Serum hormone levels in women with chronic pain

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Lisa D Buck

Balliol College, University of Oxford

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Abstract

Title: Serum Hormone Levels in Women with Chronic Pain, The Women In Pain Study, Oxford (WIPSOx).

Introduction:
Regular menstrual cycles in post-menarchal women reflect a healthy hypothalamic-pituitary-ovarian (HPO) axis. It is well known that psychological stress can disrupt this axis both acutely and chronically leading to cycle irregularity or a period of amenorrhoea. High levels of opiate use, both for chronic pain and recreationally, can lead to suppression of ovarian endocrine function, amenorrhoea or even premature ovarian insufficiency. However, it is not known whether chronic pain in itself is a sufficient stressor to disrupt HPO axis activity.

Aim of Investigation
This observational study aimed to investigate the extent to which hormone production is altered in women with chronic pain and to explore whether clinical symptoms relate to this suppression.

Methods:
Local ethical approval was obtained (Oxford REC 15/SC/0077).

Pre-menopausal women (aged 18 – 50) with chronic (>6 months) pelvic or musculoskeletal pain were recruited by advertisement. At a single research visit, subjects completed a detailed questionnaire, were weighed and measured and blood was drawn. All samples were analysed to determine levels of estradiol, progesterone, testosterone, follicle stimulating hormone (FSH), cortisol, Vitamin D and sex hormone binding globulin (SHBG).

Questionnaire data collected included: menstrual data; number and regularity of periods in the previous 6 months; date of last menstrual period; length of cycle; measures of psychological distress: Beck Depression scale (BDI), State-Trait Anxiety Inventory Scale (STAI), measures of sleep disturbance and current and past stressful events.

Results:

Menstrual cycles:

33% of the cohort described irregular or absent cycles in the last 6 months. Comparing women with regular cycles to those with irregular/absent cycles, there was no significant difference in age; duration of pain; severity of pain; state anxiety, or depression. There were also no significant differences in any of these variables when women with regular cycles were compared to those with amenorrhoea for the last six months.

Serum hormone levels:

Four women were clinically hypoestrogenic and eight women were hypoandrogenic. However, forty-eight of the women had estradiol levels in the lowest quartile of the normal range and forty-eight had testosterone levels in the lowest quartile.

Conclusions:

This study found high rates of menstrual cycle disturbance in a cohort of women with chronic pain who were of reproductive age. In line with this, low levels of estradiol and testosterone were present in approximately half of the cohort. Such changes in serum hormone levels of women with chronic pain may have a significant impact on their morbidity and potentially mortality. Health consequences include reduced fertility; increased risk of cardiovascular disease and osteoporosis; psychological sequelae; and potentially an exacerbation of pain symptoms.

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Abbreviations

ACOG	American College of Obstetricians and Gynaecologists
ACTH	Adrenocorticotropic Hormone
ASRM	American Society for Reproductive Medicine
BBC	British Broadcasting Corporation
BCG	Bromocresol Green
BCP	Bromocresol Purple
BD	Becton Dickson
BDI	Beck Depression Inventory
BDI-II	Revised Beck Depression Inventory
BMI	Body Mass Index
CRF	Case Report Form
CMIA	Chemiluminescent Microparticole Immunoassay
CRH	Corticotropin Releasing Hormone
CVD	Cardiovascular Disease
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone Sulphate
DSM-IV	Doagnostic and Statistical Manual of Mental Disorders
E1	Estrone
E2	Estradiol
E3	Estriol
E4	Estetrol
FB	Facebook
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GnRH	Gonadotropin Releasing Hormone
GP	General Practitioner
HPA	Hypothalamic Pituitary-adrenal axis
HPO	Hypothalamic Pituitary-ovarian axis
HRT	Hormone Replacement Therapy
HTA	Human Tissue Act
IASP	International Association for the Study of Pain
IU/L	International Units Per Litre
IUS	Intrauterine System
LH	Luteinising Hormone
LNG	Levonorgestrel
MPQ	McGill Pain Questionnaire
MSK	Muskuloskeletal
NICE	National Institute of Clinical Excellence
NHS	National Health Service
NMOL/L	Nanomoles Per Litre
NRS	Numerical Rating Scale
PCS	Pain Catastrophising Scale
PCOS	Polycystic Ovary Syndrome

PMOL/L	Picomoles Per Litre
PSQI	Pittsburgh Sleep Quality Index
REC	Research Ethics Committee
RCOG	Royal College of Obstetricians and Gynaecologists
RLU	Relative Light Units
SF-MPQ2	Short Form McGill Pain Questionnaire (modified version)
SHBG	Sex Hormone Binding Globulin
SRRS	Social Readjustment Rating Scale
STAI	Stait Trait Anxiety Index
SPSS®	Statistical Package for the Social Sciences
SST™	Serum Seperator Tube
TMJ	Temporomandibular Joint
VAS	Visual Analogue Scale
WIPSOx	Women in Pain Study, Oxford

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1 Introduction

The observational study (WIPSOx) reported within this thesis aims to investigate the extent to which hormone production is altered in women with chronic pain, and to explore whether clinical symptoms relate to this suppression.

This introductory chapter will define chronic pain, and outline the hypothalamic pituitary ovarian (HPO) and hypothalamic pituitary adrenal (HPA) axes, and regulation and possible causes of menstrual cycle disruption, together with the role of sex hormones in health. The psychosocial factors that may contribute to the experience of pain and how they can be measured will be explored, and a review of the evidence suggesting that pain may suppress hormone levels will be considered. Finally, the rationale and design of the study will be presented.

1.1 Pain

Pain as defined by the International Association for the Study of Pain is *"an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"* (IASP Task Force 2012).

Pain is regarded as chronic when it lasts or recurs for more than three to six months (International Association for the Study of Pain 2017) persisting past the normal healing time of tissue injury (Turk and Okifuji 2009), or exists in the absence of any such injury.

Chronic pain is considered to be a major public health issue, contributing substantially to morbidity, mortality, and disability, placing serious demands on the healthcare system and the wider national economy (Simon 2012). In 2016

the estimated prevalence of chronic pain in the United Kingdom was 28 million adults, or 43% of the adult population (Fayaz *et al* 2016) and is most likely to continue to increase.

The prevalence of the most common forms of chronic pain is higher among women than men, as well as female-specific pain conditions such as dysmenorrhoea and endometriosis. As this pain is frequently cyclical or intermittent, it is only considered chronic once it has been present for six months (IASP Task Force 2012).

An increasing body of evidence from multiple epidemiologic studies clearly demonstrates that the population prevalence of many chronic pain conditions, including back, abdominal, oral and musculoskeletal (MSK) pain, fibromyalgia, osteoarthiritis, temporomandibular joint (TMJ) disorder, migraine, and headache is far greater for women than it is for men (Smith *et al* 2001, Tsang *et al* 2008, Fillingim *et al* 2009, Bartley and Fillingim 2013).

All chronic pain conditions can be debilitating, with the potential to impact on ones quality of life, affecting sleep, mental health, mobility, sexual activity and finances. In 2009 the Chief Medical Officers report stated that pain reduced quality of life more than almost any other condition (Donaldson 2009).

1.2 Psychosocial factors and pain

As pain is identified as both a sensory and an emotional experience (IASP 1994) it is not surprising that chronic pain and mental health disorders frequently co-occur (Osterweis 1987, RCOG 2012), with psychological and emotional factors often determining, or greatly influencing, the experience of pain (Gureje 2007). Various psychological and social measures, together with

techniques to assess pain in adults will be used within this study and are discussed below.

1.2.1 State and Trait Anxiety Inventory (STAI)

The STAI (Spielberger *et al* 1983) was developed as a self-reporting research instrument for investigating not only the presence and severity of anxiety as a symptom (State Anxiety Scale), but also the propensity to be anxious (Trait Anxiety Scale) in adults. The measure has forty items divided into the two subscales: the State Anxiety Scale, evaluates the current, “right now” state of anxiety, whereas the Trait Anxiety Scale evaluates how “prone” one is to anxiety. Each item is rated on a four-point scale from “Almost Never” to “Almost Always”, with higher scores indicating a greater level of anxiety. Despite a caution regarding the relatively poor validity of the scale, particularly the Trait Anxiety Scale for differentiating anxious from depressed states (Julian 2011), it is among the most widely used measure of general anxiety.

1.2.2 Beck Depression Inventory (BDI)

The BDI (BECK *et al* 1961) was developed to measure the behavioural manifestations of depression in adults and adolescents from age thirteen. The instrument contains items that measure the cognitive, somatic and affective symptoms of depression.

This was revised to the BDI-II (Beck, Steer and Brown 1996) to be more consistent with the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria for depression (American Psychiatric Association 1994).

This self-reporting measure consists of twenty-one items to assess symptoms and intensity of depression. Each item has a list of four statements ranging from not present (0) to severe (3) about a particular symptom.

The BDI is amongst the most used self-rating scales for measuring depression worldwide. Advantages are its reliability, internal consistency, content validity and validity in differentiating between depressed and non-depressed subjects (Richter 1998).

1.2.3 Pain Catastrophising Scale (PCS)

Those who catastrophise report more negative pain-related thoughts, more emotional distress, and more pain than non-catastrophisers (Sullivan *et al* 1995). The most consistent finding within the literature is that catastrosphising is associated with a heightened pain experience (Sullivan *et al* 2001). The relationship between catatrophising and pain has been observed in both clinical and experimental investigations and across diverse patient groups including arthritis (Keefe *et al* 1989) low back pain (Jensen *et al* 1991), dental and dental hygiene (Chaves and Brown 1987, Sullivan and Neish 1997), and those experiencing headache (Ukestad and Wittrock 1996, Bedard *et al* 1997).

The PCS (Sullivan *et al* 1995) was developed to focus specifically on assessing catastrophising in clinical and non-clinical populations. The PCS contains thirteen items that are grouped under three subscales: helplessness, rumination and magnification where each item is rated on a five-point scale, 0 to 4 (not at all - all the time). The internal consistency and validity of the scale has been demonstrated (Osman *et al* 1997) and it has become the reference standard psychometric tool for pain catastrophising.

1.2.4 Social Readjustment Rating Scale (SRRS)

Associations are known to exist between the experience of life events and the occurrence of psychological distress in some psychiatric disorders (Turner and Wheaton 1995). Approaches to assess major life events include a checklist approach or structured interviews. The former represents the traditional and still dominant method for gathering data on event exposure to major life events (Herbert and Cohen 1997). Checklist measures include the Schedule of Recent Experiences, developed in the 1950s, or the Social Readjustment Rating Scale (Holmes and Rahe 1967), an elaboration of this instrument developed by Holmes & Rahe.

The SRSS is a forty-three item scale identifying changes in life style and life events of individuals. The greater the number of changes is represented as the amount of environmental stress an individual has experienced, increasing the likelihood that some kind of physical or emotional illness will occur.

This measure, despite being a traditional method is still used extensively by those measuring stressful life events. However, it does have its own limitations. The checklist scale identifies only the occurrence of the event, whereas interviewing techniques assess this together with the magnitude, the nature of event and any relations between combinations of events. However, interview methods are costly and time consuming.

1.2.5 Sleep Quality

Numerous studies have provided evidence to suggest that chronic pain and insomnia frequently co-occur (Menefee *et al* 2000, Kundermann *et al* 2004, Smith and Haythornthwaite 2004, Ohayon 2005, Kelly *et al* 2011). Sleep

disturbance and poor quality sleep may result from ‘actual’ physical discomfort, or from ‘other’ aspects of physical illness that are related to conditions such as depression and anxiety (Carpenter and Andrykowski, 1998, Soldatos and Paparrigopoulos 2005, Finan and Smith 2013).

The PSQI (Buysse *et al* 1989) is a self-rated questionnaire that was specifically designed to measure the patterns and quality of sleep in adults. It differentiates “poor” from “good” sleep quality by measuring seven components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month. Each component generates a score with the sum of each equal to the total ‘global’ score.

A recent systematic review by Mollayeva et al (2016) found that the PSQI was the only standardised clinical instrument that covers a broad range of indicators relevant to sleep quality. Despite being developed with no specific population in mind the PSQI is the most commonly used measure for sleep dysfunction in clinical and research settings (Mollayeva *et al* 2016).

1.3 Assessing pain and unpleasantness

1.3.1 Numerical rating scale (NRS)

Despite the vast body of literature, few papers actually recommend the use of one pain measurement scale over the other. The NRS for pain is a one-dimensional measure that provides an estimate of pain intensity in adults, including those with chronic pain. Respondents are asked to indicate a value on a single 11-point numeric scale that best describes their pain intensity. At

either end of the scale are terms describing pain severity extremes of “no pain” and “worst pain imaginable”.

The pain NRS is considered to be a valid and reliable scale to measure pain intensity in adults with chronic pain with good sensitivity(Hawker *et al* 2011; Hjermstad *et al* 2011). Strengths include its simplicity in scoring and that it is easy to administer (Jensen et al 1986, Williamson and Hoggart 2005). Furthermore, chronic pain patients prefer the NRS over other measures of pain intensity, including the pain Visual Analogue Scale (VAS) due to comprehensibility and ease of completion (Williams *et al* 2000).

A disadvantage of the pain NRS is that it evaluates only one component of the pain experience, pain intensity. It does not evaluate the complexity and multiple dimensions of the pain experience. To overcome this the McGill Pain Questionnaire (Melzack 1975) may be used alongside.

1.3.2 The McGill Pain Questionnaire (MPQ)

The MPQ was developed for adults with chronic pain to measure not only the intensity, but also the quality of the patient’s pain. The SF-MPQ-2 (Dworkin *et al* 2009) is a shortened version of the original MPQ and a modified version of the short form MPQ (Melzack 1987).

The shorter versions were developed for use in research settings where time may be limited and more information is required above a single score of intensity, such as what a VAS or NRS provide. This more recent modified version (SF-MPQ-2) has the additional advantage of including descriptors relevant to neuropathic pain. It consists of twenty-two descriptors within four

subscales: continuous pain; intermittent pain; neuropathic pain, and affective pain. The total score is the mean of the subscale scores.

A disadvantage of traditional pain assessment methods such as VAS and NRS was the assumption that pain was a one-dimensional experience that can be measured with a single item scale (Melzack 1975). The design of the SF-MPQ-2 captures the qualitative aspects of pain and multidimensional varieties of unpleasantness within the pain experience. The McGill Pain Questionnaires are considered the 'gold standard' in the measurement of the various qualities within chronic pain. The SF-MPQ-2 is considered a psychometrically sound, reliable and valid tool (Dworkin *et al* 2009, Katz and Melzack 2011), with good discriminative capacity (Katz and Melzack 2011).

1.4 Neuroendocrine systems

1.4.1 Hypothalamic pituitary adrenal (HPA) axis

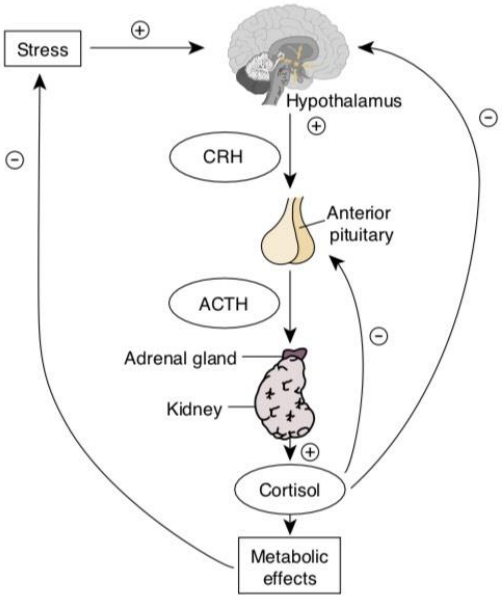
The HPA axis refers to the hypothalamus, pituitary gland and the adrenals (see figure 1). Together these glands control our reactions to stress and have a central role in regulating many homeostatic systems in the body, including the metabolic, cardiovascular, immune, reproductive and central nervous systems (Besedovsky *et al* 2008). Activation of this system initiates the release of corticotrophin releasing hormone (CRH) in response to various stimuli, including both physical and psychological stress (Hiller-Sturmhofel and Bartke 1998).

1.4.2 Hypothalamic pituitary-ovarian (HPO) axis

The HPO axis refers to the hypothalamus, pituitary gland, and ovaries in women. This system regulates female reproduction by the release of sex hormones to control uterine and ovarian function. It is has been described by Hiller-Sturmhöfel & Bartke (1998) as a '*regulatory hormonal cascade'* whose activities are controlled by both negative and positive feedback mechanisms (Figure 1).

Regular menstrual cycles in post-menarchal women reflect a healthy HPO axis. It is well known that psychological stress can disrupt this axis both acutely and chronically leading to cycle irregularity or a period of amenorrhoea (Carpenter 1994, Gordon *et al* 2017). High levels of opiate use, both for chronic pain and when used recreationally can interfere with the normal functioning of the HPO axis, leading to suppression of ovarian endocrine function, amenorrhoea or even premature ovarian insufficiency (Daniell 2008).

The HPA Axis



Female HPO Axis

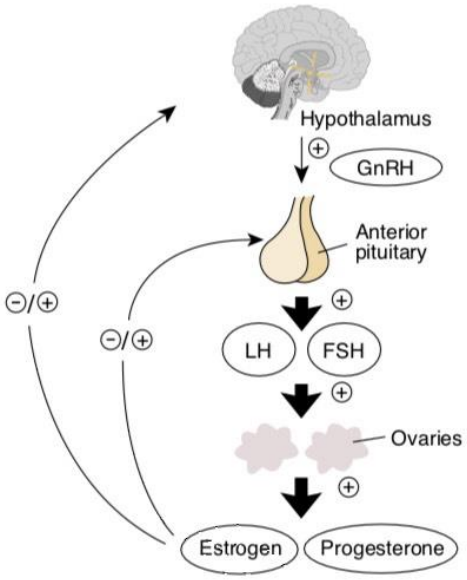


Figure 1: Diagram of the HPA and female HPO axis, from Hiller-Sturmhöfel & Bartke (1998). (+) = stimulates; (−) = inhibits; ACTH = adrenocorticotropic hormone; CRH = corticotropin-releasing hormone; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; HPA = hypothalamic pituitary adrenal; HPO = hypothalamic pituitary ovarian; LH = luteinizing hormone.

In both the HPO and HPA axes, the hypothalamus secretes releasing hormones in a pulsatile manner (CRH or GnRH), acting on the pituitary gland. In response to this stimulus the pituitary gland releases adrenocorticotropic hormone (ACTH) or gonadotropins (LH and FSH). In the HPA axis ACTH activates the adrenal glands to release cortisol, generating its metabolic effects in a variety of end organs, as well as affecting the hypothalamus and pituitary gland through a negative feedback to achieve homeostasis.

In the HPO axis, LH and FSH in women stimulate the ovaries to produce estrogens and progesterone. Those hormones also affect the hypothalamus and pituitary gland in a negative feedback loop, except during the mid-point of the menstrual cycle when a positive feedback loop involving oestradiol and LH initiates ovulation.

1.5 The menstrual cycle

A normal menstrual cycle is a clear indicator of the reproductive health status of a woman. Typically the duration of a woman's menstrual cycle is twenty-eight days and is regular, although a normal regular menstrual cycle length can range from twenty-one to thirty five days (ACOG 2012), see figure 2. The variability of length, regularity and frequency is high in the first five years after the menarche and in the last five years or so before the menopause (Fraser and Inceboz 2000). Because the menstrual cycle can be observed as bleeding it may be used as a indicator of ovarian function, thus assumptions can be made about levels of hormones secreted at different phases. However, it is well established that there are both inter- and intra- subject variations in the length of the total cycle as well as actual amounts of estrogens and progestagens (Bellem *et al* 2011).

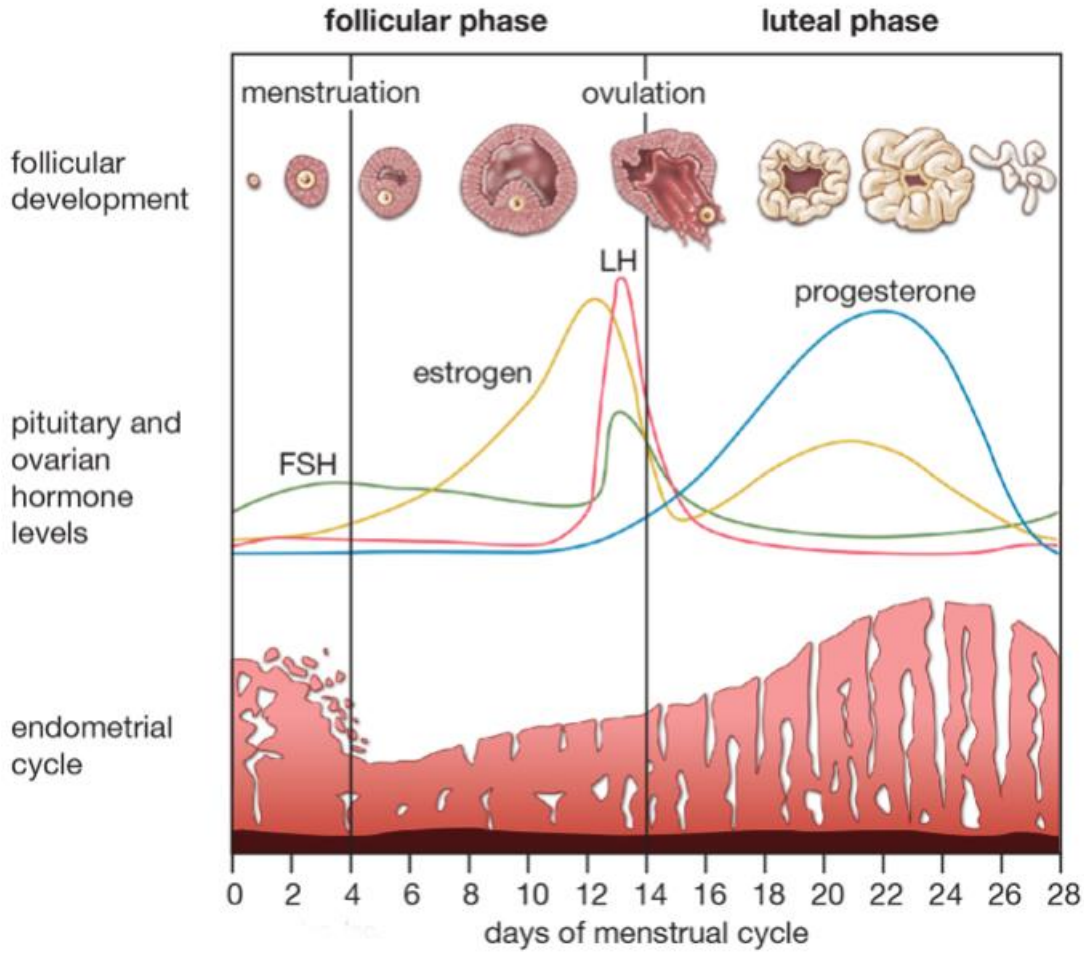


Figure 2: Cyclical changes during a woman's normal ovulatory menstrual cycle. Image from Encyclopaedia Britannica (*The menstrual cycle* 2008).

1.6 Mechanisms which may disturb the menstrual cycle

The absence or cessation of menstruation is defined as amenorrhoea, classified as either primary, the absence of menarche by the age of sixteen years or secondary, occurring in women who previously had an established menstrual cycle (NICE 2018). Whereas oligomenorrhoea is defined as

infrequent bleeding, as less often than every six weeks and includes a spectrum of severity up to complete amenorrhoea (Fraser and Inceboz 2000).

Once the physiological causes are excluded, such as (pregnancy, lactation and the menopause) the prevalence of amenorrhoea within the general population is approximately 3-4% (Fourman and Fazeli 2015). There are many potential causes of secondary amenorrhoea, which occur either in the hypothalamus, pituitary or the ovaries. The majority of cases fall under four conditions, hypothalamic amenorrheoa, hyperprolactiaemia, polycystic ovary syndrome (PCOS) and ovarian failure, although it may also be due to iatrogenic causes. Menstrual cycle disruption may or may not indicate low estrogen levels; this is dependent on the cause of amenorrhoea.

1.6.1 Hypothalamic causes

Hypothalamic causes occur in about one-third of cases of amenorrhoea (Hinson *et al* 2016). Psychosocial stress, weight changes, under nutrition, and excessive exercise are frequently associated with functional hypothalamic amenorrhoea (Reindollar *et al 1*986, Carpenter 1994, Hannoun *et al* 2007, Wiksten-Almströmer, Hirschberg and Hagenfeldt 2007). Reduced body fat and/or low body mass index (BMI) may impair the regulation and release of GnRH at the hypothalamus (Balen 2000, Hinson *et al* 2016) suggesting that a regular menstrual cycle may not occur if the BMI is less than 19kg/m$^{2.}$

1.6.2 Pituitary Causes

1.6.2.1 Hyperprolactinaemia

Hyperprolactinaemia is the most common pituitary cause of secondary amenorrhoea accounting for approximately one third of cases of amenorrhoea in non-pregnant women (Hinson *et al* 2016). An increased prolactin level inhibits the secretion of GnRH from the hypothalamus, resulting in a decrease in FSH and LH production leading to disturbances of the menstrual cycle.

1.6.3 Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome is the most common endocrine disturbance affecting women, accounting for around twenty per cent of cases of secondary amenorrhoea (Hinson *et al* 2016). It is characterised by signs of hyperandrogenism (hirsuitism, balding, acne) and menstrual disorders including oligomenorrhoea, dysfunctional uterine bleeding, and infertility (ASRM 2004). Unlike other causes of menstrual irregularity, PCOS is not associated with low estradiol levels.

1.6.4 Ovarian failure

Premature ovarian failure or insufficiency, also known as premature menopause occurring before the age of forty, affects around one per cent of women (Balen 2000). Whereas, ovarian failure in a woman over forty years is described as the menopause, “A normal biological stage in a woman’s life that occurs when she stops menstruating and reaches the end of her natural reproductive life” (NICE 2015, p19).

In the UK, the mean age of the natural menopause is 51 years, although this can vary between different ethnic groups (Sarri, Davies and Lumsden 2015). It may be diagnosed clinically after twelve consecutive months of amenorrhoea if no obvious pathological cause can be found (Burger 2006). Biochemically gonadotrophin concentrations (LH and FSH) will increase due to the negative feedback system in the HPO axis, and estradiol (E2) levels will decrease. A reduction in E2 can increase a woman's risk of mortality and morbidity, including cardiovascular disease (CVD), cognitive decline, dementia, and osteoporosis (Rees *et al* 2009).

1.6.5 Iatrogenic causes of amenorhoea

Iatrogenic causes may either be temporary in nature disturbing cycle control, or permanent, including surgical procedures frequently performed within gynaecology such as oophorectomy, hysterectomy and endometrial resection. Various medications, including hormonal contraceptives may be used to purposefully disrupt the menstrual cycle and induce amenorrhoea.

1.6.5.1 Mirena® Intra uterine system (IUS)

A Mirena® IUS releases levonorgestrel (LNG) 20 micrograms per day directly into the uterine cavity. The LNG is quickly absorbed into the basal layer of the endometrium, via the capillary network and into the systemic circulation (Bayer 2014), resulting in potential disruption to the menstrual cycle. A Mirena® IUS does not have a dramatic effect on systemic hormone levels, recent work by Mansour has stated that there is no reduction in systemic estradiol levels during the use of a Mirena® (Mansour D 2017).

1.6.5.2 Opioids

Opioid-induced hypogonadism is well established and appears to be a common complication in both therapeutic and illicit opioid use (Daniell 2008, Katz and Mazer 2009, Vuong *et al* 2010, Darnall *et al* 2012, Wersocki *et al* 2017) in both sexes. Exogenous opioids can exert an effect on the same receptors as endogenous opioids inhibiting or decreasing the release of GnRH, or interfering with its normal pulsatile secretion at the level of the hypothalamus, (Faletti *et al* 1999, Katz and Mazer 2009). In women this results in decreased secretion of LH and FSH being released from the pituitary, leading to inadequate production of female sex hormones.

Potential consequences of opioid-induced hypogonadism include loss of libido, infertility, fatigue, depression, anxiety, loss of muscle strength and mass, osteoporosis, and compression fractures in both men and women; impotence in men; and menstrual irregularities in women (Katz and Mazer 2009, Vuong *et al* 2010, Darnall *et al* 2012, Wersocki *et al* 2017).

1.7 Sex hormones

Sex hormones involved in the function of the female reproductive system can be divided into proteins and steroids, table 1. This section will focus only on the role of female sex hormones as the study described within this thesis investigates only women.

Table 1: Sex hormones: the protein and steroid hormones in women and their primary function.

Key: ■protein hormones, ■steroid hormones**.**

Hormone	Endocrine gland	Primary Hormone Function
Follicle stimulating hormone	Anterior pituitary gland	Stimulates follicle development and oestradiol in the ovary
Leuteinising hormone	Anterior pituitary gland	Stimulates luteinisation of ruptured follicle and progesterone produced in the ovary as well as 'triggering' ovulation.
Prolactin	Anterior pituitary gland	Controls lactation
Ostrogens **- Oestrone (E1),** **- Oestradiol (E2)** **- Oestriol (E3)**	Ovaries -granulosa cells surrounding follicles	Stimulates development of the female reproductive organs, and endometrium
Progesterone	Ovaries -theca cells Adrenal gland	Prepares endometrium for pregnancy and lactation.
Testosterone	Ovaries -theca cells Adrenal gland	Libido / sexual arousal Bone strength Muscle mass and strength

1.7.1 Protein sex hormones

1.7.1.1 Gonadotropins

The protein hormones are water-soluble and circulate freely in the blood. In females the gonadotropins FSH and LH, under the control of GnRH are secreted in a pulsatile manner from the anterior pituitary gland. Within the ovaries, FSH acts to stimulate follicle development whereas LH stimulates production of sex hormones in the ovary as well as during ovulation.

Ovarian steroids, principally estrogens, modulate the secretion of LH and FSH, which in turn regulate the menstrual cycle (Abbott 2016).

1.7.2 Steroid sex hormones

The sex steroid hormones (androgens, estrogens, and progestins) play an important role in human reproductive and sexual function (Traish *et al* 2018). They are all synthesised from cholesterol with conversions between the groups occurring readily, see figure 3: steroid pathways. Circulation in the blood stream can only occur if they are bound to a carrier protein, either a non-specific protein such as albumin, or a specific protein such as sex hormone binding globulin (SHBG). The association with serum proteins suppresses the biological activity of the steroid hormone. An inactive 'pool' is created which protects it from metabolic and chemical alterations, and provides a buffer against sudden changes in active hormone concentrations (Burton and Westphal 1972).

Testosterone and estradiol are transported within the bloodstream, bound loosely to albumin (~54%), and tightly to SHBG (~44%) (Hinson *et al* 2016). Only a very small fraction, about 1-2% is unbound or "free", and therefore biologically active and able to able to enter a cell to activate its receptor (Hammond 2016). Thus, the level of SHBG influences the bioavailability of sex hormones and regulates their access to target tissues. In women SHBG levels are usually about twice as high than those in men limiting their exposure to both androgens and estrogens (Winters 2004).

1.7.2.1 Estradiol (E2)

Estradiol is the primary female sex hormone responsible for the regulation of the female reproductive cycles. Three other naturally occurring forms of estrogen include estrone (E1) and estriol (E3), with estetrol (E4) being produced only during pregnancy. E2 is primarily synthesised within the follicles of the ovary, and in lower concentrations by other tissues including the adrenals, fat, the liver, breasts, and the brain. The major synthesis pathways are shown in figure 3 and involve the formation of androstenedione. This can be converted by aromatase into estrone and then into estradiol. Alternatively, androstenedione can be converted into testosterone, which can then be converted into estradiol. Upon menopause, production of estrogens by the ovaries cease significantly and E2 levels decrease, although postmenopausal women have relatively higher levels of oestrone E1 and E3.

1.7.2.2 Androgens

In women, androgens are secreted in small amounts by both the ovaries and the adrenal glands (Winters 2004). In pre-menopausal women testosterone is synthesised equally (25%) by the ovaries and adrenals, and by peripheral conversion (50%) of androstenedione and dehydroepiandrosterone (DHEA) within the peripheral tissues (Mazer 2002). Despite being a male hormone, testosterone is essential for women exerting its physiological effects in reproductive and non- reproductive tissues in women contributing to sexual, cardiovascular, cognitive, and musculoskeletal health (Davis and Wahlin-Jacobsen 2015).

Dehydroepiandrosterone Sulphate (DHEAS) is the sulphated form of DHEA, a weak androgen that is present in the blood of both men and women. DHEAS is produced by the adrenal cortex and may be converted by the body into stronger androgens, such as testosterone and androstenedione, or converted in small amounts to estrogen by the ovaries (Davis and Wahlin-Jacobsen 2015).

1.7.2.3 Progesterone

Progesterone in non-pregnant women is synthesised in the ovaries and adrenal glands in small amounts. Following ovulation, there is a significant rise in progesterone as the corpus luteum begins to produce progesterone in increasing amounts, preparing the endometrium for implantation of an embryo. Assessing levels of progesterone is used to evaluate function of the corpus luteum i.e. if ovulation had occurred in a menstrual cycle, as part of a full hormone profile screen. However, its interpretation requires correlation with the phase of the menstrual cycle.

1.7.3 Biosynthesis of sex steroids

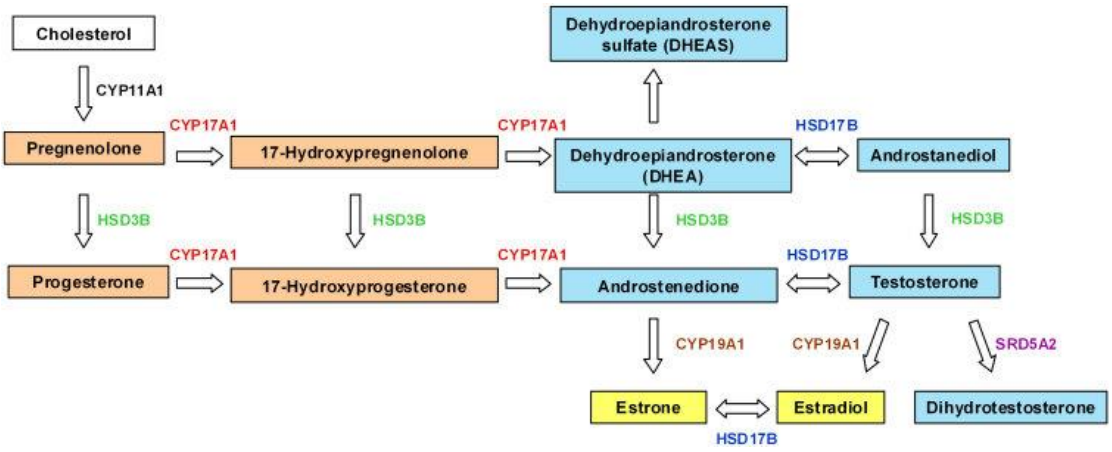


Figure 3: Major pathways in sex steroid biosynthesis. The estrogens are shown in yellow boxes, androgens, precursors to all the estrogens in blue, and progestogens in orange. The enzymes involved in the biosysnthesis of each sex hormone are shown although will not be discussed. Figure from Chen et al (Chen *et al* 2011).

1.7.4 Cortisol

Cortisol is the major glucocorticoid hormone secreted by the adrenal cortex in response to stress as the end product of the HPA axis. Physiological functions include glycogenesis, suppression of the immune system, and to aid in the metabolism of lipid, proteins, and carbohydrates. Cortisol can also suppress reproductive function at the hypothalamic, pituitary, and uterine levels via cross talk between the HPA and HPO axes, promoting the development of amenorrhea as a functional adaptation to stress (Fourman and Fazeli 2015).

Cortisol secretion is pulsatile and follows a diurnal pattern and measurements of this hormone are used as a direct measure of adrenal status. In healthy individuals cortisol plasma levels are known to peak rapidly after wakening with concentrations decreasing by about half towards the evening (Whitley *et al*

1999). This variation corresponds to the circadian rhythm and is referred to as the diurnal cortisol slope (Adam *et al* 2017).

1.8 Pain and hormones

Within the literature there is a dearth of evidence on the effects of pain on hormones levels in females. Consideration of the evidence that suppression of the HPO and HPA axis and menstrual cycle disruption may occur in the presence of chronic pain will be considered below, together with various measures to assess pain and the psychosocial factors that may be associated with chronic pain.

Evidence that HPA axis suppression occurs in association with a variety of pain conditions (Fries *et al* 2005, Petrelluzzi *et al* 2008, Vincent *et al* 2011) is well established, although the mechanisms underlying such suppression are not yet fully understood. Work by Vincent and colleagues showed that serum cortisol levels decreased with increased duration of symptoms in women with dysmenorrhoea (Vincent *et al* 2011), supporting the theory that a system "burn-out" occurs with continuous or repeated activation in response to a stressor, such as pain (Fries 2005).

However, the influence of chronic pain on the activity of the HPO axis has been investigated less. Opiates are known to have a significant effect on the HPO axis, causing significant hypogonadotrophic hypogondism in a relatively large-proportion of recreational opiod users (Daniell 2008, Vuong *et al* 2010).

Opioids have also been found to impair the synthesis of androgen from the adrenals, as seen in oophorectomised women consuming opiates who were found to have significantly reduced circulating testosterone (Daniell 2008).

Whilst work on recreational opiate users, who did not experience pain has also been found to cause suppression of the HPO axis (Vuong *et al* 2010), small studies of non-opioid users with chronic pain suggest that the pain itself may influence or change the hormonal environment (Neeck and Crofford 2000).

It is not known whether chronic pain in itself is a sufficient stressor to disrupt HPO axis activity. However, given that psychosocial stressors are known to be able to alter the regularity of a woman's menstrual cycle or cause amenorrhoea (Neeck and Crofford 2000), it would therefore be plausible that repeated episodes or continuous pain could do the same.

Hormones are primarily known for their role in controlling reproductive processes, such as regulation of the menstrual cycle and supporting pregnancy, yet they are also known to have influences on a variety of other body processes. Work in healthy women by Smith et al and more recently by Vincent et al has demonstrated the key influence of steroid hormones on pain inhibitory pathways within the central nervous system. It now appears that low levels of cortisol, estradiol and testosterone may all exacerbate pain by reducing endogenous mechanisms of analgesia (Smith 2006, Vincent *et al,* 2013, 2014).

1.9 Rationale for the proposed study

The rationale for this study was,

To further our understanding of the extent to which ovarian and adrenal function are clinically and biochemically suppressed in non-opiate using women with chronic pain.

Changes in serum hormone levels of women with chronic pain may have a significant impact on their morbidity and potentially mortality. Health consequences may include, reduced fertility; increased risk of cardiovascular disease and osteoporosis; predisposition to other chronic immune, infective and psychological conditions; and potentially exacerbation of pain symptoms. It is therefore important that we understand the extent to which HPO suppression occurs in women with chronic pain and explore which women are most likely to develop HPO and/or HPA suppression. If significant HPO axis suppression is seen in a proportion of women with chronic pain, there will be the potential for a significant change in future clinical practice.

Whilst correction of hypo-cortisolism is difficult, sex steroidal hormonal therapies are frequently used in modern day medical practice as contraceptives (Guillebaud and Mac Gregor 2013), to control peri and post-menopausal symptoms (NICE 2015) and in the management of chronic gynaecological pain (Moore and Kennedy 2012).

1.10 Research Questions

The research questions to be addressed by this study are:

1. To what extent is the hormone profile of women with chronic pain altered? Specifically what proportion of women are hypoestrogenic, hypoandrogenic and hypocortisolaemic?

2. Do clinical symptoms in women with chronic pain relate to the extent of HPO/HPA axis suppression?

1.10.1 Study objectives and outcome measures

Table 2: Objectives and outcome measures / endpoints of the WIPSOx study

Objectives	**Outcome Measures/Endpoints**
Primary Objective To evaluate the hormonal profile of women with chronic pain	Hormone profiles, obtained from serum samples
Secondary Objectives To evaluate the relationship between characteristics of symptoms and suppression of hypothalamic-pituitary-ovarian and adrenal axes	Hormone profiles, bleeding patterns and measures of pain severity and duration

1.11 Study design

Prospective, observational studies fall under the category of analytical research. This design aims to identify and evaluate causes or risk factors of disease or health-related events (Moore and Kennedy 2012) in a group (cohort) of individuals who share defined characteristics or experience.

Advantages of this approach are that a single study can examine multiple outcome variables simultaneously.

The proposed study is exploratory in nature; the data is looking at questions that have not been explored before to gain new knowledge. It is a non-interventional study; no treatment, intervention, or exposure will be administered to participants, differentiating it from experimental research. This design allows the investigator to draw inferences from a sample of a population by observing individuals without manipulation; and to measure a variety of variables that may be relevant to a condition (Mann 2003). Observational studies are considered a fundamental part of epidemiological research with the use of cohorts often being mandatory, as Song and Chung (Mann 2003) describe, it would be unethical to expose individual to risk (a disease or condition) to undertake a randomised controlled trial.

1.11.1 Limitations

To arrive at a correct study conclusion it is essential to understand any limitations of the study design. One criticism of an observational study is the possibility that the results may be influenced by unidentifiable confounding factors. However, work by Benson and Concato (Song and Chung 2010) have challenged this by showing comparable results between randomised controlled trials and observational studies.

1.11.1.1 Bias

One must consider the influence of bias, both overt and hidden when drawing inferences in observational research (Rosenbaum 1991).

Bias may occur in any design of research and reflects the potential that the sample is not representative of the population it was drawn from (Mann 2003). Furthermore, with a selective sample, a statement can only be made about a population corresponding to these selection criteria. (Mann, 2003). Röhrig et al (2009) suggests that the possibility of generalising the results, and thus raising the external validity of the study, will be greatly influenced by where the participant is recruited from. Whether participants were from specialist hospital pain clinics, or from many different primary care practices for example, and whether to perform the study at a single institution or site, or at several.

To overcome self-selection bias and ensure subjects were representative of women experiencing chronic pain the following recruitment strategies were employed:

- Involve both a hospital and community strategy to advertise the study, aiming to recruit participants who are accessing primary, secondary or tertiary medical care and those who are not receiving medical treatment.
- Offer visits at home, to avoid discriminating against disability, mobility or other issues associated with travel, childcare or fear of the clinical environment.
- Advertise using a range of mediums in a variety of locations.

1.11.1.2 Aims of thesis

The literature reviewed within this chapter suggests that it may be plausible for chronic pain to be a sufficient stressor to disrupt HPO axis activity in women. This thesis has two aims,

i. To investigate the extent to which hormone production is altered in women with chronic pain

ii. To explore whether clinical symptoms relate to this suppression.

2 Methods

This chapter will provide details of the research methods adopted to address the research questions, together with the means of collecting data for analysis.

2.1 Study Design

The research strategy that was adopted was a prospective, observational study named The Women in Pain Study, Oxford (WIPSOx). All study documentation was produced by the WIPSOx study nurse[1] who also set-up and led the study.

2.2 Ethical and regulatory considerations

The study was conducted in conformity with The Declaration of Helsinki (Gandevia and Tovell 1964) and The European Union Clinical Trials Directive (European Union 2001), which requires clinical trials to be conducted according to the principles of Good Clinical Practice (GCP). Ethical approval for this study was granted by the Oxford Research Ethics Committee B, REC reference: 15/SC/0077.

2.3 Study Participants

Women with any chronic pain condition such as pelvic pain and women with chronic musculoskeletal (MSK) pain (back, hip or knee or fibromyalgia) were recruited. Defined and selective inclusion and exclusion criteria were applied to ensure the selected sample would be representative of the study population, see table 3: Inclusion and exclusion criteria.

[1] All study nurse duties were undertaken by the author, Lisa Buck, BSc (Hons) Midwifery.

2.3.1 Inclusion and Exclusion Criteria

The participant was included if they were willing and able to give informed consent for participation in the study. All participants must have experienced pelvic or musculoskeletal pain for more than 50% days/month for at least the last 6 months, were female and be of reproductive age and between 18-50 years old. This age range was of a wide range to focus analysis on reproductive age women, taking into account the difficulties recruiting to studies seeking this group of women who do not use exogenous hormones or are currently, or have recently been pregnant/breastfeeding. However, in view of some women experiencing menopause earlier than the UK average or experiencing premature ovarian failure, it was decided that those with a raised FSH (within the laboratory menopausal range) would be excluded from the hormone analysis.

The participant did not enter the study if any of the exclusion criteria applied at time of screening. The one exception were those women with pelvic pain. They were not excluded if they had a Mirena® IUS in situ as this is frequently used as a treatment for pelvic pain (Moore and Kennedy 2012). Furthermore, recent work by Mansour has stated that there is no reduction in systemic estradiol levels during the use of a Mirena® (Mansour D 2017).

Table 3: Study inclusion and exclusion criteria.

Inclusion criteria	**Exclusion criteria**
Female	Using exogenous hormones for any reason (including contraception*, hormone replacement therapy (HRT), or for the treatment of pain). *Women with pelvic pain were included if they had a Mirena® IUS
Willing and able to give informed consent for participation in the study.	Using mild opiates (e.g. codeine phosphate, tramadol) for fifty per cent of the days within a month.
Aged 18-50 years old.	Used strong or recreational opiates within the last six months.
Experienced pelvic or MSK pain for more than fifty per cent of the days within a month for at least the last six months.	Had a confirmed or suspected pregnancy, or had been pregnant within the last six months
	Currently breast-feeding
	Previous hysterectomy or bilateral oophorectomy
	Any other issue (social, psychological or physical) that may cause harm or affect the participant's ability to participate in the study, e.g. needle phobia, language barrier requiring translation, recent surgery.

2.3.2 Screening and Eligibility Assessment

The Women in Pain Study, Oxford (WIPSOx) study nurse screened potential study participants from July 2015 to December 2017. Eligibility was assessed prior to the research visit against the study inclusion and exclusion criteria on a study screening form. Those that met the inclusion criteria then had a study visit booked. The study-screening form and consent form can be found in Appendices 1 and 2 respectively.

The research visit took place at the University of Oxford Nuffield department of Women's and Reproductive Health, previously The Nuffield department Obstetrics and Gynaecology. This department is located at the Oxford University Hospitals NHS Foundation Trust Women's Centre in Oxford.

Home visits were also arranged to take into account any individual needs such as childcare or mobility issues, extreme fatigue, and concerns about returning to the hospital where a neonatal bereavement took place. Pregnancy tests were offered to potential subjects who were unsure if they might be pregnant. If positive, indicating pregnancy exclusion from the study would have been discussed and participants thanked for their time, interest and support to the study.

2.3.3 Subject recruitment

Study participants were recruited by advertising the study and its need for participants by posters that were displayed in both clinical and public areas of the Oxford University Hospital NHS foundation Trust, and on its website. Following written permission, posters were also displayed within community health care and rehabilitation settings such as GP practices, physiotherapy,

chiropractic and osteopathy clinics, complementary therapy clinics, gyms, and swimming pools. A radio interview was also organised with the BBC on Radio 4 Women's hour. The Primary Investigator was interviewed by the Women's Hour host on the prevalence of chronic pain within the female population and outlined the WIPSOx study.

The Oxford B ethics committee gave permission for approved advertising material to be used via social media. A social media recruitment campaign was set up using Facebook (FB) as an online platform to share the study with pain support groups. Permission to 'post' and share the study on FB was sought and obtained from the administrator of the online support groups.

2.3.3.1 Informed Consent

Written and verbal versions of the 'Participant Information and Informed Consent' leaflet were presented to the participant detailing: the exact nature of the study; what it would involve; implications and constraints of the protocol; and any risks involved in taking part (Appendix 3). The participant was given as much time as they wished to consider the information, and the opportunity to question the investigator, their GP or other independent parties to decide whether they will participate in the study. Furthermore, it was clearly stated that they were free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant personally signed and dated the Informed Consent form before any study specific procedures were performed.

A copy of the signed Informed Consent form was given to the participant with the original being retained at the study site.

2.3.3.2 Participant confidentiality

The study nurse ensured that the participants' anonymity was maintained at all times, with each participant identified only by a unique study ID on the study case report form (CRF) and on the electronic database. All documentation and data was stored securely and only accessible by study staff and authorised personnel. The study complied with the Data Protection Act, The European Union General Data Protection Regulations and in accordance with Universities policy[2]

2.3.3.3 Participant safeguarding

Due to the sensitive nature of some of the questions participants were given time to discuss any concerns with the study nurse at the end of the visit. Each participant was also provided with the study contact details should they wish to discuss anything further. If the participant disclosed any other information and needed support the contact details of relevant agencies, such as The Samaritans, Oxford Rape Crisis, Oxford Domestic Abuse Services and Relate were given.

2.4 Data collection

A detailed self-reporting questionnaire was completed at a single, dedicated research appointment at the John Radcliffe Hospital or in a community setting.

[2] University of Oxford policy on the ethical conduct of research involving human participants and personal data requirements.

On completion of the questionnaire, participants were weighed and measured and their BMI calculated.

The participant's age, date of birth, date of visit, and time that venepuncture was performed was recorded on their participant CRF. Phlebotomy was performed at the end of the study visit and will be discussed further in the section on blood collection.

2.4.1 Study Questionnaire

The study questionnaire (Appendix 4) was completed with the study nurse present in case further explanation or clarification was required. Data collected via the study questionnaire included pain duration, severity and quality of the pain within the last month, together with questions assessing presence or history of other pain symptoms.

The short Form McGill Pain Questionnaire 2 (SF-MPQ-2) assessed intensity and quality of the respondent's pain, whereas The Numerical Rating Scale (NRS) for pain was used to measure pain in that current moment, the strongest pain in the last four weeks, and how strong the pain was on average in the past four weeks.

The NRS is a one-dimensional measure that provides an estimate of pain intensity in adults, including those with chronic pain. Participants were asked to indicate a value on a single eleven-point numerical scale that best described their pain intensity. The pain severity scale had terms describing pain extremes of *"no pain"* and *"worst pain imaginable"*.

Questions seeking information on the occurrence or absence of a menstrual cycle in the previous six months, the date of their last menstrual period, length

of their cycle and regularity of their periods (if present) in the last six months were included. Questions pertaining to other chronic medical conditions, current and previous medication use including analgesics, contraceptives and complimentary or alternative therapies and questions on relevant past obstetric and gynaecological history were also included.

The widely accepted IASP definition of pain identifies that *Pain is an unpleasant sensory and emotional experience (*IASP 2012*)*, therefore various psychological measures were included within the data collection. Depression was assessed using the revised Beck Depression scale (Beck *et al* 1961), anxiety with the State-Trait Anxiety Inventory Scale (Spielberger *et al 1*983). Current and past stressful events were identified with the Holmes and Rahe Social Readjustment Rating Scale (Holmes and Rahe 1967), and Pain catastrophising was measured with the Pain Catastrophising Scale (Sullivan *et al* 1995). Sleep disturbance measures were obtained with The Pittsburgh Sleep Quality Index (Buysse *et al* 1989).

2.4.1.1 Blood collection

Following written and verbal consent approximately twenty millilitres of peripheral venous blood was collected from the participant's antecubital vein. A Becton Dickson (BD) Vacutainer® one-use holder and a BD Vacutainer® Eclipse™ blood collection needle were used with two gold top BD Vacutainer™ SST™ II Advance Tubes. This type of tube is lined with silica particles that when thoroughly mixed with blood activates clot formation and a serum gel separator. The gel is composed of inert components, which are part of a polyester-based blood proprietary formulation which separates the serum and the blood clot during centrifugation (BD 2019). Once filled the tubes were

inverted five to ten times to activate clotting and allowed to stand for thirty minutes at room temperature to allow the blood to clot. Care was taken to avoid haemolysis.

Venepuncture was performed at the end of the study visit so any discomfort from this did not interfere with the response to questions on pain. Blood samples were taken in accordance with local infection control and health and safety policies.

2.4.1.2 Preparation of serum samples

Following the research visit the study nurse transported the blood samples to the Laboratory within the Nuffield Department of Women's and Reproductive Health for processing and storage.

The blood was centrifuged for ten minutes at 3000 revolutions per minute (RPM) at 2186 gx, at four degrees Celsius using a Becman Coulter™ Avanti™ J-20 XP centrifuge. The supernatant (serum) was then divided into two aliquots of 2 ml with a graduated 2ml pipette. The samples were labelled before being transferred to the freezer, identifiable only by subject number, date and time. All samples were stored at -80^{o}C until the end of the study in accordance with the Human Tissue Act 2004 (HTA 2004). Dr Tim James, Head of Biomedical Science, Oxford University Hospital NHS Foundation Trust, approved the study protocol.

2.5 Serum steroid hormones and testing

Serum samples were submitted to the Clinical Biochemistry laboratory at Oxford University Hospital, Oxford for analysis. Samples were batch analysed for estradiol, progesterone, testosterone, follicle stimulating hormone, cortisol,

albumin, Dehydroepiandrosterone sulfate, sex-hormone binding globulin and Vitamin D levels.

2.5.1.1 Immunoassays

All serum samples except albumin were immunoassays analysed using the Abbott ARCHITECT i-2000SR (Abbott 2016) which uses Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. The biological principles of the procedure are described for testosterone below. Specific reagents and the number of steps in all of the immunoassay are listed in table 4.

1. Serum sample, anti-testosterone (mouse, monoclonal) coated paramagnetic microparticles, testosterone acridinium-labeled conjugate and assay diluent are combined to create the reaction mixture.

2. Testosterone present in the sample competes with the testosterone acridinium-labeled conjugate for binding with anti- testosterone (mouse, monoclonal) coated microparticles to form an antigen-antibody complex.

2. After washing, Pre-Trigger and Trigger Solutions were added to the reaction mixture; the resulting chemiluminescent reaction was measured as relative light units (RLUs).

4. An inverse relationship was seen between the amount of hormone in the sample and the RLU's detected by the ARCHITECT iSystem optics.

Exceptions to this step were seen in the SHBG and Vitamin D immunoassays.

The concentration of SHBG within the sample was determined by comparing the chemiluminescent signal in the reaction to the ARCHITECT SHBG calibration. Whereas, an indirect relationship exists between the amount of vitamin D in the sample and the RLUs detected by the ARCHITECT i System optics.

Table 4: Type of immunoassays and specific reagents used for E2, testosterone, albumin, progesterone, Vitamin D, SHBG, DHEA-S, FSH, and cortisol. A delayed immunoassay involved a second incubation (and washing in E2 and DHEA-S immunoassays), a two-step immunoassay added a acridinium-labeled conjugate following washing, such as anti-SHBG acridinium-labeled conjugate for the SHBG immunoassay).

Hormone	**Type of immunoassay**	**Monoclonal**	**Antibody**
Estradiol	CMIA delayed one-step	Rabbit	Anti-estradiol
Progesterone	CMIA, one-step	Sheep Mouse	Anti-progesterone
Testosterone	CMIA, one-step	Mouse	Anti-testosterone
FSH	CMIA, two-step	None	Anti-β FSH coated micro particles
Cortisol	CMIA delayed one-step	None	Anti-cortisol coated paramagnetic micro particles
DHEA-S	CMIA delayed one-step	None	Anti-DHEA-S coated micro particles
SHBG	CMIA, two-step	None	Anti-SHBG coated micro particles
25-hydroxy Vitamin D	CMIA delayed two-step	None	Anti-vitamin D coated micro particles

Spectrophotometry

Serum albumin can be measured by one of two methods, both of which utilise a colour change, forming a coloured complex by a dye binding to human albumin. The absorbance of the complex at a specific unit of length (nm) is directly proportional to the albumin concentration within the sample. In this case abumin was assayed using the dye binding method bromocresol purple (BCP) on the Abbott ARCHITECT c8000.

In the BCP method the absorbance of the complex is measured at 604 nm and is considered more specific than the commonly used bromocresol green (BCG) method, which measures the absorbance at 628 nm (Bachmann *et al* 2017).

2.5.1.2 Inter assay variation

When many samples are to be tested, it is necessary that samples be run on multiple assay plates. To express the precision, or repeatability, of immunoassay test results, measures of the Coefficient of Variability (CV), the Inter-Assay CV(%) is calculated (Salimetrics, 2019). Each assay plate is run with its own calibrators and controls with known concentrations of the analyte, and a high, mid and low value are also included on each plate. The inter-assay CV is an expression of plate-to-plate consistency that is calculated from the mean values for the high and low controls on each plate (as illustrated in table five. From local results as a general guideline, to gauge the overall reliability of your immunoassay results, the inter-assay %CV should be less than 10% (table 5).

Table 5: inter assay variation, quality control samples (low, med, high values) for estradiol, testosterone, albumin, progesterone, Vitamin D, SHBG, DHEA-S, FSH, and cortisol. Precision summary for all the methods based on the last data from January – March 2019, Clinical Biochemistry Laboratory University of Oxford OUH NHS Foundation Trust.

Test	**low IQC**			**mid IQC**			**high IQC**		
	mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Estradiol	191.2	13.00	6.8	785.2	21.3	2.7	1786.7	33.38	1.9
Testosterone	1.48	0.08	5.5	13.54	0.52	3.80	33.1	0.12	3.4
Albumin	28.3	0.41	1.4	no mid qc	no mid qc	no mid qc	52.4	0.62	1.2
Progesterone	1.70	0.15	8.9	32.70	1.51	4.60	59.0	3.21	5.4
Vit D	20.8	1.55	7.5	54.1	2.4	4.5	99.9	3.51	3.5
SHBG	20.0	1.51	7.6	44.4	3.3	7.5	59.9	4.47	7.5
DHEA	0.7	0.03	4.7	4.7	0.1	2.5	19.9	0.39	2.6
FSH	5.0	0.14	2.8	17.4	0.5	2.7	38.5	1.03	2.7
Cortisol	114.0	4.76	4.2	417.4	10.5	2.5	867.4	27.20	3.1

The study participant and their GP were informed of all test results. Any abnormal results identified to have clinical significance were highlighted to the participant and they were advised to discuss this further with their General Practitioner.

2.6 Data management

To maintain accuracy and quality assurance a double data entry technique was employed when entering data into the study database.

2.7 Statistical methods

Data was analysed at the end of the study using the statistical software SPSS®, version 25 (Chicago, Illinois, USA) and Graphpad Prism (San Diego, California, USA). Data checks and cleaning was undertaken prior to any analysis.

Data cleaning was undertaken to identify and correct incomplete or inaccurate records from the database. Any ‘dirty’ data was replaced, modified or deleted to complete the process. Initially unwanted observations, duplicates or irrelevant observations and structural errors such as typographical errors were corrected or removed from the dataset.

For missing numeric data, the values were flagged with an indicator variable (999 in this case) to highlight the observation was missing. The original missing values were then filled with zero to meet the requirement of no missing values.

To identify potential outliers a careful examination of the data set was performed using histograms to visualise the shape of the distribution and scatter plot graphs. Values that were considered outliers were not omitted.

Data explorations were performed using frequency checks, descriptive statistics, normality tests, and where appropriate graphically with histograms for a visual inspection of the distribution. Data was analysed to produce descriptive statistics for both groups covering the range of hormone levels, proportions of women who are amenorrhoeic, hypo-estrogenic, hypoandrogenic and hypocortisolaemic. Initial correlation analyses were undertaken to identify any relationships between serum hormone levels and the severity and duration of symptoms.

3 The influence of chronic pain on menstrual cycle regularity

3.1 Introduction

It is well known that a variety of psychological and physical stressors, including sudden weight loss (Carpenter 1994, Golden and Shenker 1994, Fourman and Fazeli 2015), over-exercising (Loucks *et al 1*989, Carpenter 1994, Fourman and Fazeli 2015), psychological stress (Carpenter 1994, Fourman and Fazeli 2015), chronic disease (Fourman and Fazeli 2015), and opiate use can lead to menstrual cycle irregularity or a period of amenorrhoea. High levels of opiate use for chronic non-cancer pain (Daniell 2008, Rhodin *et al* 2010, Seyfried and Hester 2012, Wersocki *et al* 2017) and recreational use can lead to suppression of ovarian endocrine function, amennorrhoea or even premature ovarian insufficiency (Sofsky *et al* 1975, Vuong *et al* 2010).

Given that psychosocial stressors are known to be sufficient to alter menstrual regularity and even produce amenorrhoea it would not be surprising that repeated or continuous episodes of pain, which is both a psychological and physical stressor, may sufficiently disrupt the HPO axis.

I therefore put forward the following hypothesis,

'Chronic pain itself is a sufficient stressor to impact HPO activity and thus have an impact on menstrual cycle regularity.'

The analysis described here uses menstrual cycle length and regularity as a surrogate for a healthy HPO axis.

3.2 Methods

3.2.1.1 Cohort

The WIPSOx study flow diagram illustrating the number of study participants screened, recruited onto the study and entered into the data analysis for this chapter is shown in figure 4.The demographics and characteristics of the study participant's, types of pain experienced, medical conditions and comorbidities reported by the cohort (n=100) are shown in table six.

Table 6: Study participant's demographics and characteristics. Data are n (%) unless otherwise indicated; N is equal to the total number of participants in the group.

	N (%)
Age (years) 18-15 26-35 36-45 45-50	8/8 25/25 43/43 24/24
Ethnic group White Black Asian Other Not known	 91/91 1/1 5/5 2/2 1/1
Duration of pain (yrs) **≤1** **2-5** **6-10** **11-20** **>20**	 11/11 29/29 25/25 24/24 11/11
Days in pain / month 7-13 14-20 >21 Daily Unknown	 6/6 17/17 13/13 60/60 4/4

Table 6: continued.

	N (%)
Types of pain	
Pelvic	29/29
Musculoskeletal	71/71

Location of pain	
Abdomen	22/22
Arm	27/27
Back	70/70
Bladder	3/3
Buttock	6/6
Breast	2/2
Leg	26/26
Chest	19/19
Facial	7/7
Groin	3/3
Jaw	7/7
Neck	33/33
Ankle	17/17
Elbow	14/14
Hip	35/35
Knee	43/43
Feet/toes	31/31
Hands/fingers	22/22
Shoulder	57/57
Pelvis	22/22

	Median / IQ range
Severity of pain	8 / 1
Psychological measures	
Anxiety (trait)	44.0 / 16
Depression (BDI)	12 / 11
Sleep (PSQI) score	7 / 7
Stress (SRRS) score	132 / 115
BMI	23.9 / 6.5

Table 6: continued.

	N / %
Medical conditions & co-morbidities	
Asthma	23/23
Chronic fatigue syndrome/Myalgic encephalomyelitis	9/9
Chrons disease	1/1
Fibromyalgia	18/18
Diabetes	1/1
Eczema	16/16
Ehlers-Danlos syndrome	2/2
Endometriosis	7/7
Fibroids	5/5
Interstitial cystitis/painful bladder syndrome	10/10
Irritable bowel syndrome (IBS)	27/27
Glandular fever	15/15
Migraine	30/30
Osteoarthritis	11/11
Scoliosis	16/16
Thyroid disease	3/3
Psychological:	
Anxiety	27/27
Depression	39/39
PTSD	1/1

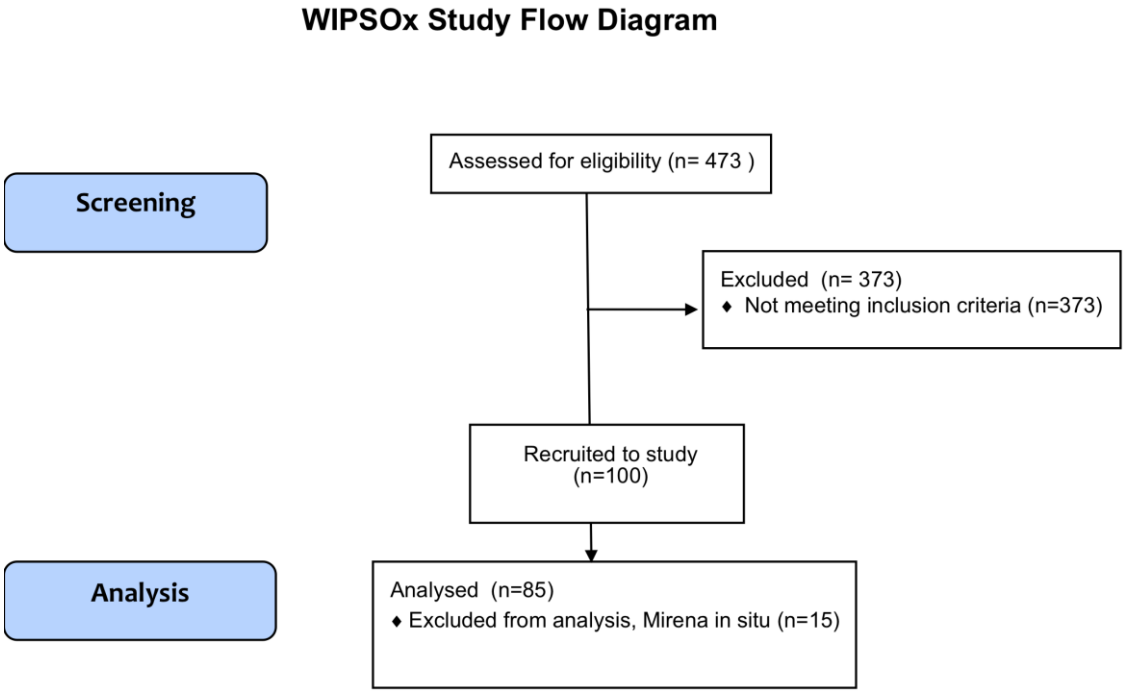


Figure 4: WIPSOx study flow diagram: number of study participants screened, recruited onto the study and entered into the data analysis for this chapter.

3.2.2 Statistical Methods

Data were analysed to produce descriptive statistics exploring the age of the cohort, severity and duration of pain experienced, psychological variables, sleep quality, catatastrophising and body mass index.

The Shapiro-Wilk normality test was used to assess the distribution of the data. Non-parametric tests were undertaken to identify any possible relationships between those with regular menstrual cycles and those with an irregular or absent cycle.

3.3 Results

Seven of the nine variables studied were of a non-normal distribution so the median and inter quartile range for all the data has been reported.

Table 7: Descriptive statistics of the cohort identifying minimum, maximum, median and inter quartile range. Variables included age; duration of pain in years, severity of pain 0-10; trait anxiety (STAI); sleep quality using the Pitsburgh Sleep Quality Index (PSQI); stress using the Social Readjustment Rating Scale (SRSS); Pain Catatrophising Scale (PCS) measured pain catastrophising behaviour and Body Mass Index (BMI)., n=100.

	Median	IQ Range	Min	Max
Age	38	14	19	50
Duration of pain (yrs)	7	11	1	26
Strongest pain (0-10)	8	1	3	10
Trait Anxiety	46	14	23	71
BDI Score	13	10	0	33
PSQI Score	8	7	1	19
PCS Score	19	13	0	52
BMI Score	24.0	6.4	17.6	56.6
SRRS Score	12	445	132	109

Fifteen women who had a Mirena® intra uterine system were excluded from the analysis on menstrual cycle regularity. Work by Mansour found that during the first ninety days after Mirena® placement amenorrhea and infrequent bleeding is experienced by 0% and 11%, increasing to 16% and 57% by the end of the first year of use respectively (Mansour 2017). Therefore, the total number of subjects used in further analysis in this chapter is eighty-five.

3.3.1.1 Regularity and length of menstrual cycles within the cohort

Table 8: Regularity and length of menstrual cycles. Number of women within the cohort (n=85) with a regular menstrual cycle, irregular menstrual cycle, or reported absent cycles within the last six months. Extremely regular (period starts 1-2 days before or after it is expected), very regular (period starts 3-4 days before or after it is expected), regular (period starts 5-7 days before or after it is expected), somewhat irregular (period starts 8-20 days before or after it is expected), irregular (period starts more than 20 days before or after it is expected).

	Number of women (n)	**Proportion of cohort (%)**
Regular cycles Extremely regular Very regular Regular	57 14 23 20	67 16.4 27.0 23.5
Irregular cycles Somewhat irregular Irregular	19 8 11	22.4 9.4 12.9
Amenorrhoea	9	10.6
LMP 1-6 months ago 7-12 months ago >12 months ago Unknown	 74 5 2 4	
Cycle length (days) <24 24-31 32-38 39-50 Too irregular to estimate Amenorrhoea	 8 49 9 4 4 11	

A comparison of women with regular cycles to those with irregular/absent cycles was undertaken using a Mood's Median Test. The scores of the variables measured in all subjects, irrespective of their menstrual cycle regularity are presented in table nine below. Variables include age, duration of pain (years), severity of pain (1-10), anxiety (trait) depression (BDI), Stress (SRRS), catatrophising (PCS), sleep (PSQI) and BMI.

The median age of the sample was forty years old. Nineteen women (22.4%) described irregular menstrual cycles in the last six months and nine women (10.6%) described an absence of their cycle (amenorrhoea) within the last six months.

When comparing women with regular cycles to those with irregular/absent cycles I found no statistically significant differences in any of the following variables, age; duration of pain; severity of pain; trait anxiety; depression; sleep; stress score; catastrophising or body mass index (Table:10).

A second comparison was undertaken comparing those with regular cycles to those with absent cycles, also shown in table ten. This comparison found when women with regular cycles were compared to those with absent cycles (for the last 6 months) the variable of body mass index was found to be significant. Those with a higher BMI had more regular periods.

It must be noted that the sample size of the group with absent cycles was very small, n=9. However, it was observed that there was not even a trend towards statistical significance for any of the other variables measured in this group.

Table 9: Variables measured within the cohort. Age; duration of pain in years, severity of pain (0-10); trait anxiety; sleep quality using the Pitsburgh Sleep Quality Index (PSQI); stress using the Social Readjustment Rating Scale (SRRS); Pain Catastrophising Scale (PCS) and Body Mass Index (BMI). The median and Inter Quartile range is reported with the minimum and maximum for each variable, n=85

	Median	**IQ Range**	**Minimum**	**Maximum**
Age	40	13	22	50
Duration of pain (yrs)	9	12	1	36
Severity of pain (0-10)	8	1	3	10
Trait	44	17	23	71
BDI	11	11	0	33
PSQI	7	8	1	19
SRRS	131	110	12	445
PCS	19	14	0	52
BMI	23.9	5.7	17.6	45.2

Table 10: Comparison 1, women with regular cycles vs those with irregular / absent cycles (amenorrhoea), comparison 2, regular cycles vs those with absent cycles. Analysed using Mood's Median Test (Independent samples median test) p = <0.05, confidence interval = 95%. Variables measured, age; duration of pain in years, severity of pain (0-10); trait anxiety; sleep quality using the Pitsburgh Sleep Quality Index (PSQI); stress using the Social Readjustment Rating Scale (SRRS); Pain Catastrophising Scale (PCS) and Body Mass Index (BMI). The median is reported for each variable, n=85.

	Regular cycles n=57	**Irregular cycles n=19**	**Absent cycles n=9**	**Regular vs Irregular/absent p value / significance**	**Regular vs absent p value / significance**
Age	40	38	46	NS	NS
Duration of Pain (yrs)	9	6.5	9.5	NS	NS
Strongest Pain (0-10)	9	9	8	NS	NS
Trait anxiety	45.4	44	43	NS	NS
BDI	11	15	11	NS	NS
PSQI	7	6.5	6	NS	NS
SRRS	125	128	135	NS	NS
PCS	19.3	19.8	15.1	NS	NS
BMI	24	24.9	22.6	NS	0.040*

*A p of 0.04 (BMI) would not of been significant if corrected for multiple comparisons.

3.4 Discussion

These data have identified that a large proportion of women within the cohort (33%) have disrupted menstrual cycles. The analysis found a one and a half fold increase in rates of irregular cycles and a three-fold increase in rates of amenorrhoea when compared against published data for healthy women. This is a novel finding and given the high prevalence of chronic pain in women of reproductive age is potentially of clinical importance.

These findings have implications for the health and well-being of women with chronic pain. For women wishing to conceive, irregular cycles will reduce the number of fertile days per year, whilst marked suppression of ovarian estrogen secretion in those with amenorrhoea increases the risks of osteoporosis and cardiovascular disease in the long-term.

Despite having measured a variety of variables none was responsible for disrupted menstrual cycles overall, and only one of the measured variables (BMI) was solely responsible for this disruption when women with regular cycles were compared to those with amenorrhoea. Moreover, this significant difference may have occurred by chance because correction for multiple comparisons was not undertaken. Furthermore, the sample size of this group was very small, n=9. However, there was not even a trend towards statistical significance for any of the other variables measured in this group.

There are likely to be multiple mechanisms that can generate amenorrhoea or menstrual cycle irregularity including a raised or reduced BMI, psychological stress or increased age.

However, with the exception of BMI no differences between measured 'stressors' were found across the regular, irregular or absent cycle groups.

Within the cohort there was a wide spread of BMI scores. We know both an increase and decrease of BMI can cause menstrual cycle irregularities and evidence suggests that a regular menstrual cycle will not occur if the BMI is less than 19kg/m^2 as fat is considered essential for the HPO axis to function normally (Balen 2000).

It is likely that both high and low BMI scores accounted for menstrual cycle disruption within the cohort although BMI was found to be the only variable that was statistically significant within the amenorrhoeic group, although this group was small (n=9) and the median BMI was greater than 19kg/m^2.

It is surprising to have not seen age relating to menstrual cycle disruption as some of the older subjects within the cohort may have been experiencing peri-menopausal changes to their menstrual cycle or had actually experienced early menopause. However, without an endocrine profile it is hard to remove the age factor. Within the absent cycle group women were generally older, although this is a small sample size and serum FSH levels would be required to pick out those that had experienced an early menopause.

It is also surprising that the psychological factors measured do not relate to amenorrhoea rates given that we know that psychological stress is sufficient to cause menstrual cycle disruption and amenorrhoea in healthy populations.

It may be that the levels of psychological stress within the cohort as a whole are so high that we do not have sufficient variation to see any differences between those with, and those without amenorrhoea because they are all at the higher end.

This data in isolation identifies a disruption of the menstrual cycle of a large proportion of women within the cohort that is likely to be multifactorial. However, studying this data any further without knowing the underlying mechanism is very difficult. It is essential to have endocrine profiles to be able to exclude those women who are actually already menopausal from ovarian failure. I would also potentially need a larger sample size, with a larger

variation in scores to identify some of these variables, and to be able to account for multiple variables at the same time. Furthermore, by looking at the endocrine profiles of the cohort I would have a larger spread of data than I have just by looking at the crude breakdown of the three groups; regular, irregular and absent. Hormone levels would provide a wider spread of measured ranges that I would then be able to use for correlation analyses.

Enquiring about menstrual cycle disruption is a clinically useful question and could be a quick screening tool within clinical practice to flag up women that we are concerned about. Although to actually help us understand the mechanisms in more detail just asking about cycle disruption is not enough. To be able to really understand the research question in more detail, *is menstrual cycle regularity disrupted in women with chronic pain?* One would need to explore the endocrine profiles of the cohort instead of arbitrarily looking at their cycles.

3.5 Conclusion

This data demonstrates for the first time that women with chronic pain have menstrual cycle disruption compared to healthy controls.

Disruption of the menstrual cycle is likely to reflect a disturbance of Hypo Pituitary Ovarian axis activity. Unfortunately, it is not possible to explain the mechanism for this, or is it a clear clinical predictor using the regularity of a woman's menstrual cycle. There is a need for hormone profiles to interpret this data further and so exploration of the participant's hormone profiles will therefore be the focus of the next chapter.

4 The influence of chronic pain on endocrine profiles

4.1 Introduction

The previous chapter has shown that women with chronic pain have menstrual cycle disruption. The data in isolation identified disruption of the menstrual cycle in 33% of the cohort. Twenty-eight of the eighty-five participants reported irregular cycles or amenorrhoea within the last six months although nothing was found that was able to predict this.

It is essential to have the endocrine profiles to help us understand in more detail whether there is a single pathway that chronic pain is acting through such as hypogonadotrophic hypogonadism even if there are multiple contributory factors generating this. To explore this further hormone levels were studied from the endocrine profiles taken from the study subjects

4.2 Aims

The research question this chapter aim to address is,

What are the hormone profiles of women with chronic pain? I therefore put forward the following hypothesis, '*Chronic Pain is a sufficient stressor to impact the hypothalamic pituitary ovarian (HPO) axis resulting in endocrine disruption.'*

Firstly, I aim to identify the proportion of women with chronic pain who have an endocrine profile that is biochemically hypoestrogenic, hypocortisolaemic and hypoandrogenic.

Secondly, do any of the clinical features that have been measured (age, duration and severity of pain, BDI, STAI, PSQI, SRRS, PCS and BMI) relate to this suppression that then could be used to help predict which women are at risk of endocrine disruption.

4.3 Methods

4.3.1.1 Cohort

Serum samples were collected from the study subjects, n = 100. However, four samples were reported by the laboratory as insufficient and were unable to be analysed. Furthermore, eleven women had an elevated follicle stimulating hormone (FSH) level >30 IU/L. This elevated FSH level is a diagnostic indicator of ovarian failure or menopause, so these women were excluded from any further statistical analysis. Therefore the total number of samples used in this analysis of endocrine profiles was eighty-five.

Within the eleven who were excluded six of these eleven women were clinically hypoestrogenic (estradiol <77 pmol/L), three women had estradiol levels in the lowest quartile, and the remaining two had levels within the normal range.

Thirteen women within the cohort reported that they were using a Mirena® IUS. In view of this an initial comparison analysis will be performed to explore any differences in estradiol, testosterone and cortisol levels in those with a Mirena® IUS in situ and those without.

4.3.2 Statistical methods

Data were analysed to produce descriptive statistics exploring the range of hormone levels and the proportion of women who are amenorrhoeic, hypo-estrogenic, hypoandrogenic and hypocortisolaemic. To calculate the proportions of estradiol, testosterone and cortisol the interquartile range was calculated based on the laboratory reference ranges for each hormone (Table 9). Correlation analyses were used to identify any relationships between serum hormone levels and the severity and duration of symptoms.

To identify potential factors or reasons for any hypogonadotrophism a comparison test was undertaken for a range of variables. The medians of subjects who are below the normal laboratory range (hypoestrogenic, hypoandrogenic or hypocortisolaemic) and in the lowest quartile will be compared against those with normal values in the 'other' (medium and upper) quartiles. The variables that will be explored include, age; duration of pain (in years); severity of pain (0-10); trait anxiety (STAI); depression (BDI, Beck Depression Index); sleep score (PSQI); stress (SRSS); catastrophising and Body Mass Index (BMI). Subsequent comparison analyses were used to investigate if it was possible to predict the hypoestrogenic, hypoandrogenic and hypocortisolaemic subjects based on their menstrual cycle.

Due to the variation of estradiol across the days of a normal menstrual cycle a comparison of estradiol levels above and below the median in women with irregular menstrual cycles and women with amenorrhoea was calculated for age, duration and severity of pain, trait anxiety (STAI), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and body mass index (BMI).

The Shapiro-Wilk normality test was used to assess the distribution of the data. Non-parametric tests were undertaken for non-normal distributions and the median and IQ range reported. A p value of <0.05 was considered significant.

Summary of the statistical tests that will be undertaken within this chapter:

1. Comparison of subject with Mirena® IUS (n=13) and without (n=72), Man-Whitney U Test.
2. Proportions of hormones estradiol, testosterone and cortisol – descriptive statistics
3. Relationship between between E2 and testosterone – Spearman's Rank correlation
4. Potential factors for hypogonadotrophism – Mood's Median Test[3]
5. Cycle variation of E2: median split comparison in women with irregular cycles and amenorrhoea – Independent samples comparison test.
6. Predicting hypoestrogenism, hypoandrogenism and hypocortisolaemia based on the regularity of the menstrual cycle – Mood's Median Test: Independent samples comparison test.

[3] Mood's Median Test (non-parametric test) is a special case of Pearson's chi-squared test.

The analysis presented here uses normal laboratory references ranges, provided by the testing laboratory where the samples were analysed, shown in table 11: below.

Table 11: Biochemistry reference ranges (*female only values stated) from the Clinical Biochemistry Laboratory, Oxford University Hospital NHS Foundation Trust, Oxford.

Analyte	**Reference range**
Albumin	32-50 g/L
Cortisol	Before 10am: 101.2 – 535.7 nmol/L After 5pm: 79 – 477.8 nmol/L
Dehydroepiandrodosterone Sulphate* (DHEAS)	15-29 yrs: 1.33 – 12.46 µmol/L 30-39 yrs: 1.66 – 11.83 µmol/L 40-49 yrs: 0.96 – 12.66 µmol/L 50-59 yrs: 0.00 – 10.37 µmol/L
Follicle Stimulating Hormone* (FSH)	Pre-menopausal follicular/luteal phase: 1.0-8.0 IU/L. Ovulatory peak: up to 20 IU/L Post-menopausal >30 IU/L
Luteinising Hormone* (LH)	Pre-menopausal follicular/luteal phase: 2-12 IU/L Ovulatory peak: up to 20-90 IU/L Post-menopausal >30 IU/L
Estradiol* (E2)	Follicular phase: 77-920 pmol/L Mid-cycle peak: <2400 pmol/L Luteal phase: 77-1145 pmol/L
Progesterone*	Post-menopausal/follicular phase: <2 mol/L Luteal progesterone of > 25 nmol/L Usually indicates ovulation.
Sex Hormone Binding Globulin* (SHBG)	18-49 years: 34-148 nmol/L ≥50 yrs" 16-118 nmol/L
Testosterone* (Total)	>14 yrs: 0.5-2.6 nmol/L
25-hydroxyvitamin D	>50 nmol/L – vitamin D sufficiency 30-50 nmol/L – vitamin deficiency <30 nmol/L – severe vitamin D deficiency

The laboratory reported testosterone as <0.5 if it is below the normal laboratory range (<0.5 – 2.6 nmol/L), thus indicating a woman is hypoandrogenic. For the purposes of this analysis all values reported as <0.5 have been changed to 0.4 as the less than symbol (<) is not recognized by the statistical software package SPSS®.

Furthermore, cortisol levels were corrected for the time of day that they were taken, before 10am or after 5pm.

4.4 Results

4.4.1 Comparison of subjects with and without a Mirena® IUS.

A comparison analysis was performed to explore any differences in estradiol, testosterone, cortisol and FSH levels in those with a Mirena® IUS in situ and those without. All samples were included in this analysis, n=85. A Mann-Whitney U Test found that there was no statistical difference in estradiol, testosterone, cortisol and FSH levels between the two groups, (table: 12). Therefore, the serum results from women who had a Mirena® in situ were used in all further analysis.

Table 12: Study subjects with 'yes' and without 'no' a Mirena® ('Yes/No') were compared with a Mann-Whitney U Test. Median and IQ range were calculated for each group. A p value of less than 0.05 was considered significant.

Median / IQ range	Total cohort n=85	'NO' Mirena® n=72	'YES' Mirena® n=13	Significance
Estradiol	295 / 295	285 / 267	308 / 440	0.903 NS
Testosterone	0.9 / 0.5	0.9 / 0.5	1.0 / 0.6	0.377 NS
Cortisol	186 / 97	180.5 / 91	204 / 141	0.650 NS
FSH	4.85 / 3.9	4.85 / 3.9	4.0 / 3.5	0.211 NS

4.4.2 Proportions of estradiol, testosterone and cortisol

Of the eighty-five subjects four women (4.7%) were biochemically hypoestrogenic and 8 women (9.4%) were biochemically hypoandrogenic when compared against the laboratory reference ranges. Two women (2.4%) were found to be both hypoestrogenic and hypoandrogenic, and one women was both hypoandrogenic and hypocortisolaemic (1.2%).

All of these cases have been described further in tables thirteen to sisteen, in which the participant's age, location, duration, severity and days per month in pain have been described in detail. Psychological scores, anxiety (trait) and depression (DBI), stress, catastrophising and sleep scores together with BMI and LMP.

Table 13: Characteristics of those subjects who were biochemically hypoestrogenic with subject's age (yrs), duration (yrs) and severity of pain (1-10), anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and BMI, n=2.

Characteristic	**Hypo estrogenic subject 1**	**Hypo estrogenic subject 2**
Age	23	50
Location of pain(s)		
Days of pain/month	Daily	Daily
Duration of pain (yrs)	7	2
Severity of pain (0-10)	10	8
Trait score	51	23
BDI score	13	4
PSQI	11	5
SRSS	-	13
PCS	27	9
BMI	24.0	24.9
Last period (months ago)	1-6	>12

Table 14: Characteristics of those subjects who were biochemically hypoandrogenic with subject's age (yrs), duration (yrs) and severity of pain (1-10), anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and BMI, n=5.

Characteristic	**Hypo androgenic subject 1**	**Hypo androgenic subject 2**	**Hypo androgenic subject 3**	**Hypo androgenic subject 4**	**Hypo androgenic subject 5**
Age	44	48	36	44	43
Location of pain(s)	Back (scoliosis)	Joint pain: hip, wrist, shoulder (Osteoarthritis)	Arm, face, ear, jaw (radicular neuropathic pain)	Head, abdomen, breast (migraine, breast adenoma).	Groin
Days of pain/month	daily	14-20	daily	daily	Daily
Duration of pain (yrs)	3	4	1	4	13
Severity of pain (0-10)	8	6	6	9	9
Trait score	57	43	41	45	61
BDI score	25	1	16	16	33
PSQI	14	5	6	11	15
SRSS	81	35	110	124	51
PCS	17	4	21	12	36
BMI	19.3	20.9	20.3	18.6	18.9

Table 15: Characteristics of those subjects who were biochemically hypocortisolaemic with subject's age (yrs), duration (yrs) and severity of pain (1-10), anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and BMI, n=.3

Characteristic	**Hypo Cortisolaemic subject 1**	**Hypo Cortisolaemic subject 2**	**Hypo Cortisolaemic subject 3**
Age	25	38	34
Location of pain(s)	Back, neck, jaw (4 fractured vertebrae)	Shoulder, neck, back, joints (spinal deformity)	Back, neck, wrist
Days of pain/month	Daily	7-13	>21
Duration of pain (yrs)	1	20	10
Severity of pain (0-10)	9	8	8
Trait score	48	42	67
BDI score	8	3	31
PSQI	6	5	9
SRSS	96	233	152
PCS	22	25	19
BMI	20.8	27.0	37.3

Table 16: Characteristics of those subjects who were biochemically hypo estrogenic and hypoandrogenic (n=2) and hypoandrogenic and hypocortisolaemic (n=1) with subject's age (yrs), duration (yrs) and severity of pain (1-10), anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and BMI, n=.3

Characteristic	**Hypo estrogenic and hypo androgenic subject 1**	**Hypo estrogenic and hypo androgenic subject 2**	**Hypo androgenic and hypo cortisolaemic subject 1**
Age	26	46	33
Location of pain(s)	Fibromyalgia	Shoulder, hips, foot	Back, neck, head, joint pain: shoulder, hip.
Days of pain/month	14-20	1-6	daily
Duration of pain (yrs)	2	10	5
Severity of pain (0-10)	8	7	8
Trait score	54	61	54
BDI score	25	18	16
PSQI	14	10	19
SRRS	73	194	292
PCS	16	7	15
BMI	17.6	23.5	19.0

A further 56.5% of the women (n=48) had estradiol levels in the lowest quartile of the normal range, and 47% (n=40) had testosterone levels in the lowest quartile. Four (4.7%) women were hypocortisolaemic with 39 (46 %) women within the lowest quartile. See figures 5, 6 and 7.

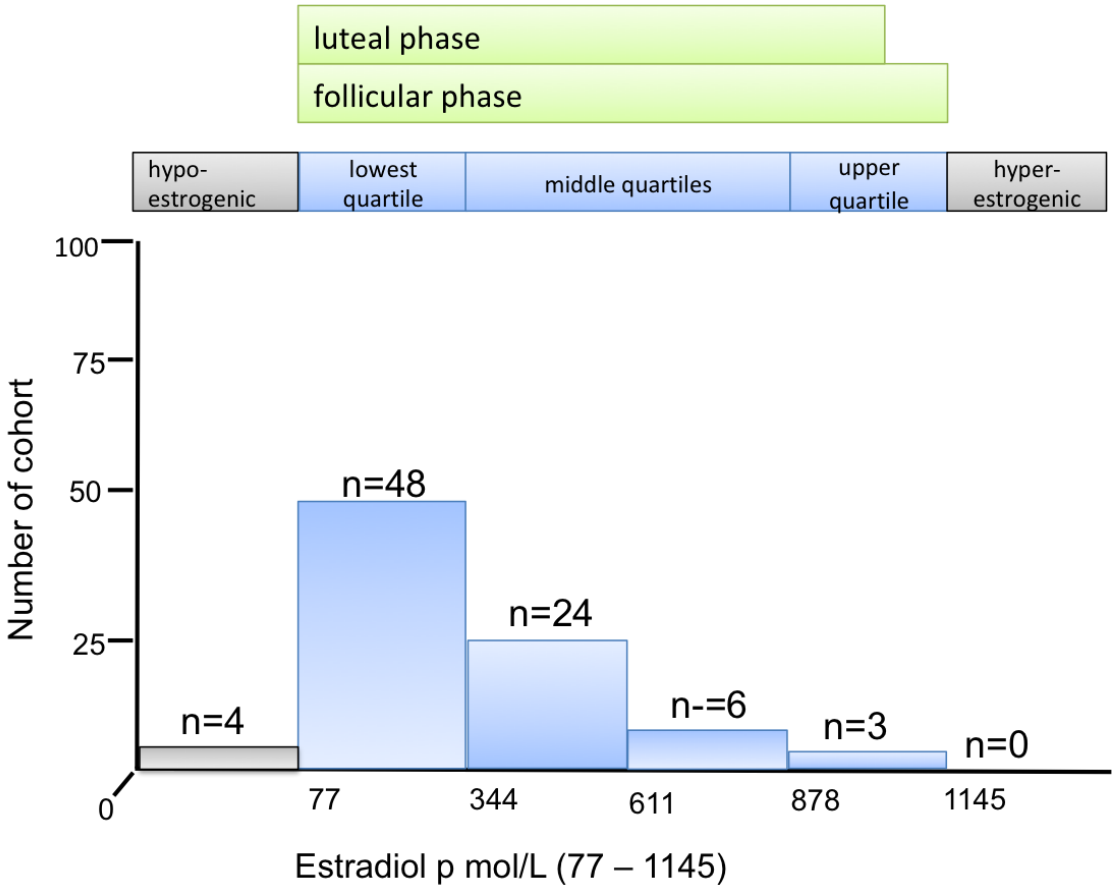


Figure 5: Graphical representation of estradiol levels and number of subjects from each quartile of the cohort, n=85.The follicular and luteal phases of the menstrual cycle are also shown. The normal reference range for estradiol is 77-1145 pmol/L across the menstrual cycle.

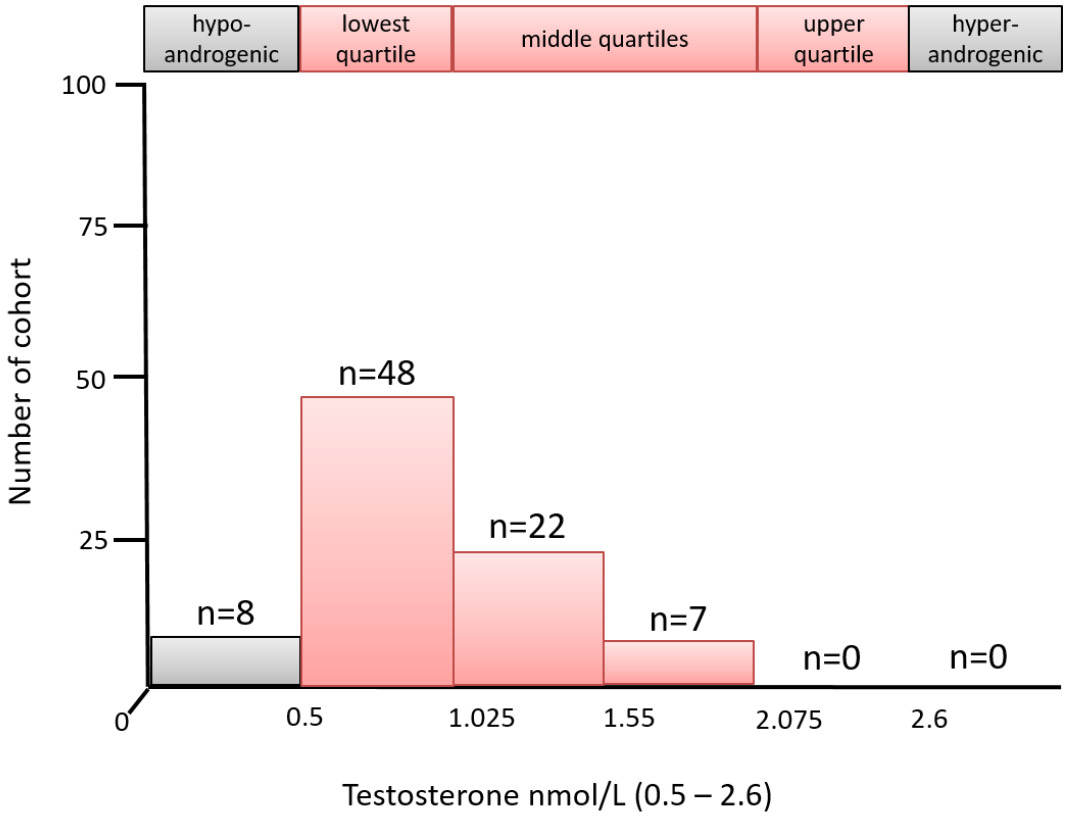


Figure 6: Graphical representation of testosterone levels and number of subjects from each quartile of the cohort, n=85. The normal reference range for testosterone is 0.5-2.6 nmol/L.

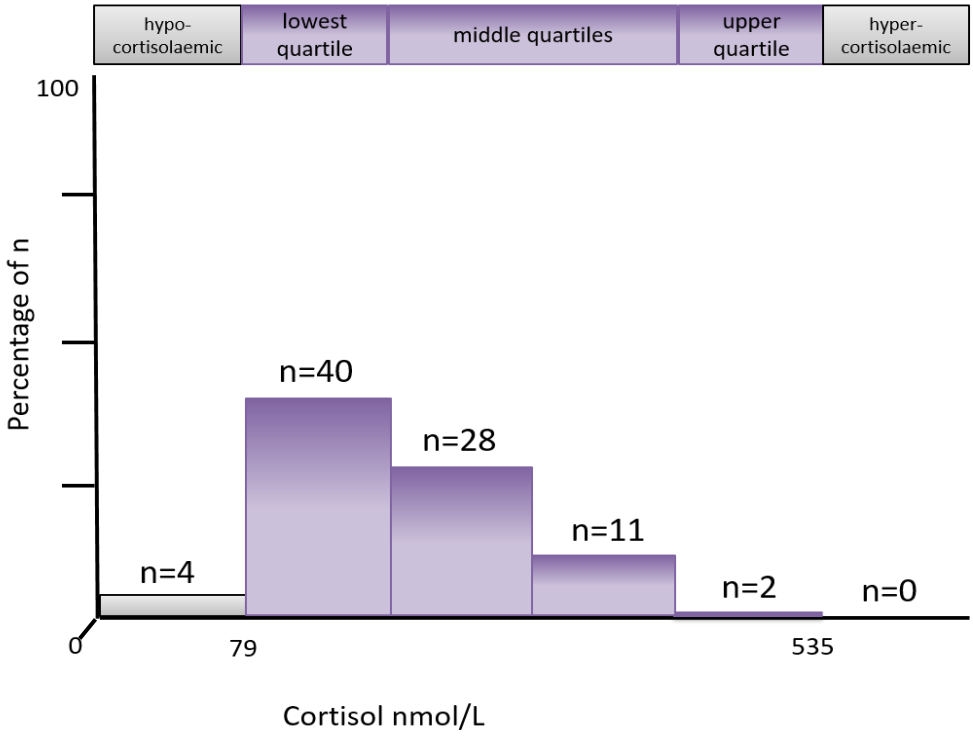


Figure 7: Graphical representation of cortisol levels and number of subjects from each quartile of the cohort, n=85. The normal reference range for cortisol is 79-535.7 nmol/L. (Cortisol levels were normalised for the time of day, before 10am or after 5pm or after as shown in table 9).

4.4.3 Correlation between estradiol and testosterone.

A Spearman's Rank correlation, non-parametric test was used to measure the strength of association between estradiol and testosterone

This analysis found that there was no significant correlation (p=0.876, rho= -0.017) between estradiol and testosterone serum levels in women with regular, irregular or absent cycles, or any consistent pattern found within the cohort. The majority of the cohort had low testosterone and low estradiol with over 50% of the cohort being hypoandrogenic, hypoestrogenic or falling within the lowest quartile. There was still no significant correlation (p= 0.806, rho= -0.064) when women with regular cycles or a Mirena® in situ were excluded.

4.4.4 Potential factors for the hypogonadotrophism identified

This analysis explores whether there are other factors or reasons for the hypogonadotrophism identified within the cohort?'

Mood's Median Test compared Hormone levels that fell below the normal range (hypo samples) and those from the lowest quartile against those in the 'other' quartiles (medium and upper quartiles) were compared for the following variables, age; duration and severity of pain, trait anxiety, depression, sleep score, stress, catastrophising and BMI.

Because of the small sample size of the hypoestrogenic, hypoandrogenic and hypocortisolaemic groups they were combined with samples from their respective lowest quartile for statistical analysis. The statistical test was repeated for estradiol, testosterone, and cortisol.

A Mood's Median Test was performed to compare the medians of those women who were hypoestrogenic, hypoandrogenic and hypocortisolaemic and in the lowest quartile against those in the 'other' quartiles (middle and upper quartiles) in each hormone, for nine of variables. Variables included: age (yrs), duration (yrs) and severity of pain (1-10), anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and BMI. Significance level=0.05, Confidence Interval level=95, n=85.

Comparing subjects who were hypoestrogenic and in the lowest quartile against those who had normal serum estrogen levels found no significant statistical differences in any of the following variables; age, duration of pain, severity of pain, trait anxiety, depression, body mass index, sleep, or catastrophising. This was also the case for those subjects who were hypocortisolaemic.

However, when comparing subjects who were hypoandrogenic against those who had normal serum androgen levels (testosterone) age was the only difference that was found to be statistically significant, p=0.000, median age was 42.5 years old (Figure 8, graph B)..

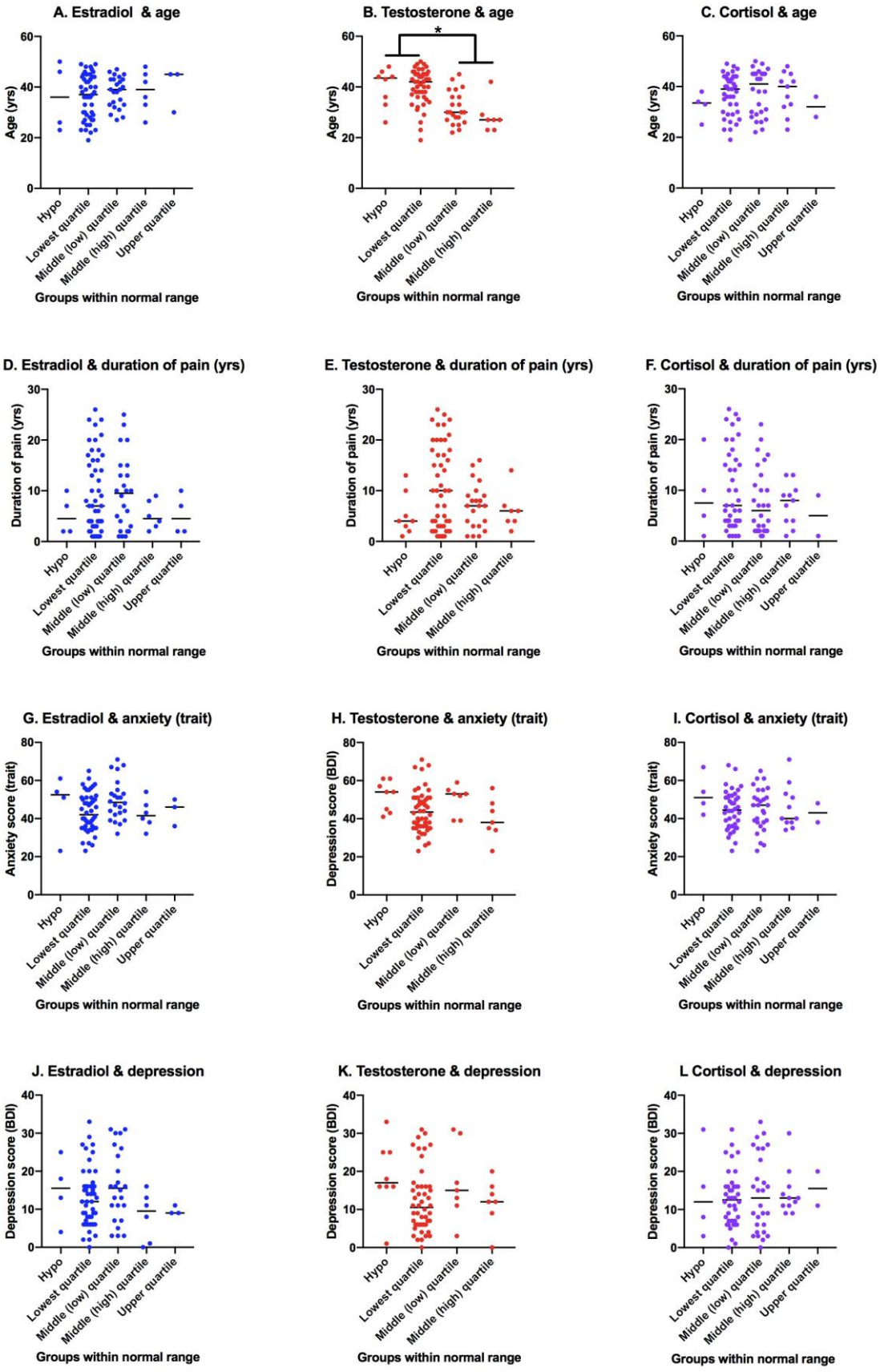


Figure 8: Scatter plots illustrating the spread of values for age (yrs), duration of pain (1-10), anxiety and depression in the following five groups: hypo, lowest, quartile middle quartiles and upper quartile for estradiol, testosterone and cortisol (Graphs A-I).

Graph B: Independent Median Test for testosterone (samples who were hypo, lowest and 'other' quartiles) Comparison of subjects who were hypoandrogenic and in the lowest quartile against those who were in the 'other' quartiles, n=85. Significance level=0.05, Confidence Interval level=95. NS = not statistically significant.*Other quartiles = medium and upper quartiles combined.

The scatter plot graphs show that there was no variation in the duration of pain across the three hormones by plotting them in five groups; hypo, lower quartile, middle quartiles, and upper quartile. Pain severity clustered at the upper end of the 1-10 rating scale for all three hormones. However, more variation was evident within the psychological scores than the measures of stress and sleep in the five groups across the three hormones.

4.4.5 Cyclical variation of E2: Median split and comparison

A Mood's Median Test was then undertaken to test the null hypothesis, *"The medians of age, duration and severity of pain, anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising (PCS) and body mass index (BMI) are the same above and below the median in the absent and irregular cycle group of estradiol samples".*

The median estradiol level of the irregular and absent menstrual cycle group was 181, n=27. The samples were divided into two groups, those above the median and those below the median (table: 17).

Table 17: Median Test to compare the group above and below the median of estradiol for age, duration and severity of pain, anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and BMI in women with irregular and absent cycles (amenorrhoea), n=27, Significance level 0.05, confidence interval level =95.

Median / IQ range	**Estradiol: Irregular + absent cycles combined n=27**	**Above median n=14**	**Below median n=13**	**Estradiol Irregular + absent cycle Comparison (p=)**
Age	29/17	42/17	27/7	0.004 S
Duration of pain (yrs)	4/7	2.75/7	6.5/9	0.082 NS
Severity of pain (0-10)	8/1.0	8/1.3	8.5/1.5	0.645 NS
Trait anxiety	47/14	47/14	48/15	0.555 NS
BDI	15/11	14.5/8	15/17	0.384 NS
PSQI	8/6	9.5/6	8/7	0.567 NS
SRSS	135/90	146.5/94	127/139	0.501 NS
PCS	21/13	23/21	21/11	0.330 NS
BMI	24.9/9.6	23.0/17	24.9/12.8	0.520 NS

Comparing estradiol samples above and below the median for age, duration and severity of pain, anxiety (trait), depression (BDI), sleep (PSQI), stress (SRSS), catastrophising and body mass index (BMI), in the irregular and absent cycle group found that age was the only statistically significant variable, median=181, p=0.004.

4.4.5.1 Predictor comparison

(Median Test to compare two independent samples)

Those with a Mirena® were removed to look again at those who were hypoestrogenic, hypoandrogenic and hypocortisolaemic. *'Is it possible to predict these states based on menstrual cycle groups?"* Can those subjects who are hypoestrogenic, hypoandrogenic or hypocortisolaemic be predicted by the regularity of their menstrual cycle?

A Mood's Median Test, comparing two independent samples was undertaken to test the null hypothesis *"The medians of estradiol, testosterone and cortisol are the same across the categories of regular and irregular+absent cycles."* The test was then repeated with regular vs absent cycles. Both Median tests were undertaken for each hormone, estradiol, testosterone and cortisol.

Comparison test 1: Regular cycles vs irregular+absent cycles

Comparison test 2: Regular vs absent cycles

Because of the sample size of the absent menstrual cycle groups they were combined with samples from the irregular menstrual cycle group for statistical analysis.

However, for the purposes of illustration in the tables below (tables 18-20) they have been separated. Removing those with a Mirena® reduced the number of subjects for this analysis to n=72.

Table 18: Descriptive statistics for estradiol in regular, irregular and absent menstrual cycle groups. Median tests to compare differences across regular vs irregular+absent and regular vs absent menstrual cycle groups. Significance level =0.05, confidence interval level =95.

Estradiol	Regular n=56	Irregular n=12	Absent n=4	Regular vs irregular cycles + absent cycles	Regular vs absent cycles
Median	317.5	175.5	192.0	0.395 NS	0.607 NS
IQ range	268	190	382		
Min	36	98	36		
Max	966	362	530		

Table 19: Descriptive statistics for total testosterone in regular, irregular and absent menstrual cycle groups. Median tests to compare differences across regular vs irregular+absent and regular vs absent menstrual cycle groups. Significance level =0.05, confidence interval level =95.

Testosterone	Regular n=56	Irregular n=12	Absent n=4	Regular vs irregular cycles + absent cycles	Regular vs absent cycles
Median	0.9	1.2	0.65	0.103 NS	0.865 NS
IQ range	0.3	0.7	1.1		
Min	0.4	0.5	0.4		
Max	1.6	2.0	1.8		

Table 20: Descriptive statistics for cortisol in regular, irregular and absent menstrual cycle groups. Median tests to compare differences across regular vs irregular+absent and regular vs absent menstrual cycle groups. Significance level =0.05, confidence interval level =95.

Cortisol	Regular n=56	Irregular n=12	Absent n=4	Regular vs irregular cycles + absent cycles	Regular vs absent cycles
Median	180.5	229.5	171.0	0.777 NS	0.607 NS
IQ range	77	115	49		
Min	34	49	129		
Max	459	435	193		

Comparing those subjects with a regular menstrual cycle against those who had an irregular and absent or absent cycle found no significant difference in their estradiol, testosterone or cortisol results in either comparison. Therefore, asking about a woman's menstrual cycle is not enough; it cannot be used to predict which women will be hypoestrogenic, hypoandrogenic or hypocortisolaemic.

4.5 Discussion

This is the first study to our knowledge to examine HPO axis suppression in women with chronic pain conditions who are not consuming opiates. These data have identified that a significant proportion of women with chronic pain have a disturbance to their endocrine profile, which may have long-term implications for their physical health, cardiovascular health and fertility.

This has major public health implications that need to be taken into consideration in clinical practice.

This chapter explored the endocrine profiles of all subjects after removing those women who were actually already menopausal from ovarian failure. These analyses found low levels of estradiol and testosterone in over half of the cohort of women, sixty one per cent and sixty five point eight per cent respectively. Thus for the majority of women with low / or very low estradiol levels the underlying mechanism is not hypogonadism.

Such changes in serum hormone levels in women may have a significant impact on their morbidity and potentially mortality. Health consequences include, reduced fertility; increased risk of cardiovascular disease and osteoporosis; psychological sequelae; and potential exacerbation of pain symptoms as previously discussed in chapter one.

Despite having measured a variety of variables (age, duration and severity of pain, anxiety (trait), depression, sleep, stress, catastrophising and BMI) to explore possible factors or reasons for the hypogonadotrophism identified within the cohort there was no significant difference between those that did have a suppressed endocrine profile and those that did not.

The only significant difference found between the hypestrogenic, hypoandrogenic and hypocortisolaemic groups was age, whereby the median age was significantly higher in those who were hypoandrogenic and those in the lowest quartile when compared against those in the middle and upper quartiles (42.5 vs 29, p=0.000). However, it is already known that androgen levels decrease with increasing age (Spencer *et al* 2007) and is to be expected, this is therefore unlikely to be pain related.

When looking in detail at the comparison tables very little differences were seen across the groups with very low levels and those that were normal. This is a surprising result given what we know from the literature that a variety of psychosocial and physical stressors can impact the HPO axis by altering menstrual cycle regularity, as previously discussed. It may well be that the whole cohort have such high levels of pain severity, pain duration, anxiety, stress, depression and sleep disturbance that it is not possible to dichotomise a group.

When exploring the data in detail, some of the variables measured could be a driver for endocrine suppression although the sample size is not big enough to capture this. There is no statistical difference in severity of pain, across the three hormone groups. The whole cohort had severe pain, the median was eight, the IQ range was one, the maximum was ten and the minimum was three. Whereas if the cohort was bigger and had a larger variation in scores it may be possible to identify some of the factors causing the hypogonadotrophism.

This analysis has shown that it is not possible to identify, out of the factors we have measured except the pain, what is causing suppression of the HPO axis to help predict those women who are at risk of endocrine suppression based on psychological, social or physical variables.

Despite being unable to identify a predictor to identify those subjects who were hypoestrogenic, hypoandrogenic or hypocortisolaemic by the regularity of their menstrual cycle, or the duration/severity of their pain it has highlighted the important fact that we must not assume, that the longer a patient has been in

pain, or the more severe the pain or levels of anxiety are equal to the level of endocrine suppression.

These data have also shown that it is not possible to predict from the clinical data alone which women will have endocrine suppression, or to see how predictive it is for the level of suppression by the regularity of their menstrual cycle. A simple question “how regular is your menstrual cycle?” is unable to predict which women are hypoestrogenic, hypoandrogenic or hypocortisolaemic based on the analysis of the endocrine samples and menstrual data together.

A linear regression model would be a subsequent analysis to evaluate the variables that may influence hormone profiles, while accounting for other confounding variables that may be associated. However, building this type of model is not within the scope of this thesis because of the sample size. Due to the nature of estradiol across the menstrual cycle the analysis would only be able to use those sample with a regular menstrual cycle. This would then split the E2 sample reducing the size that is required for each outcome variable in this type of model. To perform this in the future a larger sample size would be required.

4.6 Conclusions

These data demonstrates that disruption of the hypothalamic pituitary ovarian axis is common in women with chronic pain. In women of reproductive age with chronic pain low levels of estradiol and testosterone were observed in over half of the cohort.

These findings have implications for the health and wellbeing of women living with chronic pain and suggest that assessing endocrine status or at least enquiring about menstrual cycle regularity within clinical practice should be considered.

5 Discussion

This observation study has provided information on the ‘real world’ to help contribute to this under researched area in the field of pain research. Since I began this work further emphasis has been made on gender and sex differences in the field of pain, raising awareness and recognition of pain issues affecting women worldwide.

It is hoped these findings will contribute to further our understanding of the extent to which ovarian and adrenal function are suppressed in non-opiate using women with chronic pain. To help formulate hypotheses to be tested in future experiments, and the design of future clinical trials to inform changes in clinical practice.

5.1 Limitations

5.1.1.1 Sample size

One of the limiting factors of this thesis is the sample size that I was able to achieve. This is something that is very difficult within this field and it became apparent very early on that recruiting a large sample size was very challenging. I therefore looked into all avenues and decided upon a variety of approaches including, traditional recruitment posters in healthcare and community locations, the World Wide Web, and patient support groups via social media to advertise and raise awareness of the study. Not only to advertise widely, it was essential so the results could be generalised to the female population, and ensure it was a fair representation of the chronic pain population.

Of 473 women who contacted the WIPSOx study nurse 100 were eligible to participate. The data in response to each recruitment method is shown in figure 9.

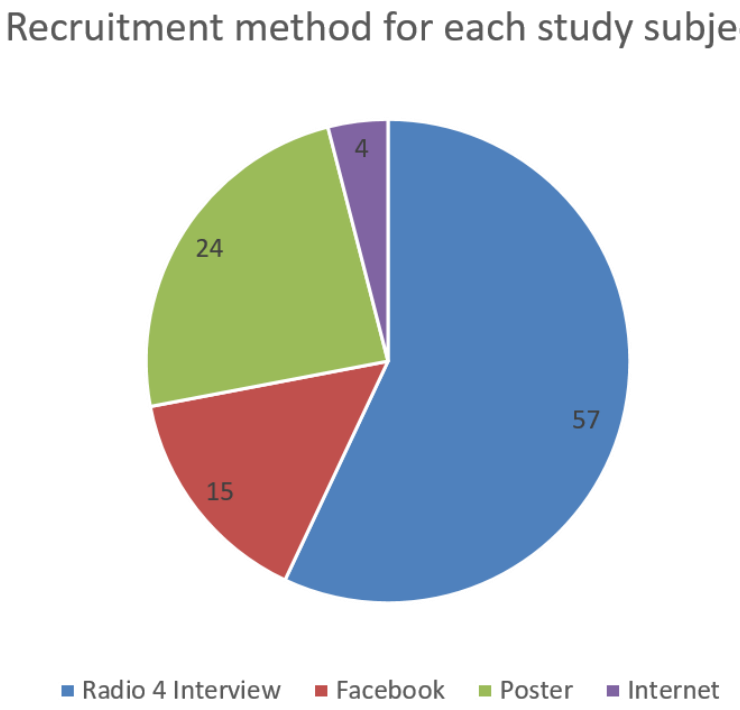


Figure 9: Recruitment method for each subject that participated in the WIPSOx study.

I also managed to organise a spot on BBC Radio 4 Women's Hour, persuading my supervisor to be interviewed about the study and put out a recruitment plea. Despite this I was only able to recruit one hundred participants within the time frame and funding that was allocated for recruitment. This was a limiting factor of the study and clearly if there had been many more participants it may have been possible to identify further significances. Furthermore, to understand what is actually causing suppression of the HPO axis there is a need for further analysis with a much larger sample size as previously discussed.

5.1.1.2 Serum hormone samples

The serum samples provided by the participants provided information about the concentration of the hormone at a single point in time. Ideally, measuring bioavailable steroid hormones over at least one complete menstrual cycle is considered to provide the most accurate information on the role of the measured hormone on body tissues and disease (Bellem *et al* 2011). However, this depends on the subject having one complete menstrual cycle and it was hypothesised that a significant proportion of the cohort would not. Therefore, this method of testing hormones was not possible and so a single sample was taken.

The age of the sample would also be re-considered if this study was to be repeated. In view of the wide peri-menopausal age range the study age may be limitied to a maximum age of 45, or even perhaps 35 years old to limit the number of subjects with menstrual cycle disturbances or biochemical changes due to peri-menopause or menopause.

Furthermore, women with Mirena® IUS would also not be included if this study were to be repeated or in any further work in this area. The difficulties recruiting women based on past studies within the department were not seen during the recruitment phase for this study. Women had to be excluded from parts of the analysis due to the side effect of menstrual cycle irregularities that the Mirena® system offers, thus limiting the findings.

One further point to consider is that women may have participated in the hope of obtaining a hormone profile to help explain symptoms they may have been experiencing, potentially accounting for the increased proportion of ammenorrehoea within the cohort. However, this is merely an assumption.

This study focused on women with chronic pelvic or musculoskeletal pain, however, it is hoped that the findings of this study will translate into further work of direct clinical benefit to women with a variety of chronic pain conditions. In addition, by undertaking this work I believe highlighted to the many women in pain that this is an important area within the scientific community, research *is* taking place, thereby giving them recognition and hope.

6 Conclusions and Future Work

The study discussed within this thesis aimed to assess the hormonal environment of women with chronic pain, and to establish whether a relationship exists between disease characteristics and the extent of HPA/HPO axis suppression.

I have answered the research questions I set out to answer and found that chronic pain is a sufficient stressor to impact HPO activity and thus have an impact on menstrual cycle regularity. High rates of menstrual cycle disruption (33%) were found within a cohort of women with chronic pain conditions, which is a three-fold increase in rates of amenorrhoea when compared against rates in the normal population. This is a novel finding and given the high prevalence of chronic pain in women of reproductive age is potentially of clinical importance. I have also shown that simply asking the question regarding menstrual cycle regularity is not predictive of endocrine disruption, biological tests are needed.

These data have also identified that a significant proportion of women with chronic pain have disruption to their endocrine profile with low levels of estradiol and testosterone seen in over half of the cohort. Such changes to serum hormones levels have the potential to have long-term implications for a woman's physical, cardiovascular health, fertility, and well-being.

These findings have significant public health implications for women with chronic pain that need to be taken into consideration in clinical practice.

6.1 Future work

I hope that the findings of this study will help direct further studies exploring the benefit of public health strategies in women with chronic pain, for example,

- A health economics analysis on this data together with the United Kingdom Bio bank to identify rates of fractures and cardiovascular mortality in the chronic pain population.

- Long term health issues analysis exploring the provision of Vitamin D and Calcium for women with hypoestrogenism and the consideration of hormonal therapies. In particular, an analysis to identify what proportion of women in the cohort had hypovitaminosis D, and what proportion of those in the lowest E2 quartile had a Vitamin D insufficiency.

7 Appendices

7.1 Appendix 1: Study screening form

WIPSOx

Serum hormone levels in women with chronic pain

Screening Form

Date:

Name:

☎ 🖱

Inclusion Criteria:		Meets Criteria (Y/N)
Age *(18-50)*		
Fluent English		
Site of pain *(Pelvis, back, knee, hip, general body)*		
Duration of pain *(≥6 months)*		
Average days/month in pain *(≥50%/15 days)*		

All met: Yes ☐ No ☐

Exclusion Criteria:		Meets Criteria (Y/N)
Pregnant/Breast-feeding *(confirmed or suspected pregnancy)*		
Using exogenous hormones *(Mirena allowed)*		
Average days/month mild opiates *(Codeine, Tramadol ≥50%/15 days)*		
Recent use of strong opiates *(within 6 months)*		
Previous hysterectomy or BSO		
Recreational opiate use		

None apply: Yes No

Suitable: **Yes** **No**

↓

Recruited: Yes ☐ No ☐

PIS sent: Yes ☐ Email ☐ Post ☐

Appt arranged: Yes ☐ No ☐

Date:

Time:

Location: OUH ☐ Community Visit ☐

Name of GP: Dr

Surgery Address:

GP Letter sent: Yes ☐

7.2 Appendix 2: Study consent form

WIPSOx

Oxford University Hospitals NHS
NHS Foundation Trust

Serum hormone levels in women with chronic pain

Consent Form

	Initials
1. I confirm I am over 18 years of age and have read and understood the patient information sheet version 3.0 dated 09/10/15 for the above study and have had the opportunity to ask questions and had them answered satisfactorily.	
2. I agree that my participation is voluntary and that I am free to withdraw without giving any reason, without any medical care or legal rights being affected.	
3. I agree to donate a blood sample of up to 20 mls of blood.	
4. I agree that my given sample is taken for the purpose of research is a gift to the University of Oxford. If a commercial product were developed as a result of this study, I, nor my heirs would profit financially.	
5. I understand that the blood sample is for research purposes and is not intended to be diagnostic in any way. However in the event that any result may be of medical concern my GP will be notified.	
6. I agree that data collected during the study can be looked at by authorised individuals from the University of Oxford and local NHS Trusts.	
7. I agree that samples collected may be analysed locally or transferred and analysed at a study collaborators facility.	
8.I agree that my GP will be informed of my participation in the study	
9. I agree to take part in this study.	
10. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers.	
This is optional 11. I agree to allow genetic studies to be performed on the blood sample in the future.	**Optional**
This is optional 12. I agree to be contacted in the future about related studies approved by an ethics committee.	**Optional**
This is optional 13. I agree to have my blood sample stored and used in future related studies worldwide, which might include transfer to a relevant Research Tissue Bank, and the researcher and their collaborators might have access to my anonymised samples and data in the future. Retained samples can be preserved for many years and will be disposed of in accordance with the law and government guidance in effect at that date	**Optional**

_____	_____	_____
Printed name of patient	Date	Signature of patient
_____	_____	_____
Printed name of person explaining consent	Date	Signature of person explaining consent

7.3 Appendix 3: Participant information sheet

Oxford University Hospitals NHS
NHS Foundation Trust

WIPSOx

Serum hormone levels in women with chronic pain

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.

- Part 2 gives you more detailed information about the conduct of the study.

Please ask us if you would like more information or if there is anything that is not clear. Talk to others about the study if you wish and take time to decide whether or not you would like to participate.

Thank you for reading this information sheet.

Part 1

1. **Study title:**

Women in Pain Studies, Oxford (WIPSOx1): Determination of serum hormone levels in women with chronic pain.

2. **What is the purpose of the study?**

Chronic pain is a major public health issue with approximately 7.8 million people in the UK living with chronic pain. Most chronic pain conditions are more common in women and have been reported to worsen particularly during childbearing years. Chronic pain impacts on all areas of life; including sleep, emotional wellbeing, sexual function, employment and other physical activities.

It is well known that experiencing either physical or psychological stress can alter the menstrual cycle in women. For example, periods can become irregular or even stop after rapid weight loss or during very stressful events such as exams. Chronic pain itself is associated with a significant amount of stress and people suffering with chronic pain are known to have changes in how the system that produces the stress response works, often resulting in low levels of the hormone cortisol. However, as yet no one has investigated what effect chronic pain has on the production of the hormones that control the menstrual cycle and reproductive function (including estrogen, progesterone and testosterone). Whilst altered levels of some of these hormones (e.g. progesterone and follicle stimulating hormone (FSH)) would only be a problem if a woman was trying to become pregnant, persistent low levels of estrogen are associated with long-term health risks such as osteoporosis (brittle bones), heart disease and stroke. Moreover, from the results of our previous research we believe that low levels of estrogen, testosterone and cortisol may worsen the experience of pain.

The purpose of this research is to determine the levels of a variety of hormones in women with chronic pain who are of childbearing age. This study aims to investigate the extent to which hormone production is altered in women with chronic pain and whether we can identify scientific clinical features that relate to these changes. This would allow us to identify which women with chronic pain may require further investigations and treatment (for example with hormone replacement and Vitamin D) to prevent long term complications. Additionally, these results will inform the design of future research investigating whether hormones can be used to reduce the suffering associated with chronic pain in women.

3. **Why have I been invited?**

You belong to a group of women who have the type of chronic pain that we are interested in and are of reproductive age. We aim to recruit one hundred women aged 18 – 50 years with chronic pelvic pain and one hundred women with chronic musculoskeletal (joint, muscle or bone) pain.

4. **Do I have to take part?**

No, it is your choice. Your participation in this study is entirely voluntary. If you decide to take part you will be given this information sheet to keep and at the study visit you will be asked to sign a consent form. If you agree to take part but later change your mind, you are free to withdraw at any time without giving a reason and with no implication whatsoever on your future care.

5. Who is organising and funding the research?

The Chief Investigator is Dr.Katy Vincent, an Academic Gynaecologist and the research is being carried out by the Nuffield Department of Obstetrics and Gynaecology, which is part of the University of Oxford. The study is funded by the Medical Research Fund and is sponsored by The University of Oxford.

6. Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the National Research Ethics Service Committee South Central - Oxford B**.**

7. What will happen to me if I take part?

If you are interested in taking part we will need to see you just once for approximately one hour. The appointment can be at the Nuffield Department of Obstetrics and Gynaecology at the Oxford University Hospital NHS Trust (John Radcliffe Hospital), or at home if this is more convenient for you.

At this appointment the study will be explained again in detail and you will be asked to sign a consent form. Before you sign the consent form, you should ask questions about anything that you do not understand. Once you have signed the consent form you will be given a copy for you to keep. Furthermore, you can still change your mind and withdraw from the study at any time and without giving a reason; this will not affect any current or future medical care. If you would like more time to consider the study a future appointment can be arranged with you.

If you consent to take part, you will then be asked to complete a detailed set of questionnaires to assess the clinical measures we are interested in. This should take about 30 minutes. A blood sample of approximately 20 mls (4 teaspoons) will be taken from your arm during the research appointment in the Oxford University Hospital. You will then have the opportunity to discuss any issues that these questions have raised with the study nurse who will refer on to the investigating researcher if necessary. Before leaving, we will ask you whether you would be interested in being contacted about future ethically approved related studies.

Part 2

8. What will happen if I don't want to carry on with the research?

Taking part in this research is completely voluntary. Your medical care will not be affected if you decide to not take part, or if you withdraw from the study at any point. You are not obliged to give any reason for this. If you do choose not to carry on with the research, we will ask your permission to use any data or samples that we have already collected from you, however, all identifying information will be removed from this data. We will offer you the choice of allowing your data and blood sample, if it has already been collected, to be used within the study, or to have all your data and your blood sample withdrawn from the study.

9. What are the possible disadvantages and risks of taking part?

You will be asked how your pain affects your day to day life. You may find answering some of these questions difficult or embarrassing, but you will not have to answer all of them if you do not wish to. Sometimes people find it distressing to think about the past or about how much their life has been influenced by their pain. We will ask your permission to contact your GP if we feel that your level of distress is very high after completing the questionnaire.

You may have some discomfort and bruising at the site where the blood sample is taken or inflammation of the vein and possible infection when the blood sample is taken, however this is rare All samples will be taken by a nurse trained in taking blood who will do her best to minimize discomfort, and care will be taken to avoid complications. The blood sample taken will be unnamed and will only be used for the purposes of this, or future ethically approved, studies.

10. What are the possible benefits of taking part?

There is no direct medical benefit for you taking part in this study. However, the information we obtain from this study may help us to treat patients with chronic pain better in the future. After we analyse all the samples at the end of the study, which may take up to a year, we will write to both you and your GP with the results. If your hormone levels were outside of the normal range, we may suggest you consider having these rechecked with your GP.

11. What will happen to the samples taken in this study?

The blood samples will be used primarily to measure the amounts of hormones in the blood. However, if you consent, some blood will also be stored and used for future approved studies on chronic pain. You do not have to consent to this in order to be involved in the main study, however.

The blood sample will be taken by the study doctor/nurse. The study doctor will be responsible for the samples and will ensure they are kept securely and confidentially, the samples will be unnamed and labelled only using your unique study identification number. The study doctor/nurse will retain a record of which study participant was assigned which unique study number.

The investigators will be responsible for the samples and will ensure they are kept securely until they are all used up, which may take up to several years. This will allow the researchers to study future questions about chronic pain. You will be asked to give consent to your samples being transferred to a research tissue bank, all samples will be anonymised and non-identifiable. The researchers and their collaborators (i.e. direct or general researchers, commercial companies) may have access to your anonymised samples and data for other scientific research.

Your anonymised samples may be analysed here in our laboratory or they may be transferred to a third party/study collaborator for analysis at their facility. Retained samples can be preserved for many years. However, it may eventually become necessary to dispose of samples. This will be done in accordance with the law and government guidance in effect at that date.

The samples will be considered a gift to the University of Oxford so you would not benefit from any commercial product that might be developed using the samples. The samples may be used for future studies if ethical approval is granted.

12. Is there reimbursement for expenses?

No payment will be given to study participants, however we will endeavour to arrange visits at your convenience e.g. if you are attending the hospital or are in the area for another reason. If this is not possible the cost of using public transport or additional car parking costs will be reimbursed to you.

13. What if something goes wrong?

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact the research team, the Chief Investigator is Dr Katy Vincent and her telephone number is 01865 221021 or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224, or email ctrg@admin.ox.ac.uk. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure.

The University has a specialist insurance policy in place which would operate in the event of any harm that may come to you as a result of your involvement in this research.

14. Is there a contact point where I can seek independent information about taking part in research?

The hospital PALS Office (Patient Advice and Liaison Service) can be contacted. They will give you advice about how to contact someone for independent advice. Their website is www.pals.nhs.uk. The PALS office at the John Radcliffe Hospital site can be contacted on 01865 741166. Alternatively you can get information about public involvement in research studies from the patient organization – INVOLVE. Their website address is www.invo.org.uk .

15. Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research and your participation in the study will be kept strictly confidential and data and blood samples will be fully anonymised using your unique study identification number.

Blood samples will also be referred to only by the study identity number, and your name will not be available to the laboratory investigators. You will be identified in our computers not by name, but by a study identity number.

All information will be retained in a secure environment for future analysis and will be stored in an anonymous format at the John Radcliffe Hospital under the custodianship of the University of Oxford. The information will be stored for 5 years and may be used in future research as our understanding of chronic pain grows.

Responsible members of the University of Oxford or the Oxford University Hospitals NHS Trust may be given access to data for monitoring and/or audit of the study to ensure we are carrying out the study correctly.

16. Involvement of your General Practitioner (GP)

We will write to your General Practitioner both to inform them that you are taking part in this study and of the results of your hormone levels at the end of the study. Additionally, we may contact your GP after completion of your questionnaire if we have any specific concerns about your wellbeing.

17. What will happen to the results of the research study?

The results of this study will be published in a professional medical journal and presented at conferences. Any participant in the study will not be able to be identified in any report or publication. If you are interested in the results of this study we could provide you with a copy of articles or papers after publication.

18. Contact for further information

Thank you for taking the time to read and consider this information sheet. If you think you would like to be involved in the study or would like any further information please contact Lisa Buck, research nurse, by:

- email: Lisa.buck@obs-gyn.ox.ac.uk
- telephone: **01865 221120 / 07802 861 666**

  You can also contact Lisa using her pager by telephoning the John Radcliffe hospital/Oxford University Hospital on **Tel 01865 741166** and ask them to **bleep 4569**
- post: Lisa Buck, WIPSOx, Level 3, Nuffield Department of Obstetrics &Gynaecology, John Radcliffe Hospital, Women's Centre, Oxford OX3 9DU.

If for any reason you would like to speak with the chief investigator please contact Katy Vincent (NDOG Pain Fellow) on Katy.vincent@obs-gyn.ox.ac.uk or 01865 221021.

19. I am interested in being part of the study, what should I do now?

Please contact the study nurse by phone, bleep or email as above, and let her know that you are interested in taking part.

The study team will be able to answer any further questions you may have, and to arrange to discuss the study further at a time that is convenient for you.

7.4 Appendix 4: Study questionnaire

Oxford University Hospitals NHS
NHS Foundation Trust

WIPSOx

Serum hormone levels in women with chronic pain

Study Questionnaire

Subject number:

Age:

Date:

Women with chronic pain experience a variety of different symptoms, therefore some of the following questions may not appear to be relevant to you. However, please try to answer the questionnaire as completely as possible. If any of the questions are unclear please ask us for clarification. Any information provided in this questionnaire and throughout the study will remain confidential.

Questions about your pain

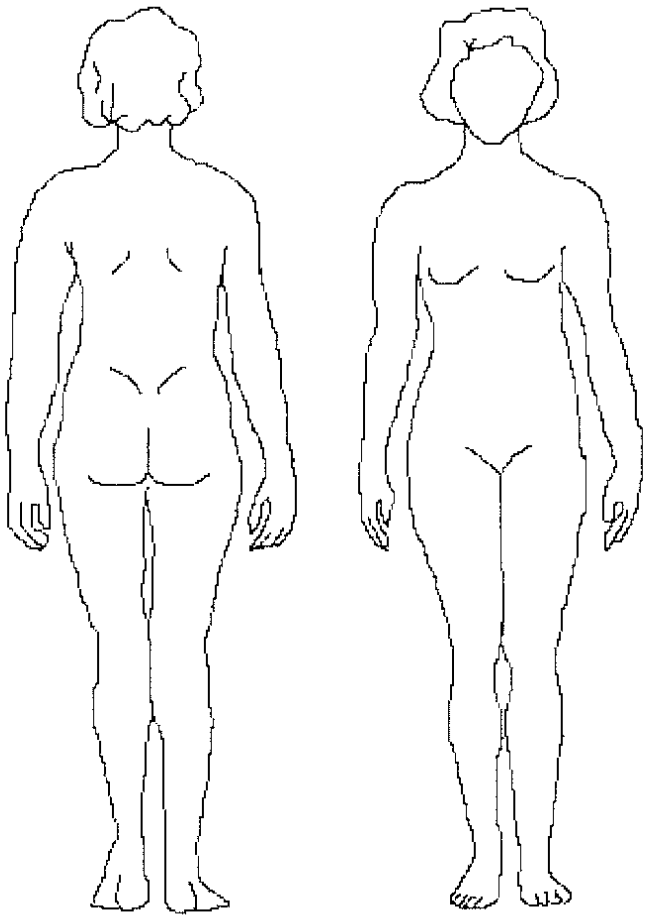
Many women with chronic pain experience their pain in a number of different places. Please use the red pen provided to mark on the body maps below the area where you experience your worst pelvic or muscle/bone/joint pain.

Does this pain radiate to other regions of your body?

Yes ☐ No ☐

If yes, please draw in red the direction in which the pain radiates.

Please then use the black pen provided to mark all other areas where you experience pain as a regular occurrence. Please number these according to how much they bother you with 1 being the most bothersome. Use these numbers when you come to complete the table in section 4.



The following questions are about the pain in the area you marked as red.

How would you assess your pain now, at this moment?

0	1	2	3	4	5	6	7	8	9	10

none maximum

How strong was the strongest pain during the past 4 weeks?

0	1	2	3	4	5	6	7	8	9	10

none maximum

How strong was the pain during the past 4 weeks on average?

0	1	2	3	4	5	6	7	8	9	10

none maximum

How many days in a month do you have this pain?

less than 7	7-13	14-20	more than 21	Every day
(less than a week)	*(1-2 weeks)*	*(2-3 weeks)*	*(more than 3 weeks)*	
☐	☐	☐	☐	☐

On days when the pain is present, how many hours during the day and night do you have this pain?

1-2 hours	2-6 hours	6-12 hours	12-18 hours	18-24 hours
☐	☐	☐	☐	☐

How long have you had the pain (in years or months if less than 1 year)?

How old were you when the pain first started?

Please select the picture that best describes the course of your pain:

 Persistent pain with slight fluctuations ☐

 Persistent pain with pain attacks ☐

 Pain attacks without pain between them ☐

 Pain attacks with pain between them ☐

Do you suffer from a burning sensation (e.g. stinging nettles) in the marked areas?

never	*hardly noticed*	*slightly*	*moderately*	*strongly*	*very strongly*
☐	☐	☐	☐	☐	☐

Do you have a tingling or pricking sensation in the area of your pain (like crawling ants or electrical tingling)?

never	*hardly noticed*	*slightly*	*moderately*	*strongly*	*very strongly*
☐	☐	☐	☐	☐	☐

Is light touching (clothing, a blanket) in this area painful?

never	*hardly noticed*	*slightly*	*moderately*	*strongly*	*very strongly*
☐	☐	☐	☐	☐	☐

Do you have sudden pain attacks in the area of your pain, like electric shocks?

never	*hardly noticed*	*slightly*	*moderately*	*strongly*	*very strongly*
☐	☐	☐	☐	☐	☐

Is cold or heat (bath water) in this area occasionally painful?

never	*hardly noticed*	*slightly*	*moderately*	*strongly*	*very strongly*
☐	☐	☐	☐	☐	☐

Do you suffer from a sensation of numbness in the areas that you marked?

never	*hardly noticed*	*slightly*	*moderately*	*strongly*	*very strongly*
☐	☐	☐	☐	☐	☐

Does slight pressure in this area, e.g. with a finger, trigger pain?

never	*hardly noticed*	*slightly*	*moderately*	*strongly*	*very strongly*
☐	☐	☐	☐	☐	☐

Here are a list of words that describe some of the different qualities of pain and related symptoms. Please circle the numbers that best describe the intensity of the each of the pain and related symptoms you felt during the past week. Use 0 if the word does not describe your pain or related symptoms.

1	Throbbing pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
2	Shooting pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
3	Stabbing pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
4	Sharp pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
5	Cramping pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
6	Gnawing pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
7	Hot-burning pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
8	Aching pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
9	Heavy pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
10	Tender	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
11	Splitting pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
12	Tiring-exhausting	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
13	Sickening	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
14	Fearful	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
15	Punishing-cruel	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
16	Electric-shock pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*

17	Cold-freezing pain	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
18	Piercing	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
19	Pain caused by light touch	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
20	Itching	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
21	Tingling or 'pins and needles"	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*
22	Numbness	*none*	0	1	2	3	4	5	6	7	8	9	10	*worst possible*

Everyone experiences painful situations at some point in their lives. Such experiences may include headaches, tooth pain, joint, or muscle pain. People are often exposed to situations that may cause pain such as illness, injury, dental procedures, or surgery. We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Using the scale, please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

	Not at all	To a slight degree	To a moderate degree	To a great degree	All the time
I worry all the time about whether the pain will end	☐	☐	☐	☐	☐
I feel I can't go on	☐	☐	☐	☐	☐
It's terrible and I think it's never going to get any better	☐	☐	☐	☐	☐
It's awful and I feel that it overwhelms me	☐	☐	☐	☐	☐
I feel I can't stand it anymore	☐	☐	☐	☐	☐
I become afraid that the pain will get worse	☐	☐	☐	☐	☐
I keep thinking of other painful events	☐	☐	☐	☐	☐
I anxiously want the pain to go away	☐	☐	☐	☐	☐
I can't seem to keep it out of my mind	☐	☐	☐	☐	☐
I keep thinking about how much it hurts	☐	☐	☐	☐	☐
I keep thinking about how badly I want the pain to stop	☐	☐	☐	☐	☐
There's nothing I can do to reduce the intensity of the pain	☐	☐	☐	☐	☐
I wonder whether something serious may happen	☐	☐	☐	☐	☐

Questions about your menstrual cycle

How old were you when you had your first menstrual period?

Have there been any times in your life when you haven't had periods for any reason? If so, please can you tell us why this was, when it occurred and for how many months/years you didn't have a period.

Reasons why you might not have had periods	Age(s) that you stopped having periods	How long did the episode(s) last (months/years)?
Using hormonal contraception that stops periods (e.g. back to back pills, injectable progesterone, implant, Mirena coil)		
Pregnant		
Breastfeeding		
Using hormonal treatment of pain (e.g. Zoladex/Prostap, Mirena, back to back pills)		
Significant stresS		
Significant weight loss/very underweight		
Significant weight gain/very overweight		
Early menopause diagnosed		

Other		

Have you had any periods in the last 3 months? (We mean bleeding for which you needed a tampon or sanitary pad, NOT discharge (spotting) for which you needed a panty liner only)

☐ Yes

☐ No

If no, why do you think this is?

Approximately how many periods have you had over the last 12 months? _____

When was your last period?

☐ 1-6 months ago ☐ 7-12 months ago ☐ Over 12 months ago

When was the first day of your last menstrual period (LMP)?

LMP___/___/_____ ☐ Uncertain

DD MM YYYY

If you have had periods in the last 6 months, please answer the following 3 questions about your recent periods.

Were your periods in the last 6 months regular?

☐ extremely regular (period starts 1-2 days before or after it is expected)

☐ very regular (period starts 3-4 days before or after it is expected)

☐ regular (period starts 5-7 days before or after it is expected)

☐ somewhat irregular (period starts 8-20 days before or after it is expected)

☐ irregular (period starts more than 20 days before or after it is expected)

In the last 6 months, how many days were there between the first day of one period and the first day of the next <u>on average</u>? *(Not including spotting)*

☐ < 24 days

☐ 24-31 days

☐ 32-38 days

☐ 39-50 days

☐ 51+ days

☐ Too irregular to estimate

A variety of different pains can be associated with the menstrual cycle. Please mark on the line how badly you suffered with each of the following types of pelvic/lower back pain on average over the last 6 months:

Pain just before your period:

0	1	2	3	4	5	6	7	8	9	10

no pain worst pain imaginable

Pain with your period:

0	1	2	3	4	5	6	7	8	9	10

no pain worst pain imaginable

Pain just after your period has finished:

0	1	2	3	4	5	6	7	8	9	10

no pain worst pain imaginable

Pain with ovulation (mid-cycle):

0	1	2	3	4	5	6	7	8	9	10

no pain worst pain imaginable

Has there been a time in your life when you typically had pelvic/lower back pain during your periods?

☐ Yes

☐ No

If you have ever had pain of more than 3/10 with your periods (e.g. you would have circled any of the shaded boxes on the scale below), please fill out the table below:

Pain with your period:

0	1	2	3	4	5	6	7	8	9	10

no pain worst pain imaginable

What age were you when the period pain started?	How severe was the pain on average during this time (0-10 scale as above)?	How many periods did you have a year at this time?	How many years did you have period pain for at this time?

N.B. you only need to fill out one row of this table if you have had period pain since your periods started or if there has never been a time when you didn't have periods or when your periods were pain-free.

Questions about medicines you have used

Please tell us about any pain medications, over-the-counter or prescription, that you have used at least once a week for a period of 3 months or longer.

PAIN RELIEF DRUG TABLE

Type of drug	Ever used? ✓*if yes*	Currently taking? ✓*if yes*	At what age did you first take this drug regularly?	For what pain was this medication used?	How many days per week?	How many tablets per week?	In total, how long have you used this drug?
Paracetamol/acetaminophen	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years
Aspirin (325 mg or more/tablet)	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years
Ibuprofen (e.g., Neurofen, Brufen)	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years
Celebrex, Vioxx (COX-2 inhibitors)	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years
Other anti-inflammatory analgesics (naproxen, mefanamic acid, Aleve, Naprosyn, Relafen, Ketoprofen, Anaprox)	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years
Strong (narcotic) analgesics (hydrocodone +paracetamol, codeine+paracetamol, morphine, codeine, oxycodone, hydrocodone, Demerol)	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both	☐ 1 ☐ 2-3 ☐ 4-5	☐ 1-2 ☐ 3-5 ☐ 6-14	____ months

				☐ Other pain	☐ 6+	☐ 15+	____ years
Other pain-killing drugs aimed at the nerves/central nervous system (amitriptyline, nortriptyline, gabapentin, pregabalin, lamotrigine, duloxetine)	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years
Muscle relaxants (diazepam/temazepam, buscopan)	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years
Herbal medicines	☐	☐	____	☐ Pelvic pain ☐ Muscle/joint pain ☐ Both ☐ Other pain	☐ 1 ☐ 2-3 ☐ 4-5 ☐ 6+	☐ 1-2 ☐ 3-5 ☐ 6-14 ☐ 15+	____ months ____ years

Have you EVER taken prescription drugs for more than 3 months, excluding hormone treatments and pain medications?

☐ Yes → please fill out the Prescription Drug Table below before proceeding to the next section

☐ No → please go to question 4.1 in the next section

PRESCRIPTION DRUG TABLE

Type of drug	Have you ever taken this drug every day for over a month?	At what age did you first take this drug every day for over a month?	In total, how many years you have taken this drug? Please estimate, and enter "0 total years" if less than 1 year.	Are you currently taking this drug every day?	Please write down the specific name of the drug you have used most recently if known:
	✓ *if yes*	*Age 1st*	*Years taken:*	✓ *if yes*	*Name of drug:*
a. Diuretic (water pill)	☐	__ __	__ __	☐	
b. Diabetic tablets	☐	__ __	__ __	☐	
c. Insulin	☐	__ __	__ __	☐	
d. Thyroid drugs	☐	__ __	__ __	☐	
e. Drugs for epilepsy	☐	__ __	__ __	☐	
f. Sleeping tablets / tranquilisers	☐	__ __	__ __	☐	
g. Anti-depressants	☐	__ __	__ __	☐	
h. Other drugs to treat mental illness	☐	__ __	__ __	☐	
i. Drugs for osteoporosis ("brittle bones")	☐	__ __	__ __	☐	
j. Drugs for rheumatoid arthritis	☐	__ __	__ __	☐	
k. Antibiotics for a month or more	☐	__ __	__ __	☐	
l. Antacids	☐	__ __	__ __	☐	
m. Drugs for stomach ulcer / gastritis	☐	__ __	__ __	☐	
n. Drugs for high cholesterol	☐	__ __	__ __	☐	
o. Drugs for allergies (antihistamines)	☐	__ __	__ __	☐	
p. Steroids (oral, inhaled, or nasal)	☐	__ __	__ __	☐	
q. Chemotherapy for cancer	☐	__ __	__ __	☐	
r. Tamoxifen for cancer	☐	__ __	__ __	☐	
s. Blood pressure drugs	☐	__ __	__ __	☐	
t. Drugs for angina (chest pain)	☐	__ __	__ __	☐	
u. Other drugs for a heart condition	☐	__ __	__ __	☐	

v. Inhaler for asthma	☐	__ __	__ __	☐	
w. Warfarin / heparin to thin blood	☐	__ __	__ __	☐	
x. Migraine tablets/injections	☐	__ __	__ __	☐	
Other 1: ……	☐	__ __	__ __	☐	
Other 2: ……	☐	__ __	__ __	☐	
Other 3: ……	☐	__ __	__ __	☐	
Other 4: ……	☐	__ __	__ __	☐	

Questions about your general health

Have you ever had any of the following medical conditions and if so, at what age were you first diagnosed by a doctor?

Medical Condition			Age diagnosed	Medical Condition			Age diagnosed
No	Yes			No	Yes		
☐	☐	Ankylosing Spondylitis		☐	☐	Mitral valve prolapse	
☐	☐	Anxiety requiring medication or therapy		☐	☐	Multiple Sclerosis	
☐	☐	Asthma		☐	☐	Osteoarthritis	
☐	☐	Cardiovascular disease		☐	☐	Painful bladder/interstitial cystitis (NOT bacterial bladder infection)	
☐	☐	Crohn's Disease		☐	☐	Pelvic Inflammatory Disease (PID)	
☐	☐	Chronic Fatigue Syndrome (CFS) / Myalgic encephalomyelitis (ME)		☐	☐	Polycystic Ovary Syndrome	
☐	☐	Deafness/difficulty hearing		☐	☐	Rheumatoid Arthritis	
☐	☐	Depression requiring medication or therapy		☐	☐	Scoliosis (curvature of the spine)	
☐	☐	Diabetes requiring diet control		☐	☐	Spine problems (excluding scoliosis)	
☐	☐	Diabetes requiring insulin or tablets		☐	☐	Sjogren's syndrome	
☐	☐	Eczema		☐	☐	SLE (Lupus)	
☐	☐	Fibroid uterus		☐	☐	Thyroid disease	
☐	☐	Fibromyalgia		☐	☐	Ulcerative Colitis	
☐	☐	Glandular Fever		☐	☐	Other (*Please specify*):	
☐	☐	Graves' Disease				1.	
☐	☐	Hashimoto's disease				2.	

☐	☐	High blood pressure				3.	
☐	☐	Irritable Bowel Syndrome (IBS)				4.	
☐	☐	Migraine				5.	

We would like to know a bit more about the other pains you experience regularly. Please complete the table below with respect to each different pain you marked on the body maps in section 1.

Pain severity:

0	1	2	3	4	5	6	7	8	9	10

no pain worst pain imaginable

Number	Site/type of pain (e.g. head, hand)	Diagnosis (e.g. migraine, CRPS etc or none if you have not been given a diagnosis)	What age were you when this pain started?	How severe is the pain on average (0-10 scale as above)?	How many days a month do you experience this pain?	How many hours does the pain last when it is present?

We would also like to know if you used to experience other regular pain. Please complete the table below for any other pain you experienced for 3 months or more that has now resolved.

Site/type of pain (e.g. head, hand)	Diagnosis (e.g. migraine, CRPS etc or none if you have not been given a diagnosis)	What age were you when this pain started?	How many years did this pain last for (or give number of months if <1 year)?	How severe was the pain on average when you experienced it (0-10 scale as above)?	How many days a month was the pain present when you experienced it?	How many hours did the pain last when it was present?

Questions about your sleep

The following questions relate to your usual sleep habits during the past month *only*. Your answers should indicate the most accurate reply for the *majority* of days and nights in the past month. Please answer all the questions.

During the past month when have you usually gone to bed at night?

Usual bedtime: _____

During the past month, how long in minutes has it usually taken you to fall asleep each night?

Number of minutes: _____

During the past month when have you usually got up in the morning?

Usual getting up time: _____

During the past month how many hours of *actual* sleep did you get at night?

Hours of sleep per night_____

For each of the remaining questions, mark the one best response. Please answer *all* the questions.

During the past month, how often have you had trouble sleeping because you…	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
cannot get to sleep within 30 minutes				
wake up in the middle of the night or early morning				
have to get up to use the bathroom				
cannot breathe comfortably				
cough or snore loudly				

feel too cold				
feel too hot				
had bad dreams				
have pain				
Other reason(s): please describe				

	Very good	Fairly good	Fairly bad	Very bad
During the past month, how would you rate your sleep quality overall?				

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
During the past month, how often have you taken medicine (prescribed or “over the counter”) to help you sleep?				
During the past month, how often have you had trouble staying awake while driving, eating meals or engaging in social activity?				

	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?				

Questions about pregnancy

Have you ever been pregnant or tried to get pregnant? Yes ☐ No ☐

If no, then go on to section 7.

Did you ever have problems getting pregnant? Yes ☐ No ☐

If yes, please tell us about these problems and any investigations and treatments that you had:

Please tell us what happened in each of your pregnancies?

		Outcome				
Date	How long did it take to get pregnant?	Termination	Miscarriage	Ectopic	Stillbirth	Live birth

Please tell us a bit more about each of the live births?

		Type of delivery							
Date	No of weeks	Normal delivery	Ventouse (suction)	Forceps	Caesarean	Cut or tear?	Pain relief?	Alive and well now?	Anything else you would like to tell us or think we should know?

Did you suffer from postnatal depression? Yes ☐ No ☐

Questions about your mood

Suffering with pain can influence your mood and your mood can also influence how you feel about the pain. The following questions are designed to assess different aspects of mood, so please try to answer them all even if they appear repetitive. There are no right or wrong answers.

Below are a set of statements people have used to describe themselves. Please circle the appropriate number to indicate how much each statement applies to how you *generally* feel:

	Not at all	Somewhat	Moderately	Very well
I feel pleasant	1	2	3	4
I feel nervous and restless	1	2	3	4
I feel satisfied with myself	1	2	3	4
I wish I could be as happy as others seem to be	1	2	3	4
I feel like a failure	1	2	3	4
I feel rested	1	2	3	4
I am “cool, calm and collected”	1	2	3	4
I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
I worry too much over something that doesn’t really matter	1	2	3	4
I am happy	1	2	3	4
I have disturbing thoughts	1	2	3	4
I lack self-confidence	1	2	3	4
I feel secure	1	2	3	4
I make decisions easily	1	2	3	4
I feel inadequate	1	2	3	4
I am content	1	2	3	4
Some unimportant thought runs through my head and bothers me	1	2	3	4
I take disappointments so keenly that I can’t put them out of my mind	1	2	3	4
I am a steady person	1	2	3	4
I get in a state of tension or turmoil as I think over my recent concerns or interests	1	2	3	4

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right now*, that is, *at this moment in time*. Do not spend too much time on each statement but give the answer which seems to describe your present feelings best.

	Not at all	Somewhat	Moderately	Very much
I feel calm	1	2	3	4
I feel secure	1	2	3	4
I am tense	1	2	3	4
I feel strained	1	2	3	4
I feel at ease	1	2	3	4
I feel upset	1	2	3	4
I am presently worrying over possible misfortunes	1	2	3	4
I feel satisfied	1	2	3	4
I feel frightened	1	2	3	4
I feel comfortable	1	2	3	4
I feel self-confident	1	2	3	4
I feel nervous	1	2	3	4
I am jittery	1	2	3	4
I feel indecisive	1	2	3	4
I am relaxed	1	2	3	4
I feel content	1	2	3	4
I am worried	1	2	3	4
I feel confused	1	2	3	4
I feel steady	1	2	3	4
I feel pleasant	1	2	3	4

From the statements below choose one statement from among the group of four in each question that best describes how you have been feeling during the past few days. Circle the number beside your choice.

1	0 I do not feel sad. 1 I feel sad. 2 I am sad all the time and I can't snap out of it. 3 I am so sad or unhappy that I can't stand it.	8	0 I don't feel I am any worse than anybody else. 1 I am critical of myself for my weaknesses or mistakes. 2 I blame myself all the time for my faults. 3 I blame myself for everything bad that happens.
2	0 I am not particularly discouraged about the future. 1 I feel discouraged about the future. 2 I feel I have nothing to look forward to. 3 I feel that the future is hopeless and that things cannot improve.	9	0 I don't have any thoughts of killing myself. 1 I have thoughts of killing myself, but I would not carry them out. 2 I would like to kill myself. 3 I would kill myself if I had the chance.
3	0 I do not feel like a failure. 1 I feel I have failed more than the average person. 2 As I look back on my life, all I can see is a lot of failure. 3 I feel I am a complete failure as a person.	10	0 I don't cry any more than usual. 1 I cry more now than I used to. 2 I cry all the time now. 3 I used to be able to cry, but now I can't cry even though I want to.
4	0 I get as much satisfaction out of things as I used to. 1 I don't enjoy things the way I used to. 2 I don't get any real satisfaction out of anything anymore. 3 I am dissatisfied or bored with everything.	11	0 I am no more irritated by things than I ever am. 1 I am slightly more irritated now than usual. 2 I am quite annoyed or irritated a good deal of the time. 3 I feel irritated all the time now.
5	0 I don't feel particularly guilty. 1 I feel guilty a good part of the time. 2 I feel quite guilty most of the time. 3 I feel guilty all of the time.	12	0 I have not lost interest in other people. 1 I am less interested in other people than I used to be. 2 I have lost most of my interest in other people. 3 I have lost all of my interest in other people.
6	0 I don't feel I am being punished.	13	0 I make decisions about as well as I ever could.

	1 I feel I may be punished. 2 I expect to be punished. 3 I feel I am being punished.		1 I put off making decisions more than I used to. 2 I have greater difficulty in making decisions than before. 3 I can't make decisions at all anymore.
7	0 I don't feel disappointed in myself. 1 I am disappointed in myself. 2 I am disgusted with myself. 3 I hate myself.	14	0 I don't feel that I look any worse than I used to. 1 I am worried that I am looking old or unattractive. 2 I feel that there are permanent changes in my appearance that make me look unattractive. 3 I believe that I look ugly.

0 I can work about as well as before. 1 It takes an extra effort to get started at doing something. 2 I have to push myself very hard to do anything. 3 I can't do any work at all.	19	0 I haven't lost much weight, if any, lately. 1 I have lost more than five pounds. 2 I have lost more than ten pounds. 3 I have lost more than fifteen pounds. (Score 0 if you have been purposely trying to lose weight.)
0 I can sleep as well as usual. 1 I don't sleep as well as I used to. 2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep. 3 I wake up several hours earlier than I used to and cannot get back to sleep.	20	0 I am no more worried about my health than usual. 1 I am worried about physical problems such as aches and pains, or upset stomach, or constipation. 2 I am very worried about physical problems, and it's hard to think of much else. 3 I am so worried about my physical problems that I cannot think about anything else.
0 I don't get more tired than usual. 1 I get tired more easily than I used to. 2 I get tired from doing almost anything. 3 I am too tired to do anything.	21	0 I have not noticed any recent change in my interest in sex. 1 I am less interested in sex than I used to be. 2 I am much less interested in sex now. 3 I have lost interested in sex completely.
0 My appetite is no worse than usual. 1 My appetite is not as good as it used to be. 2 My appetite is much worse now. 3 I have no appetite at all anymore.		

Questions about your experiences of stress

We know that life events can be stressful and that it may take time to readjust. Please place a X in the box next to each of the life events below that have happened to you during the last year.

		Has happened to me during the last year
1	Death of spouse	
2	Divorce	
3	Marital separation	
4	Detention in jail or other institution	
5	Death of a close family member	
6	Major personal injury or illness	
7	Marriage	
8	Being fired at work	
9	Marital reconciliation	
10	Retirement from work	
11	Major change in the health or behaviour of a family member	
12	Pregnancy	
13	Sexual difficulties	
14	Gaining a new family member (i.e. birth, adoption, older adult moving in etc.)	
15	Major business readjustment	
16	Major change in financial state (i.e. a lot worse or better off than usual)	
17	Death of a close friend	
18	Changing to a different line of work	
19	Major change in the number of arguments with spouse (i.e. either a lot more or a lot less than usual regarding child rearing, personal habits etc)	
20	Taking on a mortgage (for home, business, etc.)	
21	Foreclosure on a mortgage or loan	
22	Major change in responsibilities at work (i.e. promotion, demotion etc.)	
23	Son or daughter leaving home	

24	In-law troubles	
25	Outstanding personal achievement	
26	Spouse beginning or ceasing work outside the home	
27	Beginning or ceasing formal schooling	
28	Major change in living condition (new home, remodelling, deterioration of home or neighbourhood etc.)	
29	Revision of personal habits (dress, manners, associations, quitting smoking etc.)	
30	Troubles with the boss	
31	Major changes in working hours or conditions	
32	Changes in residence	
33	Changing to a new school	
34	Major change in usual type and/or amount of recreation	
35	Major change in church activity (i.e. a lot more or less than usual)	
36	Major change in social activities (clubs, movies, visiting etc.)	
37	Taking on a loan	
38	Major change in sleeping habits (a lot more or a lot less than usual)	
39	Major change in number of family get-togethers	
40	Major change in eating habits (a lot more or less food intake, or very different meal hours or surroundings)	
41	Vacation	
42	Major holidays (i.e. Christmas, Diwali, Eid etc.)	
43	Minor violations of the law (i.e. traffic offences, disturbing the peace etc.)	

Unfortunately some people also experience very traumatic events during their life. Listed below are a number of difficult or stressful things that sometimes happen to people. For each event check one or more of the boxes to the right to indicate that: a) it <u>happened to you</u> personally; b) you <u>witnessed it</u> happen to someone else; c) you <u>learned about it</u> happening to a close family member or close friend; d) you were exposed to it as <u>part of your job</u>; e) you are <u>not sure</u> if it fits; or f) it <u>doesn't apply</u> to you. Be sure to consider your entire life (growing up as well as adulthood) as you go through the list of events.

If you have never revealed these before and wish to discuss them further please talk to the nurse at the end of the appointment.

	Event	Happened to me	Witnessed it	Learned about it	Part of my job	Not sure	Doesn't apply
1	Natural disaster (e.g. flood, hurricane, tornado, earthquake)						
2	Fire or explosion						
3	Transportation accident (e.g. car accident, boat accident, train wreck, plane crash)						
4	Serious accident at work, home or during recreational activity						
5	Exposure to toxic substance (e.g. dangerous chemicals, radiation)						
6	Physical assault (e.g. being attacked, hit, slapped, kicked, beaten up)						
7	Assault with a weapon (e.g. being shot, stabbed, threatened with a knife, gun, bomb)						
8	Sexual assault (rape, attempted rape, made to perform any type of sexual act through force or threat of harm)						
9	Other unwanted or uncomfortable sexual experience						
10	Combat or exposure to a war-zone (in the military or as a civilian)						
11	Captivity (e.g. being kidnapped, abducted, held hostage, prisoner of war)						

12	Life-threatening illness or injury						
13	Severe human suffering						
14	Sudden violent death (e.g. murder, suicide)						
15	Sudden accidental death						
16	Serious injury, harm or death you caused to someone else						
17	Any other very stressful event or experience						

If you answered happened to you or witnessed it for any of the events listed above then please complete the items below, otherwise please go on to section 9.

If more than one event happened, please choose the one that is most troublesome to you now.

The event you experienced was……and occurred on……..

Below is a list of problems and complaints that people sometimes have in response to stressful life experiences. Please read each one carefully, then mark the box to the right to indicate how much you have been bothered by the problem in the past month.

	Bothered by	Not at all	A little bit	Moderately	Quite a bit	Extremely
1	Repeated disturbing memories, thoughts or images of the stressful experience?					
2	Repeated, disturbing dreams of the stressful experience?					
3	Suddenly acting or feeling as if the stressful experience were happening again (as if you were reliving it)?					
4	Feeling very upset when something reminded you of the stressful experience?					
5	Having physical reactions (e.g. heart pounding, trouble breathing, or sweating) when something reminded you of the stressful experience?					
6	Avoiding thinking about or talking about the stressful experience or avoiding having feelings related to it?					
7	Avoiding activities or situations because they remind you of the stressful experience?					
8	Trouble remembering important parts of the stressful experience?					
9	Loss of interest in activities that you used to enjoy?					

10	Feeling distant or cut off from other people?					
11	Feeling emotionally numb or being unable to have loving feelings for those close to you?					
12	Feeling as if your future will somehow be cut short?					
13	Trouble falling or staying asleep?					
14	Feeling irritable or having angry outbursts?					
15	Having difficulty concentrating?					
16	Being “super alert” or watchful or on guard?					
17	Feeling jumpy or easily startled?					

Demographic questions

What is your date of birth? __ __ / __ __ / __ __ __ __

DD MM YYYY

How would you describe your ethnic origin?

☐ White ⟶ ☐ North/West European ☐ East European ☐ South European

☐ North American ☐ Other: _____ *(please specify)*

☐ Asian/Pacific/Oriental

☐ Black ⟶ ☐ Black African ☐ African American ☐ Black Caribbean

☐ American Indian or Alaskan native

☐ Native Hawaiian or other Pacific Islanders

☐ Mixed Race

☐ Other: _____ *(please specify)*

Do you consider yourself to be Spanish/Hispanic/Latina? ☐ No ☐ Yes

What is your major ancestry? (Please check one).

☐ Scandinavian ☐ African

☐ Irish / Celtic / British ☐ Asian

☐ Other Northern European ☐ Southern European / Mediterranean

☐ Eastern European ☐ Other (Please specify): _____

☐ South American ☐ Don't know

☐ Central American or Caribbean

Are you currently in school?

☐ No

☐ Yes

What is the highest level of education you have attained (with certificate)?

☐ Primary school

☐ Lower secondary/middle school

☐ Upper secondary/high school

☐ Post-secondary not university / some college or vocational school

☐ University

☐ Postgraduate

Since 18 years of age, what is the most that you have weighed (*not including pregnancy and the 12 months following pregnancy*)?

_____ kg or if you prefer, _____ pounds or _____ stones

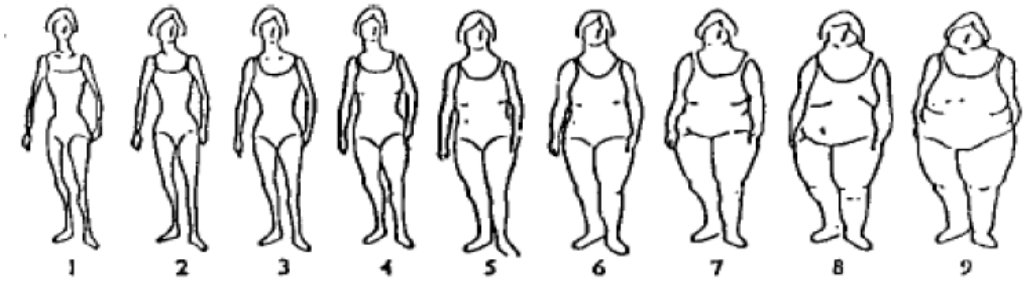
How old were you when you weighed that amount? _____

Since 18 years of age, what is the least that you have weighed?

_____ kg or if you prefer, _____ pounds or _____ stones

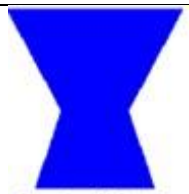
How old were you when you weighed that amount? _____

Which picture best depicts your shape at each age? For each age grouping (*e.g. 10-14, 15-19*), please mark under the number of the figure that best describes you at that age.



10 to 14									
15 to 19									
20 to 24									
25 to 29									
30 to 34									
35 to 39									
40 to 44									
45+									

F12. The shapes and descriptions below describe 4 typical female body shapes. Which shape best describes your body at each age? For each age grouping, please place a check mark under the shape that best describes you at that age. Please leave blank any age groups, which you have not reached yet.

	 Rectangle "Straight"	 Triangle Up "Pear"	 Inverted Triangle "Apple"	 Hourglass
	The circumferences of your chest and hips are about the same and you have little to no waist; when you gain weight, it distributes evenly, although with excess, your stomach may protrude.	Your hip circumference is greater than your chest, and your waist is not prominent; when you gain weight it tends to be disproportionately in your hips, rear, and thighs.	Your chest circumference is greater than your hips and your waist is not prominent; when you gain weight, it tends to be disproportionately in your upper arms, shoulders (back), and chest (not necessarily the breasts).	The circumferences of your chest and hips are about the same but you have a pronounced waist; when you gain weight it is distributed on your shoulders, chest, hips, and rear before affecting your waist and stomach.
15 to 19				
20 to 29				
30 to 39				
40 to 49				
50+				

At age 18, what was your natural hair color? (Please check one).

☐ Red ☐ Dark brown

☐ Blonde ☐ Black

☐ Light brown

What is your eye color? (Please check one).

☐ Blue ☐ Hazel

☐ Gray ☐ Brown

☐ Green

During the last 12 months, what was your average time per week spent on each of the following recreational activities?

	Zero	1-4 min	5-19 min	20-59 min	One hour	1-1.5 hours	2-3 hours	4-6 hours	7-10 hours	11+ hours
Walking or hiking outdoors (include walking to work)										
Jogging (slower than 10 minutes/mile)										
Running (10 minutes/mile or faster)										
Bicycling (include stationary machine)										
Calisthenics/aerobics/aerobic dance/rowing machine										
Tennis, squash, racquetball										
Lap swimming										
Other aerobic recreation (e.g., lawn mowing)										

Have you smoked more than 100 cigarettes during your lifetime?

☐ No

☐ Yes

If yes: How old were you when you first started smoking? _____ years old

Do you smoke currently?

☐ No, I stopped smoking at age _____

☐ Yes, and I smoke about _____ cigarettes per week

Do you drink any alcohol?

☐ No

☐ Yes

If yes: During an average week, how much do you drink of each of the following?
(*Please note exact numbers, <u>not</u> ranges such as 1-3*)

Type of alcohol (serving size)	Average number of each drink per week
Beer/lager/cider (half pints (284 ml)	……
Sherry/vermouth/port (50 ml)	……
Wine (175 ml)	……
Spirits (25 ml)	……
Other (*Please specify*)	……

Thank you for your time and cooperation in answering these questions.

If you have any comments or questions about this questionnaire or would like to talk through any of the issues raised, please speak to the nurse before you leave.

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