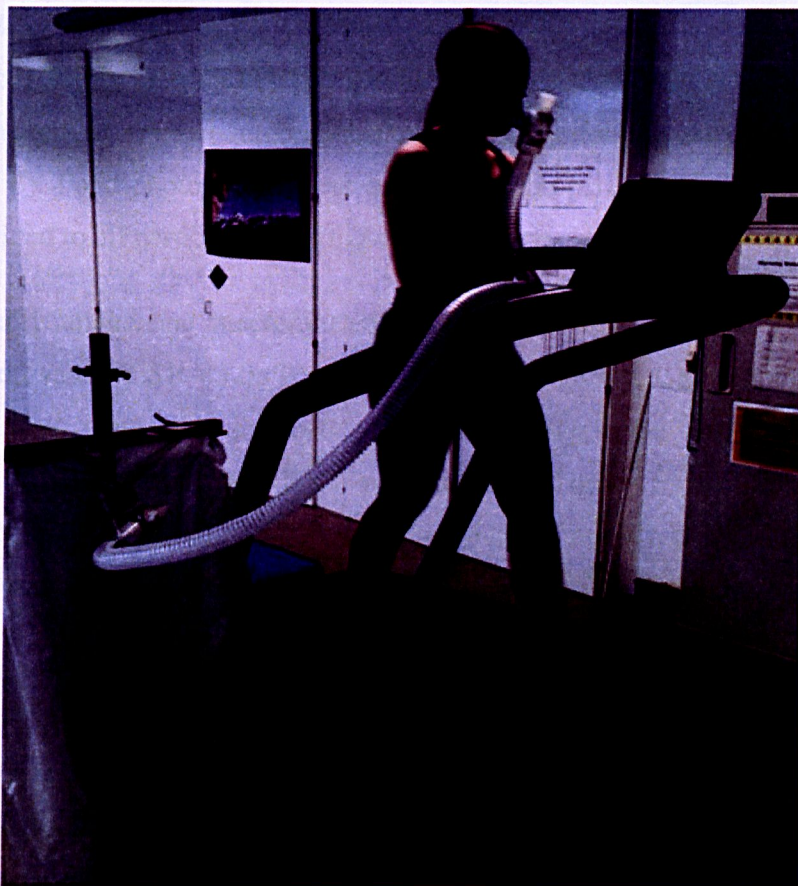


Figure 2.7: Breath samples collected in a Douglas bag during exercise sessions

2.2.12: Premenstrual Syndrome (PMS) symptoms

Prospective analysis of PMS symptoms was required during study 3. Menstrual Distress Questionnaire (MDQ) Form T was administered every other day throughout the duration of the study and enabled the recording of symptoms related to the previous 24 hours (appendix 14). The current investigation required PMS symptoms to be at least 30% greater during the luteal phase compared to the follicular phase at baseline (cycle 1) to confirm eligibility to the study.

Following the completion of the intervention, PMS symptom scores were collated and averaged according to each cycle phase and the questionnaire instructions. The luteal phase was defined as up to 10 days prior to menstruation including the first day of menstruation. The follicular phase began from the end of menstruation and was defined by at least 5-7 asymptomatic days before the luteal phase. These definitions were flexible in order to account for variations in phase and total cycle duration between participants.

2.2.13: Dietary Intake

The 24 hour Triple Pass dietary recall interview technique using food photographs, was conducted by trained interviewers once weekly for the duration of study 3 (appendix 3). Food photographs were used to aid the participant recall process and were provided to all participants during each dietary recall interview.

Mean energy intake and macronutrient composition were analysed using dietary analysis software, WISP V3.0 (Tinuviel Software, Anglesey, UK). Foods consumed by participants that were not represented by existing codes within WISP V3.0 (Tinuviel Software, Anglesey, UK), were estimated using recipes from standard methodologies and where applicable, up-to-date nutrition information from individual food products was obtained from food labels.

2.2.14: Data Analysis of hormone concentrations: Cobas e411

All plasma samples were thawed and analysed for oestradiol (E2, 03000079, Roche, UK), progesterone (Progesterone, 12145383, Roche, UK) and or insulin (Insulin, 12017547, Roche, UK) concentrations by electrochemiluminescence technology using a Cobas e411 semi-automated analyser (Roche diagnostics, Burgess Hill, UK) (Figure 2.8). Oestradiol was measured in pg/ml, progesterone in ng/ml and insulin in μ U/ml with data processing and calculations of AUC conducted using the same procedures as the glucose AUC data.

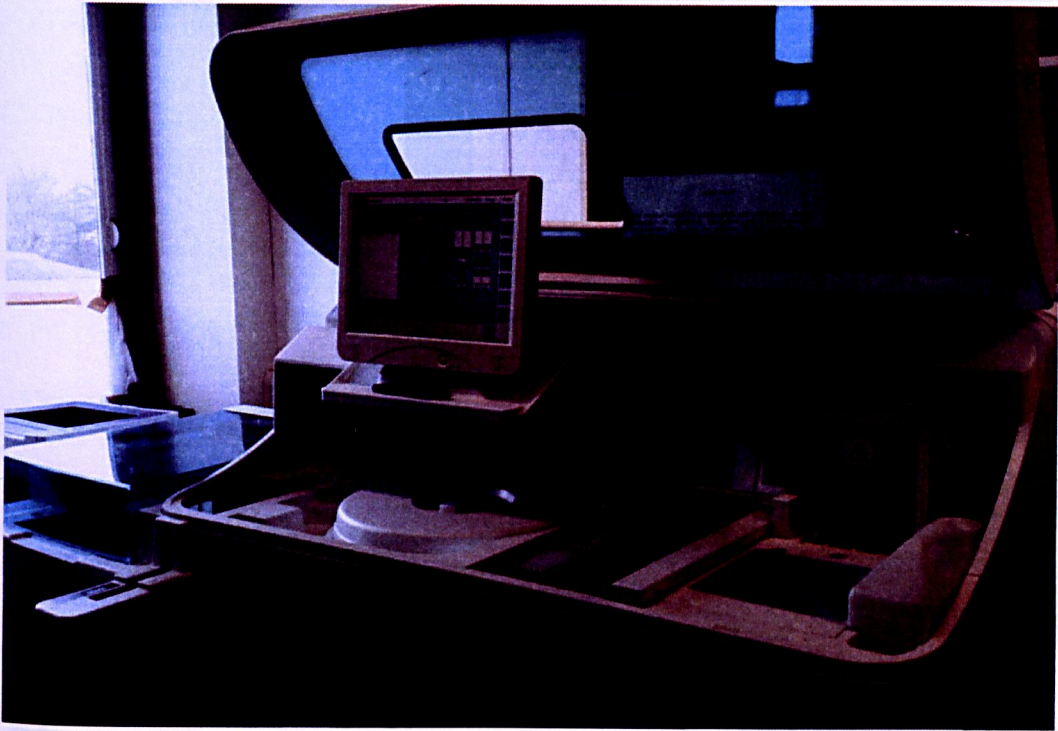


Figure 2.8: Cobas e411 semi-automated analyser (Roche diagnostics, Burgess Hill, UK)

The Cobas e411 uses electrochemiluminescence (ECL) technology to measure oestradiol, progesterone and insulin content in the plasma samples. ECL is a process by which highly reactive species are produced from stable precursors at an electrode, reacting to each other to produce light. After the sample (participant plasma) is flushed through the cell, a magnet is turned on and a voltage applied to capture the microparticles with immune complexes. The ECL reaction is initiated and measured through light emission by a photomultiplier; this detects and converts the ECL signal into an electrical signal which allows the final assay result to be calculated (Roche diagnostics, Burgess Hill, UK). The Cobas e411 conducts two different assay principles: Sandwich and Competitive Assays. Oestradiol, progesterone and insulin assays were conducted using the competitive assay technique as these are low molecular weight analytes. The competitive assay is divided into three stages (Figure 2.9).

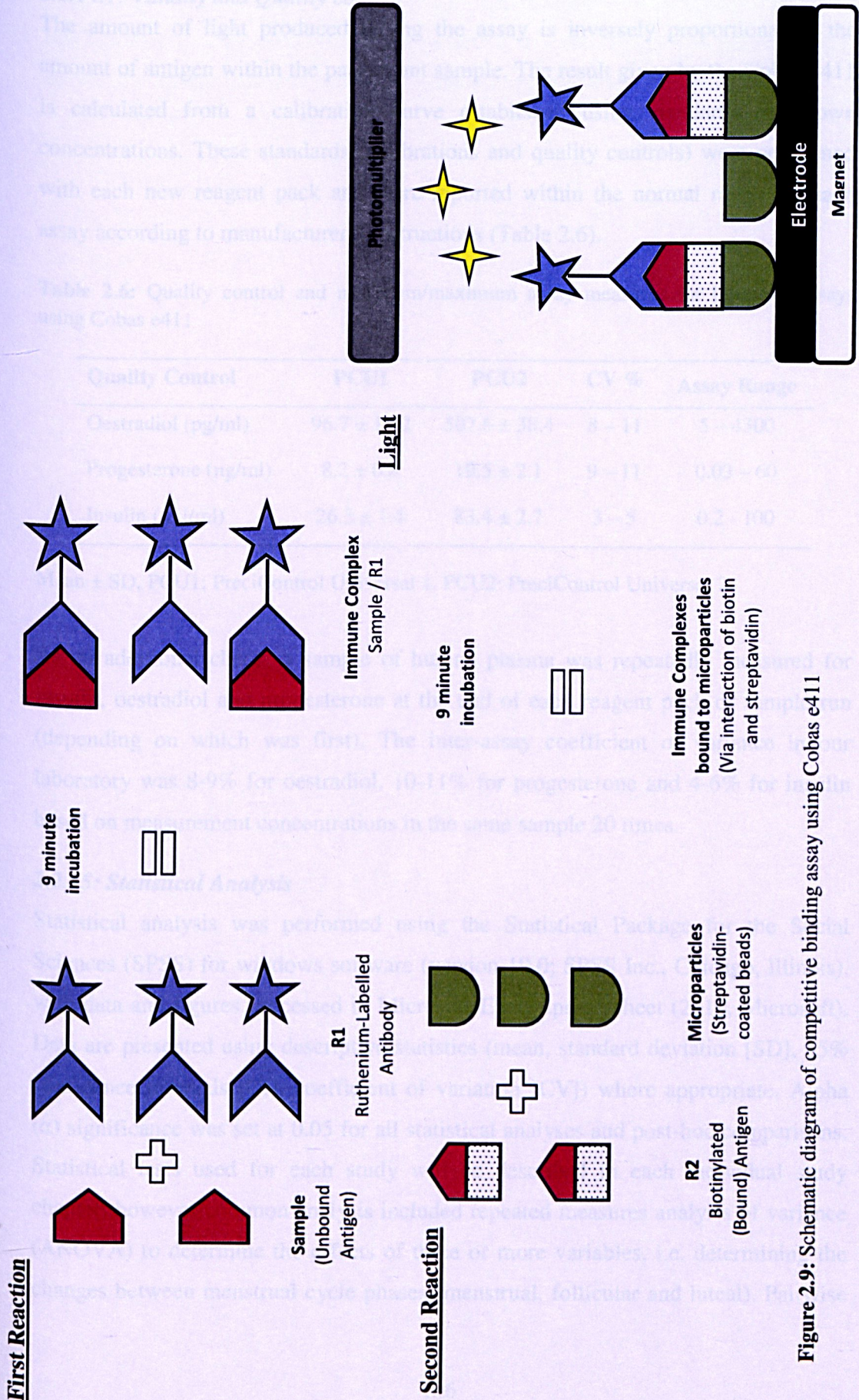


Figure 2.9: Schematic diagram of competitive binding assay using Cobas e411

2.2.14.1: Validity and Quality control

The amount of light produced during the assay is inversely proportional to the amount of antigen within the participant sample. The result given by the Cobas e411 is calculated from a calibration curve established using standards of known concentrations. These standards (calibrations and quality controls) were performed with each new reagent pack and were reported within the normal range for each assay according to manufacturer's instructions (Table 2.6).

Table 2.6: Quality control and minimum/maximum assay measures for hormone assays using Cobas e411

Quality Control	PCU1	PCU2	CV %	Assay Range
Oestradiol (pg/ml)	96.7 ± 10.2	507.6 ± 38.4	8 – 11	5 – 4300
Progesterone (ng/ml)	8.2 ± 0.8	19.5 ± 2.1	9 – 11	0.03 – 60
Insulin (μU/ml)	26.3 ± 1.4	83.4 ± 2.7	3 – 5	0.2 - 100

Mean ± SD, PCU1: PreciControl Universal 1, PCU2: PreciControl Universal 2

As an additional check, a sample of human plasma was repeatedly measured for insulin, oestradiol and progesterone at the end of each reagent pack or sample run (depending on which was first). The inter-assay coefficient of variance in our laboratory was 8-9% for oestradiol, 10-11% for progesterone and 4-6% for insulin based on measurement concentrations in the same sample 20 times.

2.2.15: Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for windows software (version 19.0; SPSS Inc., Chicago, Illinois), with data and figures processed in Microsoft Excel spread sheet (2010, Microsoft). Data are presented using descriptive statistics (mean, standard deviation [SD], 95% confidence intervals [CI], coefficient of variation [CV]) where appropriate. Alpha (α) significance was set at 0.05 for all statistical analyses and post-hoc comparisons. Statistical tests used for each study will be described in each individual study chapter; however common analysis included repeated measures analysis of variance (ANOVA) to determine the effects of three or more variables, i.e. determining the changes between menstrual cycle phases (menstrual, follicular and luteal). Pairwise

comparisons were carried out where a significant difference was observed using the Bonferroni test. Pearson's correlation co-efficient were also conducted to identify significant correlations between sex hormones and some study outcome measures.

Chapter 3: Glucose Homeostasis and Insulin Sensitivity during the Menstrual Cycle

3.1: Abstract

3.1.1: Introduction

Current evidence concerning the effects of oestrogen and progesterone during the menstrual cycle on glucose response and insulin sensitivity is conflicting. The aim of this study is therefore to investigate the glucose and insulin response to a 75g glucose load and insulin sensitivity during the menstrual cycle.

3.1.2: Methods

Venous blood samples were collected in 18 regularly menstruating women (age, 28 ± 6 y) to determine oestrogen and progesterone concentrations for one complete menstrual cycle, for accurate menstrual phase definition. A 75g OGTT was performed on one day during each of the three distinct menstrual cycle phases (menstrual, follicular and luteal) throughout a second menstrual cycle. Venous blood samples were taken before each OGTT to confirm oestrogen and progesterone concentrations.

3.1.3: Results

Menstrual cycle phases (menstrual, follicular and luteal) were successfully confirmed by oestradiol and progesterone concentrations and did not significantly differ between the two menstrual cycles. Glucose AUC was significantly increased during the luteal phase (148.2 ± 59.8 mmol/L; 170.5 ± 85.1 mmol/L; 183.2 ± 96.3 mmol/L; menstrual, follicular and luteal). Insulin AUC was significantly increased during the luteal phase (3339.9 ± 1462.0 μ U/ml; 3658.7 ± 1699.0 μ U/ml; 3896.0 ± 1836.7 μ U/ml) ($P < 0.05$). However, insulin sensitivity using the insulin sensitivity index did not significantly differ between the three cycle phases.

3.1.4: Conclusion

These findings indicate that fluctuations in oestrogen and progesterone during the luteal menstrual cycle may affect glycaemic response but not insulin sensitivity.

3.2: Introduction

The prevalence of overweight and obesity in women is increasing and as a consequence, escalating numbers of young pre-menopausal women are developing symptoms of the metabolic syndrome and in particular insulin resistance (IR). A number of recent clinical investigations have shown strong correlations between sex hormones oestrogen and progesterone and aspects of the metabolic syndrome, including changes in IR, obesity and lipid biomarkers (Manning *et al.*, 2001; Salpeter *et al.*, 2006).

Current evidence suggests that both oestrogen and progesterone may be implicated in glucose homeostasis and insulin sensitivity with early studies demonstrating that oestrogen promotes insulin sensitivity (Hansen *et al.*, 1996; Latour *et al.*, 2001) but may not alter insulin responsiveness to a glucose load (Cooper *et al.*, 2007; Toth *et al.*, 2008). A lack of oestrogen has been reported to result in the development of insulin resistance as seen in ovariectomized models and postmenopausal women, which can be reversed following oestrogen therapy (Latour *et al.*, 2001; Van Pelt *et al.*, 2003). This is potentially due to an increase in GLUT 4 protein content (Saengsirisuwan *et al.*, 2009) and an increase in oestrogen-stimulated insulin signalling by AKTSer 473 phosphorylation in the muscle (Salehzadeh *et al.*, 2011).

Progesterone however, appears to promote insulin resistance via a decrease in glucose uptake, particularly evidenced in pregnant women when concentrations are high and which can lead to the development of gestational diabetes (Kalkhoff *et al.*, 1970; Kirwan *et al.*, 2002; Nagira *et al.*, 2006). In addition, cell culture models report that progesterone reduces GLUT4 content in skeletal muscle and appears to impair contraction-stimulated glucose uptake during exercise (Campbell and Febbraio, 2002). A study administering combined hormonal contraceptives (50-500µg progesterone: 30-40 µg oestrogen) has reported significant decreases in insulin sensitivity (Godsland *et al.*, 1992), indicating a combination of oestrogen and progesterone may also result in significant changes in glucose homeostasis and insulin sensitivity.

Research to date has yielded conflicting reports when evaluating the effects of normal fluctuations of endogenous sex hormones in regularly menstruating women

on glucose response and insulin sensitivity (Bingley *et al.*, 2008; Diamond *et al.*, 1993; Jarrett and Graver, 1968; Pulido and Salazar, 1999; Toth *et al.*, 1987; Trout *et al.*, 2007; Valdes and Elkind-Hirsch, 1991; Yeung *et al.*, 2010; Ykijarvinen, 1984). Studies using the intravenous glucose tolerance test (IVGTT) or the euglycemic clamp test have reported significant decreases in insulin sensitivity during the luteal phase of the menstrual cycle in some (Pulido and Salazar, 1999; Valdes and Elkind-Hirsch, 1991) but not all of the research to date (Bingley *et al.*, 2008; Diamond *et al.*, 1993; Toth *et al.*, 1987; Ykijarvinen, 1984). Studies implementing OGTT to determine glucose tolerance report a decrease in the luteal phase in regularly menstruating women (Jarrett and Graver, 1968; Peppler *et al.*, 1978).

In addition, alternative data modelling methods to determine insulin resistance such as the homeostasis model of insulin resistance (HOMA-IR) suggest significant increases in insulin resistance occurs during the luteal phase of the menstrual cycle (Yeung *et al.*, 2010). The study also reports a positive association with both oestrogen and progesterone, although the observed population were largely overweight or obese (Yeung *et al.*, 2010). However, there have been studies that report no significant differences in fasting blood glucose or insulin during the menstrual cycle, which would suggest that not all fasting samples and insulin sensitivity indexes record significant variations (Lundman *et al.*, 1994; Okey, 1925; Reinke *et al.*, 1972).

The inconsistent results reported between studies may be explained by failures to control for factors that affect these responses before testing. Diet, alcohol intake, exercise and weight are all factors that could explain the inconsistencies seen in the previous research. Moreover, many studies do not accurately measure endogenous sex hormone concentrations to confirm menstrual cycle phase, instead favouring to approximate cycle days (Bonora *et al.*, 1987; Diamond *et al.*, 1993; Diamond *et al.*, 1989; Goldman and Eckerlin, 1970; Macdonald and Crossley, 1970; Spellacy, 1967). Cycle duration and phase duration vary considerably between women (Chiazze *et al.*, 1968; Creinin *et al.*, 2004), therefore subjective and standardised methods of estimating menstrual cycle phase may result in missed fluctuations in sex hormones and incorrect cycle phase definition. These methods reduce the ability to draw

accurate associations between oestrogen and progesterone and their effects on glucose response and insulin sensitivity.

The aim of this study was therefore to determine the effects of fluctuations in endogenous sex hormones during the menstrual cycle on glucose response and insulin sensitivity. Based on the previous reviewed literature it is hypothesised that glucose and insulin response and insulin resistance will increase during the luteal phase of the menstrual cycle. Three oral glucose tolerance tests (OGTT) were performed during one complete menstrual cycle, having identified cycle phases using oestrogen (oestradiol) and progesterone hormone concentrations over a previous complete menstrual cycle.

3.3: Methods

3.3.1: Participant Characteristics

Eighteen regularly menstruating (21 – 35 d) pre-menopausal women were recruited to the study (Table 3.1).

Table 3.1: Participant baseline characteristics

Characteristics	Participant Mean
Age (y)	28 ± 6
Height (m)	1.66 ± 0.07
Body mass (kg)	62.1 ± 10.8
BMI (kg/m ²)	22.4 ± 3.0
Body fat (%)	27.7 ± 7.1
Mean ± SD, BMI: Body Mass Index	

Participants were required to be aged between 18-40 y, BMI 18.5 - 30kg/m² and fasting blood glucose <6.1mmol/l. Participants were excluded if they reported irregular menstrual cycles for three months prior to testing, were on any form of hormone regulated contraception, were pregnant, breastfeeding, had given birth within the previous two years or were diabetic. Ethical approval was obtained from the University Research Ethics Committee at Oxford Brookes University (UREC registration number: 100470) in accordance with the Declaration of Helsinki.

Written informed consent was obtained from each participant before the study began. Participants were members of the public from the Oxford area and were considered healthy following the completion of a series of health questionnaires.

3.4: Experimental Protocol

The study was conducted over two complete menstrual cycles (cycle A and B); the first cycle (cycle A) collected oestradiol and progesterone concentrations to pre-define the three individual menstrual cycle phases (menstrual, follicular and luteal). This was followed by a second cycle (cycle B) during which a 2 hour oral glucose tolerance test (OGTT) was performed on three occasions corresponding to the three cycle phases identified during cycle A.

3.4.1: Cycle A: Hormone profile

Participants contacted the researcher at the first sign of menstrual bleeding (day 1). Venous blood samples (6ml) were taken from each participant every other week day morning (before 1000 h) for one complete menstrual cycle. Venous blood samples were collected into EDTA-containing tubes (K2E 10.8mg, Becton Dickinson, UK) and stored on ice. All blood tubes were centrifuged at 4000rpm for 10 min at 4 °C and aliquots of plasma were stored at -80 °C for later analysis. Following hormone analysis the menstrual, follicular and luteal phases were identified in each participant.

3.4.2: Cycle B: Oral Glucose Tolerance Test

A dietary record was taken for 24 hours preceding the first OGTT and was matched prior to subsequent visits. Participants were instructed to refrain from consuming alcohol or caffeine and from undertaking any exercise for 24 hours prior to testing. Participants arrived at the laboratory following a 12 hour overnight fast and had a venous blood sample (6 ml) taken to confirm oestradiol and progesterone concentrations. Capillary blood samples (approx. 305 µl) were then taken by finger-prick using single-use lancing system (Unistik 3, Owen Munford, Woodstock, UK) at -5 and 0 min. Participants then ingested a glucose beverage consisting of 75g glucose (Lloyds Pharmacy Ltd, UK) made up with water to a volume of 250 ml. Further finger-prick blood samples (approx. 305 µl) were collected at 15, 30, 45, 60, 90 and 120 min post glucose load consumption. Of the 305 µl blood sample, 5 µl

was used immediately to measure blood glucose using an automatic blood glucose analyser (Glucose 201+, HemoCue AB, Sweden); 300 μ l was collected into Microcuvette CB 300 (Sarstedt, Nümbrecht, Germany) and stored on ice. All blood tubes were centrifuged at 4000rpm for 10 min at 4 °C and aliquots of plasma were stored at -80 °C.

3.5: Biochemical Analysis

Where appropriate, plasma samples were thawed and analysed using a Cobas e411, semi-automated analyser (Roche, UK) for insulin (Insulin, 02369877, Roche, UK), oestradiol (E2, 03000079, Roche, UK) and progesterone (Progesterone, 12145383, Roche, UK).

3.6: Calculations

ISI (Matsuda and DeFronzo, 1999), IGI (Seltzer *et al.*, 1967), DI (Bergman *et al.*, 1981) and HOMA-IR (Matthews *et al.*, 1985) equations were used to determine insulin sensitivity or insulin resistance across the different menstrual cycle phases.

3.7: Statistical Analysis

Power analysis calculations based on results from previous studies (Jarrett and Graver, 1968) demonstrated that a total sample size of 16 participants was required in order to have the power (0.8) to detect a significant effect (0.772) in glucose response. Taking into account the ~10% drop out rate associated with longitudinal studies a total sample size of 18 participants were recruited.

Data analysis was performed using SPSS for windows software (version 19.0; SPSS Inc., Chicago, IL). Data are expressed as means \pm SD unless otherwise stated. Plasma oestradiol and progesterone concentrations were analysed using a one-way repeated measures analysis of variance (ANOVA) to determine significant differences in hormone concentrations during the three menstrual phases. Plasma glucose and insulin concentrations including area under the curve (AUC) during different menstrual phases, as well as whole body ISI, IGI, DI and HOMA-IR were also compared using one-way repeated measures ANOVA. Pairwise comparisons were carried out where a significant difference was observed using the Bonferroni test. Pearson's correlation co-efficient were conducted to identify possible

correlations between both sex hormones and measures of glucose and insulin as well as body composition. Significance was accepted at the $P < 0.05$ level.

3.8: Results

3.8.1: Menstrual Cycle Duration

All 18 participants had regular ovulatory menstrual cycles based on oestradiol and progesterone concentrations (27 ± 3 d) (Table 3.2). Menstrual cycle phase duration varied considerably between participants, menstrual phase duration ranged from 4-8 d, follicular phase 6-12 d and luteal phase 9-16 d. Cycle days identified as individual phases ranged from days 1- 8 (menstrual), 7-20 (follicular) and 13-31 (luteal) of the menstrual cycle. There was no significant difference between baseline and test cycle duration within participants.

Table 3.2: Menstrual cycle duration (days) for all participants during baseline and test cycle

Participant	Baseline	Test Cycle
1	26	27
2	31	31
3	26	26
4	25	23
5	30	24
6	28	28
7	26	28
8	24	27
9	26	28
10	24	22
11	34	33
12	28	23
13	26	27
14	23	24
15	28	28
16	28	27
17	28	27
18	32	28
Group Average	27 ± 3	27 ± 3

3.8.2: Oestradiol and progesterone concentrations

Variations in hormone concentrations were evident in the current cohort of participants when analysing individual hormone profiles (Figure 3.1 A and B). Peak oestradiol concentrations differed by 42.1% (increase of 91pg/ml) during the follicular phase (day 14 versus day 18), with peak progesterone concentrations (day 21 versus day 27) differing by 35.2% (increase of 62µg/ml) during the luteal phase when compared to standard test days used in previous literature.

Oestradiol and progesterone concentrations during cycle B were not significantly different compared to cycle A. Oestradiol hormone concentrations during both menstrual cycles (Figure 3.1: A and B) were significantly higher during the follicular and luteal phases as compared to menstrual, with oestradiol concentrations significantly higher during the follicular phase compared with luteal ($P < 0.05$). Progesterone concentrations were significantly elevated during the luteal phase when compared to the follicular and menstrual phases (Table 3.3).

Table 3.3: Plasma hormone concentrations during the menstrual, follicular and luteal phases

	Cycle A		Cycle B	
	Oestradiol (pg/ml)	Progesterone (ng/ml)	Oestradiol (pg/ml)	Progesterone (ng/ml)
Menstrual (MP)	50 ± 13 ^a	0.77 ± 0.36 ^a	48 ± 21 ^a	0.66 ± 0.32 ^a
Follicular (FP)	313 ± 93 ^b	0.88 ± 0.44 ^a	289 ± 98 ^b	0.96 ± 0.61 ^a
Luteal (LP)	156 ± 68 ^c	14.13 ± 4.82 ^b	126 ± 44 ^c	11.88 ± 6.56 ^b

Mean ± SD, Within each column, values with different superscripts are significantly different from each other ($P < 0.05$)

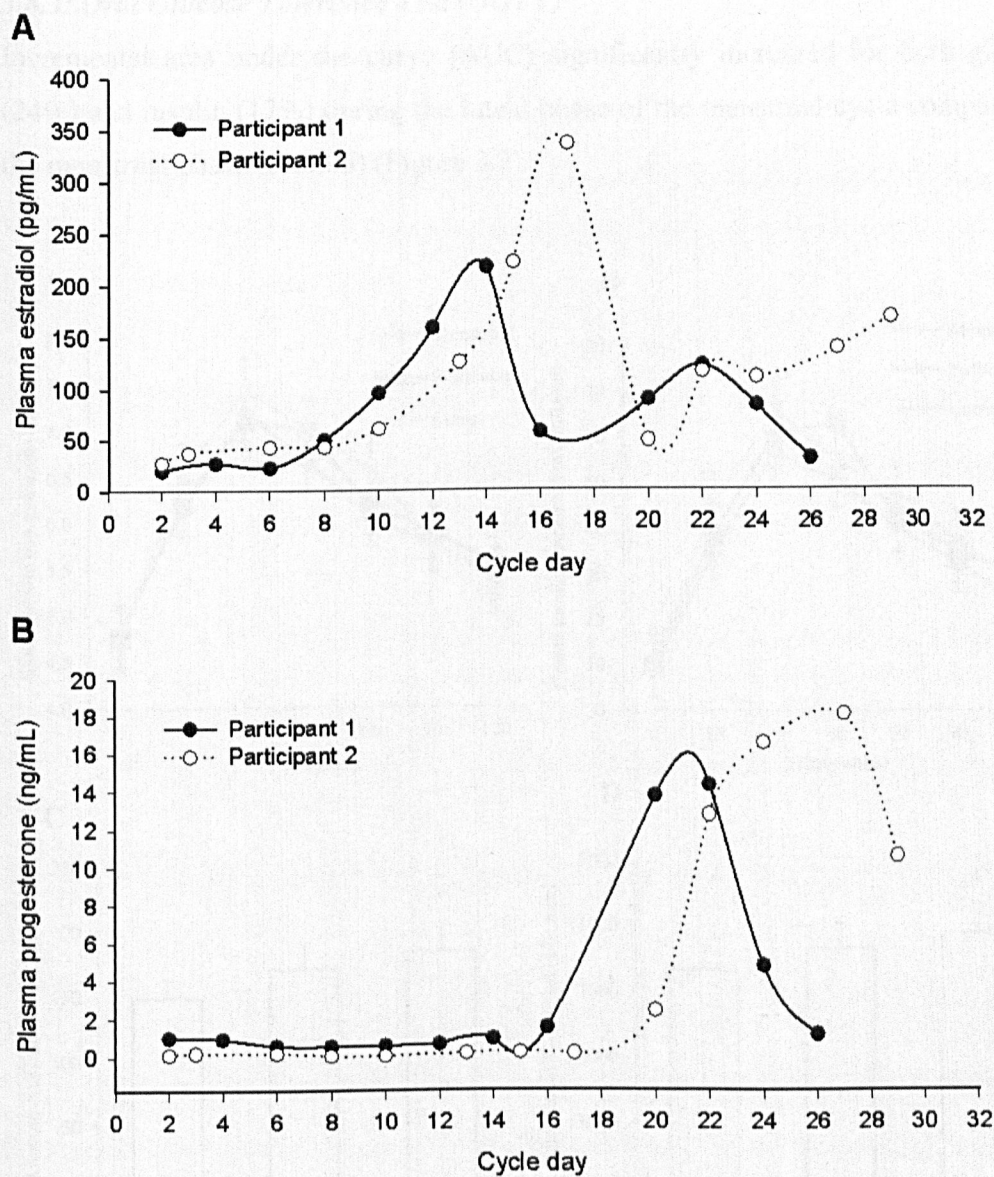


Figure 3.1: Representative hormone profiles of oestradiol (A) and progesterone (B) for two participants during one complete menstrual cycle

3.8.3: Oral Glucose Tolerance Test (OGTT)

Incremental area under the curve (AUC) significantly increased for both glucose (24%) and insulin (17%) during the luteal phase of the menstrual cycle compared to the menstrual phase ($P < 0.05$) (Figure 3.2).

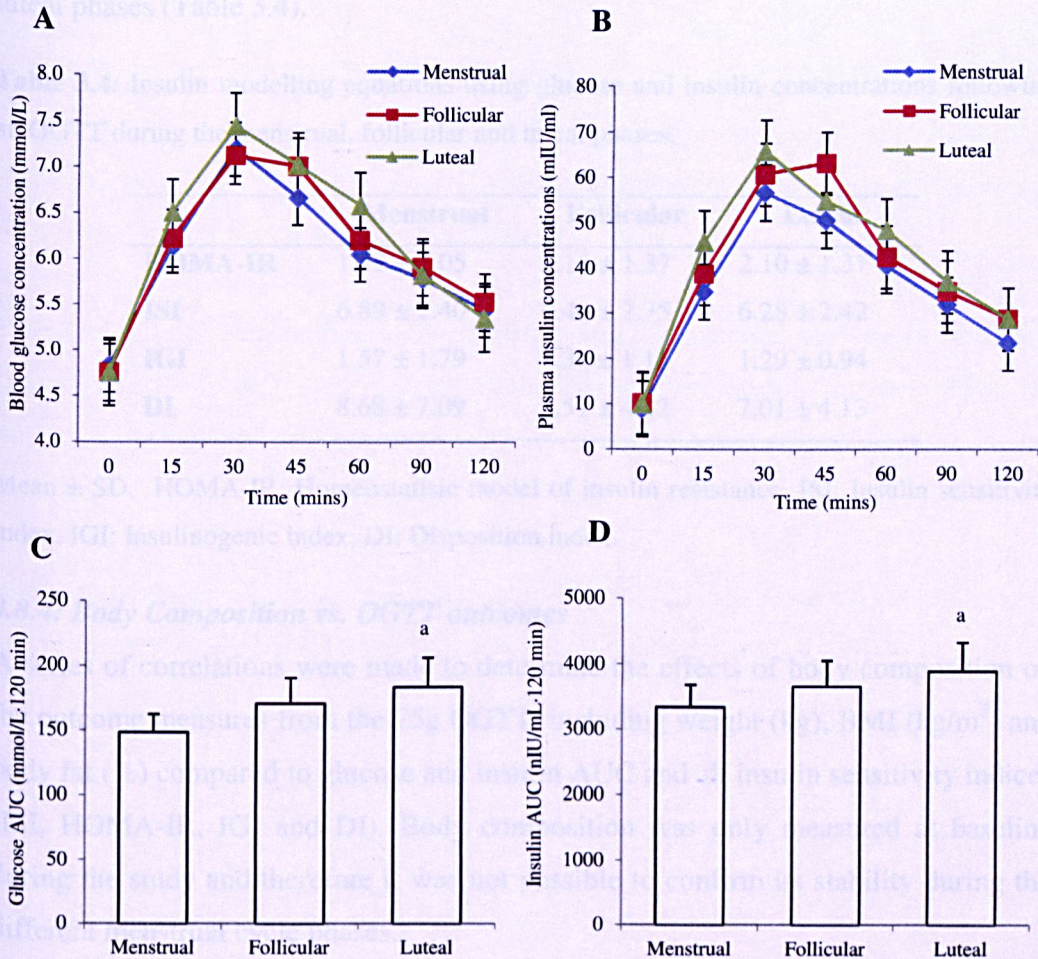


Figure 3.2: Glucose (A) and insulin (B) plasma concentrations and glucose (C) and insulin (D) area under the curve (AUC) during the OGTT in the menstrual, follicular and luteal phases during cycle B. ^a = Significantly increased from menstrual phase ($P < 0.05$), (Mean \pm SD)

The glucose profiles of all 18 participants during all three phases of the menstrual cycle were indicative of normal glucose tolerance profiles. Blood glucose concentrations returned to baseline or near baseline (± 1 mmol/L) following the two hour glucose test.

In addition, there was no significant difference in insulin sensitivity index (ISI), insulinogenic index (IGI) and disposition index (DI) between the three menstrual cycle phases (Table 3.4). Insulin resistance was also calculated using fasting glucose and fasting insulin concentrations to determine HOMA-IR (Matthews *et al.*, 1985). HOMA-IR was also not significantly different between the menstrual, follicular and luteal phases (Table 3.4).

Table 3.4: Insulin modelling equations using glucose and insulin concentrations following an OGTT during the menstrual, follicular and luteal phases.

	Menstrual	Follicular	Luteal
HOMA-IR	1.92 ± 1.05	2.14 ± 1.37	2.10 ± 1.37
ISI	6.89 ± 2.40	6.40 ± 2.75	6.28 ± 2.42
IGI	1.57 ± 1.79	1.36 ± 1.11	1.29 ± 0.94
DI	8.68 ± 7.09	7.51 ± 4.42	7.01 ± 4.13

Mean ± SD, HOMA-IR; Homeostatic model of insulin resistance, ISI; Insulin sensitivity index, IGI: Insulinogenic index, DI; Disposition index.

3.8.4: Body Composition vs. OGTT outcomes

A series of correlations were made to determine the effects of body composition on the outcome measures from the 75g OGTT, including weight (kg), BMI (kg/m²) and body fat (%) compared to glucose and insulin AUC and all insulin sensitivity indices (ISI, HOMA-IR, IGI and DI). Body composition was only measured at baseline during the study and therefore it was not possible to confirm its stability during the different menstrual cycle phases.

The results indicate a significant positive correlation between BMI, BF% and weight (kg) and InAUC and HOMA-IR with a negative correlation for ISI (Table 3.5). However there was no significant correlation between body composition measures and sex hormone concentrations.

Table 3.5: Correlation (R) between body composition measures (weight, body fat and BMI) and glucose and insulin measures following 75g OGTT

	Glu AUC	In AUC	ISI	HOMA	IGI	DI	E2	Prog
Weight (kg)	0.22	0.48*	-0.57*	0.65*	0.31*	-0.24*	-0.17	-0.05
BF %	0.45*	0.39*	-0.48*	0.52*	0.20	-0.27	-0.12	-0.64
BMI	0.16	0.45*	-0.61*	0.75*	0.38*	-0.18	-0.23	-0.05

Glu AUC: Glucose area under the curve, In AUC: Insulin area under the curve, ISI: Insulin sensitivity index, HOMA: Homeostatic model of insulin resistance, IGI: Insulinogenic index, DI: Deposition index, E2: Oestradiol, Prog: Progesterone, BF%: Body fat percentage, BMI: Body mass index, *= Significant correlation (P<0.01).

Correlations between sex hormone concentrations and glucose and insulin AUC and insulin sensitivity measures indicate no significant relationship between either sex hormone during the whole menstrual cycle or within each individual phase (Table 3.6).

Table 3.6: Correlation (R) between sex hormones (oestrogen and progesterone) and glucose and insulin measures following 75g OGTT during different menstrual cycle phases

Phase	Menstrual		Follicular		Luteal		Whole Cycle	
	E2	Prog	E2	Prog	E2	Prog	E2	Prog
Glu AUC	0.03	0.31	0.07	-0.34	0.39	-0.38	0.16	-0.04
In AUC	-0.34	-0.21	-0.08	0.08	-0.18	0.08	-0.02	0.02
ISI	0.37	-0.29	0.00	-0.20	0.39	-0.20	0.05	0.05
HOMA	-0.25	-0.19	-0.24	0.37	-0.37	0.37	-0.09	0.04
IGI	-0.30	-0.13	-0.25	0.17	-0.26	0.17	-0.16	-0.01
DI	-0.25	-0.64	-0.13	0.04	0.05	0.04	-0.11	-0.01

E2: Oestradiol, Prog: Progesterone, Glu AUC: Glucose area under the curve, In AUC: Insulin area under the curve, ISI: Insulin sensitivity index, HOMA: Homeostatic model of insulin resistance, IGI: Insulinogenic index, DI: Deposition index

3.9: Discussion

The study reported here accurately maps menstrual cycle phase before and during testing using plasma analysis of oestradiol and progesterone concentrations. The study demonstrated a significant increase in glucose and insulin AUC following the consumption of a 75g glucose load during the luteal phase compared to the menstrual phase of the menstrual cycle. The significant increases in glucose are consistent with a number of earlier observations in studies implementing the OGTT technique (Ezenwaka *et al.*, 1993; Jarrett and Graver, 1968; Peppler *et al.*, 1978).

However, there has been conflicting evidence reported in both glucose metabolism and insulin sensitivity measures during the menstrual cycle in studies implementing either IVGTT or euglycemic hyperinsulinemic clamp techniques (Bingley *et al.*, 2008; Diamond *et al.*, 1993; Diamond *et al.*, 1989). Yki-järvinen *et al.* (1984) implemented the IVGTT technique in seven women during the follicular and luteal phase of one menstrual cycle. The study reports no significant difference in glucose metabolism or insulin sensitivity between the two phases, with no relationship between insulin-stimulated glucose uptake and either sex hormone reported (Ykijarvinen, 1984). In contrast, other studies have reported lower insulin sensitivity during the luteal phase compared to the follicular phase in healthy menstruating women (Ezenwaka *et al.*, 1993; Pulido and Salazar, 1999; Trout *et al.*, 2007; Valdes and Elkind-Hirsch, 1991). However, studies using the euglycemic hyperinsulinemic clamp technique report no significant differences in glucose response or insulin sensitivity across the menstrual cycle in five (Diamond *et al.*, 1993) and six (Toth *et al.*, 1987) women respectively. This suggests that there is conflicting evidence surrounding changes in glucose homeostasis and insulin sensitivity during the menstrual cycle which could be attributed to variations in techniques and methods implemented.

The current investigation reports no significant difference in data modelling methods such as ISI (Matsuda and DeFronzo, 1999), IGI (Seltzer *et al.*, 1967), DI (Bergman *et al.*, 1981) and HOMA-IR (Matthews *et al.*, 1985) to determine insulin sensitivity or insulin resistance across the different menstrual cycle phases. These results support previous studies that have used data modelling or fasting measures of analysis to indicate insulin sensitivity and resistance (Okey, 1925; Reinke *et al.*, 1972). In

contrast, a more recent study provides a conflicting data set when analysing HOMA-IR values in 257 women and report insulin resistance is positively associated with both oestradiol and progesterone (Yeung *et al.*, 2010). The study also reports lower HOMA-IR values when compared to the current investigation (1.35-1.55 vs. 1.92-2.14); this may be due to the superior participant numbers in the study conducted by Yeung *et al* (2010) or the differences in body composition between the studies and therefore may have had underlying variations in insulin response at baseline.

The results also identify a significant positive correlation between BMI, BF% and weight (kg) and InAUC and HOMA-IR with a negative correlation for ISI ($P < 0.01$). This suggests that an increase in body composition (BMI, weight or body fat %) correlates with an increase in insulin AUC and insulin resistance (HOMA-IR) and a decrease in insulin sensitivity (ISI). These results support a number of studies linking obesity and increased body composition with insulin resistance (Kahn and Flier, 2000). Although the results indicate a correlation between body composition and insulin sensitivity measurements, the study does not report significant differences between menstrual cycle phases. In addition, there was no correlation between body composition and sex hormone concentrations, suggesting body weight in the current population of women is not indicative of variations in sex hormone concentrations

The strengths of the current investigation are observed in the analysis of oestrogen and progesterone plasma concentrations. This unique aspect of mapping individual participant hormone concentrations during the menstrual cycle enables significant changes in both oestradiol and progesterone during different cycle phases (menstrual, follicular and luteal) to be identified and pre-determines appropriate testing days in a second menstrual cycle. Oestradiol and progesterone concentrations were not significantly different and showed little variation (%CV 8.9 and 10.2 respectively) between cycle A and B. This highlights the reproducibility of the methodology implemented and is effective in limiting the number of test days that need to be excluded where participants have been tested in the incorrect phase. This method also allows accurate conclusions to be made surrounding changes in glycaemic response and insulin sensitivity during different phases of the menstrual cycle which no previous study has detailed.

3.9.1: Possible mechanisms

As the current results report significant increases in glucose and insulin AUC during the luteal phase of the menstrual cycle, this would suggest that a combination of both oestrogen and progesterone or progesterone alone may drive these changes, particularly as progesterone is only present in low concentrations during the other phases.

3.9.2: Oestrogen

Oestrogen has two known receptors that exert its physiological signalling ($ER\alpha$ and $ER\beta$). Both receptors are located within the skeletal muscle cell, which is the tissue responsible for the largest quantity of glucose uptake (Ropero *et al.*, 2008). It is well established that insulin secreted from the pancreas stimulates the insulin receptors present at the skeletal muscle cell membrane, thus triggering a cascade of events that leads to the translocation of GLUT 4 from the intracellular vesicle to the cell membrane, causing an increase in glucose uptake (Saltiel and Kahn, 2001).

However, it has been reported that both $ER\alpha$ and $ER\beta$ play a significant role in the expression of the GLUT 4 glucose transporters, with $ER\alpha$ increasing GLUT 4 translocation and $ER\beta$ inhibiting GLUT 4 gene expression in the muscle (Barros *et al.*, 2009). $ER\alpha$ is therefore associated with more positive insulin action (Gorres *et al.*, 2011) and $ER\beta$ with impaired insulin function (Foryst-Ludwig and Kintscher, 2010). Consequently, the ratio of $ER\alpha$ and $ER\beta$ may play a pivotal role in the regulation of insulin stimulated glucose uptake (Foryst-Ludwig and Kintscher, 2010; Ropero *et al.*, 2008).

There is evidence to report no significant differences in $ER\alpha$ mRNA or $ER\beta$ mRNA between pre- and postmenopausal women in adipose tissue (Shin *et al.*, 2007). However, a study using ovariectomized rats reports a lack of oestrogen may increase $ER\alpha$ expression in the liver, as ER expression may be regulated via feedback of oestrogen concentrations (Hao *et al.*, 2010), with increased ER expression in phases of high oestrogen concentrations (Sakaguchi *et al.*, 2003). Subsequently the results reported by Shin *et al.* (2007) may be related exclusively to adipose tissue. In addition, $ER\alpha$ has been reported to be the dominant ER in premenopausal women, in the myometrium (Sakaguchi *et al.*, 2003). As high concentrations of oestrogen are

reported to increase ER expression and ER α is the most dominant ER in premenopausal women (Sakaguchi *et al.*, 2003) and the positive effects ER α has in insulin stimulated glucose uptake (Gorres *et al.*, 2011), it is unsurprising that the current investigation reports no significant difference in glucose or insulin during the follicular phase when oestrogen concentrations are high. As such the increase in glucose and insulin during the luteal phase reported in the current investigation therefore may be due to an increase in progesterone concentrations rather than oestrogen. However, the inter-individual differences in ER α : ER β content in insulin-sensitive tissue may also possibly explain the changes in OGTT response between the various menstrual phases (Shin *et al.*, 2007). Furthermore the regulation of ER expression during different menstrual cycle phases is unknown and therefore requires further investigation

3.9.3: Progesterone

Evidence has been reported to suggest progesterone promotes insulin resistance and therefore could explain the changes in glucose and insulin AUC reported during the luteal phase in the current investigation. Rodent models have suggested that progesterone concentrations could disrupt the diverse signalling cascade associated within insulin stimulated glucose uptake (Wada *et al.*, 2010). In particular, progesterone appears to suppress the PI-3 kinase mediated pathway, leading to a decrease in IRS-1 expression within the adipose tissue cells and the inhibited metabolic signalling of insulin between Akt and GLUT4 translocation (Wada *et al.*, 2010). Progesterone therapy has also been reported to cause a reduction in GLUT4 content in skeletal muscle and subsequently impair contraction-stimulated glucose uptake during exercise (Campbell and Febbraio, 2002). In addition, neither oestradiol nor progesterone treatment is reported to cause changes in pancreatic insulin content in ovariectomised (OVX) rats. The evidence suggests oestradiol acts directly on the pancreas by stimulating insulin release, whereas progesterone is more likely to affect insulin response through an increase in insulin resistance (Sutter-Dub, 1979). Previous research also reports an increase in the concentration of progesterone receptors during the late follicular phase just before ovulation with a gradual fall in receptor concentrations reported by the late luteal phase just before menstruation (Ingamells *et al.*, 1996). This suggests that oestrogen and progesterone

may concurrently affect insulin response. Studies have shown that oestrogen can induce progesterone receptor changes by increasing islet progesterone receptor binding and that this may increase the sensitivity of progesterone's metabolic effects (Elseifi *et al.*, 1981).

As progesterone is only present in significant concentrations during the luteal phase, the insulin resistant effects previously reported (Campbell and Febbraio, 2002; Wada *et al.*, 2010) can only significantly occur during this phase. The results of the current investigation suggest glucose and insulin AUC increased during luteal phase, indicating that the elevated progesterone concentrations may have reduced effective insulin stimulated glucose uptake.

3.9.4: Sex hormone binding globulin (SHBG)

Although the current study measured oestradiol (oestrogen) and progesterone hormone concentrations, further investigations should include measurements of sex hormone binding globulin (SHBG). SHBG is a major glycoprotein which binds sex steroids including oestrogen and testosterone within plasma, regulating the distribution between free and protein bound steroids and modulating their access to target tissues (Hammond, 2002; Kahn *et al.*, 2002; Selby, 1990). Reports have identified low SHBG plasma concentrations as an indicator of central adiposity and an increased risk of type 2 diabetes development (Pugeat *et al.*, 2010; Sutton-Tyrrell *et al.*, 2005).

One investigation has reported a significant increase in SBHG concentrations during the luteal phase compared to the follicular phase (55.1 vs. 51.4 nmol/L), indicating that the percentage of unbound oestrogen may be increased during the follicular phase (Thys-Jacobs *et al.*, 2008). Subsequently, although the absolute amount of oestrogen may not differ, a greater percentage of SHBG bound oestrogen may exist during the luteal phase when significant changes in glucose and insulin are being reported. As a result, if unbound sex steroids have the greatest effect on metabolic pathways then oestrogen may not be the dominant hormone driving the changes in the insulin stimulated glucose uptake pathway reported during the luteal phase. With this in mind, rather than oestrogen and progesterone directly affecting the glucose and insulin responses, the possible changes reported may therefore occur as a result

of a cascade of events including the availability of SHBG plasma concentrations as this determines the quantity of free or unbound hormones.

The current data report an increase in glucose and insulin response during the luteal phase only, suggesting that the changes are phase specific. As insulin resistance develops from a gradual decline in effective uptake and response to glucose by increased insulin concentrations. Then the small increase reported in the current study highlights the potential vulnerability some women face to the development of impaired glucose homeostasis and insulin resistance. Particularly in those women who are more predisposed or genetically vulnerable to developing conditions such as type 2 diabetes.

3.10: Conclusion

The findings presented in this study indicate glucose and insulin AUC significantly increases during the luteal phase compared with the menstrual phase of the menstrual cycle following the ingestion of a 75g glucose beverage, using the OGTT method. The unique aspect presented in this study is the superior experimental design used to determine menstrual cycle phases in a Baseline cycle to predefine testing days in a second menstrual cycle. As the current study reports an increase in glucose and insulin AUC during the luteal phase, this suggests that both oestrogen and progesterone or progesterone alone drive the reported changes.

It would therefore appear that the physiological fluctuations in oestrogen and progesterone during the menstrual cycle in young pre-menopausal women need to be considered when investigating glucose response and insulin resistance. In particular, the long term implications of impaired glucose and insulin control require the subtle influence of factors such as oestrogen and progesterone to be explored in much greater detail.

Chapter 4: Energy Expenditure and Substrate Oxidation during the Menstrual Cycle at Rest and during Exercise

4.1: Abstract

4.1.1: Introduction

There is conflicting evidence concerning the potential effects of fluctuating oestrogen and progesterone during the menstrual cycle on energy expenditure and substrate oxidation. The aim of the current study is therefore to investigate energy expenditure and substrate oxidation at rest and during exercise within the three phases of the menstrual cycle.

4.1.2: Methods

Nineteen participants undertook resting measures (REST) and ten for exercise (EX). Oestrogen and progesterone concentrations were collected for one complete menstrual cycle for accurate menstrual phase definition. In a second menstrual cycle, the REST group had fasting energy expenditure measured on one day during each of the three distinct menstrual cycle phases (menstrual, follicular and luteal). The EX group had energy expenditure measures made during three moderate intensity aerobic exercise sessions (30 minutes, 70-80% age predicted HR max) corresponding to the three menstrual cycle phases.

4.1.3: Results

Plasma oestradiol and progesterone concentrations did not significantly differ between the two cycles in either the REST or EX group. The REST group showed a significant decrease in CHO oxidation (0.12 ± 0.01 vs. 0.09 ± 0.01 vs. 0.10 ± 0.01 , g/min, menstrual, follicular and luteal, $P < 0.05$) and a marginal increase in fat oxidation (0.047 ± 0.004 vs. 0.054 ± 0.003 vs. 0.051 ± 0.003 , g/min; $P = 0.06$; menstrual, follicular and luteal) during the follicular phase. There was no difference in energy expenditure. The EX group showed no difference in energy expenditure or substrate oxidation between cycle phases.

4.1.4: Conclusion

The results indicate CHO oxidation decreases and fat oxidation increases during the follicular phase of the menstrual cycle at rest only. Oestrogen therefore may mediate

these effects as it is the only hormone present in high concentrations during this phase. Neither sex hormone appears to have an effect on energy expenditure or substrate oxidation during exercise.

4.2: Introduction

There are a number of factors that have been reported to influence energy expenditure and substrate oxidation including, gender (Tarnopolsky, 2008), exercise duration and intensity (Venables *et al.*, 2005), training status (Jeukendrup *et al.*, 1997) and diet (Goedecke *et al.*, 2000). It has been reported that during exercise, males display a significant increase in carbohydrate (CHO) oxidation matched at the same relative exercise intensity compared to women, who display an increase in fat oxidation (Tarnopolsky *et al.*, 1990). It has been proposed that sex hormones may play a significant role in determining the differences in energy expenditure and substrate oxidation reported between males and females (Tarnopolsky, 2008).

Previous studies have attempted to determine the effects of sex hormones on substrate oxidation in rodent studies. Ovariectomized rats were treated with daily oestrogen (2 µg), oestrogen (2 µg) and progesterone (2 mg) or a control vehicle for 8 days. Following 60 minutes of exercise, it was reported that the oestrogen only treatment group significantly increased fat oxidation compared to the oestrogen and progesterone group (Hatta *et al.*, 1988). Additional rodent studies have further suggested that oestrogen only administration results in an increase in fat and a decrease in CHO oxidation (Ellis *et al.*, 1994; Kendrick and Ellis, 1991; Rooney *et al.*, 1993), with oestrogen reported to increase free fatty acid availability through lipolysis and a decrease in adipocyte lipoprotein lipase (LPL) activity (Ellis *et al.*, 1994). However, progesterone administration has also been reported to decrease CHO oxidation through increasing liver glycogen storage and decreasing gluconeogenesis (Kalkhoff, 1982; Matute and Kalkhoff, 1973), suggesting these sex hormones may work concurrently.

However, currently there is limited information known about the effects of fluctuating sex hormone concentrations on energy expenditure and substrate oxidation during different phases of the menstrual cycle. Particularly as fluctuations in sex hormone concentrations during the menstrual cycle are small in comparison to differences between genders. Previous research investigating resting metabolic rate (RMR) during the menstrual cycle has produced conflicting and inconsistent data with studies suggesting RMR significantly increases during the luteal phase (Bisdee *et al.*, 1989b; Curtis *et al.*, 1996; Hitchcock, 1929; Meijer *et al.*, 1992; Snell, 1920;

Solomon *et al.*, 1982; Wakeham, 1923; Webb, 1986), and a significant increase in fat oxidation and a decrease in CHO oxidation during the luteal phase also reported (Bisdee *et al.*, 1989a; Snell, 1920; Wakeham, 1923). In contrast, other studies have demonstrated no significant difference across the three phases of the menstrual cycle at rest (Blunt, 1921; Henry *et al.*, 2003; Howe *et al.*, 1993; Piers *et al.*, 1995; Weststrate, 1993; Wiltshire, 1921). Similarly during exercise, there is strong evidence to suggest that fat oxidation increases and CHO oxidation decreases during the luteal phase at different exercise intensities (Fu *et al.*, 2009; Hackney, 1999; Hackney *et al.*, 1994; Hackney *et al.*, 2000; Wenz *et al.*, 1997). However there are a number of studies that report no significant differences in substrate oxidation during the menstrual cycle (Horton *et al.*, 2002; Mclay *et al.*, 2007; Vaiksaar *et al.*, 2011).

The inconsistent data may be in part related to variations in methodological practices implemented during the aforementioned studies. In particular, many of the earlier studies did not report hormone (oestrogen and progesterone) concentration data. This may account for some of the variations in results as the methods used to distinguish menstrual cycle phases and hormone concentrations are inconsistent and subjective measures. By determining oestrogen and progesterone concentrations during the menstrual cycle (menstrual, follicular and luteal phases), pre-defined days can be selected to perform accurate measurements of energy expenditure and substrate oxidation.

The aim of the present study was to investigate the effects of oestrogen and progesterone throughout the menstrual cycle, on energy expenditure and substrate oxidation at rest and during exercise. It was hypothesised that elevated oestrogen concentrations during the follicular phase cause an increase in energy expenditure as well as fat oxidation. Measurements of oestrogen (oestradiol) and progesterone concentrations were made for the duration of one complete menstrual cycle, to pre-define the three cycle phases (menstrual, follicular and luteal). This method establishes hormone concentrations of both oestrogen (oestradiol) and progesterone before measurements of energy expenditure and substrate oxidation are performed during a second cycle.

4.3: Methods

4.3.1: Baseline characteristics

Ethical approval was obtained from the University Research Ethics Committee at Oxford Brookes University (UREC registration number: 100470 and 110571). Written informed consent was obtained from each participant prior to taking part. Participants were aged between 18-40 y, BMI 18.5 - 30kg/m² and had a fasting blood glucose <6.1mmol/l. Participants were excluded if they reported irregular menstrual cycles for three months prior to the beginning of the study, were anaemic or diabetic. Exclusions were also made based on factors that influence sex hormone concentrations including any form of hormone regulated contraception, pregnancy, breastfeeding or having given birth within the previous two years. Participants were members of the public from the Oxford area and were considered healthy following the completion of a series of health questionnaires. Twenty nine regularly menstruating (21 – 40 d) pre-menopausal women were recruited, 19 women took part in the resting measures, and 10 were recruited for the exercise measures (Table 4.1).

Table 4.1: Baseline characteristics of participants in resting and exercise group

Characteristic	REST Group	EX Group
Age (y)	27 ± 6	27 ± 6
Height (m)	1.66 ± 0.07	1.65 ± 0.02
Body mass (kg)	63.2 ± 11.5	67.8 ± 12.9
BMI (kg/m ²)	22.8 ± 3.3	24.8 ± 4.5
Body fat (%)	28.3 ± 7.4	30.7 ± 9.1

Mean ± SD, REST Group n= 19, EX Group n= 10, BMI: Body Mass Index

The investigation was divided into study A: resting (REST) and B, exercise (EX), and was conducted over two complete menstrual cycles. During cycle 1 (study A and B) plasma oestradiol and progesterone concentrations were collected to pre-define the three individual menstrual cycle phases (menstrual, follicular and luteal) were

determined. During cycle 2, fasting measures of RMR were recorded on three occasions in the REST group. Participants in the EX group undertook 3 x 30 minutes aerobic exercise sessions (70-80% age predicted heart rate (HR) max) during which energy expenditure and substrate oxidation were calculated from VO_2 and VCO_2 rates using validated equations. Measures taken in both REST and EX groups corresponded to the three individual menstrual cycle phases.

4.4: Experimental Protocol

4.4.1: Cycle 1: REST and EX group

Participants contacted the researcher at the first sign of menstrual bleeding (day 1). Venous blood samples (6ml) were taken from each participant every other week day for one complete menstrual cycle. Venous blood samples were collected into EDTA-containing tubes (K2E 10.8mg, Becton Dickinson, UK) and stored on ice, centrifuged at 4000 rpm for 10 minutes at 4°C and aliquots of plasma frozen at -80°C until analysis. Following hormone analysis the three cycle phases were identified (menstrual, follicular and luteal).

4.4.2: Cycle 2: Rest

A dietary intake record was taken for 24 hours preceding the first RMR measurement and was matched prior to subsequent visits. Participants (n=19) were instructed to refrain from consuming alcohol and caffeine and from undertaking any exercise for 24 hours prior to testing. Participants arrived at the laboratory following a 10-12 hour overnight fast and had a venous blood sample (6ml) taken to confirm oestradiol and progesterone. Participants were rested supine for 30 minutes to allow for stabilisation and acclimatization to the canopy and instrument noise. Respiratory gas was then collected for 30 minutes by a ventilated hood system (Deltatrac Datex, Helsinki, Finland) and average RMR, fat and CHO oxidation rates were then calculated (Jeukendrup and Wallis, 2005).

4.4.3: Cycle 2: Exercise

The same pre-test control protocol as the REST group was implemented 24 hours before testing, including dietary intake replication and refrained consumption of alcohol or caffeine. Participants (n=10) arrived at the laboratory at least four hours post-prandial and had a venous blood sample (6ml) taken to confirm oestradiol and

progesterone concentrations. The supervised exercise regime was conducted on a treadmill within the Human Performance Laboratory at Oxford Brookes University. The walking exercise included a 5 minute warm-up (50% heart rate, HR max); 30 minutes (70-80% HR max) and a 2-minute cool down (50% HR max). Respiratory gases were collected using a Douglas bag for two minutes every ten minutes whilst participants were exercising (70-80% HR max) and a measure of rate of perceived exertion (RPE) was taken (Borg, 1970). HR was measured during all exercise sessions using a Polar FS1 HR Monitor (Polar Electro, Finland). A sample of expired air was analysed (Servomex Gas Analyser, 1440C, Servomex Group Ltd, UK) for oxygen and carbon dioxide concentrations and total volume expired. Gas concentrations were then used to calculate oxygen uptake (VO_2 , l/min) and carbon dioxide production (VCO_2 , l/min) allowing CHO and fat oxidation (g/min) to be calculated (Jeukendrup and Wallis, 2005).

4.5: Biochemical Analysis

Where appropriate, plasma samples were thawed and analysed using a Cobas e411, semi-automated analyser (Roche, UK) for oestradiol (E2, 03000079, Roche, UK) and progesterone (Progesterone, 12145383, Roche, UK).

4.6: Calculations

Oxygen uptake (VO_2 , l/min) and carbon dioxide (VCO_2 , l/min) production was calculated following indirect calorimetry at rest and during exercise. As a result, mean energy expenditure, CHO and fat oxidation rates were then calculated using validated equations (Jeukendrup and Wallis, 2005).

$$\text{Energy Expenditure (kcal)} = (3.87 \times \text{VO}_2) + (1.2 \times \text{VCO}_2)$$

$$\text{Carbohydrate Oxidation: CHO (g/min)} = (4.21 \times \text{VCO}_2) - (2.96 \times \text{VO}_2)$$

$$\text{Fat Oxidation: FAT (g/min)} = (1.695 \times \text{VO}_2) - (1.70 \times \text{VCO}_2)$$

$$\text{Fat Oxidation (\%EE)} = \frac{(\text{FAT} \times 9.75)}{\text{EE}} \times 100$$

$$\text{Carbohydrate Oxidation (\%EE)} = 100 - \text{FAT (\%EE)}$$

4.7: Statistical Analysis

Power analysis calculations using data from a previous study (Howe *et al.*, 1993) showed that in order to have the power (0.8) to detect a moderate significant effect (0.724) in energy expenditure, 18 participants were required, however 19 participants were recruited in order to allow for any unexpected drop-outs.

Data analysis was performed using SPSS for windows software (version 19.0; SPSS Inc., Chicago, IL). Data are expressed as means \pm SD unless otherwise stated. Plasma oestradiol and progesterone concentrations were analysed using a one-way repeated measures analysis of variance (ANOVA) to determine significant differences in hormone concentrations during the three menstrual phases (menstrual, follicular and luteal). Energy expenditure and substrate oxidation at rest and during exercise as well as all other exercise measures across the three phases of the menstrual cycle were analysed using repeated measures ANOVA. Pairwise comparisons were carried out if a significant difference was observed using the Bonferroni test. Significance was accepted at the $P < 0.05$ level.

4.8: Results

4.8.1: Menstrual Cycle Duration

All 18 participants had regular ovulatory menstrual cycles based on oestradiol and progesterone concentrations (27 ± 3 d) (Table 4.2). Menstrual cycle phase duration varied considerably between participants, menstrual phase duration ranged from 4-8 d, follicular phase 6-12 d and luteal phase 9-16 d. Cycle days identified as individual phases ranged from days 1- 8 (menstrual), 7-20 (follicular) and 13-31 (luteal) of the menstrual cycle. There was no significant difference between baseline and test cycle duration within participants.

Table 4.2: Menstrual cycle duration (days) in both EX and CON during baseline and test cycle

Participant	Baseline	Test Cycle
1	28	29
2	28	26
3	28	27
4	23	24
5	28	28
6	21	20
7	29	29
8	30	29
9	30	27
10	32	33
11	26	27
12	31	31
13	26	26
14	25	23
15	30	24
16	28	28
17	26	28
18	24	27
19	26	28
20	24	22
21	34	34
22	34	33
23	28	23
24	26	27
25	23	24
26	28	28
27	28	27
28	28	27
29	32	28
Group Average	28 ± 3	27 ± 3

Mean ± SD

4.8.2: Oestradiol and Progesterone concentrations

All 29 participants (REST and EX) displayed regular, ovulatory menstrual cycles based on oestradiol and progesterone concentrations (27 ± 6 d). Menstrual cycle phase duration varied considerably between participants (menstrual phase, 4 - 8 d; follicular phase, 6 - 12 d and luteal phase, 9 - 16 d).

Oestradiol and progesterone concentrations during cycle 1 were not significantly different between groups or compared to cycle 2 in either group (REST or EX). Oestradiol hormone concentrations during cycle 1 and 2 were significantly higher during the follicular and luteal phases as compared to menstrual, with oestradiol concentrations significantly higher during the follicular phase compared with luteal ($P < 0.05$) in both groups. Progesterone concentrations were significantly elevated during the luteal phase when compared to the follicular and menstrual phases in both groups (Table 4.3).

Table 4.3: Plasma sex hormone concentrations during the menstrual, follicular and luteal phases in both resting and exercising participants (REST and EX).

	Baseline		REST group		EX group	
	Cycle 1		Cycle 2		Cycle 2	
	Oestradiol (pg/ml)	Progesterone (ng/ml)	Oestradiol (pg/ml)	Progesterone (ng/ml)	Oestradiol (pg/ml)	Progesterone (ng/ml)
Menstrual (MP)	50 ± 3 ^a	0.78 ± 0.08 ^a	44 ± 4 ^a	0.60 ± 0.07 ^a	43 ± 5 ^a	0.65 ± 0.12 ^a
Follicular (FP)	327 ± 26 ^b	0.91 ± 0.11 ^a	290 ± 22 ^b	0.98 ± 0.13 ^a	308 ± 37 ^b	0.59 ± 0.08 ^b
Luteal (LP)	155 ± 16 ^c	14.09 ± 1.10 ^b	139 ± 8 ^c	12.29 ± 1.17 ^b	155 ± 21 ^c	13.47 ± 2.54 ^c

Mean ± SD; Baseline n=29; REST group n= 19; EX group n=10; Within each column, values with different superscripts are significantly different from each other (P < 0.05).

4.8.2: Resting

There was no difference in VO_2 or VCO_2 between the menstrual, follicular or luteal phases (Table 4.4). Resting metabolic rate (RMR, kcal/day) and energy expenditure (kcal/min) were not significantly different between phases. CHO oxidation was significantly decreased ($P<0.05$) and a marginal increase in fat oxidation was reported during the follicular phase ($P<0.06$).

Table 4.4: RMR and substrate oxidation during menstrual, follicular and luteal phases at rest

	Menstrual (MP)	Follicular (FP)	Luteal (LP)
VO_2 (l/min)	0.18 ± 0.03	0.18 ± 0.03	0.18 ± 0.03
VCO_2 (l/min)	0.15 ± 0.03	0.15 ± 0.03	0.15 ± 0.03
RMR (kcal/day)	1268 ± 228	1255 ± 192	1281 ± 196
RMR / BW (kcal/kg)	20 ± 2	20 ± 1	20 ± 1
EE (kcal/min)	0.90 ± 0.16	0.89 ± 0.14	0.91 ± 0.14
Fat oxidation (g/min)	0.047 ± 0.015	0.054 ± 0.011^b	0.051 ± 0.014
CHO oxidation (g/min)	0.12 ± 0.04	0.09 ± 0.03^a	0.10 ± 0.05
Fat oxidation (%)	51 ± 14	59 ± 11^a	56 ± 16
CHO oxidation (%)	49 ± 14	41 ± 11^a	44 ± 16

Mean \pm SD, REST group n=19, VO_2 : Volume of oxygen consumed; VCO_2 : Volume of carbon dioxide produced; RMR: Resting Metabolic Rate; BW: Body Weight; EE: Energy Expenditure; CHO: Carbohydrate; ^a Significantly different from MP ($P<0.05$), ^b Trending difference from MP ($P<0.06$)

4.8.3: Exercise

There was no difference in VO_2 or VCO_2 between the menstrual, follicular or luteal phases (Table 4.5) and no difference in HR or RPE between phases. This indicates exercise intensity and perceived intensity was the same during each of the three test days. In addition, no significant difference was reported in energy expenditure (kcal/min), CHO or fat oxidation (g/min or %) between the three menstrual cycle phases (menstrual, follicular and luteal).

Table 4.5: Energy expenditure and substrate oxidation during exercise in the menstrual, follicular and luteal phases

	Menstrual (MP)	Follicular (FP)	Luteal (LP)
VO_2 (l/min)	1.53 ± 0.25	1.52 ± 0.23	1.58 ± 0.31
VCO_2 (l/min)	1.35 ± 0.21	1.38 ± 0.23	1.43 ± 0.31
HR (bpm)	147 ± 11	145 ± 11	144 ± 8
EE (kcal/min)	7.58 ± 1.21	7.57 ± 1.16	7.84 ± 1.55
Fat oxidation (g/min)	0.29 ± 0.18	0.24 ± 0.17	0.24 ± 0.16
CHO oxidation (g/min)	1.17 ± 0.39	1.29 ± 0.45	1.36 ± 0.51
Fat oxidation (%)	36 ± 22	31 ± 24	30 ± 20
CHO oxidation (%)	64 ± 22	69 ± 24	70 ± 20
RPE	13 ± 2	13 ± 2	13 ± 2
Speed (kph)	5.8 ± 0.4	5.8 ± 0.4	5.8 ± 0.4
Incline (%)	5.9 ± 1.0	5.9 ± 1.0	5.9 ± 1.0

Mean \pm SD, EX group n=10; VO_2 : Volume of oxygen consumed; VCO_2 : Volume of carbon dioxide produced; HR: Heart Rate; EE: Energy Expenditure; CHO: Carbohydrate; RPE: Rate of Perceived Exertion

4.9: Discussion

The current study aimed to investigate energy expenditure and substrate oxidation at rest and whilst exercising during the three phases of the menstrual cycle (menstrual, follicular and luteal).

4.9.1: Study A: Rest

The current study is the first to report a decrease in CHO and an increase in fat oxidation combined with no significant differences in RMR at rest during the follicular phase. These results are supported by previous studies investigating RMR (Henry *et al.*, 2003; Howe *et al.*, 1993; Piers *et al.*, 1995; Weststrate, 1993) and substrate oxidation (Bisdee *et al.*, 1989a) at rest separately, but there are none that show the current combination of results.

However, there are also a number of previous studies that report changes in energy expenditure during the luteal phase when both oestrogen and progesterone concentrations are high (Bisdee *et al.*, 1989b; Curtis *et al.*, 1996; Hitchcock, 1929; Meijer *et al.*, 1992; Snell, 1920; Solomon *et al.*, 1982; Wakeham, 1923; Webb, 1986). In addition, a number of investigations report increases in fat oxidation during the luteal phase (Matsuo *et al.*, 1999; Matsuo *et al.*, 1998), with some studies reporting no differences between menstrual cycle phases (Piers *et al.*, 1995; Toth *et al.*, 1999; Weststrate, 1993) which is contradictory to the current investigation. The differences between studies may be in response to variations in methodologies implemented and sample size, with studies testing during only two menstrual cycle phases, follicular and luteal (Matsuo *et al.*, 1999; Matsuo *et al.*, 1998) compared to three phases in the current investigation (menstrual, follicular and luteal) and therefore the exact concentrations of both sex hormones is unclear.

As the results in the current study report a decrease in CHO oxidation and an increase in fat oxidation during the follicular phase; this suggests that oestrogen may be responsible as this hormone is present in high concentrations. It has been suggested that oestrogen via oestrogen receptor alpha (ER α) may act through a number of specific pathways to stimulate fat oxidation (Oosthuyse and Bosch, 2012). The first ER α stimulated pathway includes the up regulation of peroxisome proliferation activator receptors (PPAR, α and δ). These receptors stimulate the transport proteins and enzymes essential for fatty acid uptake including, fatty acid

translocase (FAT/CD36), cytosolic fatty acid binding protein (FABPc) and fatty acid transport protein (FATP). The up regulation of PPAR (α and δ) subsequently results in an increase in long chain fatty acid (LCFA) uptake into the skeletal muscle. This causes an increase in β -oxidation and the formation of acetyl-CoA, which results in a decrease in glucose oxidation (Oosthuyse and Bosch, 2012). This theory is supported by reports in ovariectomized rats infused with oestrogen only which result in an increase in PPAR expression. Rats infused with progesterone only or a combination of oestrogen and progesterone display a blunted PPAR expression in skeletal muscle (Campbell *et al.*, 2003).

Secondly, oestrogen also increases skeletal muscle lipoprotein lipase (LPL) activity, which results in an increase in very low density lipoprotein triacylglycerol (VLDL-TG) breakdown. This breakdown increases LCFA formation and these FA can then be transported into the cell resulting in an increased uptake. The third pathway occurs within adipose tissue, where $ER\alpha$ is reported to down regulate adipogenesis and promote LCFA mobilisation. This increase in LCFA mobilisation increases LCFA availability which may lead to an increased uptake in the skeletal muscle cells (Oosthuyse and Bosch, 2012).

As such, the mechanistic evidence currently suggests that oestrogen may be responsible for the increases fat oxidation reported in the current study as it is present in high concentrations. Although oestrogen remains elevated during the luteal phase, the concomitant effect of progesterone seem to blunt oestrogen's response (Oosthuyse and Bosch, 2012) and as such may explain why there are no significant changes during in energy expenditure or substrate oxidation reported during this phase.

4.9.2: Study B: Exercise

The results in the current study report no significant difference in energy expenditure or substrate oxidation following 30 minutes exercise at 70-80% predicted HR max (approx. 60-65% VO_2 max) in the three menstrual cycle phases. These results concur with previous studies implementing similar exercise measures, with no between cycle phase differences in respiratory exchange ratio (RER) reported in 6 moderately trained women during a 90-minute cycling bout at 60% VO_2 max (Nicklas *et al.*, 1989). Likewise, no significant differences were

reported during exercise at 50% (Braun *et al.*, 2000; Horton *et al.*, 2002), 70% (Galliven *et al.*, 1997; Vaiksaar *et al.*, 2011), 80% VO_2 max (Desouza *et al.*, 1990) or following a 16km time-trial (McLay *et al.*, 2007).

However, the aforementioned studies, in addition to the results from the current investigation, are contrary to a large number of other studies that have reported significant increases in fat oxidation during the luteal phase following exercise (Fu *et al.*, 2009; Hackney, 1999; Hackney *et al.*, 1994; Hackney *et al.*, 2000; Ruby *et al.*, 1997; Wenz *et al.*, 1997; Zderic *et al.*, 2001). It could be argued that the differences reported between the current investigation and previous literature during different phases of the menstrual cycle can be explained by the variations in exercise duration and mode since the exercise intensity for these studies also averaged 60-65% VO_2 max (Fu *et al.*, 2009; Hackney, 1999; Hackney *et al.*, 1994; Hackney *et al.*, 2000; Ruby *et al.*, 1997; Wenz *et al.*, 1997; Zderic *et al.*, 2001). Participants in these studies exercised between 60-90 minutes in duration, whilst the current study required participants to exercise for 30 minutes only. Participants in four of these studies were also required to perform cycling exercises (Fu *et al.*, 2009; Hackney, 1999; Wenz *et al.*, 1997; Zderic *et al.*, 2001), whereas the current study required a brisk treadmill walk only. It has been recently demonstrated that exercise mode or type does affect substrate oxidation, with significantly higher fat oxidation following a running exercise compared to a cycling exercise (Cheneviere *et al.*, 2010). Two studies reported similar energy expenditure and substrate oxidation rates during treadmill exercise; however these exercise sessions were longer (60 minutes) and included anovulatory participants (Hackney *et al.*, 1994; Hackney *et al.*, 2000).

A further explanation could be that the current exercise group had measurements of energy expenditure and substrate oxidation taken 4 hours post-prandial whereas previous exercise studies report measures following an overnight fast (Hackney, 1999). As fasting measurements of energy expenditure have been reported to predominantly utilize FAs (Jeukendrup, 2002), the post-prandial measures taken during the current exercise group may diminish any potential increases in fat oxidation that may be reported as a result of the content in the pre-exercise meal. Previous evidence has reported high glycaemic index pre-exercise meals increase

CHO oxidation whereas low GI pre-exercise meals increase fat oxidation at varying exercise intensities (Stevenson *et al.*, 2009; Stevenson *et al.*, 2006; Wong *et al.*, 2008; Wu *et al.*, 2003). Although the current investigation implemented strict pre-test control procedures, with repeated dietary intake before each trial, it is unclear what effect the last meal consumed may have had on the results.

4.9.3: Differences between resting and exercise measures

The current investigation reports a decrease in CHO and an increase in fat oxidation at rest but not whilst exercising during the luteal phase of the menstrual cycle. The increase in fat oxidation following an overnight fast at rest is unsurprising as FAs are the predominant fuel used by skeletal muscle during fasting (Jeukendrup, 2002). However the differences reported may also be due to the elevated energy expenditure that occurs as a result of the walking exercise. This may have reduced any potential changes in substrate oxidation that may have been reported, as a result of sex hormones during different phases of the menstrual cycle. The differences may also be due to the alterations in pre-trial protocols, with resting measures performed postprandial, following an overnight fast whereas exercise sessions were conducted following 4 hours post-prandial.

In addition, energy expenditure and substrate oxidation measures were conducted in a reduced number of participants within the EX group compared to the REST group (n=10 vs. n=19). Although the number of participants in the EX group was similar to many of the previous investigations (Galliven *et al.*, 1997; Mclay *et al.*, 2007; Ruby *et al.*, 1997), this does reduce the ability to draw concrete conclusions regarding the effects of oestrogen and progesterone on energy expenditure and substrate oxidation during exercise.

4.9.4: Methodological limitations

The study reported here is the first to accurately identify menstrual cycle phase before and during testing, using plasma analysis of oestradiol and progesterone concentrations. This improved methodology enables significant changes in both oestradiol and progesterone concentrations during different cycle phases (menstrual, follicular and luteal) to be identified and to pre-define appropriate testing days in a second menstrual cycle. Accurate cycle phase identification is scarcely reported in previous studies, with authors favouring less accurate

measures to distinguish menstrual cycle phase. These include BBT as an indicator of ovulation or standard cycle duration of 28 days, with day 14 as a guide midpoint (Desouza *et al.*, 1990; Horton *et al.*, 2002; Matsuo *et al.*, 1999). In addition, although some previous investigations report significant changes during the luteal phase (Desouza *et al.*, 1990; Hackney, 1999; Matsuo *et al.*, 1999; Zderic *et al.*, 2001), the terminology used to describe different menstrual cycle phases can vary between studies. The follicular phase for example is used to describe two distinctly different fluctuations in both oestradiol and progesterone hormone concentrations. Early follicular demonstrates low oestrogen and low progesterone concentrations with late follicular demonstrating high oestrogen and low progesterone concentrations.

The current investigation accurately determined cycle phase based on oestradiol and progesterone hormone concentrations (menstrual, follicular and luteal) reporting no significant difference between groups. Previous studies that have similarly distinguished between these three phases demonstrate similar results to the current investigation (Horton *et al.*, 2006), reporting no significant difference in energy expenditure or substrate oxidation during exercise.

4.10: Conclusion

The current investigation reports a significant decrease in CHO and a marginal increase in fat oxidation during the follicular phase at rest, but with no difference in RMR. In addition, no difference is reported in energy expenditure or substrate oxidation during moderate intensity exercise. Changes in substrate oxidation at rest during the follicular phase suggest that oestrogen may mediate the increase in fat oxidation but that other rate limiting steps such as FA availability may supersede its effects during exercise. It would therefore appear that high oestrogen concentrations during the follicular phase may need to be considered when investigating energy expenditure and substrate oxidation in women.

Chapter 5: The Effects of an Exercise Intervention on Premenstrual Syndrome (PMS) and Dietary Intake in Sedentary Women: A Randomised Control Trial

5.1: Abstract

5.1.1: Introduction

Exercise is currently recommended as a tool to manage PMS symptoms; however few studies have confirmed its effectiveness. In addition, energy and carbohydrate (CHO) intake have been reported to increase during the luteal phase in women with PMS (Wurtman *et al.*, 1989). The current study investigated whether a moderate intensity exercise intervention had any effect on PMS symptoms and Quality of Life (QoL) and whether any such effect is associated with changes in oestrogen and progesterone concentrations. In addition, the study investigated whether energy and macronutrient intake within cycle phases was affected by the moderately intensive exercise regime.

5.1.2: Methods

Twenty-five sedentary women, identified as suffering from PMS symptoms, were recruited to a randomised control trial spanning four menstrual cycles, the first serving as a baseline followed by three cycles of intervention. Participants were randomly assigned to either an exercise (EX) group (n=13), which involved three supervised 30-minute moderate-intensity (70-80% HR max) treadmill walking exercise sessions per week, or to a control (CON) group (n=12), which involved attending a 90-minute, one-to-one meeting once per week with the investigator. PMS symptoms were evaluated every other day by a validated symptom questionnaire (Moos, 1968) and dietary intake was assessed by a 24-hour dietary recall interview once per week. Venous blood samples were collected three times per week during Cycle 1 and 4 to determine menstrual cycle phase according to plasma oestradiol and progesterone concentrations.

5.1.3: Results

The EX group demonstrated a reduction in PMS symptoms following the exercise intervention (pain, water retention, autonomic reaction and impaired concentration; $P<0.05$), whereas no difference was observed in the CON group.

The EX group reported a 13% reduction in the number of 'unhealthy' QoL days during cycle 4. No differences were observed in plasma oestradiol or progesterone concentrations between cycle 1 and 4, in either group. There was no difference in energy intake (2487 vs. 2457 kcal) or macronutrient intake (%TEI) (Protein: 14.4% vs. 14.6%; Fat: 35.1% vs. 34.5%; CHO: 48.5% vs. 49.4%; NMES: 17.4% vs. 16.1%), as a result of the exercise intervention (EX vs. CON). However, averaged data of the three cycles of the intervention period reported CHO intake (%TEI) was significantly lower during the luteal phase, as compared to the menstrual (45.5% vs. 50.1%) and follicular (45.5 vs. 49.9%) phases in the EX group. No difference was found between menstrual cycle phases in the CON group.

5.1.4: Conclusion

Moderate-intensity exercise over three months reduces symptoms of PMS and improves QoL; however this does not appear to be mediated by changes in plasma oestradiol or progesterone concentrations. In addition, CHO intake (%TEI) during the luteal phase is reduced following a 3 month moderate-intensity exercise programme but does not affect overall energy intake. The results suggest moderate-intensity exercise provides some PMS symptom relief and therefore may be an effective symptom management tool. In addition the decrease in CHO intake during the luteal phase has potentially wider implications for weight management and therefore further studies need to determine the exact duration and intensity required for the most effective symptom relief.

5.2: Introduction

Premenstrual Syndrome (PMS) is the common term used to describe a cluster of symptoms associated with the luteal phase (premenstrual phase) of the menstrual cycle that manifests with distressing physical, behavioural and psychological symptoms. It is estimated that PMS affects up to 75-85% of women of reproductive age (Halbreich *et al.*, 2007), with symptoms having adverse effects on Quality of Life (QoL) (Yonkers *et al.*, 2008). The types and severity of symptoms experienced varies between women but the most common symptoms include dysphoria, mood swings, pain, fatigue, headaches, irritability, tension, aggression, bloating, breast tenderness, backache, water retention and food cravings (American College of Obstetricians & Gynecologists, 2000).

The exact aetiology of PMS remains uncertain and multifactorial, however symptoms occur during the luteal phase of the menstrual cycle, when an increase in both oestrogen and progesterone hormone concentrations occur, with progesterone at its peak (Stoddard *et al.*, 2007). There have therefore been a number of hypotheses suggesting that women with PMS may display an increased sensitivity to normal fluctuations in oestrogen and progesterone concentrations during the menstrual cycle (American College of Obstetricians & Gynecologists, 2000). In particular, cyclical fluctuations in sex hormones have been reported to alter brain neurotransmitters that act on the hypothalamus, which in turn causes a decrease in serotonin concentrations which are associated with depression (Frackiewicz and Shiovitz, 2001; Meltzer, 1989; Rapkin *et al.*, 1987). In addition, women who use hormonal contraceptives or experience anovulatory menstrual cycles are reported to have lower incidences of PMS symptoms (Freeman, 1997; Shangold *et al.*, 1990).

As a result of this uncertainty, various treatments have been proposed to alleviate symptoms of PMS. The UK Royal College of Obstetricians and Gynaecologists (RCOG) (2007) currently gives general advice about exercise, diet and stress reduction before starting hormonal treatment as a first line of symptom management with the combined pill or low dose selective serotonin reuptake inhibitors (SSRIs) also used in treatment (Royal College of Obstetricians and Gynaecologists, 2007). However, despite lifestyle based interventions being

recommended, very little research has been conducted to successfully support its inclusion.

Exercise or physical activity is currently recommended for general populations to provide positive overall health benefits. An improved psychological well-being in healthy and clinical populations following exercise participation (Hassmen *et al.*, 2000), that can provide important mental health benefits (Department of Health, 2004) has also been previously reported. Observational research has suggested that women who participate in regular exercise report fewer PMS symptoms compared to sedentary women, with those who exercise vigorously also reporting a decrease in symptoms (Aganoff and Boyle, 1994; Choi and Salmon, 1995). However, women who perform regular high intensity exercise do experience a greater frequency of anovulatory cycles, with lower steroid hormone concentrations, which may account for the reduction in symptoms experienced (Shangold *et al.*, 1990).

With this in mind, only four studies have investigated the effects of an exercise intervention on PMS symptoms (Prior *et al.*, 1986; Prior *et al.*, 1987; Steege and Blumenthal, 1993; Stoddard *et al.*, 2007). All four intervention studies report significant beneficial effects of aerobic exercise. The most recent was a 24 week moderate intensity exercise intervention conducted in 14 sedentary women with PMS symptoms measured by questionnaires. The study reports significant decreases in symptoms following the exercise regime (Stoddard *et al.*, 2007). However the methodologies implemented in all of these previous studies were unable to control for factors that are known to affect the prevalence and severity of PMS such as age or age since menarche (Daley, 2009). The investigations were also unable to provide an assessment of ovulation status, an important factor as ovulatory cycles have a greater effect on PMS symptoms compared to anovulatory cycles (Shangold *et al.*, 1990).

Similarly, sex hormone concentrations before and after the interventions were not detailed explicitly in any of these studies and those that did, recorded concentrations in urine up to four days following excretion (Stoddard *et al.*, 2007). As PMS occurs during periods of high oestrogen and progesterone, reporting concentrations of these hormones may allow potential mechanisms of

action to be proposed. Previous research has reported PMS symptoms to be associated with higher progesterone concentrations compared to non-PMS women (Redei and Freeman, 1995). Whilst others indicate that the absolute concentrations of sex hormones may not differ (Stoddard *et al.*, 2007) or that women who suffer from PMS may be more susceptible to changes in hormone concentrations and their proposed metabolic effects (Schmidt *et al.*, 1988). As such, the inclusion of sex hormone concentrations would allow any significant effects of exercise on PMS symptoms and QoL to be attributed appropriately.

In addition, dietary intake has been reported to vary across different phases of the menstrual cycle, demonstrating significant increases in energy intake during the luteal phase (Dalvit-Mcphillips, 1983; Dalvit, 1981). Conditions associated with hormone fluctuations during the menstrual cycle, such as PMS, may predispose women to changes or exacerbations in appetite control and energy intake. Previous evidence suggests women who suffer from PMS report significantly increased energy intake during the luteal phase as compared to the follicular phase (Brzezinski *et al.*, 1990; Cross *et al.*, 2001; Johnson *et al.*, 1995; Wurtman *et al.*, 1989), with a significant proportion of this being contributed by an increase in carbohydrate (CHO) intake (Wurtman *et al.*, 1989). However, this is not universally reported, with some studies reporting an increase in both CHO and fat during the luteal phase (Brzezinski *et al.*, 1990; Cross *et al.*, 2001; Johnson *et al.*, 1995) or no significant differences across the menstrual cycle (Bryant *et al.*, 2006).

Interestingly, when analysing the dietary intake by food groups, a significant increase in cakes, desserts and high sugar food categories during the luteal phase is reported (Cross *et al.*, 2001). This could explain some of the increases in CHO intake, as well as an increase in non-milk extrinsic sugars (NMES) during the luteal phase which are present in many of these products (Bryant *et al.*, 2006). NMES are sugars that are not naturally incorporated into the cellular structure of the food, but have been added to potentially enhance the flavour (Health Education Authority, 1999). In addition, dietary intake has been reported to be suppressed following exercise and this could result in a decrease in energy or CHO intake in PMS women (Blundell *et al.*, 2003). There is evidence that sex

hormones may affect CHO oxidation, with differences recorded during the three phases of the menstrual cycle (Foryst-Ludwig and Kintscher, 2010). To understand the alterations in substrate oxidation of women with PMS it is important to understand the potential influence that fluctuating sex hormones during the menstrual cycle and the effects of exercise may have on dietary behaviour and the long-term consequences, since overconsumption can lead to the development of obesity and type 2 diabetes.

As a result, a study has been designed to investigate the effects of moderate intensity-exercise on PMS symptoms and QoL and whether any such effect is mediated by changes in plasma oestrogen and progesterone concentrations. It is hypothesised that PMS symptoms will reduce and QoL scores will increase following the exercise intervention. In addition, the exercise intervention will also cause a reduction in sex hormone concentrations and therefore may be responsible for some of the reduction in PMS symptoms reported. The study also aims to investigate changes in energy, macronutrient and NMES intake that may additionally occur in women suffering from PMS with the comparison to a sedentary control group.

5.3: Methods

5.3.1: Participant Recruitment

Ethical approval was obtained from the University Research Ethics Committee at Oxford Brookes University (UREC registration number: 110571) in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant before the study began.

Seventy four members of the public from the Oxford area responded to participant recruitment advertisements. All women were invited to undertake a screening visit to determine their eligibility to the study. Participants were required to be aged between 18-40 y, BMI 18.5 - 30kg/m², fasting blood glucose <6.1mmol/l, sedentary (<1hr exercise per week) and to suffer from PMS symptoms during at least three previous menstrual cycles. Participants were excluded if they reported irregular menstrual cycles for three months prior to the beginning of the study,

were anaemic or diabetic. Exclusions were also made if participants were taking any form of hormone regulated contraception, pregnant, breastfeeding or had given birth within the previous two years.

5.3.2: Screening

All potential participants (n=60) completed a screening visit to determine their eligibility to the study and signed a screening informed consent form. To determine PMS eligibility, the Menstrual Distress Questionnaire (MDQ) Form C (Moos, 1968) and the Premenstrual Syndrome Screening Tool (PSST) (Steiner *et al.*, 2003) were completed to retrospectively measure symptoms that occurred during the most recently completed menstrual cycle. To ensure participants met the strict inclusion criteria, PMS mood scores using MDQ, were required to be at least 30% greater between the follicular phase and the luteal phase. PSST required potential participants to score at least 5 moderate to severe symptoms during the luteal phase of the most recent menstrual cycle, with symptoms affecting normal every-day activities in at least one category. The participants were considered healthy, except from suffering with PMS, following the completion of a series of health questionnaires. Participants were blinded to the exact hypothesis and informed that the nature of the study was to determine hormone concentrations in women with PMS before and after exercise.

Of the 60 women that undertook the screening visit, 25 regularly menstruating (21 – 40 d) pre-menopausal women were recruited to the randomized controlled trial to determine the effects of an exercise intervention on PMS symptoms and QoL. Participant baseline anthropometry data were recorded during screening visits and included height measured to the nearest centimetre using a free-standing stadiometer (Seca 217, Birmingham, UK), body weight measured to the nearest 0.05kg using calibrated anthropometric weighing scales (Seca 877, Birmingham, UK) and body composition measured using bio-electrical impedance segmental body composition analyser (Model BC-418 MA, Tanita UK Ltd., Yiewsley, UK) (Table 5.1). All participants indicated they performed aerobic exercise less than 1 hour per week following the completion of the habitual physical activity questionnaire during screening (appendix 12).

Table 5.1: Baseline characteristics of participants in exercise (EX) and control (CON) group

Characteristics	EX group	CON group	Combined
Age (y)	27 ± 6	28 ± 7	27 ± 6
Height (m)	1.64 ± 0.04	1.65 ± 0.07	1.64 ± 0.05
Body mass (kg)	67.1 ± 12.3	66.8 ± 16.3	67.0 ± 13.8
BMI (kg/m ²)	24.8 ± 4.3	24.3 ± 4.2	24.6 ± 4.1
Body fat (%)	30.9 ± 8.6	29.4 ± 8.3	30.2 ± 8.3

Mean ± SD, EX Group n= 11, CON Group n= 9, Combined n= 20, BMI: Body Mass Index

Eligible participants (n=25) were randomised into either the EX or CON group using numbers automatically assigned to groups by an online randomisation calculator (www.randomizer.com). Of the 25 participants that were eligible for the study, 20 participants completed all four menstrual cycles. Reasons for exclusion or drop-out included irregular menstrual cycles, prescribed hormonal contraceptive use during Baseline and time commitment (2 exercises, 3 controls).

5.4: Experimental Protocol

The study was conducted over four complete and consecutive menstrual cycles (Cycle 1: Baseline; Cycle 2, 3 and 4: Intervention).

5.4.1: Cycle 1: Baseline

Participants from both groups (EX and CON) contacted the researcher at the first sign of menstrual bleeding (day 1). Venous blood samples (6ml) were then taken from each participant three times a week for one complete menstrual cycle. Venous blood samples were collected into EDTA-containing tubes (K2E 10.8mg, Becton Dickinson, UK), stored on ice, centrifuged at 4000 rpm for 10 minutes at 4°C and aliquots of plasma frozen at -80°C until analysis. PMS symptoms were recorded every other day by the participant using prospective form T of the MDQ (Moos, 1968). 24 hour dietary recall interviews were conducted by the investigator once weekly and quality of life (HR-QoL) (Centers for Disease

Control and Prevention, 2000) questionnaires were self-completed once at the end of each menstrual cycle.

5.4.2: Cycle 2 and 3: Intervention

5.4.2.1: Exercise group (EX)

Participants were required to undertake 3 x 30 minute supervised aerobic exercise sessions per week for two menstrual cycles (approx. 8 weeks). The exercise was conducted using a treadmill and was of moderate intensity 70-80% predicted maximum heart rate (220 bpm - age). The exercise regime included a 5 minute warm-up (50% HR max), 30 minutes walking exercise (70-80% HR max) and a 2-minute cool down (50% HR max). Rate of perceived exertion (RPE) and HR were monitored every 10 minutes throughout each exercise session. Symptomatic questionnaires were continuously collected together with dietary intake and QoL questionnaires as per Cycle 1. In addition, total cycle duration was recorded at the end of each menstrual cycle to monitor regularity.

5.4.2.2: Control group (CON)

To minimise the effects of participant-investigator interaction in the EX group, participants in the CON group undertook a 90-minute one-to-one meeting with the investigator for two menstrual cycles (approx. 8 weeks). Participants were required to attend weekly sessions during which body composition measurements were taken (Tanita BC-418MA segmental body composition analyser) and a 24 hour dietary recall interview was conducted by the investigator. Symptomatic questionnaires were continuously collected together with QoL questionnaires as per Cycle 1. In addition, total cycle duration was recorded at the end of each menstrual cycle. The participants continued daily life as normal, ensuring a sedentary lifestyle was maintained.

5.4.3: Cycle 4: Intervention

All EX participants continued the exercise programme for one further menstrual cycle (approx. 4 weeks, 12 weeks total). CON participants repeated Cycle 1 protocol for one further menstrual cycle. Venous blood samples (6ml) were taken and processed, along with PMS symptoms, dietary intake and QoL also recorded as during Cycle 1 in both groups (EX and CON).

5.5: Biochemical Analysis

Where appropriate, plasma samples were thawed and analysed using a Cobas e411, semi-automated analyser (Roche, UK) for oestradiol (E2, 03000079, Roche, UK) and progesterone (Progesterone, 12145383, Roche, UK). Following hormone analysis, oestradiol and progesterone hormone concentrations and the three cycle phases were identified (menstrual, follicular and luteal).

5.6: Statistical Analysis

Data analysis was performed using SPSS for Windows software (version 19.0; SPSS Inc., Chicago, IL). Data are expressed as means \pm SD and means plus 95% Confidence Intervals (CI) where indicated. Plasma oestradiol and progesterone concentrations were analysed using a one-way repeated measures analysis of variance (ANOVA) to determine significant differences in hormone concentrations during the three menstrual cycle phases (menstrual, follicular and luteal). Differences in PMS symptom scores and QoL, between groups, cycles and phases, were analysed using repeated measures ANOVA. Dietary intake data were coded using Wisp V3.0 (Tinuviel Software, Anglesey, UK) dietary analysis software and analysed by repeated measures ANOVA to determine between cycle and within cycle phase differences. Significance was accepted at the $P < 0.05$ level.

5.7: Results

5.7.1: Menstrual Cycle Duration

All twenty participants recorded regular menstrual cycles as defined by total cycle duration during all four menstrual cycles. The mean cycle duration was longer in the CON group compared to the EX group during cycle 1 (28 ± 3 vs. 31 ± 6 ; $P=0.057$); however this difference was small, not statistically significant and was not replicated during cycle 4 (27 ± 3 vs. 30 ± 4 ; $P=0.07$) (Table 5.2).

Table 5.2: Menstrual cycle duration (days) in both EX and CON during all four experimental cycles

	EX Group	CON Group	Combined
Cycle 1	28 ± 3	31 ± 6	29 ± 5
Cycle 2	27 ± 4	29 ± 5	28 ± 4
Cycle 3	27 ± 4	29 ± 4	28 ± 4
Cycle 4	27 ± 3	30 ± 4	29 ± 4

Mean \pm SD, EX group $n=11$; CON group $n=9$

Variation in cycle length was minimal within participants but average total cycle duration between participants ranged between 20 – 37 days. This variation highlights the importance of mapping each participant menstrual cycle when investigating factors that may be affected by cycle phase (Table 5.3).

Table 5.3: Menstrual cycle duration (days) for all participants during all four experimental cycles

Participant	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Mean
1	28	33	27	29	29 ± 3
2	39	37	35	35	37 ± 2
3	28	26	28	26	27 ± 1
4	28	26	30	27	28 ± 2
5	27	26	28	26	27 ± 1
6	40	36	34	34	36 ± 3
7	34	32	27	34	32 ± 3
8	34	35	31	33	33 ± 2
9	28	27	26	28	27 ± 1
10	26	27	27	27	27 ± 1
11	23	25	21	24	23 ± 2
12	22	24	27	28	25 ± 3
13	28	28	26	28	28 ± 1
14	29	27	28	28	28 ± 1
15	21	20	19	20	20 ± 1
16	28	25	25	27	26 ± 2
17	29	25	25	29	27 ± 2
18	30	28	27	29	29 ± 1
19	30	34	29	27	30 ± 3
20	32	27	33	33	31 ± 3

Mean ± SD

5.7.2: Exercise

There was no difference in speed (kph) or incline (%) between the three cycles of exercise intervention (Table 5.4).

Table 5.4: Exercise measures in the EX group during the three cycles of intervention during Cycle 2, 3 and 4

	Cycle 2	Cycle 3	Cycle 4
Heart Rate (bpm)	146 ± 11	145 ± 11	144 ± 8
RPE	13 ± 3	13 ± 2	13 ± 2
Speed (kph)	5.8 ± 0.4	5.8 ± 0.4	5.8 ± 0.4
Incline (%)	5.9 ± 1.0	5.9 ± 1.0	5.9 ± 1.0

Mean ± SD, EX group n=11; RPE: Rate of perceived exertion

5.7.3: PMS Symptom Scores

There was no difference in symptom scores between EX and CON during cycle 1, as measured by percentage change or actual scores during the premenstrual phase (Table 5.5), with participants displaying at least a 30% greater symptom score between the follicular phase and the luteal phase during cycle 1.

Table 5.5: PMS symptom scores between the follicular phase and luteal phase in Cycle 1 in both the EX and CON group

Symptom Categories	EX Group			CON Group		
	FP	LP	% change	FP	LP	% change
Pain	46 ± 6	65 ± 9	43 ± 23	49 ± 7	67 ± 11	38 ± 27
Water Retention	45 ± 6	67 ± 18	54 ± 56	44 ± 7	66 ± 14	53 ± 30
Autonomic Reaction	48 ± 5	64 ± 15	34 ± 34	48 ± 5	65 ± 19	33 ± 33
Negative Affect	42 ± 7	61 ± 15	47 ± 29	42 ± 5	60 ± 15	43 ± 25
Impaired concentration	46 ± 6	62 ± 12	43 ± 18	43 ± 6	60 ± 79	40 ± 29
Behaviour Change	46 ± 7	62 ± 23	35 ± 45	48 ± 10	64 ± 23	34 ± 34
Arousal	41 ± 12	50 ± 10	32 ± 30	55 ± 15	48 ± 10	37 ± 39
Control	46 ± 6	44 ± 5	1 ± 7	43 ± 0	44 ± 4	2 ± 8

Mean ± SD; EX group n=11, CON group n=9. FP: Follicular phase, LP: Luteal phase, % change: Difference in symptom scores between FP and LP expressed as a percentage, Symptoms clusters; **Pain**; muscle stiffness, headache, cramps, backache, fatigue, general aches and pain; **Water retention**; weight gain, skin blemish or disorder, painful or tender breasts, swelling (breasts or abdomen); **Autonomic reaction**; dizziness or faintness, cold sweats, nausea or vomiting, hot flashes; **Negative affect**; loneliness, anxiety, mood swings, crying, irritability, tension, feeling sad or blue, restlessness; **Impaired concentration**; insomnia, forgetfulness, confusion, poor judgement, difficulty concentrating, distractible, minor accidents, poor motor coordination; **Behaviour change**; poor school or work performance, take naps or stay in bed, stay at home, avoid social activities, decreased efficiency; **Arousal**; affectionate, orderliness, excitement, feelings of well-being, bursts of energy or activity; **Control**; feelings of suffocation, chest pains, ringing in the ears, heart pounding, numbness or tingling, blind spots or fuzzy vision.

Baseline vs. screening (Prospective vs. Retrospective comparison)

Screening questionnaire symptom scores were compared to baseline prospective scores (% change from follicular to luteal vs. actual luteal scores) in both EX and CON groups to determine if the participants continued to be eligible for PMS diagnosis. There was no significant difference in symptoms between EX or CON groups at screening. There was no significant difference in mean % change scores recorded in each symptom category between retrospective or prospective symptom analyses in either group (Table 5.6).

Table 5.6: Percentage change between follicular and luteal phases during both screening (MDQ Form C) and baseline cycle (MDQ Form T) for diagnosis of PMS

% change	EX group		CON group	
	Screening	Baseline	Screening	Baseline
Pain	43 ± 19	43 ± 23	46 ± 28	38 ± 27
Water Retention	48 ± 25	54 ± 56	45 ± 38	53 ± 30
Autonomic Reaction	34 ± 16	34 ± 34	30 ± 32	33 ± 33
Negative Affect	46 ± 17	47 ± 29	54 ± 32	43 ± 25
Impaired concentration	36 ± 41	43 ± 18	38 ± 35	40 ± 29
Behaviour Change	49 ± 21	35 ± 45	47 ± 51	34 ± 34
Arousal	37 ± 59	32 ± 30	32 ± 32	37 ± 39
Control	1 ± 10	1 ± 7	2 ± 14	2 ± 8

Individual participant data report variations in mean % change scores (all symptoms) in some participants between screening and baseline (Table 5.7). Although there was no change in the average symptom scores between groups, some participants recorded a lower % change in symptoms at baseline compared to the retrospective screening.

Table 5.7: Participant symptoms scores (% change), between follicular and luteal phases during screening (MDQ Form C) and baseline (MDQ Form T) between groups

Participant	EX group		CON group	
	Screening	Baseline	Screening	Baseline
1	62 ± 40	33 ± 30	51 ± 35	39 ± 16
2	36 ± 28	40 ± 30	32 ± 29	30 ± 21
3	35 ± 20	41 ± 26	72 ± 54	35 ± 29
4	39 ± 40	94 ± 55	44 ± 40	30 ± 28
5	50 ± 37	28 ± 27	33 ± 17	33 ± 32
6	62 ± 42	37 ± 32	56 ± 26	34 ± 33
7	32± 14	36 ± 29	41 ± 44	35 ± 47
8	39 ± 36	62 ± 60	30 ± 20	69 ± 35
9	41 ± 30	38 ± 24	30 ± 37	55 ± 32
10	31 ± 18	27 ± 17		
11	43 ± 35	36 ± 36		

This highlights the differences between retrospective and prospective symptom analysis and the potential consequences this may have in correctly diagnosing women with PMS. Despite some differences reported within participants between retrospective (Form C) and prospective (Form T) analysis, all participants maintained eligibility for PMS diagnosis with at least a 30% change in symptoms scores between follicular and luteal phases using either screening or baseline data.

To determine the effects of the exercise intervention on PMS symptoms, symptom scores (8 categories) recorded in Cycle 1 and 4 in each group (EX and CON) were analysed. A repeated measures ANOVA was performed to determine the effects of Time (Cycle 1 vs. Cycle 4) x Group (EX and CON) for all PMS Symptoms (8 categories). The analysis (Figure 5.1) revealed a decrease in symptom scores as a result of time (Cycle 1 vs. Cycle 4, $P<0.01$) and this was marginally different ($P=0.083$) between groups (EX vs. CON).

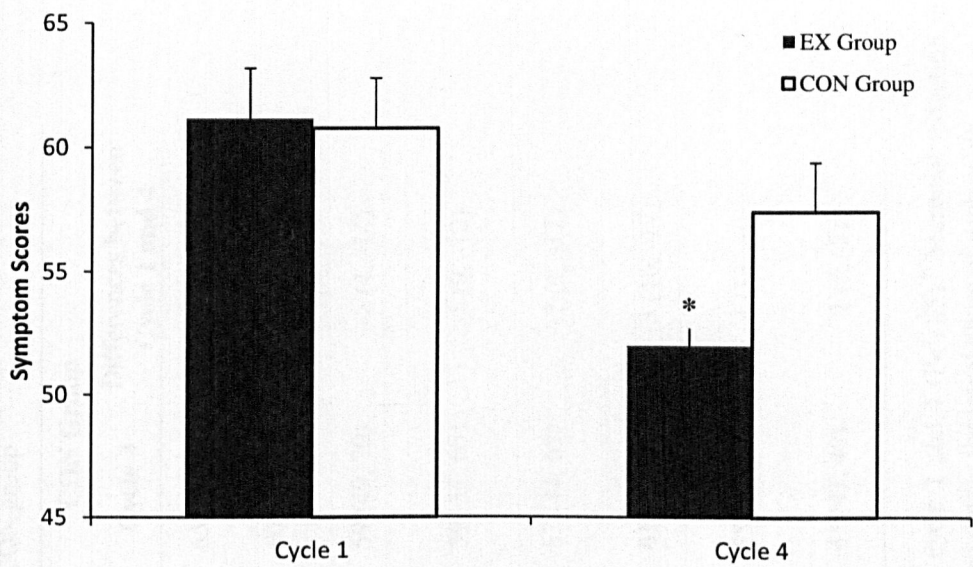


Figure 5.1: Mean total symptom score during the luteal phase of Cycle 1 and 4 in the EX and CON groups (* $P=0.083$)

Further analysis demonstrated symptoms experienced as a percentage change between Cycle 1 and 4 was significantly different in the EX compared to the CON group; with a reported 15% decrease in total symptoms experienced within the EX group compared to a 3.5% decrease in the CON group.

Changes in individual symptom categories between cycles 1 and 4 within each group (EX and CON) were also analysed (Table 5.8). Participants assigned to the EX group showed a reduction in some PMS symptoms between cycle 1 and 4. These include pain, water retention, autonomic reaction and impaired concentration ($P<0.05$), with a marginal reduction in negative affect ($P=0.074$) and behaviour change ($P=0.082$). The CON group showed no reduction in any of the symptom categories between cycle 1 and 4.

Table 5.8: PMS symptom scores for individual symptom categories between Cycle 1 and 4 in both EX and CON group

Symptom Category (Score range)	EX Group		CON Group	
	Cycle 1	Cycle 4	Differences between Cycle 1 and 4	Differences between Cycle 1 and 4
Pain (36-136)	65 (59, 71)	58 (50, 67)	-7 (-2, -13) ^a	-1 (10, -13)
Water Retention (35-141)	67 (57, 77)	56 (47, 65)	-11 (-1, -21) ^a	-7 (2, -15)
Autonomic Reaction (42-141)	64 (53, 74)	52 (43, 62)	-11 (-4, -19) ^a	-5 (4, -14)
Negative Affect (36-112)	61 (52, 71)	51 (44, 58)	-10 (1, -22) ^b	-1 (9, -12)
Impaired Concentration (37-158)	65 (56, 74)	50 (42, 58)	-15 (-4, -26) ^a	-7 (4, -17)
Behaviour Change (37-160)	62 (48, 77)	52 (44, 59)	-11(2, -22) ^b	-3 (10, -17)
Arousal (29-127)	45 (34, 56)	49 (35, 63)	4 (15, -7)	4 (13, -4)
Control (42-236)	44 (41, 47)	45 (41, 48)	1 (5, -4)	1 (5, -3)

Mean (95% CI), EX group n=11, CON group n=9; ^a Significant reduction in symptom severity between cycle 1 and 4 (P<0.05). ^b Marginal reduction in symptom severity between cycle 1 and 4 (P<0.08). Scores correspond to the severity of symptoms experienced during the luteal phase of each cycle

Additional analysis was completed using PMS symptoms recorded in the EX group only to determine the effects of the intervention in each study cycle (C1 – 4) and phase (menstrual, follicular and luteal). As PMS scores were not recorded throughout cycle 2 and 3 for the CON group, comparative analysis between groups for all cycles was inappropriate in this instance.

A 3 phase (menstrual, follicular and luteal), 4 cycle (C1, C2, C3, C4) within subject factors repeated measures ANOVA was conducted for all 8 symptom categories (pain, water retention, autonomic reaction, negative affect, impaired concentration, behaviour change, arousal and control). There were no differences in average scores during each cycle, but there were significant phase related changes for symptoms pain, water retention, autonomic reaction, negative affect and impaired concentration ($P < 0.05$).

Table 5.9: PMS symptoms scores during all phases and all 4 cycles in the exercise group (EX)

Symptom	Cycle 1 (Baseline)				Cycle 2				Cycle 3				Cycle 4				Significance for all cycles
	MP	FP	LP	MP	FP	LP	MP	FP	LP	MP	FP	LP	MP	FP	LP	FP	
Pain	46 ± 10	46 ± 6	65 ± 9	51 ± 10	51 ± 10	53 ± 10	50 ± 6	51 ± 11	60 ± 13	50 ± 9	51 ± 10	58 ± 13	51 ± 10	58 ± 13	51 ± 10	58 ± 13	MP < LP*
Water Retention	47 ± 10	45 ± 6	67 ± 17	46 ± 5	50 ± 11	52 ± 18	48 ± 4	48 ± 5	57 ± 13	49 ± 14	49 ± 10	56 ± 16	49 ± 10	56 ± 16	49 ± 10	56 ± 16	MP < LP*
Autonomic Reaction	47 ± 4	48 ± 5	64 ± 15	52 ± 2	49 ± 7	53 ± 10	48 ± 8	46 ± 3	53 ± 16	49 ± 9	48 ± 5	52 ± 12	49 ± 9	48 ± 5	52 ± 12	48 ± 5	MP < LP* FP < LP*
Negative Affect	48 ± 6	42 ± 6	61 ± 15	45 ± 5	46 ± 7	55 ± 19	48 ± 11	45 ± 10	49 ± 11	52 ± 17	44 ± 10	51 ± 10	52 ± 17	44 ± 10	51 ± 10	44 ± 10	MP < LP* FP < LP*
Impaired Concentration	49 ± 7	46 ± 6	65 ± 12	53 ± 5	50 ± 9	55 ± 14	48 ± 12	46 ± 5	50 ± 12	48 ± 13	47 ± 10	50 ± 14	48 ± 13	47 ± 10	50 ± 14	47 ± 10	MP < LP* FP < LP*
Behaviour Change	49 ± 6	46 ± 7	62 ± 23	52 ± 4	54 ± 11	57 ± 21	50 ± 9	45 ± 8	47 ± 12	50 ± 8	47 ± 9	52 ± 13	50 ± 8	47 ± 9	52 ± 13	47 ± 9	NS
Arousal	42 ± 8	41 ± 12	50 ± 10	42 ± 8	48 ± 15	53 ± 19	44 ± 14	49 ± 16	45 ± 17	41 ± 14	47 ± 20	49 ± 23	41 ± 14	47 ± 20	49 ± 23	47 ± 20	NS
Control	44 ± 4	46 ± 6	44 ± 5	45 ± 3	43 ± 0	42 ± 1	45 ± 3	45 ± 7	44 ± 4	45 ± 3	43 ± 0	45 ± 5	45 ± 3	43 ± 0	45 ± 5	43 ± 0	NS

Mean ± SD, EX group n= 11, MP – Menstrual Phase, FP – Follicular Phase, LP – Luteal Phase, *Significance – P < 0.05, NS – Not significant

Cycle by phase analysis also indicated significant differences in 4 symptom categories (pain, water retention, negative affect and impaired concentration, $P < 0.05$). Further analysis determining the differences in luteal phase symptom scores between cycles revealed significant reductions between cycle 1 and 2 in pain and water retention symptom categories (Figure 5.2 A and B).

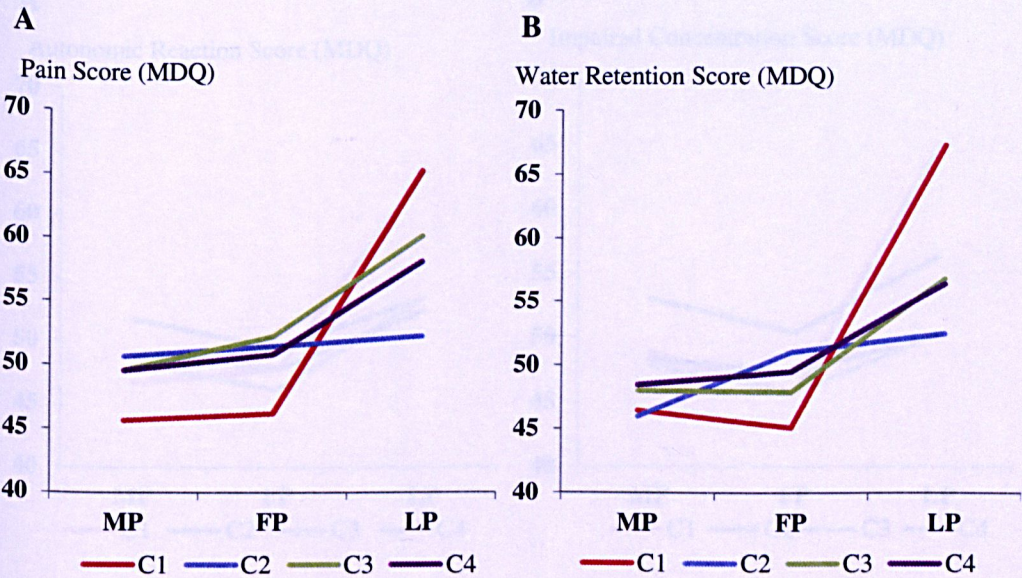


Figure 5.2: Pain (A) and Water Retention (B) symptom scores recorded during the menstrual, follicular and luteal phases in cycles 1 – 4 for the EX group. Pain and Water Retention symptom scores in Cycle 1 were significantly elevated compared to Cycle 2 ($P < 0.05$). MP – Menstrual phase, FP – Follicular phase, LP – Luteal phase, C1 – Cycle 1, C2 – Cycle 2, C3 – Cycle 3, C4 – Cycle 4.

MDQ symptoms vs. DSM 5 symptoms

Individual MDQ symptom scores were then reanalysed to determine if the participants remained PMS symptomatic according to the symptoms required to reach the DSM 5 definition (Table 5.10). The MDQ symptoms included in this analysis were: loneliness, crying, feeling sad, irritability, mood swings, anxiety, tension, restlessness, poor work performance, stay at home, avoid socialising, decreased efficiency, concentration, distractible, naps, weight gain, insomnia, confusion, stiffness, headache, backaches, aches and pains, painful breasts and

Autonomic reaction was significantly different between cycle 1 and 4 (Figure 5.3 A), with impaired concentration symptoms scores significantly reduced in cycle 2, 3 and 4 compared to cycle 1 (Figure 5.3 B). There were no significant differences in all other symptom categories (negative affect, behaviour change, arousal and control).

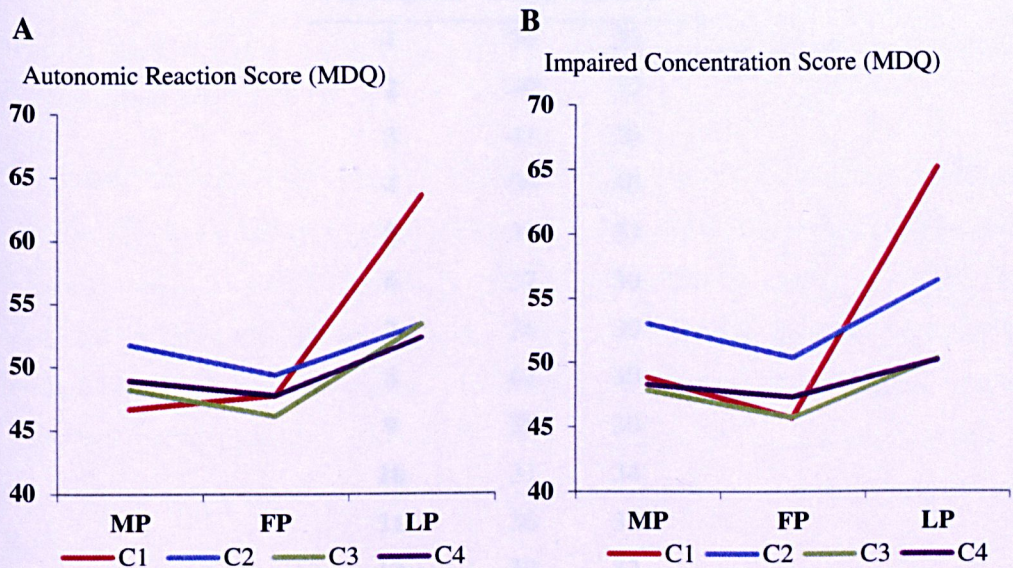


Figure 5.3: Autonomic Reaction (A) and Impaired Concentration (B) symptom scores recorded during the menstrual, follicular and luteal phases in Cycles 1 – 4 for the EX group. Autonomic Reaction Cycle 1 score significantly elevated compared to Cycle 4 ($P < 0.05$). Impaired Concentration Cycle 1 score significantly elevated compared to Cycle 2, 3 and 4 ($P < 0.05$). MP – Menstrual phase, FP – Follicular phase, LP – Luteal phase, C1 – Cycle 1, C2 – Cycle 2, C3 – Cycle 3, C4 – Cycle 4.

MDQ symptoms vs. DSM 5 symptoms

Individual MDQ symptom scores were then reanalysed to determine if the participants remained PMS symptomatic according to the symptoms required to reach the DSM 5 definition (Table 5.10). The MDQ symptoms included in this analysis were: loneliness, crying, feeling sad, irritability, mood swings, anxiety, tension, restlessness, poor work performance, stay at home, avoid socialising, decreased efficiency, concentration, distractible, naps, weight gain, insomnia, confusion, stiffness, headache, backache, aches and pains, painful breasts and

swelling. These symptoms corresponded the most effectively to the 11 DSM 5 symptom categories.

Table 5.10: Percentage change in symptom scores between follicular and luteal phases during two prospective baseline cycles comparing MDQ and DSM5.

Participant	MDQ	DSM5
1	33	33
2	40	30
3	41	30
4	94	40
5	32	34
6	37	30
7	36	30
8	62	30
9	38	30
10	31	34
11	36	37
12	39	32
13	30	34
14	35	30
15	30	31
16	33	33
17	34	35
18	35	40
19	69	36
20	55	38

The results indicated that all participants remain PMS symptomatic at baseline (30% difference between follicular and luteal phase) using only the symptoms required for the DSM 5 criteria diagnosis.

A series of paired sample t-tests were conducted to determine if there were any differences in symptom scores between baseline and cycle 4 in each group (EX and CON) using only those symptoms required for DSM 5 diagnosis (Table 5.11).

Table 5.11: Symptom scores between cycle 1 (baseline) and cycle 4 using DSM5 criteria symptoms.

	C1	C4
EX Group	36 ± 3	23 ± 4
CON Group	39 ± 6	35 ± 3

Mean ± SD, C1: Cycle 1 or Baseline, C4: Cycle 4, EX: Exercise Group n=11, CON: Control Group n=9

The ANOVA results indicate no significant difference in symptom scores at baseline (cycle 1), between groups (EX and CON). Symptom scores significantly reduced between cycle 1 (baseline) and cycle 4 in the EX group ($P<0.05$), but were not in the CON group ($P=0.065$). These results indicate that the exercise intervention reduced symptoms of PMS using both the original MCQ questionnaire designed by Moos (1960) and the individual symptoms reported in the DSM 5 guidelines for PMS diagnosis (American Psychiatric Association, 2013).

5.7.4: Quality of Life

Quality of life (QoL) scores were measured using HR-QoL questionnaire (Centres, 2000) and divided into 2 categories: unhealthy days and healthy days. Participants were instructed to determine the number of days during the last menstrual cycle that impairments to QoL were experienced. Non-parametric data were analysed by Chi-squared test, examining the differences in the number of days recorded between groups (EX vs. CON) and between cycles (1 vs. 4).

Table 5.12: QoL category scores representing the number of days reported during Cycle 1 and 4 in both EX and CON groups

QoL Categories	EX group		CON group	
	Cycle 1	Cycle 4	Cycle 1	Cycle 4
Unhealthy days	13 ± 6	9 ± 6	10 ± 7	11 ± 7
Healthy days	17 ± 6	21 ± 6	20 ± 7	19 ± 7

Mean ± SD, EX group n=11, CON group n=9;

At baseline (Cycle 1), the number of healthy or unhealthy days recorded were not significantly different between EX and CON (Table 5.12). A reduction in the number of unhealthy days and an increase in healthy days were reported in the EX group between Cycle 1 and Cycle 4 but this was not significant. However in public health terms, this translates to a 13% decrease in the number of unhealthy days experienced (13 – 9; 4 days) and subsequently increases the number of healthy days from 17 to 21 (4 days).

5.7.5: Oestrogen and Progesterone concentrations

Complete sex hormone profiles were recorded for 18 of the 20 participants. Two participants were unable to provide complete cycle 1 and 4 hormone measurements due to insufficient vein access on several occasions throughout the study and therefore their hormone concentrations were not analysed.

Both groups displayed regular menstrual cycle patterns with ovulatory cycles in both cycle 1 and 4 as measured by progesterone concentrations. Oestradiol concentrations were significantly higher during the follicular and luteal phases compared to the menstrual, with oestradiol concentrations significantly higher during the follicular phase compared to the luteal ($P < 0.05$) (Table 5.13).

Table 5.13: Plasma oestradiol hormone concentrations for menstrual, follicular and luteal phases during Cycle 1 and 4 in the EX and CON group

Oestradiol (pg/ml)	Cycle 1		Cycle 4	
	EX Group	CON Group	EX Group	CON Group
Menstrual (MP)	46 ± 10 ^a	50 ± 12 ^a	40 ± 12 ^a	47 ± 12 ^a
Follicular (FP)	319 ± 100 ^b	314 ± 100 ^b	292 ± 84 ^b	296 ± 81 ^b
Luteal (LP)	137 ± 38 ^c	154 ± 44 ^c	143 ± 44 ^c	170 ± 63 ^c

Mean ± SD, EX group n=10, CON group n=8; within each column, values with different superscripts are significantly different from each other ($P < 0.05$)

Progesterone concentrations were significantly elevated during the luteal phase compared to the follicular and menstrual phases (Table 5.14), in both cycles (1 and 4) and both groups (EX and CON).

Table 5.14: Plasma progesterone hormone concentrations for menstrual, follicular and luteal phases during Cycle 1 and 4 in the EX and CON group

Progesterone (ng/ml)	Cycle 1		Cycle 4	
	EX Group	CON Group	EX Group	CON Group
Menstrual (MP)	0.6 ± 0.2 ^a	0.6 ± 0.3 ^a	0.6 ± 0.3 ^a	0.6 ± 0.3 ^a
Follicular (FP)	0.7 ± 0.1 ^a	0.7 ± 0.4 ^a	0.8 ± 0.2 ^a	0.7 ± 0.4 ^a
Luteal (LP)	12.6 ± 5.2 ^b	15.6 ± 3.2 ^b	13.6 ± 5.7 ^b	15.6 ± 3.2 ^b

Mean ± SD, EX group n=10, CON group n=8; within each column, values with different superscripts are significantly different from each other (P < 0.05)

There was no difference in oestradiol or progesterone concentrations between cycle 1 and 4 in either group; however progesterone AUC during cycle 1 was lower in the EX compared to the CON group (P<0.05). Neither oestradiol nor progesterone (AUC) was significantly different between groups in cycle 4 (Table 5.15).

Table 5.15: Oestradiol and progesterone AUC concentrations during Cycle 1 and 4 in both EX and CON groups

	Cycle 1		Cycle 4	
	EX Group	CON Group	EX Group	CON Group
Oestradiol AUC	3252 ± 899	3816 ± 1305	3224 ± 1170	3442 ± 1019
Progesterone AUC	97.1 ± 55.9 ^a	148.7 ± 40.5	106.6 ± 70.3	117.9 ± 41.1

Mean ± SD, EX group n=10, CON group n=8; ^a = significantly different from CON; P<0.05

5.7.6: Dietary Intake

Dietary intake records were recorded in all participants (n=20) by conducting 24-hr dietary recall interviews. Interviews were conducted during all three phases of the menstrual cycle and represented both weekday and weekend dietary intake.

Table 5.16: The number of weekday and weekend (days) 24 hr. dietary recalls conducted in both EX and CON groups

	EX Group		CON Group	
	Weekday	Weekend	Weekday	Weekend
Cycle 1	28	9	25	9
Cycle 2	29	8	26	6
Cycle 3	32	6	20	9
Cycle 4	29	10	23	8
% TR	78	22	75	25

EX group n=11, CON group n=9, (%TR); Percentage of total recorded

There were a greater number of dietary recall interviews conducted during weekdays as compared to weekend days in both groups (Table 5.16), but this did not differ between groups or between phases (Tables 5.17 and 5.18) as expressed as a proportion of the total number of dietary recall interviews conducted.

Table 5.17: The number of weekday and weekend (days) 24 hr. dietary recalls conducted in during the menstrual, follicular and luteal phases in the EX group

	Menstrual		Follicular		Luteal	
	Weekday	Weekend	Weekday	Weekend	Weekday	Weekend
Cycle 1	9	2	7	4	12	3
Cycle 2	9	2	8	3	12	3
Cycle 3	9	2	8	3	15	1
Cycle 4	9	2	9	2	11	6
% TR	82	18	73	27	79	21

EX group n=11, CON group n=9, (%TR); Percentage of total recorded

The total number of 24 hr. dietary recalls conducted was greater in the EX group (Table 5.17) compared to the CON group (Table 5.18) but this was due to the differences in participant numbers between the groups.

Table 5.18: The number of weekday and weekend (days) 24 hr. dietary recalls conducted during the menstrual, follicular and luteal phases in the CON group

	Menstrual		Follicular		Luteal	
	Weekday	Weekend	Weekday	Weekend	Weekday	Weekend
Cycle 1	6	3	5	4	14	2
Cycle 2	7	2	8	1	11	3
Cycle 3	7	1	5	4	7	6
Cycle 4	7	2	7	2	9	4
% TR	77	23	69	31	76	24

EX group n=11, CON group n=9, (%TR); Percentage of total recorded

5.7.6.1: Average dietary intake: Cycle 1

There was no difference in energy intake or macronutrient intake during cycle 1 between EX and CON; however CHO intake (%TEI) was significantly higher in the CON group as compared to EX group (Table 5.19).

Table 5.19: Energy and macronutrient intake during Cycle 1 in both EX and CON groups

	EX Group	CON Group	Difference (EX vs. CON)
Energy (kcal/day)	2415 (2091, 2739)	2630 (2272, 2988)	215.1 (-267.6, 697.8)
Protein (g/day)	84.7 (75.3, 94.0)	84.2 (73.9, 94.5)	-0.5 (-14.4, 13.5)
(% TEI)	15 (13, 17)	14 (12, 15)	-1 (-4, 1)
Fat (g/day)	94.5 (80.3, 108.8)	96.2 (80.4, 111.9)	1.6 (-19.6, 22.9)
(% TEI)	35 (33, 38)	33 (30, 36)	-2 (-6, 2)
CHO (g/day)	309.8 (253.3, 366.4)	375.9 (313.5, 438.5)	66.1 (-18.1, 150.4)
(% TEI)	50 (47, 54)	56 (52, 59)	6 (1, 11) ^a
NMES (g/day)	116.3 (76.4, 156.1)	164.4 (120.3, 208.5)	48.2 (-11.3, 107.6)
(% TEI)	17 (13, 21)	23 (18, 27)	5 (-1, 11)

Mean (95% CI), EX group n=11, CON group n=9; CHO; Carbohydrate, NMES; Non-Milk Extrinsic Sugars, %TEI; Percentage total energy intake. ^a P<0.05

5.7.6.2: Average dietary intake between menstrual cycle phases: Cycle 1

There was no difference in energy intake, macronutrient intake or macronutrient intake as a proportion of total energy intake (%TEI) between menstrual cycle phases during cycle 1 in the EX group (menstrual, follicular and luteal) (Table 5.20). The CON group displayed significant decreases in energy, CHO and NMES intake during the menstrual phase compared to the follicular and luteal phase during cycle 1. In addition, protein intake was significantly reduced during the menstrual phase compared to the follicular, with fat intake also lower during the menstrual as compared to the luteal phase. However, there was no difference in any macronutrient intake when expressed as a %TEI during the menstrual cycle phases in the CON group (Table 5.21, Figure 5.4 and 5.5).

Table 5.20: Energy and macronutrient intake during the menstrual, follicular and luteal phases of Cycle 1 in the EX group

	Menstrual	MP vs. FP	Follicular	FP vs. LP	Luteal	LP vs. MP
Energy (kcal/day)	2127 (1574, 2679)	-263 (-1118, 592)	2390 (1888, 2891)	-340 (1339, 659)	2730 (2208, 3251)	603 (-452, 1658)
Protein (g/day)	82.4 (62.1, 102.7)	0.1 (-32.7, 32.9)	82.3 (67.9, 96.6)	-7.2 (-29.9, 15.6)	89.4 (76.1, 102.8)	7.1 (-26.3, 40.5)
(% TEI)	16 (14, 18)	1 (-5, 6)	15 (11, 19)	1 (-4, 7)	14 (11, 17)	-2 (-6, 2)
Fat (g/day)	88.0 (51.2, 121.9)	-0.8 (-41.9, 40.4)	88.8 (67.0, 110.6)	-18.0 (-52.9, 16.9)	106.8 (84.9, 128.8)	18.8 (-20.6, 58.2)
(% TEI)	36 (29, 43)	2 (-4, 9)	34 (29, 38)	-3 (11, 4)	37 (30, 43)	1 (-10, 11)
CHO (g/day)	252.5 (190.7, 314.3)	-79.9 (-194.1, 34.1)	332.4 (239.7, 425.1)	-12.2 (-234.8, 210.4)	344.6 (217.9, 471.4)	92.2 (-114.9, 299.3)
(% TEI)	46 (39, 53)	-5 (-12, 1)	51 (45, 57)	6 (-7, 20)	45 (38, 52)	-1 (-16, 14)
NMES (g/day)	70.5 (33.2, 107.8)	-81.5 (-188.8, 25.9)	151.9 (58.8, 245.0)	25.6 (-107.7, 158.9)	126.3 (77.5, 175.2)	55.9 (-24.3, 136.0)
(% TEI)	11 (7, 16)	-10 (-20, 1)	21 (12, 30)	4 (-8, 16)	17 (13, 21)	5 (-2, 12)

Mean (95% CI), EX group n=11; CHO; Carbohydrate, NMES; Non-Milk Extrinsic Sugars, %TEI; Proportion of total energy intake as a percentage, MP vs. FP; menstrual phase versus follicular phase, FP vs. LP; follicular phase versus luteal phase, LP vs. MP; luteal phase versus menstrual phase

Table 5.21: Energy and macronutrient intake during the menstrual, follicular and luteal phases of Cycle 1 in the CON group

	Menstrual	MP vs. FP	Follicular	FP vs. LP	Luteal	LP vs. MP
Energy (kcal/day)	2167 (1498, 2836)	-752 (-1493, -10) *	2919 (1875, 3963)	113 (-690, 917)	2805 (2142, 3469)	638 (294, 982) *
Protein (g)	72.3 (51.3, 93.3)	-23.9 (-46.8, -0.9) *	96.2 (69.1, 123.3)	12.1 (-40.3, 64.6)	84.1 (64.4, 103.8)	11.7 (-25.8, 49.2)
(% TEI)	14 (10, 18)	-1 (-6, 4)	15 (9, 20)	3 (-6, 11)	12 (10, 14)	-2 (-7, 4)
Fat (g)	80.7 (60.0, 101.3)	-24.8 (-66.0, 16.4)	105.5 (56.1, 154.9)	3.1 (-51.1, 57.3)	102.4 (81.8, 122.9)	21.7 (-0.39, 43.8) *
(% TEI)	35 (30, 39)	3 (-4, 9)	32 (25, 39)	-1 (-13, 10)	33 (30, 37)	-1 (-10, 8)
CHO (g)	304.7 (180.7, 428.7)	-118.2 (-224.5, -11.8) *	422.9 (261.9, 583.8)	22.5 (-90.1, 135.2)	400.3 (275.5, 528.1)	95.6 (29.9, 161.3) *
(% TEI)	51 (42, 57)	-3 (-6, 1)	54 (48, 59)	1 (-7, 9)	53 (47, 59)	2 (-6, 10)
NMES (g)	106.5 (23.3, 189.7)	-92.8 (-179.0, -6.6) *	199.3 (78.5, 320.1)	11.8 (-78.8, 102.4)	187.5 (98.1, 276.9)	81.0 (29.5, 132.5) *
(% TEI)	16 (7, 26)	-8 (-18, 2)	24 (14, 34)	0 (-11, 11)	24 (17, 30)	8 (-2, 17)

Mean (95% CI), CON group n=9; CHO; Carbohydrate, NMES; Non-Milk Extrinsic Sugars, %TEI; Proportion of total energy intake as a percentage, MP vs. FP; menstrual phase versus follicular phase, FP vs. LP; follicular phase versus luteal phase, LP vs. MP; luteal phase versus menstrual phase *P<0.05

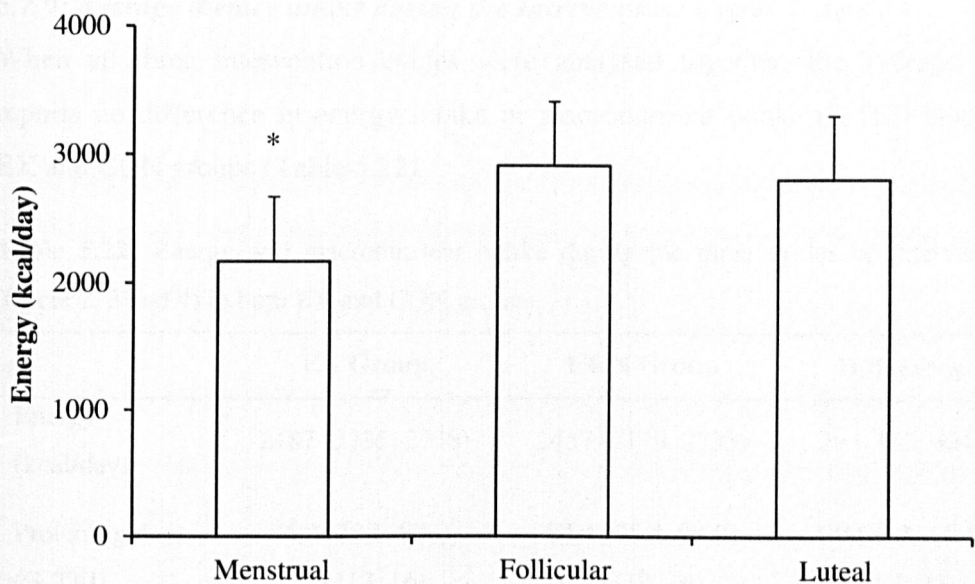


Figure 5.4: Average energy intake during the menstrual, follicular and luteal phase in the CON group during cycle. * Significantly reduced intake compared to follicular and luteal phases, $P < 0.05$.

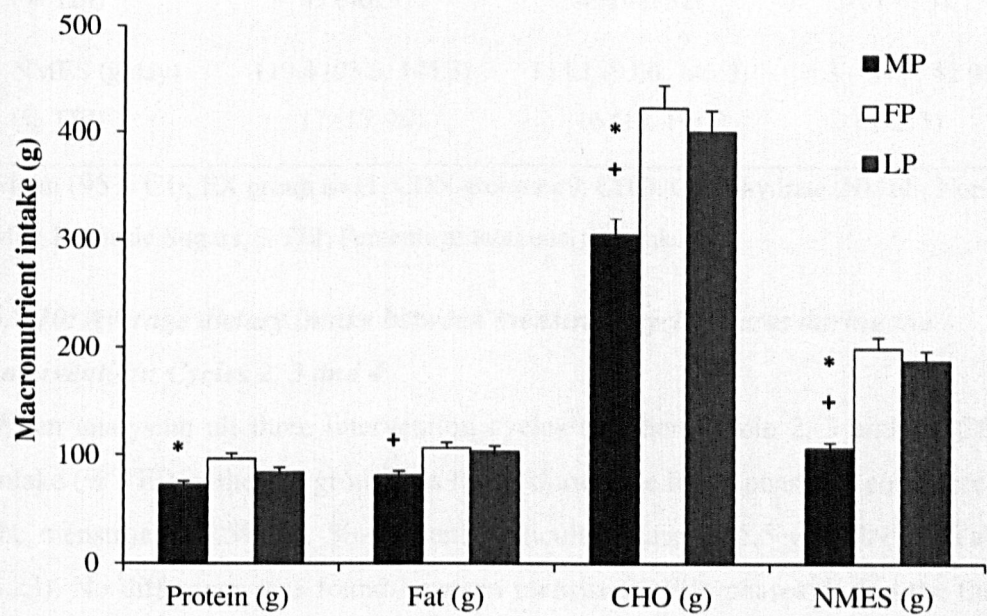


Figure 5.5: Average macronutrient intake during the menstrual (MP), follicular (FP) and luteal phase (LP) in the CON group during cycle 1. * MP significantly reduced intake vs. FP, + MP significantly reduced intake vs. LP, $P < 0.05$.

5.7.9: Average dietary intake during the Intervention: Cycles 2, 3 and 4

When all three intervention cycles were analysed together, the average data reports no difference in energy intake or macronutrient intake (%TEI) between EX and CON groups (Table 5.22).

Table 5.22: Energy and macronutrient intake during the three cycles of intervention (Cycle 2, 3 and 4) in both EX and CON groups

	EX Group	CON Group	Difference
Energy (kcal/day)	2487 (2235, 2738)	2457 (2179, 2735)	29 (-345, 404)
Protein (g/day)	86.3 (78.5, 94.0)	84.4 (75.8, 93.0)	1.9 (-9.7, 13.4)
(% TEI)	14 (13, 16)	15 (13, 16)	0 (-2, 1)
Fat (g/day)	97.7 (86.0, 109.3)	95.5 (82.6, 108.4)	2.2 (-15.2, 19.5)
(% TEI)	35 (33, 37)	35 (32, 37)	1 (-3, 4)
CHO (g/day)	325.3 (282.9, 367.7)	320.4 (282.1, 358.7)	-4.9 (-62.0, 52.2)
(% TEI)	49 (46, 51)	49 (47, 52)	-1 (-4, 3)
NMES (g/day)	119.4 (93.5, 145.3)	115.1 (93.6, 145.3)	4.3 (-34.3, 42.9)
(% TEI)	17 (15, 20)	16 (13, 19)	1 (-2, 5)

Mean (95% CI), EX group n=11, CON group n=9; CHO; Carbohydrate, NMES; Non-Milk Extrinsic Sugars, %TEI; Percentage total energy intake

5.7.10: Average dietary intake between menstrual cycle phases during the Intervention: Cycles 2, 3 and 4

When analysing all three intervention cycles together (Cycle 2, 3 and 4), CHO intake (% TEI) in the EX group was lower during the luteal phase, as compared to the menstrual (45.5% vs. 50.1%) and follicular phases (45.5 vs. 49.9%) (Table 5.23). No difference was found between menstrual cycle phases during the three cycles of the intervention in the CON group (Table 5.24).

Table 5.23: Energy and macronutrient intake in the EX group during three cycles of intervention (Cycle 2, 3 and 4) divided into the menstrual, follicular and luteal phases

	Menstrual	MP vs. FP	Follicular	FP vs. LP	Luteal	LP vs. MP
Energy (kcal/day)	2506 (2130, 2882)	21 (-410, 452)	2486 (2187, 2784)	18 (-357, 394)	2467 (2154, 2781)	-39 (-433, 355)
Protein (g/day)	87.1 (72.0, 102.1)	3.8 (-12.2, 19.8)	83.2 (73.9, 92.6)	-5.3 (-20.9, 10.4)	88.5 (74.5, 102.5)	1.4 (-18.2, 21.1)
(% TEI)	14 (13, 16)	0 (-2, 2)	14 (13, 15)	-1 (-3, 2)	15 (13, 17)	0 (-2, 3)
Fat (g/day)	99.6 (79.6, 119.6)	8.6 (-12.6, 29.7)	91.1 (77.2, 104.9)	-11.3 (-29.7, 7.2)	102.3 (87.4, 117.2)	2.7 (-18.4, 23.8)
(% TEI)	35 (31, 38)	1 (-3, 5)	34 (30, 37)	-4 (-8, 0)	37 (34, 41)	3 (-2, 7)
CHO (g/day)	328.2 (277.3, 379.1)	-6.0 (-70.1, 58.1)	334.2 (286.8, 381.6)	35.3 (-19.6, 90.3)	298.8 (258.0, 339.6)	-29.3 (-90.3, 19.6)
(% TEI)	50 (47, 53)	0 (-4, 4)	50 (47, 53)	4 (0, 9) *	46 (42, 49)	-5 (-9, 0) *
NMES (g/day)	127.1 (91.7, 162.5)	15.6 (-33.2, 64.4)	111.5 (81.9, 141.1)	-8.1 (-48.7, 32.5)	119.6 (89.3, 149.9)	-7.5 (-43.8, 28.8)
(% TEI)	19 (16, 23)	3 (-1, 8)	16 (13, 19)	-2 (-6, 3)	17 (14, 21)	-2 (-6, 2)

Mean (95% CI), EX group n=11; CHO; Carbohydrate, NMES; Non-Milk Extrinsic Sugars, %TEI; Percentage total energy intake, MP vs. FP; menstrual phase vs. follicular phase, FP vs. LP; follicular phase vs. luteal phase, LP vs. MP; luteal phase vs. menstrual phase; *P<0.05

Table 5.24: Energy and macronutrient intake in the CON group during three cycles of intervention (Cycle 2, 3 and 4) divided into the menstrual, follicular and luteal phases

	Menstrual	MP vs. FP	Follicular	FP vs. LP	Luteal	LP vs. MP
Energy (kcal/day)	2495 (2088, 2902)	109 (-258, 475)	2386 (2041, 2732)	-103 (-461, 255)	2490 (2083, 2896)	-5 (-454, 443)
Protein (g/day)	82.4 (71.5, 93.3)	2.9 (-10.7, 16.6)	79.4 (68.7, 90.2)	-12.0 (-29.0, 5.0)	91.4 (76.9, 105.9)	9.1 (-7.9, 26.1)
(% TEI)	14 (13, 16)	0 (-2, 2)	14 (13, 15)	-2 (-5, 1)	16 (14, 18)	2 (-1, 5)
Fat (g/day)	94.2 (72.2, 116.1)	1.9 (-19.4, 23.4)	92.2 (77.3, 107.1)	-8.1 (-31.5, 15.4)	100.3 (80.0, 120.5)	6.1 (-19.7, 31.9)
(% TEI)	33 (30, 37)	-2 (-6, 3)	35 (32, 39)	0 (-5, 5)	35 (32, 39)	2 (3, 7)
CHO (g/day)	340.3 (279.3, 401.2)	17.2 (-37.8, 72.1)	323.1 (264.5, 381.7)	10.6 (-36.6, 57.8)	312.5 (249.1, 376.0)	-27.7 (-100.0, 44.6)
(% TEI)	51 (48, 55)	1 (-3, 6)	50 (47, 54)	3 (-2, 8)	47 (43, 51)	-4 (-11, 2)
NMES (g/day)	128.3 (78.9, 177.8)	15.9 (-30.8, 62.7)	112.4 (69.7, 155.1)	7.6 (-23.1, 38.4)	104.7 (64.5, 144.9)	-23.6 (-78.7, 31.5)
(% TEI)	18 (14, 22)	1 (-3, 6)	16 (13, 20)	2 (-2, 6)	14 (10, 18)	-3 (-9, 2)

Mean (95% CI), CON group n=9; CHO; Carbohydrate, NMES; Non-Milk Extrinsic Sugars, %TEI; Percentage total energy intake, MP vs. FP; menstrual phase vs. follicular phase, FP vs. LP; follicular phase vs. luteal phase, LP vs. MP; luteal phase vs. menstrual phase

5.8: Discussion

The current randomised controlled trial aimed to investigate the potential effects of regular moderately intensive exercise on symptoms of PMS, QoL and dietary intake for three menstrual cycles over approximately 12 weeks.

5.8.1: PMS Symptoms and QoL

The results indicate a generalised decrease in symptom severity between Cycle 1 and 4, with a marginal-difference between EX and CON groups ($P=0.083$). Significant reductions were reported in pain, water retention, autonomic reaction and impaired concentration symptom scores during the intervention cycles (cycles 2, 3, and 4) compared to baseline in the EX group. In public health terms; a 15% decrease in total PMS symptom severity scores between Cycle 1 and 4 following the exercise intervention is reported. The results also indicate a similar reduction in symptom scores following the exercise intervention when analysing only symptoms present in the DSM 5 (American Psychiatric Association, 2013) definition of PMS. In addition, a 13% decrease in the total number of 'unhealthy days' experienced in the EX group, following the intervention (4 days), was reported.

The reduction in PMS symptoms reported in the present study concur with previous studies that have investigated the effects of exercise on PMS symptoms (Prior *et al.*, 1986; Prior *et al.*, 1987; Steege and Blumenthal, 1993; Stoddard *et al.*, 2007). Prior *et al.* (1986; 1987) reported significant decreases in PMS symptoms following an exercise conditioning regime for 3 and 6 months ($n=6$ and 8, respectively). Breast tenderness and fluid retention symptoms were most notably reduced in both studies. In addition, Steege and Blumenthal (1993) reported significant reductions in PMS symptoms following 12 weeks of aerobic training but not for strength training. This indicates that the type of exercise may play a potentially significant role in reducing PMS symptoms. In the most recent study, 14 women were enrolled in a 24 week programme involving a moderately intensive training regime. Water retention, breast tenderness, pain, tension and concentration were significantly improved between baseline and post-intervention (Stoddard *et al.*, 2007). The current results report similar decreases in pain, water retention, autonomic reaction and impaired concentration.

In addition, the current study is the first to report symptoms from a sedentary PMS control group for direct comparison, which previous studies have not. Symptoms reported in the current CON group were not significantly different from the EX group during Cycle 1, indicating that the severity of PMS symptoms reported at baseline was similar between groups, and therefore allows direct comparisons to be made following the exercise intervention. The results presented in the current investigation consistently demonstrate no significant differences in any of the PMS symptom categories between Cycle 1 and 4 in the CON group and therefore indicate that the aerobic exercise intervention reduces both physical and psychological symptoms of PMS.

5.8.2: Oestrogen and Progesterone

The exact aetiology of PMS currently remains unknown, however it has been suggested that women who suffer from PMS are sensitive to the normal fluctuations in oestrogen and progesterone during the menstrual cycle which may be a contributing factor (American College of Obstetricians & Gynecologists, 2000). Women who use hormonal contraceptives or experience anovulatory menstrual cycles also report fewer PMS symptoms (Freeman, 1997; Shangold *et al.*, 1990), further contributing to the aforementioned increased sensitivity hypothesis.

It is unlikely that the dramatic fall in sex hormone concentrations reported towards the end of the luteal phase are the main cause for the symptom development, as PMS symptoms can be recorded anytime from ovulation onwards. Indeed, the occurrence of ovulation itself is also essential for PMS by definition to occur, highlighting the essential role of progesterone within this syndrome (American College of Obstetricians & Gynecologists, 2000). However, it currently remains unclear as to whether the exact concentration of both oestrogen and progesterone hormones changes between women with and those without PMS. Recent evidence from Thys-Jacob *et al* (2008) reports no significant differences in sex hormone concentrations, whereas (1996) and Seippel and Backstrom (1998) report significantly elevated oestrogen and reduced progesterone concentrations in women with PMS compared to controls.

One potential mechanism hypothesised in the current investigation was that the exercise intervention may decrease PMS symptoms by reducing oestrogen (oestradiol) and progesterone hormone concentrations. Previous evidence in female athletes has suggested that vigorous exercise regimes can cause changes in hormone profiles which can often lead to amenorrhea or secondary amenorrhea (Speed, 2007). Similarly, in studies employing comparable moderate exercise regimes to the current investigation a reduction in sex hormone concentrations following exercise is also reported (Bullen *et al.*, 1984; Keizer *et al.*, 1987; Morris *et al.*, 1999), whilst others have indicated no significant differences following exercise (Prior *et al.*, 1987; Ronkainen *et al.*, 1985). A recent study reports significant decreases in oestrogen and progesterone concentrations following a moderately intensive exercise regime in women with PMS (Stoddard *et al.*, 2007).

However the current investigation reports oestrogen and progesterone were not significantly different between cycle 1 and 4 in the EX group, with all participants reporting ovulatory cycles, demonstrated by progesterone concentrations of <2.0 pg/ml during the luteal phase. This indicates that the reduction in PMS symptoms reported in the EX group may not be mediated by oestrogen (oestradiol) or progesterone concentrations (absolute or AUC) and therefore may be as a result of other indirect mechanisms.

5.8.3: β -endorphins and Cortisol

As plasma concentrations of oestrogen and progesterone do not appear to mediate the reduction in PMS symptoms following an exercise intervention, it may be that the interaction between exercise and a decrease in PMS symptoms are mediated by an alteration in either β -endorphin activity or cortisol. β -endorphin are neuropeptides, 31-amino acids long, cleaved from proopiomelanocortin (POMC) along with adrenocorticotrophic hormone (ACTH) (Hirsch and Millington, 1991), are secreted by the pituitary gland and have receptors located in the hypothalamus and limbic systems of the brain, areas associated with pain, emotion and behaviour (Dalayeun *et al.*, 1993). β -endorphin is also secreted in response to stress and those present within plasma reduce pain by inhibiting the electrical responses to sensory nerves (Dalayeun *et al.*, 1993).

The role of β -endorphins in the pathophysiology of PMS was first suggested in the 1980's (Reid and Yen, 1981), with evidence to suggest that women with PMS have lower β -endorphin concentrations compared to non-PMS women (Chuong *et al.*, 1985). β -endorphin concentrations also display a cyclical variation, with a notable reduction during the luteal phase of ovulatory cycles, which is not reported during anovulatory cycles (Chuong *et al.*, 1985).

Exercise has been previously reported to cause an increase in β -endorphin secretions from the pituitary gland into the plasma (Bortz *et al.*, 1981; Farrell *et al.*, 1982). It is hypothesised that exercise increases β -endorphin activity, by increasing its in neurotransmitter activity (Shangold *et al.*, 1990). This increase in neurotransmitter activity interacts within the hypothalamic pituitary adrenal axis (HPA) resulting in a down-regulating pituitary secretions of FSH (Gonzalez-Ortiz *et al.*, 1998) and LH (Chuong *et al.*, 1985). This down-regulation may result in unsuccessful follicular development and consequently reduces ovarian oestrogen and progesterone secretions (Drinkwater *et al.*, 1986).

Increased β -endorphin concentrations have been reported following aerobic exercise (e.g. cycling and running) at intensities of $>70\%$ of VO_2 max, (Goldfarb *et al.*, 1990; Kraemer *et al.*, 1989), with similar results reported in women exercising at more moderate intensities following 8 weeks of 30 minutes, 3 times per week treadmill running (Heitkamp *et al.*, 1998). Subsequently, participating in an exercise regime could result in a reduction in PMS symptoms associated with pain, emotion and behaviour by increasing β -endorphin secretions.

Cortisol is a derivative of progesterone and progestagens, secreted from the adrenal cortex via the hypothalamic-pituitary-adrenal axis and has been reported to increase in response to stressors including physical activity (Hatta *et al.*, 2013; Stupnicki and Obminski, 1992). Previous evidence has reported an altered cortisol profile in women that suffer with PMS during the follicular phase compared to women without PMS (Parry *et al.*, 2000). As cortisol has been previously linked with mood, showing a direct correlation with depressed mood and poor performance in non-clinical populations (Van Honk *et al.*, 2003), concentrations of this hormone may be strongly linked with some of the symptoms reported in women suffering from PMS.

However, although heightened concentrations of cortisol are reported in women suffering with PMS (Parry *et al.*, 1991; Walder *et al.*, 2012), others report a decrease in cortisol concentrations (Girdler *et al.*, 2001; Odber *et al.*, 1998) or typical cortisol profile compared to healthy controls (Haskett *et al.*, 1984; Rabin *et al.*, 1990; Steiner *et al.*, 1984). A recent study measuring plasma β -endorphin in women with PMS (n=27) vs. control (n=27) similarly indicate significantly lower resting β -endorphin and cortisol in the PMS group during the luteal phase compared to the control group (Straneva *et al.*, 2002).

If physical activity were to cause increased cortisol concentrations in women with PMS, this could potentially lead to a decrease in many of the depressive mood symptoms that women report as a result of PMS. A recent review has indicated that there is a clear relationship between exercise participation and improvements in mood (Salmon, 2001) and exercise participation is currently recommended by the National Institute for Health and Clinical Excellence as a method of treating depression or low mood (National Institute for Health and Clinical Excellence, 2007).

The current investigation reports no significant change in either oestrogen or progesterone concentrations in either the EX or CON group, indicating both groups maintained ovulatory cycles. As a result, participants in the current exercise intervention (3 x 30 minutes moderate intensity) may have increased their β -endorphin and or cortisol concentrations through the increase in exercise and this may explain the decrease in PMS symptoms associated with pain, emotion, behaviour and mood reported in the exercise cohort. However, further analysis of β -endorphin and cortisol concentrations is required to confirm this hypothesis.

5.8.4: Dietary Intake

Dietary intake was recorded once weekly for the duration of the present investigation to determine the effects of moderate intensity exercise on dietary intake during the whole intervention and within each of the three menstrual cycle phases.

5.8.4.1: Dietary intake during Cycle 1

The results demonstrate no difference in energy intake or macronutrient intake (g/day or %TEI) during the three phases of cycle 1 in the EX group. Energy (kcal/day), fat (g/day), CHO (g/day) and NMES (g/day) intake in the CON group during cycle 1, was significantly reduced during the menstrual phase compared to the follicular and luteal phase, with protein intake significantly reduced during the menstrual phase compared to the follicular phase only. However, macronutrient intake as a %TEI did not significantly differ between cycle phases in the CON group during cycle 1.

The results in the current investigation do not support the previous literature which indicates a significant increase in energy and macronutrient intake during different phases of the menstrual cycle in women with PMS (Brzezinski *et al.*, 1990; Cross *et al.*, 2001; Wurtman *et al.*, 1989) and may be due to methodological limitations. The most notable limitation in the present study includes the analysis of only one baseline menstrual cycle for dietary intake data recorded. Dietary intake recall interviews were first conducted during the menstrual phase in cycle 1 and therefore may be subject to the greatest quantity of underreporting. Although participants were informed of the dietary recall interview process before data collection began, underreporting is heavily associated with all dietary recall methods (Illner *et al.*, 2012) and therefore future studies may need to collect more dietary intake data to determine any baseline phase changes.

5.8.4.2: Average dietary intake during the intervention (Cycle 2, 3 and 4)

There was no difference in energy or macronutrient intake (g/day or %TEI) as a result of the 3 cycles (Cycle 2, 3 and 4) of intervention in either group (EX vs. CON). Previous evidence has reported uncertainty concerning changes in energy intake following exercise. In the short-term (1-2 days), intense bouts of exercise can induce anorexia and a suppression of hunger (Blundell *et al.*, 2003). Both short and medium (7-16 days) term studies demonstrating no compensation in energy intake following exercise induced negative energy balance (Blundell *et al.*, 2003; King *et al.*, 1997). However, it has been reported that compensation of up to 30% of the exercise-induced energy deficit occurs, irrespective of gender (Whybrow *et al.*, 2008). Macronutrient selection has similarly demonstrated no consistent change

following short or medium term exercise; however the data is limited with variations in methodology (Blair *et al.*, 1981; Pate *et al.*, 1990). A study conducted in 13 moderately active women reported an increase in fat and protein intake immediately following a 75-minute high intensity walking exercise bout compared to an inactive control, but the study also reported an increase in daily CHO intake following the high-intensity exercise, suggesting no overall change in macronutrient selection as a result of the exercise (Pomerleau *et al.*, 2004). Long-term exercise interventions of > 1 month however, report small but significant decreases in energy intake (Andersson *et al.*, 1991), with evidence of an increase in fat and a decrease in CHO intake following 5-10 weeks of aerobic exercise (Ambler *et al.*, 1998; Johnson *et al.*, 1972). As the present study does not report significant changes in energy or macronutrient intake as a result of the 3 cycles of intervention in either the EX or CON group, this suggests that the exercise intervention did not affect overall dietary intake in PMS women. This may be as a result of the low intensity and frequency of the exercise in the current intervention compared to the aforementioned studies, which may not have been sufficient enough to over-ride the naturally increased energy intake reported in PMS women (Brzezinski *et al.*, 1990; Wurtman *et al.*, 1989).

However, the current study does report CHO intake as a %TEI was significantly lower during the luteal phase compared to the menstrual and follicular phases during the 3 cycles of intervention in the EX group, which was not replicated in the CON group. This indicates that exercise may reduce macronutrient intake in a more phase specific manner, which may be in response to the reported reduction in PMS related symptoms experienced rather than the direct effects of increasing exercise levels. Previous investigations have however reported significant increases in macronutrient intake during the luteal phase in women with PMS, but not as a result of exercise (Brzezinski *et al.*, 1990; Cross *et al.*, 2001; Wurtman *et al.*, 1989). It is believed that an increase in CHO intake in women with PMS may be in response to a decrease in serotonin concentrations also reported during the luteal phase. Low serotonin concentrations have been strongly associated with depression and some PMS symptoms (Frackiewicz and Shiovitz, 2001; Meltzer, 1989; Rapkin *et al.*, 1987). The reduction in serotonin concentrations reported within the luteal phase may stimulate appetite in an attempt to increase its concentrations by increasing insulin secretions

and the plasma tryptophan ratio through CHO intake (Wurtman *et al.*, 1989; Wurtman and Wurtman, 1995). In addition, a previous research study conducted in 79 women hypothesised that low blood glucose concentrations may cause an increase in PMS related symptoms during the luteal phase. Participants were instructed to commence a three-hourly CHO intake regime in an attempt to maintain steady blood glucose concentrations and resulted in a decrease in PMS symptoms (Dalton and Holton, 1992).

Serotonin is heavily implicated as a potential mechanism to cause an increase in mood. A previous investigation conducted by Chaouloff *et al.* (1985) reports increased tryptophan and 5-HIAA (serotonin metabolite) following a running exercise regime in rats. It is believed that exercise increases motor activity which causes the firing rates of serotonin neurons to rise, in turn increasing the synthesis and release of serotonin within the brain (Jacobs and Fornal, 1999; Rueter and Jacobs, 1996). In addition, tryptophan concentrations have been reported to increase after an exercise regime (Chaouloff *et al.*, 1986; Melancon *et al.*, 2012). As such the potential effects of exercise induced increases in serotonin concentrations may have overridden any changes in CHO intake required to stimulate serotonin concentrations to provide symptom relief as previously reported (Dalton and Holton, 1992).

Although previous evidence has yielded a decrease in symptoms following an increase in CHO intake during the luteal phase, the current investigation does not support this and reports a decrease in CHO intake during the luteal phase alongside a decrease in PMS symptoms. This suggests that the exercise regime implemented may over-ride the possible symptom related phase changes in dietary intake.

5.8.5: Methodological limitations

The data collected from the present investigation complement a number of previous intervention studies that have investigated the effects of exercise on symptoms of PMS (Prior *et al.*, 1986; Prior *et al.*, 1987; Steege and Blumenthal, 1993; Stoddard *et al.*, 2007). However, as the decrease in all PMS symptoms was trending towards a statistically significant result between EX and CON groups ($P=0.083$), further data collection is required to confirm the previous available evidence. Based on the results from the current investigation, a further three (total of 23) participants would

need to be recruited to detect a significant effect in total PMS symptom scores between groups.

A potential limitation of the current investigation includes the recruitment of women who were self-diagnosed PMS sufferers, with symptoms confirmed following prospective symptom scores collected during cycle 1, and additional retrospective analysis during screening. This methodological approach was implemented in order to reduce participant attrition and drop-out rate associated with intervention studies and is similar to previous study methodologies (Stoddard *et al.*, 2007). However, the current guidelines promote the use of two baseline cycles before PMS diagnosis is confirmed (Royal College of Obstetricians and Gynaecologists, 2007). Future studies therefore may need to consider implementing two baseline menstrual cycles or recruiting participants that have clinically diagnosed PMS to confirm the reduction in PMS symptoms following an exercise intervention.

A strength and novel aspect of the current investigation is the inclusion of oestrogen (oestradiol) and progesterone hormone concentration and AUC data. This allowed any significant effects of the exercise intervention to be attributed appropriately. Previous studies have made little attempt to control for factors that are known to affect the prevalence and severity of PMS (Daley, 2009). In particular, the studies were unable to provide an assessment of ovulation status, an important factor as ovulatory cycles have a greater effect on PMS symptoms compared to anovulatory cycles (Shangold *et al.*, 1990). In addition, neither menstrual cycle duration nor sex hormone concentrations before or after interventions were explicitly detailed within the studies. However, to identify absolute oestrogen and progesterone secretion changes during the menstrual cycle, more frequent blood sampling would be required than the current implemented methodology (3 x weekly). In addition, changes in sex hormone concentrations may occur only following more intense exercise than that prescribed in the current investigation (Morris *et al.*, 1999). Also the exact interaction between sex hormones and exercise may occur at a cellular level including receptor sensitivity rather than absolute sex hormone concentrations and therefore warrants further investigation. There is evidence to suggest that physical activity increases the sensitivity or activation of ER and PR present within the skeletal muscle fibres or within the skeletal muscle myotubes (Aizawa *et al.*,

2007). A 7 week, 1 hour per day endurance training study in rats previously reported increased expression of ER mRNA in gastrocnemius muscle after training (Lemoine *et al.*, 2002). However it is currently unclear to what extent this occurs during moderate intensity exercise such as that prescribed in the current investigation.

In addition, sex hormone binding globulin (SHBG) regulates the distribution between free and protein bound hormones testosterone, oestrogen and androgens, modulating their access to target tissues (Kahn *et al.*, 2002; Selby, 1990). The availability of oestrogen within plasma is limited, with 66% bound to SHBG and 30% bound to albumin, leaving only 1-3% free (Dunn *et al.*, 1981). The 'free' proportion is able to pass through the blood-brain barrier, which excludes the SHBG bound hormone complex (Toniolo *et al.*, 1994; Yaffe *et al.*, 2000). The albumin bound oestrogen is only loosely associated and as such can easily enter target cells similarly to 'free' or unbound oestrogen (Pazol *et al.*, 2004). The unbound and albumin bound hormone complex are collectively known as the 'bio-available' portion and are thought to be the most active part of the total hormone concentration present in plasma (Bellem *et al.*, 2011). The methodology used in the current investigation measured the total amount of oestrogen in the plasma sample and was unable to differentiate between bound and unbound oestrogen. Previous evidence has shown oestrogen and progesterone concentrations significantly decrease, with SHBG transiently increasing following an exercise and restricted calorie dietary intake intervention (Williams *et al.*, 2010). With this in mind, rather than oestrogen and progesterone directly affecting PMS symptoms, the possible changes therefore may be more likely to occur as a result of a cascade of events including the availability of SHBG plasma concentrations. Subsequently, further investigations could include measurements of SHBG during the menstrual cycle as this would determine the concentrations of free and protein bound steroid hormones available and therefore their potential effects on PMS symptom severity.

Recording accurate dietary intake from participants within a research setting can be difficult to achieve (Schoeller, 1990) therefore the dietary intake data recorded may be subject to inconsistencies that are associated with short-medium duration research studies. Dietary intake in the current investigation was recorded using 24 hour dietary recall interviews once per week for 3 menstrual cycles (approx. 12 weeks).

Participants therefore may have been subjected to participant fatigue and the dietary intake results may reflect habitual behaviour rather than in response to the exercise intervention (Stubbs *et al.*, 1998). However, the individual raw data of the current cohort of women displayed a normal distribution in dietary intake, indicating that good dietary recall data were collected. Women were also of normal weight therefore the least likely to underreport dietary intake (Plankey *et al.*, 1997). In addition, the methodology implemented to record dietary intake (24 hour dietary recall) used additional food portion photographs (Nelson, 2002) to assist in portion size recall, which is a primary reason for underestimation in dietary intake research. The changes in CHO intake reported in the current study have significant long-term health implications should these differences continue to be reported over time, particularly if the increased intake is not compensated for during the other two phases (menstrual and follicular). By identifying phases or times when dietary intake may change (increase or decrease) appropriate weight management can occur and potentially reduce the development of overweight and obesity.

5.9: Conclusion

The results of the current investigation indicate a decrease in PMS symptoms and a 13% decrease in the number of unhealthy days experienced following 30 minutes of moderate intensity (70-80% HR max) exercise three times a week for 3 menstrual cycles. The reduced symptoms however do not appear to be mediated by changes in plasma oestrogen (oestradiol) or progesterone concentrations. In addition, CHO intake was significantly reduced during the luteal phase of the exercise intervention, indicating that aerobic exercise may cause changes in dietary intake regimes in women with PMS. As excessive CHO consumption is associated with the development of type 2 diabetes and many other health diseases, a reduction in CHO intake as reported in the current investigation could further improve the overall health and well-being of PMS sufferers. The combination of dietary intake and exercise therefore could result in a further decrease in symptoms experienced.

Additional research in a larger sample size is required to determine the exact quantity and intensity of exercise needed to have the greatest effect on PMS symptomatology and to determine the potential mechanisms by which these actions may occur. The current study provides strong evidence using a unique hormone

analysis methodology, to support further investigation into the effects of lifestyle interventions, such as exercise and dietary intake. The development of this further research will provide greater symptom relief and management guidelines for PMS sufferers, whilst improving overall well-being and QoL.

Chapter 6: Concluding Remarks and Recommendations for Future Work

The following chapter discusses this series of studies in a wider context and when taken together, the results have far reaching implications in many aspects of women's health.

6.1: General Discussion

It is widely accepted that the loss of sex hormones after the menopause is strongly linked with cancer, osteoporosis, insulin resistance and obesity (Abildgaard *et al.*, 2013; Carr, 2003), a greater understanding of the effects that oestrogen and progesterone may have on women's health and the long term consequences and implications this may cause is of paramount importance. However there is currently very little appreciation for the contributing effect of changes in sex hormone concentrations that are experienced during the most reproductive phase of a women's life cycle on the development of some of these health diseases. Investigating the effects of temporary fluctuations in sex hormone concentration during the menstrual cycle may shed light on how these hormones interact within metabolic pathways and potentially their contribution to the development of a number of metabolic health diseases (including insulin resistance, obesity and metabolic syndrome). The current series of studies aimed to determine the effects of the natural fluctuation in oestrogen and progesterone concentrations during the menstrual cycle on energy regulation, glucose regulation and premenstrual syndrome (PMS).

Previous evidence has indicated that changes in oestrogen and progesterone during the menstrual cycle may be responsible for the variation in energy expenditure and substrate oxidation reported between genders (Tarnopolsky, 2008) and these potential variations may have significant implications for overall energy balance. Rodent models suggest that oestrogen is the dominant hormone driving the increase in preferential fat oxidation reported in women compared with men (Campbell and Febbraio, 2001; Campbell *et al.*, 2003). It has been suggested that oestrogen elicits its effects through its oestrogen receptor (ER) and that activation of these results in an increase in activated protein kinase (AMPK) concentrations (D'eon *et al.*, 2008).

As a result, this causes an increase in FAT/CD36 translocation and a decrease in malonyl CoA concentrations, which in turn causes an increase in long chain fatty acid (LCFA) transportation. As malonyl CoA concentrations reduce, this also inhibits the pyruvate dehydrogenase (PDH) complex, which is responsible for the formation of acetyl CoA through pyruvate breakdown (Jeukendrup, 2002). This reduces carbohydrate (CHO) oxidation and as such results in an increase in fat oxidation.

The work presented in chapter 5 reports carbohydrate (CHO) oxidation significantly decreased ($P < 0.05$) and fat oxidation increased ($P < 0.06$) during the follicular phase of the menstrual cycle during resting measures, with no significant difference in RMR. Previous evidence has suggested that at rest carnitine palmitoyltransferase I (CPT1) is inhibited by an increase in malonyl CoA (Winder *et al.*, 1989) resulting in preferential CHO oxidation. As the current investigation reports a reduction in CHO oxidation during the follicular phase at rest, this suggests oestrogen may interact within the normal oxidation pathways resulting in an increase in fat oxidation rate during the follicular phase when oestrogen is present in high concentrations.

However, the current investigation also reports no significant differences during different menstrual cycle phases in either energy expenditure or substrate oxidation during exercise. Although exercise intensity plays a significant role in fat and CHO oxidation rates, with an increase in fat oxidation reported until approximately 65% VO_2 max (Achten and Jeukendrup, 2004), as the current study employed an exercise intensity which resulted in an increase in fat oxidation compared to resting measures, this suggests that the potential effects of oestrogen on oxidation pathways as reported during rest, may be reduced during exercise. Equally, the results reported during exercise may be affected by lower participant numbers, variations in pre-exercise meal consumption between participants or other rate limiting steps during fat oxidation that may have reduced the subtle effects of elevated oestrogen concentrations.

The current investigation (chapter 5) also reports no significant change in energy expenditure during the menstrual cycle at rest or during exercise, but does report an increase in fat oxidation during the follicular phase. This suggests that fat storage

could be reduced during the follicular phase and therefore an increase in fat intake would result in oxidation rather than storage. Previous evidence does report increases in dietary intake during the menstrual cycle, but predominantly during the luteal phase (Cross *et al.*, 2001; Dalvit-Mcphillips, 1983). As the luteal phase demonstrates no significant difference in substrate oxidation, this could potentially result in an increase in weight gain during this phase if fat intake is increased, as fat may be stored rather than oxidised.

As efficient CHO oxidation relies on the availability of glucose to enter the cell and proceed through glycolysis, one rate limiting step of effective insulin-stimulated glucose uptake at rest is appropriate insulin receptor function (Chang *et al.*, 2004; Saltiel and Kahn, 2001). The significant reduction in CHO oxidation reported at rest in the present study (chapter 5) could potentially be a result of changes in glucose uptake or insulin sensitivity. Previous evidence suggests oestrogen plays a positive role in glucose response and insulin sensitivity (Bryzgalova *et al.*, 2006), by increasing AMPK concentrations which also increase GLUT 4 translocation, and result in effective glucose uptake (D'eon *et al.*, 2008; Ropero *et al.*, 2008; Spangenburg *et al.*, 2012). Whereas, progesterone is reported to promote insulin resistance through a decrease in GLUT 4 translocation, via an increase in IRS-1 degradation (Ricort *et al.*, 1995; Sasaoka *et al.*, 2005) resulting in a decrease in effective glucose uptake as insulin decreases (Wada *et al.*, 2010).

The study in chapter 3 reports an increase in glucose and insulin response (area under the curve, AUC) ($P < 0.05$) during the luteal phase of the menstrual cycle following an oral glucose tolerance test (OGTT). Insulin sensitivity surrogate indexes were applied to the data and indicate a reduction in insulin sensitivity during the luteal phase but this was not significantly different between menstrual cycle phases. These results were reported during elevated concentrations of both oestrogen and progesterone; however progesterone is present only in low concentrations during the previous two phases (menstrual and follicular respectively). This indicates that progesterone may be the driving hormone which causes the changes in glucose and insulin response during the luteal phase of the menstrual cycle.

The results also identify a significant positive correlation between BMI, BF% and weight (kg) and InAUC and HOMA-IR with a negative correlation for ISI ($P < 0.01$). This suggests that an increase in body composition (BMI, weight or body fat %) correlates with an increase in insulin AUC and insulin resistance (HOMA-IR) and a decrease in insulin sensitivity (ISI). These results are supportive of a number of studies linking obesity and increased body composition with insulin resistance (Kahn and Flier, 2000). Although the results indicate a correlation between body composition and insulin sensitivity measurements the study fails to report significant differences between menstrual cycle phases. In addition there was no correlation between body composition and sex hormone concentrations, suggesting heavier weight women do not display an increase in sex hormone concentrations compared to smaller weight women. However, body composition was only measured at baseline during the current study and therefore we can only assume that it remained stable during the different menstrual cycle phases. As such future studies should measure body composition during the different phases of the menstrual cycle, given its implications in insulin resistance and correlate this alongside sex hormone concentrations to determine its overall effects in glucose and insulin response during different menstrual cycle phases.

An increase in glucose and insulin response as reported in chapter 3 could result in an increased glucose availability therefore stimulating CHO oxidation. However the results in chapter 4 do not support this hypothesis as no significant increase in CHO was reported during the luteal phase. As oestrogen is still elevated during the luteal phase (although not at its peak), it could be hypothesised that this may result in a continual preference for fat oxidation at rest. However, as progesterone concentrations are also elevated during the luteal phase, the increase in glucose and insulin response may result in an increase in CHO oxidation. As both hormones are present in elevated concentrations during the luteal phase, the two stimulated oxidation pathways may cancel each other out and result in similar oxidation rates to the menstrual phase when neither hormone is elevated. This may explain why there is no significant difference in oxidation rates between the menstrual and luteal phases and follicular and luteal phases in chapter 4.

The results of these two studies (chapter 3 and 4) suggest changes in CHO and fat oxidation and glucose and insulin response occur as a result of fluctuating oestrogen and progesterone concentrations during the menstrual cycle. These could, over prolonged periods of time have significant implications in energy balance and glucose homeostasis. The changes reported in this series of studies highlights the cyclical nature of the changes in substrate oxidation, glucose and insulin response, occurring only during phases of elevated concentrations of sex hormones. A reduction in glucose tolerance and insulin sensitivity is similarly reported during pregnancy, when both oestrogen and progesterone concentrations are elevated which can result in a reduction in efficient insulin stimulated glucose uptake in skeletal muscle which often leads to the development of gestational diabetes (Kalkhoff *et al.*, 1970; Kirwan *et al.*, 2002; Ruchat *et al.*, 2012). Likewise, women who take hormonal contraceptives report changes in CHO oxidation (Dorflinger, 2002; Kahn *et al.*, 2003), with a decrease in glucose tolerance and an increase in insulin resistance reported (Godsland *et al.*, 1992; Reaven, 2005). However, the exact response is dependent on the dose and combination of hormones contained within the hormonal contraceptive administered (Lopez *et al.*, 2009).

The results have highlighted the significant wider public health implications, particularly as a decrease in insulin sensitivity is heavily implicated in the aetiology and development of type 2 diabetes. By determining the effects of elevated oestrogen and progesterone during the menstrual cycle on insulin-stimulated glucose uptake, this could have significant implications for the estimated 1.3 million women that suffer from type 2 diabetes in the UK and the many more who exhibit some degree of insulin resistance (Diabetes UK, 2011). Similarly, a reduction in effective glucose uptake also increases the risk-factors associated with developing other co-morbidities linked with the metabolic syndrome including obesity.

These two studies (chapter 3 and 4) are the first to accurately report concentrations of oestrogen and progesterone during a baseline menstrual cycle, to define the three menstrual cycle phases and to provide a reliable and consistent indication of cycle phase during a second menstrual cycle. Previous evidence has indicated that cycle duration and cycle phase duration varied significantly within women (Chiazze *et al.*, 1968; Creinin *et al.*, 2004; Jukic *et al.*, 2008) and therefore the method implemented

in the current series of studies minimised the requirement for repeated testing measures due to incorrect phase assignment. It also provided an accurate record of the concentration of each hormone during the different cycle phases. This methodology demonstrates that determining sex hormone concentrations is essential in accurately defining menstrual cycle phases.

Following the determination of this reproducible methodology in chapter 3 and 4, it was hypothesised that this method could be implemented using a population that experience significant health implications in response to changes in oestrogen and progesterone concentrations during the menstrual cycle. Women suffering from PMS report physical and psychological symptoms such as pain, food cravings, bloating, water retention and impaired concentration during the luteal phase of ovulatory menstrual cycles (American College of Obstetricians & Gynecologists, 2000; Royal College of Obstetricians and Gynaecologists, 2007). These symptoms have significant effects on health and quality of life (QoL), displaying detrimental effects on personal and work relationships, including reduced work productivity and absenteeism which have significant economic cost to employers and individuals (American College of Obstetricians & Gynecologists, 2000; Royal College of Obstetricians and Gynaecologists, 2007). Given that 75-85% of women of reproductive age are reported to suffer with PMS related symptoms (Halbreich, 2003), lifestyle interventions such as exercise have the potential to improve symptom management and create an improved sense of well-being and QoL in a large population of women.

As a result, a randomised controlled trial was designed to determine the effects of an exercise intervention on symptoms of PMS and QoL, using the established oestrogen and progesterone methodology during the menstrual cycle to determine cycle phases and any possible mechanisms behind the potential outcome. This randomised control trial reports a significant decrease ($P < 0.05$) in some PMS symptoms between baseline and post-intervention following an exercise regime. This reduction is the equivalent to a 15% decrease in reported symptoms. In addition, the number of unhealthy days experienced was reduced by 4 following the exercise intervention although this was not significant. These results are comparable to the previous limited evidence which reports decreases in PMS symptoms following an exercise

intervention (Prior *et al.*, 1986; Prior *et al.*, 1987; Steege and Blumenthal, 1993; Stoddard *et al.*, 2007); however these studies failed to implement a non-active control group for direct comparison.

Participating in an exercise regime stimulates glucose uptake through insulin-dependent and insulin-independent pathways, lowering blood glucose concentrations and reducing insulin resistance. With exercise regimes regularly reported to reduce the development of gestational diabetes in pregnant women (Artal, 2003; Ruchat *et al.*, 2012), the exercise intervention conducted in chapter 6 could have far wider health implications than just reducing the most common PMS symptoms reported, such as lowering the risk of type 2 diabetes development. In addition, the exercise regime implemented in chapter 5 included 3 x 30 minutes of moderate-intensity exercise per week.

The exercise regime implemented in the current investigation was chosen based on the limited previous exercise intervention studies conducted in women suffering from PMS (Prior *et al.*, 1986; Prior *et al.*, 1987; Steege and Blumenthal, 1993; Stoddard *et al.*, 2007). In order to determine the effects on an exercise intervention on symptoms of PMS more clearly the inclusion criteria required only previously sedentary women to be recruited. A moderate intensity walking exercise was chosen in an attempt to increase attendance and reduce drop-out that can be associated with this type of study design (Stoddard *et al.*, 2007). However the current national guidelines for physical activity recommend that adults should undertake 30 minutes of moderate-intensity exercise five times per week (Department of Health, 2011). This indicates that although a reduction in PMS symptoms and an increase in QoL was recorded in the current intervention, an extra two 30 minute sessions per week would be required to establish the wider positive health outcomes associated with physical activity (Department of Health, 2011).

In addition, the women recruited to this study were of an average age of 27 (± 6 y) with a BMI of 24.6 (± 4.1 kg/m²), therefore this fairly young and healthy group of women may not be representative of the general PMS population. Previous evidence reports the prevalence of PMS increases in obese women (Masho *et al.*, 2005). As such, future studies should recruit PMS suffering women of a wider age range and

different BMI categories to determine if obesity levels play a significant role in the prevalence of PMS symptoms following an exercise intervention. Furthermore, this would also shed light on the effects of increased physical activity has on dietary intake, overall body weight and subsequent health disease developments in women suffering with PMS.

A secondary aim of this study was to investigate the effects of the exercise intervention on dietary intake in women with PMS and to determine if there were any menstrual cycle phase related changes. Previous literature has reported significant increases in energy intake during the luteal phase of the menstrual cycle in women suffering from PMS (Brzezinski *et al.*, 1990; Cross *et al.*, 2001; Johnson *et al.*, 1994; Wurtman *et al.*, 1989). Evidence also suggests that an increase in CHO intake during the luteal phase may be in response to a reduced serotonin concentration during this phase (Dalton and Holton, 1992; Wurtman, 1993). The current investigation reported a significant decrease in carbohydrate intake (%TEI, $P < 0.05$) within the luteal phase of the three cycles of intervention in the exercise group compared to the menstrual and follicular phases. No significant changes were reported in the control group. These results suggest that the previously reported requirement for an increase in CHO consumption to provide adequate symptom relief (Dalton and Holton, 1992), may have been reduced as a result of the exercise intervention in the current investigation, as a reduction in both PMS symptoms and CHO intake was recorded (chapter 5).

Previous evidence has suggested low blood glucose concentrations are correlated to increased PMS symptom (Dalton and Holton, 1992) and that an increase in CHO intake during the luteal phase is in response to lower serotonin and tryptophan concentrations (Dalton and Holton, 1992; Wurtman *et al.*, 1989; Wurtman and Wurtman, 1995). The studies indicate that an increase in CHO intake reduces the prevalence of PMS symptoms. A decrease in PMS symptoms was only reported in 54% of women (aged 38.9 ± 8.7 y) who increased their CHO intake and adapted their dietary intake patterns to a three-hourly intake regime (Dalton and Holton, 1992). In addition, the study reported only 19% of women who undertook this dietary intake regime required no further treatment for their PMS symptoms.

However, the participants in the current investigation did not report any significant changes in dietary intake during the luteal phase of the menstrual cycle at baseline and reported a decrease in CHO intake during the luteal phase following the exercise regime. This suggests that the evidence reported by Dalton and Holton (1992) and Wurtman *et al* (1989) and Wurtman and Wurtman (1995) would not apply to the women in the current study as no increase in CHO intake occurred yet a decrease in PMS symptoms was reported. The importance of maintaining efficient blood glucose is evidenced in the plethora of work investigating glycaemic index/response and type 2 diabetes (Chiu *et al.*, 2011; Wolever, 2003); however evidence of its effects in reducing PMS symptoms is minimal. As the women in the current investigation were of a normal BMI range ($24.6 \pm 4.1 \text{ kg/m}^2$), reported fasting blood glucose of $< 6.0 \text{ mmol}$ and did not report any adverse health, it is unlikely that Dalton's theory of low blood sugar causing an increase in PMS symptoms is applicable to this population. However blood glucose profiles were not performed and therefore we are unable to completely rule out Dalton's theory. Future studies should therefore investigate blood glucose response in women suffering from PMS pre- and post-exercise intervention to determine if a change in blood glucose profile may also be contributing to a reduction in PMS symptoms. This could have positive health implications involving overall energy balance and the development of type 2 diabetes.

The results reported in this thesis have increased the knowledge and understanding of the implications that the regular fluctuations of oestrogen and progesterone during the menstrual cycle have on energy regulation, glycaemic control and premenstrual syndrome. As such, further investigations need to be conducted using larger sample sizes to determine the potential long-term adverse effects of fluctuations in oestrogen and progesterone on health and well-being in women.

6.2: Recommendations for Future Work

1. Menstrual cycle phase should be taken into consideration when using female participants.

The evidence presented in this thesis indicates phase related changes occur particularly during glucose tolerance, glycaemic index and energy expenditure investigations. Therefore future studies conducted in these subject areas should consider recording menstrual cycle phase in female participants to ensure changes in sex hormone concentrations are not contributing to the results. This may also have significant clinical implications when diagnosing type 2 diabetes if OGTT are initially taken during the luteal phase of the menstrual cycle.

2. A comprehensive series of further studies should be initiated to determine the mechanistic pathways of sex hormones and their effects on glucose and insulin response.

The results from chapter 3 report elevated glucose and insulin AUC during the luteal phase however the results in conjunction with previous literature is unable to fully indicate by what mechanism this may occur. Consequently, determining ER ratio in skeletal muscle and measuring GLUT4 content in response to different oestrogen and progesterone hormone concentrations, using human muscle biopsies or adipose tissue may provide some mechanistic details.

3. The study described in chapter 5 should be extended from a pilot to full intervention to evaluate the effects of exercise on PMS symptoms.

A full intervention study would allow a greater number of participants to be recruited to determine the effects in a larger proportion of PMS suffering women. In addition, a full intervention could determine the exact intensity, frequency and mode of exercise that is optimum to reduce PMS symptoms and dietary intake.

4. Concentrations of β -endorphins and serotonin, or its metabolite 5-hydroxyindoleacetic acid (5-HIAA), should be measured in studies investigating exercise and PMS.

Given their potential mechanistic implications in the aetiology of PMS (Royal College of Obstetricians and Gynaecologists, 2007), measuring these neurotransmitters would provide causality evidence for the reduction in PMS symptoms reported following an exercise intervention.

5. Measurements of SHBG should be taken in studies investigating sex hormones during the menstrual cycle.

SHBG regulates the distribution between free and bound sex hormones and modulates their access to target tissues (Kahn *et al.*, 2002). As the bio-available portion of the sex hormone is reported to be the most active part of total hormone concentration within the plasma (Bellem *et al.*, 2011) it is therefore important to determine the ratio of free to bound sex hormones.

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2. Quality of Life Questionnaire

Quality of Life Questionnaire

Subject no. Date

1. Would you say in general that your health is (please tick)

Excellent	
Very good	
Good	
Fair	
Poor	

2. Now thinking about your physical health, which includes physical illness and injury, for how many days during the past 30 days, was your physical health not good?

Number of days	
----------------	--

3. Now thinking about your mental health, which includes stress, depression and problems with emotion, for how many days during the past 30 days was your mental health not good?

Number of days	
----------------	--

If both Question 2 and 3= “None”, please move on to Question 5

During the past 30 days, for about how many days did poor physical or mental health keep you from doing your usual activities, such as self-care, work or recreation?

Number of days	
----------------	--

4. Are you LIMITED in any way in any activities because of an impairment or health problem? (Please tick)

Yes	
No	

If Q5= No please move on to Question 10

If Q5= Yes please move on to Question 6

5. What is the MAJOR impairment or health problem that limits your activities?

.....

6. For HOW LONG have your activities been limited because of your major impairment or health problem? (Please fill in appropriate box)

Days	
Weeks	
Months	
Years	

7. Because of any impairment or health problem, do you need the help of other persons in handling your PERSONAL needs, such as eating, bathing, dressing or getting around the house? (Please tick)

Yes	
-----	--

No	
----	--

8. Because of any impairment or health problem, do you need the help of other persons in handling your ROUTINE needs, such as everyday household chores, doing necessary business, shopping or getting around for other purposes? (Please tick)

Yes	
No	

9. During the past 30 days, for about how many days did PAIN make it hard for you to do you usual activities, such as self-care, work or recreation?

Number of days	
----------------	--

10. During the past 30 days, for about how many days have you felt SAD, BLUE or DEPRESSED?

Number of days	
----------------	--

11. During the past 30 days, for about how many days have you felt WORRIED, TENSE or ANXIOUS?

Number of days	
----------------	--

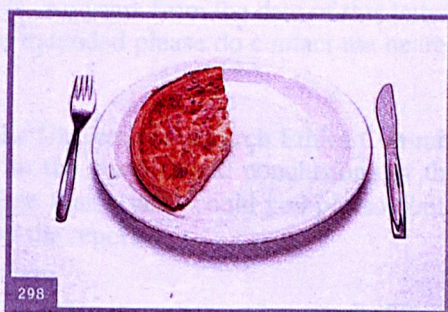
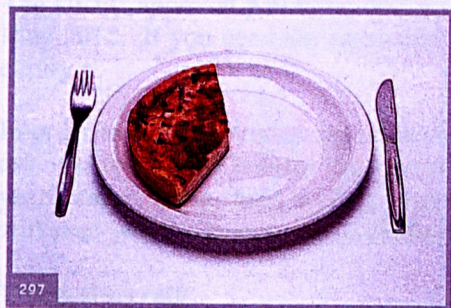
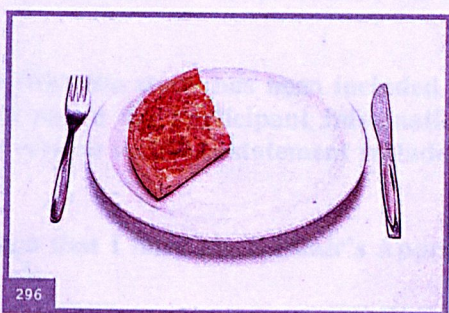
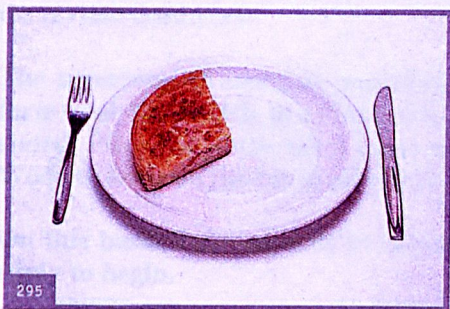
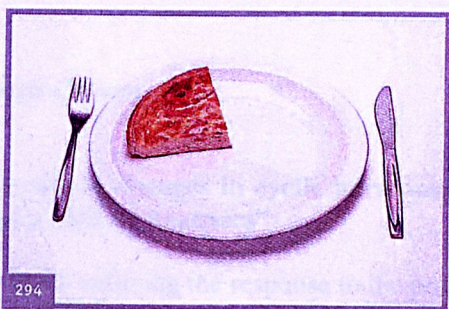
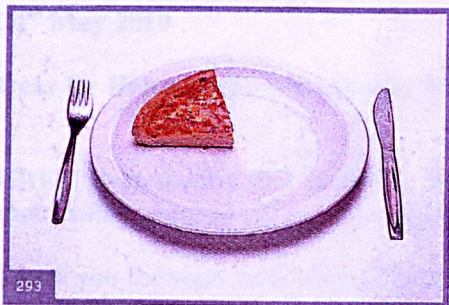
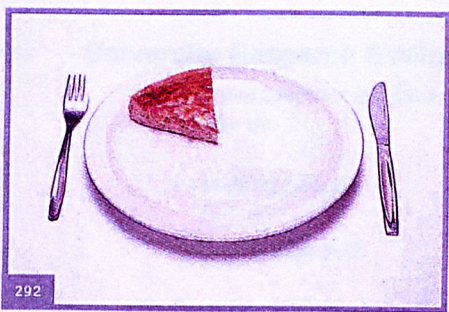
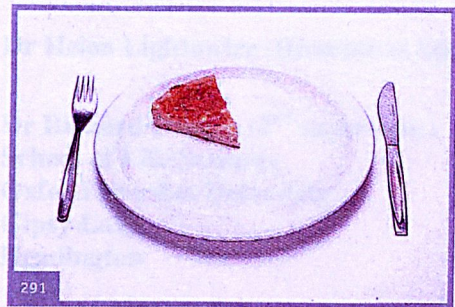
12. During the past 30 days, for about how many days have you felt you did NOT get ENOUGH REST or SLEEP?

Number of days	
----------------	--

13. During the past 30 days, for about how many days have you felt VERY HEALTHY AND FULL OF ENERGY?

Number of days	
----------------	--

4. Example of Food Photographs



5. UREC Ethical Approval Letter - 100470

Dr Helen Lightowler (Director of Studies)

University Research Ethics Committee

Dr Richard Craven (2nd supervisor)
School of Life Sciences
Oxford Brookes University.
Gipsy Lane
Headington

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t. +44 (0)1865 483484

ethics@brookes.ac.uk

24th May 2010

Dear Dr Helen Lightowler and Dr Richard Craven

UREC Registration No: 100470 - "Influence of changes in cyclic hormones on energy metabolism, carbohydrate metabolism and taste preferences"

Thank you for your email of the 20th May 2010 outlining the response to the points raised in my previous letter about the study for your **PhD student Sarah Hillier**, and for attaching the revised documents.

The screening of anaemic participants from the study has been included in the E2U form and the health questionnaire, but not in the Participant Information Sheet as stated. Please send the most up to date version with this statement included to Louise Wood to keep on file for audit purposes.

On this basis, I am pleased to inform you that I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so the 20th May 2012. If you need the approval to be extended please do contact me nearer the time of expiry.

In order to monitor studies approved by the University Research Ethics Committee, we will ask you to provide a (very brief) report on the conduct and conclusions of the study in a year's time. If the study is completed in less than a year, could you please contact me and I will send you the appropriate guidelines for the report.

Yours sincerely

Dr Elizabeth T Hurren

Chair of the University Research Ethics Committee

cc Jill Organ, Graduate Office

Louise Wood, Ethics Officer

6. UREC Ethical Approval Letter - 110571

**Dr Richard Craven, Director of Studies,
Dr Michelle Venables, Second Supervisor and
Dr Simon Wheeler, Third Supervisor
School of Life Sciences
Oxford Brookes University
Gipsy Lane
Headington**

University Research Ethics Committee

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25th July 2011

Dear Dr Richard Craven, Dr Michelle Venables and Dr Simon Wheeler

UREC Registration No: 110571: "The effects of an exercise intervention on premenstrual syndrome (PMS) in sedentary women"

Thank you for your email of the 22nd July 2011 outlining the response to the points raised in my previous letter about the PhD study of your research student Sarah Hillier, and attaching the revised documents.

I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin provided that the daily blood samples will be centrifuged and stored at - 80 degrees C, and that the Human Tissue Authority has confirmed to the satisfaction of the supervisory team that as the plasma contains no cells this study will not require an HTA licence.

The UREC approval period for this study is two years from the date of this letter, so the 25th July 2013. If you need the approval to be extended please do contact me nearer the time of expiry.

In order to monitor studies approved by the University Research Ethics Committee, we will ask you to provide a (very brief) report on the conduct and conclusions of the study in a year's time. If the study is completed in less than a year, could you please contact me and I will send you the appropriate guidelines for the report.

Yours sincerely

**Dr Elizabeth T Hurren
Chair of the University Research Ethics Committee**

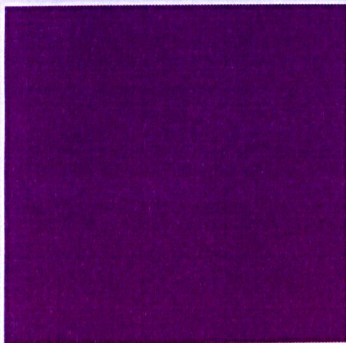
**cc Sarah Hillier, Research Student
Jill Organ, Graduate Office
Louise Wood, UREC administrator**

7. Participant Recruitment Posters

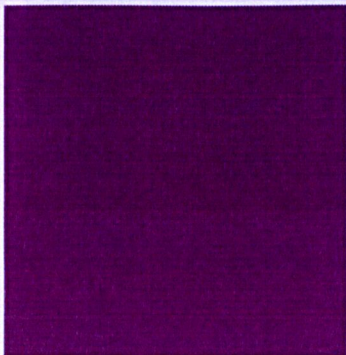
**MENSTRUAL
CYCLE STUDY**

An opportunity to participate in cutting edge research

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Are you female?
Aged 18-40 years old?
Don't use any form of
hormonal contraception?
Currently don't suffer from
diabetes?



You are invited to take
part in a study on glucose
response and energy
balance during the
menstrual cycle

Earn £30 Amazon
Vouchers by attending
weekly appointments

For further information call
Sarah on
01865 483283 Or email
shillier@brookes.ac.uk
This research has been
approved by Oxford Brookes
University Research Ethics
committee.

8. Information Sheet Given in Chapter 3 and 4

Information Sheet

Full title of project – Influence of changes in cyclic hormones on energy metabolism, carbohydrate metabolism and taste preference.

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the Study?

Current literature demonstrates scientific uncertainty with regards to changes in glycaemic and insulin response, energy metabolism and taste preferences during the menstrual cycle. The aim of this research is to determine the relationship between hormones within the menstrual cycle (oestrogen and progesterone) and energy metabolism, glycaemic and insulin response and taste preferences for sugar and salt.

Why have I been invited to participate?

The study is looking for non-smoking female volunteers, all with a BMI $\leq 30\text{kg/m}^2$. All volunteers will be between the ages of 18-40 years, with no known diabetes or impaired glucose tolerance and a fasting blood glucose measurement of less than 6.1mmol/L. Volunteers must not be suffering from anaemia. All volunteers must have 3 previous regular menstrual cycles and not be experiencing hot flushes. Volunteers must not be pregnant or have given birth within the previous 2 years or are still breastfeeding. All volunteers must not be taking any form of hormonal contraception. You have been asked to volunteer because you fall into these categories.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you are a student choosing to either take part or not take part in the study or to withdraw at any time, this will have no impact on your marks, assessment or future studies.

What will happen to me if I take part?

The research will be divided up into three sections:

The first part of the study will involve one blood sample being taken during the morning of each test day. Blood samples will be taken from a vein located in the arm. Test days will be 2 - 3 days apart accommodating for the weekends and will occur for one complete menstrual cycle. The tests will take no longer than 15 minutes to complete.

The second part the study will investigate your response to an Oral Glucose Tolerance Test (OGTT) on three separate occasions throughout one complete menstrual cycle; the exact

days will be dependent on the results from the first part of the study. Twenty four hours prior to the first test day you will be instructed to record a food diary detailing all food and drinks consumed. The foods consumed in this period will then need to be replicated 24 hours prior to the following 5 test sessions. On each test day you are invited to come to the lab (S403) in a fasted state (10-12 hours) where your weight, height and body fat percentage measurements will be taken. A sample of venous blood will be taken to monitor hormone levels. An OGTT will then be performed for the next 3 hours. Finger-prick blood samples will be taken at -5, 0 minutes before the consumption of a glucose solution is given. Finger-prick blood samples will then be taken at 15, 30, 45, 60, 90, 120, 150 and 180 minutes in relation to before glucose solution consumption (-5 and 0 minutes) to test glucose response and insulin hormone. Each OGTT measurement requires approximately 300µl of blood which will be obtained by a finger prick. The two other test days will be completed using the same procedure. The test will take approximately 3-3.5 hours to complete. You will be able to use your laptop to perform quiet office type work whilst participating in the study.

The third part of the study will investigate your resting metabolic rate (RMR) and your response to a selection of sugar and salt solutions of differing concentrations on three separate occasions throughout one complete menstrual cycle. The exact test days again will be dependent on the results from the first part of the study. On each test day you are invited to come to the lab in a fasted state (10-12 hours) where your weight, height and body fat percentage measurements will be taken. A sample of venous blood will then be taken to monitor hormone levels. You will then be asked to lie still under a large transparent doomed shape hood and canopy for 30 minutes whilst breathing at a normal rate allowing respiration samples to be measured. The hood and canopy allow us to measure your metabolism accurately without the discomfort of a mouthpiece and nose clip. This indirect calorimetry measurement will allow your RMR to be calculated. Once this is complete you then will be presented with a selection of sugar and salt solutions to taste and record your preference ratings. The test should take no longer than 1.5 hours to complete. The other two test days will be completed with the same procedure. At the end of each test you will then be given a snack to eat (e.g. cereal bar, fruit or crisps).

What are the possible risks in taking part?

There are no major risks. The only risk will be slight bruising of the arm and fingertip from which the blood has been taken but that should disappear within a few days. The bruising will not prevent you from doing your regular daily activities.

What are the possible benefits from taking part?

Taking part in this study will provide you with information about your menstrual cycle, body mass index (Stupnicki and Obminski) and body composition including percentage body fat. The study will also provide information concerning resting metabolic rate, glucose metabolism and insulin response. You will be provided with a summary of the key results of the study upon request. You will also receive £20 book vouchers on completion of the study.

Will what I say in this study be kept confidential?

All information collected about you will be kept strictly confidential (subject to legal limitations). Access to the data will be by the researchers working on this study only. Access to computer files will be by password only and data, codes and identifying

information will be kept in locked filing cabinets. Data generated by the study must be retained in accordance with the University's policy on Academic Integrity. The data generated in the course of the research must be kept securely in paper or electronic form for a period of five years after the completion of a research project. Due to the small number of volunteers taking part in this study it may be difficult to maintain anonymity of the volunteers, though every effort will be made by the research team to ensure participant confidentiality.

What will happen to the results of the research study?

The results of this research will be published in a research journal. Your name or identity will not be recognisable from this. If you wish to obtain a copy of the published research you can do so by contacting the researchers at the address, phone number or email address given below following the completion of the study.

Who is organising and funding the research?

The study is being conducted by a PhD research student of the Functional Food Centre at Oxford Brookes University. This research is being completed and funded by the School of Life Sciences in Oxford Brookes University.

Who has reviewed the study?

This research has been approved by the University Research Ethics Committee at Oxford Brookes University.

Contact for further information:

Sarah Hillier
PhD Research Student

Dr. Richard Craven
Principal Lecturer in Physiology

Email: shillier@brookes.ac.uk or rpcraven@brookes.ac.uk
Tel: +44 (0)1865 483283

If you require further information please do not hesitate to contact me.

If you have any concerns about the way in which the study has been conducted, you should contact the Chair of the University Research Ethics Committee on ethics@brookes.ac.uk

Thank you for taking time to read this information sheet.

9. Information Sheet Given in Chapter 5

Participant Information Sheet

Investigating the differences in sex hormones during the menstrual cycle in women with premenstrual syndrome (PMS)

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the Study?

Current literature demonstrates scientific uncertainty with regards to changes in oestrogen and progesterone hormone concentrations during the menstrual cycle. The latest research suggests women who suffer from PMS may have higher concentrations in these hormones, particularly progesterone. Women who regularly exercise are reported as having lower concentrations in these menstrual cycle hormones. The aim of this research is to determine the effects of exercise on sex hormone concentrations (oestrogen and progesterone) during the menstrual cycle in women with PMS.

Participants will be randomly divided into two equally important testing groups. Once enrolled onto the study you will be allocated to one of these groups. The first group (1) will include exercise; the second group (2) will be non-exercise.

Who are we looking for?

The study is looking for non-smoking female participants, who have suffered from moderate symptoms of PMS for at least 3 previous cycles, all with a BMI greater than 18.5 kg/m² and less than 30kg/m². Participants should not currently be performing aerobic exercise for more than 1 hour per week, this includes any exercise that increases your heart rate (pulse), makes you out of breath or sweat. All participants will be between the ages of 18-40 years, with no known diabetes or impaired glucose tolerance. Participants must not be suffering from anaemia. All participants should have 3 previous regular menstrual cycles. Participants should not be pregnant or have been pregnant within the previous 2 years or are still breastfeeding. All participants should not be taking any form of hormonal contraception.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you are a student choosing to either take part or not take part in the study or to withdraw at any time, this will have no impact on your marks, assessment or participation in future studies.

What is involved if I take part?

You will be given this participant information sheet and a consent form to sign. You will then be invited to a screening session where we will be able to determine if you are eligible for the study or not.

Once agreeing to take part in the screening session you will be asked to fill in a series of questionnaires including one on your PMS status in the Functional Food Centre, Oxford Brookes University. These will give us an indication of your health and any medications that may affect the parameters measured. Screening of your height and weight will be taken at this stage. A blood glucose measurement will also be taken; this involves a small finger-prick blood sample. If there is any indication that you do not meet the inclusion criteria, regrettably you will not be able to continue to take part in the study at this stage. However if you are eligible to participate you will be asked to sign a study consent form and then be randomly assigned to either group 1 or 2.

The study will be divided into 3 stages:

Stage 1: The first part of the study is the same for groups 1 and 2 and will involve one 6ml blood sample being taken during the morning of each test day for one complete menstrual cycle (≈ 4 weeks). Blood samples will be taken from a vein located in the arm. You will also be asked to fill out a questionnaire about your PMS symptoms. Test days will be 3 times a week accommodating for weekends and will occur for one complete menstrual cycle. A dietary recall interview will be conducted on 3 of the test days to record food intake for the previous 24 hours. The tests will take no longer than 20 minutes to complete.

Stage 2: Group 1 participants will take part in a two cycle (≈ 8 weeks) exercise training programme. This will involve 3 x 30 minute exercise sessions per week at a medium intensity on a bike or treadmill. Medium intensity means you will experience a noticeable rise in heart rate and you may sweat but will be able to carry out a conversation whilst completing the exercise. A 5 minute stretching and warm up session will also take place at this time. A dietary recall interview will be conducted on 3 of the test days during the cycle to record food intake for the previous 24 hours (6 days total for both cycles). A maximum of 60 minutes is required for each exercise session.

Group 2 participants will take part in a two cycle (≈ 8 weeks) supervised nutrition workshop programme. You will be asked to attend weekly 90 minute sessions during which body composition measurements will be taken, in addition dietary recall interviews will be conducted on 3 of the test days to record food intake for the previous 24 hours (6 days total for both cycles).

Stage 3: Group 1 will continue to take part in the exercise regime for an additional cycle (≈ 4 weeks). This will include a 6ml venous blood sample being taken and you will be asked to fill in a questionnaire about your PMS symptoms and quality of life. Dietary recall interviews will also be conducted on 3 of the test days during the cycle to record food intake for the previous 24 hours. In addition three of the exercise sessions will be selected to record measurements of expired air. This involves breathing in and out of a mouth piece whilst exercising. Diet intake will be replicated the day before each of these test days.

Group 2 participants will repeat the testing performed during stage 1. Blood samples will be taken three times a week for one complete menstrual cycle (≈ 4 weeks), with dietary recall interviews being conducted on 3 of the test days to record food intake for the previous 24 hours. In addition you will be asked to fill in a questionnaire about your PMS symptoms and quality of life. A summary of the study, including what measurements will be taken can be seen below:

	Week 1 (Days 1 – 7)	Week 2 (Days 7-14)	Week 3 (Days 14-21)	Week 4 (Days 21 – 28)
Stage 1 (4 weeks)	3x Lab visits = 3x B, 1x DR	3x Lab visits = 3x B, 1x DR	3x Lab visits = 3x B	3x Lab visits = 3x B, 1x DR
Stage 2 (8 weeks)	Group 1: 3x 30 min exercise 1x DR Group 2: 1x 90min nutrition 1x DR	Group 1: 3x 30 min exercise 1x DR Group 2: 1x 90min nutrition 1x DR	Group 1: 3x 30 min exercise Group 2: 1x 90min nutrition	Group 1: 3x 30 min exercise 1x DR Group 2: 1x 90min nutrition 1x DR
Stage 3 (4 weeks)	Group 1: 3x Lab visits = 3x 30 min exercise 3x B, 1x DR 1x EA Group 2: 3x Lab visits = 3x B, 1x DR	Group 1: 3x Lab visits = 3x 30 min exercise 3x B, 1x DR 1x EA Group 2: 3x Lab visits = 3x B, 1x DR	Group 1: 3x Lab visits = 3x 30 min exercise 3x B Group 2: 3x Lab visits = 3x B	Group 1: 3x Lab visits = 3x 30 min exercise 3x B, 1x DR 1x EA Group 2: 3x Lab visits = 3x B, 1x DR

B = Blood sample, DR = Dietary Recall interview, EA = Expired air

What are the possible risks in taking part?

There are no major risks. The only risk will be slight bruising of the arm from which the blood has been taken but that should disappear within a few days. The bruising will not prevent you from doing your regular daily activities.

What are the possible benefits from taking part?

Taking part in this study will provide you with information about your menstrual cycle, body mass index (Stupnicki and Obminski) and body composition including percentage body fat. You will be provided with a summary of the key results of the study upon request. You will also receive £150 on completion of all four stages of the study.

Will what I say in this study be kept confidential?

All information collected about you will be kept strictly confidential (i.e. subject to legal limitations). Access to the data will be by the researchers working on this study only. Access to computer files will be by password only and data, codes and identifying information will be kept in locked filing cabinets. Data generated by the study must be retained in accordance with the University's policy on Academic Integrity and will comply with the Data Protection Act in the UK at all times. The data generated in the course of the research will be kept securely in paper or electronic form for a period of ten years after the completion of a research project. Due to the small number of participants taking part in this study it may be difficult to maintain anonymity of the participants, though every effort will be made by the research team to ensure participant confidentiality.

What should I do if I want to take part?

If you would like to take part in this research study you can do so by contacting the researchers at the address, phone number or email address given below.

What will happen to the results of the research study?

Following the completion of this study, you will receive information regarding anthropometric, body composition measurements and blood parameters; these will be basic physical, body composition measurements and not diagnostic information. The results of this research will be published in a research journal. Your name or identity will not be recognisable from this. If you wish to obtain a copy of the published research you can do so by contacting the researchers at the address, phone number or email address given below following the completion of the study.

Who is organising and funding the research?

The study is being conducted by a PhD research student and members of staff of the Functional Food Centre at Oxford Brookes University. This research is being completed and funded by the School of Life Sciences at Oxford Brookes University.

Who has reviewed the study?

This research has been approved by the University Research Ethics Committee at Oxford Brookes University.

Contact for further information:

Sarah E Hillier

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**Functional Food Centre,
Oxford Brookes University.**

If you require further information please do not hesitate to contact me.

If you have any concerns about the way in which the study has been conducted, you should contact the Chair of the University Research Ethics Committee on ethics@brookes.ac.uk

Thank you for taking time to read this information sheet.

10. Consent Form

Consent form

Contacts:

Sarah Hillier, PhD Research Student
Dr Richard Craven, Principal Lecturer in Physiology
Dr Michelle Venables, Early Career Research Fellow
Dr Simon Wheeler, Lecturer in Nutrition

School of Life Sciences
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Tel: 01865 483283
Email: shillier@brookes.ac.uk / rpcraven@brookes.ac.uk /
michelle.venables@brookes.ac.uk /simon.wheeler@brookes.ac.uk

Please INITIAL the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	<input type="checkbox"/>	<input type="checkbox"/>
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	<input type="checkbox"/>	<input type="checkbox"/>
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, or to withdraw any unprocessed data previously supplied.	<input type="checkbox"/>	<input type="checkbox"/>
4. I understand that confidentiality of information provided can only be protected within the limits of the law.	<input type="checkbox"/>	<input type="checkbox"/>
5. I agree to take part in the above research screening day.	<input type="checkbox"/>	<input type="checkbox"/>
6. I agree to take part in the above research by giving blood samples as described in the Participant Information sheet	<input type="checkbox"/>	<input type="checkbox"/>

Name of ParticipantDate
(block capitals)

Signature
Contact number: email:.....

Name of ResearcherDate
(block capitals)
Signature

11. Health Questionnaire

Health Questionnaire

(Please circle as appropriate)

- Are you allergic to any foods? Yes or No
If yes which one(s)? _____
- Do you have a genetic or metabolic disease? Yes or No
- Do you suffer from anaemia? Yes or No
- Are you taking any medication? Yes or No
If yes, which one(s)? _____
- Are you a smoker? Yes or No
If yes, cigarettes/day: _____
- Are you following a special diet? Yes or No
If yes, which one(s)? _____
- Do you exercise or participate in any sports? Yes or No
How often a week? _____ Duration: _____ Intensity: _____

Menstrual Cycle: please answer the following questions:

- Do you use oral contraceptive pills? Yes or No
If yes, which type of pill? _____
- Have you experienced regular menstrual cycles for the previous 3 cycles? Yes or No
- Have you given birth within the last 2 years? Yes or No
If yes, are you still lactating / breastfeeding? Yes or No
- What is the average length of your menstrual cycle? (I.e. From beginning of one menstruation to the beginning of the next) _____
- What is the average length of menstruation? (No. of days bleeding) _____
- Number of days since the end of your last menstruation? _____

12. Habitual Physical Activity Questionnaire

Habitual Physical Activity Questionnaire

Name: Subject No: Date:

Please circle the response most applicable to each of the statements or answer the questions. All of the results will be strictly confidential and will be available only to the researcher. This is a previously validated questionnaire.

Thank You

What is your main occupation?
.....

1. At work I sit

never seldom sometimes often always
2. At work I stand

never seldom sometimes often always
3. At work I walk

never seldom sometimes often always
4. At work I lift heavy loads

never seldom sometimes often always
5. After work I'm tired

very often sometimes seldom never often
6. At work I sweat

very often sometimes seldom never often
7. In comparison with others of my own age, I think my work is physically

much heavier as heavy lighter much

heavier lighter
8. Do you play sport?

Yes No

If Yes:

Which sport do you play most frequently?

How many hours a week?

<1 1-2 2-3 3-4 >4

How many months a year?

<1 1-2 2-3 3-4 >4

If you play a second sport:

Which sport is it?						
How many hours a week?		<1	1-2	2-3	3-4	>4
How many months a year?		<1	1-2	2-3	3-4	>4
9.	In comparison with others of my own age, I think my physical activity during leisure time is	much more	more	the same	less	much less
10.	During leisure time I sweat	very	often	sometimes	seldom	never often
11.	During leisure time I play sport	never	seldom	sometimes	often	very
12.	During leisure time I watch Television	never often	seldom	sometimes	often	very
13.	During leisure time I walk	never often	seldom	sometimes	often	very
14.	During leisure time I cycle	never often	seldom	sometimes	often	very
15.	How many minutes do you walk and/or cycle per day to and from work, school and shopping?	<5	5-15	15-30	30-45	>45

13. Premenstrual Symptom Screening Tool (PSST) – Chapter 5

Premenstrual Symptom Screening Tool (PSST)

Name _____ Date _____ Subject No. _____

The premenstrual symptoms screening tool (PSST)

(please mark an "X" in the appropriate box)

Do you experience some or any of the following premenstrual symptoms which start before your period and stop within a few days of bleeding?

Symptom	Not at all	Mild	Moderate	Severe
1. Anger/irritability				
2. Anxiety/tension				
3. Tearful/increased sensitivity to rejection				
4. Depressed mood/hopelessness				
5. Decreased interest in work activities				
6. Decreased interest in home activities				
7. Decreased interest in social activities				
8. Difficulty concentrating				
9. Fatigue/lack of energy				
10. Overeating/food cravings				
11. Insomnia				
12. Hypersomnia (needing more sleep)				
13. Feeling overwhelmed or out of control				
14. Physical symptoms: breast tenderness, headaches, joint/muscle pain, bloating, weight gain				

Have your symptoms, as listed above, interfered with:

	Not at all	Mild	Moderate	Severe
A. Your work efficiency or productivity				
B. Your relationships with coworkers				
C. Your relationships with your family				
D. Your social life activities				
E. Your home responsibilities				

14. Menstrual Distress Questionnaire (MDQ), Form T

Menstrual Distress Questionnaire (Form T – Today)

Name _____ Age _____ Marital Status _____

Usual Occupation _____ Today's Date _____

Directions:

The list below shows common symptoms and feelings associated with menstruation. For each item, choose the descriptive category from the box below that best describes what you are feeling today. That is, for each item, decide whether you have "no experience of symptom" or whether your experience is "present mild", "present moderate", "present strong", or "present severe". Then write the number of the category in the space provided. If none of the categories exactly describes your experience, choose the one that most closely matches what you feel.

Be sure to rate every item on the form for each day on which symptoms are to be recorded. When you have completed the prescribed number of forms, return them to the person who gave them to you.

Descriptive Categories: 0 No experience of symptom; 1 Present, mild;
2 Present, moderate; 3 Present, strong; 4 Present, severe

- | | |
|--------------------------------------|---|
| 1. _____ Muscle stiffness | 23. _____ Insomnia |
| 2. _____ Headache | 24. _____ Forgetfulness |
| 3. _____ Cramps | 25. _____ Confusion |
| 4. _____ Backache | 26. _____ Poor judgment |
| 5. _____ Fatigue | 27. _____ Difficulty concentrating |
| 6. _____ General aches and pains | 28. _____ Distractible |
| 7. _____ Weight gain | 29. _____ Minor Accidents |
| 8. _____ Skin blemish or disorder | 30. _____ Poor motor coordination |
| 9. _____ Painful or tender breasts | 31. _____ Poor school or work performance |
| 10. _____ Swelling (breasts abdomen) | 32. _____ Take naps, stay in bed |
| 11. _____ Dizziness, faintness | 33. _____ Stay at home |
| 12. _____ Cold sweats | 34. _____ Avoid social activities |
| 13. _____ Nausea, vomiting | 35. _____ Decreased efficiency |
| 14. _____ Hot flashes | 36. _____ Affectionate |
| 15. _____ Loneliness | 37. _____ Orderliness |
| 16. _____ Anxiety | 38. _____ Excitement |
| 17. _____ Mood swings | 39. _____ Feelings of well-being |
| 18. _____ Crying | 40. _____ Bursts of energy, activity |
| 19. _____ Irritability | 41. _____ Feelings of suffocation |
| 20. _____ Tension | 42. _____ Chest pains |
| 21. _____ Feeling sad or blue | 43. _____ Ringing in the ears |
| 22. _____ Restlessness | 44. _____ Heart pounding |
| | 45. _____ Numbness, tingling |
| | 46. _____ Blind spots, fuzzy vision |
| | 47. Do you have your period
(menstrual flow) today? _____ Yes _____ No |

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