Associations of Mood, Perceived Stress, Cognitive Dietary Restraint and the Menstrual Cycle in Healthy New Zealand Females.

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Abstract

Background: Subclinical ovulatory disturbances (SOD) are the initial adaptations of the female reproductive axis to stress. Previous research suggests that psychological stressors, such as perceived stress and cognitive dietary restraint (CDR), may contribute to the occurrence of subclinical ovulatory disturbed cycles (SOD cycles) in healthy menstruating females; however, no studies have investigated these associations in the New Zealand context. The reduction in sex steroid hormones that occurs with SOD cycles may also contribute to variations in psychological factors throughout the menstrual cycle. Although previous research has investigated changes in mood and perceived stress between menstrual cycle phases, mixed results have been reported between studies and none have considered the ovulatory status of participants when investigating these changes. Therefore, the primary aim of the present study was to determine if females who presented with at least two SOD menstrual cycles (anovulation or luteal phase defects) displayed higher perceived stress and CDR than females who presented with at least two ovulatory menstrual cycles over a three-cycle period. A secondary aim was to investigate if there were variations in mood, perceived stress and CDR throughout the menstrual cycle in females with different menstrual cycle presentations (ovulatory versus SOD cycles).

Methods: Ninety-seven naturally menstruating females aged 30.5 ± 5.6 years participated in this prospective cohort study over five menstrual cycles. Participants completed the Profile of Mood States Questionnaire (POMS-Q), Perceived Stress Scale (PSS) and the Three Factor Eating Questionnaire (TFEQ) to measure mood, perceived stress and CDR during the early follicular, late follicular and mid-luteal phases of the menstrual cycle. Urinary luteinising hormone testing and mid-luteal phase plasma progesterone levels were measured to classify cycles as ovulatory (mid-luteal progesterone > 10 nmol/L) or SOD (mid-luteal progesterone ≤ 10 nmol/L) and to verify menstrual cycle phase. Two-tailed independent samples t-tests were used to compare mean perceived stress and CDR scores between ovulatory and SOD participants. Linear mixed models with participants as the random effect and baseline gynaecological age, percentage body fat and scaled metabolic equivalent of task (MET) minutes per week were used to analyse the effects of menstrual cycle phase, ovulatory status and their interaction on scores of mood, perceived stress and CDR.

Results: The average perceived stress score of all females was 24.7 ± 5.5 and the average CDR score was 14.0 ± 4.1. There was no difference in perceived stress ($t = 0.5$, df = 84, $p = 0.606$) or CDR ($t = -1.7$, df = 84, $p = 0.100$) scores between ovulatory ($n = 68$) and SOD ($n = 18$) groups. Furthermore, linear mixed models showed no individual or interaction effects of menstrual cycle phase and ovulatory status on mood, perceived stress or CDR ($p > 0.006$ for all variables).

Conclusion: The results of this study suggest that healthy menstruating females presenting with at least two SOD cycles may not have higher perceived stress or CDR than females presenting with at least two ovulatory cycles over a three-cycle period. Furthermore, no group-level effects of menstrual cycle phase and ovulatory status on mood, perceived stress or CDR were observed. However, large inter-individual variability was seen throughout the menstrual cycle. Further research is needed to develop and improve methods used in menstrual cycle research.

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Table of Contents

List of Tables

List of Figures

Abbreviations

Abbreviation	Meaning
BMI	Body Mass Index
CDR	Cognitive Dietary Restraint
FSH	Follicle-Stimulating Hormone
LH	Luteinising Hormone
MET	Metabolic Equivalent of Task
POMS-Q	Profile of Mood States Questionnaire
PSS	Perceived Stress Scale
SOD	Subclinical Ovulatory Disturbances
SOD cycle	Subclinical Ovulatory Disturbed cycle
TFEQ	Three Factor Eating Questionnaire
TFEQ-R	Three Factor Eating Questionnaire Restraint Subscale

Terminology Disclaimer

The use of the term ‘female’ throughout this thesis refers to an individual’s biological sex rather than their social construct or gender identity. Participants in this project were provided the option of identifying their sex and gender. From this data, all participants in the present study indicated that they identified as female (sex) and women (gender). Therefore, the use of the term female was considered appropriate for this thesis, especially as the focus of this research is on the menstrual cycle, a biological process that is a feature of the female sex.

Chapter 1: Purpose

1.0 Introduction

The hypothalamic-pituitary-ovarian axis is a precisely controlled feedback system that regulates female reproduction (Reed & Carr, 2015). This regulation of female reproduction is coordinated through the cyclic release of gonadotrophic and sex steroid hormones (Reed & Carr, 2015). It is the interactions of these hormones, typically over the course of 21 to 35 days, that facilitate the key activities within the fertile menstrual cycle, including follicular development, ovulation, thickening of the endometrium for possible implantation and shedding of the endometrium if the ovum is not fertilised (Reed & Carr, 2015). Broadly, the menstrual cycle is described in two phases: the follicular phase and the luteal phase. The variations in sex steroid hormones and corresponding activities previously noted that can occur throughout the menstrual cycle can be more accurately defined through four sub-phases: (1) the early follicular phase, (2) the late follicular phase, (3) the ovulatory phase and (4) the mid-luteal phase (De Jonge et al., 2019; Elliott-Sale et al., 2021).

When a female is subjected to physical, psychological, emotional or social stress to an extent that the conditions are not suitable to sustain pregnancy, their reproductive axis and physiology may become suppressed (Nepomnaschy et al., 2004; Prior et al., 2022; Schliep et al., 2015; Wade et al., 1996; Williams et al., 2007). The suppression and regaining of a fertile menstrual cycle is a protective response that occurs along a continuum of severity, with both adaptations occurring naturally (Bullen et al., 1985; De Souza et al., 2010; Prior et al., 1982). The initial adaptations of the female reproductive axis to stress are subclinical ovulatory disturbances (SOD), which present as normal-length menstrual cycles with anovulation or luteal phase defects (Bedford et al., 2010; Prior et al., 1990a). As a result of subclinical ovulatory disturbed cycles (SOD cycles) occurring first along the reproductive axis suppression continuum, their estimated prevalence is high. Previously, in a large cross-sectional study in Norway, 37% of females with normal menstrual cycle lengths had an anovulatory cycle, identified by a serum progesterone level of less than 9.54 nmol/L during the luteal phase (Prior et al., 2015). Furthermore, results from a two-year prospective cohort study in Canada reported a 61% prevalence of at least one anovulatory cycle and an 82% prevalence of at least one cycle with a short luteal phase length in regularly menstruating females, using the least-squares method of quantitative basal body temperature analysis (Bedford et al., 2010). Cumulatively, this data indicates that it may be common for healthy, regularly menstruating females to experience sporadic SOD cycles.

Despite their high prevalence, detection of SOD cycles can be difficult because the overt symptoms of reproductive axis disorders, such as changes to menstrual cycle length or an absence of menstrual bleeding, are not always present (Bedford et al., 2010; Prior et al., 2022; Prior et al., 1990a). Previous

research investigating the prevalence of SOD cycles have typically used least-squares quantitative basal temperature analysis to determine evidence of luteal activity (Bedford et al., 2010; Prior et al., 1990a). However, the use of mid-luteal venous progesterone levels, combined with the tracking of menstrual cycle length and urinary luteinising hormone (LH) testing has been proposed as a valid method of detecting SOD cycles (Practice Committees of the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility, 2021). The silent occurrence of SOD cycles is concerning, given that research suggests that females who routinely present with SOD cycles may have an increased risk of bone mineral loss (Bedford et al., 2010; Li et al., 2013; Prior et al., 1990a). A meta-analysis of prospective cohort studies reported a weighted mean difference in percentage annual spinal bone mineral density change of -0.86% in females who presented with frequent SOD cycles, compared with females who experienced fewer SOD cycles (Li et al., 2013). It may be proposed that if the experience of SOD cycles is consistent or a regular occurrence throughout their reproductive years, this loss of bone mineral density may place females at an increased risk of bone fractures (Melton III et al., 1993). An additional consequence of SOD cycles is the adverse impact on fertility, with spontaneous fertile cycles requiring both ovulation and adequate progesterone levels in the luteal phase (Mesen & Young, 2015). Research suggests that infertility is a common experience among New Zealand females (Righarts et al., 2021; van Roode et al., 2015). Although there are likely multiple factors influencing the prevalence of infertility, the occurrence of SOD cycles in healthy females is a factor worth considering. However, the current understanding of the prevalence of SOD cycles in New Zealand females and the lifestyle factors that may be contributing to them is limited.

A significant proportion of previous research investigating lifestyle factors that may contribute to female reproductive axis suppression has traditionally focused on physical and nutritional stressors, such as overtraining and problematic low energy availability (Bullen et al., 1985; De Souza et al., 2021; De Souza et al., 2010; Mountjoy et al., 2023). As a result, there is a strong consensus within the literature that overtraining and problematic low energy availability can contribute to subclinical and clinical menstrual cycle impairment (Mountjoy et al., 2023). However, there is currently limited understanding of how psychological stressors, such as perceived stress and cognitive dietary restraint (CDR), contribute to menstrual cycle disturbances. Perceived stress refers to the degree to which an individual appraises situations they experience as stressful (Cohen et al., 1983), while CDR refers to an intentional cognitive effort to restrict food intake, in an attempt to control body weight (Herman & Mack, 1975; Stunkard & Messick, 1985). There is growing evidence to suggest that high levels of perceived stress and CDR may contribute to SOD cycles in healthy females (Bedford et al., 2010; Prior et al., 2022; Schliep et al., 2015). Previous research has suggested that these changes may be driven by interactions between the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-ovarian axis (Ferin, 1999). Specifically, it has been proposed that increased stress can upregulate the hypothalamic-pituitary-adrenal axis, which may downregulate the hypothalamic-pituitary-ovarian axis, resulting in

suppression of ovarian activity and subsequently, a reduction in sex steroid hormones (Ferin, 1999). However, few studies have used venous blood measurement to verify sex steroid hormone levels and ovarian activity when investigating associations between perceived stress, CDR, and SOD in healthy menstruating females. Therefore, the extent to which perceived stress and CDR may differ between healthy menstruating females who present with consistent ovulatory or SOD cycles is still an area that requires investigation. Furthermore, no studies have been completed in New Zealand females.

The occurrence of SOD cycles in healthy menstruating females will be associated with reductions in sex steroid hormones throughout the menstrual cycle, particularly during the luteal phase (Schliep et al., 2014; Strott et al., 1970). Given that psychological stressors have been associated with an increased prevalence of SOD cycles in healthy menstruating females (Bedford et al., 2010; Prior et al., 2022; Schliep et al., 2015), it is plausible that the reductions in sex steroid hormones caused by SOD cycles may also influence changes in psychological mood states throughout the menstrual cycle. Although variations in mood and perceived stress throughout the menstrual cycle in healthy females have been explored in previous research (Jain et al., 2023; Romans et al., 2012a), to the best of the author's knowledge, no studies to date have considered the ovulatory status (and corresponding sex steroid hormone profiles) of participants when investigating these changes.

The failure of previous research to consider ovulatory status when researching variations in mood and perceived stress throughout the menstrual cycle in healthy females could be a contributing factor to the significant variability in reported results (Brown & Lewis, 1993; Jain et al., 2023; Montero-López et al., 2018; Romans et al., 2012a). An additional consideration is that previous research has frequently reported on changes in mood states and perceived stress in the late luteal and early-to-mid-follicular phases of the menstrual cycle (Brown & Lewis, 1993; Kikuchi et al., 2010; Reed et al., 2008; Symonds et al., 2004). The focus on the late luteal and early-to-mid-follicular phases of the menstrual cycle, during which sex steroid hormones are relatively low (Elliott-Sale et al., 2021), does not appropriately encapsulate the four distinct hormonal profiles that occur throughout the menstrual cycle (early follicular phase, late follicular phase, ovulatory phase and mid-luteal phase) (De Jonge et al., 2019; Elliott-Sale et al., 2021). Furthermore, the strong focus on reporting of negative emotions within some of the mood studies (Gonda et al., 2008; Kikuchi et al., 2010; Ross et al., 2003; Symonds et al., 2004) may further perpetuate negative stereotypes, such as the idea that negative mood changes in females are attributable to menstruation (Koeske & Koeske, 1975). To avoid perpetuating a biased narrative, research that investigates variations in both positive and negative mood states across the four distinct hormonal profiles within the menstrual cycle is needed. A final consideration that is worth noting is that, to the best of the author's knowledge, no research to date has investigated the influence of sex steroid hormone concentrations throughout the menstrual cycle on CDR scores in healthy females. Given research has reported that females experience increased cravings for foods high in sugar, salt and

fat during the luteal phase of the menstrual cycle (Souza et al., 2018; Yukie et al., 2020), and that energy intake may be increased during this time (Tucker et al., 2024), it is plausible that females could also experience changes in their cognitive efforts to restrict food intake. Therefore, research is required to investigate if this association exists.

1.1 Research Aims and Objectives

1.1.1 Aims

The primary aim of the present study was to determine if females who presented with at least two SOD menstrual cycles (anovulation or luteal phase defects) displayed higher perceived stress and CDR than females who presented with at least two ovulatory menstrual cycles over a three-cycle period. A secondary aim was to investigate if there were variations in mood, perceived stress and CDR throughout the menstrual cycle in females with different menstrual cycle presentations (ovulatory versus SOD cycles).

1.1.2 Objectives

1. To determine if there is a difference in perceived stress (on the Perceived Stress Scale [PSS]) and CDR (on the restraint subscale of the Three Factor Eating Questionnaire [TFEQ-R]) between females who have two or more ovulatory cycles and females who have two or more SOD cycles over the three-cycle testing period.
2. To determine if there are differences in mood, perceived stress and CDR in the early follicular, late follicular and mid-luteal phases of the menstrual cycle in females who have an ovulatory cycle versus females who have an SOD cycle.

1.1.3 Hypotheses

1. Females who have two or more SOD cycles will have higher scores for perceived stress and CDR than females who have two or more ovulatory cycles.
2. Females will present with higher scores of negative mood, perceived stress and CDR in the mid-luteal phase of the menstrual cycle, compared with the early follicular and late follicular phases, while positive mood scores will be higher during the late follicular phase than the mid-luteal phase. Due to reduced variability in sex steroid hormones, any phase effect on mood, perceived stress and CDR will be weaker in females who have an SOD cycle.

1.2 Structure of Thesis

This thesis begins with an introduction to SOD cycles in healthy females and the psychological stressors they may be associated with, concluding with the aims, objectives and hypotheses of the present study. The following chapter will provide a comprehensive review of previous literature investigating variations in mood and perceived stress throughout the menstrual cycle and the influence of high perceived stress and CDR on the physiological functioning of the menstrual cycle, including their association with SOD. Chapter three is a manuscript of the present study, which provides details of the methodology used and results. Finally, chapter four provides a summary of the implications of the research, strengths, limitations and recommendations for future research.

1.3 Researcher's Contributions

Table 1.1. *Summary of Researcher's Contributions to the Study.*

Researcher	**Contribution**
Caitlin McKenzie MSc Nutrition and Dietetics Student	Primary author of thesis and manuscript Statistical analysis Interpretation of results
Dr Claire Badenhorst Primary supervisor Associate Professor School of Sport, Exercise and Nutrition	Designed study Ethics application Data collection Provided advice and revised thesis
Dr Robyn Lawrence Co-supervisor Lecturer in Nutrition and Dietetics	Provided advice and revised thesis
Rebecca Paul Research Officer	Data collection and management
Dr Karen Mumme Statistician	Preparation and cleaning of data Assisted with statistical analysis
Dr Beatrix Jones Associate Professor Statistics	Assisted with statistical analysis

Chapter 2: Literature Review

2.0 Introduction

The menstrual cycle is a dynamic and complex sequence of physiological processes that are regulated by the hypothalamic-pituitary-ovarian axis and result in fluctuations of sex steroid hormones. These fluctuations in sex steroid hormones have been associated with physiological, psychological and behavioural changes throughout the menstrual cycle; particularly during the late luteal phase, with previous research having reported that up to 80% of females experience at least one physical or psychological symptom during this time (Hantsoo et al., 2022; Schoep et al., 2019; Wittchen et al., 2002). Furthermore, psychological factors such as perceived stress and cognitive dietary restraint (CDR), a form of chronic psychological stress, may suppress physiological functions of the menstrual cycle (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994; Bedford et al., 2010; Garcia de Leon et al., 2023; Prior et al., 2022; Schliep et al., 2015). This literature review begins with an overview of the menstrual cycle, including the classification of the menstrual cycle phases. The review will then provide a critical summary of variations in two psychological factors (mood and perceived stress) throughout the menstrual cycle and the effects of two psychological factors (perceived stress and CDR) on the menstrual cycle itself, with a particular focus on subclinical ovulatory disturbances (SOD).

2.1 Methods

To understand current knowledge surrounding (1) variations in mood and perceived stress across the menstrual cycle and (2) the influence of perceived stress and CDR on menstrual cycle functioning, the author searched PubMed, Google Scholar, Scopus and Discover Massey University Library online databases for relevant articles. The following search terms were used:

- “Premenopausal women” OR “menstruating women” OR “women” OR “females”
- “Menstrual cycle” OR “menstrual cycle phase” OR “menstruation” OR “follicular phase” OR “ovulatory phase” OR “luteal phase”
- “Perceived stress” OR “mood”
- “Cognitive dietary restraint” OR “dietary restraint”
- “Ovulation” OR “anovulation” OR “subclinical ovulatory disturbance” OR “luteal phase defect” OR “luteal phase length” OR “progesterone” OR “estrogen” OR “oestrogen” OR “menstrual cycle length”

The titles and abstracts of the identified articles were screened to determine relevance. Included articles focus on healthy, premenopausal females (human or other primate), who had not been diagnosed with reproductive disorders (such as premenstrual dysphoric disorder or polycystic ovary syndrome) or psychiatric disorders (such as depression).

2.2 Menstrual Cycle Overview

Although considerable individual variability exists (Bakos et al., 1994; Bull et al., 2019; Chiazze et al., 1968; Fehring et al., 2006; Najmabadi et al., 2020), a typical menstrual cycle length ranges from 21-35 days, and broadly can be divided into two phases: the follicular phase and the luteal phase. The follicular phase is the initial phase of the menstrual cycle, occurring from the first day of menstruation and lasting until the day before ovulation, when the ovum is released. Mainstream communications about the menstrual cycle suggest that ovulation happens on day 14; however, as with other menstrual cycle characteristics, research has demonstrated that ovulation can occur within a broad range of cycle days (Bull et al., 2019; Najmabadi et al., 2020; Soumpasis et al., 2020). The day after ovulation marks the start of the luteal phase, which ends the day before menstruation of the next cycle. The menstrual cycle can be further broken down into four sub-phases, which reflect the unique fluctuations in sex steroid hormones (oestrogen and progesterone) occurring in the follicular and luteal phases of the menstrual cycle. The four sub-phases are: (1) the early follicular phase, (2) the late follicular phase, (3) the ovulatory phase and (4) the mid-luteal phase (De Jonge et al., 2019; Elliott-Sale et al., 2021) (Figure 2.1).

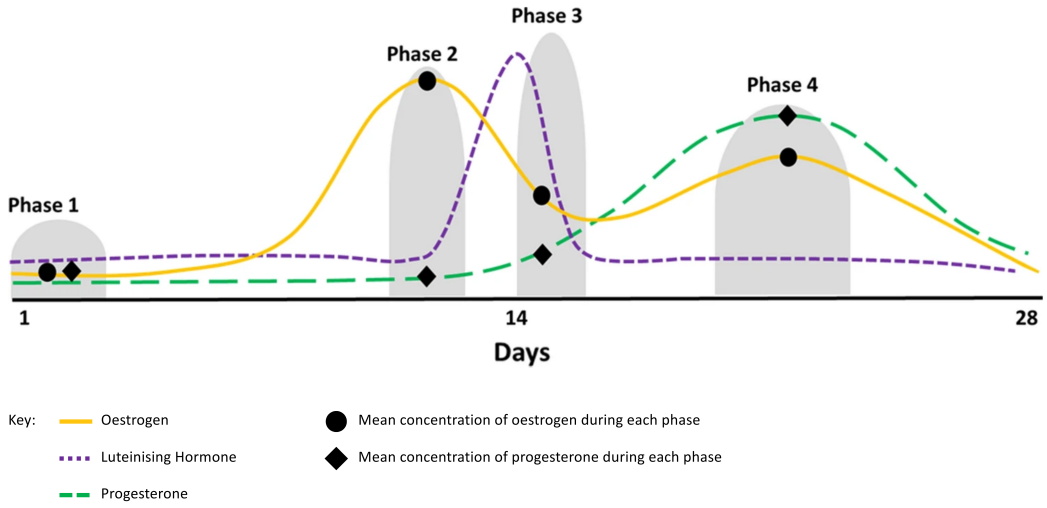

Figure 2.1. *A diagram of sex steroid hormone fluctuations and patterns used to define the sub-phases throughout an idealised 28-day menstrual cycle, originally published in Elliott-Sale et al. (2021).*

The duration of the four menstrual cycle sub-phases is highly variable, both between females and between cycles for an individual (Fehring et al., 2006). However, in general, the early follicular phase can be defined as the first days of the follicular phase, when menstrual bleeding is present, and is characterised by low concentrations of both sex steroid hormones (Anckaert et al., 2021). During this phase, the pituitary gland releases follicle-stimulating hormone (FSH), which stimulates the recruitment of a cohort of follicles within the ovaries, one of which will mature into the dominant follicle (McGee

& Hsueh, 2000). As the dominant follicle matures, it releases oestrogen in proportion to its growth. The increasing levels of oestrogen result in the progressive thickening of the endometrial lining. The concentration of oestrogen peaks approximately 34-36 hr prior to ovulation (Hoff et al., 1983), which is typically considered the hormonal characteristic of the late follicular phase. A sufficient concentration of oestrogen (greater than 200 pg/mL), that is sustained for approximately 50 hr, stimulates a feedforward signal on the hypothalamus to increase the release of luteinising hormone (LH) from the pituitary gland (Young & Jaffe, 1976). Approximately 10-12 hr after the concentration of LH peaks, ovulation typically occurs (Pauerstein et al., 1978), and this is characterised by the release of an ovum from the mature follicle in the ovary. Following ovulation, oestrogen drops to a medium concentration, while progesterone remains low (Anckaert et al., 2021). The remnants of the mature follicle in the ovary form the corpus luteum, which releases both sex steroid hormones, but primarily progesterone. The concentration of progesterone typically peaks seven days after ovulation, which is considered a hormonal characteristic of the mid-luteal phase (Anckaert et al., 2021; Elliott-Sale et al., 2021). During this time, oestrogen may reach moderate levels and its secondary peak (Anckaert et al., 2021). In the absence of fertilisation, the corpus luteum function will begin to decline approximately 9-11 days after ovulation (Reed & Carr, 2015). While the mechanism is still unknown, the breakdown of the corpus luteum causes a decline in sex steroid hormones. The decline in progesterone initiates spontaneous decidualisation of the endometrial cells, resulting in menstruation and the commencement of the subsequent cycle (Reed & Carr, 2015).

2.3 Variations in Psychological Factors Throughout the Menstrual Cycle

The menstrual cycle is a physiological mechanism that may contribute to psychological variability in females. Mood and perceived stress are both psychological factors that have been reported to fluctuate throughout the different phases of the menstrual cycle (Gonda et al., 2008; Jain et al., 2023; Reed et al., 2008; Romans et al., 2012b; Ross et al., 2003; Symonds et al., 2004), and therefore, may be influenced by variations in sex steroid hormone concentrations. This section of the literature review will present a critical overview of previous research that explores the variations in mood and perceived stress throughout the menstrual cycle in healthy females without Premenstrual Dysphoric Disorder, highlighting relevant methodological issues, differences between studies and gaps in the literature.

2.3.1 Mood

Multiple previous studies have reported changes in mood across menstrual cycle phases (Gonda et al., 2008; Reed et al., 2008; Romans et al., 2012b; Ross et al., 2003; Symonds et al., 2004). However, due to the considerable heterogeneity in the components of mood that have been measured and hormonal variations both between females and within studies, a consensus on specific mood changes and the magnitude of these changes is yet to be established. A prospective study of 63 females measured mood

over three menstrual cycles, using the 51-item Symptom Distress Checklist (Gonda et al., 2008). Menstrual cycle phase was determined using calendar-based counting and was then transformed into an idealised 28-day cycle pattern. The researchers reported that over three menstrual cycles, females in the late luteal phase of their cycle scored significantly higher for measures of anxiety, depression, somatisation, interpersonal sensitivity, obsessive-compulsive symptoms and neuroticism, and exhibited poorer perception of self-image than in the follicular phase of their cycle, an effect that was independent of any changes in physical symptoms (Gonda et al., 2008). Similarly, a prospective cohort study that measured mood scores of 15 females over two menstrual cycles reported significantly higher peak scores on negative mood subscales during the late luteal phase compared with the follicular phase. However, no significant differences in positive mood scores were found (Reed et al., 2008). A key strength of this prospective cohort study was that menstrual cycle phases were verified by the collection of venous blood samples and analysis of sex steroid hormones in participants. The researchers also used urinary LH testing to identify the day of ovulation; however, they did not confirm ovulatory status using the serum progesterone measurements that were collected. Another prospective study of 76 females measuring daily mood over six months showed that only two of the twelve measured mood items (sadness and irritability) were associated with menstrual cycle phase (determined by calendar-based counting) (Romans et al., 2012b). Specifically, sadness was reported to be greater in the premenstrual phase compared with the mid-cycle or menses phase, while irritability was reportedly greater in both the premenstrual and menses phases compared with the mid-cycle phase. However, within this study, the researchers noted that for both sadness and irritability, along with the remaining mood items, the influence of perceived stress, physical health and social support on mood were more significant than menstrual cycle phase (Romans et al., 2012b). Complementary to much of the previous research, in a prospective study of 15 females over one menstrual cycle, Symonds et al. (2004) reported that hedonic scores (associated with psychological traits such as elevated mood, enjoyment and optimism) were lower in the late luteal phase compared with the mid-follicular phase, indicating poorer mood during the late luteal phase. Additionally, in a prospective survey measuring symptom changes across the menstrual cycle over a 70-day period in 181 females, Ross et al. (2003) demonstrated that negative affect was greatest in the late luteal and menstrual phases. However, it should be noted that these previous studies used calendar-based counting methods to determine menstrual cycle phase and not objective methods (such as serum or plasma sex steroid hormone measurements). In contrast to most of the research discussed, a prospective cohort study of 13 females over one menstrual cycle showed no difference in Profile of Mood States Questionnaire (POMS-Q) score between the follicular and premenstrual phases of the menstrual cycle (Kikuchi et al., 2010). Serum sex steroid hormones were measured in this study and results showed normal oestrogen and progesterone concentrations in both the follicular and premenstrual phases of the menstrual cycle. Taken together, the above literature suggests negative mood states may be more prevalent during the late luteal phase of the menstrual cycle,

although there is high variability between studies on the types of negative mood that are measured and the extent to which they are affected.

The variability in results from studies investigating mood changes across the menstrual cycle could be attributed to several methodological differences and limitations between studies. Firstly, previous literature shows considerable diversity in the tools used to measure mood (Table 2.1), which has contributed to inconsistencies in the components of mood measured. As a result of this variability in the components of mood measured, the quantification of mood changes across the menstrual cycle is constrained. Furthermore, many of the tools used in the reported studies focus on the measurement of negative mood states (Table 2.1), which may perpetuate negative menstrual cycle stereotypes. If mood is influenced by fluctuating sex steroid hormones, it would be expected that positive mood states are also more prevalent during certain cycle phases. Therefore, future research should measure and report on changes to both positive and negative mood to avoid biasing and stereotyping menstrual cycle research and provide a more accurate overview of the unique cyclical changes that females experience each month.

Another prevalent methodological limitation is the use of calendar-based counting methods as the sole determiner of menstrual cycle phase by most studies (Gonda et al., 2008; Romans et al., 2012b; Ross et al., 2003; Symonds et al., 2004). Calendar-based counting is an indirect method of menstrual cycle verification that uses participants' self-reported day one of menstruation as the first day of the cycle and counts the days thereafter to estimate menstrual cycle phase. The days of the menstrual cycle and phase classifications are based on predefined menstrual cycle phases (discussed in 2.2 Menstrual Cycle Overview) and are not verified against individual sex steroid hormone levels. Importantly, research has reported that this method is not an accurate predictor of menstrual cycle phase, as it does not account for the high level of inter-individual variability in sex steroid hormones seen throughout the literature (Schaumberg et al., 2017; Wideman et al., 2013). Furthermore, calendar-based counting cannot detect variations in menstrual cycle presentations or SOD (such as anovulation and luteal phase defects), which may occur within menstrual cycles of normal length (21-35 days) (Prior et al., 1990a). While indirect methods of menstrual cycle phase verification provide a convenient and affordable option, research has reported that a combined approach, including menstrual mapping, urinary LH testing and venous blood sampling of sex steroid hormones, is required for researchers to accurately verify menstrual cycle phase and individual sex steroid hormone profiles (Schaumberg et al., 2017). Therefore, future research should consider the use of validated tools to measure mood across menstrual cycle phases and ensure that both menstrual cycle phase and menstrual cycle status are accurately verified by measuring sex steroid hormone levels in order to understand and quantify the effect of variations in sex steroid hormones on mood in healthy females.

Finally, there are inconsistencies in the definitions of menstrual cycle phases, the number of phases within a menstrual cycle that data is collected in and the number of menstrual cycles data is collected (Table 2.1). Recommendations for female health research suggest that researchers should measure across four menstrual cycle phases (early follicular phase, late follicular phase, ovulatory phase and mid-luteal phase) to enable comparisons of the four distinct hormonal phases of the menstrual cycle (Elliott-Sale et al., 2021). However, all of the studies included in the present review compare data between two (Kikuchi et al., 2010; Reed et al., 2008; Symonds et al., 2004) or three (Gonda et al., 2008; Romans et al., 2012b; Ross et al., 2003) menstrual cycle phases. A notable trend within these studies was the focus on comparing the early-to-mid-follicular phase with the late luteal phase. However, few studies compare mood between high sex steroid hormone phases (late follicular and mid-luteal phases) and low sex steroid hormone phases (late luteal and early follicular phases). If variations in sex steroid hormone concentrations do influence mood states in healthy females, it is logical to compare both high and low hormone phases, as these may correspond to the most significant differences in mood changes. Furthermore, the length of the included studies varies considerably, with data collection ranging from across one menstrual cycle to across six cycles. Given that a female may experience between-cycle variability in characteristics such as menstrual cycle length (Chiazze et al., 1968; Creinin et al., 2004), menstrual cycle phase length (Fehring et al., 2006), sex steroid hormone concentrations (Schliep et al., 2014) and ovulation (such as anovulation and luteal phase defects) (Crawford et al., 2017; Schliep et al., 2014), data collection over a greater number of menstrual cycles may provide a more accurate overview of differences in mood across phases of the menstrual cycle, trends that can occur between cycles and trends that may occur with different menstrual cycle presentations. The methodological issues discussed highlight the need for high-quality, long-term studies that compare mood in each of the four hormonal phases of the menstrual cycle, using standardised definitions of these phases that are verified through measurement of sex steroid hormones.

Although few mechanisms have been proposed to explain mood fluctuations throughout the menstrual cycle, some researchers have suggested that the effect of variations in sex steroid hormones on neurotransmitter levels may be a contributing mechanism (Kale et al., 2025). Previous research has reported that progesterone has the ability to increase dopamine secretion (Dluzen & Ramirez, 1991), while oestrogen may upregulate the expression of the rate-limiting enzyme in cerebral serotonin synthesis, tryptophan hydroxylase, thereby increasing cerebral serotonin production (Hiroi & Handa, 2013). Oestrogen has also been shown to exert modulatory effects on tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis (Maharjan et al., 2005). Although depletion of serotonin and dopamine has been a longstanding proposed mechanism for clinical mood disorders such as depression (Hirschfeld, 2000), it should be noted that a meta-analysis showed that depletion of serotonin and dopamine did not influence mood in healthy participants (Ruhé et al., 2007). Furthermore, studies in healthy females have not shown significant differences in serotonin (Kikuchi et al., 2010; Rapkin et al.,

1987) or dopamine (Nordström et al., 1998) between the follicular and luteal phases of the menstrual cycle. Interestingly, Kikuchi et al. (2010) reported that females with lower oestrogen in the premenstrual phase presented with lower serotonin, and that these participants showed increased tension-anxiety and fatigue during this phase of the menstrual cycle. This indicates there may be an association between low oestrogen, low serotonin, and mood; however, the natural fluctuations in oestrogen throughout the menstrual cycle may not be significant enough to influence serotonin concentrations.

2.3.2 Perceived Stress

Perceived stress is the degree to which an individual appraises situations they experience as stressful (Cohen et al., 1983). As with mood, stress has been hypothesised to fluctuate throughout the menstrual cycle and therefore, could be influenced by variations in sex steroid hormones. However, there is no consensus regarding how perceived stress may be influenced within the menstrual cycle or between females. A prospective cohort study of 397 females used the perceived stress scale (PSS) to compare perceived stress between two menstrual cycle phases: (1) the combined menstrual and late luteal phase and (2) the late follicular phase (Jain et al., 2023). Cycle phases were determined using the calendar-based counting method. Results showed that ratings of perceived stress were significantly higher in the late luteal and menstrual phase compared with the late follicular phase. These results were somewhat contradictory to those of a prospective cohort study of 104 females from Brown and Lewis (1993), who used the Hassles and Uplifts Scale to measure perceived stress in the premenstrual phase compared with the postmenstrual phase (determined by calendar-based counting) over one menstrual cycle. The researchers reported that perceived stress during the premenstrual phase was only higher than the postmenstrual phase for participants who experienced high levels of premenstrual symptoms such as pain, breast tenderness and food cravings. Conversely, those with low premenstrual symptomology showed no significant differences in perceived stress between phases. Consistent with the results of Brown and Lewis (1993) in females without high premenstrual symptomology, a cross-sectional study of 42 females that compared PSS scores between females who were self-reported to be in the follicular (postmenstrual) phase of the menstrual cycle (n = 24) with females who were self-reported to be in the luteal (premenstrual) phase of the menstrual cycle (n = 18) reported no significant differences in perceived stress between phases (Montero-López et al., 2018). However, this study is significantly limited by its cross-sectional study design, the fact that menstrual cycle phase was self-reported and the lack of sex steroid hormone measurement that would appropriately verify menstrual cycle phase. These limitations mean that it is unknown whether participants' sex steroid hormone concentrations replicated the typical profiles expected during the premenstrual and postmenstrual phases, and therefore, may not meaningfully reflect the effect of sex steroid hormones on perceived stress. An additional issue with self-reporting menstrual cycle phase is that it may increase the risk of menstrual cycle stereotyping among participants (such as implicitly reporting greater stress in the premenstrual phase due to pre-

existing beliefs that stress is greater in this phase), particularly given that participants were not blinded to the purpose of the study. Informing participants of the phase of their cycle during data collection may be a factor to consider in research study designs, as previous research has demonstrated that when participants were inaccurately told they were in the premenstrual phase of their cycle, they reported more symptoms than those who were not (Klebanov & Jemmott, 1992; Ruble, 1977). This indicates that prior expectations or beliefs regarding the menstrual cycle can influence reported symptoms.

It is evident that the literature investigating the variations in perceived stress across the menstrual cycle is limited in both quantity and quality, which may explain the contradictory results that have been reported. In addition to the methodological shortcomings already highlighted, all studies in the present review used calendar-based counting methods to determine menstrual cycle phase, which as discussed in section 2.3.1, cannot accurately predict menstrual cycle phase or reflect the high level of inter-individual variability in sex steroid hormones that have been suggested as individual factors that could affect stress (Schaumberg et al., 2017; Wideman et al., 2013). Furthermore, to the best of the author's knowledge, all studies to date have collected data across one menstrual cycle only and compared perceived stress between two menstrual cycle phases, instead of reporting changes across all distinct hormonal phases within the menstrual cycle. To gain consensus on the relationship between perceived stress and menstrual cycle phase, further research that addresses the multitude of methodological issues is needed. This can be achieved through studies that use the PSS to prospectively assess perceived stress throughout the menstrual cycle, over more than one cycle, whilst using methods that enable menstrual cycle phase and sex steroid hormone concentrations to be verified.

While research that investigates perceived stress using subjective, validated scales is limited, studies have also measured variations in cortisol throughout the menstrual cycle (Hamidovic et al., 2020). Cortisol is released from the adrenal glands during a stress response (Tsigos & Chrousos, 2002) and, consequently, has been used as a biomarker for stress in research (Hellhammer et al., 2009). A recent meta-analysis of 35 studies comparing cortisol at any point within the follicular and luteal phases of the menstrual cycle reported higher cortisol levels in females during the follicular phase of the menstrual cycle (Hamidovic et al., 2020). Studies were only included in the meta-analysis if they had healthy, premenopausal, human female participants; used a valid form of menstrual cycle phase verification, such as urinary LH testing, basal body temperature or sex steroid hormone measurement; and compared cortisol between at least two menstrual cycle phases to help control for the methodological constraints throughout much of the research in this area. However, the researchers reported that their study was potentially underpowered to detect the diurnal effects of cortisol, noting that the effect of morning menstrual phase cortisol was significant, but afternoon menstrual phase cortisol was not. The results of this meta-analysis suggest that cortisol may be influenced by sex steroid hormone variations throughout the menstrual cycle. However, the results from this review do not support the hypothesis that cortisol

modulates the potential relationship between higher perceived stress in the late luteal phase of the menstrual cycle.

2.4 The Menstrual Cycle: A Continuum

The previous sections of this literature review have discussed the changes in mood and perceived stress that may occur within a typically defined eumenorrheic menstrual cycle. However, research has demonstrated that menstrual cycle physiology is a continuum, of which the first disturbances to occur are SOD (Bedford et al., 2010; Prior et al., 1990a). As a result of SOD occurring first along the continuum of menstrual cycle disturbances, their estimated prevalence may be high, although there is considerable variability in the reported prevalence among published studies. A meta-analysis of 436 healthy females from six studies reported that the prevalence of SOD ranged from 13% to 82% over a one to four year period (Li et al., 2013). A recent prospective cohort study showed a 55% prevalence of at least one short luteal phase length (less than 10 days) and a 17% prevalence of at least one anovulatory cycle over a one year period in a cohort of 53 healthy females (694 cycles) who were pre-screened to have two normal-length (21 to 36 days) and normal-ovulatory (luteal phase more than 10 days) cycles prior to enrollment (Henry et al., 2024). These studies suggest that it may be common for healthy females to show different menstrual cycle presentations between cycles. As most of the research investigating mood and perceived stress throughout the menstrual cycle did not verify participant serum sex steroid hormone levels, it is possible that, given the high estimated prevalence of SOD, some of the data collected in these studies were in participants with anovulatory or luteal phase-deficient cycles. To date, there is limited research that has studied variations in mood and perceived stress between different menstrual cycle presentations. However, as sex steroid hormones are proposed to influence mood and stress, different menstrual cycle presentations, such as SOD, may also result in different mood and stress levels between menstrual cycles.

Subclinical ovulatory disturbances are defined as anovulation or luteal phase defects that occur within normal-length menstrual cycles and result in a menstrual cycle with lower levels of progesterone (Prior et al., 1990a). A cycle is considered anovulatory when there is an absence of ovulation, meaning an ovum is not released from an ovary. Clinically, an indication of anovulation is the failure of naturally occurring metabolites of oestrogen (estrone conjugates) and LH to rise mid-cycle (Santoro et al., 2003). Luteal phase defects describe menstrual cycles that present with either short or inadequate luteal phases within an ovulatory cycle. A short luteal phase is when the corpus luteum is present for less than ten days, and as a result, progesterone levels in the second half of the menstrual cycle are only elevated for ten days or less (Practice Committees of the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility, 2021). An inadequate luteal phase occurs when there are low progesterone levels in the second half of the menstrual cycle. Clinically, this may be

defined as mid-luteal venous blood progesterone levels of less than 9.54 nmol/L (Prior et al., 2015). Importantly, subclinical ovulatory disturbed cycles (SOD cycles) are difficult to detect, as they can occur without changes to overall cycle length (Bedford et al., 2010; Henry et al., 2024; Prior et al., 2022; Prior et al., 1990a), which is a key feature females are told to monitor in order to detect menstrual cycle disturbances.

2.5 Psychological Factors Affecting the Menstrual Cycle

Previous research has reported associations between psychological stressors and SOD cycles. The next section of the present literature review will discuss the relationship between two forms of psychological stress, perceived stress and CDR, and sex steroid hormones concentrations in the context of the menstrual cycle physiological continuum.

2.5.1 Perceived Stress

It is generally recognised that psychological stress can have widespread and significant impacts on human health (Adam et al., 2017; Barry et al., 2020). Various physiological systems have been reported to be impacted by psychological stress, including the immune system (Segerstrom & Miller, 2004), cardiovascular system (Liu et al., 2017; Richardson et al., 2012), gastrointestinal system (Diao et al., 2023) and the female reproductive system (Nepomnaschy et al., 2004; Prior et al., 2022; Schliep et al., 2015). With regard to the interaction between menstrual cycle presentation and psychological stress, a direct link is yet to be established. However, psychological stress has been hypothesised to downregulate the hypothalamic-pituitary-ovarian axis by the hypothalamic-pituitary-adrenal axis, a key component of the human stress system (Ferin, 1999). Within the hypothalamic-pituitary-adrenal axis, corticotropin-releasing hormone is the primary regulator of the stress response and is secreted from the hypothalamus (Chrousos & Gold, 1992). During the stress response, the increase in corticotropin-releasing hormone stimulates the secretion of adrenocorticotropic hormone from the pituitary gland, which will subsequently increase the release of cortisol from the adrenal cortex (Tsigos & Chrousos, 2002). This response may be further stimulated by arginine-vasopressin (Chrousos & Gold, 1992). The activation of the hypothalamic-pituitary-adrenal axis and subsequent rise in corticotropin-releasing hormone during the stress response may inhibit gonadotrophin-releasing hormone production (Chen et al., 1992). A reduction in gonadotrophin-releasing hormone can impair LH and FSH production and pulsatility (Berga et al., 1989), leading to reduced recruitment of follicles in the ovaries, impaired production of oestrogen from a developing dominant follicle, and therefore, reduced likelihood of ovulation (Pohl et al., 1983).

The influence of psychological stress on the menstrual cycle has been investigated in both animal models and human research. An experimental trial in adult female cynomolgus monkeys investigated

the individual and combined effects of metabolic and psychosocial stressors on menstrual cycle abnormalities (defined as cycles greater than 44 days in length or that were anovulatory) (Williams et al., 2007). Following two control cycles, 27 monkeys with normal menstrual cycles were randomised into one of three groups for a further two experimental cycles: (1) moving into a different cage next to unknown monkeys (a mild psychosocial stressor), (2) inducing moderate energy imbalance through dietary restriction and exercise or (3) moving into a different cage plus dietary restriction and exercise. In the first group, only one of the eight monkeys displayed menstrual cycle abnormalities during the second experimental cycle. Similarly, one of the nine monkeys in the second group displayed menstrual cycle abnormalities during both experimental cycles. These were significantly less than the menstrual cycle abnormalities reported in the third group (exposed to the combination of psychosocial and metabolic stressors), where seven of the ten monkeys displayed menstrual cycle abnormalities during either of the experimental cycles, including one who displayed abnormalities in both experimental cycles. The researchers noted that the menstrual cycle abnormalities in the monkeys subjected to the combination of psychosocial and metabolic stressors were predominantly characterised by increased cycle length and follicular phase length, although shortening of luteal phase length was also observed (Williams et al., 2007). These results suggest that a combination of stressors may act synergistically to impair menstrual cycle function in female cynomolgus monkeys.

While the stressors implemented in the study by Williams et al. (2007) may not fully encapsulate the types of stress, particularly psychosocial, that human females experience, studies in human females have reported associations between high psychological stress and impairment of sex steroid hormones. A prospective cohort study of 259 healthy females aged 18-44 years used both daily diaries and the PSS (14-item at baseline and 4-item at four clinic visits) to evaluate the association between perceived stress and reproductive function across two menstrual cycles (Schliep et al., 2015). Reproductive function was determined by serum sex steroid hormone concentrations, which were measured at up to eight visits during each menstrual cycle (e.g., menstruation, mid-follicular phase, late follicular phase, LH and FSH surge, expected ovulation, early luteal phase, mid-luteal phase and late luteal phase). Females with higher daily stress (measured using daily diaries) showed higher FSH and lower oestradiol, free oestradiol, LH and luteal phase progesterone compared with females reporting lower daily stress. However, there were no significant associations between baseline PSS scores (4-item or 14-item) and sex steroid hormones (Schliep et al., 2015). Complementary to these findings, a one-year prospective cohort study of 24 females aged 18-39 years reported that high urinary cortisol (a commonly used biomarker of stress) was associated with lower pregnanediol glucuronide (a urinary metabolite of progesterone) in the mid-luteal phase (Nepomnaschy et al., 2004). While further research is needed, these studies suggest that high stress may result in disruptions to the menstrual cycle and subsequently, changes in sex steroid hormone concentrations throughout the cycle.

It is worth noting that the evidence examining the relationship between stress and ovulation, menstrual cycle length and menstrual cycle phase length is conflicting. The study by Schliep et al. (2015) showed that high versus low daily stress was associated with higher odds of anovulation. Specifically, for each unit increase in daily stress level, the odds of having an anovulatory episode increased by 70%. However, there was no significant association between perceived stress and anovulation using the 4-item or 14-item PSS. In alignment with the result of daily stress increasing the odds of anovulation, Prior et al. (2022) compared a cohort of 301 menstruating females aged 19-35 years (control group) with a cohort of 112 menstruating females of the same age group during the 2019 coronavirus pandemic, to understand the influence of pandemic-related stress on ovulation. Over one menstrual cycle, this preliminary analysis showed that the pandemic cohort displayed an increase in negative mood (e.g., anxiety, depression, frustration) and outside stressors compared with the control cohort. Importantly, while both groups had similar menstrual cycle and bleeding lengths, the pandemic group had significantly more anovulatory cycles, indicating multidimensional stresses (such as those related to the pandemic) may influence SOD without changing overall cycle length (Prior et al., 2022). Another study during the pandemic retrospectively measured perceived stress within the first 6-12 months of the coronavirus pandemic (August 2020 to February 2021), using an online survey (Garcia de Leon et al., 2023). The survey asked 1,866 premenopausal females, who were reported to be naturally menstruating, if they had experienced any changes to their menstrual cycle since the beginning of the 2019 coronavirus pandemic or the middle of March 2020. Those who reported changes to their menstrual cycle were then prompted to answer further questions relating to the specific changes they had experienced. The results showed that females with higher levels of perceived stress, as measured by the Coronavirus Health Impact Survey, were more likely to report menstrual cycle disturbances (such as changes in menstrual symptoms, increased menstruation length and fewer or more menstrual bleeds) since the start of the pandemic, suggesting that females experiencing higher stress during the pandemic may have had an increased risk of menstrual cycle irregularities (Garcia de Leon et al., 2023). However, asking participants retrospectively if they experienced menstrual cycle changes during the pandemic is likely a source of both recall and confirmation bias. Furthermore, rather than analysing the effect of perceived stress on changes to individual menstrual cycle characteristics, as done by other researchers investigating this relationship (Fenster et al., 1999; Nagma et al., 2015; Nepomnaschy et al., 2004; Prior et al., 2022; Schliep et al., 2015), all menstrual cycle changes were grouped together for a single analysis, which may overestimate the potential influence of stress on the menstrual cycle.

Interestingly and in contrast to these studies, a prospective cohort study reported no association between workplace psychological stress and ovulatory function in 276 healthy premenopausal females aged 18-39 years (Fenster et al., 1999). Furthermore, job stress was not reported to be correlated with cycle variability, a short luteal phase (equal to or fewer than ten days in length), a long follicular phase (equal to or greater than 24 days in length), a long menses (equal to or greater than eight days in length) or a

long cycle length (equal to or greater than 36 days in length). However, within this study, it was noted that females working highly stressful jobs had a two-fold increase in their risk of having a short cycle length (equal to or fewer than 24 days in length) compared with females who experienced lower job stress (Fenster et al., 1999). Although this study had participants complete daily menstrual diaries and urine samples (to measure oestrogen and progesterone metabolites) for an average of five menstrual cycles, this study was limited by the measurement of workplace psychological stress (using the Job Content Questionnaire) at baseline only. Without repeated measures across the period of data collection, females who experienced changes in workplace stress throughout the study may have been misclassified in the subsequent analysis. Similarly, the study by Schliep et al. (2015) that was previously mentioned also reported no relationship between baseline PSS score and menstrual cycle function, despite finding sex steroid hormone and ovulatory impairment in healthy females reporting higher levels of daily stress, indicating that perceived stress can vary within an individual. The single baseline measurements of stress that were used in these studies may not be representative of changes in perceived stress over time, which may have been a contributing factor to the lack of association between baseline perceived stress and menstrual cycle variations reported in the two studies. Furthermore, in a cross-sectional study of 100 female university students aged 19-23 years, limited associations between perceived stress and menstrual cycle function were reported (Nagma et al., 2015). Participants in this study completed the PSS, a menstrual cycle history questionnaire and recorded their menstrual blood loss and bleeding length for one menstrual cycle. Females with high perceived stress (PSS greater than 20) were more likely to report irregular menstrual cycles (defined as acyclical bleeding at irregular intervals) than females with low perceived stress (PSS less than 20). However, unlike the study by Fenster et al. (1999), there was no relationship between perceived stress and short-length cycles (less than 21 days). Furthermore, long cycle lengths (greater than 35 days), hypomenorrhea (light menstrual bleeding), menorrhagia (heavy menstrual bleeding) and dysmenorrhea (painful menstrual periods) showed no relationship to perceived stress (Nagma et al., 2015). This study was limited in that the cross-sectional design does not capture inter-cycle variability within females. Furthermore, the length of menstrual cycle phases, presence of ovulation and sex steroid hormone concentrations were not measured; therefore, it is unclear whether SOD were present in this population. As a result, the findings may not encapsulate how stress might affect the menstrual cycle, and specifically, the effect stress may have on SOD cycles in healthy females remains a gap in the literature.

Much like the research regarding variations in psychological factors throughout the menstrual cycle, methodological limitations within the current body of literature preclude a causal relationship between perceived stress and the menstrual cycle continuum from being established. Further research that implements a prospective design to measure perceived stress over more than one menstrual cycle is needed. Future research should also use standardised, validated scales to measure perceived stress and menstrual cycle characteristics, with urinary LH testing and venous blood sex steroid hormone

measurement to identify SOD cycles. It is important to recognise and acknowledge that females often experience several psychological and psychosocial stressors concurrently throughout their lives, which may contribute to increased perceived stress. These stressors can be acute or chronic and can result from a variety of sources, including competing work and family responsibilities (Gilbert-Ouimet et al., 2020) and body image pressures (such as a high drive for thinness) (Gibbs et al., 2011). One form of chronic stress experienced by some females is CDR, which will be discussed in the next section of this literature review.

2.5.2 Cognitive Dietary Restraint (CDR)

Cognitive dietary restraint refers to an intentional cognitive effort to restrict food intake, in an attempt to control body weight (Herman & Mack, 1975; Stunkard & Messick, 1985). Largely independent from dieting, CDR is not necessarily correlated with physical behaviours, such as reduced energy intake. Instead, it is a psychological concept, where those who score highly on restraint scales tend to have a heightened preoccupation with food or body weight (Lowe & Kleifield, 1988).

Cognitive dietary restraint has been hypothesised to influence the menstrual cycle, with previous research having shown an association between high CDR scores and menstrual cycle disturbances. This research suggests that CDR predominantly impairs the luteal and ovulatory phases of the menstrual cycle, as females with high CDR scores have consistently displayed shorter luteal phases and a higher prevalence of anovulation than females with low CDR scores, despite having similar menstrual cycle lengths and energy intakes (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994; Bedford et al., 2010). This was demonstrated by Barr, Janelle, et al. (1994), who prospectively assessed ovulatory function using the least-squares method of basal body temperature analysis in 45 normally menstruating females aged 20-40 years. Participants were classified as high (baseline Three Factor Eating Questionnaire (TFEQ) score equal to or greater than 13) or low (baseline TFEQ score below the sample median of 7) CDR for the analysis. The study reported that, over six menstrual cycles, females with higher baseline CDR scores had more anovulatory cycles, shorter luteal phase lengths and lower luteal phase indexes (luteal phase length/cycle length), while total cycle length and energy intake were not significantly different between females with different CDR scores (Barr, Janelle, et al., 1994). Another prospective study of 27 ovulatory females who had a mean age of 41 years reported similar results (Barr, Prior, et al., 1994). Over a period of three menstrual cycles, females who were in the highest tertile of CDR score on the TFEQ had shorter luteal phase lengths (measured using least-squares quantitative temperature analysis) than females in the lowest tertile of CDR score. As with the study by Barr, Janelle, et al. (1994), no differences in total cycle length or energy intake were reported (Barr, Prior, et al., 1994). The results of both of these studies have been supported by a two-year longitudinal study in 123 females aged 19-35 years (Bedford et al., 2010). Participants' ovulatory function was assessed using daily basal

body temperature records (least-squares quantitative temperature analysis) and they were grouped for the analysis based on their CDR score (high or low on the TFEQ), which was measured at baseline and during two follow-up visits (at 6-12 months and 18-30 months). As with the previous studies, there was no association between CDR score and total cycle length or energy intake but the percentage of SOD cycles (anovulation or short luteal phase length) was 56% in females with higher CDR, compared with 34% in females with lower CDR over the two-year period (Bedford et al., 2010). In contrast to these studies, a two-year prospective cohort study among 225 females aged 21-40 years showed no association between CDR score (measured on the TFEQ) and SOD cycles (measured by salivary progesterone measurements and urinary LH tests) (Waugh et al., 2007). The difference in results to Barr, Janelle, et al. (1994), Barr, Prior, et al. (1994) and Bedford et al. (2010) could be attributed to differences in the study population. The researchers of this study grouped participants into tertiles of CDR score to analyse the proportion of females with three or more SOD cycles. As there was a low proportion of females (~7%) who had three or more SOD cycles, differences in the groups may have been difficult to detect. Furthermore, participants had body mass index (BMI) values that ranged from 16-39 kg/m^2, compared with 18-25 kg/m^2 (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994) and 18-30 kg/m^2 (Bedford et al., 2010) in previous studies, and BMI was shown to be positively associated with CDR score. Therefore, BMI may have been a confounding factor in these results.

It should be noted that CDR (a psychological stress relating to food), but not total energy intake, was found to be associated with a higher prevalence of SOD cycles in these previous studies (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994; Bedford et al., 2010). Despite these previous results, severe or prolonged restriction of total energy intake has been reported as a contributing factor to problematic low energy availability (Mountjoy et al., 2023), which has been reported as a causal factor in the development of severe menstrual cycle disturbances, such as amenorrhea and oligomenorrhea (Reed et al., 2015). Furthermore, increased energy intake over a twelve-month period has been reported to improve menstrual function in females with amenorrhea and oligomenorrhea (De Souza et al., 2021). It may, therefore, be proposed that in the absence of severe energy intake restriction, the presence of high psychological stress relating to food intake (CDR) may result in subtle, subclinical menstrual cycle disturbances. However, for more severe changes in the menstrual cycle (such as amenorrhea and oligomenorrhea) to occur, energy restriction may need to occur simultaneously with CDR. To the author's knowledge, there has been only one study to date that has reported a difference in energy intake between restrained and unrestrained eaters in the context of menstrual cycle disturbances. This was a prospective cohort study of 22 females, which reported that females with high CDR (above the 75^{th} percentile on the TFEQ) had lower daily energy intakes (by approximately 500 calories) and shorter luteal phase lengths and menstrual cycle durations over one menstrual cycle than females with low CDR (below 25^{th} percentile on the TFEQ) (Schweiger et al., 1992). Although these results are indicative of SOD rather than clinical menstrual cycle impairment, it is worth noting that the study did not measure

energy availability (the amount of energy available for physiological processes after accounting for energy expended during exercise (Mountjoy et al., 2023)), which, if low and prolonged, is considered a causal factor in the development of clinical menstrual cycle disturbances (Williams et al., 2001). Previous research has suggested that energy availabilities below 30 kcal/kg FFM/day are associated with disruption of luteinising hormone pulsatility (Loucks & Thuma, 2003). Other studies have reported that there is not a threshold of energy availability that induces menstrual cycle disturbances, but that the prevalence (not severity) increases as energy availability decreases (Lieberman et al., 2018; Williams et al., 2015). One possible reason for the presence of SOD cycles rather than clinical menstrual cycle disturbances in participants with lower energy intakes in the study by Schweiger et al. (1992) is that these participants may have had sufficient energy availability despite having lower daily energy intakes. Another consideration is that the study was only conducted over one menstrual cycle, and therefore, did not capture any inter-cycle variability that may have occurred within participants. Furthermore, it may take months to years of persistent low energy availability for clinical menstrual cycle disturbances to occur (Williams et al., 2001), and with just one cycle of data collected, it is not possible to determine if the high CDR group had consistently lower energy intake. Future research should measure both CDR and energy availability over more than one menstrual cycle to understand if a combination of high CDR and severe energy restriction may contribute to clinical menstrual cycle disturbances.

There have been studies that have reported a higher prevalence of clinical menstrual cycle disturbances in females with high CDR; however, they did not measure energy intake among participants. A prospective cohort study of 84 premenopausal females aged 18-35 years compared self-reported menstrual status (amenorrhoeic, oligomenorrheic or eumenorrheic), corroborated by daily urinary estrone-3-glucuronide (metabolite of oestrogen) and pregnanediol-3-glucuronide (metabolite of progesterone), between females with high (TFEQ score equal to or greater than nine) versus normal (TFEQ score less than nine) CDR and across quartiles of CDR scores on the TFEQ (Vescovi et al., 2008). Compared with normal CDR, high CDR was associated with a greater prevalence of oligomenorrhoea. The prevalence of oligomenorrhoea also increased across the CDR quartiles. In alignment with Vescovi et al. (2008), in a cross-sectional study of 596 female university students, McLean and Barr (2003) reported a higher prevalence of self-reported menstrual cycle irregularity in females with high CDR (scores of 13 to 21 on the TFEQ) (n = 145) than females with low dietary restraint (scores of 0 to 5 on the TFEQ) (n = 189). However, no differences in self-reported menstrual cycle length were observed, and importantly, self-reported menstrual cycle irregularity was assessed retrospectively at a single time point, rather than prospectively over multiple full menstrual cycles. Furthermore, it appears there was no predefined criterion for menstrual cycle irregularity, leaving it up to the participants' interpretation. These two factors mean the study results may have been influenced by recall bias among participants.

With regard to the mechanism by which CDR may contribute to subtle, subclinical menstrual cycle disturbances, research investigating this is currently limited. Previous studies have reported that females with high CDR have higher levels of cortisol (Anderson et al., 2002; McLean et al., 2001; Rutters et al., 2009), which may impair hypothalamic-pituitary-ovarian axis functioning. However, the average and median cortisol concentrations that were reported in the high CDR groups in these three studies were all within the normal ranges for healthy females, raising the question of whether the increased cortisol would be sufficient to induce SOD. Furthermore, the study by Bedford et al. (2010), which reported a higher prevalence of SOD cycles in females with high CDR, reported that 24 hr urinary free cortisol was positively associated with high CDR scores but not with the prevalence of SOD cycles. This suggests cortisol may not directly contribute to SOD cycles, and that alternative factors may play a role in downregulating the hypothalamic-pituitary-ovarian axis when the hypothalamic-pituitary-adrenal axis is upregulated. Investigating these potential mechanisms may be a consideration for future research. Furthermore, given that previous research suggests females with different sex steroid hormone presentations can display different levels of CDR, future research could investigate how CDR may change within females with variations in sex steroid hormones throughout the menstrual cycle. To the author's knowledge, no studies to date have explored this relationship.

2.6 Methods used to Determine Mood, Perceived Stress and CDR

Within the previous literature that has been discussed throughout this review, a variety of different tools have been used to measure mood, perceived stress and CDR in study populations. Of the studies measuring variations in mood throughout the menstrual cycle, eleven different instruments were used (Table 2.1). As shown in Table 2.1, there is considerable diversity in both the components of mood assessed in each tool and the timeframe it was assessed across, with some tools measuring transient mood states and others measuring mood in the past week or month. Furthermore, of the surveys and tools used, many had different scoring systems and one study used a non-validated tool (Romans et al., 2012b). The lack of standardisation in tools used to measure mood variations across the menstrual cycle between various studies precludes any comparison between their outcomes, thereby limiting our understanding of this relationship. Further studies using standardised and validated methods of mood measurement are required to understand whether mood is influenced by sex steroid hormones throughout the menstrual cycle, or whether the associations identified to date are due to differences in the tools used to collect mood data throughout the menstrual cycle.

Compared with mood, there is considerably less diversity in tools used to measure perceived stress, both in studies measuring variations in perceived stress across the menstrual cycle (Table 2.1) and studies measuring the influence of perceived stress on menstrual cycle functioning (Table 2.2). The most commonly used measure of perceived stress was the PSS, which has been validated in previous

research (Andreou et al., 2011; Cohen, 1988; Cohen et al., 1983; Lesage et al., 2012). Previous studies have recommended the use of the 10-item PSS in research, as it has demonstrated the strongest internal reliability (Cohen, 1988) and discriminative sensitivity (Lesage et al., 2012) of the three PSS versions. However, other studies have reported minimal differences between the 10-item and 14-item scales, with researchers concluding both are suitable for use in research and healthcare (Andreou et al., 2011). It is generally agreed that the 4-item version is the least acceptable scale (Andreou et al., 2011; Cohen, 1988; Lesage et al., 2012); however, it has been proposed as an appropriate measure in situations that require a shorter instrument, such as to reduce time or respondent burden (Cohen, 1988). Within the studies that used the PSS, there is variability in the version of the scale used. As discussed with mood, this limits the comparison of results between studies, as the differences in scoring systems typically prevent review studies from performing meta-analyses. As with mood, the use of standardised methods and tools to measure perceived stress in menstrual cycle research would improve our understanding of the extent to which perceived stress is influenced by sex steroid hormones throughout the menstrual cycle and how perceived stress may influence sex steroid hormones and menstrual cycle disturbances.

Unlike tools used to measure mood and perceived stress, studies measuring the influence of CDR on sex steroid hormones and menstrual cycle disturbances are consistent in the tool used. The dietary restraint subscale from the validated TFEQ (TFEQ-R) (Stunkard & Messick, 1985) was used across all studies included in the present review, with two studies using an additional measure of dietary restraint: the Dutch Eating Behaviour Questionnaire (Schweiger et al., 1992) and the Restraint Scale (Anderson et al., 2002). While the consistency in the use of the TEFQ-R to measure CDR helps with the comparison of results between studies, the use of different cut-off points to define low versus high CDR among the literature discussed in section 2.5.2 may limit the comparison of their results. Furthermore, the limited number of studies investigating the relationship between CDR and the menstrual cycle and the methodological limitations and differences between studies discussed in section 2.5.2 limits the ability to fully comprehend and explain the relationship that may exist. Further research investigating longitudinal differences in CDR between different menstrual cycle presentations (ovulatory versus SOD) and throughout the menstrual cycle is needed, using sex steroid hormone verification.

Table 2.1. *Tools Used to Measure Variations in Mood and Perceived Stress throughout the Menstrual Cycle.*

	Study	Study Design	Instrument Used	Details of Instrument	Mood States/Stress Assessed
Mood	(Gonda et al., 2008)	Prospective cohort study in Hungary of 63 females with a mean age of 26.7 ± 0.66 years, across three menstrual cycles. Data was collected across three menstrual cycle phases: early follicular (3-4 days after onset of menstruation), late follicular (8-10 days after onset of menstruation) and late luteal (2-3 days before the expected beginning of the next cycle).	State Anxiety Scale of the State Trait Anxiety Inventory	20 items scored 1-4, range of scores 20-80.	Transient feelings of anxiety.
			Symptom Checklist-51	51 items scored 1-3, comprising of 5 subscales. Total score range from 0-153.	Interpersonal sensitivity, depression, anxiety, obsessive compulsive, somatisation.
			Zung Self-Rating Depression Scale	20 items scored 1-4, range of scores 20-80.	Frequency of depressive symptomology (physical and psychological components).
	(Kikuchi et al., 2010)	Prospective cohort study in Japan of 13 females aged 19-30 years across one menstrual cycle.	POMS-Q	30 items scored 0-4, comprising of 10 subscales.	Vigour (positive); tension–anxiety; depression–dejection; anger–hostility; fatigue; and confusion (all negative).

	Study	Study Design	Instrument Used	Details of Instrument	Mood States/Stress Assessed
		Data was collected across two menstrual cycle phases: follicular (days 5-10 of an idealised 28 day cycle) and premenstrual (days 20-25 of an idealised 28 day cycle).			
	(Reed et al., 2008)	Prospective cohort study in the United States of America of 15 females with a mean age of 30 ± 6.1 years, across two menstrual cycles. Data was collected across two menstrual cycle phases: follicular (6-10 days after onset of menstruation) and late luteal (1-5 days before onset of menstruation).	Modified Daily Ratings Form	24 items scored 1-6, range of scores 1-144	Transient mood states (positive and negative).
			Beck Depression Inventory II	21 items scored 0-3, range of scores 0-63.	Aspects of depression.
			State Anxiety Scale of the State Trait Anxiety Inventory	20 items scored 1-4, range of scores 20-80.	Transient feelings of anxiety.
			POMS-Q	72 items scored 0-4, composing of 10 subscales.	Tension–anxiety, depression–dejection, anger–hostility, vigour, fatigue, confusion, friendliness, elation, arousal, positive mood.
	(Romans et al., 2012b)	Prospective cohort study in Canada of 76 females with a mean age of 30.8 ±	Daily Life Questionnaire	Non-validated tool developed for the purpose of the study. Mood portion of the tool includes 4 positive visual analogue	Transient mood states (positive and negative).

	Study	Study Design	Instrument Used	Details of Instrument	Mood States/Stress Assessed
		7.7 years, across four to six menstrual cycles, over six months. Data was collected across three menstrual cycle phases: menses (onset to cessation of menstruation), mid-cycle (all non-menses and premenstrual days) and premenstrual (the 5 days prior to menses).		scale mood items and 4 negative visual analogue scale mood items.	
	(Ross et al., 2003)	Prospective cohort study in Australia of 181 females with a mean age of 29.9 ± 8.6 years, over 70 days. Data was collected across three menstrual cycle phases: follicular (days 7-11 following menstruation),	Modified Moos Menstrual Distress Questionnaire	34 items, scored 0-3, each representing menstrual symptoms, some of which relate to mood.	Negative affect, arousal, control.

	Study	Study Design	Instrument Used	Details of Instrument	Mood States/Stress Assessed
		premenstrual (days 24-28 preceding menstruation) and menstrual (duration of menstruation).			
	(Symonds et al., 2004)	Prospective cohort study of 15 females with a mean age of 20.7 years, over one menstrual cycle. Data was collected across two menstrual cycle phases: follicular (7-9 days following onset of menstruation) and luteal (5-7 days prior to onset of menstruation).	Short Form Beck Depression Inventory	13 items scored 0-3, range of scores 0-39.	Affective and cognitive aspects of depression.
			Altman Self-Rating Mania Scale	5 items scored 0-4, ranges of scores 0-20.	Manic and hypomanic symptoms.
			UWIST Mood Adjective Checklist	48 items/adjectives scored 0-3, ranges of scores 0-144.	Transient arousal, stress, hedonia.
Perceived Stress	(Brown & Lewis, 1993)	Prospective cohort study of 104 females with a mean age of 32.9 ± 5.04 years, over one menstrual cycle. Data was collected across two menstrual cycle	Modified Hassles and Uplifts Scale	140 items (65 hassles, 75 uplifts) scored 0-3, ranges of scores 0-420.	Perceived stress over previous week.

	Study	Study Design	Instrument Used	Details of Instrument	Mood States/Stress Assessed
		phases: premenstrual (4 days prior to onset of menses) and postmenstrual (4-7 days following onset of menstruation).			
	(Jain et al., 2023)	Prospective cohort study in India of 397 females with a mean age of 20.7 ± 1.9 years, over one menstrual cycle. Data was collected across two menstrual cycle phases: combined menstrual and late luteal phase (days 21 until first day of next menstruation) and late follicular phase (days 11-20 of menstrual cycle).	10-item PSS	10 items scored 0-4, ranges of scores 0-40.	Perceived stress.
	(Montero-López et al., 2018)	Cross-sectional study in Spain of 42 females with a mean age of 33.6 ± 7.75	14-item PSS	14 items scored 0-4, range of scores 0-56.	Perceived stress.

	Study	Study Design	Instrument Used	Details of Instrument	Mood States/Stress Assessed
		years, which compared females self-reported to be in the follicular (cycle days 2-8) and luteal (cycle days 18-26) phases of the menstrual cycle.			

Table 2.2. *Tools Used to Measure Perceived Stress in Studies Investigating the Effect of Perceived Stress on the Menstrual Cycle.*

Study	Study Design	Instrument Used	Details of Instrument	Type of Stress Assessed
(Fenster et al., 1999)	Prospective cohort study in the United States of America of 276 females aged 18-39 years, over five menstrual cycles.	Modified Job Content Questionnaire	13 items within three subscales, scored on a 4-point Likert scale.	Workplace stress: job demand (job pace and pressure), job control (skills needed to perform a job and freedom in decision making) and social support at work.
		Participants were directly asked if their job was stressful.	4-point Likert scale.	Perception of job stress.
(Garcia de Leon et al., 2023)	Online retrospective survey in Canada of 1,866 premenopausal females aged 25-69 years.	Coronavirus Health Impact Survey	10 items scored 1-5, range of scores 10-50.	Pandemic-related psychological distress and resilience.
(Nagma et al., 2015)	Cross-sectional study of 100 females with a mean age of 20.9 years.	14-item PSS	14 items scored 0-4, range of scores 0-56.	Perceived stress during the last month.
(Prior et al., 2022)	Comparison of two single-cycle prospective cohort studies.	Menstrual Cycle Diary	No additional details are currently available as the article was published in short form.	Data still to be published by authors.
(Schliep et al., 2015)	Prospective cohort study in the United States of America of 259 females aged 18-44 years, over two menstrual cycles.	Daily Diary	Prospective record of stress levels, recorded daily. Scored [1] not stressful, [2] a little stressful, [3] very stressful.	Daily perceived stress.
		4-item PSS	4 items scored 0-4, range of scores 0-16.	Perceived stress over the previous week.
		14-item PSS	14 items scored 0-4, range of scores 0-56.	Baseline perceived stress.

2.7 Conclusion

The variations in sex steroid hormones that occur within a typically defined eumenorrheic menstrual cycle may be associated with changes in mood and perceived stress throughout the menstrual cycle. Previous research in healthy females suggests that negative mood states and perceived stress may be highest during the late luteal phase of the menstrual cycle (Gonda et al., 2008; Jain et al., 2023; Reed et al., 2008; Ross et al., 2003; Symonds et al., 2004). However, due to considerable heterogeneity in tools used to measure mood and perceived stress, the present understanding of the specific mood changes throughout the menstrual cycle and the magnitude of these changes is still an ongoing area of investigation. Furthermore, the sole use of calendar-based counting methods to determine menstrual cycle phase limits our understanding of phase-specific sex steroid hormone concentrations on mood changes and perceived stress. Therefore, the validity of future menstrual cycle research that is investigating mood changes and perceived stress can be improved by adopting the three-step method of menstrual cycle phase verification. This method combines calendar-based counting of menstrual cycle length with urinary LH testing and direct measurement of venous blood sex steroid hormones to define menstrual cycle phases (Schaumberg et al., 2017).

It is worth noting that research that has investigated variations in mood and perceived stress has focused on changes within an eumenorrheic menstrual cycle. However, it has been proposed that menstrual cycle physiology occurs along a continuum of clinical entities, of which the first disturbances to occur are SOD cycles (Bedford et al., 2010; Prior et al., 1990a). The sex steroid hormone concentrations in the phases of SOD cycles (characterised by low levels of mid-luteal phase progesterone) have also been proposed to result in different psychological symptoms between menstrual cycles and between females.

Previous research has reported a higher prevalence of SOD cycles among females with higher perceived stress and CDR (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994; Bedford et al., 2010; Prior et al., 2022; Schliep et al., 2015). However, few studies have used venous blood measurement of progesterone to verify ovulatory status when investigating associations between perceived stress, CDR, and SOD in healthy menstruating females. Therefore, the extent to which perceived stress and CDR may differ between healthy menstruating females who present with consistent ovulatory or SOD cycles is still an area that requires investigation.

Chapter 3: Manuscript

3.0 Abstract

Background: Previous research suggests that psychological stressors, such as perceived stress and cognitive dietary restraint (CDR), may contribute to subclinical ovulatory disturbances (SOD) in healthy menstruating females. Furthermore, it is proposed that variations in sex steroid hormones throughout the menstrual cycle may influence mood, perceived stress and CDR. This study aimed to determine if females who presented with at least two subclinical ovulatory disturbed cycles (SOD cycles) displayed higher perceived stress and CDR than females who presented with at least two ovulatory cycles over a three-cycle period. A secondary aim was to investigate if there were variations in mood, perceived stress and CDR throughout the menstrual cycle.

Methods: Ninety-seven naturally menstruating females participated in this prospective cohort study over five menstrual cycles. Mood, perceived stress and CDR were measured during the early follicular, late follicular and mid-luteal phases of the menstrual cycle. Urinary luteinising hormone (LH) testing and mid-luteal phase plasma progesterone levels were measured to classify cycles as ovulatory (mid-luteal progesterone > 10 nmol/L) or SOD (mid-luteal progesterone ≤ 10 nmol/L) and to verify menstrual cycle phase. Two-tailed independent samples t-tests were used to compare mean perceived stress and CDR scores between ovulatory and SOD participants. Linear mixed models were used to analyse the effects of menstrual cycle phase, ovulatory status and their interaction on scores of mood, perceived stress and CDR.

Results: The average perceived stress and CDR scores of all females were 24.7 ± 5.5 and 14.0 ± 4.1, respectively. There were no differences in perceived stress ($t = 0.5$, df = 84, $p = 0.606$) or CDR ($t = -1.7$, df = 84, $p = 0.100$) between ovulatory ($n = 68$) and SOD ($n = 18$) groups. Furthermore, linear mixed models showed no individual or interaction effects of menstrual cycle phase and ovulatory status on mood, perceived stress or CDR.

Conclusion: The results showed that healthy menstruating females who presented with at least two SOD cycles did not have higher perceived stress or CDR than females who had at least two ovulatory cycles over a three-cycle period. Furthermore, the results suggest no group-level effects of menstrual cycle phase and ovulatory status on mood, perceived stress or CDR.

3.1 Introduction

Stress is a universal experience that can have a widespread impact on human health (Adam et al., 2017; Barry et al., 2020). Females of reproductive age may experience a variety of stressors, including those of physical, psychological, emotional and social nature (Bonazza et al., 2023; Mountjoy et al., 2023; Poitras et al., 2024). The influence of various forms of stress on the female hypothalamic-pituitary-ovarian axis has been reported, primarily in the context of its contribution to functional hypothalamic amenorrhea (Bonazza et al., 2023; Mountjoy et al., 2023). However, functional hypothalamic reproductive axis suppression may occur within a spectrum of clinical entities, of which subclinical ovulatory disturbances (SOD), inclusive of anovulation and luteal phase defects, are the first physiological adaptations to occur (Bedford et al., 2010; Prior et al., 2015; Prior et al., 1990a).

Anovulation refers to the failure of naturally occurring metabolites of oestrogen (estrone conjugates) and luteinising hormone (LH) to rise mid-cycle, precluding the ovary from releasing an ovum (Santoro et al., 2003). Luteal phase defects describe menstrual cycles that present with either short (luteal phase less than 10 days in length) or inadequate (mid-luteal progesterone less than 9.54 nmol/L) luteal phases (Practice Committees of the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility, 2021; Prior et al., 2015). Although the concentration of oestrogen in subclinical ovulatory disturbed cycles (SOD cycles) is sufficient to maintain normal-length menstrual cycles, progesterone levels are insufficient to preserve pregnancy (Prior et al., 2015; Prior et al., 1990a). With infertility rates reported to be up to 26% among New Zealand females (van Roode et al., 2015), understanding lifestyle factors that may contribute to SOD cycles is a needed area of research.

Previous research has reported that psychological stressors, such as cognitive dietary restraint (CDR) and perceived stress, may contribute to SOD cycles in otherwise healthy females (Bedford et al., 2010; Prior et al., 2022; Schliep et al., 2015). Cognitive dietary restraint is a form of chronic psychological stress that refers to an intentional cognitive effort to restrict food intake (Herman & Mack, 1975; Stunkard & Messick, 1985). In a two-year longitudinal study that measured monthly ovulatory status, a 56% prevalence of SOD cycles in females with high CDR (scores higher than the median) was reported. The prevalence of SOD cycles in females with high CDR was exacerbated when compared with females who had low CDR (34% SOD prevalence), despite no differences in reported energy intake between the groups (Bedford et al., 2010). Similarly, research has also noted that perceived stress, the degree to which an individual appraises situations they experience as stressful (Cohen et al., 1983), may be associated with ovulatory disturbances (Prior et al., 2022; Schliep et al., 2015). In a preliminary publication, it was recently reported that a cohort of females studied during the 2019 coronavirus pandemic had a 63% prevalence of SOD cycles, approximately 50% of which were anovulatory (Prior et al., 2022). The prevalence of these SOD cycles was higher when compared with a cohort of females

who were studied 13 years prior and displayed a 10% prevalence of SOD cycles. Furthermore, females studied during the pandemic showed higher levels of negative mood states and stress, but no differences in menstrual cycle length were observed between the two groups (Prior et al., 2022). Subsequently, these results indicate that multidimensional stressors experienced by females during the coronavirus pandemic may have been associated with ovulatory disturbances within normal-length menstrual cycles. Although this research suggests an association between psychological stressors and SOD cycles, few studies have measured venous blood progesterone during the mid-luteal phase to verify ovulatory status; a method that is important for the identification of luteal phase deficiency (Practice Committees of the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility, 2021). Furthermore, as no studies have been completed in New Zealand, the extent to which perceived stress and CDR may differ between healthy menstruating New Zealand females who present with consistent ovulatory or SOD cycles is still an area that requires investigation.

The occurrence of SOD cycles in healthy menstruating females will be associated with variations in sex steroid hormones throughout the menstrual cycle, particularly in the luteal phase (Schliep et al., 2014; Strott et al., 1970). With previous research reporting that SOD cycles are more common in females experiencing psychological stressors (Bedford et al., 2010; Prior et al., 2022; Schliep et al., 2015), it is worth considering if the reduction in sex steroid hormones experienced during these SOD cycles may also contribute to differences in psychological symptoms, such as mood, perceived stress and CDR, throughout the menstrual cycle. Although there have been inconsistencies in reported results, previous research suggests that females may experience changes to mood and perceived stress throughout the menstrual cycle (Jain et al., 2023; Romans et al., 2012a). As such, results from this previous research have suggested that variations in sex steroid hormone concentrations throughout the menstrual cycle may influence mood and perceived stress. To the best of the author's knowledge, no studies to date have measured CDR throughout the menstrual cycle or considered the ovulatory status (and corresponding sex steroid hormone profiles) of participants when investigating these changes. Therefore, present understanding of whether variations in sex steroid hormones, both in SOD cycles and at different menstrual cycle phases, contribute to differences in mood, perceived stress and CDR is still an area in need of investigation.

The primary aim of the present study was to determine if females who presented with at least two SOD menstrual cycles (anovulation or luteal phase defects) displayed higher perceived stress and CDR than females who presented with at least two ovulatory menstrual cycles over a three-cycle period. A secondary aim was to investigate if there were variations in mood, perceived stress and CDR throughout the menstrual cycle in females with different menstrual cycle presentations (ovulatory versus SOD cycles).

3.2 Methodology

The present study was a prospective cohort design, conducted in naturally menstruating females living in Auckland, New Zealand. Data collection commenced in July 2023 and ended in March 2025.

3.2.1 Participants and Recruitment

A total of 97 naturally menstruating females aged 30.5 ± 5.6 years participated in the present study. Convenience sampling was used for participant recruitment, with the study advertised through social media (researcher accounts and community groups), email, physical flyers distributed across Massey University Albany Campus and word-of-mouth. Participants were first screened for eligibility through an online Qualtrics^{XM} survey, and if eligible, were asked to read the attached information sheet and register for the project. To be eligible, participants had to be aged 18-40 years and living in Auckland, New Zealand. Participants were excluded from the study if they were pregnant or breastfeeding (or had been in the past 12 months); had taken any form of hormonal contraception in the past six months; were current smokers; had been medically diagnosed with inflammatory bowel disease, coeliac disease, gastric ulcers, polycystic ovary syndrome, blood in urine or melena; had a history of reproductive surgery affecting sex steroid hormones or had taken iron supplements in the past two months. The study received ethics approval from the Massey University Southern Human Ethics Committee (SOA 22/56).

3.2.2 Study Procedures and Protocols

Following registration, participants attended their baseline visit at Massey University Albany Campus research facility, where they were provided with a full explanation of the project and written informed consent was obtained. Participants then completed three surveys: a demographic questionnaire that captured age, ethnicity and medical history data; a menstrual cycle history survey that was based on recommendations from Schmalenberger et al. (2021) and the New Zealand Short Form Physical Activity Questionnaire (Craig et al., 2017; Moy et al., 2008). To conclude the session, participants were provided with two cycles' worth of urinary ovulation tests (Baby4You) and provided researchers with an estimated start date of their next menstrual bleed.

On the first day of their next menstrual bleed, participants notified the researchers and commenced menstrual cycle tracking and data collection for five menstrual cycles. Menstrual cycle tracking within the study consisted of daily questionnaires to determine onset of menstrual bleeding. Ovulation testing began on day eight of the individual participant's cycle and continued until either a positive result was displayed or day 21 was reached. For participants with cycle lengths longer than 30 days, ovulation testing began on day 10 and ended when there was a positive result or when day 24 to 25 of their cycle

was reached. The first two cycles were used to determine menstrual cycle length and approximate dates for testing in the next three menstrual cycles.

Testing in the third cycle occurred in the early follicular phase (days two to five of the menstrual cycle) and the mid-luteal phase (either seven days after a positive ovulation test or five days before their next scheduled period). Testing in the fourth cycle occurred in the late follicular phase (one to two days prior to predicted ovulation, based on the timing from the previous cycle's urinary ovulation test) and the mid-luteal phase. Testing in the fifth cycle occurred in the mid-luteal phase only. Blood samples were collected at all five visits. During the early follicular, late follicular and fifth cycle's mid-luteal phases, testing included the completion of three validated questionnaires that measured mood (Profile of Mood States Questionnaire [POMS-Q]), perceived stress (Perceived Stress Scale [PSS]) and CDR (restraint subscale of the Three Factor Eating Questionnaire [TFEQ-R]).

3.2.3 Body Composition Measurements

Body composition measurements were completed at baseline and in the fifth cycle's mid-luteal phase visit (the final visit). Height was measured to the nearest centimetre using a stadiometer. Body composition was determined from a bioelectrical impedance analysis (InBody 230). The data of interest for this project were body mass (kg), fat mass (kg), percentage fat mass (%) and lean muscle mass (kg). Body Mass Index (BMI) was calculated by dividing the participant's body mass (kg) by their height (m) squared.

3.2.4 Venous Blood Sex Steroid Hormone Analysis

Venous blood samples were collected at all five visits (during cycles three, four and five) by a trained phlebotomist from the antecubital vein. For venous blood collection, participants were in a seated and rested position. The blood samples were collected with a 23 G butterfly needle into two 8 ml plasma separator tubes. Samples were inverted slowly eight times immediately following collection. Within 30 minutes of collection, the blood samples were centrifuged at 4° C, 2000 RFC for 10 minutes. The plasma supernatant was removed and divided into 1 ml and 2 ml aliquots that were stored at -80° C until analysis. Batches of 100 plasma samples were periodically sent to Auckland LabPlus for the analysis of oestradiol (pmol/L) and progesterone (nmol/L). The mid-luteal progesterone measurements that were prioritised in cycles three, four and five were used to confirm the ovulatory status of each participant's cycle. A cycle was considered ovulatory if mid-luteal progesterone was greater than 10 nmol/L. A cycle was considered SOD if there was evidence of luteal phase deficiency (mid-luteal progesterone between 3-10 nmol/L) or anovulation (mid-luteal progesterone less than 3 nmol/L) (Prior et al., 2015).

3.2.5 Questionnaires

Perceived Stress Scale (PSS)

The PSS is a 14-item scale designed to measure perceived stress over the past month, which has been validated for use in research with female participants (Andreou et al., 2011; Cohen et al., 1983). The tool consists of seven positive items and seven negative items, which are self-scored on a four-point Likert scale, ranging from 0 (never) to 4 (very often) (see Appendix A). Total scores are obtained by reversing scores on the positive items and then adding the scores of all items. Therefore, a higher score on this scale indicates a higher level of perceived stress. Previous research that has used the 14-item PSS to investigate the relationship between perceived stress and ovulatory status has used the median score for their study population to distinguish between participants with high and low perceived stress, although the researchers did not report the value of the median score (Schliep et al., 2015). Another study used a score of 20 (Nagma et al., 2015) to identify high perceived stress, while other research has classified stress as low, medium or high based on thirds of the 56-point scale (Cohen et al., 1983). The present study analysed PSS scores continuously, with participants grouped by their ovulatory status.

Profile of Mood States Questionnaire (POMS-Q)

The abbreviated POMS-Q is a validated 40-item questionnaire that is designed to measure feelings of tension, anger, fatigue, depression, esteem-related affect, vigour and confusion at a given moment (Grove & Prapavessis, 1992). The questionnaire has been used in previous research investigating the relationship between mood and menstrual cycle phase (Kikuchi et al., 2010; Reed et al., 2008). Each item is self-rated on a five-point Likert scale, ranging from 0 (not at all) to 4 (extremely). Scores are calculated by subtracting positive mood item scores (esteem-related affect and vigour) from negative mood item scores (tension, anger, fatigue, depression and confusion). Consequently, a higher POMS-Q score indicates a more negative mood state, while a lower POMS-Q score is suggestive of a more positive mood state. The present study analysed scores of each mood item continuously, with participants grouped by their ovulatory status.

Three Factor Eating Questionnaire (TFEQ)

The TFEQ is a questionnaire that measures three dimensions of eating behaviour: CDR (factor one, disinhibition (factor two) and hunger (factor three). The original questionnaire consists of 51 self-scored items that each relate to one of the three dimensions of eating behaviour (Stunkard & Messick, 1985), and this has been used in much of the previous research investigating the relationship between CDR and SOD (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994; Bedford et al., 2010; McLean & Barr, 2003; Waugh et al., 2007). The present study used a revised 18-item version of the scale, which has been validated in previous research (Karlsson et al., 2000). The items are primarily measured on a four-point scale, with six items measuring CDR, nine measuring uncontrolled eating and three measuring

emotional eating. However, given that the primary objectives of the present study relate to CDR, only participant scores from the CDR scale (TFEQ-R) will be included in the analysis. A version of the TFEQ-R scale is presented in Appendix A.

Scores on the TFEQ-R scale range from six points to 28 points, with a higher score indicating a higher level of CDR. Previous research has used the median CDR scores of their study population (approximately 7 out of 21 points) as the cut-off point to distinguish between participants with high and low CDR (Barr, Janelle, et al., 1994; Bedford et al., 2010). Other studies have compared the upper tertile (scores of 13 or greater) and lower tertile (scores of 5 or lower) of CDR scores (Barr, Prior, et al., 1994; McLean & Barr, 2003; Waugh et al., 2007). The present study analysed TFEQ-R scores continuously, with participants grouped by their ovulatory status.

3.2.6 Statistical Analysis

Statistical analysis was completed using IBM SPSS Statistics, version 30.0 (IBM Corporation, New York, USA). Based on a 48% estimated prevalence of SOD cycles (De Souza et al., 1998), a 5% type I error rate and a 10% absolute error rate, it was calculated that 96 participants would be required to complete the study. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to identify the distribution of the data, with $p > 0.05$ for either test treated as normally distributed. Data that did not meet the criteria for normality were log-transformed and retested. Parametric data were reported as mean ± standard deviation and non-parametric data were reported as median (25^{th}, 75^{th} percentiles). Categorical variables were presented as numbers (percentages).

For objective one, the ovulatory status of participants was determined using the mid-luteal progesterone values and luteal phase lengths obtained during cycles three, four and five. Participants were allocated into the ovulatory group if they had two or more ovulatory cycles, or the SOD group if they had two or more SOD cycles. Participants were excluded from the objective one analysis if they provided fewer than two cycles of sex steroid hormone data or had an equal number of ovulatory and SOD cycles. Levene's test was used to test for homogeneity of variance between ovulatory and SOD groups, with $p > 0.05$ assuming equal variances. As there were no significant within-person differences in perceived stress and CDR scores, an average of each participant's score was used for the objective one analysis (Table 3.4). Two-sided independent samples t-tests (for parametric data) and Mann-Whitney U tests (for non-parametric data) were used for comparisons between ovulatory and SOD participants. Wilcoxon signed-rank tests were used to compare baseline and final visit body composition values. A p-value less than 0.05 was considered significant.

For objective two, a linear mixed model was used to analyse the effect of menstrual cycle phase and ovulatory status on mood, perceived stress and CDR. The original model included fixed effects for cycle phase, ovulatory status and their interaction. The mid-luteal phase was chosen as the reference category for cycle phase because it was the phase hypothesised to have the most significant differences in mood, perceived stress and CDR scores. For ovulatory status, ovulatory cycles were the reference category. The original model also included a random intercept for participants, repeated measures for cycle phase and covariates of baseline gynaecological age, percentage body fat and scaled total metabolic equivalents of task (MET) minutes per week. The MET minutes were scaled (z-scored) due to the large range of scores among participants (Table 3.1). The model was estimated using Restricted Maximum Likelihood, and the covariance structure was compound symmetry. The output showed that ovulatory status or its interaction with menstrual cycle phase did not significantly affect the model. A simplified model, which excluded ovulatory status, was then analysed. A comparison of the two models using an ANOVA showed no significant differences between the two models ($p > 0.05$), suggesting that ovulatory status did not improve the model. Therefore, it was decided to present the simple linear mixed model analysis in the manuscript. For objective two, a Bonferroni correction was applied to adjust for the nine statistical tests performed. Therefore, a p-value less than 0.006 was considered significant.

3.3 Results

3.3.1 Participant Characteristics

A total of 694 females completed the screening questionnaire for the study, of which 56% met the eligibility criteria and were invited to register. Of the 115 participants who completed the baseline survey, 97 females provided data for at least two menstrual cycles (84% compliance rate). Reasons for withdrawal included medical (n = 6), pregnancy (n = 2) and other lifestyle factors (n = 10). Participants who provided at least two cycles of sex steroid hormone data during the mid-luteal phase were able to be further grouped into ovulatory (n = 68) and SOD (n = 18) subgroups for the objective one analysis. Eleven participants were unable to be classified due to providing fewer than two cycles of sex steroid hormone data or providing two cycles of data that fell into different classifications. Participants who were unable to be classified have been included at the 'all participants' level only. Further details on participants' progression from screening to data collection are provided in Figure 3.1.

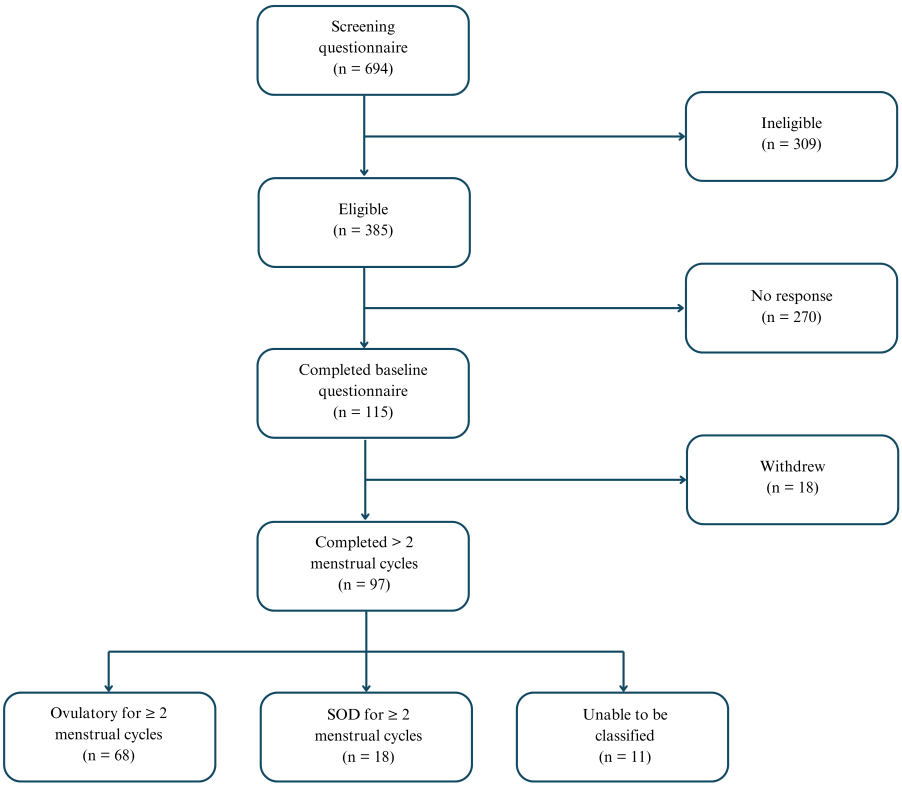

Figure 3.1. *Flow chart of the number of participants screened for the project to the final sample size and data set of the project.*

Table 3.1 presents the baseline demographic, physical and lifestyle characteristics of study participants and a comparison of these characteristics between ovulatory and SOD participants. Compared with the ovulatory group, the SOD group was younger in both age ($t = 2.3$, df = 84, $p = 0.025$) and gynaecological age ($t = 2.5$, df = 83, $p = 0.014$). The groups did not differ in any of the physical activity measures from the New Zealand Short Form Physical Activity questionnaire or any of the body composition measures reported in Table 3.1. Overall, a large proportion of the study participants had no children (78.1%), were of New Zealand European or Asian ethnicity (90.8%), were university educated (88.7%) and reported following no specific dietary pattern (72.2%). As a result, there were low participant numbers within most of the categories of ethnicity, education and dietary requirements, precluding a reliable statistical comparison between ovulatory and SOD groups.

Table 3.1 *Bivariate Analysis of Baseline Demographic, Physical and Lifestyle Characteristics in Healthy Menstruating Females by Ovulatory Status.*

Characteristic	**All Participants (*n* = 97)**	**Ovulatory[a] (*n* = 68)**	**SOD[b] (*n* = 18)**	***p*[c]**
Age (years)	30.5 ± 5.6	31.4 ± 5.2	28.2 ± 5.7	0.025*
Gynaecological Age (years) Missing (n = 1)	17.7 ± 5.7	18.8 ± 5.2	15.2 ± 5.9	0.014*
Number of Children Missing (n = 1)	0.0 (0.0, 0.0)	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.151
Ethnicity (n (%))				
Māori	7 (7.2)	4 (5.9)	2 (11.1)	
Pacific Peoples	1 (1.0)	1 (1.5)	0 (0.0)	
Asian	18 (18.6)	10 (14.7)	4 (22.2)	
Middle Eastern Latin American or African	1 (1.0)	1 (1.5)	0 (0.0)	
New Zealand European	70 (72.2)	52 (76.5)	12 (66.7)	
Highest Education (n (%))				
Highschool	8 (8.2)	4 (5.9)	0 (0.0)	
Polytechnic	3 (3.1)	2 (2.9)	1 (5.6)	
University	86 (88.7)	62 (91.2)	17 (94.4)	
Dietary Requirements (n (%)) [d]				
Vegan	3 (3.1)	3 (4.4)	0 (0.0)	
Vegetarian	6 (6.2)	3 (4.4)	3 (16.7)	
Gluten/Wheat Free	3 (3.1)	3 (4.4)	0 (0.0)	
Dairy Free/Low Lactose	4 (4.1)	3 (4.4)	1 (5.6)	
Low FODMAP	1 (1.0)	0 (0.0)	0 (0.0)	
Low Glycaemic Index	1 (1.0)	1 (1.5)	0 (0.0)	
Intermittent Fasting	3 (3.1)	2 (2.9)	0 (0.0)	
Allergy/Intolerance	7 (7.1)	6 (8.8)	1 (5.6)	
Other	2 (2.1)	1 (1.5)	0 (0.0)	
No Specific Diet	70 (72.2)	49 (72.1)	13 (72.2)	

Characteristic	All Participants (n = 97)	Ovulatory[a] (n = 68)	SOD[b] (n = 18)	p[c]
Physical Activity (MET min/week)[e]				
Vigorous	960 (120, 1,920)	1,020 (240, 1,920)	1,080 (360, 2,880)	0.380
Moderate	540 (240, 1,200)	540 (240, 1,245)	720 (360, 1,260)	0.425
Walking Missing (n = 2)	693 (330, 1,386)	594 (330, 1,386)	1,386 (693, 2,475)	0.058
Total	2,622 (1,587, 4,617)	2,676 (1,375, 4,622)	3,262 (2,403, 5,346)	0.109
Body Composition				
Missing (n = 14)				
Height (cm)	167.0 (162.0, 170.0)	167.0 (162.0, 170.0)	166.0 (161.8, 170.3)	0.695
Body Mass (kg)	68.4 (60.3, 75.6)	68.4 (60.6, 75.6)	67.7 (58.7, 72.9)	0.665
Body Mass Index (kg/m^2)	24.4 (22.0, 26.4)	24.4 (21.9, 26.9)	24.0 (22.4, 25.6)	0.696
Muscle Mass (kg)	26.1 (24.0, 28.9)	26.2 (24.3, 28.7)	25.5 (21.4, 28.8)	0.217
Fat Mass (kg)	20.0 (14.5, 24.1)	20.3 (15.3, 24.1)	18.8 (14.8, 28.5)	0.909
Percentage Fat Mass (%)	29.6 (22.4, 34.9)	30.6 (22.5, 34.5)	28.4 (21.9, 36.9)	0.727

Data are presented as mean ± standard deviation for parametric scale variables, median (25th, 75th percentiles) for non-parametric scale variables or n (valid %) for categorical variables.

[a] Participants were classified as ovulatory if at least two menstrual cycles were ovulatory and luteal phase sufficient (mid-luteal progesterone > 10 nmol/L and luteal phase length ≥ 10 days).

[b] Participants were classified as SOD if at least two menstrual cycles were anovulatory (mid-luteal progesterone < 3 nmol/L) or luteal phase deficient (mid-luteal progesterone between 3-10 nmol/L or luteal phase length < 10 days).

[c] Level of significance of difference between females classified as ovulatory and SOD using Independent Samples t-test (two-sided) (parametric scale variables) or Mann-Whitney U test (non-parametric scale variables). * Indicates significant difference in variable between ovulatory and SOD groups ($p < 0.05$).

[d] The total percentage of participants from each category is greater than 100%, due to participants having the option to select more than one dietary requirement.

[e] Metabolic Equivalent of Task (MET) minutes of vigorous physical activity, moderate physical activity and walking per week. Calculated using the Short Form New Zealand Physical Activity Questionnaire (Craig et al., 2003).

3.3.2 Menstrual Cycle Characteristics

A comparison of menstrual cycle characteristics between ovulatory and SOD groups is presented in Table 3.2. Compared with the ovulatory group, the SOD group had lower progesterone in the late follicular and mid-luteal phases of the menstrual cycle and lower oestrogen in the late follicular phase of the menstrual cycle. No significant differences in total menstrual cycle length, menstrual bleed length, follicular phase length, luteal phase length, early follicular phase progesterone, early follicular phase oestrogen or mid-luteal phase oestrogen between the ovulatory and SOD groups were observed.

Table 3.2. *Bivariate Analysis of Menstrual Cycle Characteristics of Healthy Menstruating Females by Ovulatory Status.*

Variable	All Participants (*n* = 97)	Ovulatory [a] (*n* = 68)	SOD [b] (*n* = 18)	*p* [c]
Menstrual Cycle Length (days)				
Total	28.0 (26.0, 31.0)	28.0 (26.0, 30.0)	30.0 (23.0, 35.0)	0.082
Menstrual Bleed	5.0 (4.0, 6.0)	5.0 (4.0, 6.0)	5.0 (4.0, 6.0)	0.650
Follicular Phase	14.0 (12.0, 15.0)	14.0 (12.0, 15.0)	13.0 (11.0, 15.8)	0.736
Luteal Phase	14.0 (13.0, 17.0)	14.0 (13.0, 16.0)	14.5 (9.0, 21.0)	0.852
Progesterone (nmol/L)				
Early Follicular Phase	0.7 (0.5, 1.0)	0.7 (0.5, 1.0)	0.6 (0.6, 1.0)	0.972
Late Follicular Phase	0.7 (0.4, 2.4)	0.9 (0.4, 2.7)	0.5 (0.4, 0.6)	0.023*
Mid-Luteal Phase	29.2 (13.8, 47.9)	33.4 (23.2, 54.9)	2.9 (0.7, 20.7)	<0.001*
Oestrogen (pmol/L)				
Early Follicular Phase	129.0 (91.5, 162.0)	128.0 (97.0, 162.3)	136.0 (83.0, 169.0)	0.769
Late Follicular Phase	457.0 (238.8, 916.3)	700.0 (302.0, 976.0)	221.5 (159.8, 316.5)	<0.001*
Mid-Luteal Phase	425.0 (338.5, 626.0)	436.0 (356.8, 662.3)	337.0 (305.3, 561.5)	0.057

Data are presented as median (25^{th}, 75th percentiles).

[a] Participants were classified as ovulatory if at least two menstrual cycles were ovulatory and luteal phase sufficient (mid-luteal progesterone > 10 nmol/L and luteal phase length ≥ 10 days).

[b] Participants were classified as SOD if at least two menstrual cycles were anovulatory (mid-luteal progesterone < 3 nmol/L) or luteal phase deficient (mid-luteal progesterone between 3-10 nmol/L or luteal phase length < 10 days).

[c] Level of significance of difference between females classified as ovulatory and SOD using Mann-Whitney U test. * Indicates significant difference in variable between ovulatory and SOD females ($p < 0.05$).

3.3.3 Body Composition

The change in body composition variables of participants between baseline and the final study visit is presented in Table 3.3. There were no significant changes in body mass, body mass index, muscle mass, fat mass or percentage fat mass between baseline and the final study visit for ovulatory or SOD groups.

Table 3.3. *Change in Body Composition Variables of Healthy Menstruating Females between Baseline and Final Study Visit.*

Variable	Change [c]	*p* [d]
Body Mass (kg)		
All Participants (*n* = 74)	0.2 (-1.2, 1.6)	0.598
Ovulatory [a] (*n* = 55)	0.3 (-0.9, 1.7)	0.231
SOD [b] (*n* = 17)	-0.5 (-1.9, 1.1)	0.518
Body Mass Index (kg/m^2)		
All Participants (*n* = 74)	0.1 (-0.5, 0.5)	0.550
Ovulatory [a] (*n* = 55)	0.1 (-0.3, 0.6)	0.196
SOD [b] (*n* = 17)	-0.2 (-0.7, 0.4)	0.605
Muscle Mass (kg)		
All Participants (*n* = 74)	0.1 (-0.5, 0.6)	0.680
Ovulatory [a] (*n* = 55)	0.1 (-0.4, 0.6)	0.441
SOD [b] (*n* = 17)	0.1 (-0.6, 0.4)	0.979

Variable	Change [c]	p [d]
Fat Mass (kg)		
All Participants (n = 74)	0.3 (-1.0, 1.5)	0.443
Ovulatory [a] (n = 55)	0.3 (-0.9, 1.5)	0.254
SOD [b] (n = 17)	-0.2 (-1.4, 1.3)	0.758
Percentage Fat Mass (%)		
All Participants (n = 74)	0.1 (-1.0, 1.5)	0.630
Ovulatory [a] (n = 55)	0.1 (-1.0, 1.7)	0.592
SOD [b] (n = 17)	-0.0 (-1.8, 1.8)	0.906

Data are presented as median (25^{th}, 75th percentiles).

[a] Participants were classified as ovulatory if at least two menstrual cycles were ovulatory and luteal phase sufficient (mid-luteal progesterone > 10 nmol/L and luteal phase length ≥ 10 days).

[b] Participants were classified as SOD if at least two menstrual cycles were anovulatory (mid-luteal progesterone < 3 nmol/L) or luteal phase deficient (mid-luteal progesterone between 3-10 nmol/L or luteal phase length < 10 days).

[c] Change in body composition values between baseline and final visit were calculated by subtracting participants' baseline values from their final visit values.

[d] Level of significance of difference between baseline and final visit values using Wilcoxon Signed Ranks test.

3.3.4 Perceived Stress and CDR by Ovulatory Status

The mean scores of perceived stress and CDR for ovulatory and SOD groups are presented in Table 3.4. The mean scores of perceived stress were not different between ovulatory and SOD groups (t = 0.5, df = 84, p = 0.606). Furthermore, ovulatory and SOD groups showed no difference in mean CDR scores (t = -1.7, df = 84, p = 0.100).

Table 3.4. *Bivariate Analysis of Perceived Stress and CDR Scores of Healthy Menstruating Females by Ovulatory Status.*

Variable	All Participants (n = 97)	Ovulatory [a] (n = 68)	SOD [b] (n = 18)	p [c]
Perceived Stress	24.7 ± 5.5	24.9 ± 5.6	24.1 ± 5.1	0.606
CDR	14.0 ± 4.1	13.6 ± 4.0	15.3 ± 4.2	0.100

Data are presented as mean ± SD.

[a] Participants were classified as ovulatory if at least two menstrual cycles were ovulatory and luteal phase sufficient (mid-luteal progesterone > 10 nmol/L and luteal phase length ≥ 10 days).

[b] Participants were classified as SOD if at least two menstrual cycles were anovulatory (mid-luteal progesterone < 3 nmol/L) or luteal phase deficient (mid-luteal progesterone between 3-10 nmol/L or luteal phase length < 10 days).

[c] Level of significance of difference between baseline and final visit values using an Independent Samples t-test (two-tailed).

3.3.5 Mood, Perceived Stress and CDR by Menstrual Cycle Phase and Ovulatory Status

Table 3.5 presents the median scores of the seven POMS-Q mood items, perceived stress and CDR from all participants. To explore the effect of mood, perceived stress and CDR by menstrual cycle phase and ovulatory status, participants were grouped by their ovulatory status for each menstrual cycle. Gynaecological age was added as a covariate, as the results of the present study showed that participants

with SOD cycles had lower gynaecological age. Percentage fat mass and scaled total metabolic equivalent of task (MET) minutes per week were also included as covariates, as previous research has reported a higher prevalence of SOD cycles in females with lower percentage body fat (Bingzheng et al., 2024) and higher exercise levels (De Souza et al., 1998; De Souza et al., 2010). The original linear mixed model analysis is presented in Appendix B and showed no significant effect of menstrual cycle phase, ovulatory status or their interaction on scores of mood, perceived stress or CDR ($p > 0.006$). There was no effect of baseline gynaecological age, percentage fat mass or scaled total MET minutes per week. A simplified linear mixed model, without ovulatory status (and its interaction with cycle phase), is presented in Table 3.6. There were no significant effects of menstrual cycle phase, baseline gynaecological age, baseline percentage body fat or baseline scaled MET minutes per week on scores of mood, perceived stress or CDR ($p > 0.006$). Table 3.7 provides a summary of the random effects of this model for objective two. Of mood scores from the POMS-Q, tension, anger, fatigue and depression had greater residual variance than participant variance, indicating greater within-participant variance in scores between menstrual cycle phases. Perceived stress also had greater residual variance than participant variance. For CDR, there was greater participant variance than residual variance, indicating greater between-participant variance in scores between menstrual cycle phases.

Table 3.5 *Median Scores of Mood, Perceived Stress and CDR in Healthy Menstruating Females by Cycle Ovulatory Status.*

Variable	All Participants (n = 246 cycles) [a]	Ovulatory [b] (n = 177 cycles)	SOD [c] (n = 48 cycles)
Tension	4.0 (2.0, 7.0)	5.0 (2.0, 8.0)	3.0 (1.0, 5.0)
Anger	1.0 (0.0, 3.0)	1.0 (0.0, 3.5)	1.0 (0.0, 3.0)
Fatigue	5.0 (3.0, 8.5)	6.0 (2.0, 9.0)	5.0 (3.0, 7.0)
Depression	1.0 (0.0, 3.5)	1.0 (0.0, 3.0)	2.0 (0.0, 4.0)
Esteem	15.0 (13.0, 17.0)	15.0 (13.0, 17.0)	14.0 (13.0, 16.0)
Vigour	6.0 (4.0, 9.0)	6.0 (4.0, 9.0)	6.0 (3.0, 7.0)
Confusion	3.0 (1.0, 5.0)	3.0 (1.0, 5.0)	3.0 (1.0, 5.0)
Perceived Stress	24.0 (20.0, 29.0)	25.0 (20.0, 29.0)	23.0 (19.0, 29.0)
CDR	14.0 (11.0, 17.0)	13.0 (11.0, 16.0)	15.0 (12.0, 16.0)

Data are presented as median (25^{th}, 75^{th} percentile).

[a] Twenty-one cycles were unable to be classified as ovulatory or SOD and have been included at the 'all participants' level only.

[b] Cycles were classified as ovulatory if they were ovulatory and luteal phase sufficient (mid-luteal progesterone > 10 nmol/L and luteal phase length ≥ 10 days).

[c] Cycles were classified as SOD if they were anovulatory (mid-luteal progesterone < 3 nmol/L) or luteal phase deficient (mid-luteal progesterone between 3-10 nmol/L or luteal phase length < 10 days).

Table 3.6 *Summary of Fixed Effects from a Linear Mixed Model to Evaluate the Effect of Menstrual Cycle Phase on Mood, Perceived Stress and CDR.*

Fixed Effects	Parameter Estimate (β)	95% Confidence Interval		*p* [d]
		Lower Bound	Upper Bound	
Tension [a]				
Intercept	4.38	1.05	7.70	0.013
Cycle Phase				
Early Follicular vs Mid-luteal	0.21	-0.77	1.19	0.674
Late Follicular vs Mid-luteal	-0.58	-1.58	0.40	0.250
Covariates				
Gynaecological Age	-0.01	-0.14	0.12	0.926
Percentage Fat Mass	0.03	-0.06	0.11	0.559
Total MET (z-score) [c]	0.08	-0.62	0.79	0.820
Anger [a]				
Intercept	0.91	-1.42	3.24	0.452
Cycle Phase				
Early Follicular vs Mid-luteal	0.41	-0.38	1.21	0.309
Late Follicular vs Mid-luteal	-0.41	-1.22	0.39	0.316
Covariates				
Gynaecological Age	0.06	-0.03	0.15	0.200
Percentage Fat Mass	0.00	-0.05	0.06	0.877
Total MET (z-score) [c]	0.19	-0.30	0.68	0.463
Fatigue [a]				
Intercept	2.53	-0.71	5.77	0.135
Cycle Phase				
Early Follicular vs Mid-luteal	0.72	-0.25	1.69	0.150
Late Follicular vs Mid-luteal	-0.07	-1.06	0.90	0.881
Covariates				
Gynaecological Age	0.10	-0.02	0.23	0.118
Percentage Fat Mass	0.04	-0.04	0.12	0.360
Total MET (z-score) [c]	0.10	-0.58	0.79	0.775
Depression [a]				
Intercept	1.26	-1.62	4.13	0.400
Cycle Phase				
Early Follicular vs Mid-luteal	0.15	-0.80	1.10	0.760
Late Follicular vs Mid-luteal	-0.58	-1.54	0.37	0.233
Covariates				
Gynaecological Age	-0.02	-0.13	0.09	0.713
Percentage Fat Mass	0.06	-0.02	0.13	0.140
Total MET (z-score) [c]	0.14	-0.47	0.74	0.665
Esteem [a]				
Intercept	15.66	13.02	18.30	<0.001
Cycle Phase				
Early Follicular vs Mid-luteal	-0.79	-1.49	-0.09	0.030
Late Follicular vs Mid-luteal	-0.02	-0.73	0.68	0.949
Covariates				
Gynaecological Age	0.01	-0.09	0.12	0.786

Fixed Effects	Parameter Estimate (β)	95% Confidence Interval		p [d]
		Lower Bound	Upper Bound	
Percentage Fat Mass	-0.02	-0.09	0.04	0.496
Total MET (z-score) [c]	-0.09	-0.65	0.48	0.769
Vigour [a]				
Intercept	10.34	7.31	13.37	<0.001
Cycle Phase				
Early Follicular vs Mid-luteal	-0.83	-1.64	-0.02	0.048
Late Follicular vs Mid-luteal	0.14	-0.68	0.96	0.744
Covariates				
Gynaecological Age	-0.04	-0.16	0.08	0.523
Percentage Fat Mass	-0.09	-0.17	-0.01	0.024
Total MET (z-score) [c]	0.29	-0.35	0.94	0.383
Confusion [a]				
Intercept	4.06	1.79	6.32	<0.001
Cycle Phase				
Early Follicular vs Mid-luteal	0.19	-0.46	0.84	0.567
Late Follicular vs Mid-luteal	-0.01	-0.67	0.64	0.983
Covariates				
Gynaecological Age	-0.05	-0.14	0.03	0.236
Percentage Fat Mass	0.01	-0.05	0.07	0.715
Total MET (z-score) [c]	-0.20	-0.68	0.28	0.413
Perceived Stress [b]				
Intercept	20.85	15.51	26.19	<0.001
Cycle Phase				
Early Follicular vs Mid-luteal	0.47	-1.12	2.08	0.563
Late Follicular vs Mid-luteal	-0.44	-2.07	1.17	0.596
Covariates				
Gynaecological Age	-0.05	-0.26	0.16	0.632
Percentage Fat Mass	0.16	0.02	0.29	0.026
Total MET (z-score) [c]	0.18	-0.95	1.31	0.759
CDR [a]				
Intercept	10.84	6.75	14.92	<0.001
Cycle Phase				
Early Follicular vs Mid-luteal	-0.40	-1.10	0.31	0.270
Late Follicular vs Mid-luteal	0.23	-0.48	0.95	0.523
Covariates				
Gynaecological Age	0.04	-0.12	0.20	0.604
Percentage Fat Mass	0.08	-0.03	0.18	0.150
Total MET (z-score) [c]	0.79	-0.10	1.67	0.089

[a] n (participants) = 82; n (cycles included in analysis) = 209; n (cycles missing data) = 37.

[b] n (participants) = 82; n (cycles included in analysis) = 210; n (cycles missing data) = 36.

[c] Scaled (z-score) total metabolic equivalent of task minutes per week.

[d] Level of significance is $p < 0.006$ with Bonferroni Correction.

Table 3.7 *Summary of Random Effects from a Linear Mixed Model to Evaluate the Effect of Menstrual Cycle Phase on Mood, Perceived Stress and CDR.*

	Participant		Residual	
	Variance	**SD**	**Variance**	**SD**
Tension [a]	6.88	2.62	8.37	2.89
Anger [a]	2.75	1.66	5.56	2.34
Fatigue [a]	6.46	2.54	8.13	2.85
Depression [a]	4.44	2.11	7.82	2.80
Esteem [a]	4.82	2.20	4.22	2.06
Vigour [a]	6.30	2.51	5.70	2.34
Confusion [a]	3.30	1.82	3.64	1.91
Perceived Stress [b]	17.49	4.18	22.35	4.73
CDR [a]	14.49	3.81	4.19	2.05

[a] *n* (participants) = 82; *n* (cycles included in analysis) = 209; *n* (cycles missing data) = 37.

[b] *n* (participants) = 82; *n* (cycles included in analysis) = 210; *n* (cycles missing data) = 36.

3.4 Discussion

The results of this study showed that the occurrence of two or more SOD cycles was not associated with higher perceived stress or CDR. Furthermore, menstrual cycle phase, ovulatory status and their interaction were not associated with variations in any of the seven mood items, perceived stress or CDR. To the best of the author's knowledge, the present study was the first to investigate CDR scores throughout the menstrual cycle and to consider the possible interaction effects of ovulatory status with menstrual cycle phase on mood, perceived stress and CDR.

3.4.1 Perceived Stress by Ovulatory Status

In contrast to the hypothesis of the present study, perceived stress scores were not higher in the SOD group than in the ovulatory group. To the best of the author's knowledge, no previous studies have compared PSS scores in healthy menstruating females with different ovulatory statuses. However, one previous study did use the PSS to investigate whether higher perceived stress was associated with an increased likelihood of ovulatory disturbances (Schliep et al., 2015). In alignment with the results of the present study, Schliep et al. (2015) reported that higher scores on the 4-item PSS over four menstrual cycles and the 14-item PSS at baseline were not associated with an increased odds of anovulation in healthy menstruating females, when analysed continuously or by median split. However, when the same study analysed daily stress diary scores as an alternative measure of perceived stress, each unit increase in daily stress was associated with a 70% increased odds of anovulation (Schliep et al., 2015). It is worth noting that the daily stress diary used in the study by Schliep et al. (2015) has not been previously validated, and the researchers did not report strong agreement or correlation between the 4-item or 14-item PSS and the daily stress diary. Although there may be benefits to the use of a daily stress diary, such as improved external validity, reduced recall bias and improved understanding of within-person

variability (Bolger et al., 2003), future research could benefit from the validation of this tool within healthy females.

When considering alternative measures of perceived stress, previous literature investigating its relationship with ovulatory status in healthy menstruating females has reported conflicting results. Consistent with the results of the present study, Fenster et al. (1999) showed no association between baseline job-related stress and risk of anovulation or a short luteal phase in 276 healthy menstruating females over five menstrual cycles. In contrast, preliminary results from Prior et al. (2022) showed a 63% prevalence of SOD cycles in a cohort of 112 healthy menstruating females studied during the coronavirus pandemic, compared with a 10% prevalence of SOD cycles in a cohort of 301 healthy menstruating females studied 13 years prior. The pandemic cohort also displayed higher scores of negative mood and external stress, supporting the hypothesis that stress may influence ovulatory activity. It should be noted that the coronavirus pandemic was a significant external stressor (Salari et al., 2020) that may have resulted in higher perceived stress than what females may typically experience in their daily lives. Therefore, it is also possible that ovulatory activity may be disrupted by perceived stress if it is associated with significant external stressors (e.g., the coronavirus pandemic) versus high perceived stress reported within typical daily living and activities. Within the available literature, higher PSS scores (within the upper third of the 56-point scale) are considered to be indicative of higher perceived stress (Cohen et al., 1983). It is worth noting that only one participant in the present study met this criterion, with the mean score of all participants (24.7) instead considered to be indicative of a moderate level of stress. Therefore, it is possible that the degree of stress experienced by females in the present study was not significant enough to influence ovulatory function. Investigating females who report higher levels of perceived stress or who have experienced significant external stress may be a consideration for future research to better understand the association between SOD cycles and perceived stress.

3.4.2 CDR by Ovulatory Status

The present study showed that CDR scores were not significantly different between ovulatory and SOD groups, a finding that contrasts the majority of previous literature investigating this topic. Over a two-year period, Bedford et al. (2010) showed that females who had a prevalence of SOD cycles above the median of 39% had a higher average TFEQ-R score (8.7) than females who had a prevalence of SOD cycles below the median (7.1). Similarly, Barr, Prior, et al. (1994) reported that luteal phase length was shorter in females in the highest tertile of TFEQ-R scores (8.6 days), compared with the females in the lowest tertile of TFEQ-R scores (10.8 days). Finally, Barr, Janelle, et al. (1994) reported that, compared with females below the median, females with TFEQ-R scores greater than 13 had shorter luteal phase lengths (10.7 days versus 7.4 days, respectively) and fewer ovulatory cycles (5.0 cycles versus 3.6

cycles, respectively). Interestingly, the mean TFEQ-R score of all participants in the present study (14.0) was higher than the scores from previous research that showed an association between SOD and CDR, which ranged from 7.9-10.0 (Barr, Prior, et al., 1994; Bedford et al., 2010). It should be acknowledged that the present study used a revised version of the TFEQ-R subscale, with a maximum score of 24 rather than a maximum score of 21 used in previous studies. However, when considering the average scores as a proportion of the maximum, participants in this study still had higher average scores than in previous research. Therefore, the finding that CDR scores were not significantly different between ovulatory and SOD groups in the present study was surprising.

One possible reason for the discrepancy in results between the present study and those of Barr, Janelle, et al. (1994), Barr, Prior, et al. (1994) and Bedford et al. (2010) is the grouping of participants. Previous research has typically analysed SOD on a continuous basis, whereas the present study investigated the differences in average TFEQ-R score among females with two or more ovulatory cycles and females with two or more SOD cycles. Importantly, there were few participants who met the latter criteria, resulting in 18.6% of participants in the SOD subgroup. Although the SOD subgroup in the present study was small, the percentage prevalence of at least two SOD cycles over a three-cycle period aligns with a previous study by De Souza et al. (1998), who reported a 25.7% percentage prevalence of at least two SOD cycles over a three-cycle period. Comparable to the results reported by this study, Waugh et al. (2007) also found no difference in baseline TFEQ-R scores between ovulatory and SOD females who were grouped similarly to participants in the present study. Specifically, Waugh et al. (2007) classified participants who had three or more SOD cycles as SOD, and participants who had fewer than three SOD cycles as ovulatory. Here, it is worth noting that grouping participants according to their frequency of SOD or ovulatory cycles likely resulted in a markedly lower proportion of study participants within the SOD subgroup (7%). This lower proportion of participants in the SOD group within the study by Waugh et al. (2007) may have been further exacerbated by their choice to use three SOD cycles for their participant grouping, compared with two cycles that were used in the present study. As SOD cycles may occur sporadically (Li et al., 2013; Prior et al., 2015), the grouping of participants used in both studies is a probable factor that contributed to a lower proportion of the study cohort being classified into the SOD subgroup. The small sample size in the SOD group may have prevented the detection of potential differences in CDR between females who had more frequent SOD cycles and females who had more frequent ovulatory cycles.

A further consideration is that the mean gynaecological age of participants in the present study (17.7 years) was much older than participants in previous research, which has ranged from 9.7-15.0 years (Barr, Janelle, et al., 1994; Bedford et al., 2010). The results of the present study and previous research have reported higher gynaecological age in participants with more frequent ovulatory cycles (Bedford et al., 2010). Therefore, females with higher gynaecological age may be less likely to experience SOD

cycles until perimenopause, where more frequent SOD cycles have been reported (Metcalf, 1979; Prior & Hitchcock, 2011). As a result of the higher gynaecological age of participants in the present study, the level of CDR may not have been high enough to contribute to the occurrence of SOD cycles. Instead, it may be proposed that higher levels of CDR or the presence of physical stress, such as restriction of energy intake, may be required to contribute to SOD cycles in females with higher gynaecological age (prior to perimenopause).

3.4.3 Mood and Perceived Stress by Menstrual Cycle Phase

At the group level, none of the seven POMS-Q mood items were associated with menstrual cycle phase in the present study. When considering previous research, two studies have used the POMS-Q to prospectively measure mood between the follicular and late luteal phases of the menstrual cycle in healthy females (Kikuchi et al., 2010; Reed et al., 2008). In alignment with the present study, Kikuchi et al. (2010) did not find a difference in scores from any of the POMS-Q mood items between the follicular and late luteal phases of one menstrual cycle in 13 menstruating females. Conversely, in a group of 15 healthy females, Reed et al. (2008) reported that scores of negative mood items were higher in the late luteal phase compared with the mid-follicular phase, while scores of positive mood items were higher in the mid-follicular phase. Both the present study and that of Kikuchi et al. (2010) analysed POMS-Q mood items individually, while Reed et al. (2008) grouped all negative and positive mood items together for the analysis. This grouping of negative and positive mood items together may have increased the statistical power, which may have increased the likelihood of finding a statistically significant result. A further consideration is that Reed et al. (2008) measured mood in the late luteal phase, whereas the present study measured mood in the mid-luteal phase. The timing of the mid-luteal phase progesterone measurement in the current study was chosen to enable the identification of SOD cycles (Practice Committees of the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility, 2021), and aligned with recommendations from previous literature on when to collect data to define the distinct sex steroid hormone profiles (Elliott-Sale et al., 2021). However, the mid-luteal phase may not align with the time point in the menstrual cycle where females report the most severe mood changes and psychological symptoms (Backstrom et al., 1983; Meaden et al., 2005; Pearlstein et al., 2005). Therefore, the results of the present study and the previous literature discussed highlight that there may be a discrepancy in the menstrual cycle subphase that recommendations suggest to collect data to determine hormone peaks and define ovulatory status (mid-luteal phase) and the timepoint where females report psychological symptoms (late luteal phase). Previous research in females with premenstrual syndrome and premenstrual dysphoric disorder has suggested that the sex steroid hormone patterns of females with these conditions do not differ from healthy females (Backstrom et al., 1983). Instead, it has been hypothesised that their neuroendocrine thresholds may be more sensitive to the cyclical variations in oestrogen and progesterone, which may

be more prominent during the final five days of the luteal phase (Backstrom et al., 1983; Nappi et al., 2022). Therefore, it is worth considering that it may not be high sex steroid hormones that contribute to changing mood states; rather, the withdrawal effect of declining sex steroid hormones in the late luteal phase. This concept of considering the impact of daily hormone variations and the transitions between menstrual cycle subphases has been raised previously within an exercise context. Bruinvels et al. (2022) suggested that the collection of data daily, particularly during the between-phase transitions, may provide a better understanding of how individuals respond to the dynamic changes in sex steroid hormone ratios throughout the menstrual cycle, rather than measuring a small number of static time points across the menstrual cycle subphases. To address this consideration, it may be important for future research to measure daily mood throughout the menstrual cycle to capture the possible effect of between-phase transitions, rather than treating each menstrual cycle phase as homogenous. A final consideration is that although the comparison of three menstrual cycle phases (early follicular, late follicular and mid-luteal) over three cycles in the present study captured a greater variation in sex steroid hormone profiles than in previous research, data was only collected in each cycle phase once, rather than being repeated in each cycle. Although this methodology reduced participant burden, it did not control for alternative factors that may have influenced participant mood and stress, such as changes to university or work schedules, life events or social stressors (O'Dougherty et al., 2012; Väisänen et al., 2024). To improve the reliability and validity of future research, measurement in all three cycle phases of a given menstrual cycle across multiple cycles should be considered.

Regarding perceived stress, this study also showed no significant group-level effect of menstrual cycle phase on PSS scores. Similar to previous research investigating mood variations throughout the menstrual cycle, studies that have analysed perceived stress have also reported mixed results. In a single-cycle study of 397 females, scores on the PSS were reported to be higher in the combined late luteal and menstrual phase of the menstrual cycle compared with the late follicular phase (Jain et al., 2023). However, this finding was not supported by the results of the present study or those of a cross-sectional study by Montero-López et al. (2018), which reported no significant difference in PSS score between females in the follicular and luteal phases of the menstrual cycle. It should be noted that the study by Jain et al. (2023) compared two menstrual cycle phases over a single menstrual cycle, whereas the present study compared three menstrual cycle phases over three cycles. Without the collection of data over more than one menstrual cycle phase, it is not possible to determine whether the differences observed by Jain et al. (2023) were a true relationship or simply due to chance. Furthermore, as mentioned with the association between menstrual cycle phase and mood states, Jain et al. (2023) reported a difference in stress in the late luteal phase, whereas the present study measured stress in the mid-luteal phase. This discrepancy in measurement timing may be a contributing factor to the differences observed, highlighting the previous recommendation that future research may need to

consider measuring daily perceived stress and mood throughout the mid-luteal and late luteal phases of the menstrual cycle, alongside mid-luteal progesterone.

3.5 Conclusion

In conclusion, 18.6% of healthy menstruating females in the present study experienced two or more SOD cycles. There were no significant differences in mean scores of perceived stress or CDR between ovulatory and SOD groups, despite CDR scores in the present study being higher than reported in previous research. Compared with the ovulatory group, the SOD group was younger in both chronological age and gynaecological age, which suggests that lower gynaecological age may be a contributing factor to SOD cycles in healthy menstruating females in this study. Furthermore, menstrual cycle phase was not shown to influence mood, perceived stress or CDR scores. This remained true when considering the interaction between menstrual cycle phase and ovulatory status, and when adjusted for baseline gynaecological age, percentage body fat and total MET minutes.

Given that SOD cycles have been associated with adverse effects on fertility and bone health (Bedford et al., 2010; Li et al., 2013; Prior et al., 1990a), understanding lifestyle factors that may be associated with these cycles in healthy menstruating females remains an important area of research. Future research should consider longitudinal designs with larger sample sizes to improve the reliability of the results. Moreover, further research into the optimal timing of data collection that aligns with sex steroid hormone variations throughout the menstrual cycle is essential to improve our understanding of the interactions between menstrual cycle phase and ovulatory status with mood, perceived stress and CDR.

Chapter 4: Conclusions and Recommendations

4.0 Achievement of Aims and Hypotheses

The primary aim of the present study was to determine if females who presented with at least two subclinical ovulatory disturbed cycles (SOD cycles) (anovulation or luteal phase defects) displayed higher perceived stress and CDR than females who presented with at least two ovulatory menstrual cycles over a three-cycle period. A secondary aim was to investigate if there were variations in mood, perceived stress and CDR throughout the menstrual cycle in females with different menstrual cycle presentations (ovulatory versus SOD cycles).

The results of the present study did not support the hypothesis that females who had two or more SOD cycles would report higher scores for perceived stress and CDR than females who had two or more ovulatory cycles. Although these results dispute much of the previous literature investigating the relations between perceived stress, CDR and ovulatory status (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994; Bedford et al., 2010; Prior et al., 2022; Schliep et al., 2015), this study took a novel approach in the direction by which this relationship was analysed by comparing perceived stress and CDR scores between females with at least two ovulatory cycles and females with at least two SOD cycles. In contrast, most previous research has analysed whether high versus low perceived stress and CDR are associated with an increased prevalence of SOD cycles. This approach has raised important methodological considerations for future research that will be outlined towards the end of this chapter.

Regarding variations in mood, perceived stress and CDR throughout the menstrual cycle, it was hypothesised that females would present with higher scores of negative mood, perceived stress and CDR in the mid-luteal phase of the menstrual cycle, compared with the early follicular and late follicular phases, while positive mood scores would be higher during the late follicular phase than the mid-luteal phase. Furthermore, due to less variability in sex steroid hormones, it was hypothesised that any phase effect for mood, perceived stress and CDR would be weaker in females who had an SOD cycle. However, the present study showed no effect of menstrual cycle phase, ovulatory status or their interaction on scores of mood, perceived stress or CDR. Although there is not yet a clear consensus on how sex steroid hormone variations may influence psychological symptoms throughout the menstrual cycle, some previous research has reported poorer mood and higher stress in the late luteal phase (Gonda et al., 2008; Jain et al., 2023; Reed et al., 2008; Ross et al., 2003; Symonds et al., 2004). This discrepancy in results between the present study and previous research poses an interesting question of whether the effect of sex steroid hormones on psychological symptoms may be more prominent during menstrual cycle sub-phase transitions, where sex steroid hormone levels are changing, than within a sub-phase, where sex steroid hormone levels may be more stable. Further research measuring daily

psychological symptoms and sex steroid hormone levels throughout the late luteal phase (where oestrogen and progesterone levels are declining), rather than testing at a single point in the mid-luteal phase (where sex steroid hormones are consistently high) and early follicular phase (where sex steroid hormones and consistently low), may be warranted to improve collective understanding of how variations in sex steroid hormones may influence mood, perceived stress and CDR.

4.1 Strengths

To the best of the author's knowledge, the present study was the first to examine the influence of perceived stress and CDR on subclinical ovulatory disturbances (SOD) in New Zealand females. Furthermore, no studies to date have considered the ovulatory status (and corresponding sex steroid hormone profiles) of participants when researching variations in psychological factors throughout the menstrual cycle. To the best of the author's knowledge, the present study was also the first to explore the influence of menstrual cycle phase on CDR scores in healthy females.

To reach an acceptable level of significance, the original statistical power calculations estimated that 96 participants were required. Ninety-seven females were included in the final analysis, which meant the study was appropriately powered to test for statistical significance. Along with the collection of data across three menstrual cycles, the sample size provided a large number of cycles (n = 246) for the final analysis.

The collection of data in three phases of the menstrual cycle was also a strength of the present study, as previously, few studies have investigated changes in mood and perceived stress across more than two menstrual cycle phases (Gonda et al., 2008; Romans et al., 2012b; Ross et al., 2003). The collection of data in the early follicular, late follicular and mid-luteal phases enabled comparison between three of the four distinct subphases of the menstrual cycle, encompassing both low and high sex steroid hormone profiles. However, future research could consider measuring daily mood, perceived stress and sex steroid hormone variations to capture the potential effects of declining sex steroid hormones throughout the late luteal phase on these psychological factors.

A final strength of the present study was the use of the three-step method to verify menstrual cycle phases and ovulatory status. This method involved calendar-based counting of menstrual cycle length, urinary luteinising hormone (LH) testing and direct measurement of venous blood sex steroid hormones (Schaumberg et al., 2017). A large majority of the studies that have previously reported on changes in mood and perceived stress throughout the menstrual cycle have used only calendar-based counting methods to determine menstrual cycle phase (Brown & Lewis, 1993; Gonda et al., 2008; Jain et al., 2023; Montero-López et al., 2018; Romans et al., 2012b; Ross et al., 2003; Symonds et al., 2004). The

sole use of the calendar-based counting method does not capture intra-individual or inter-individual variability in sex steroid hormones that can occur within and between menstrual cycles and cannot confirm ovulation (Schaumberg et al., 2017; Wideman et al., 2013). The measurement of sex steroid hormones in the present study meant that participants' cycle phase could be accurately classified, thereby improving the validity of the results. Furthermore, the measurement of plasma progesterone to verify urinary LH testing improved the identification of SOD cycles. Of note, 38 (15.4%) cycles in the present study returned positive urinary LH tests but had plasma progesterone levels indicative of anovulation or luteal phase deficiency, a hormonal profile that has been described in previous research and has been defined as a luteinised unruptured follicle (Brown, 2011). Menstrual cycles with a luteinised unruptured follicle are characterised by peak levels of oestrogen and LH that are typical of an ovulatory cycle, but progesterone levels that fail to rise during the mid-luteal phase due to the absence of follicle rupture (Brown, 2011). As the occurrence of a typical LH peak results in a positive urinary LH test, mid-luteal progesterone measurement is required to identify cycles with luteinised unruptured follicles, thereby demonstrating the importance of measuring sex steroid hormones in order to verify ovulatory status.

4.2 Limitations

Although the total sample size (n = 97) provided sufficient statistical power, a small number of participants exhibited at least two SOD cycles (n = 18) throughout the study period. Although an 18.6% prevalence of at least two SOD cycles is a relatively high proportion from a clinical perspective, statistically, it provided a small sample of females for the SOD subgroup analyses. In future, a larger sample size or a longer study period may facilitate a larger SOD subgroup for the analyses, which in turn may reduce the risk of type II errors.

The characteristics of the study population should also be considered a limitation. Firstly, convenience sampling was used to recruit participants, which may have resulted in self-selection bias. It is possible that females who volunteered to participate in the study may have had a greater interest in learning about their menstrual cycle or differed in characteristics such as eating and physical activity behaviours, body composition or health literacy. Therefore, the study results may not be fully representative of the New Zealand population. The physical characteristics of the females in the study may also limit the generalisability of the results. The study sample was primarily of New Zealand European (72.2%) or Asian (18.6%) ethnicity, which is not wholly reflective of the ethnic diversity within the New Zealand population (Stats NZ, 2024). To improve the generalisability, future research could benefit from greater recruitment of Māori and Pacific females, who were underrepresented in the present study. A further consideration is that most participants were within the healthy body mass index (BMI) range. Previous research has reported a positive association between BMI and both stress (Tenk et al., 2018) and CDR

(Barr, Janelle, et al., 1994; Bedford et al., 2010; Waugh et al., 2007; Zerón-Rugerio et al., 2022). The homogeneity within the sample of the present study may limit the applicability of results to the wider New Zealand population, where the most recent healthy survey data reports a mean BMI of 28.6 kg/m^2 and a 34.9% prevalence of a BMI greater than 30.0 kg/m^2 in adult females (Ministry of Health, 2024). Therefore, future research could consider the inclusion of a wider BMI range to improve the generalisability of the results.

Beyond the study sample, the present study had some methodological limitations that should be considered. Although the measurement of plasma sex steroid hormones is considered a strength of the present study, it should be noted that there are limitations with the use of a single phase-specific measurement. Progesterone is secreted in pulses, with previous research reporting ranges of 2.3 ng/ml to 40.1 ng/ml over 24 hr in healthy females during the mid-luteal and late luteal phases of the menstrual cycle (Filicori et al., 1984). Single-timepoint measurements may not capture peak concentrations of sex steroid hormones in respective phases. This may increase the variability of sex steroid hormone levels within a menstrual cycle phase and reduce the likelihood of finding a statistically significant result. It should be acknowledged that single phase-specific measurement of plasma sex steroid hormones is a relatively cost-effective option with lower participant burden than continuous biochemical monitoring. However, to account for the pulsatility of progesterone secretion, future research could consider a sum of three daily mid-luteal progesterone values to classify ovulatory status, whereby a total value less than 30 ng/mL would be indicative of an SOD cycle (Practice Committees of the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility, 2021).

A final methodological limitation was that daily energy intake was not recorded throughout the study or when phase-specific testing was completed. Although this helped to minimise participant burden, there is an established association between energy restriction (for example, through reduced dietary intake) and menstrual cycle impairment (Mountjoy et al., 2023). Previous research has shown a higher prevalence of SOD cycles in females with higher CDR, which is independent of changes to dietary intake (Barr, Janelle, et al., 1994; Barr, Prior, et al., 1994; Bedford et al., 2010). However, other studies have reported lower energy intake among females with higher CDR (Rideout et al., 2004; Schweiger et al., 1992), indicating energy intake may be a factor worth controlling for within analyses of CDR and SOD. Therefore, future research would benefit from collecting baseline and final visit three-day dietary records to estimate usual energy intake.

4.3 Recommendations and Future Directions for Research

The present study has provided valuable insights into the associations of ovulatory function and menstrual cycle phase with mood, perceived stress and CDR in healthy naturally menstruating females.

Despite not finding significantly different results between ovulatory and SOD participants or between menstrual cycle phases, the study has highlighted some of the complexities associated with conducting menstrual cycle research and the need for future research to consider different methodologies within a longitudinal study design. Recommendations for future research and practical applications of the outcomes from the present study are summarised below.

4.3.1 Recommendations for Future Research

- As the finding that scores of perceived stress and CDR were not significantly different between ovulatory and SOD groups disputes much of the previous research, continued investigation of this topic remains an important area of research. A larger sample size over a longer study period may increase the size of participants with SOD cycles, which would improve the statistical power when comparing scores of perceived stress and CDR between ovulatory and SOD participants.
- Since the cohort of the present study was primarily of New Zealand European or Asian ethnicity, future research should consider strategies to increase recruitment and participation of Māori and Pacific peoples. These strategies could be developed through consultation and partnership with Māori and Pacific stakeholders, ensuring the ethnic diversity of the study cohort reflects that of Aotearoa New Zealand. Such efforts would improve the generalisability and applicability of future research outcomes.
- To account for the pulsatility of progesterone secretion, future research should consider the use of three daily mid-luteal progesterone values to improve accuracy when classifying menstrual cycles as ovulatory or SOD.
- Given that the present study did not show poorer mood or perceived stress in the mid-luteal phase of the menstrual cycle, future research could consider measuring daily mood and perceived stress throughout the entire luteal phase to identify if there is an effect of declining sex steroid hormones (e.g., during the late luteal phase) on psychological symptoms.
- Energy intake was not measured in the present study. As inadequate energy intake is a known contributor to problematic low energy availability (Mountjoy et al., 2023), a causal factor of hypothalamic-pituitary-ovarian axis impairment (Reed et al., 2015), future research should measure energy intake to control for this potentially confounding factor for CDR scores. This could be achieved through a three-day dietary record at baseline and final visit to measure both between-group and within-group differences, while minimising participant burden.
- A final consideration for future research is the inclusion of females taking hormonal contraceptives, given their common use among New Zealand females (Thomas et al., 2023). As the present study aimed to measure mood, perceived stress and CDR among naturally menstruating females, those taking hormonal contraceptives were excluded. However,

comparison of results from naturally menstruating females with dynamic and biphasic sex steroid hormone variations to hormonal contraceptive users, who have medically suppressed endogenous hormones, may be an interesting area for future research to consider.

4.3.2 Practical Applications of Research Outcomes

- Although significant group-level variations in mood throughout the menstrual cycle were not evident, the results of the present study suggest that there is a high level of inter-individual variability in mood states throughout the menstrual cycle. It is important to note that this inter-individual variability in mood states may have reduced the significance of the group results. Therefore, while there is not currently enough evidence to support the provision of standardised advice regarding psychological symptoms to females based on their menstrual cycle phase, clinical practitioners should be mindful that effects may occur at an individual level. Practical advice should be provided on an individual basis, informed by patient-reported symptoms.
- Further to the above recommendation, it is important for females to understand their unique menstrual cycle and the variations in psychological symptoms that may occur throughout it. This could be achieved through the use of mobile applications to track an individual's menstrual cycle and their associated symptoms.
- Given that the present study showed an 18.6% prevalence of two or more SOD cycles over a three-cycle period, clinical practitioners should be aware that these cycles are relatively common among healthy menstruating females, particularly in females who are of lower gynaecological age. Routine mid-luteal phase venous blood progesterone testing should be considered in females who may be at risk of frequent SOD cycles to help prevent adverse health consequences, including effects on fertility and bone health (Bedford et al., 2010; Li et al., 2013; Prior et al., 1990a).

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Appendices

Appendix A: Questionnaires

Abbreviated Profile of Mood States Questionnaire (POMS-Q), published by Grove and Prapavessis (1992)[a]

Abbreviated POMS (Revised Version)

Name:_____ Date:

Below is a list of words that describe feelings people have. Please **CIRCLE THE NUMBER THAT BEST DESCRIBES HOW YOU FEEL RIGHT NOW**.

	Not At All	A Little	Moderately	Quite a Lot	Extremely
Tense	0	1	2	3	4
Angry	0	1	2	3	4
Worn Out	0	1	2	3	4
Unhappy	0	1	2	3	4
Proud	0	1	2	3	4
Lively	0	1	2	3	4
Confused	0	1	2	3	4
Sad	0	1	2	3	4
Active	0	1	2	3	4
On-edge	0	1	2	3	4
Grouchy	0	1	2	3	4
Ashamed	0	1	2	3	4
Energetic	0	1	2	3	4
Hopeless	0	1	2	3	4
Uneasy	0	1	2	3	4
Restless	0	1	2	3	4
Unable to Concentrate	0	1	2	3	4
Fatigued	0	1	2	3	4
Competent	0	1	2	3	4
Annoyed	0	1	2	3	4
Discouraged	0	1	2	3	4
Resentful	0	1	2	3	4

	Not At All	A Little	Moderately	Quite a Lot	Extremely
Nervous	0	1	2	3	4
Miserable	0	1	2	3	4
Confident	0	1	2	3	4
Bitter	0	1	2	3	4
Exhausted	0	1	2	3	4
Anxious	0	1	2	3	4
Helpless	0	1	2	3	4
Weary	0	1	2	3	4
Satisfied	0	1	2	3	4
Bewildered	0	1	2	3	4
Furious	0	1	2	3	4
Full of Pep	0	1	2	3	4
Worthless	0	1	2	3	4
Forgetful	0	1	2	3	4
Vigorous	0	1	2	3	4
Uncertain about things	0	1	2	3	4
Bushed	0	1	2	3	4
Embarrassed	0	1	2	3	4

THANK YOU FOR YOUR COOPERATION

PLEASE BE SURE YOU HAVE ANSWERED EVERY ITEM

Abbreviated POMS (Revised Version)

***** SCORING KEY *****

Scores for the seven subscales in the abbreviated POMS are calculated by summing the numerical ratings for items that contribute to each subscale. The correspondence between items and subscales is shown below.

Item	Scale	Not At All	A Little	Moderately	Quite a Lot	Extremely
Tense	**TEN**	0	1	2	3	4
Angry	**ANG**	0	1	2	3	4
Worn Out	**FAT**	0	1	2	3	4
Unhappy	**DEP**	0	1	2	3	4
Proud	**ERA**	0	1	2	3	4

Item	Scale	Not At All	A Little	Moderately	Quite a Lot	Extremely
Lively	**VIG**	0	1	2	3	4
Confused	**CON**	0	1	2	3	4
Sad	**DEP**	0	1	2	3	4
Active	**VIG**	0	1	2	3	4
On-edge	**TEN**	0	1	2	3	4
Grouchy	**ANG**	0	1	2	3	4
Ashamed	**ERA**	Reverse-score this item [0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0]				
Energetic	**VIG**	0	1	2	3	4
Hopeless	**DEP**	0	1	2	3	4
Uneasy	**TEN**	0	1	2	3	4
Restless	**TEN**	0	1	2	3	4
Unable to Concentrate	**CON**	0	1	2	3	4
Fatigued	**FAT**	0	1	2	3	4
Competent	**ERA**	0	1	2	3	4
Annoyed	**ANG**	0	1	2	3	4
Discouraged	**DEP**	0	1	2	3	4
Resentful	**ANG**	0	1	2	3	4
Nervous	**TEN**	0	1	2	3	4
Miserable	**DEP**	0	1	2	3	4
Confident	**ERA**	0	1	2	3	4
Bitter	**ANG**	0	1	2	3	4
Exhausted	**FAT**	0	1	2	3	4
Anxious	**TEN**	0	1	2	3	4
Helpless	**DEP**	0	1	2	3	4
Weary	**FAT**	0	1	2	3	4
Satisfied	**ERA**	0	1	2	3	4
Bewildered	**CON**	0	1	2	3	4
Furious	**ANG**	0	1	2	3	4
Full of Pep	**VIG**	0	1	2	3	4
Worthless	**DEP**	0	1	2	3	4
Forgetful	**CON**	0	1	2	3	4
Vigorous	**VIG**	0	1	2	3	4
Uncertain about things	**CON**	0	1	2	3	4
Bushed	**FAT**	0	1	2	3	4
Embarrassed	**ERA**	Reverse-score this item [0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0]				

TEN = Tension	Note that 2 of the items on the Esteem-related Affect (ERA) subscale are reverse-scored prior to being combined with the other items. **Total Mood Disturbance (TMD)** is calculated by summing the totals for the negative subscales and then subtracting the totals for the positive subscales: TMD = [TEN+DEP+ANG+FAT+CON] – [VIG+ERA]. A constant (e.g., 100) can be added to the TMD formula in order to eliminate negative scores.
ANG = Anger	
FAT = Fatigue	
DEP = Depression	
ERA = Esteem-related Affect	
VIG = Vigour	
CON = Confusion	

[a] Grove, J.R., & Prapavessis, H. (1992). Preliminary evidence for the reliability and validity of an abbreviated Profile of Mood States. *International Journal of Sport Psychology, 23*(2), 93-109.

Perceived Stress Scale, published by Cohen et al. (1983)[b]

Items and Instructions for Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate *how often* you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

For each question, choose from the following alternatives:

0. never
1. almost never
2. sometimes
3. fairly often
4. very often

1. In the last month, how often have you been upset because of something that happened unexpectedly?
2. In the last month, how often have you felt that you were unable to control the important things in your life?
3. In the last month, how often have you felt nervous and "stressed"?
4. [a] In the last month, how often have you dealt successfully with irritating life hassles?
5. [a] In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?
6. [a] In the last month, how often have you felt confident about your ability to handle your personal problems?
7. [a] In the last month, how often have you felt that things were going your way?

8. In the last month, how often have you found that you could not cope with all the things that you had to do?
9. [a] In the last month, how often have you been able to control irritations in your life?
10. [a] In the last month, how often have you felt that you were on top of things?
11. In the last month, how often have you been angered because of things that happened that were outside of your control?
12. In the last month, how often have you found yourself thinking about things that you have to accomplish?
13. [a] In the last month, how often have you been able to control the way you spend your time?
14. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

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[a] Scored in the reverse direction

[b] Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behaviour, 24*(4) 385-396.

Cognitive Dietary Restraint Subscale of the Three Factor Eating Questionnaire (TFEQ), published by Karlsson et al. (2000)[c]

Cognitive Restraint

1. I deliberately take small helpings as a means of controlling my weight.
   definitely true / mostly true / mostly false / definitely false
2. I consciously hold back at meals in order not to gain weight.
   definitely true / mostly true / mostly false / definitely false
3. I do not eat some foods because they make me fat.
   definitely true / mostly true / mostly false / definitely false
4. How frequently do you avoid 'stocking up' on tempting foods?
   almost never / seldom / usually / almost always
5. How likely are you to consciously eat less than you want?
   unlikely / slightly likely / moderately likely / very likely
6. On a scale of 1 to 8, where 1 means no restraint in eating (eating whatever you want, whenever you want it) and 8 means total restraint (constantly limiting food intake and never 'giving in'), what number would you give yourself?
   eat whatever I want, whenever I want it / constantly limiting food intake, never 'giving in'

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[c] Karlsson, J., Persson, L. O., Sjöström, L., & Sullivan, M. (2000). Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. *International Journal of Obesity, 24*(12), 1715-1725.

Appendix B: Supplementary Results (Objective Two)

Table 0.1 *Summary of Fixed Effects from a Linear Mixed Model to Evaluate the Effect of Menstrual Cycle Phase, Ovulatory Status and Their Interaction on Mood, Perceived Stress and CDR.*

	Parameter Estimate (β)	Standard Error	df	*t*	95% Confidence Interval		*p* [a]
					Lower Bound	Upper Bound	
Tension (*n* = 79 participants, 196 cycles)							
Intercept	5.30	1.85	84.81	2.87	1.72	8.85	0.005
Ovulatory Status [b]							
SOD vs Ovulatory	-1.45	0.98	166.79	-1.47	-3.37	0.44	0.143
Cycle Phase							
Early Follicular vs Mid-luteal	0.07	0.60	128.41	0.12	-1.09	1.25	0.903
Late Follicular vs Mid-luteal	-0.57	0.59	128.17	-0.97	-1.73	0.56	0.332
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	0.94	1.27	136.45	0.74	-1.54	3.39	0.462
SOD × Late Follicular vs Ovulatory × Mid-luteal	0.04	1.36	146.31	0.03	-2.58	2.68	0.977
Covariates							
Gynaecological Age	-0.03	0.07	80.27	-0.47	-0.17	0.11	0.643
Percentage Body Fat	0.02	0.05	79.10	0.51	-0.06	0.11	0.611
Total MET (z-score) [c]	0.04	0.38	72.62	0.10	-0.69	0.76	0.924
Anger (*n* = 79 participants, 196 cycles)							
Intercept	1.43	1.29	78.13	1.11	-1.04	3.90	0.271
Ovulatory Status [b]							
SOD vs Ovulatory	-1.01	0.79	176.52	-1.27	-2.52	0.54	0.206
Cycle Phase							
Early Follicular vs Mid-luteal	0.56	0.50	127.40	1.11	-0.41	1.54	0.270
Late Follicular vs Mid-luteal	0.71	0.49	127.35	-1.43	-1.67	0.25	0.156
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	-0.02	1.05	140.50	-0.02	-2.07	2.01	0.986

	Parameter Estimate (β)	Standard Error	df	*t*	95% Confidence Interval		*p* [a]
					Lower Bound	Upper Bound	
SOD × Late Follicular vs Ovulatory × Mid-luteal	1.09	1.11	153.23	0.97	-1.07	3.25	0.332
Covariates							
Gynaecological Age	0.04	0.05	72.95	0.83	-0.06	0.14	0.408
Percentage Body Fat	0.01	0.03	72.73	0.24	-0.05	0.07	0.813
Total MET (z-score) [c]	0.16	0.26	64.52	0.61	-0.34	0.65	0.545
Fatigue (*n* = 79 participants, 196 cycles)							
Intercept	1.90	1.73	81.26	1.10	-1.43	5.23	0.276
Ovulatory Status [b]							
SOD vs Ovulatory	0.02	0.94	166.95	0.02	-1.78	1.84	0.981
Cycle Phase							
Early Follicular vs Mid-luteal	0.92	0.58	125.81	1.59	-0.20	2.04	0.115
Late Follicular vs Mid-luteal	-0.04	0.57	125.58	-0.08	-1.14	1.05	0.938
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	-0.68	1.22	134.61	-0.56	-3.05	1.67	0.567
SOD × Late Follicular vs Ovulatory × Mid-luteal	-0.85	1.30	145.11	-0.66	-3.36	1.66	0.513
Covariates							
Gynaecological Age	0.13	0.07	76.69	1.89	0.00	0.26	0.063
Percentage Body Fat	0.04	0.04	75.61	1.02	-0.04	0.13	0.310
Total MET (z-score) [c]	0.20	0.35	68.99	0.58	-0.48	0.88	0.567
Depression (*n* = 79 participants, 196 cycles)							
Intercept	1.85	1.61	75.45	1.15	-1.24	4.94	0.252
Ovulatory Status [b]							
SOD vs Ovulatory	-0.86	0.94	171.07	-0.92	-2.66	0.95	0.357
Cycle Phase							
Early Follicular vs Mid-luteal	0.19	0.59	122.80	0.32	-0.95	1.33	0.748
Late Follicular vs Mid-luteal	-0.80	0.57	122.65	-1.39	-1.93	0.31	0.166
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	-0.30	1.23	134.18	-0.24	-2.68	2.09	0.807
SOD × Late Follicular vs Ovulatory × Mid-luteal	0.85	1.31	146.52	0.65	-1.66	3.41	0.517

	Parameter Estimate (β)	Standard Error	df	*t*	95% Confidence Interval		*p* [a]
					Lower Bound	Upper Bound	
Covariates							
Gynaecological Age	-0.04	0.06	70.65	-0.70	-0.17	0.08	0.484
Percentage Body Fat	0.06	0.04	69.98	1.45	-0.02	0.14	0.153
Total MET (z-score) [c]	0.10	0.32	62.68	0.32	-0.52	0.73	0.750
Esteem (*n* = 79 participants, 196 cycles)							
Intercept	15.62	1.43	81.97	10.92	12.86	18.37	<0.001
Ovulatory Status [b]							
SOD vs Ovulatory	0.22	0.73	161.83	0.30	-1.22	1.61	0.763
Cycle Phase							
Early Follicular vs Mid-luteal	-0.74	0.44	124.72	-1.69	-1.60	0.11	0.093
Late Follicular vs Mid-luteal	0.14	0.43	124.46	0.32	-0.70	0.97	0.752
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	0.01	0.93	131.97	0.01	-1.78	1.82	0.992
SOD × Late Follicular vs Ovulatory × Mid-luteal	-0.66	1.00	141.39	-0.66	-2.59	1.27	0.511
Covariates							
Gynaecological Age	0.02	0.06	77.68	0.43	-0.08	0.13	0.669
Percentage Body Fat	-0.03	0.04	76.38	-0.89	-0.10	0.04	0.376
Total MET (z-score) [c]	-0.06	0.29	70.53	-0.21	-0.62	0.50	0.825
Vigour (*n* = 79 participants, 196 cycles)							
Intercept	10.95	1.66	83.89	6.58	7.74	14.15	<0.001
Ovulatory Status [b]							
SOD vs Ovulatory	-1.01	0.82	160.76	-1.23	-2.64	0.57	0.221
Cycle Phase							
Early Follicular vs Mid-luteal	-0.91	0.50	125.87	-1.83	-1.87	0.05	0.070
Late Follicular vs Mid-luteal	0.04	0.49	125.60	0.09	-0.91	0.98	0.932
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	0.29	1.05	132.51	0.28	-1.73	2.35	0.781
SOD × Late Follicular vs Ovulatory × Mid-luteal	0.16	1.13	141.38	0.14	-2.00	2.36	0.886
Covariates							

	Parameter Estimate (β)	Standard Error	df	*t*	95% Confidence Interval		*p* [a]
					Lower Bound	Upper Bound	
Gynaecological Age	-0.05	0.07	79.68	-0.76	-0.18	0.08	0.452
Percentage Body Fat	-0.10	0.04	78.33	-2.35	-0.18	-0.02	0.021
Total MET (z-score) [c]	0.24	0.34	72.71	0.72	-0.41	0.90	0.476
Confusion (*n* = 79 participants, 196 cycles)							
Intercept	4.06	1.24	81.68	3.28	1.66	6.44	0.002
Ovulatory Status [b]							
SOD vs Ovulatory	0.33	0.67	166.79	0.49	-0.98	1.62	0.623
Cycle Phase							
Early Follicular vs Mid-luteal	0.37	0.41	126.07	0.90	-0.43	1.16	0.372
Late Follicular vs Mid-luteal	0.08	0.40	125.85	0.20	-0.71	0.86	0.839
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	-0.68	0.87	134.75	-0.79	-2.36	1.00	0.432
SOD × Late Follicular vs Ovulatory × Mid-luteal	-0.41	0.93	145.14	-0.44	-2.19	1.40	0.659
Covariates							
Gynaecological Age	-0.06	0.05	77.12	-1.22	-0.15	0.03	0.225
Percentage Body Fat	0.01	0.03	76.02	0.36	-0.05	0.07	0.722
Total MET (z-score) [c]	-0.20	0.25	69.44	-0.79	-0.68	0.29	0.431
Perceived Stress (*n* = 80 participants, 197 cycles)							
Intercept	22.37	2.95	87.06	7.58	16.69	28.05	<0.001
Ovulatory Status [b]							
SOD vs Ovulatory	-2.97	1.62	170.02	-1.83	-6.09	0.18	0.069
Cycle Phase							
Early Follicular vs Mid-luteal	0.31	1.00	130.45	0.31	-1.64	2.25	0.754
Late Follicular vs Mid-luteal	-0.89	0.98	130.24	-0.90	-2.80	1.01	0.368
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	0.07	2.10	141.09	0.03	-3.97	4.15	0.974
SOD × Late Follicular vs Ovulatory × Mid-luteal	1.71	2.25	149.42	0.76	-2.63	6.06	0.449
Covariates							
Gynaecological Age	-0.10	0.12	83.61	-0.84	-0.32	0.12	0.404

	Parameter Estimate (β)	Standard Error	df	*t*	95% Confidence Interval		*p* [a]
					Lower Bound	Upper Bound	
Percentage Body Fat	0.16	0.07	81.05	2.16	0.02	0.30	0.033
Total MET (z-score) [c]	0.14	0.60	74.17	0.24	-1.01	1.30	0.810
CDR (*n* = 80 participants, 196 cycles)							
Intercept	10.95	2.19	81.26	5.01	6.74	15.18	<0.001
Ovulatory Status [b]							
SOD vs Ovulatory	-0.09	0.77	136.69	-0.12	-1.57	1.39	0.907
Cycle Phase							
Early Follicular vs Mid-luteal	-0.31	0.44	117.52	-0.70	-1.16	0.54	0.484
Late Follicular vs Mid-luteal	0.41	0.44	117.52	0.93	-0.44	1.25	0.352
Ovulatory Status × Cycle Phase							
SOD × Early Follicular vs Ovulatory × Mid-luteal	-0.35	0.94	121.10	-0.37	-2.15	1.47	0.713
SOD × Late Follicular vs Ovulatory × Mid-luteal	-0.54	1.02	124.93	-0.53	-2.51	1.44	0.597
Covariates							
Gynaecological Age	0.02	0.09	79.11	0.24	-0.15	0.19	0.808
Percentage Body Fat	0.09	0.05	76.98	1.63	-0.02	0.20	0.108
Total MET (z-score) [c]	0.82	0.46	74.27	1.78	-0.07	1.71	0.079

[a] Level of significance was $p < 0.006$ with Bonferroni Correction.

[b] Participants were classified according to their ovulatory status for each cycle. Participants were classified as ovulatory if a given menstrual cycle was ovulatory and luteal phase sufficient (mid-luteal progesterone > 10 nmol/L and luteal phase length ≥ 10 days). Participants were classified as SOD if a given menstrual cycle was anovulatory (mid-luteal progesterone < 3 nmol/L) or luteal phase deficient (mid-luteal progesterone between 3-10 nmol/L or luteal phase length < 10 days).

[c] Scaled (z-score) total metabolic equivalent of task minutes per week