Read in the name of thy Sustainer who has created–
Created man out of a germ cell!
Read: for thy Sustainer is the Most Bountiful One
who has taught [man] the use of the pen –
taught man what he did not know

Quran 96:1-5.

THE COMPARTMENTAL DISTRIBUTION OF FLUID AND ELECTROLYTES IN RELATION TO THE SYMPTOMATOLOGY OF THE OVARIAN CYCLE AND PREMENSTRUAL SYNDROME

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DOCTOR OF PHILOSOPHY

by

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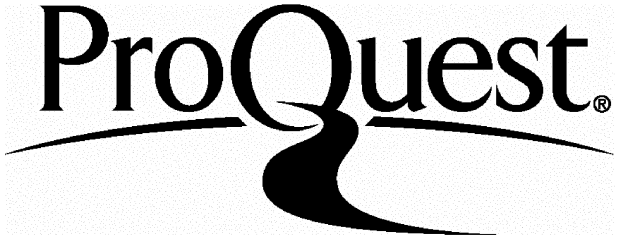

ABSTRACT

The aims of this thesis were to study fluid, electrolyte and hormonal changes during the menstrual cycle and to determine their relationship to symptoms in patients suffering from Premenstrual Syndrome (PMS). The fluid and electrolyte changes were assessed in relationship to psychological and somatic symptoms, but particularly bloatedness, which has long been considered to be due to water retention or fluid shifts. The first study demonstrated atrial natriuretic peptide (ANP) to be significantly lower in patients compared to controls throughout the menstrual cycle, with a significant *decrease* in the luteal phase of the patients. Mid–luteal ANP concentrations showed a strong negative correlation with PMS symptoms. Cycle simulation with hormones resulted in a significant fall in ANP during oestrogen replacement only. Vascular permeability taken as the 0–10 minute albumin change before and after application of venous pressure, demonstrated a significant increase in the patients in the luteal phase, suggesting increased fluid permeability.

Total body water, extracellular fluid, and plasma volume demonstrated no significant change in either group from follicular to luteal phases. There was a follicular to luteal phase *decrease* in total body exchangeable sodium in both patients and controls. This *decrease* was significantly different when compared between patients and controls. Creatinine and urea significantly *increased*, whereas the urinary volume significantly *decreased* in patients compared to controls in the luteal phase. Follicle stimulating hormone and luteinising hormones were both

significantly *lower*, whilst the oestrogen concentration was significantly *higher* in the luteal phase when compared to the follicular phase in patients.

There was no significant relationship of symptoms to any parameter measured apart from the finding that weight change from the follicular to luteal phase being related to breast and bloatedness scores in patients. The results presented contradict the widely held view that PMS is associated with substantial sodium and water retention or fluid shifts. However possible changes of vascular permeability and exchangeable sodium were detected in the patients which is likely to be related to the hormonal changes demonstrated in the studies.

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CHAPTER 1

PREMENSTRUAL SYNDROME

1.1 Introduction

The aetiology of Premenstrual Syndrome (PMS) still remains elusive. In the fifty years, since Frank (1931) first recognised it as a medical condition, an enormous amount of research has been undertaken; unfortunately we still have not determined its aetiology, nor do we have a uniform working definition. We are no nearer to finding a cure or effective treatment for this condition. Not surprisingly a multitude of treatment approaches has been proposed ranging from hypnosis, herbal treatment, acupuncture on the one hand to radical pelvic surgery at the other extreme. Probably no one specific cause accounts for every PMS symptom and it is more likely to be a multifactorial disorder. An interaction of hormonal, psychological, behaviourial and environmental factors seems probable.

In this introductory chapter the historical background, definition and existing pathogenic theories will be discussed, followed briefly by the current treatment available, and concluding with the aims of this thesis. Diagnosis of PMS is discussed in further detail in a separate chapter.

1.2 Historical Background

"Premenstrual Syndrome" (though not under its present name), has been recognised since ancient times as a condition affecting women intermittently and being related to the menstrual cycle. Hippocrates commented that "women are

subject to intermittent agitations and as a result the agitated blood finds its way from the head to the uterus whence it is expelled" (Ricci, 1950). This no doubt describes premenstrual changes and implies symptoms are relieved by the appearance of menstruation. Over the past decades, apart from speculations and erroneous suggestions no known functional significance other than the production of the ovum has been attributed to the ovarian cycle. With the isolation of crystalline oestrone from the urine of pregnant women by Doisy, Thayer and Velor in 1929 a new era in reproductive endocrinology was initiated. This coincided with the publication of a book by Frank in 1929 "The Female Sex Hormone" based on lectures delivered by him at the University of Illinois School of Medicine in December 1928, in which he first coined the term "Premenstrual Tension" (Frank, 1929). In a paper published in 1931 he described a spectrum of mild to severe "premenstrual tension" and went on to describe premenstrual aggravation of certain systemic disorders such as epilepsy and bronchial asthma (Frank, 1931).

A very apt description of this condition was noted by Frank (1931) in the above paper, "The group of women to whom I refer especially complain of a feeling of indescribable tension from ten to seven days preceding menstruation which, in most instances, continues until the time that the menstrual flow occurs. These patients complain of unrest, irritability, like jumping out of their skin and a desire to find relief by foolish and ill considered actions. Their personal suffering is intense and manifests itself in many reckless and sometimes reprehensible reactions. Not only do they realise their own suffering, but they feel conscience stricken toward their husbands and families, knowing well that they are

unbearable in their attitudes and reactions. Within an hour or two of the onset of the menstrual flow complete relief from both physical and mental tension occurs." This description still holds true and typifies the syndrome of PMS. The cause of this symptom complex suggested by Frank (1931) was an excess of hormone accumulation and could be treated with venesection and permanently improved in the severest cases by reduction in the amount of female sex hormone in the circulation, brought about by radiotherapy treatment to produce amenorrhoea. It is interesting to note that other treatment options included theobromine sodio-salicyclate, calcium lactate, various saline laxatives and caffeine preparations, most of these are now strongly advised against, in women suffering from PMS (Frank, 1931).

Thomas (1933) described women with premenstrual water retention reporting weight gains of 12-14 pounds being relieved on the first day of menstruation with diuresis of 4.5 litres. Other descriptions of premenstrual oedema come from Sweeney (1934). He found 30% of 42 healthy women gained 1.4 kg (3 lbs) or more in the premenstrual phase and complained of tightness of clothing and stiffness of hands. In 1950 Morton studied 29 patients, all of whom had transient emotional instability and most had breast pain, increase in appetite, abdominal bloatedness, weight gain and lower abdominal pain. He suggested, while studying female prison inmates, that most crimes (62%) were committed in the premenstrual phase of the menstrual cycle (Morton et al, 1953). Many other researchers since have continued to describe aspects of this condition and have postulated as to the cause – and yet no conclusive definition, aetiology or treatment exists. In the last six decades PMS has become a condition which has

not only stimulated a great degree of scientific interest, both sceptic and zealous, but has also gained immense public and media interest.

1.3 Definitions and Symptomatology

1.3.1 Existing Terminologies of "Premenstrual Syndrome"

The name by which this condition should be designated has still not been accepted universally. Various terminologies such as "Premenstrual Tension" as coined by Frank (1931), "Premenstrual Tension Syndrome" (Abraham, 1981), "Premenstrual Changes", "Primary Recurrent Premenstrual Tension Disorder" and Secondary Recurrent Tension Disorder" (Steiner et al, 1980), "Premenstrual Syndrome" with further sub classification of "Primary Premenstrual Syndrome" (PMS) and "Secondary Premenstrual Syndrome" exist (O'Brien, 1987).

"Premenstrual Syndrome" pedantically may be considered as a misnomer. The definition of "syndrome" implies a specific collection of signs and symptoms. Symptoms vary from woman to woman and there are no objective physical signs. However for each woman the symptoms tend to remain the same and follow a set pattern and thus may possibly be referred as "Premenstrual Syndrome".

1.3.2 Phases of the Menstrual Cycle

Phases of the cycle have been variously described.

Premenstrual Phase or Premenstruum

This has been generally accepted as the week before the onset of menstruation.

Luteal Phase

This phase lasts from ovulation till the onset of the periods.

Paramenstruum

This has been described as the period of four days before, to the fourth day of menstruation (Dalton, 1984).

Dalton et al (1984) defined seven phases of the menstrual cycle while both Moos (1968) and Taylor (1979a) divided the cycle into menstrual, intermenstrual and premenstrual phase. Rubinow et al (1986) compared the week before menstruation to the week after menstruation. Steiner et al (1980) compared one follicular phase score (day 9) to that of one luteal phase score (day 26). O'Brien et al (1980) described four phases while Hammarback et al (1985) used a preovulatory period (5-10) and premenstrual period (5-10). Casper et al (1987) compared two specific days in the follicular and the luteal phases of the menstrual cycle. Sanders et al (1983) on the other hand described six phases in the cycle.

1.3.3 Definition of "Premenstrual Syndrome"

There is no uniform consensus regarding the definition. The definition will necessarily dictate the diagnosis. Definitions and criteria for diagnosis vary amongst researchers. Systematic evaluation and comparison of prevalence, symptoms and results are therefore handicapped. As no objective biochemical marker to define PMS has yet been discovered, the definition has to be based on subjective criteria alone. Vague, liberal definitions will obviously result in an apparently increased prevalence and minor premenstrual symptoms may be

diagnosed as PMS. This is clearly demonstrated in a study by Sutherland & Stewart (1965) and Pennington (1957) where 97 and 95% of women respectively were diagnosed as suffering from PMS. On the other hand studies in which more strict diagnostic criteria were used resulted in a much lower prevalence (Coppen & Kessel, 1963, Rees, 1953). The Premenstrual syndrome Workshop at the Royal College of Obstetricians and Gynaecologists (Taylor, 1983) defined PMS as follows:

> "A woman can be said to be suffering from premenstrual syndrome (PMS) if she complains of regularly recurring psychological or somatic symptoms, or both, which occur specifically during the luteal phase of the cycle. The symptoms must be relieved by the onset of, or during, menstruation; there must be a symptom free week following menstruation."

This definition covers the cyclicity of the condition, and the complete relief of symptoms following menstruation and thus excludes women who also have an underlying psychological disorder. However some women may suffer from PMS in addition to a psychological disorder, and the above definition would wrongly exclude these women. In addition the severity of the symptoms is not taken into account; again diagnosis based on this definition may include women suffering from minor premenstrual changes. Reid and Yen (1981) in their definition took severity into account.

> "Premenstrual syndrome may be defined as the cyclical recurrence, in the luteal phase of the menstrual cycle, of any combination of distressing physical, psychological and/or behavioural changes of sufficient severity to

result in deterioration of interpersonal relationships and/or interference with normal activities."

The National Institute for Mental Health (NIMH) in the USA has delineated two criteria for the definition of PMS and has categorised severity as follows:

1. A marked increase (30%) in the intensity of symptoms measured intermenstrually (from days 5 through 10 of the follicular phase), as compared to those measured premenstrually (within 6 days prior to menstruation).
2. Documentation of these changes for at least 2 consecutive cycles.

The figure of 30% has been chosen arbitrarily and not in any scientific manner. It was, however, the first attempt to put a number to quantifying severity.

A further definition put forward by Steiner et al (1980) has not only included severity and timing of symptoms but has also taken into account their nature (Table 1.1). Although this definition is rigid and encompasses both severity and cyclicity, it mainly deals with psychological symptoms there being a dearth of somatic symptomatology. This may result in excluding those patients who mainly complain of physical symptoms. There is also controversy about the degree of symptom relief that has to be obtained post menstrually. Absence of symptoms in the follicular phase would clearly define a group of patients, however many patients tend to have some symptoms in all phases of the cycle and a small number of women may have continuous mild to severe, psychological or somatic background symptoms on top of which they may suffer from premenstrual aggravation. Dalton (1984) states that there should be an absence of symptoms in the postmenstruum. Reid et al (1985) have stated in their definition "the

cyclical recurrence of symptoms in the *luteal phase..*", whilst Steiner, Haskett and Carroll (1980) in the above definition also clearly specify that lack of symptoms is essential for the diagnosis of "Primary Premenstrual Tension Disorder". If the patient also meets the criteria for the diagnosis of a psychiatric disorder she is labelled as "Secondary Premenstrual Tension Disorder". To avoid confusion O'Brien (1987) has divided these women into two separate groups:

Primary Premenstrual Syndrome

A disorder of non-specific somatic, psychological and/or behavioral symptoms recurring in the premenstrual phase of the menstrual cycle. Symptoms must resolve completely by the end of menstruation, leaving a symptom-free week. The symptoms should be of sufficient severity to produce social, family or occupational disruption. Symptoms must have occurred in at least four of the six previous menstrual cycles.

Secondary Premenstrual Syndrome

A disorder of non-specific somatic, psychological or behavioural symptoms recurring in the premenstrual phase of the menstrual cycle. Although symptoms remain following menstruation, they should significantly *improve* by the end of menstruation and this improvement should be sustained for at least a week. The symptoms should be of sufficient severity to produce social, family or occupational disruption. Symptoms must have occurred in at least four of the six previous menstrual cycles.

This definition includes all major aspects essential for the diagnosis of PMS; recurring premenstrual symptoms implying cyclical hormonal changes, severity indicated by the disruption of normal day to day life, and the recognition that PMS may be superimposed secondarily on another underlying psychological or somatic disease. It is also apparent that in previous studies the grouping together of these two types of PMS patients may have accounted for the disparity in the results.

1.3.4 Symptomatology of PMS

Only a few of the previous definitions dictate the specific nature of the symptoms. This comes as no surprise because as many as 200 symptoms have been reported in PMS. Most researchers agree that the symptoms can be divided into two broad groups, a) Psychological and behavioural symptoms, and b) Somatic or physical symptoms. The symptoms tend to remain constant in an individual patient, although the severity may fluctuate from cycle to cycle.

1.3.4.a Psychological Symptoms

The commonest psychological symptoms reported are irritability, depression, aggression, tension and tiredness. Many women complain of poor concentration, coordination and clumsiness. It has been claimed that a larger proportion of women committing suicide did so during the premenstrual phase (MacKinnon & MacKinnon, 1956). It has also been suggested that women with PMS have a higher incidence of underlying neurosis (Coppen & Kessel, 1963) and that a greater frequency of previously undetected psychological and marital

Table 1.1

Criteria for the Diagnosis of Premenstrual Tension Disorder (Steiner, Haskett and Carroll) (1980)

---

1. At least five of the following are required for a definite diagnosis and four for a probable one, as a part of a current episode:
   - a. Irritable, hostile, angry, short fused
   - b. Tense, restless, jittery
   - c. Decreased efficiency, fatigue
   - d. Dysphoric, marked emotionality, crying
   - e. Lower motor communication, clumsy, prone to accidents (e.g. cut finger, break dish)
   - f. Distractible, confused, forgetful, difficulty with concentration, lowered judgment
   - g. Change in eating habits (e.g. craving, overeating)
   - h. Marked change in libido

2. Overall disturbance is so severe that at least one of the following is present:
   - a. Serious impairment socially, with family, at home, at school or work
   - b. Patient sought or was referred for help from someone or took medication (especially tranquillisers and/or diuretics) at least once during a premenstrual cycle

3. Premenstrual dysphoric symptoms for at least six preceding menstrual cycles
4. Symptoms only during the premenstrual period, with relief soon after onset of menstruation.

---

problems exists in comparison with asymptomatic women. However no studies have reanalysed this after division of patients into the primary and secondary disorder. Mira et al (1985) demonstrated significantly higher scores on the State and Trait Anxiety Scales in both phases of the menstrual cycle in the PMS sufferers, the luteal phase score being significantly higher when compared to the follicular phase. Interestingly, premenstrual changes in feeding, social withdrawal, restlessness, and aggression have also been noted in non-human primates (Hausfater & Skoblick, 1985).

1.3.4.b Somatic Symptoms

The diversity of somatic or physical symptom is equally wide, but once more there are common symptoms such as bloatedness and abdominal distension, feeling of weight gain and "water retention", premenstrual pelvic pain, breast pain and tenderness (cyclical mastalgia), headache and many more. The physical symptoms of bloatedness has not been consistently associated with weight gain. Faratian et al (1984) in a study of 52 women demonstrated no significant change in the body weight and no change in abdominal dimensions. Women frequently complain of symptoms of "water retention" in the absence of premenstrual weight gain, sodium or water retention. Andersch et al (1978a) demonstrated that there was no premenstrual increase in the total body water, but did suggest a redistribution of fluid which may account for the feeling of bloatedness. They have not presented data to support this.

As the number of presenting somatic symptoms of PMS is enormous it is important to distinguish PMS symptoms from other medical conditions. Common

conditions which may be confused with PMS may include endometriosis, pelvic pain syndrome, idiopathic oedema and perimenopausal symptoms. It is also essential to differentiate the cyclical mastalgia of PMS from other causes of breast pain such as breast carcinoma.

1.3.5 Subcategorisation of PMS

The large number of symptoms which women may present leads one to speculate on the possibility of PMS being divided into various subcategories based on the predominant type of symptomatology. The subdivision of women suffering from PMS, into Primary and Secondary PMS has already been discussed above. The most widely used questionnaire both in its original and modified form is the Moos' Menstrual Distress Questionnaire which, on factor analysis of 47 items, classified symptoms into seven main symptom groups: pain, negative affect, water retention, behavioural change, concentration, autonomic reaction, arousal as well as an eighth control scale which actually measured the patients' tendency to complain and was not intended to record PMS symptoms. The 47 questions are listed in Table 1.2 under the appropriate headings. Another study by Claire and Wiggins (1979) analysed data from 500 British women using a modified 35 item MDQ omitting the arousal and control scales and actually identified 5 of the first 6 symptom factors identified in Moos' original study. It has been claimed by some researchers that the development of the Moos' Menstrual Distress Questionnaire involved a non specific group of women, based on a population who were not typically complaining of PMS. Half of Moos' normative sample were taking oral contraceptives. Despite this its widespread use and subsequent validation in its use

in PMS by many authors has made it the most widely used questionnaire in PMS research.

Other subcategorisations have been suggested by Abraham (1981) established on symptom-based subsyndromes, implying that each group has a single aetiology. He classified premenstrual tension syndrome into four subsyndromes:

PMT-A: Nervous tension, mood swings, irritability, anxiety

PMT-H: Weight gain, swelling of extremities, breast tenderness, abdominal bloating

PMT-C: Headache, fatigue, craving for sweets, increased appetite, heart pounding, dizziness, fainting

PMT-D: Depression, forgetfulness, confusion, crying, insomnia

It would be ideal if PMS could be rigidly subgrouped thus, but most patients present with a combination of symptoms and may have symptoms from all the above groups, which then makes a nonsense of the sub categorisation. Additionally, there is no rationale for the grouping of the subgroup.

Halbreich and Endicott (1982) devised a 95 item Premenstrual Assessment Form which allowed for categorisation of at least 18 subsyndromes. They allowed for bidirectionality of change when considering the same symptom thus providing a method for selecting patients with similar symptomatology. However this questionnaire mainly catered for the behavioural and psychological symptoms but appears to be favoured by psychiatrists who research PMS.

1.4 Measurement of PMS

The problem that all investigators face in diagnosing PMS, is the lack of consistent biochemical or physical change which can be measured. The potential means which could possibly assist in the diagnosis of PMS include history taking, psychiatric assessment, physical objective measurements such as weight gain and bloatedness assessment, biochemical and hormonal tests, and most importantly the methods of self assessment. No biochemical test or hormonal measurement available has been shown to date to be conclusive in the diagnosis of PMS. Tests such as thyroid function tests are performed to exclude thyroid disorder, prolactin levels (particularly if the patient is complaining of headaches) to exclude pituitary adenomas, and luteinising hormone (LH) and follicle stimulating hormone (FSH) to exclude polycystic ovarian disease or indeed perimenopausal symptoms. The history and self assessment methods are the most important in the diagnosis of PMS. The very initial reports on PMS were purely dependent on case histories (Frank, 1931). With progress in research, many questionnaires have been devised in an attempt to find a more reliable diagnostic tool. One of the first attempts at keeping a systematic record of both psychological and physiological changes associated with the menstrual cycle were by McCance et al in 1937. This was interestingly a prospectively administered questionnaire and these authors implied the need for long term assessment to determine the periodicity of certain symptoms. For instance the association of altered sexual libido with the menstrual cycle was only possible after analysis of 8 years questionnaires!

Table 1.2

Moos' Menstrual Distress Questionnaire

1)	Pain		5)	Impaired Concentration	
	1.	Muscle stiffness		23.	Insomnia
	5.	Headache		27.	Forgetfulness
	10.	Cramps		31.	Confusion
	14.	Backache		32.	Poor judgment
	19.	Fatigue		36.	Difficulty concentrating
	21.	General aches and pains		40.	Distractible
				44.	Minor accidents
2)	**Water retention**			46.	Poor motor coordination
	2.	Weight gain			
	6.	Skin blemish/disorder			
	11.	Painful/tender breasts	6)	**Behaviour Change**	
	15.	Swelling		24.	Poor school or work performance
				28.	Take naps, stay in bed
3)	**Autonomic Reactions**			33.	Stay at home
	3.	Dizziness, faintness		37.	Avoid social activities
	7.	Cold sweats		41.	Decreased efficiency
	12.	Nausea, vomiting			
	16.	Hot flashes	7)	**Arousal**	
				25.	Affectionate
4)	**Negative Affect**			29.	Orderliness
	4.	Loneliness		34.	Excitement
	8.	Anxiety		38.	Feeling of well being
	9.	Mood swings		42.	Bursts of energy, activity
	13.	Crying			
	17.	Irritability	8)	**Control**	
	18.	Tension		26.	Feelings of suffocation
	20.	Feeling sad or blue		30.	Chest pain
	22.	Restlessness		35.	Ringing in the ears
				39.	Heart pounding
				43.	Numbness, tingling
				45.	Blind spots, fuzzy vision

Some of the questionnaires which have been developed are solely for retrospective assessment and others for prospective assessment, while some of these can be used for both types of measurement.

1.4.1 Retrospective Assessment

Many authors claim that retrospective methods of assessment tends to overestimate symptoms (McCance et al, 1939, Sampson & Prescott, 1981, Abplanalp et al, 1979). The most widely used methodological tool is the Moos' Menstrual Distress Questionnaire (MDQ-C) which as mentioned earlier consists of 47 questions, originally rated from 1-6, but, now the more recent version rates each question from 0-4. However a number of studies have shown discrepancy between retrospective and prospective ratings of symptoms (Abplanalp, 1979, McCance et al, 1937, Moos, 1968, Rouse, 1978). Another retrospective methodological tool was developed by Halbreich et al (1982) for the initial screening of PMS patients. It has 95 items scored on a six point scale of severity of change in the symptoms ranging from "no change" to "extreme change". The diagnostic criteria (DC) is a questionnaire produced by Steiner, Haskett and Carroll (1980) which is based mainly on psychological symptoms.

1.4.2 Prospective Assessment

Both the above forms have modified versions which are able to measure daily symptom ratings. The Moos' Menstrual Distress Questionnaire for prospective rating is called MDQ-T form, the T denoting "today". Halbreich et al (1982) also developed a 21 item PAF Daily Rating Form to measure symptoms

prospectively. It is laid out in a manner such that the woman has a visual perception of the rhythmicity of the symptoms and therefore it may cause bias in her symptom rating especially when she approaches the period. Steiner, Haskett & Carroll (1980) developed a self-rating scale (PMRS) which consists of 36 questions, indicating either the presence or absence of a symptom. There are only 4 items in this questionnaire which relate to physical symptoms. Reid (1985) devised a more complicated chart known as the Prospective Record of the Impact and Severity of Menstrual symptoms (PRISM), which included both daily weights and symptom scoring, rated from one to three.

A further methodological tool devised for objective symptom measurement has been the Visual Analogue Scale. Analogue scales have been used for rating subjective feelings as early as 1921 (Hayes & Patterson, 1921). The application of the visual analogue scale as a methodological tool in PMS was first made by O'Brien (1979). It consists of a 100 mm line at either end of which are either opposing adjectives (bipolar scale) or an adjective extending from maximum to the minimum intensity possible, that is, zero (unipolar scale). Typically recognisable symptoms of PMS (O'Brien, 1979) or disguised symptom adjectives of PMS (Faratian, 1984) may be used giving equally comparable results.

Simple menstrual/mood charts are frequently employed outside research. These give a representation of symptom type and timing but not severity. The PMT–Cator is a simple and novel version of this. This tool devised by Magos and Studd (1988) is a simple rotating disc called the PMT-Cator on which patients can insert their own specific symptoms, rating them from 0 to 3. As the disc rotates it covers the previous records thus preventing comparison with previous days. On

separating the discs at the end of the month, an instant graphical record is available. This method is likely to be insensitive for research purposes because of the limited range of the rating scale but of value in the clinical setting.

1.4.3 Analysis of Symptoms

There have been innumerable methods devised to quantify symptom changes. These have ranged from simple methods such as measuring the mean change of scores in the different phases of the menstrual cycle to more complicated statistical curve fitting and time series analysis.

Rubinow accepted a change of 30% or more to make a diagnosis of PMS, a criterion subsequently accepted by the National Institute of Mental Health in the United States. Others have looked at differences in the mean symptoms during the two phases of the menstrual cycle. Some authors on the other hand have compared maximum scores in the luteal phase to the scores in the follicular phase. The "premenstrual severity index" has been used by O'Brien (1980). Hammarback (1989) performed non parametric statistics between the two phases and if $p<0.05$ the diagnosis was taken as PMS.

Sampson and Jenner (1977) analysed daily symptom scoring using a least mean square method of fitting sine waves. A more sophisticated method has been the time-series analysis, for analysing the cyclicity of symptoms. Modified Triggs' trend analysis has been used by Magos and Studd (1986a) while another method has been the spectral frequency analysis methods by Severino et al (1989). These methods utilise moving averages and curve fitting. Recently menstrual symptoms have also been analysed by Fourier transformation (Metcalf et al, 1989).

1.5 Pathogenesis of Premenstrual Syndrome

The possibility of determining one aetiological factor as being responsible for all aspects of PMS is highly unlikely. It is more likely to be a multifactorial psychoneuroendocrine disorder. In order to discuss the various theories it is convenient to divide the existing evidence into three broad categories, namely a) biochemical, b) psychological, and c) socio–behavioural.

1.5.1 Biochemical

PMS is a cyclical condition related to the ovarian cycle and the hypothalamic pituitary axis. Not surprisingly various researchers have tried to elucidate a hormonal imbalance or derangement in women suffering from PMS.

1.5.1.a Oestrogen and Progesterone

Both oestrogen and progesterone have been implicated in the aetiology of PMS. Frank et al (1931) suggested that excess of oestrogen could cause PMS. In an interesting study by Morton (1950) 28 patients were recruited in whom daily basal temperatures, endometrial biopsy, vaginal smears, and urinary hormones were assessed. They suggested an oestrogen–progesterone imbalance based on the fact that the basal temperatures failed to show a rise at mid-cycle and that the endometrium demonstrated a proliferative or hyperplastic picture on biopsy, as opposed to that characteristic of the luteal phase. The vaginal smears indicated a persistence of cornified cells throughout the cycle which they suggested was an indication of oestrogen-progesterone imbalance with a relative excess of oestrogen. Backstrom et al (1976) studying only 7 patients with PMS noted that

in the luteal phase, the concentrations of progesterone and FSH were slightly lower in women with PMS, whilst the level of oestrogen was slightly higher, again suggesting an imbalance in the oestrogen to progesterone ratio. Dalton (1984) suggested progesterone deficiency as the cause of PMS, which has been supported by other researchers (Dalton, 1984, Backstrom et al, 1976, Dennerstein et al, 1985, Munday et al, 1977, 1981, Taylor, 1979b). A classical study performed by Israel in 1938 also suggested an altered oestrogen–progesterone ratio. Dalton (1984) has been a staunch protagonist of progesterone deficiency as the cause for PMS symptoms and has treated many women with natural progesterone. However, a double blind placebo controlled study has never been published to demonstrate superiority of "natural" progesterone over placebo. In fact no definite improvement of premenstrual symptoms was demonstrated by treatment of PMS with progesterone vaginal suppositories (Andersch & Hahn, 1985, Maddocks et al, 1986, Sampson, 1979, Van Der Meeret al, 1983). It has also been suggested that higher doses of progesterone suppositories may improve the PMS symptoms. A recent study by Myers et al, (1987) showed a decrease in plasma progesterone concentrations in the luteal phase in PMS women. On the other hand Ying et al (1987) in a study of infertile women, showed no increase in PMS symptoms amongst those women who had histologically proven luteal phase deficiency.

Results to date are conflicting as there are others who have shown no difference in the progesterone concentrations between PMS and control women (Taylor, 1979b, O'Brien et al, 1980, Backstrom et al, 1983). In fact O'Brien (1980) suggested a possibility of increased progesterone levels as the cause for PMS symptoms. Although the maximum concentrations of progesterone were found to

be similar in PMS patients and controls by Watts et al (1985), the mean concentration of progesterone was noted to rise earlier in the PMS group suggesting that patients with PMS ovulated earlier than controls. Follicular growth was found to be significantly lower in the PMS patients in association with lower oestradiol concentrations. They thus concluded that ovulation occurred prematurely in women suffering with PMS. Magos and Studd (1986b) in a study using menopausal women as a model, in whom cyclical oestrogen and progesterone were administered in a double blind placebo controlled manner, demonstrated that PMS symptoms could be generated in the progestogen phase of the cycle. An earlier study associated with hormonal administration concluded the opposite i.e. a relative lack of progestogen with oestrogen dominance could be related to increased irritability (Cullberg, 1972). Most of the studies reporting changes in progesterone are faced with a basic methodological problem in that progesterone levels have been shown to fluctuate by almost as much as 30ng/ml in as little as 30 minutes and O'Brien (unpublished study) has demonstrated pulsatile secretion within 10 minute intervals. Thus studies reporting progesterone levels on a daily basis may be meaningless as they may represent either trough or peak values. The amplitude of these pulses is greater than the progesterone differences which have been previously claimed.

When considering oestrogen excess as a cause for PMS it becomes difficult to explain mid-luteal phase well being in many women, when the oestrogen levels are known to peak (Backstrom et al, 1983). In fact this is the point in the cycle when the oestrogen–progesterone ratio is greatest. In an earlier study by Backstrom and Carstensen (1974) higher levels of oestrogen are demonstrated in

PMS sufferers on days 5-2 before the onset of menstruation in association with lower levels of progesterone on days 6-3 before menstruation, thus oestrogen-progesterone ratios were shown to be significantly higher on days 6-3 before menstruation in women demonstrating anxiety as their main symptom. In a later study by Backstrom et al (1976), more frequent sampling during the menstrual cycle revealed that the oestrogen levels in the PMS patients were lower in the early part of the luteal phase, whereas they became significantly higher than the controls on day 5 before the menstruation till day 1 following menstruation. However, premenstrual symptoms at their worst coincide with the decline in the circulating oestradiol concentrations (Reid & Yen, 1983).

The role of oestrogen in inducing the symptoms of PMS, in as yet an undetermined manner, is even more evident from animal experiments, where oestrogen and progestogen have been shown to alter the electrical and chemical properties of the neuronal cells of the hypothalamus. Oestradiol has been observed in a number of hypothalamic areas of the female rat brain and it has been shown that priming with oestradiol for several days causes the monoaminooxidase levels to decrease. Monoamine oxidase is an enzyme necessary in the degradation of serotonin and catecholamine. Inhibitors of monoaminooxidase are used in the treatment of behavioural problems and depression (Luine et al, 1977). It has been demonstrated that if, following oestrogen priming of the female rat brain, progesterone is then added there is a rapid increase in the MAO activity. Research by Terasawa & Sawyer (1969) demonstrated that discharge rates of hypothalamic neurons in rats altered during the oestrous cycle in the female rat. Other animal studies have shown increased

neuronal excitability (Nabekura et al, 1986) and actual demonstration of increase in neuronal exo-endocytotic pits within 1 minute of perfusion of physiological levels of 17-ß oestradiol (Garcia–Segura et al, 1987). This suggests that oestrogen may be responsible for some of the symptoms associated with PMS. In fact oestrogen has been shown to provoke epileptic seizures within 15 minutes when intravenously injected (Logothetis, 1959).

The actual role of gonadal steroids in the production of PMS symptoms remains undetermined, but it is no doubt involved in symptom production in a complex manner interacting with the central neurotransmitters to produce some of the effects on mood and behaviour, sleep and temperature regulation and locally on breast tissue and pelvic organs.

1.5.1.b Vitamin Deficiency

It has been suggested as early as 1943 that vitamin B deficiency may be a cause of PMS (Biskind, 1943). Adams et al (1973) showed that Vitamin B6 reduced the depression associated with oral contraceptive pill. Vitamin B6 is a co-factor in the synthesis of various neurotransmitters such as dopamine and serotonin in the hypothalamus (Brush et al, 1977, 1979). A clinical trial using pyridoxine for the treatment of PMS by Day (1979b) has suggested improvement. Others have actually measured plasma levels of pyridoxal phosphate (PLP) in a small series of PMS sufferers and have not found any difference from those of controls (Ritchie et al, 1986). The role of Vitamin B in the metabolism of oestrogen has been questioned when Zondek & Brezezinski (1948) demonstrated normal oestrogen metabolism in women with severe Vitamin B deficiency.

Rose (1978) suggested that by inducing hepatic enzymes, oestrogen might lead to a relative deficiency of Vitamin B6 thus resulting in decreased biosynthesis of serotonin from tryptophan resulting in depression. Recent controlled studies relating to Vitamin B6 therapy have failed to demonstrate any clinical benefit (Hagen et al, 1985, Kendall & Schnurr, 1987). In a study by Kendall and Schnurr (1987), each PMS subject received 2 consecutive months of pyridoxine or placebo following an untreated baseline cycle. Symptoms of pain, water retention, negative affect, arousal and impaired concentration on the Moos' Menstrual Distress Questionnaire were no better on pyridoxine and they concluded that it was not effective in the treatment of PMS. Conversely, data from a larger study by Williams et al (1985) suggested PMS to be improved compared to placebo.

Vitamin A deficiency was first proposed as a cause for PMS by Simkin (1947). Large doses of Vitamin A were being used for hyperthyroidism and it was found that this incidentally cured the symptoms of PMS in one patient. Subsequently a relationship was described between Vitamin A and oestrogen metabolism; cure of 30 PMS patients was claimed by Argonz and Abinzano (1950) in one uncontrolled trial.

There are reports of Vitamin E (dl-alphatocopherol) in the treatment of premenstrual mastalgia. Initial studies suggested good results with the use of α-tocopherol therapy (London et al, 1981). Further studies have shown that alphatocopherol treatment was not significantly better than placebo (London et al, 1983).

1.5.1.c Essential Fatty Acids and Prostaglandins

Horrobin (1983) first noted improvement in premenstrual syndrome in volunteers who were being studied for another purpose (eczema) and were being treated by essential fatty acids. Essential fatty acids such as linoleic acid and arachidonic acid are required in the synthesis of prostaglandin E_2, gammalinolenic acid is required for the synthesis of prostaglandin E_1, and eicosapentaenoic acid to prostacyclin. The improvement of PMS symptoms with evening primrose oil (Efamol) has been shown by some workers (Massil et al, 1987). Essential fatty acid levels were measured in the phospholipid fraction of plasma and were compared to controls. A significant decrease in n-6 fatty acids gamma-linolenic acid (GLA) and dihomogamma-linolenic acid (DGLA) were noted but there was no deficiency of linoleic acid, suggesting a partial deficiency of the enzyme delta 6 saturase rather than dietary deficiency present at all phases of the menstrual cycle (Brush, 1984). Other studies by Kindahl et al (1976), and Jordan and Pokoly (1977), showed no significant changes in the prostaglandin (PG) levels in the luteal phase of the cycle. In a study by Jakubowicz et al (1984) the prostaglandin E_2, $PGF_{2\alpha}$ and $PGF_{2\alpha}$ metabolites were found to be significantly lower throughout the menstrual cycle in affected women when compared with controls. Although PGE_1 was not measured it was suggested in their paper an excess of PGE_1 was the possible cause of PMS, which resulted from the depletion of the precursors required for the synthesis of the other prostaglandins.

It may be naive to presume that prostaglandin or fatty acid deficiency to be the sole aetiological factor responsible for the genesis of PMS symptoms.

Horrobin (1983) has stated that for the functioning of the n-6 biosynthetic pathway, various co-factors are required in adequate levels, such as pyridoxine. niacin, zinc and magnesium. It is possible that the deficiency of prostaglandins or fatty acids may result in increased sensitivity to the ovarian steroids (O'Brien & Massil, 1990).

1.5.1.d Prolactin

The initial suggestion that prolactin may be associated in the pathophysiological mechanism of PMS was also proposed by Horrobin (1979). Halbreich et al (1976) have demonstrated higher premenstrual prolactin levels in the luteal phase in women suffering from PMS, though these levels remained within the normal range. At the same time another study published by Benedek–Jaszmann (1976) revealed bromocryptine to be effective in the treatment of PMS. Prolactin is released episodically with circadian periodicity and peak levels during sleep with marked individual fluctuations from day to day; there is peaking of PRL levels at ovulation with higher levels in the follicular levels of the menstrual cycle (Kaulhausen et al, 1978, Simkin, 1963, Franchimont, 1976). Higher levels of prolactin in the luteal phase in PMS patients have also been demonstrated by Andersch (1978b) and Cole et al (1975). Other studies have not reported any differences in prolactin (O'Brien & Symonds, 1982, Andersen, 1977). Varma (1984) also found no differences in prolactin levels or in electrolytes in patients and controls during the menstrual cycle.

There have been studies suggesting that prolactin is elevated in PMS and dopaminergic agents have been suggested to play a role in PMS. Thus pyridoxal

phosphate deficiency results in impaired dopaminergic activity. Others have shown that Vitamin B_6 does not seem to have any effect on prolactin secretion (Canales, 1976, Peters, 1978, Hussami, 1978, Tolis, 1977).

1.5.1.e Opioids

Wardlaw et al (1982) have demonstrated that when the ovarian steroid concentrations were low such as during menstruation or at ovariectomy, the ß endorphin concentration became undetectable. They further demonstrated that replacement of oestrogen and progesterone in ovariectomized monkeys resulted in high levels of endogenous opioid peptides, thus confirming for the first time a direct link between gonadal steroid secretion and central endogenous opioid peptides. More recently opioids have been implicated in the aetiology of PMS. Various neurotransmitters and small peptides have been shown to influence behaviour (Bloom et al, 1976, Reid, 1983, 1986b, Goldstein, 1976). Reid et al (1983) have demonstrated an increase in the concentration of prolactin and decrease in growth hormone (GH) and luteinising hormone (LH) on intravenous administration of ß-endorphin. Also naloxone, the opiate receptor antagonist caused the LH concentration to increase both in the frequency and amplitude of its pulsatile release (Quigley & Yen, 1980). Chuong et al (1985) demonstrated significantly lower levels of ß-endorphin in the luteal phase in PMS subjects when compared to asymptomatic controls. This has been further verified by Facchinetti et al in 1987.

1.5.1.f Serotonin

A deficiency of serotonin has been implicated in the pathophysiology of PMS (Rapkin, 1987). Administration of specific serotonin uptake inhibitor (Fluoxetine) has shown to improve psychological and physical symptoms in PMS (Wood et al, 1992).

1.5.1.g Fluid Retention and Renin-Angiotensin System and Aldosterone

Fluid retention has long been postulated as a mechanism for the genesis of PMS symptoms (Greenhill & Freed, 1941). Thomas in 1933 reported cyclically recurring oedema in two cases associated with abdominal fullness, coma and convulsions, which disappeared with the diuresis at the onset of menstruation. Frank (1931) suggested the use of cathartics with caffeine to reduce the excessive oestrogen from the circulation. Various studies have shown weight gain, suggesting water retention and have noted good correlation with severity of symptoms (Bickers, 1951, Bruce & Russell, 1962, Bickers & Richmond, 1952). Bickers & Wood (1951) reproduced PMS symptoms with pitressin injections and treatment with ammonium chloride, a diuretic, resulted in the decrease of the symptoms of PMS. A later study by Bickers & Richmond (1952) suggested "water toxaemia" as the cause of PMS and successfully treated 22 patient with a compound called pyrilamine 8 bromo theophyllinate. Bruce and Russell (1962) studied 10 patients in a metabolic ward and demonstrated a small but significant weight gain and corresponding water and sodium retention. Five of the ten patients were however suffering from depressive disorders, four patients were phobic and one was schizophrenic. This group of psychiatrically ill patients would therefore not

represent true PMS. Herzberg (1971) studied electrolyte and water distribution in eleven nuns with PMS and demonstrated no change in total exchangeable sodium nor were there any significant changes in the distribution of sodium between the intracellular and extracellular spaces. A significant decrease in total body water was noted from the follicular to the luteal phase. This was not compared to controls and thus one cannot draw conclusions from this study. A later study by Andersch et al (1978) comparing PMS women to controls demonstrated no significant change in either total body water or total body potassium, although in the luteal phase water/potassium ratio in litres per mol of potassium was significantly higher in patients than in the controls. They suggested a redistribution of fluids accounted for the symptoms of bloatedness. To date there is no study which has compared total body water, extracellular fluid volume, plasma volume, and exchangeable sodium simultaneously between patients and controls. Klein and Carey (1957) performed serial measurements of total body sodium during the menstrual cycle in normal women. They concluded that there were no cyclic changes in sodium balance or sodium retention in the normal menstrual cycle.

The redistribution of fluids has been indirectly investigated by determining capillary permeability. Jones et al (1966) showed increased capillary permeability in women during the luteal phase of the menstrual cycle when compared to men. Wong (1972) demonstrated increased capillary coefficient in women with bloatedness compared to women without symptoms. More recently Oian (1987) investigating transcapillary dynamics demonstrated a reduction in colloidal and interstitial osmotic pressure in the luteal phase. They suggested that this may be

due to a dilutional effect as a result of redistribution of body fluids because of a reduction in total body protein mass and change in metabolism of plasma proteins during the luteal phase of the menstrual cycle. A recent study on vascular permeability on women undergoing hormonal therapy for in–vitro fertilisation have shown increased passage of fluids across capillary membranes (Tollan, 1990).

Over the years the role of renin-angiotensin system and aldosterone have been thoroughly investigated in disorders implicating blood volume and electrolyte changes. The renin-angiotensin system is responsible for not only blood volume control but also vasoconstriction and control of blood pressure (Symonds, 1988). Electrolyte homeostasis is also maintained by this system. It is therefore reasonable to consider the renin-angiotensin-aldosterone system to be involved in the pathogenesis of the "water retention" which is said to occur with PMS. Changes in this system have been extensively investigated in an attempt to find the cause of PMS.

Reich et al (1962) demonstrated in 6 normal volunteer women that aldosterone levels peaked in the last week of the menstrual cycle. Some studies have shown that salt restriction may result in hyperaldosteronism but Crabbe et al (1958) demonstrated that very severe sodium restriction is required for many days to cause noticeable hyperaldosteronism. This was also shown by Cox et al in 1959. A further study to determine aldosterone secretory rates in the normal menstrual cycle was also performed by Gray et al (1968). They also suppressed ovulation and determined total body water and 3 hour sodium space on these subjects. Aldosterone levels were found to be increased in the mid-luteal phase when compared to the follicular phase of the normal menstrual cycle, but 5 out

of the 6 women with suppression of their menstrual cycles did not show increased aldosterone secretion in the mid luteal phase. Total body water and sodium space did not change in normal subjects from the follicular to the luteal phases of the normal menstrual cycle. It has been suggested that this increased excretion of aldosterone may be because of compensatory physiological change in response to

i) increase in the glomerular secretion rate of sodium,

ii) an exaggeration of the diurnal translocation of fluid which occurs as a result of upright posture, and

iii) an indirect effect of ovarian hormones such as progesterone, which is a natriuretic.

Michelakis (1975) demonstrated an increase in plasma renin activity and aldosterone in the luteal phase of the cycle provided ovulation occurred. However if ovulation failed to occur then there was no rise noted. Plasma renin has also been shown to be increased in the late luteal phase (Brown et al, 1964). Aldosterone itself has been suggested as one of the aetiological factors in the genesis of PMS. Stress is known to increase the secretion of aldosterone (Venning et al, 1957). Higher aldosterone levels have been found by Chiorboli in 1966 in women with PMS compared to controls. A correlation between mood and aldosterone levels has been suggested (Jenner et al, 1967). O'Brien et al (1980) showed higher aldosterone levels in both PMS and asymptomatic controls in the postovulatory phase. However it was higher in the asymptomatic group although not statistically significant. In this study no significant correlation was noted between mood and aldosterone concentrations.

1.5.1.h Atrial natriuretic peptide

Recently atrial natriuretic peptide (ANP) has received considerable attention as it appears to offer an additional cardiovascular regulatory mechanism. ANP reflects the central circulating volume. It causes natriuresis, is vasodilatory and inhibits the renin-angiotensin system. It is also inhibitory to aldosterone and anti-diuretic hormone (Atlas & Laragh, 1986).

It is a hormone secreted by the atria in the form of alpha, beta and gamma ANP in different proportions in different individuals. In the human being the 28-amino acid alpha ANP is the dominant form. Its pathophysiological role is slowly being unfolded. It is known to increase in essential hypertension, atrial distension and increased in certain patients with heart failure, ascitic cirrhosis and nephrotic syndrome and also in idiopathic oedema.

ANP has also been shown to be increased in severe pre-eclampsia when compared to normal pregnancy (Thomsen et al, 1987, Miyamoto et al, 1989). Tan et al (1987) demonstrated no differences during the different phases of the normal menstrual cycle. Davidson and colleagues (1988) compared the normal menstrual cycle to that of PMS and again found no differences. The number of patients in this study were small and the diagnostic criteria used for defining PMS were unclear. Further investigation is required to draw any conclusive evidence.

1.5.1.i Hypoglycaemia

"Food craving" especially for sweets and carbohydrates is a symptom of PMS which has been well documented. It has been suggested that hypoglycaemia or altered glucose metabolism could be an aetiological factor

(Morton, 1953, Bertoli et al, 1980). More recently Reid at al (1986a) demonstrated that there were no significant differences in the oral glucose tolerance or secretion of insulin during the menstrual cycle.

1.5.1.j Thyroid Dysfunction

It has been suggested PMS may be related to abnormal thyroid function (Roy Byrne et al, 1987). Abnormal thyroid stimulating hormone responses have been demonstrated in response to thyroid releasing hormone stimulation tests in PMS patients when compared to controls. However no follicular to luteal phase differences were observed (Roy Byrne et al, 1987).

1.5.2 Psychiatry and Premenstrual Syndrome

As no biochemical aetiological factor has been as yet discovered many theoreticians have suggested that PMS is related to certain personality traits and others have proposed PMS as a psychiatric disorder. Coppen and Kessel (1963) in their study on mildly symptomatic women, found a significant correlation between neuroticism as measured by the Maudsley Personality Inventory and premenstrual complaints, but not with dysmenorrhoea. Watts et al (1980) also found significantly higher scores in PMS women on the State-Trait Anxiety Inventory and Eysenck Personality Inventory (EPI neuroticism scale). This study did not agree with the psychoanalytical theory of women with PMS being less able to accept the feminine role, thus showing a negative attitude towards menstruation (Berry & McGuire, 1972). Ruble (1977) demonstrated experimentally, that the artificial suggestion as to the phase of cycle the women were in, could alter her

symptomatology. Similarly, that social expectancies could alter the perception of menstrual cycle symptoms has also been claimed by Sommer (1973), Parlee (1973, 1974) and Olasov et al (1987).

Gannon (1981) in a critical review, concluded that there was no conclusive evidence to support a psychological cause for menstrual distress. A later study by Stout and Steege (1985) also showed that women of certain "personality type" did not suffer from PMS. Slade (1984) noted in a study on 118 women that physical symptoms peaked premenstrually whilst psychological symptoms occurred randomly. These women were unaware of the of the purpose of the study and its link to the menstrual cycle. A further study by Trunnell (1988) noted significantly higher scores of depression in PMS in the luteal phase, but similar scores to non-PMS women in the follicular phase of the menstrual cycle. This suggests that women with PMS are not all psychiatrically ill patients, though they may have superimposed psychiatric symptoms.

It has been also suggested by McClure et al (1971) that those women who suffer from bipolar premenstrual symptoms have an increased tendency to have bipolar affective disorder. Rees (1953) also demonstrated a higher incidence of PMS in psychiatric and psychosomatic disorders. Dalton (1959) published a paper citing increased incidence of admissions to psychiatric wards during the premenstrual phase of the menstrual cycle. The incidence of suicides also appears to be higher in the premenstrual phase of the menstrual cycle (MacKinnon & MacKinnon, 1956). Wetzel et al (1975) found a significantly higher percentage of college students complaining of premenstrual affective symptoms developing depressive disorders during a 4 year follow up study when compared to college

students not complaining of premenstrual affective symptoms. There appeared to be some evidence of an association between premenstrual affective symptoms and the occurrence of depressive disorders.

1.5.3 Socio-Behavioural Factors

An enormous amount of research has been performed investigating the effect of of socio-cultural factors in relation to PMS (Abraham & Mira, 1989). A comprehensive discussion is not within the scope of this thesis. It has been suggested that premenstrual attitudes and behaviour reflect a complex interplay of cultural and social experience. However there is no clear cut evidence that those women with negative attitudes may be more likely to experience PMS symptoms.

1.6 Idiopathic Oedema

This condition is often confused with PMS and therefore will be briefly described so as to differentiate it from PMS. It is a condition in which oedema occurs without evidence of cardiovascular, renal, hepatic or neurologic disease. Most cases described are females and it appears to be associated with diuretic intake and in some patients with excess carbohydrate intake (MacGregor et al, 1979). MacGregor et al (1979) demonstrated increased oedema in 9 out of 10 patients on stopping diuretics, returning to normal as the plasma renin activity and urinary aldosterone returned to normal. An interesting case study by Hill et al (1960) also demonstrated hyperaldosteronism in a woman with idiopathic

oedema induced by recurrent continual diuretic therapy. It is a condition which is seen to occur more commonly in women probably as a result of diuretic abuse.

1.7 Treatment of PMS

Innumerable and varied treatment regimens have been tried over the years and some of the important methods of treatment PMS will be outlined briefly. In discussing treatment of PMS, it is of importance to take into consideration the very high placebo response which PMS patients are known to have (Day, 1979a, Magos et al, 1986c).

A true double blind crossover study may not be ideal for some trial designs because

1) the wash out period may be long (essential fatty acids)
2) other effects of the drug may be so obvious that it is difficult to obtain a true placebo effect (drugs inhibiting menstrual cycle).
3) it would be unethical to perform sham operations to assess oophorectomy.

Another problem is related to the methodology used, as it becomes difficult to compare placebo studies if different methods are used to measure the placebo response. Treatment can be conveniently divided into

i) Non-drug methods, such as relaxation techniques, yoga, exercise, acupuncture and other similar methods,

ii) Non-hormonal drugs, such as diuretics, pyridoxine, essential fatty acids, essential minerals, alpha tocopherol and others,

iii) Hormonal manipulation which includes

a) Medical

b) Surgical methods.

1.7.1 Non Drug Methods

1.7.1.a Education and Counselling

Education and counselling of the patient is extremely important and has a major role to play in the management of this condition. Relaxation techniques such as yoga, exercise and even hypnosis have been found to reduce tension.

1.7.2 Non Hormonal drugs

1.7.2.a Pyridoxine

The rational for the use of pyridoxine is related to its role as a co-factor in the final steps of the synthesis of dopamine and serotonin both of which affect mood. Thus a deficiency in pyridoxine may lead to an imbalance in these neurotransmitters. However, there is no direct evidence of a link between ovarian steroidogenesis and vitamin B6 metabolism.

On the other hand there have been reports of the occurrence of severe sensory peripheral neuropathy with excess vitamin B6 administration (Kendall & Schnurr, 1987, Schaumburg et al, 1983) and thus should be prescribed with caution.

1.7.2.b Bromocryptine

Some studies have shown PMS patients to have higher prolactin levels in the luteal phase (Franchimont et al, 1976, Carroll & Steiner, 1978, Halbreich, 1986). Based on these findings bromocryptine a dopamine agonist that lowers prolactin level, has been used for the treatment of PMS. Some double blind studies have shown significant improvement but mainly in the breast symptoms (Benedek–Jaszmann & Sturtevant, 1976, Andersen et al, 1977). Kullander et al (1979) and Steiner et al (1984) however did not find improvement of any symptoms with bromocryptine when compared with placebo. It has been generally felt that those women who have significant breast symptoms especially associated with raised prolactin levels benefit from bromocryptine therapy.

1.7.2.c Prostaglandin Inhibitors

Prostaglandins have been suggested to mediate PMS symptoms based on which prostaglandin synthetase inhibitors have been used. A double blind placebo controlled cross–over trial by Wood et al (1980) demonstrated that mefenamic acid was significantly better than placebo. Further studies using mefenamic acid have shown similar results, although it appears to be most effective in women with dysmenorrhoea (Mira et al, 1975).

1.7.2.d Prostaglandin Precursors

It has been suggested that the deficiency of an enzyme delta–6–desaturase results in defective conversion from cis–linoleic acid to gamma–linolenic acid, a step in the synthesis of PGE_1 and arachidonic acid. A

study by Brush et al (1984) demonstrated low plasma levels of dihomogammalinolenic (DGLA) acids. Evening primrose oil (EPO) (72% cis–linoleic acid and 9% gammalinolenic acid) has therefore been considered as a logical treatment for PMS. Puolakka et al (1985) in a double blind cross over trial demonstrated EPO to be superior to placebo on global symptom assessment. Massil et al (1987) have also shown similar results.

1.7.2.e Diuretics

Diuretic therapy has been based on the so called "water retention" theory. There have as yet been no studies which demonstrate water retention and thus the wide use of diuretics may be unjustified and may result in the frequently described "idiopathic oedema" (MacGregor et al, 1979). Bickers and Wood (1952) treated patients complaining of "premenstrual water retention" with ammonium chloride. Studies in which diuretics were used for the treatment of PMS particularly in relation to symptoms of bloatedness, "water retention" and weight gain have shown conflicting results. Varma (1982) demonstrated 80% improvement in PMS symptoms when Navidrex–K was added to dydrogesterone in the treatment regimen. Placebo appeared to improve body swelling and psychological symptoms better than the diuretics in the placebo controlled trial performed by Coppen et al (1969b) and by Mattson et al (1974). Smith et al(1975) administering spironolactone 25 mg four times daily, found it to be no better than placebo. O'Brien et al (1979a) used spironolactone 25mg four times a day, in a double blind controlled study and demonstrated significant reduction in symptoms and weight in the PMS group of women. This improvement

in symptoms in part may be attributed to the anti-androgenic effect of spironolactone (Chapman et al, 1986, Burnet et al, 1991). A further study by Vellacott et al (1987) using 100mg as a single dose also demonstrated significant improvement in symptoms of bloatedness, though no improvement in psychological symptoms were noted.

The use of diuretics in PMS must be considered with caution, and if used at all aldosterone antagonists such spironolactone should be given in order to counteract stimulation of the renin-angiotensin-aldosterone axis.

1.7.3 Hormonal Methods

1.7.3.a Progesterone Therapy

Dalton (1984) has claimed progesterone deficiency to be the cause of PMS and progesterone treatment has been popularised as the treatment for PMS. Unfortunately most of the reports claiming treatment success have been uncontrolled. Controlled trials using progesterone have shown no difference between placebo and progesterone (Sampson, 1979, Van Der Meer et al, 1983, Andersch & Hahn, 1985, Maddocks et al, 1986). Oral micronised progesterone was reported to be more effective than placebo in single cross–over study by Dennerstein et al (1985). In this study symptoms were graded by the Moos' Menstrual Distress Questionnaire. Only the symptom of "water retention" improved when progesterone was compared to placebo. The majority of symptoms did not demonstrate any significant improvement on oral micronised progesterone. Interestingly the "control" symptoms improved with this therapy. It is not

surprising that scientific scepticism exists regarding the use of progesterone in PMS.

1.7.3.b Oral Contraception

The oral combined pill which produces anovulation should theoretically improve symptoms of PMS. Cullberg's (1972) classic study gave a wide variety of results. The high oestrogen pills appeared to worsen symptoms while the strongly progestogenic pills improved symptoms. It was concluded that an increase in the oestrogen/progesterone ratio was responsible for the symptom of irritability. Backstrom and Carstensen (1974) have also shown similar results. Different age group of women respond differently to the oral contraceptive pill (Sheldrake & Cormack, 1976). Andersch and Hahn (1981) showed the age group of 18 years did not respond beneficially to the Pill, but rather the symptoms worsened. It is difficult to assess the benefit of the Pill in PMS as most of the studies have been on the older higher dose pill, and on women incidentally using the pill rather than women with PMS using the Pill for therapy. There are no studies reported which set out to establish in a double blind manner the efficacy of newer dose contraceptive pills nor the effect of continuous pill therapy.

1.7.3.c Danazol

Day (1979a) studied women suffering from PMS by giving doses of 200 to 500mg of danazol. The symptom of breast tenderness appeared to benefit the most. Watts et al (1987) used doses of 100 to 400mgs of danazol in a placebo controlled trial. Improvement was obtained in 2 of 13 symptoms in the

placebo group and 7 of 13 in the patient group obtained by the third month of treatment. The 12 patients on danazol and one on placebo withdrew because of side effects. Due to the large number of withdrawals the 400mg group of patients was not reported on. The side effects of the higher doses of danazol required to induce anovulation thus limit the use of this drug as a first line treatment for PMS.

1.7.3.d Oestrogen Implants and Patches

Ovulation inhibition can also be attained by the use of oestradiol implants as has been shown by Magos et al (1986c). Sixty eight women were treated for up to 10 months in a placebo controlled study. Significant improvement was noted within 2 months for all six symptom clusters of the Moos' Menstrual Distress Questionnaire. The difficulty which may arise is that cyclical progestogen may cause severe "premenstrual like symptoms" as have been demonstrated in another study by Magos et al (1986b). Another problem which has been seen in symptomatic women receiving oestradiol implants, has been that of tachyphylaxis, thus resulting in the patient needing to have frequent insertion of implants in spite of high levels of serum oestradiol. There is some concern regarding the much higher non-physiological levels of serum oestradiol obtained by this route and the possibility of an association with breast carcinoma.

The alternative approach has been the administration of oestradiol patches for the treatment of PMS. This resulted in much more physiological concentrations of serum oestradiol and is thus more acceptable (Watson et al, 1988).

1.7.3.e Gonadotrophin Releasing Hormone Analogues (GnRH-Analogue)

The first study which reported on the use of GnRH-analogue in PMS was by Muse et al (1984). Eight subjects with well documented PMS received daily subcutaneous GnRH-analogue for 3 months. Marked improvement occurred in all women achieving amenorrhoea. Similar results have also been demonstrated by Hammarback et al (1988), Bancroft et al (1987) using intranasal GnRH-analogue, and West and Baird (1987) using one monthly subcutaneous depot preparations. In a placebo controlled cross over trial of buserelin by the author, significant improvement of PMS symptoms occurred (Hussain et al, 1992).

There is no doubt that inhibiting ovulation which removes the hormonal cyclical component of the menstrual cycle improves PMS symptoms markedly, but the main concern in the long term use of GnRH analogues is the side effects produced by hypooestrogenism, such as osteoporosis. Other side effects such as hot flushes, and lack of libido and atrophic vaginitis may be so distressing as to override the beneficial effects.

1.7.4 Surgical Approach

1.7.4.a Hysterectomy and Oophorectomy

Although hysterectomy alone may modulate ovarian steroidogenesis the results of a study by Backstrom et al (1981) demonstrated no effect on the intensity of the symptoms to any lasting significant degree. In a more recent study Casson et al (1990) performed hysterectomy and bilateral oophorectomy on women with severe PMS after demonstrating that ovarian suppression by danazol eliminated symptoms. Metcalf et al (1992) reported 66%

improvement in PMS symptoms following hysterectomy. They concluded that the uterus was not essential for the expression of PMS but its removal often resulted in the reduction in PMS. Casper and Powell (1990) administered low dose conjugated oestradiol and produced long term alleviation of symptoms. This is undoubtedly a radical approach which should be reserved for the more severe and intractable cases of PMS. Although suppression of ovarian function by these medical and surgical techniques have limited application in the management of PMS - the results do provide some of the first good evidence of a link between the ovarian hormone cycle and the genesis of PMS symptoms.

It is difficult to consider one aetiology being responsible for the wide spectrum of symptomatology in PMS. It is cyclical in nature and does not occur prior to puberty or postmenopausally and therefore must surely be related to hormonal changes occurring in the menstrual cycle. Theoretically as PMS is thought to occur only in ovulatory cycles, it is possible that "water retention" and "bloatedness" may be related to an imbalance between oestrogen and progesterone. It is unlikely to occur in the absence of ovulation. Other aetiological factors which have been discussed above may possibly contribute in some direct or indirect manner to produce such diverse symptoms. Vitamin B_6 for example is required as a cofactor in the synthesis of certain neurotransmitters, whilst other vitamins have been suggested to be related to the metabolism of oestrogen. Serotonin and prolactin by acting through neurotransmitters centrally, may be involved in the pathophysiology of PMS. On the other hand essential fatty acids and prostaglandins may act through sensitizing the receptors to the sex steroids. As the relationship of the symptoms of "bloatedness" and "water retention" to that

of the ovarian cycle and to PMS were being studied in this thesis the different projects under taken have concentrated solely on this aspect of PMS.

1.8 Summary

PMS is a condition which, in spite of being researched for six decades, has no identifiable biochemical or other objective means of diagnosis. Definitions are varied, and are solely dependant on subjective criteria leading to diagnostic confusion and thus making comparisons between studies difficult. It is unlikely that a single aetiological factor is responsible for PMS and it most probably results from a combination of hormonal, biochemical, environmental and socio–behavioural phenomena. Physical symptoms such as "water retention" and bloatedness form a major complaint in many of these women. There is as yet no conclusive evidence demonstrating the occurrence or absence of premenstrual fluid retention.

1.9 Aims of this thesis

1. To establish the most appropriate means for the diagnosis of premenstrual syndrome utilising already validated methodological tools.
2. To study atrial natriuretic peptide in the normal menstrual cycle and in PMS. If changes occurred to assess if these could be mimicked by administering cyclical exogenous hormones.
3. To study changes in vascular permeability occurring in the normal menstrual cycle and in PMS. To study the effect of cyclical

exogenous hormone administration on vascular permeability.

4. To detect changes in total body water, total body exchangeable sodium, extracellular fluid volume and plasma volume in the normal menstrual cycle and in premenstrual syndrome. If changes occurred to determine whether these correlated with somatic or psychological symptoms.

5. To detect whether there is a causal relationship between compartmental fluid and electrolyte and hormonal changes with that of PMS symptomatology.

CHAPTER 2

DIAGNOSIS OF PREMENSTRUAL SYNDROME

2.1 Introduction

A vast amount of research has been performed in order to obtain better methods of diagnosing Premenstrual Syndrome (PMS). At present the only method available is the subjective evaluation of symptoms. Almost every worker in this subject has produced yet another method of diagnosing and evaluating the symptoms. There appears to be a great diversity in the methodology, of symptom assessment and analysis, and therefore, not surprisingly, there is little consistency and uniformity in the diagnosis of PMS. This adds to the complexity when studying and assessing this disorder not only with regards to the diagnosis but also in the study of symptomatology and the assessment of treatment improvement.

The aim of this thesis is to study and correlate the symptomatology and changes in the compartmental fluid and electrolyte distribution in PMS. It was therefore felt that the use of an already established methodological tool and the determination of a simple technique of analysing the symptoms would be the desired approach in the diagnosis of women suffering from primary PMS.

The other important aspect in establishing the diagnosis was ensuring exclusion of those women who have psychiatric disease. This was particularly important so that analysis of data was not confounded by a large number of variables.

2.2 Exclusion of Psychological Disease

There are many questionnaires which have been designed to detect psychiatric disorder. Scales such as Macmillan's Health Opinion Survey (Passama–Nick, 1959), Gurin Mental Status Index (Gurin, 1960), were the earlier scales used in diagnosing mental disorders. More recent questionnaires have been: General Health Questionnaire or GHQ (Goldberg & Hillier, 1979), Self Reporting Questionnaire or SRQ (Harding et al, 1980) the Symptom Checklist or SCL. More specifically in order to screen for depressive disorder scales such as Beck's Depression Inventory and Leeds Depression scale have been employed. Numerous other questionnaires exist but the aim here was to select a well validated method to suit the population to be studied.

The GHQ has been well validated and a comparison to SRQ in Brazil showed they were equal in case detection with a correlation of +0.78 (Mari & Williams, 1985). The advantage of using the GHQ was that an interview technique of assessment was not required. A scaled version GHQ-28 obtained by principal components factor analysis (Goldberg & Hillier, 1992) has been shown to give equally good detection rates as the GHQ-30. The four subscales of the GHQ-28 are somatic symptoms, anxiety and insomnia, social dysfunction and severe depression. The scaled version of the GHQ when used as a screening test gives better results when scored by the simpler 'GHQ scoring method' (0-0-1-1) as opposed to the Likert method.

Based on the above it was decided that the General Health Questionnaire (Table 2.1) would be the psychometric tool used in assessing the psychiatric health of the subjects participating in the studies included in this thesis.

Table 2.1

The General Health Questionnaire-28 (Goldberg, 1979).

Have You Recently :					
A1	been feeling perfectly well and in good health?	Better than usual	Same as usual	Worse than usual	Much worse than usual
A2	been feeling in need of a good tonic?	Not at all	No more than usual	Rather more than usual	Much more than usual
A3	been feeling run down and of sorts	Not at all	No more than usual	Rather more than usual	Much more than usual
A4	felt that you are ill?	Not at all	No more than usual	Rather more than usual	Much more than usual
A5	been getting any pains in your head?	Not at all	No more than usual	Rather more than usual	Much more than usual
A6	been getting a feeling of tightness or pressure in your head?	Not at all	No more than usual	Rather more than usual	Much more than usual
A7	been having hot or cold spells?	Not at all	No more than usual	Rather more than usual	Much more than usual
B1	lost much sleep over worry?	Not at all	No more than usual	Rather more than usual	Much more than usual
B2	had difficulty in staying asleep once you are off?	Not at all	No more than usual	Rather more than usual	Much more than usual
B3	felt constantly under strain?	Not at all	No more than usual	Rather more than usual	Much more than usual
B4	been getting edgy and bad tempered?	Not at all	No more than usual	Rather more than usual	Much more than usual
B5	been getting scared or panicky for no good reason?	Not at all	No more than usual	Rather more than usual	Much more than usual
B6	found everything getting on top of you?	Not at all	No more than usual	Rather more than usual	Much more than usual
B7	been feeling nervous and strung up all the time?	Not at all	No more than usual	Rather more than usual	Much more than usual

Have You Recently

C1	been managing to keep yourself busy and occupied?	More so than usual	Same as usual	Rather less than usual	Much less than usual
C2	been taking longer over things you do?	Quicker than usual	Same as usual	Longer than usual	Much longer than usual
C3	felt on the whole you were doing things well?	Better than usual	About the same	Less well than usual	Much less well
C4	been satisfied with the way you've carried out your task?	More satisfied	About same as usual	Less satisfied than usual	Much less satisfied
c5	felt that you are playing a useful part in things?	More so than usual	Same as usual	Less useful than usual	Much less useful
C6	felt capable of making decisions about things?	More so than usual	Same as usual	Less so than usual	Much less capable
C7	been able to enjoy your normal day to day activities?	More so than usual	Same as usual	Less so than usual	Much less than usual
D1	been thinking of yourself as a worthless person?	Not at all	No more than usual	Rather more than usual	Much more than usual
D2	felt that life is entirely hopeless?	Not at all	No more than usual	Rather more than usual	Much more than usual
D3	felt that life isn't worth living?	Not at all	No more than usual	Rather more than usual	Much more than usual
D4	thought of the possibility that you might make away with yourself?	Definitely not	I don't think so	Has crossed my mind	Definitely have
D5	found at times you couldn't do anything because your nerves were too bad?	Not at all	No more than usual	Rather more than usual	Much more than usual
D6	found yourself wishing you were dead and away from it all?	Not at all	No more than usual	Rather more than usual	Much more than usual
D7	found that the idea of taking your own life kept coming into your mind?	Definitely not	I don't think so	Has crossed my mind	Definitely has

Its simplicity and ability to be used by researchers not trained in psychiatry was important. Its relative lack of precision and sensitivity does not diminish its value for the purposes of this study.

2.3 Measurement of Premenstrual Symptoms

Of the many questionnaires that have been used in the assessment of PMS, one of the most validated and widely used questionnaires has been the Moos' Menstrual Distress Questionnaire (Moos' MDQ)(Chapter 1, Table 1.2). This questionnaire may be used to assess the symptoms of the menstrual cycle both prospectively (MDQ daily questionnaire or MDQ-T) and retrospectively (MDQ-C) (Moos, 1969a). Moos questioned 839 women questioned on 47 symptoms and these were then factor analysed. This resulted in the identification of 8 factor scales (Moos, 1969a). This questionnaire has been validated by various workers (Clare & Wiggins, 1979, Rees, 1953). It has also been adapted or modified by others (Magos & Studd, 1986a). The MDQ has therefore been used in this study in its own right to validate the visual analogue scale (VAS)(Fig 2.1) which has been chosen as the methodological tool in the assessment of PMS.

The visual analogue scale has also been used extensively in recording severity of symptoms (O'Brien, 1979b, Rubinow & Roy Byrne, 1984, Casper et al, 1986). The advantages of the visual analogue scale over questionnaires are the avoidance of filling in of complicated and time consuming questionnaires, the resulting greater compliance in their completion, the greater sensitivity of technique and the simple graphic representation of symptoms. The VAS has been compared to the Self Rating Scale for Premenstrual Tension

Syndrome (Steiner et al, 1980), and the Prospective Record of the Impact and Severity of Menstrual Symptomatology by Casper & Powell (1986) and was found to be highly correlated with both scores.

Analysis of the symptoms is again another area where different techniques have been used. Simple arbitrary division of the cycle into pre and post menstrual phases and mean scores have been compared to more complicated and sophisticated trend analysis. Metcalf et al (1989, 1990) compared the more complicated Fourier fitting, a form of trend analysis to a simple difference in the mean daily scores of the premenstrual with postmenstrual phase and found a high correlation between the two methods. Whatever method is used, it should be able to determine a significant difference in the symptomatology between the pre and post menstrual phase of the menstrual cycle.

2.4 Methodology

In the first part of the study all patients and controls filled in the GHQ-28 in the follicular and the luteal phases of the menstrual cycle (Table 2.1). The follicular phase was taken as that part of the cycle falling between the day of onset of menstruation up to 14 days prior to the onset of the next menstruation. The luteal phase GHQ score was that which fell in the 7 days prior to the onset of menstruation. The GHQ (median) scores in follicular and luteal phases of the cycle are displayed in Table 2.2.

Daily Record Chart

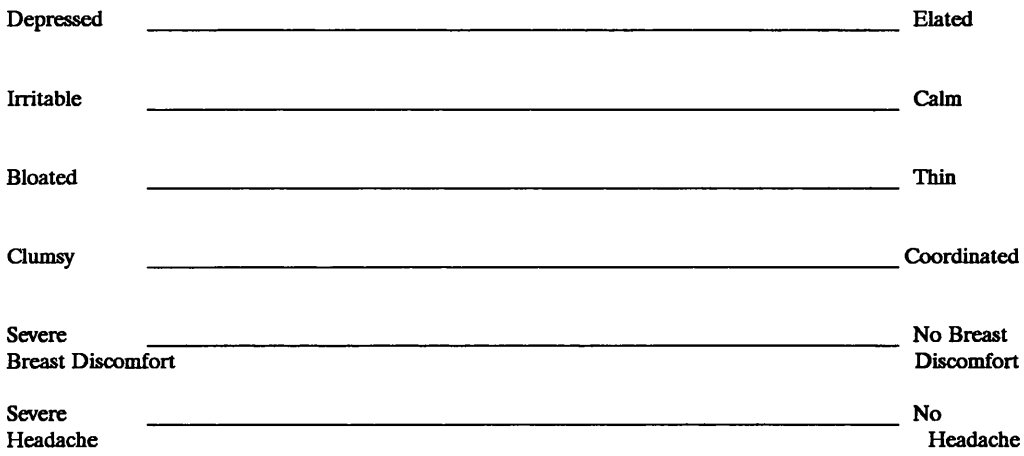


Figure 2.1

The Visual Analogue Scale consisting of three psychological and 3 somatic symptoms measured on a 100 mm linear scale.

The premenstrual symptoms were scored on the Visual Analogue Scale (VAS) (Fig 2.1). The second part of the study was to assess a method of analysing the symptom score in order to make a diagnosis of PMS. This was then validated against Moos' MDQ in the third part of the study.

Six symptoms, three physical and three psychological, were measured on a 100mm VAS (Massil & O'Brien, 1986). The physical symptoms were bloatedness, breast tenderness and headache which were on a unipolar scale while the psychological symptoms measured were depression, irritability and clumsiness on a bipolar scale.

Table 2.2

General Health Questionnaire Scores (median) in 31 patients and 22 controls.
* indicating significant p value, CI denoting confidence interval.

	F	L	CI	p value
Patients	1.0	12.0	-14.5, -18.5	<0.0001*
Controls	0.0	0.5	-1.0, 0.0	0.33
95% CI	0.001, 1.0	8.0, 16.0		
p Value	0.25	<0.00001*		

Fourteen patients were recruited from the PMS Clinic at the Royal Free Hospital and 12 comparable controls from among the hospital staff. Each subject completed a General Health Questionnaire in the pre and post menstrual phases of the menstrual cycle in order to assess any psychiatric component.

In the third part of the study 17 patients and 10 controls participated. Each of these subjects in addition to the above also filled in a daily Moos' MDQ (Table 1.2)

2.5 Analysis of Symptoms

The symptom score for all six symptoms was totalled and a mean average was calculated for each day. This was called the Global Average score (GA). A 3 point, 5 point and 7 point moving average was performed on the GA for each patient for smoothing the data (Table 2.3). This was done to eliminate the effects of extraneous causes of symptom disturbance. The Smoothed Global Average (SGA) scores during the course of one cycle for a patient (Fig 2.2) and control (Fig 2.3) have been demonstrated in Fig 2.2 & 2.3.

The maximum smoothed GA (Max SGA) which fell in the 7 days prior to menstruation was taken as the Premenstrual score while the minimum score falling before 14 days prior to the onset of menstruation was taken as the post menstrual score and the difference as the delta score (Table 2.3). The SGA delta was taken as the index of severity of PMS; thus the higher the SGA delta score the worse the PMS.

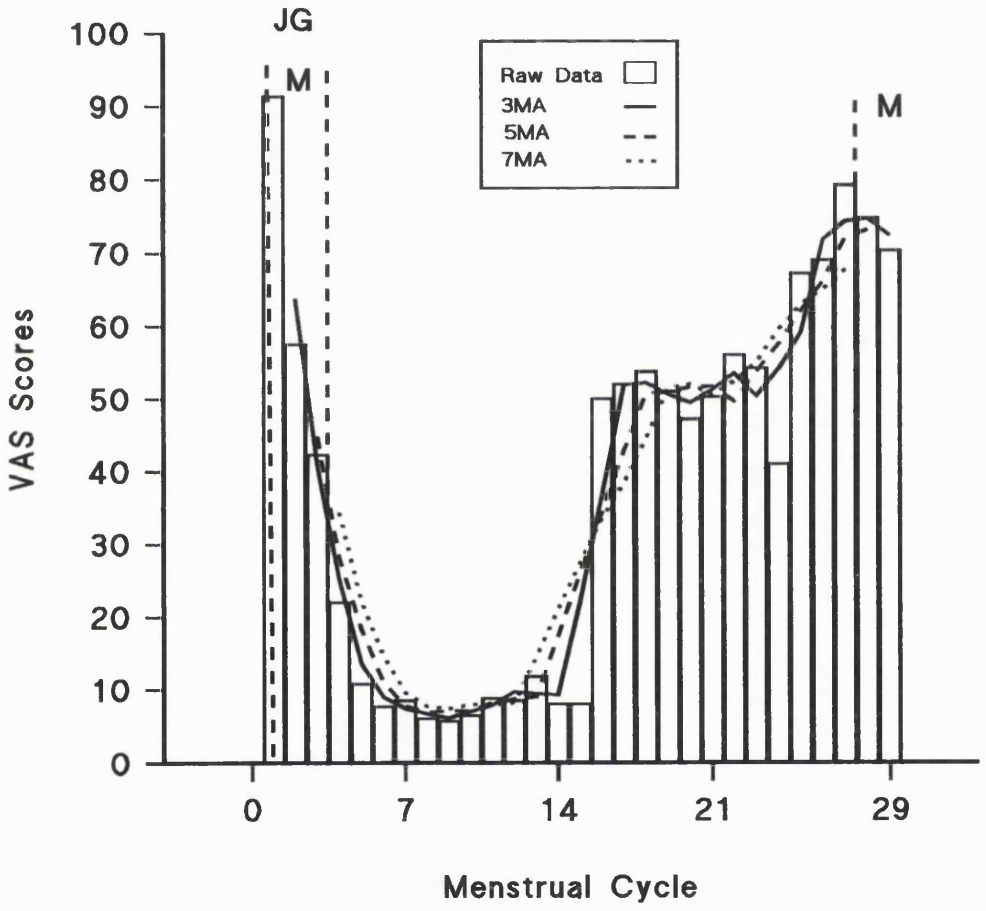


Figure 2.2

The Smoothed Global 3, 5 and 7 point moving average of the Visual Analogue Scale Scores during one cycle of a patient.

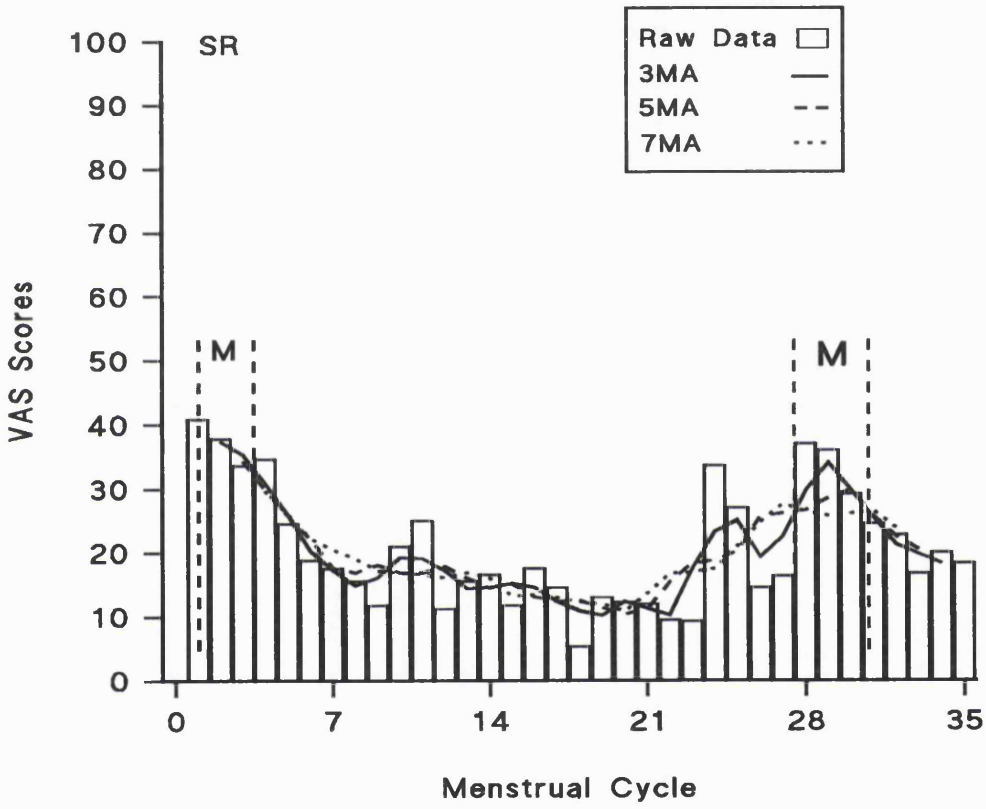


Figure 2.3

The Smoothed Global 3, 5 and 7 point moving average of the Visual Analogue Scale Scores during one cycle of a control.

Table 2.3

Global Visual Analogue Scale Score (VAS) Maximum, Minimum and Delta Scores in 14 patients and 12 controls.

	Maximum			Minimum			Delta		
Controls									
	3 MA	5 MA	7MA	3 MA	5 MA	7 MA	3 MA	5 MA	7 MA
EH	44.6	41.1	39.0	22.1	24.2	26.9	22.4	16.9	12.1
HK	30.9	40.6	41.0	9.8	24.4	22.9	21.1	16.2	18.1
SR	25.1	20.2	17.5	14.9	16.9	16.8	10.2	13.3	0.7
VD	40.8	36.0	32.5	22.4	23.2	23.7	18.4	12.8	0.8
RG	119.7	18.6	17.9	11.6	14.4	14.7	8.1	4.2	3.2
KD	32.2	28.3	25.9	6.8	9.5	11.0	25.4	18.8	14.9
MR	24.8	23.4	23.2	15.3	15.8	16.2	9.5	7.6	7.0
JH	25.0	21.7	19.8	19.3	21.2	20.4	5.7	0.5	-0.6
RS	12.9	10.1	9.0	7.3	7.5	7.4	5.6	2.7	1.6
SR	9.2	7.2	8.0	3.7	5.4	7.9	5.5	1.8	0.1
SH	24.3	18.3	13.6	0.1	0.3	0.9	24.2	18.0	12.7
LP	38.4	37.3	32.2	15.1	25.4	28.7	23.3	11.9	3.5
Patients									
OG	65.2	59.0	56.1	9.4	10.7	12.2	55.8	48.3	43.9
KR	79.1	75.8	72.9	10.4	12.1	14.5	68.6	63.6	58.4
JUS	75.1	58.3	49.4	13.3	13.8	14.0	61.7	44.5	35.4
JG	74.7	73.1	67.7	6.1	6.9	7.4	68.7	66.2	60.3
AL	78.6	71.3	62.3	10.9	12.7	14.9	67.7	58.6	47.4
GZ	74.3	59.3	72.6	24.9	30.3	34.0	49.4	28.9	38.6
SJ	69.8	66.9	67.5	28.1	29.4	30.7	41.8	37.5	36.8
BP	50.8	39.6	33.0	15.7	20.7	25.0	35.1	18.9	8.0
SG	67.7	65.9	61.6	16.1	17.6	17.8	51.6	48.4	43.8
SR	66.7	61.5	57.0	8.4	9.7	12.0	58.3	51.9	45.0
MK	54.9	53.3	52.8	14.1	15.7	19.6	40.9	37.7	33.1
LR	56.1	51.4	63.4	7.9	10.4	27.0	48.2	41.0	36.4
SM	67.8	56.3	51.0	4.9	5.5	5.5	62.9	50.8	45.5
SG	61.6	54.9	51.1	9.8	9.9	10.2	51.8	45.0	40.9

The delta scores obtained for the 3, 5 and 7 point smoothed average were then plotted (Fig 2.4). It was decided that the method which gave the clearest division between the two groups without overlap would be chosen as the method of choice. The mid-point between the highest score of the controls and the lowest of the patients would be taken as the dividing line between patients and controls.

After the method of diagnosis was established the VAS SGA delta scores would then be correlated against the MDQ SGA delta scores in order to validate the method used.

Nonparametric statistical methods, Mann Whitney's test for comparing patients and controls and Wilcoxon Rank test for comparing the follicular and luteal phases within patients and controls were used in the analysis of the GHQ scores. Spearman Rank's correlation was used in the correlation of the VAS SGA delta versus the SGA MDQ delta scores.

2.6 Results

The general characteristics of the two groups are shown in Table 2.4. The median GHQ for both patients and controls in the follicular phase were similar. In the luteal phase the GHQ scores in the patient group were much higher this being very significantly different from the controls (p value <0.00001, CI 8.0, 16.0) (Table 2.2). As patients and controls had similar follicular phase scores this difference was most likely to be due to PMS.

The SGA Max and SGA delta in the patients were significantly different from the controls, but the SGMin values were similar in the two groups. The SGA delta 3, 5, and 7 point were plotted on the same graph (Fig 2.4). The SGA delta

values obtained by the 3 point MA showed the most distinct division between the patients and controls, while the 5 and 7 point MA demonstrated an overlap. The value which was midpoint between the highest delta score of the control group and the lowest score in the patient group was taken as the threshold value. This came to a value of 30 in the group under study.

A 3 point MA was then performed on the daily MDQ scores and the max, min and delta scores (MDQ SGA max, MDQ SGA min & MDQ SGA delta) were calculated as above for each of the subjects (Table 2.5). This was then correlated against the VAS SGA Max, VAS SGA Min and VAS SGA delta scores. The VAS and MDQ SGA max and SGA delta (Spearman's rank correlation = 0.7961) scores correlated significantly whilst the SGA Min scores showed no significant correlation (Fig 2.5).

2.7 Discussion

Many women complaining of premenstrual symptoms in the past and even now often get labelled as mentally unbalanced and no note is taken of the fact that they women lead completely normal lives in the post menstrual phase of their cycle. It is very important that some method be used to exclude psychological illness before diagnosing these women to have PMS. The GHQ as has been discussed earlier is a well validated tool to determine psychological ill health in the general population. It has been proclaimed as a reliable tool to alert clinicians as to the possibility of psychological disorder.

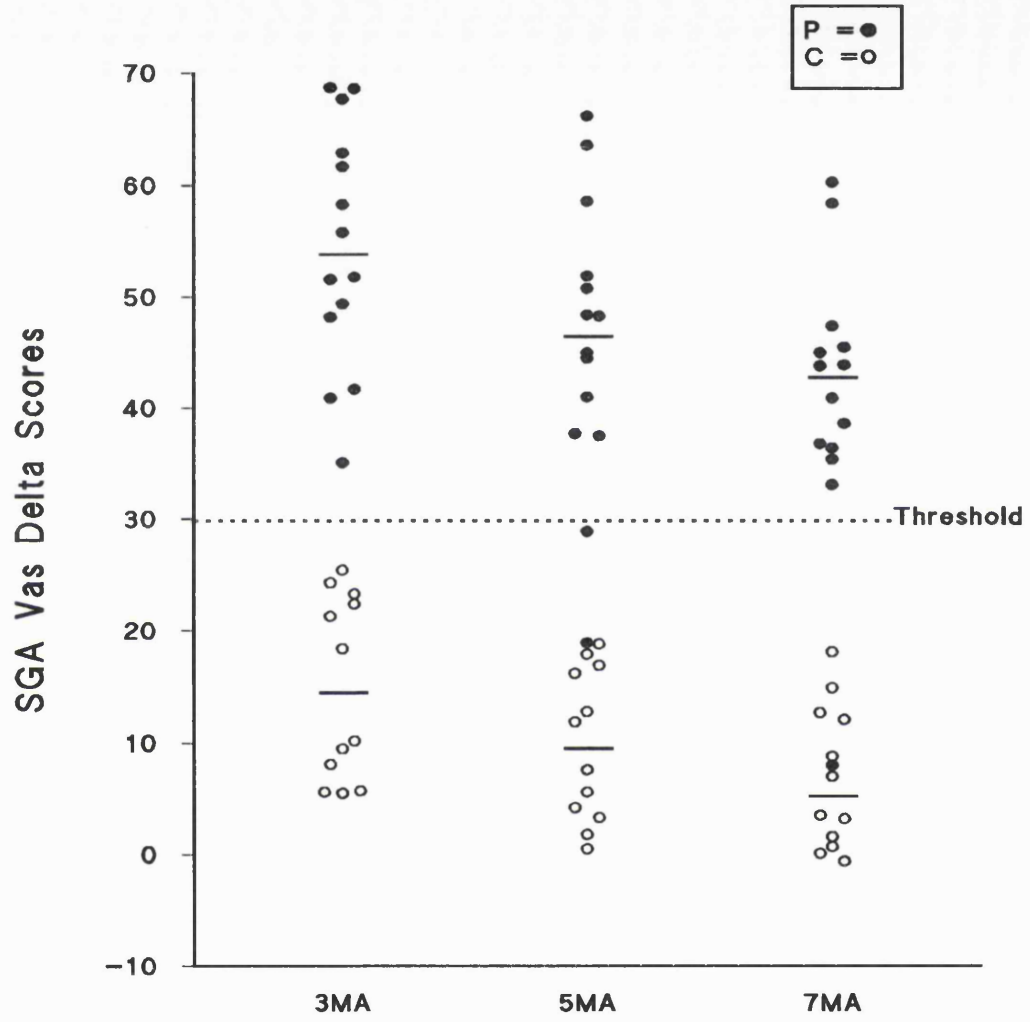


Figure 2.4

The Global Delta Visual Analogue Scale scores obtained by performing a 3, 5 and 7 point moving average in 17 patients and 10 controls.

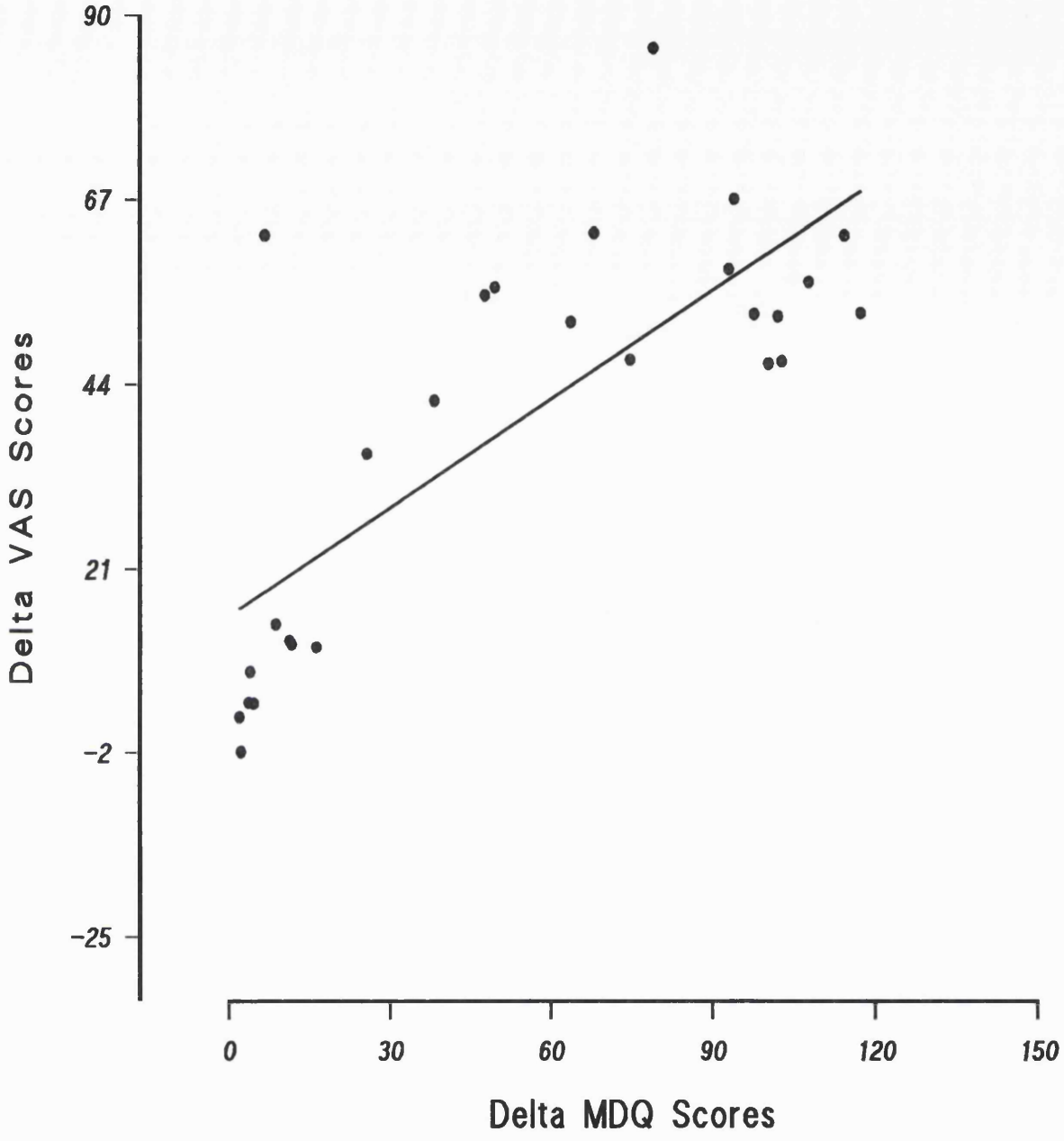


Figure 2.5

The Moos' Menstrual Distress Questionnaire score plotted against Visual analogue scale score, demonstrating a significant correlation (Spearman's Rank Correlation=0.7961). $p < 0.001$

Interestingly in this study the PMS patients demonstrated a significantly higher luteal phase score of GHQ (Table 2.2). This has led to very justified concern as to the possibility of wrongly diagnosing psychological disorders in women suffering from PMS. If a woman suffering from PMS completes a GHQ in the premenstrual phase of her cycle she is likely to be judged as being psychologically ill. Amongst the patients included in this study one woman had a follicular GHQ score of 13. Her luteal phase score two weeks later was below our threshold level for diagnosing as psychological illness. It became evident that questionnaires used to judge psychiatric disorders should be completed at specific phases of the menstrual cycle, especially in those women who may be suffering from premenstrual syndrome.

The diagnostic criteria for PMS have varied greatly in different studies and therefore have led to the reporting of prevalence rates for PMS varying from 5% to 97% The diagnosis of PMS has been imprecise and inconsistent making comparison of studies difficult. Whereas some studies have revealed a prevalence of PMS to be as low as 5% in a group of psychiatric patients (Rees, 1953), others have reported extremely high prevalence such as 97% in a group of nurses and students (Sutherland & Stewart, 1965). This indicates the need for a uniform method of diagnosing the condition so that not only accurate estimates of the prevalence of the condition may be made but also various studies may be compared.

One important objective of this study was to find a simple method of analysing symptoms such that it would offer some objective measurements thus making possible comparison of different studies. The Moos' MDQ has been used

extensively and has been validated by a large number of researchers around the world (Clare & Wiggins, 1979, Rees, 1953). It was therefore thought to be an appropriate questionnaire to use for validation of the Visual Analogue Scale (VAS) which has been used for mood assessments by Aitken & Zeally (1970). The visual analogue scale was chosen as the method for the recording of three physical and three psychological symptoms because of its simplicity in comparison to recording on the Moos' MDQ which consists of 47 questions.

Table 2.4

General characteristics of 17 patients and 10 comparable controls. Median values are shown with 95 % Confidence Interval.

	Patients	Controls	95% CI	p Value
Age	32	28.5	–3.00, 7.00	0.625
Parity	0	0	0.00, 0.00	0.918

Visual analogue scales were first used for the assessment of PMS symptoms by O'Brien (1979b). They have since been used by other workers and the method has been shown to correlate highly with self rating scales of Steiner and Haskett (Casper et al, 1986). Casper et al (1986) demonstrated the use of linear

analogue scales comprising of 3 somatic and 3 psychological symptoms. Most questionnaires are lengthy and time consuming to complete. The compliance was therefore found to be better with VAS which is most likely due to its simplicity. It has also been found to be sensitive enough to be used for research purposes. Good compliance both of the patients and controls were obtained in the projects which have been described in this thesis.

Various methods have been devised for the analysis of the symptom scores for severity and for cyclicity. Scoring methods ranging from the use of simple arithmetic means of the follicular and luteal phase score, to more complicated and sophisticated time series analyses have been performed by Magos et al (1986) using the modified Triggs Trend Analysis and Severino et al (1988) using the spectral frequency analysis method.

Both the above methods involve curve fitting techniques to moving averages of sequential data. These methods offer a uniform approach to the diagnosis both qualitatively and quantitatively. However in the study performed by Magos et al (1986d) using modified Trigg's trend analysis the first 7 days were ignored and arbitrarily eliminated. Thus some information would undoubtedly be lost and in those with shorter cycles make it somewhat difficult to interpret. Premenstrual symptom cyclicity has been studied by Metcalf et al (1990) and the symptoms were analysed by using Fourier's analysis. They noted that a simple procedure based on the difference between the premenstrual and mid follicular scores showed close agreement with the more complicated Fourier series analysis.

Table 2.5

Global Moos' Menstrual Distress Questionnaire Scores and Visual Analogue Scale scores in 17 patients and 10 controls.

	MDQ Scores			VAS Scores		
Patients	Max	Min	Delta	Max	Min	Delta
JS	121.3	14.2	117.3	67.1	14.2	52.9
JB	80.3	1.3	79.0	94.1	8.1	86.0
DK	38.7	0.3	38.3	49.4	7.2	42.2
KOS	80.6	31.0	49.6	60.3	4.3	56.1
LH	101.0	8.0	93.0	69.9	11.5	58.4
PW	57.0	9.3	47.7	66.9	11.8	55.1
HD	70.3	2.3	68.0	71.6	8.6	62.9
LS	100.3	0.0	100.3	55.5	8.9	46.6
JS	120.3	12.7	107.7	82.1	25.3	56.8
MC	94.0	0.0	94.0	73.0	5.9	67.1
AR	104.0	2.0	102.0	73.1	20.6	52.5
JG	25.7	0.0	25.7	41.7	6.3	35.4
GW	101.7	4.0	97.7	60.5	7.7	52.8
ML	111.0	8.3	102.7	49.9	3.1	46.9
LA	75.0	0.3	74.7	57.3	10.2	47.1
JR	67.7	4.0	63.7	53.9	2.1	51.8
CN	114.3	0.0	114.3	76.2	13.6	62.6
Controls						
GO	7.0	0.3	6.7	38.9	24.2	14.7
RF	16.3	5.0	11.3	29.2	17.2	12.0
KD	10.7	6.7	4.0	24.4	16.4	8.1
JM	15.0	3.3	11.7	15.3	3.7	11.6
KH	2.3	0.0	2.3	9.2	11.1	-1.9
GS	24.3	8.0	16.3	37.8	26.6	11.2
DE	2.7	0.7	2.0	26.1	23.7	2.4
SW	10.7	7.0	3.7	10.9	6.7	4.2
SS	13.0	8.3	4.7	28.0	33.8	4.1
VS	8.7	0.0	8.7	26.8	12.8	14.1

The objective of this study was to determine a simple technique for establishing the diagnosis of PMS using a combination of a simple method of smoothing data and identifying a quantitative threshold above which a diagnosis of PMS would be made.In the subjects studied the delta scores were obtained as the difference between the follicular lowest smoothed score and the smoothed highest smoothed score in the seven days prior to menstruation obtained by performing 3, 5 and 7 point moving averages (Fig 2.4). In this study the best separation of patients and controls occurred when the 3 point moving average was used. The dividing line between the lowest delta score in the patient and the highest in the control occurred at the level of 30. This agrees with the recommendations of the DSM-III-R criteria for the diagnosis of "late luteal phase dysphoric disorders". It was therefore thought to be reasonable to use a threshold of 30 delta score for diagnosing PMS.

2.8 Summary

The diagnosis of Premenstrual Syndrome is still based completely on subjective methods for which a whole host of symptom assessment tools are available. The important first step in the assessment of PMS is to exclude psychiatric disease prior to labelling a patient as suffering from PMS. The GHQ-28 has been well validated as a psychometric tool in the exclusion of psychological illness and was thus used in this study.

The VAS was used to measure six symptoms and from these a simple technique was devised to measure PMS. This involved smoothing the score using a 3 point moving average and taking the difference of the maximum score in the

luteal phase from the minimum score in the follicular phase to obtain the delta score. A delta score of 30 or more was taken as the threshold value above which a diagnosis of PMS was made. This was validated using the more established but time consuming Moos' MDQ and were found to correlate significantly.

CHAPTER 3

ATRIAL NATRIURETIC PEPTIDE IN PREMENSTRUAL SYNDROME

3.1 Introduction

In an attempt to determine the aetiology of premenstrual syndrome various endocrine systems have been previously investigated. Atrial natriuretic peptide (ANP) has been more recently studied and its biological actions of natriuresis and diuresis, together with its role as antagonist of the renin-angiotensin-aldosterone system and in suppression of vasopressin release and thirst have been determined. In order to explore the "water retention" hypothesis as an aetiological factor in the genesis of PMS symptoms, the role of ANP has been investigated and the findings are reported in this chapter. An extensive review of the biological actions of ANP is not within the scope of this chapter, thus the physiological actions of ANP will be discussed briefly.

3.2 Background

Evidence for the existence of a third humoral factor which promotes urinary sodium excretion has been present for some time. ANP, a hormone produced primarily in the cardiac atria, has in the last decade been thoroughly investigated. It was as early as 1952, that Peters (1952) suggested that an extrarenal mechanism existed for natriuresis. That the atrium was a likely site for such a control was suggested by Smith (1957). In 1956, Henry et al (1956) and later De Wardener et al (1961) proposed that expansion of vascular volume led

to a natriuretic response. It was finally in 1979 that De Bold and colleagues demonstrated that the atrial granularity altered with change in the water and electrolyte balance (De Bold et al, 1979). A further experiment by De Bold and associates in 1981 revealed that only injections of atrial tissue extracts caused natriuresis in rats, whilst that of ventricular extracts did not (De Bold, 1981).

Flynn and colleagues have since purified and sequenced ANP (Flynn et al, 1983). There are many atrial peptides, but the human atrial peptide in its biologically active form has 28 amino acid residues. The major storage form in the atrial granules is the 126 amino acid pro-ANP and the circulating form of the 28 amino acid is cleaved from pro-ANP.

ANP is released in response to increased intravascular volume. ANP is thus increased in response to head-out water immersion, head down tilt and saline infusions. Some have suggested that dietary sodium also affects the release of ANP (Sagnella et al, 1987). Others have contradicted this theory, based on the fact that very large quantities of sodium ingestion would be required to effect very small changes in the level of ANP (Weidmann et al, 1986). ANP appears to be released by both stretch and pressure receptors, the former being the more important mechanism. ANP secretion is similarly reduced when there is volume contraction, head up or upright position and diuretic administration. ANP receptors appear to occur in parallel with angiotensin II receptors. They have been found in blood vessels, adrenal gland, kidney and central nervous system (Mendelson et al,1987).

ANP antagonises the renin-angiotensin system and aldosterone and both are responsible for the maintenance of sodium and volume homeostasis.

Tan et al (1987) investigated various physiological and sampling conditions which may affect ANP concentrations. They observed no difference in the levels of ANP between the supine and sitting position or any affect of venepuncture stress. They also found no difference of ANP concentration in the different phases of the normal menstrual cycle. They noted a significant decrease in the ANP concentration levels declining significantly during their study period of 0700 to 1500 hours. Zhang et al (1989) studying the 24 hour variation of ANP have on the other hand shown highest levels to be at 2000 and apart from a drop at 1200 hours have shown little diurnal variation. Davidson et al (1988) compared normal women with patients suffering from PMS and did not find any difference in ANP concentration between the two groups. A study performed by the author investigated ANP changes in a well defined group of PMS patients and compared them to controls. The number of patients was larger and more frequent sampling was performed (Hussain et al, 1990). This study is reported below.

The aims were to investigate

1. The changes of ANP in the normal menstrual cycle.
2. To determine the ANP concentration in women with PMS.
3. To determine any difference between the PMS and control group.
4. To administer cyclical oestrogen and progesterone to menopausal women to simulate the ovarian cycle and observe the induced changes in ANP levels during therapy.
5. To determine whether there was any correlation between changes in ANP concentration and PMS symptomatolgy.

3.3 Methodology

3.3.1 General Methodology for the Premenstrual Syndrome Study

Eleven women predominantly complaining of "weight gain" and bloatedness as one of their PMS symptoms were recruited from the Premenstrual Syndrome Clinic of the Royal Free Hospital. Twelve asymptomatic women selected from the hospital staff served as a comparison group. Informed consent was obtained from each woman. The diagnostic criteria for recruitment have been described in Chapter 2. Each patient and control completed a General Health Questionnaire (GHQ) in the mid follicular (MF) and mid luteal(ML) phases of the menstrual cycle and prospective daily Visual Analogue Scales (VAS) for the previously mentioned six symptoms. No woman had a history of psychiatric disorder or suffered from cardiac, renal or any other disease. They were on no medication for at least 2 months prior to taking part in the study. They were all on a normal diet and not taking any excess or restricted salt.

Body weight was recorded after emptying the bladder, in the MF and ML phases of the cycle. Blood pressure was also measured at the same time. Serum progesterone measurements were made one week prior to the next predicted menstruation.

Blood samples for ANP measurements were taken in the follicular (days 4-9), early (days 16-18), mid (days 19-22) and late (days 23-26) luteal phases of the menstrual cycle (F, EL, ML and LL phases respectively). All samples were taken between 1000 and 1500 hours with the women resting in the sitting position. The timings of taking blood were comparable between patients and controls. The blood was collected in tubes containing sodium EDTA (20mg) and aprotinin (400

kallikrein units) and were immediately placed in ice. All samples were centrifuged within 1 hour of collection at 2000 rotations per minute for 10 minutes at 4°C. The plasma was separated and stored at -20°C until assayed (within eight weeks of collection). All samples were assayed together at the end of the study. Characteristics of patients and controls are given in Table 3.1

3.3.2 General Methodology for the Menopausal Study

Eleven menopausal women were recruited from the Menopause Clinic at the Royal Free Hospital. Characteristics of these patients are shown in Table 3.2. The recruitment criteria were that they had their last menstruation at least six months prior to participating in the study and the FSH and LH concentrations were >50 and >20 i.u. respectively. The other eligibility criteria were the same as discussed above.

Each patient had her weight and blood pressure recorded and 10ml of blood was collected for the measurement of ANP concentrations prior to starting on hormone replacement therapy. Further blood samples were taken between 14 and 16 days (oestrogenic phase) and 26 to 28 days (progestogenic phase) of starting therapy.

The hormone replacement was administered as oral therapy in the form of prempak C 0.625mg (the first 16 days consisted of only oestrogen followed by 12 days of combined oestrogen and progestogen i.e. conjugated equine oestrogens and 1 mg norethisterone respectively).

3.3.3 Assay of ANP

3.3.3.a Preparation of Assay Buffer

To 50 Mm sodium phosphate 7.8gm at pH 7.4 was added 0.2% bovine serum albumin (2gm), 10mM EDTA (3.72gm) and 0.1% Triton-X-100 (1ml) and was made up to 1 litre by adding distilled water.

3.3.3.b Extraction of ANP

The extraction of ANP was performed using C 18 Sep-Pak Cartridges (Water Associates Milford MA). The Sep-Pak was activated with 5ml of methanol and then washed with 5ml of distilled water. Then 5ml plasma was pumped through the Sep-Pak cartridge following which the cartridge was washed with 5ml of distilled water. The adsorbed ANP was then eluted with 4.5ml of 86% ethanol/4.5% acetic acid mixture into glass tubes containing 100μl of 1% bovine serum albumin. The tubes were then evaporated to dryness at 50° C under nitrogen. To dissolve the dried residue, to each tube was added 250μl from the previously prepared phosphate triton buffer.

3.3.3.c Radiolabel

The ^{125}I α-human ANP was reconstituted in 1ml assay buffer and dispensed in aliquots of 0.2ml into polypropylene vials and stored at –20°C. Each aliquot had sufficient tracer to perform one days experiment.

3.3.3.d Antiserum

The antiserum was completely dissolved in 2ml of assay buffer. This was then further diluted to 12.5ml with assay buffer and stored at 2-4°C.

3.3.3.e Standards

Human ANP was obtained from Peninsular Laboratories. The stock standard solution of 1ml contained 5ng of ANP. Two solutions of standard were prepared in assay buffer; one at a concentration of 1nM (3.07ng/ml) and the second at a concentration of 0.1nM (0.307ng/ml). To prepare 1nM standard solution, 0.61ml from the above standard was taken and made up to 1ml with 0.39ml of assay buffer and mixed thoroughly. The standard solution of 0.1nM was prepared by adding 0.9ml of buffer to 0.1ml of the 1nM standard solution.

3.3.3.f Assay Protocol

The assay was performed using the ANP assay kit Code IM 1871 supplied by Amersham International plc. The samples and standards were assayed in duplicates.

1. 50μl of tracer solution was added to each assay tube.
2. The standard tubes were prepared as follows:

0.1nM solution of ANP was added to 4 x 2 tubes in the following amounts:- 10μl (1fmol), 20μl (2fmol), 50μl (5fmol) and 100μl (10fmol).

Similarly 1nM solution was dispensed into 4 x 2 tubes; 20μl (20fmol), 50μl (50fmol), 100μl (100fmol), & 200μl (200fmol). These tubes provide the standard curve.

3. 50μl of plasma extract was added to each sample tube.
4. 50μl from the prepared antiserum solution was added to each assay tube except the blank tube.
5. In the blank tube 350μl of buffer and 50μl of radiolabelled solution was added.
6. To the zero tube was added 300μl of buffer and 50μl of antiserum solution. The volume was made up to 400μl with buffer solution. No standard was added to this tube.
7. The volume in each tube was then made up to 400μl by adding buffer.
8. The solution in each tube was then mixed.
9. The tubes were then covered and incubated at 4°C for 24 hours.

3.3.3.g Separation Procedure

Activated charcoal (Norit GSX) (0.8gm) and dextran (0.08gm, molecular weight 60,000 to 90,000) were suspended in 50ml of assay buffer, and stirred for 20 minutes at 4°C. To each assay tube 0.25ml of dextran charcoal suspension was added, stirring the suspension continuously to avoid settling. The assay tubes were then centrifuged at 2000 rpm for 15 minutes. The supernatant and charcoal were separated. The bound supernatant was counted for 200 seconds each in a

"Selektronic" gamma counter. The standard curve was then plotted as percentage bound against fmol of ANP per assay tube on a log linear paper. As there was no commercial quality control available our specimens were exchanged with Dr. Sagnella (Department of Medicine, Charing Cross and Westmininster Medical School. Results were within the range of +/-12.5%. We instigated a local quality control of the specimens assayed and were split into aliquots of high, medium and low ANP values. These gave results in the region of +/- 9.6%.

3.4 Results

3.4.1 Premenstrual Syndrome Study Results

The GHQ scores were similar in the follicular phase of the menstrual cycle in both patients and controls (Table 3.3). In the luteal phase the patients scores were significantly higher than the controls ($p<0.0006$). This was not unexpected as the questions in the GHQ and MDQ overlap.

The diagnosis of PMS was made by the method previously described in Chapter 2. Details of the VAS delta global scores are presented in Table 3.4. The so called "water retention" scores on the MDQ and the bloatedness scores on the VAS were significantly higher in the PMS patients in the luteal phase ($p<0.0007,0.003$ respectively) (Fig 3.1). In the controls the bloatedness score on the VAS was also significantly higher in the luteal phase ($p<0.02$) (Fig 3.1). Although the body weights of the patients were higher than the controls, this was not significant and no cyclical change was noted in either group. Blood pressures did not show any changes during the cycle (Table 3.1). The ANP (median) levels are shown in Table 3.5. The concentrations in the controls did not show any change during the menstrual cycle. In the PMS patients the ANP levels *decreased*

Table 3.1

Characteristics of women with premenstrual syndrome and an asymptomatic comparison group. Results are median (range) values. No statistical difference was noted between either group.

	Patients	Controls
Age	28 (23–42)	25 (19–34)
Height	1.6 (1.56–1.78)	1.61 (1.52–1.70)
Weight		
Follicular	70.7 (52.2–82.9)	61.1 (46.2–86.0)
Luteal	71.0 (52.8–83.8)	61.3 (46.0–86.0)
Blood Pressure		
Systolic		
Follicular	110 (90–120)	111 (90–140)
Luteal	108 (90–120)	107 (90–142)
Diastolic		
Follicular	70 (60–80)	73 (60-90)
Luteal	70 (60–80)	75 (62–85)

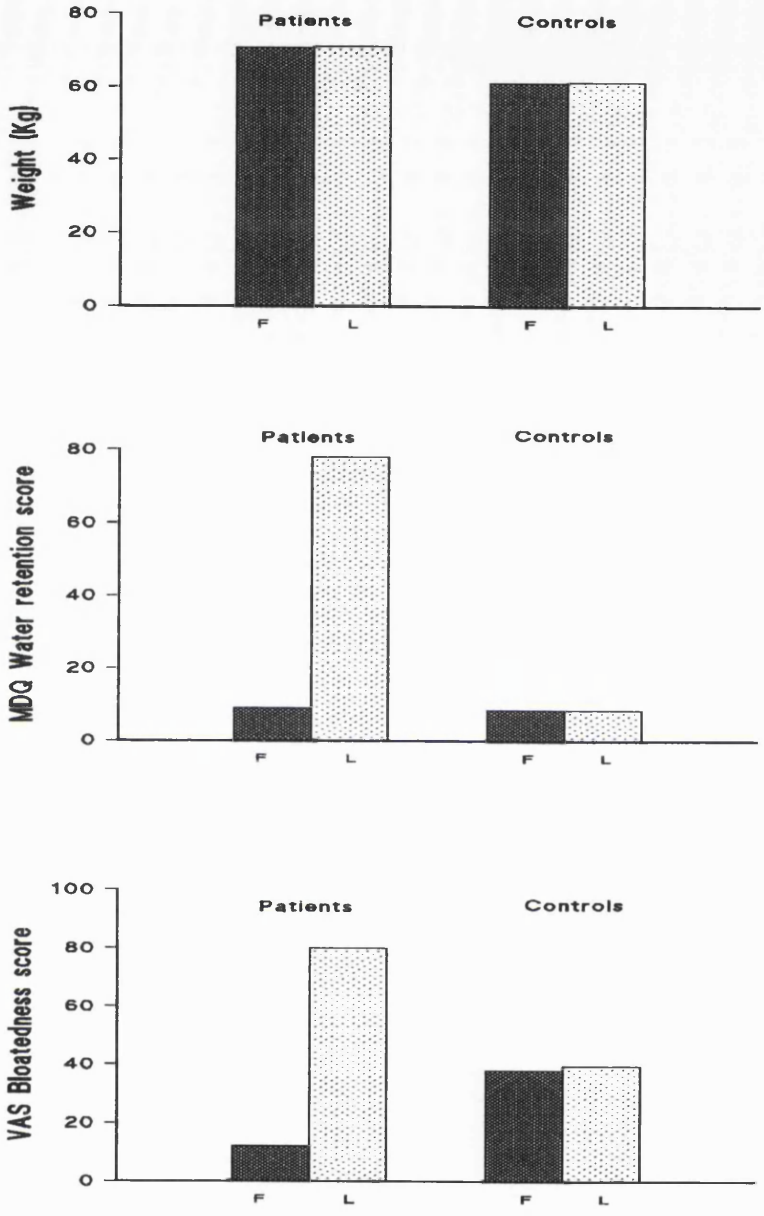


Figure 3.1

Weight (Kg), Moos' Menstrual Distress Questionnaire "Water Retention Scores" and Visual Analogue Scale scores in the follicular (F) and luteal (L) phases of the menstrual cycle. Both MDQ and VAS scores are significantly different ($p = <0.008$, $p = <0.0004$) in the L phase in spite of no difference in the actual "measured weight".

Table 3.2

Characteristics of 11 menopausal women before (Pre), and during hormone replacement therapy. E=Oestrogenic phase and EP=Oestrogen+progestogen. There were no significant differences in blood pressure.

Age		56 (38-70)
Parity		2 (0-4)
Blood Pressure		
Pre	Systolic	120 (100–150)
	Diastolic	80 (70–100)
E	Systolic	114 (94–144)
	Diastolic	72 (64-94)
EP	Systolic	112 (90-150)
	Diastolic	74 (60-90)

Table 3.3

Median General Health Questionnaire (GHQ), Moos' Menstrual Distress Questionnaire (MDQ), Visual Analogue Scale (VAS) scores in 11 PMS patients and 12 controls. Significant p values=*, p value[1] represents patients versus controls. p Value[2] represents follicular versus luteal.

	Patients	Control	p value[1]
GHQ			
F	0 (0-15)	0 (0-13)	NS
L	16 (1-27)	1.5 (0-18)	0.0006*
p Value[2]	< 0.02*	NS	
MDQ			
F	9 (0-24)	8.5 (0-50)	NS
L	78 (54-98)	13.5 (0–41)	0.0001*
p Value[2]	< 0.004*	NS	
VAS			
	Min	Max	Delta
Patients	10.9	69.9	57.6
Delta	15.2	28.0	13.1

Table 3.4

Visual Analogue Scale Global Delta scores (VAS–GD) in 11 patients and 12 controls.

Patients	VAS-GD Scores	Controls	VAS-GD Scores
AL	67.7	EH	22.4
JaS	52.9	VD	18.4
JuS	61.7	MR	9.5
JG	68.7	GO	14.7
GW	52.8	HK	21.1
JS	41.3	KD	8.1
GZ	49.4	RG	8.1
JG	35.4	KDe	25.4
JB	86.0	JH	5.7
LH	58.4	JM	11.6
JH	57.6	LP	23.7
		SR	10.2

Table 3.5

Median concentrations of Atrial Natriuretic Peptide in 11 patients and 12 controls in the follicular, early, mid and late luteal phases of the menstrual cycle. p Value[1] represents patients versus controls. p Value[2] represents follicular versus the different phases as shown below.

	Patients	Controls	p value[1]
Follicular	3.3 (1.5–10.6)	7.3 (1.5–26.3)	0.13
Early luteal	3.5 (1.6–6.8)	6.3 (3.0–12.0)	0.022
p Value[2]	0.5	0.96	
Mid luteal	2.5 (1.5–8.0)	7.25 (1.3–15.0)	0.017
p Value[2]	0.016	0.68	
Late luteal	3.0 (1.5–8.0)	7.55 (1.5–15.0)	0.031
p Value[2]	0.45	0.96	

The ANP concentrations were lower in the PMS women throughout the menstrual cycle when compared to the asymptomatic women but were significantly lower in the EL, ML, and LL phases of the cycle ($p<0.03,0.02,0.04$) (Fig 3.2).

The ANP levels in the mid-luteal phase demonstrated a negative correlation to the delta scores for irritability, bloatedness, clumsiness, breast discomfort and global scores at a significant level (Table 3.6) The delta depression and headache scores did not correlate with the ANP levels.

3.4.2 Menopausal Study Results

The characteristics of these women are shown in Table 3.2 and the median ANP concentrations Table 3.7. There was a fall in the concentration of ANP with the administration of oestrogen and progestogen (Fig 3.3). The levels of ANP though lower in the progestogen phase when compared to the pre-treatment levels, was not significantly different. The decrease in the ANP concentration from the pre-treatment to the oestrogen phase was significant ($p<0.04$, 95% C.I. 0.15, 4.65).

3.5 Discussion

Recently Kim et al (1992) have shown that ANP is synthesized in the ovary and observed changes in ovarian levels of ANP during the oestrous cycle in rats. Bidmon et al (1990) suggested modulatory effects of oestradiol on ANP production and secretion in the brain.

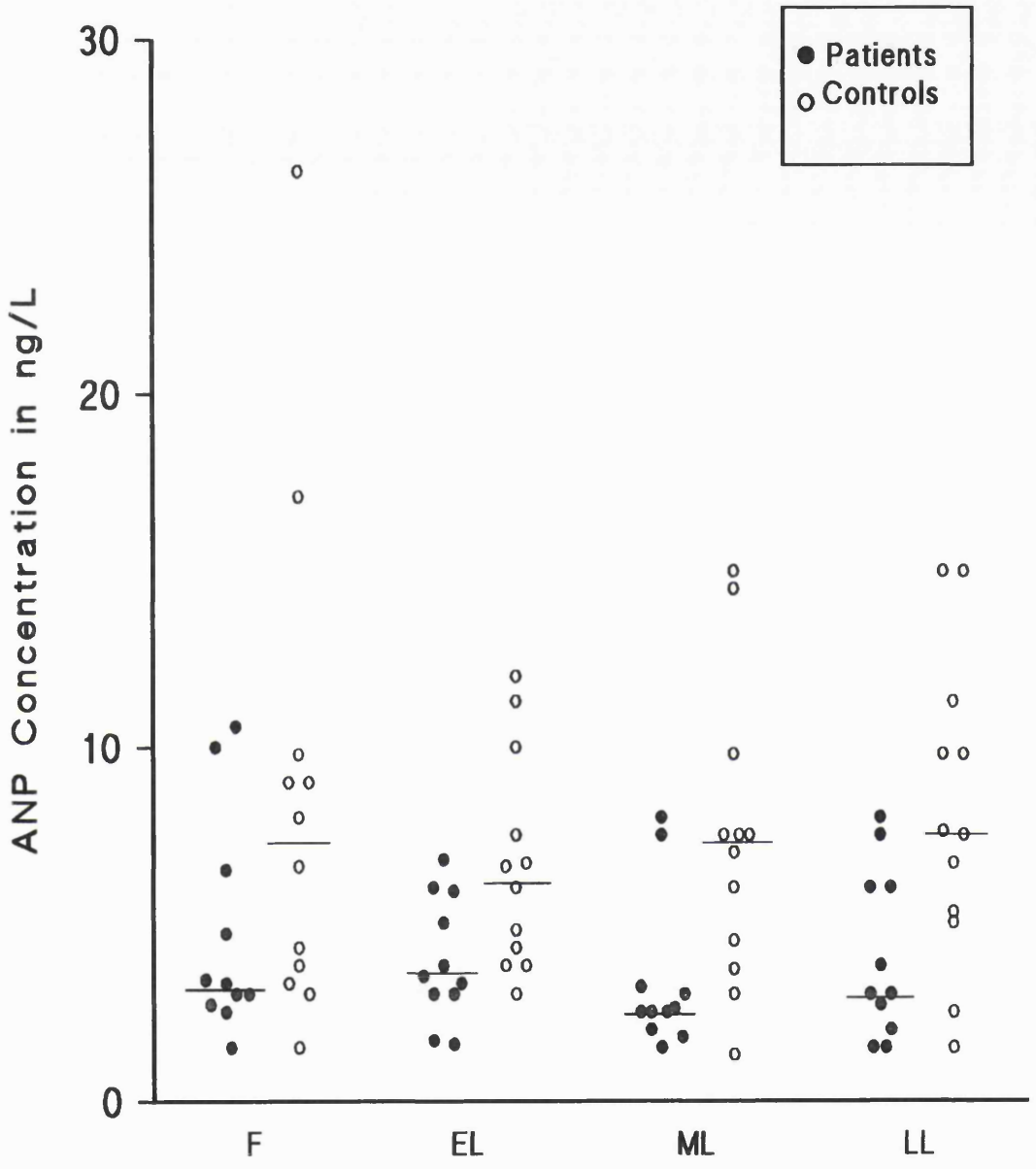


Figure 3.2

Atrial natriuretic peptide (ANP) concentrations in ng/L in 11 patients and 12 controls during the follicular (F), early (EL), mid (ML, and late (LL) luteal phases of the menstrual cycle. Median concentrations are denoted by the bars. Significantly low levels were present in the patients throughout luteal phase when compared to controls. The ML ANP concentration was significantly lower than the F-ANP in patients.

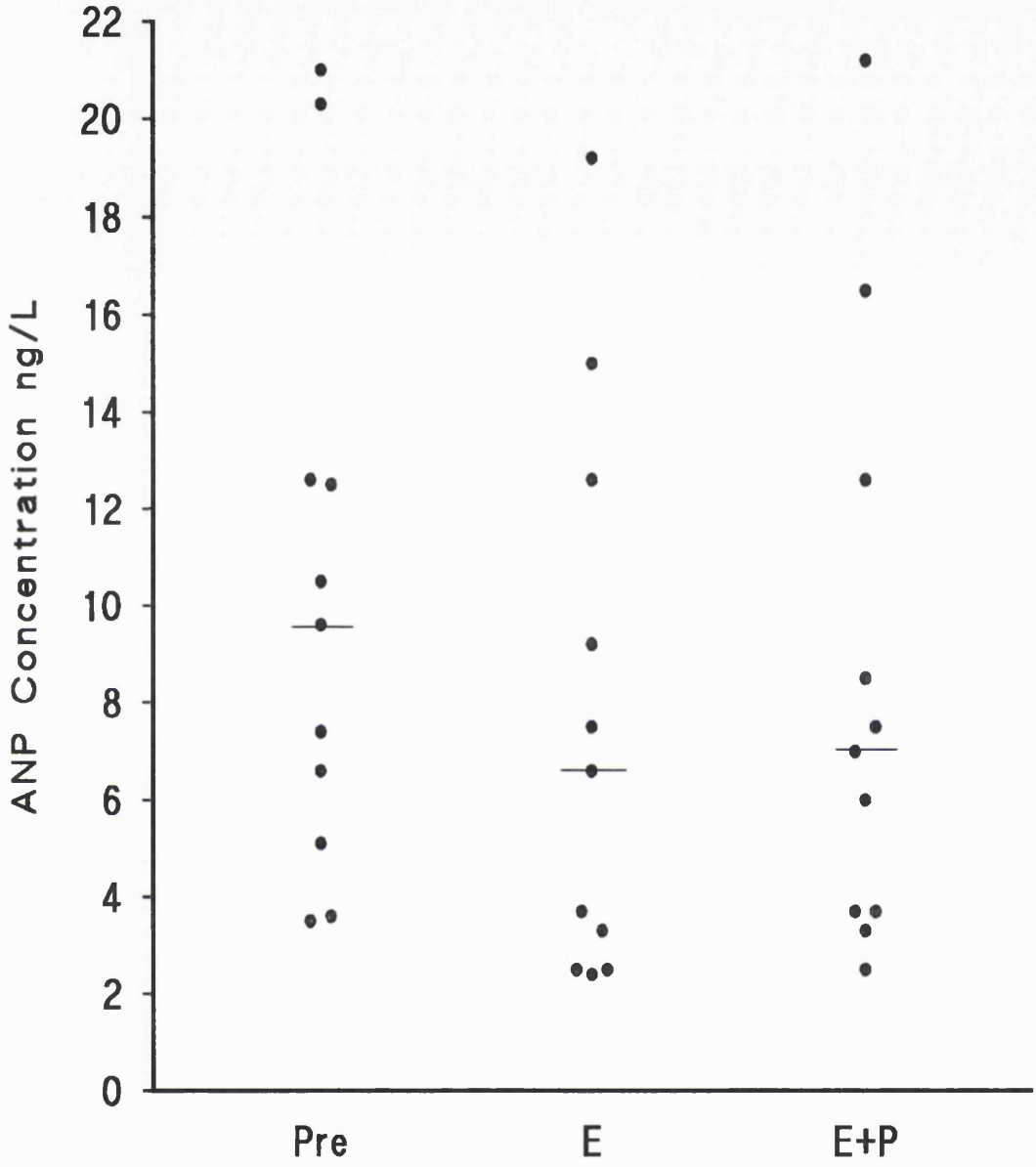


Figure 3.3

Atrial natriuretic peptide (ANP) concentrations in 11 menopausal women before (Pre), and during hormonal therapy. E=estrogen and E+P=oestrogen+progestogen. A significant decrease occurred in the ANP concentrations on administration of oestrogen. There was no further change on administration of progestogen.

Since ANP appears to play an important part in fluid and electrolyte homeostasis it was thought to be relevant to investigate its possible role in the aetiology of PMS, especially as there have been numerous claims of changes in the fluid and electrolyte homeostasis in PMS (Andersch et al, 1978a, Bruce & Russell, 1962, Herzberg, 1971, Janowsky et al, 1973). It is also an antagonist to the renin-angiotensin system. A study measuring the salivary sodium/potassium ratio in the menstrual cycle did not show any significant cyclic variation in women (Chesley & Hellman, 1957). No weight changes were noted in this study, and they suggested the claim that 30 % of women had weight increase premenstrually was probably by chance and/or that the majority of women in any case did not gain weight. In a study by Janowsky et al (1973), a definite relationship was noted between weight change, negative affect and Na/K ratio. All scores increased premenstrually and he concluded that in PMS patients the aldosterone levels were higher thus resulting in sodium retention and an alteration of the Na/K balance. ANP being an antagonist to the renin-angiotensin and aldosterone system would therefore possibly reflect these changes.

The complaint of bloatedness and premenstrual weight gain has over the years been attributed to water retention. In the study by Faratian et al (1984) attempts to measure abdominal dimensions and relate them to weight gain were made. It was interesting to note that in spite of very high bloatedness scores and the patients' perception of increased body size, there was no increase in the measured abdominal dimensions or the body weight.

Table 3.6

ANP mid luteal values correlated against symptoms measured by the Visual Analogue Scale (VAS). VAS Delta Global=GLO-D, bloatedness=Blo-D, breast=Bre-D, headache=Hea-D, depression=Dep-D, irritability=Irr-D and clumsiness=Clu-D.

Delta Scores	RS	p Value
Glo–D	–0.6364	0.001
Blo–D	–0.6235	0.001
Bre–D	–0.4136	0.05
Hea-D	–0.3227	0.133
Dep–D	–0.3182	0.139
Irr–D	–0.5712	0.004
Clu–D	–0.4533	0.03

Table 3.7

Weight and Atrial natriuretic peptide concentrations median values (range), in 11 menopausal women during hormonal therapy. The ANP concentration decreased significantly from the pre treatment phase to the oestrogenic phase (E).

	Weight	ANP
Pre	59.6 (49.2–75.1)	9.6 (3.5–21)
E	59.6 (49.3–76.0)	6.6 (2.4–19.2)
EP	59.7 (50.1–75.9)	7.0 (2.5-21.2)

In the present study there was no significant increase in weight in either patients or controls despite the patients being recruited specially because they had severe bloatedness as one of their PMS symptoms. The patients were slightly heavier than the controls but not significantly so (Table 3.1). Both groups were comparable in height and parity. The blood pressures were the same in both groups. The lower ANP in the patients was surprising. This may represent a lower plasma volume or total body sodium in the patients. The other possibility may be that in the patients in the progestogenic phase there may be an altered effect or response to aldosterone thus resulting in more salt loss and decreased ANP release, especially in the mid luteal phase. Tan et al (1987) did not find any difference in ANP during the menstrual cycle but did not take into consideration whether women were suffering from PMS. In another study measurement of ANP was made in PMS women and not compared to normal

women (Davidson et al, 1988). The criteria of defining PMS were limited. Thus there is a possibility that the "patients" they studied may have been a mixed group. The number was also very small with infrequent sampling. What was particularly interesting were the very significant negative correlations were obtained between mid luteal ANP concentration and delta scores for bloatedness, breast, irritability, clumsiness and global scores (Table 3.6, Fig 3.4).

In view of the different results obtained in the two groups studied it was felt that an investigation into ANP changes during administered oestrogen and progesterone cyclical therapy would be interesting. The menopausal women had a higher median level of ANP (9.6ng/L) than either PMS patients or controls. This is in agreement with the findings of others; this may be for hormonal reasons or because ANP rises with age (Sagnella et al, 1986). Administration of both oestrogen and progestogen resulted in a fall in ANP levels. This decrease in ANP was significant in the oestrogen only phase of the therapy. Oestrogen administration is known to stimulate the renin-angiotensin and aldosterone system (Hytten, 1970). Thus ANP concentration was expected to rise in response to the administration of oestrogen and fall in response to progestogen which is natriuretic. The decrease in ANP concentration was surprising. A fall in ANP levels was expected with the administration of progestogen, instead a rise, though not significant, was noted. Davidson et al (1988) have shown a rise in ANP when patients were treated with combined oral contraceptive therapy but not in the women on oestrogen replacement. They concluded that this may have been due to either the lower dose of oestrogen used or the effect of synthetic progestogen. In our study a higher dose of oestrogen was used.

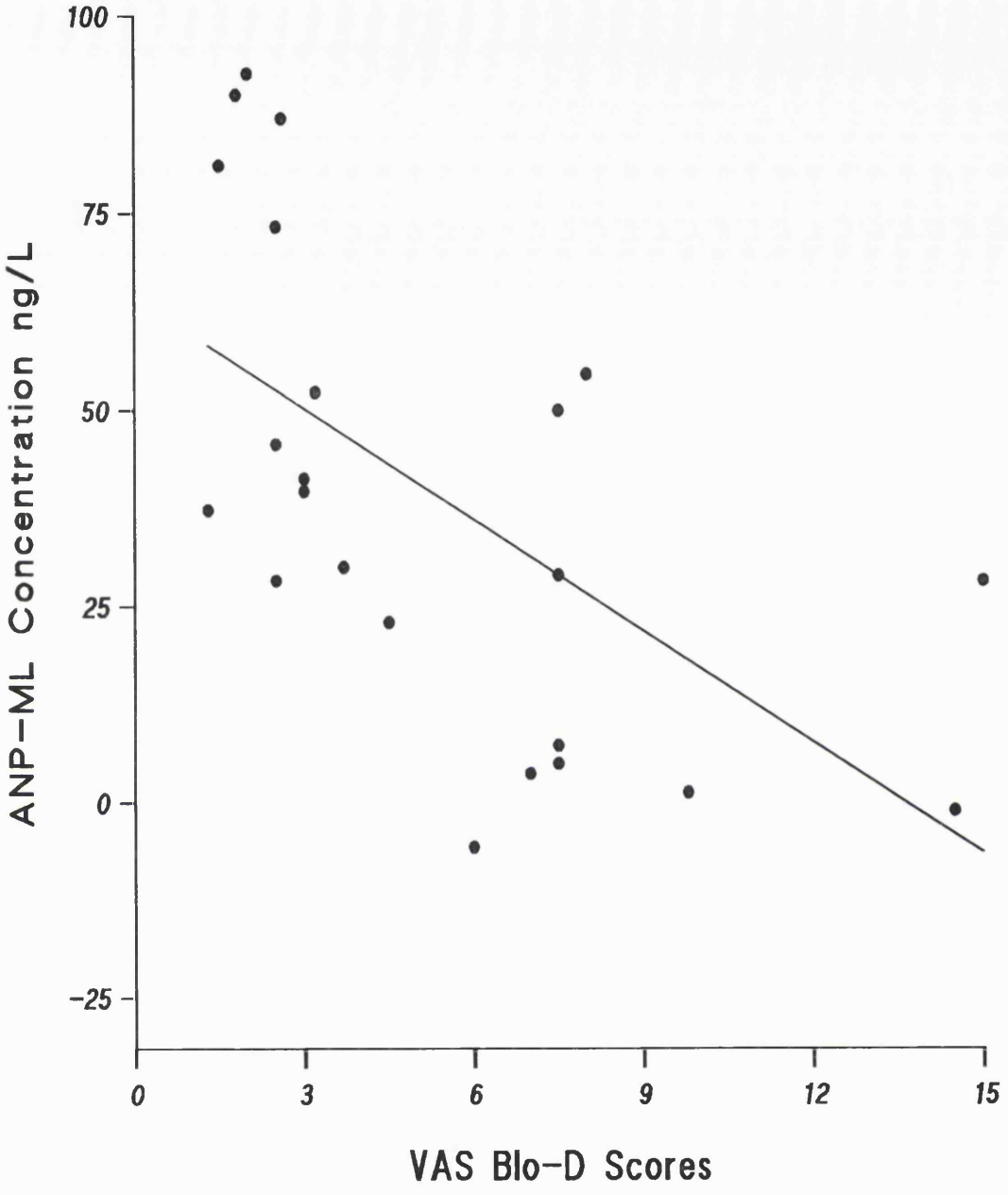


Figure 3.4

A significant correlation was demonstrated between the mid luteal Atrial natriuretic peptide (ANP) concentrations and the Visual Analogue Scale delta bloatedness scores (VAS BLO–D Scores). p value < 0.001 (R=-0.6235)

It may be that there was some fluid retention as a result of oestrogen administration and this resulted in a dilutional effect and thus a relative reduction in the ANP concentration. However in the combined phase of hormone therapy the progestogen (which is a diuretic) resulted in fluid loss thus opposing the effects of oestrogen, consequently causing a rise in ANP concentrations. The other possibility is that the effect of synthetic hormones might not truly mimic the natural hormones and may have thus resulted in these unexpected results.

3.6 Summary

Contrary to expectations atrial natriuretic peptide was found to be significantly lower in the PMS group than in the control group throughout the luteal phase of the menstrual cycle. The concentration of ANP was also noted to fall significantly in the mid-luteal phase in PMS. A strong association was detected between mid–luteal ANP concentrations and symptom scores. These results could imply either a decrease in the total body sodium or plasma or extracellular fluid volumes, which are factors which may affect the concentration of ANP. Results given in later chapters demonstrate that these volumes did not significantly alter during the menstrual cycle with the exception of total body exchangeable sodium. The effect of hormonal therapy resulted in a fall in the ANP concentrations in both the oestrogen only and the oestrogen and progestogen combined phase, the decrease being significant in the oestrogen only phase.

CHAPTER 4

VASCULAR PERMEABILITY IN PREMENSTRUAL SYNDROME

4.1 Introduction

The finding that Atrial Natriuretic Peptide (ANP) concentrations were lower in the Premenstrual Syndrome (PMS) patients than in controls and that the concentration actually reduced in the luteal phase led to the consideration that such changes could be related to, or result in, alterations in the vascular permeability, especially as ANP has an important role in fluid homeostasis (Trippodo & Barbee, 1987). The aims of this study were to investigate changes in vascular permeability using already established technique:

a) during the normal menstrual cycle

b) in a well defined group of women suffering from premenstrual syndrome and

c) in the different phases of hormone replacement therapy.

4.2 Background

Capillary permeability is a complex issue which is not fully understood. There remain certain aspects of function which are difficult to explain by the ultrastructure of capillary membranes. The first direct measurements of capillary pressures in human subjects must be credited to Landis (1930). These measurements helped to establish the validity of the Starling hypothesis of tissue fluid formation. However they also showed that increased capillary fluid filtration

resulting from increased venous pressures could not be *entirely* explained by the Starling hypothesis (1896). According to Starling's hypothesis fluid transport (F) across a semipermeable membrane is

$$F = CFC\ [(P_c - P_i) - \sigma\ (COP_p - COP_i)]$$

where P_c is the capillary pressure, P_i interstitial fluid pressure, and COP_p and COP_i are colloid osmotic pressures in plasma and interstitial fluid, respectively. *CFC* is the capillary filtration coefficient and σ is the capillary reflection coefficient for plasma proteins.

Therefore in describing capillary permeability three types of coefficients are commonly referred to. The first is hydraulic permeability or filtration coefficient which is defined as the flow of water (fluid) through a unit area of capillary wall per unit difference in hydrostatic pressure across the wall. The second coefficient is the diffusional permeability coefficient which is defined as the mass transport of a substance per unit area per unit concentration difference under conditions when the fluid flow through the capillary wall is zero. Finally the reflection coefficient, which compares the penetration of a solute with that of a solvent through a membrane during ultra filtration.

Pappenheimer (1951) suggested the pore theory for the transport of hydrophilic solutes. However he also suggested that water could pass through the capillary wall through cell membranes of the endothelium. An exclusive water permeable pathway has also been suggested by other workers (Wolf & Watson, 1989b). However it has been argued by some researchers that probably not more than 10% of capillary filtration would be accounted for by exclusive pathways (Curry et al, 1976). Various experiments conducted by

Michel (1987) in the study of fluid movement across the walls of single vessels suggested that the fluid conducting channels are not themselves sensitive to pressure increases i.e. the pores do not stretch and it is more likely that larger pores open up in response to increased venous pressures (Wolf et al, 1989a).

4.2.1 The Effects of Serum Proteins on Permeability

It is well known that plasma proteins exert osmotic pressure and increase the effectiveness of plasma expanders to exert their osmotic pressure across the capillary membranes. Albumin has been shown to reduce permeability which was specifically related to its arginine residues as exposure to alkali hampered its ability to reduce permeability (Michel, 1985). It has also been suggested that other serum proteins are required to maintain the permeability of vasculature especially to albumin itself.

4.2.2 The Effects of Hormones on Vascular Permeability

Jones et al (1966) studied capillary permeability to plasma proteins in women during the menstrual cycle. The patients were not defined as PMS patients and the controls were males. They demonstrated an increased permeability to plasma proteins in the luteal phase of the menstrual cycle.

In a further study by Wong et al (1972) an increased capillary coefficient in the luteal phase of the menstrual cycle in women complaining of premenstrual bloating was demonstrated. Increased capillary permeability has been demonstrated in ovarian stimulation cycles (Tollan et al, 1990).

4.3 Methodology

4.3.1 General Methodology

Eleven patients from the PMS clinic of the Royal Free Hospital were recruited. Twelve comparable controls were selected from amongst the hospital staff. PMS patients were defined as previously described in Chapter 2. Each patient and control completed a General Health Questionnaire (GHQ) for the follicular and the luteal phases of the menstrual cycle (Table 4.1). Each control and subject also filled in daily Visual Analogue Scales for the six symptoms ie. depression, irritability, clumsiness, bloatedness, breast symptoms and headache. Their maximum, minimum and delta Global VAS scores were calculated as given in Chapter 2 (Table 4.1). Characteristics of these patients and controls are shown in Table 4.2. The median age of the patients was 35 years and the controls 27 years, which was not significantly different on performing a Mann Whitney's test (C.I. -1.998, 9.999, $p<0.2$).

In the second part of the study, menopausal women were recruited to assess changes in vascular permeability as a result of cyclical oestrogen and progesterone replacement. Characteristics of these women are shown in Table 4.3. Twenty one women were recruited who had been menopausal for at least six months with elevated FSH(>50 i.u.) and LH(> 20 i.u.). The test for vascular permeability was then undertaken prior to initiating hormone replacement. Prempak C (0.625 mg) was given as hormone replacement therapy (conjugated equine oestrogen and norethisterone). The second measurement was made between the 14th to 16th day on oestrogen and the final measurement was made between days 26 and 28 whilst on oestrogen and progestogen.

4.3.2 Measurement of Vascular Permeability

Tests for vascular permeability were performed twice in the menstrual cycle, in the follicular phase between days 5 and 9 and the luteal phase between days 18 and 24 of the cycle. The experimental procedure for measuring vascular permeability was a modification of Landis' original technique (Landis, 1930). Measurements of albumin and of total protein were used. The subject rested for half an hour before the test was started. The test was performed in the sitting position with the arm resting at the level of the heart. A sphygmomanometer cuff was applied to the arm. A blood sample was then taken for total protein and albumin concentration. The pressure was then increased to 80mm of Hg. for 10 minutes and a second sample was taken. The pressure was then released and five minutes later a third blood sample was obtained. Albumin and total protein were measured in each sample. Permeability was assessed from the 0–10 minute change in albumin/total protein values.

This test was also performed on menopausal women on hormone replacement therapy in order to determine any effect of oestrogen and progestogen on vascular permeability. In the menopausal women the test was performed on three occasions as mentioned above.

4.3.3 Statistical Analysis

Wilcoxon Rank test for within patients or controls and Mann Whitney's test for comparison of patients and controls were used for statistical analysis.

4.4 Results

4.4.1 Vascular Permeability Study in PMS

The GHQ scores were not significantly different in the follicular phases of the menstrual cycle (Table 4.1). However, as seen before, the luteal phase scores were significantly different between the two phases of the menstrual cycle. The patients' weights when compared to the controls were not significantly different and there were no significant changes in the weights during the menstrual cycle in either the patient or the control groups. The systolic and diastolic blood pressures did not vary significantly during the menstrual cycle in the patient or control group either within or when compared to each other.

The albumin concentrations demonstrated very interesting changes both amongst the patient and control groups (Table 4.4, Fig 4.1). The increase in albumin concentration between 0 and 10 minutes was significantly higher in the patient group in the luteal phase (CI -6.5, -1.0 p value < 0.03). The opposite was noted in the controls who had a higher increase in albumin concentration from 0-10 minutes in the follicular phase (CI 1.00, 9.00 p value < 0.04) (Table 4.5).

In the patient group, the total protein changes from 0 to 10 minutes mirrored the albumin changes being significantly different in the two phases of the menstrual cycle (CI 1.00, 10.00, $p < 0.03$). The albumin changes between 0-10 minutes both in the follicular and luteal phases were significantly different from the changes in those of the control group (Table 4.5, Fig 4.1). However the total protein change from 0-10 minutes was significantly different from that of the controls only in the follicular phase.

The 10-15 minutes change in both the albumin (CI -6.500, -0.500 $p < 0.02$) and total protein (CI -11.5,-1.5 $p < 0.02$) were significantly different when the follicular phase was compared to the luteal phase amongst the patient group. In the control group albumin changes from 10-15 minutes between the follicular and luteal phase was significant (CI 0.500, 8.00, $p < 0.04$)

4.4.2 Effects of Hormonal Therapy on Vascular Permeability

The median age of the menopausal patients was 54 years (range 31–65) (Table 4.3). The median weights in the pre, oestrogen (E) and oestrogen plus progestogen (E+P) phases were 61.6, 62.7 and 61.5 kgs. These were not significantly different. The blood pressures, both systolic and diastolic did not alter during the cycle (Table 4.3). The absolute albumin levels did not vary significantly in any of the phases (Table 4.6, Fig 4.2). The total protein absolute levels or differences from 0 to 10 or 15 minutes also did not show any remarkable changes during any of the phases. The differences from 0-10, 10-15 albumin concentration did not significantly vary when the pre, oestrogen phase or the oestrogen+progestogen phase were compared (Table 4.7, Fig 4.3).

4.5 Discussion

The results demonstrate clearly that some alteration occurs in the vascular permeability to fluid during the menstrual cycle both in the patients and controls. The aim in this study was to determine changes in vascular permeability by very simply increasing the venous occlusive pressure and then measuring changes occurring in the albumin concentration.

Table 4.1

General Health Questionnaire and Visual Analogue Scale scores in 11 patients and 12 controls. Max = Maximum, Min = Minimum and Delta = Difference between the Max and Min scores. F = follicular and L = luteal scores, P = patients, C = Controls.

Name	GHQ		Visual Analogue Scale score		
P	F	L	Max	Min	Delta
JG	0	16	41.7	6.3	35.4
JB	0	6	94.1	8.1	86.0
LH	4	16	69.9	11.5	58.4
JH	4	15	69.4	11.8	57.6
KC	0	5	67.7	16.1	51.6
EP	3	7	50.8	15.7	35.1
JS	0	17	50.6	9.3	41.3
MM	0	7	67.8	4.9	61.29
SG	0	21	61.6	9.8	51.8
NA	2	5	69.8	28.1	41.7
GZ	5	18	74.3	24.9	49.4
C					
KD	0	0	32.2	6.8	25.4
RG	0	0	19.7	11.6	8.1
JH	0	0	29.0	17.2	12.7
RF	0	0	12.9	7.3	5.6
LF	1	2	14.5	10.7	3.9
NM	2	3	31.1	24.0	7.1
CT	4	3	9.2	3.7	5.5
JC	3	5	24.3	0.1	24.8
LP	0	0	44.6	22.1	22.4
JM	3	5	15.3	3.7	11.6
SR	1	3	25.1	14.9	10.2
GS	0	0	37.8	26.6	11.2

Table 4.2

Characteristics of 11 PMS patients and 12 controls. F = follicular, L = luteal. p Values between patients and controls = $^{+}$ and between F and L phases = $^{++}$.

			Patients	Controls	p Value^{+}	95% CI
Age			35	27	0.139	-1.998, 9.999
Weight	F		65.3	59.5	0.218	–3.0, 12.2
	L		65.3	59.5	0.230	–3.2, 12.2
	p value^{++}		1.00	0.683		
	95% CI		–0.1500, 0.75	–0.50, 0.250		
Blood Pressure						
	F	Sys	112	110	0.196	0.00, 18.00
	L	Sys	110	110	0.207	–2.00, 15.00
	p value^{++}		0.859	1.0		
	95% CI		–4.00, 5.00	–0.0001, 0.0001		
	F	Dias	70	70	0.57	-5.00, 8.00
	L	Dias	70	72	0.7119	–6.00, 7.00
	p value^{++}		0.529	0.554		
	95% CI		–2.0, 3.0	–2.5, 3.0		

Table 4.3

Characteristics of 21 menopausal women. Pre = before therapy, E = oestradiol phase, E+P = oestradiol + progestogens. Sys = systolic, Dias = diastolic.

Age		54		
		Pre	**E**	**E+P**
Weight (kgs)		61.6	62.7	61.5
Blood pressure	Sys	120	110	110
(mm of Hg)	Dias	78	74	78
Columns tested			**p value**	**95% CI**
Weight	Pre vs E		0.43	−2.00, 0.10
	Pre vs E+P		0.40	−0.35, 0.15
	E vs E+P		0.73	−0.30, 0.10
Blood Pressure				
Sys	Pre vs E		0.082	0.00, 6.00
	Pre vs E+P		0.256	−2.00, 8.00
	E vs E+P	0.932	−3.00, 3.00	
Dias	Pre vs E		0.120	0.000, 4.00
	Pre vs E+P		0.164	−1.00, 6.60
	E vs E+P	0.554	−2.00, 3.00	

Table 4.4

Median albumin and total protein concentrations in 12 patients and 11 controls during the menstrual cycle. p Value between patients and controls are shown.

a. Albumin Concentrations

Time	Patients	Control	p value	95% CI
Follicular				
0 min	43	44	0.406	–2.99, 1.0
10 min	51	57	0.022	–9.9, –0.9
15 min	42	43	0.82	–2.0, 2.0
Luteal				
0 min	44	45	0.06	–4.0, 0.001
10 min	55	53	0.406	–2.0, 6.00
15 min	42	42.5	0.711	–2.0, 0.999

b. Total Protein Concentration

Follicular				
0 min	69.0	69.0	0.355	–5.001, 2.002
10 min	81.0	89.0	0.039	–14.0, –0.0001
15 min	65.0	67.0	0.853	–3.998, 3.001
Luteal				
0 min	68.0	71.0	0.09	–8.001, 1.00
10 min	88.0	87.5	0.719	–7.00, 7.00
15 min	65.0	68.5	0.355	–6.99, 2.00

Table 4.5

Albumin and total protein 0–10 and 10–15 minute change during the menstrual cycle in 11 patients and 12 controls. p Values between patients and controls = $^{+}$ and between F and L phases = $^{++}$.

a) Albumin

		Patients	Controls	p Value^{+}	95% CI
F	**0-10 min**	8.0	13.0	0.009	−7.001, −0.998
L	**0–10 min**	11.0	7.5	0.036	0.002, 7.99
	p value^{++}	0.025	0.034		
	95% CI	−6.5, −1.00	1.00, 9.00		
F	**10–15**	8.0	14.5	0.006	−7.998, −2.001
L	**10–15**	13.0	10.5	0.230	−1.002, 6.001
	p Value^{++}	0.02	0.037		
	95% CI	−6.5, −0.5	0.50, 8.0		

b) Total protein

F	**0–10 min**	12.0	18.5	0.01	0.99, 10.001
L	**0–10 min**	20.0	12.5	0.123	−11.00, 2.00
	p Value^{++}	0.023	0.158		
	95% CI	1.00, 10.00	−9.5, 2.00		
F	**10–15**	13.0	21.0	0.005	−13.00, −1.998
L	**10–15**	23.0	19.5	0.185	−3.002, 6.99
	p Value^{++}	0.018	0.03		
	95% CI	−11.5, −1.5	0.50, 8.0		

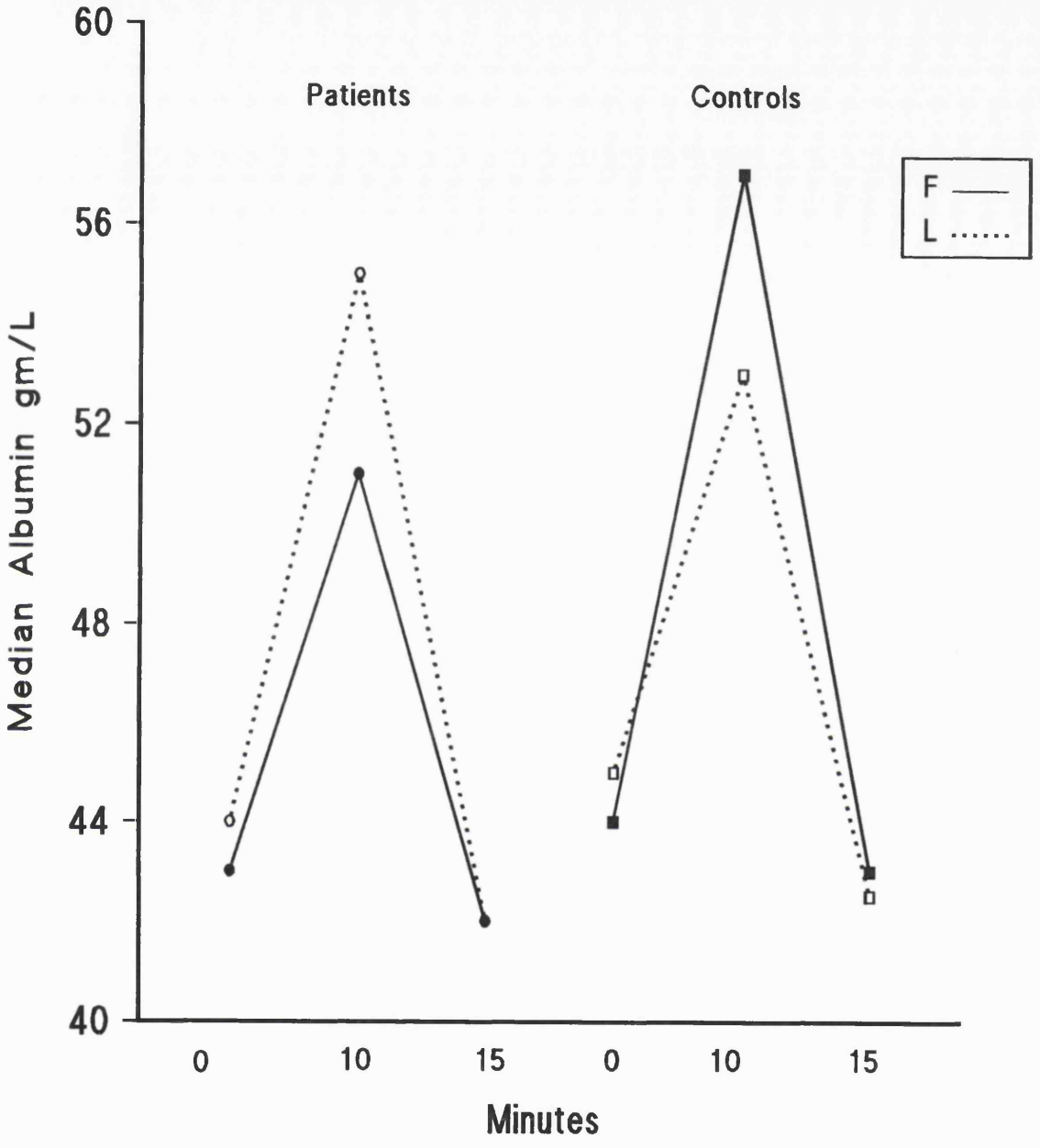


Figure 4.1

Median albumin concentrations in 11 PMS patients and 12 controls during the menstrual cycle. F = follicular and L = luteal. The 10 minute albumin concentrations between the patients and controls were significantly different in both the F and L phases; p=0.022 & p=0.039 respectively.

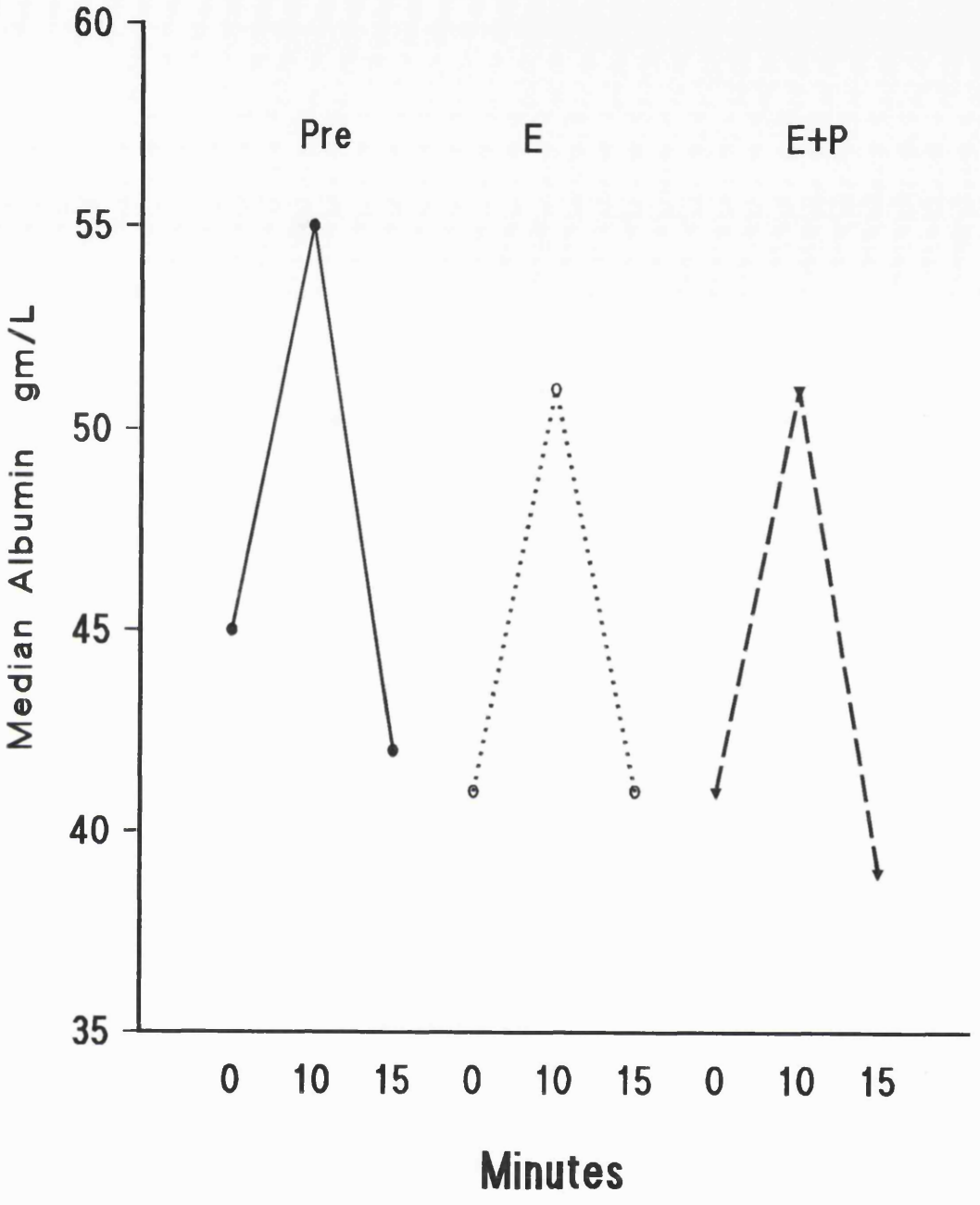


Figure 4.2

Albumin concentrations during hormonal therapy in 21 menopausal women. Pre = before therapy, E = Oestrogen phase and E+P = oestrogen + progestogen. No significant changes occurred.

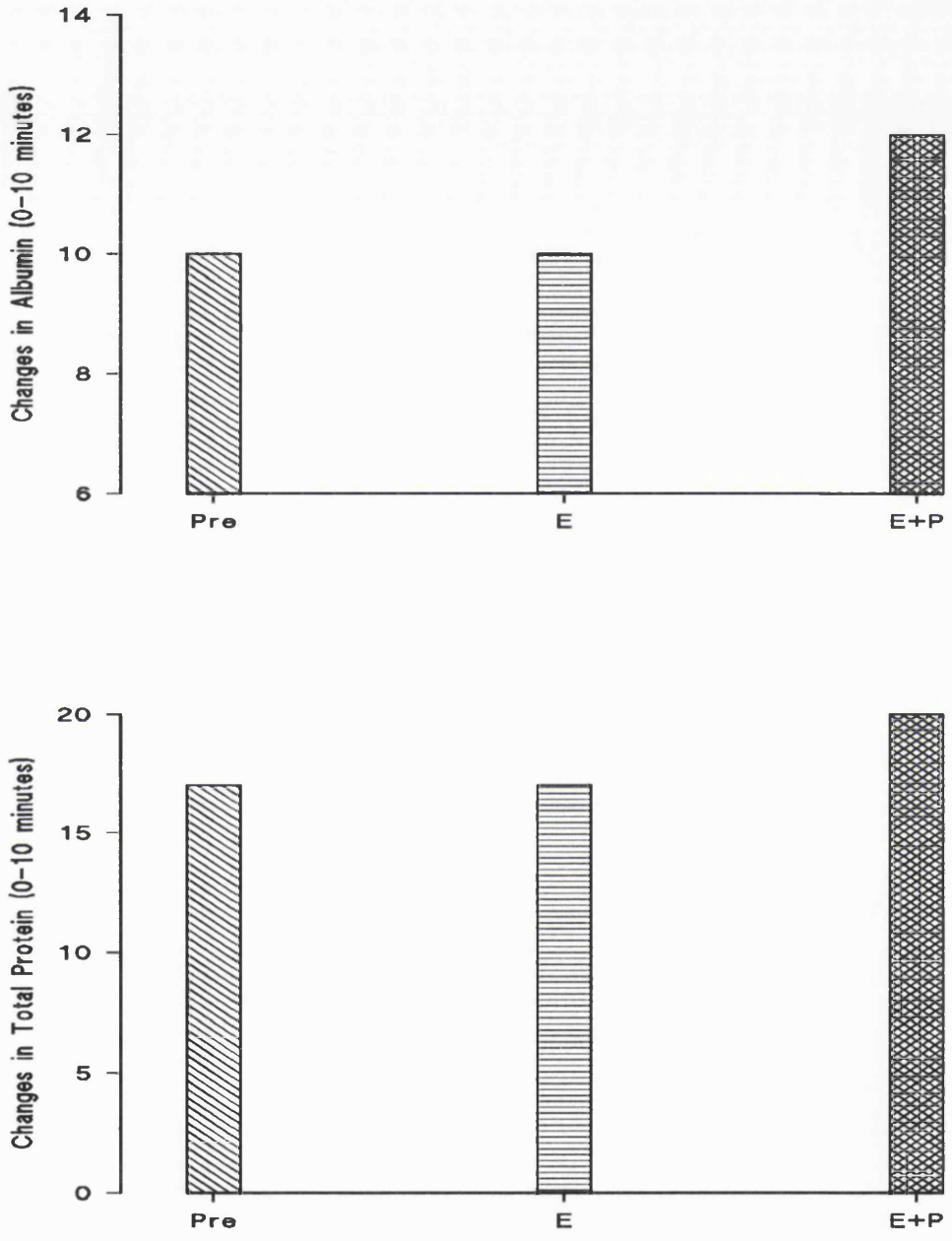


Figure 4.3
Changes in a) albumin and b) total protein concentrations from 0–10 minutes during during hormonal therapy in 21 menopausal patients. Pre = before treatment, E = oestrogen phase, E+P = oestrogen + progestogen.

Table 4.6

Median albumin and total protein concentrations in 21 menopausal women during hormonal therapy.

a) Albumin Concentrations

Time	Pre	E	E+P
0 min	45	41	41
10 min	55	51	51
15 min	42	41	39

b) Total Protein

0 min	71.0	86.0	79.0
10 min	86.0	80.0	65.0
15 min	70.0	83.0	64.0

This method of analysing vascular permeability to albumin has been described previously (Valensi et al, 1987).

The follicular-to-luteal phase differences in albumin and total protein changes from 0-10 minutes was higher in the patients than in controls (Fig. 4.4). In fact the controls on average showed a decrease in the measurement from the follicular to the luteal phase. The difference between patients and controls is illustrated in Fig 4.5 and is highly significant ($p < 0.004$). These findings suggest an increased permeability to fluid and possible transcapillary extravasation in the luteal phase in the patients. However the increased albumin concentration in the luteal phase may also imply decreased permeability to albumin. If the permeability to albumin had increased, albumin would have escaped into the interstitium and the concentration of albumin would not have increased. It is possible though that a combination of events occurs, in that both increased permeability to fluid associated with a decreased permeability to proteins occur in the luteal phase in the patients, the opposite occurring in the controls.

Jones et al (1966) suggested an increase in protein permeability in eight women in the luteal phase of the menstrual cycle. The results demonstrated in the present study suggest similarly increased permeability to albumin in the luteal phase in the normal menstrual cycle as was demonstrated in the controls. However, surprisingly, in the patients the results appeared confusing. Wong et al (1972) have shown an increased capillary coefficient, suggesting increased permeability to water in patients complaining of bloatedness. However in their study, the methodology of the diagnosis of PMS is unclear.

Table 4.7

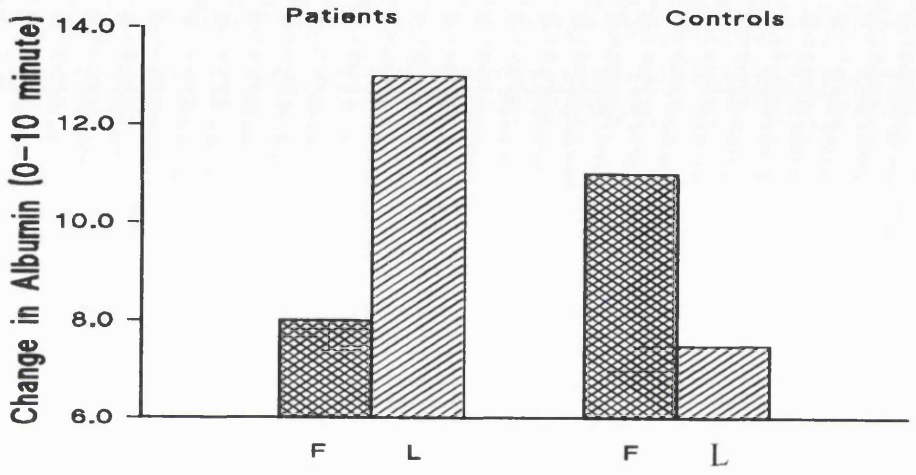
a) Albumin and b) Total protein changes from 0–10 and 10–15 minutes in menopausal women during hormonal therapy.

a. Albumin

		0–10 Minute	10–15 Minute
Pre		–10.0	11.0
E		–10.0	10.0
E+P		–12.0	13.0
Columns Tested		**p Value**	**95% CI**
Pre vs E	0–10	0.433	–5.50, 2.50
Pre vs E+P	0–10	0.808	–3.50, 3.50
E vs E+P	0–10	0.191	–1.50, 6.50
Pre vs E	10–15	0.520	–4.00, 7.00
Pre vs E+P	10–15	0.614	–6.50, 4.00
E vs E+P	10–15	0.135	–6.50, 1.00

b. Total Protein

		0–10 minute	10–15 minute
Pre		–17.0	19.0
E		–17.0	18.0
E+P		–20.0	20.0
Columns Tested		**p Value**	**95% CI**
Pre vs E	0–10	0.440	–3.00, 7.00
Pre vs E+P	0–10	0.972	–6.50, 6.50
E vs E+P	0–10	0.911	–7.00, 6.50
Pre vs E	10–15	0.872	–8.00, 6.00
Pre vs E+P	10–15	0.821	–9.00, 6.00
E vs E+P	10–15	0.972	–5.50, 5.50



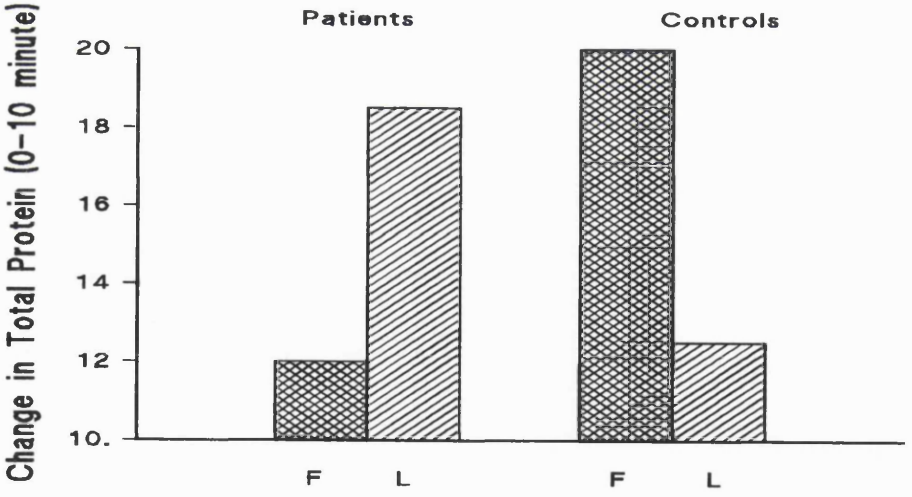


Figure 4.4

The 0-10 minute change in albumin was significantly different between follicular (F) and luteal (L) phases in patients (p=0.025) and controls (p=0.036). The 0-10 minute change in total protein was significantly different between F and L phases in patients (p=0.023), but not in controls.

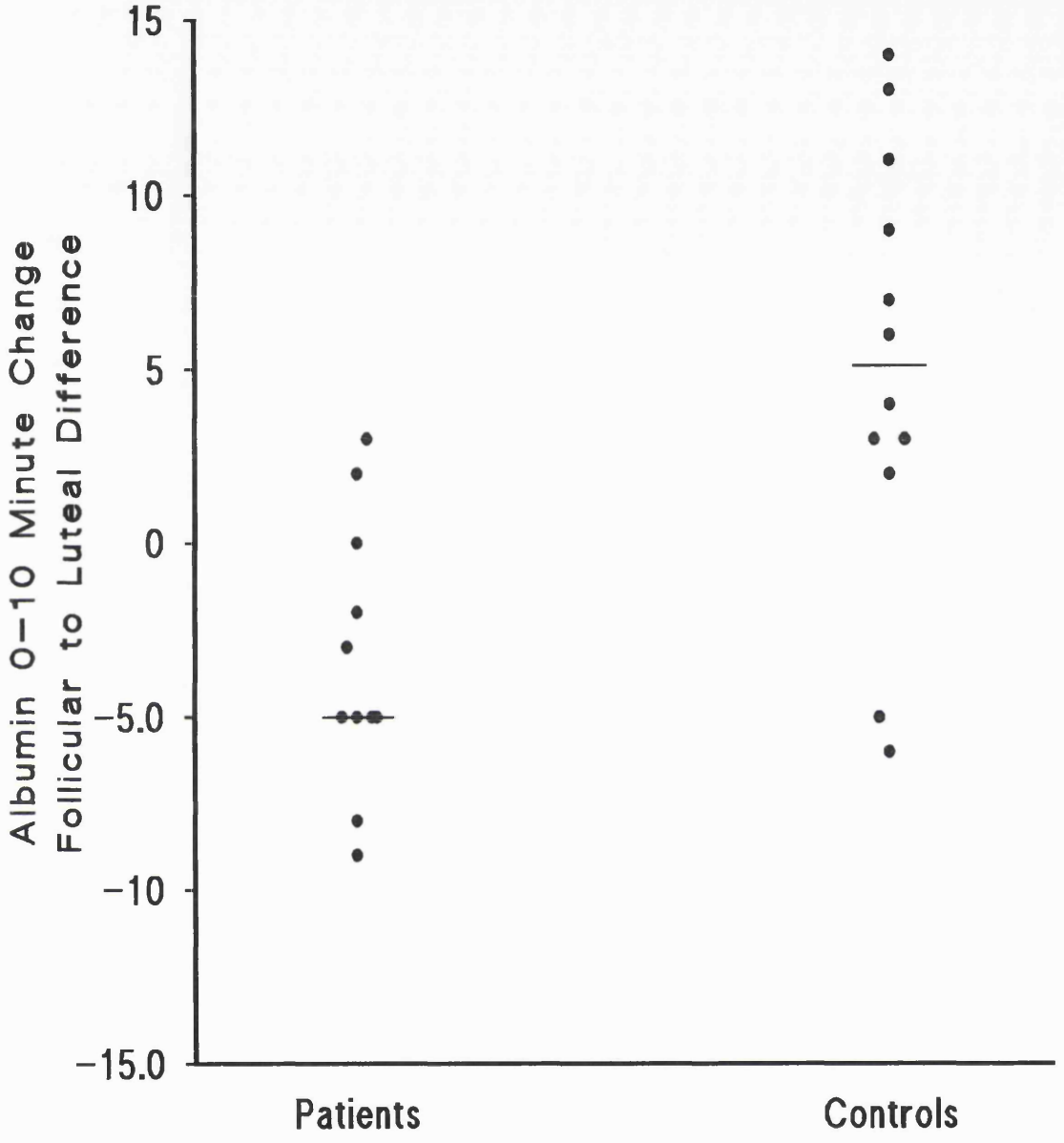


Figure 4.5

Follicular to luteal phase differences in the 0–10 minute albumin changes in patients and controls. This was significant at p value < 0.004. Bar denotes medians.

There are many factors affecting capillary permeability. Any alteration in the capillary hydrostatic pressure, intravascular colloidal osmotic pressure, interstitial colloidal osmotic pressure or lymphatic flow may affect the transcapillary fluid balance. There are factors affecting the flow of fluid and hydrophilic solutes at the ultrastructural level which also need to be considered. In this study the total protein or albumin concentration were not significantly different in either patient or control when follicular phase was compared to the luteal phase. Thus it is unlikely that the intravascular colloidal osmotic pressures were different in the different phases of the menstrual cycle. As suggested by Pappenheimer (1951), and others, at the ultrastructural level there may be an increase in the permeability to water only, which may explain the findings in the PMS patients (Pappenheimer et al, 1951, Wolf & Watson, 1989b). If so the mechanism by which this increased permeability occurs would need to be explained. Possibilities include ovarian hormonal changes. During ovarian stimulation for in vitro fertilisation, increased passage of fluids across the capillary membranes have been demonstrated (Tollan et al, 1990).

We found that in the menopausal women the albumin concentrations did not alter when the ovarian cycle was simulated with hormone replacement in menopausal women. The 0–10 minute albumin changes did not differ significantly between the pre, oestrogen only and oestrogen and progestogen combined phases of hormone replacement (Fig 4.6). It may be that the levels of oestrogen and progestogen were not high enough to affect vascular permeability.

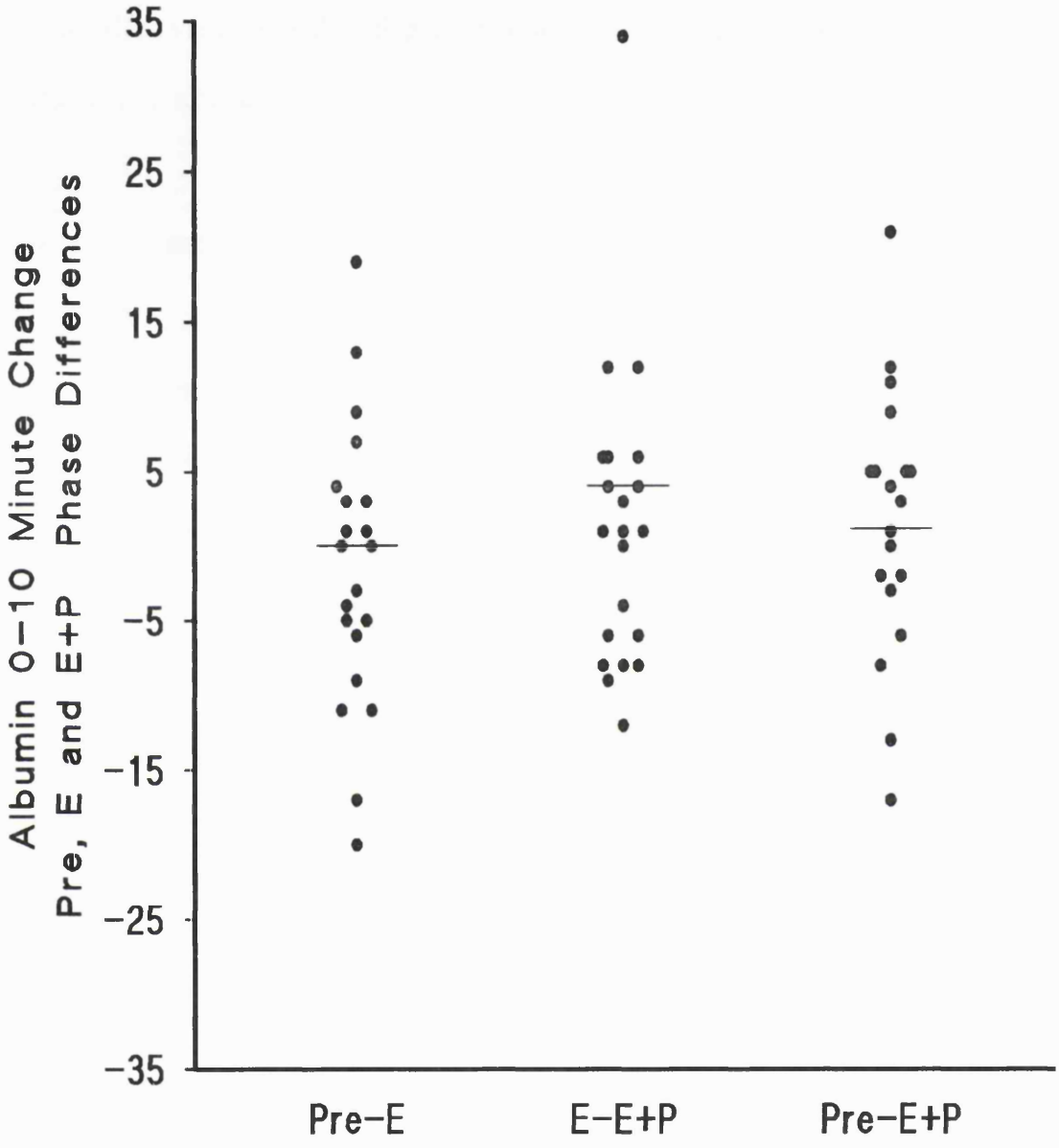


Figure 4.6

Differences between the Pre, Oestrogen and Oestrogen + progestogen phases in the 0–10 minute albumin changes in 21 menopausal patients. These were not significantly different. Bar denotes medians.

On the other hand menopausal women may not have been ideal subjects to study capillary permeability for it may be that the vascular condition is not comparable since their vascular wall collagen structure may be different due to their oestrogen deficiency status.

4.6 Summary

Vascular permeability was studied in eleven PMS patients and 12 controls by a modification of Landis' technique of venous occlusion. The changes in albumin concentration were studied. The effect of hormonal replacement on vascular permeability was also studied

The change in the 0-10 minute albumin concentration was higher in the luteal phase in the patients whilst being the opposite in the controls. Hormone replacement did not appear to affect the change in the albumin concentration in either the oestrogen or progestogen phase. These results suggest that there is increased permeability to fluids in the patients in the luteal phase but not in controls.

CHAPTER 5

THE COMPARTMENTAL DISTRIBUTION OF FLUID AND ELECTROLYTES IN PREMENSTRUAL SYNDROME

5.1 Introduction and Background Information

The somatic symptoms that have been attributed to fluid and water changes include bloatedness, perceived "increase in weight", headaches, breast tenderness and breast swelling. Water retention has been traditionally accepted as the cause for these symptoms. There has however been no conclusive evidence to suggest that premenstrual water retention actually occurs.

In this thesis different aspects of fluid and electrolyte balance have been looked at, in an attempt to explain these symptoms. Changes in the body compartments have previously been implicated and investigated but there is no definite experimental evidence to make firm conclusions. In order to make more definitive conclusions it was decided to carry out measurements on extracellular fluid volume (ECFV), plasma volume (PV), total body water (TBW) and total body exchangeable sodium (ExNa) and determine the relationship between these and somatic symptoms.

"Increase in weight premenstrually", a complaint made by PMS patients, has been thought to suggest water retention in PMS patients during the luteal phase. Bruce and Russell did not find any significant trend in weight changes in 24 psychiatric patients when leading a relatively unrestricted life (Bruce & Russell, 1962). But when 10 other psychiatrically ill patients were

studied in a metabolic ward being given a strict diet there appeared to be a gain in body weight premenstrually. This was associated with a corresponding retention of water and sodium. Urine volume and corresponding urinary sodium excretion were used as measures of water and sodium balance respectively (Bruce & Russell, 1962).

Weight gain has not been shown to be a consistent feature in PMS patients (Faratian et al, 1984, Andersch et al,1978a). Total body water has been measured by Herzberg (1971) and was found to be significantly decreased in the premenstrual phase of the cycle which is contrary to popular belief (Herzberg, 1971). This was not supported by a later study by Preece et al (1975) who measured TBW in women who had premenstrual breast pain.

Earlier experiments on the concentration of atrial natriuretic peptide (ANP) have shown that PMS patients not only had a significantly lower concentration of ANP overall, but also had a fall in the ANP concentration in the luteal phase of the menstrual cycle (Hussain et al, 1990). This therefore led the author to believe that there may actually be an increase in the plasma volume in the patients in the luteal phase of the menstrual cycle resulting in a dilutional effect and therefore a fall in the concentration of ANP. Also the plasma volume in patients may be higher in patients compared to controls, patients thus having overall lower concentration of ANP. This led on to the specific measurements of the fluid compartments of the body mentioned above; these have not been previously measured in a single research study and thus it has been impossible to make valid comparisons or draw valid conclusions of their relationships.

The aims of this section of the thesis were to determine:-

a) If detectable changes occurred in TBW, ExNa, ECFV and PV in the normal menstrual cycle.

b) If detectable changes in TBW, ExNa, ECFV and PV occurred in cycles of women who complained of PMS with significant somatic symptoms.

c) Whether any changes in a) or b) correlated with either somatic or psychological symptoms.

d) Whether there was a likely causal relationship between fluid and electrolyte changes and any of the symptoms of PMS.

5.2 Methodology

5.2.1 The Radio-isotope dilution Principle

The measurements of TBW, ECFV, PV and ExNa were undertaken by use of the radio-isotope dilution method which has been used in body composition and electrolyte studies for more than thirty years (Haxhe, 1971). The dilution principle on a relative basis allows the measurement of an unknown volume or mass by diluting in it a known volume or mass of a coloured dye or tracer isotope. Radio-isotopes and stable isotopes have been used since 1940 for measuring body compartments.

The isotope dilution method is based on the principle that when a known mass or volume of tracer is injected into an unknown mass or volume, it remains the same after mixing, when correction has been made for loss of tracer by excretion or exhalation. Thus in order to measure an unknown volume if a known concentration or amount of tracer substance is injected into this unknown volume,

and the amount to which this tracer substance dilutes after equilibrium ie. the concentration after equilibrium is measured, the unknown volume can then be calculated.

The following equation represents the above statement:

$C_1V_1 = C_2V_2$ where C_1 and V_1 are the concentration and volume of the substance before and C_2 and V_2 after dilution. Thus the unknown volume $V_2 = C_1V_1 \div C_2$ can be determined by measuring the concentration (C_2) after dilution.

5.2.2 Biological Compartments

In applying the technique to the human body factors be considered are that 1) the human body is multicompartmental and 2) a dynamic equilibrium exists between the different compartments.

Thus a state of equilibrium has to be achieved before measurements can be made. The body electrolytes can also be measured using the same principle. However in this measurement, the phenomenon of atomic exchange is responsible for the equilibration. In the measurement of exchangeable sodium for instance, the radioactive sodium tracer equilibrates with the naturally occurring sodium in the different body compartments. Some compartments readily exchange isotopes while in others exchange occurs very slowly. In compartments such as cartilage, bone and fascia sodium exchanges very slowly and true equilibration is thus not possible. It is therefore only the equilibrated sodium that can be measured, and the measurement obtained is termed as total body exchangeable sodium and not total body sodium.

5.2.3 Radio-isotope Tracers

Ideally any tracer used should possess certain properties:

a) it should be non toxic,

b) it must be able to mix and equilibrate in the compartment being measured without transfer to another compartment,

c) it should be neither metabolised nor synthesised in the body,

d) if excreted its rate of excretion should be measurable,

e) a source of easy sampling should be available eg. blood, urine.

It is difficult to find such an ideal substance as no one tracer fulfils all the above qualities. The methods and isotopes which have been used in this study have been used separately in previous studies. The important aspect of this study has been the use of multiple isotopes simultaneously for the measurement of different compartments. This led to a considerable degree of difficulty initially in accurately measuring all four isotopes when used simultaneously.

The isotopic tracers used in this study were tritiated water (^{3}HHO) for measuring total body water (TBW), ^{22}Sodium chloride ($^{22}NaCl$) for exchangeable sodium, ^{51}Chromium ethylene-diamine tetraacetic acid (^{51}Cr-EDTA) for extracellular fluid volume (ECFV), and ^{99m}Technetium labelled human serum albumin (^{99m}Tc-HSA) for the measurement of plasma volume. The physical properties of the radioisotopes are described in Table 5.1

As ^{22}Na has a half life of 2.6 years and ^{24}Na a half life of 15 hours, the use of ^{24}Na has been more popular for studies in humans in the United Kingdom. However Smith et al (1987) have suggested the use of ^{22}Na for such studies on account of it being more readily available, its cost being lower when the longer

shelf life and low administered activity were considered. Also radiation dosimetry being lower from using very low activities, implied a lower exposure risk to ionising radiation. Also of note was that the total irradiation dose from ^{22}Na, ^{51}Cr and ^{3}H precluded the use of ^{125}I-HSA (^{125}I labelled human serum albumin) and ^{99m}Tc-HSA was used as an alternative.

Table 5.1

Physical properties of radio-isotopes used in the measurement of total body water, total body exchangeable sodium, plasma volume and extracellular fluid volume respectively.

Radioisotope	Radiation	Energy	Physical Half Life
3 H	$\beta-$	0.018 MeV	12.24 years
22 Na	$\gamma\beta+$	0.51, 1.28 MeV	2.6 years
99m Tc	γ	0.14 MeV	6 hours
51 Cr	γ	0.32 MeV	27.8 Days

5.3 Protocols used for Measurement of TBW, ECFV, ExNa, PV

5.3.1 Patient Selection

Using the diagnostic criteria as described in Chapter 2, 17 patients and 10 controls were recruited for this study. These patients and controls were different to those recruited for the ANP and vascular permeability study. The measurements were carried out once in the follicular (day 5-9) and once in the luteal (day 18-26) phase of the menstrual cycle. Half the patients and controls had their first measurements performed in the follicular phase and viceversa. The General Health Questionnaire (GHQ) and Visual Analogue Scale (VAS) scores are shown in Table 5.2 and 5.3 respectively. The patient details are discussed in the result section (Table 5.4).

Table 5.2

Median General Health Questionnaire Scores in 17 patients and 10 controls

	F	L	p value	95% CI Interval
Patients	1.0	15.0	<0.0001	−17.0, −8.5
Controls	1.0	1.5	1.0	−1.0, 1.5
	Patient F	Control F		
	1.0	1.0	0.763	−2.0, 1.99
	Patient L	Control L		
	15.0	1.5	0.0001	8.0, 17.0

Table 5.3

Visual Analogue Scale scores in 17 patients and 10 controls.

Patients	Max	Min	Delta
JS	67.1	14.2	52.9
JB	94.1	8.1	86.0
DK	49.4	7.2	42.2
KOS	60.3	4.3	56.1
LH	69.9	11.5	58.4
PW	66.9	11.8	55.1
HD	71.6	8.6	62.9
LS	55.5	8.9	46.6
JS	82.1	25.3	56.8
MC	73.0	5.9	67.1
AR	73.1	20.6	52.5
JG	41.7	6.3	35.4
GW	60.5	7.7	52.8
ML	49.9	3.1	46.9
LA	57.3	10.2	47.1
JR	53.9	2.1	51.8
CN	76.2	13.6	62.6
Controls			
GO	38.9	24.2	14.7
RF	29.2	17.2	12.0
KD	24.4	16.4	8.1
JM	15.3	3.7	11.6
KH	9.2	11.1	-1.9
GS	37.8	26.6	11.2
DE	26.1	23.7	2.4
SW	10.9	6.7	4.2
SS	28.0	33.8	4.1
VS	26.8	12.8	14.1

Table 5.4

Characteristics of 17 patients and 10 controls (median values).

	Patients	Controls
Age	31.00	30.5
Height	1.63	1.65
Parity	0	0

5.3.2 General Preparation of Subjects

All subjects fasted from midnight the previous night. On the day of the study a glass of pure orange juice was allowed and taken at least 2 hours before the start of the experiment. The subject emptied her bladder and a baseline urine sample was obtained following which she was weighed in a hospital gown. The experiment started at 10 AM and the initial phase was completed at 2.00 PM during which period the patient continued to fast apart from 100 mls of orange juice given to the patient at 2 hours post radio-isotope administration. A 21 gauge butterfly cannula was inserted into the cubital vein and blood samples were obtained for background radioactivity, endocrine profile (FSH, LH, E_2, Progesterone), urea and electrolytes, following which the cannula was flushed with 50 iu heparin. Then the radioisotopes ^{3}HHO and ^{22}Na were administered orally and ^{99m} Tc-HSA and ^{51}Cr-EDTA were injected into the other arm. Blood samples were obtained at 10, 20, 30 minutes and 1, 2, 3 and 4 hours, into heparinised tubes (6 mls for each sample apart from the 4 hour sample which was 20 mls) and urine samples were collected in appropriately labelled containers; the relevance of each blood or urine sample is shown in Table 5.5.

The patient returned at the end of 24 hours the following day having fasted again from midnight. On arrival she emptied her bladder into the 22-24 hour container and was weighed again as previously following which 20 mls of blood was obtained. At this point the experiment ended for that particular phase of the menstrual cycle. The subject returned for the 2nd test in the luteal phase of her cycle when the same procedure was followed as outlined above.

Table 5.5

Tabulation of Blood and Urine samples required for the relevant test as indicated by the "x".

	TBW	PV	ECFV	ExNa
Blood				
0 minutes	x	x	x	x
10 minutes		x		
20 minutes		x		
30 minutes		x		
60 minutes		x	x	
2 hours		x	x	
3 hours			x	
4 hours	x		x	
24 hours				x
Urine				
Pre	x			x
0-4 hours	x			
22-24 hours				x
Pooled (including 4-22 h)				x

In certain cases the 2nd test did not follow within 14 days if progesterone levels indicated that ovulation had not occurred for that woman, or occasionally due to the inability of the patient to attend. Further progesterone levels were performed to establish occurrence of ovulation and thus the correct time for performing the second test. The methods used for the measurement of the different compartments will be described individually.

5.3.3 Dose Preparation

5.3.3.a Total Body Water

A dilution with water of the original stock was made to give a concentration of 20 μCi/ml.

5.3.3.b Exchangeable Sodium

A dilution with 0.9% NaCl of the original stock was made to give a concentration 1 μCi/ml.

5ml of dilution a) and 1ml of dilution b) were pipetted into a sterilin container and the volume was made up to 20ml with distilled water. This then constituted the oral doses.

5.3.3.c Extracellular Fluid Volume

A dilution of the original stock was made with sterile 0.9% saline to give a concentration of 105μCi/6.5 mls.

5.3.3.d Plasma Volume

The kit was made up to give a concentration of 600 μCi/3.5ml.

Both c) and d) were combined in one syringe. The syringe was weighed empty with hub on and again after drawing up 4mls of the ^{51}Cr-EDTA

dilution. Then 3.5 mls of ^{99m}Tc-HSA were drawn up with the same syringe which was recapped with the original hub, and weighed again.

5.3.4 Preparation of Standards

The four standards were made separately, one for each test so that cross-over correction could be performed wherever necessary.

5.3.4.a **Tritiated water:** 1 ml (20 μCi) was taken from the stock dilution and made up to 1 litre with distilled water.

5.3.4.b **^{51}Cr-EDTA** : Standard for ^{51}Cr-EDTA was prepared by withdrawing 2mls from the stock dilution and was weighed. In a 1 litre flask disodium-EDTA (stable carrier) 1.25 gm was dissolved in some distilled water to which was added the contents of the syringe containing the ECFV standard (2ml) and was made up to 1 litre by adding distilled water. The empty syringe was weighed

5.3.4.c **^{99m}Tc-HSA** : 1ml from the prepared stock solution was drawn into a syringe, capped and weighed. In a 1 litre flask half filled with distilled water 10-12 drops of 30% albumin were added (to act as a carrier) and gently swirled to avoid froth formation. To this were added the contents of the PV standard syringe (1ml) and the volume was made up to 1 litre by adding distilled water. The empty syringe was weighed.

5.3.4.d **^{22}Na** : A 500 ml flask was half filled with water to which was added 10 mls of 0.9% normal saline to act as a carrier. To this was

added 1ml from the previous stock solution (1μCi) and filled up to 500 mls with distilled water and mixed thoroughly.

5.3.5 Sample Preparation

5.3.5.a Blood Samples

The blood samples were centrifuged at no more than 2000 R.P.M. (less than 700 G) and the plasma separated using disposable safety pipettes into labelled Labco 5ml bottles.

5.3.5.b Preparation of Urine Samples

After removing 20 ml aliquots from each of the 0-4 and 22-24 hour containers the volume of urine in each container was measured and the contents then poured into the 4-22 hour container. The total in that container was then measured (to which 40 mls were added) to give the pool volume. After gently shaking, 20 mls of the pooled urine was aliquoted out and the rest was discarded.

The four urine aliquots thus obtained were needed for:

1) Predose: Background for counts remaining from previous test. Both radioactive counts and stable sodium were measured.

2) 0-4 hour: Was used to obtain total counts lost in total body water.

3) 22-24 hour: This sample represented the equilibrated sodium sample on which both radioactive counts and stable

sodium were measured. Background correction was made.

4) Pool: This was used to correct for the count lost in the sodium estimation. Both counts and stable sodium were measured and correction for background was made.

5.3.5.c Plasma Volume and Extracellular Fluid volume:

A set of LP4 counting tubes were prepared as follows:

2 ECFV standard

2 HSA standard

2 Sodium standard

2 Empty tubes (Background)

2 Pre dose

2 For each sample in time order ie. 10, 20, 30 minutes, 1, 2, 3 & 4 hours.

2 Empty tubes (background).

2 ECFV standard

2 HSA standard

2 Sodium standard

1ml of each of the sample or standard were pipetted into the appropriate tubes. The tubes were then counted as soon as possible for 1Ksec per tube. The counter was set specifically for the ^{99m}Tc and ^{51}Cr photopeaks. About two weeks later (when the ^{99m}Tc activity would have decayed to a quite insignificant amount) the tubes were then counted

again. This time the channels were set for the ^{51}Cr and ^{22}Na photopeaks and the counting time was 3 kilosecs per tube.

5.3.5.d Exchangeable Sodium

A set of LP4 tubes were prepared as follows:

1 sodium standard (1ml of standard + 2mls of distilled water)

1 Background (3 mls of distilled water)

2 Predose urine (3ml)

2 22-24 hour urine (3ml)

2 Pool urine (3ml)

1 Background (3mls of distilled water)

2 Predose plasma (3ml)

2 24 hour plasma (3ml)

1 Background (3ml A/P)

1 Sodium standard (as before)

1 Cr Standard (1ml standard + 2ml water)

After pipetting, the samples were stored for 2 weeks before counting to allow for complete decay of ^{99m}Tc and any ^{99}Mo (^{99} Molybdenum) carryover from the ^{99m}Tc generator. One counting channel was set for the ^{22}Na photopeak and a second counting channel set for the ^{51}Cr photopeak. Each sample was counted for 10 Ksecs and thus the total counting time for each exchangeable sodium estimation was about 2 days.

5.3.5.e Total Body Water

The following samples were used:-

Pre plasma

4 Hour Plasma

Pre urine

0-4 hour urine

^{3}HHO Standard

5.3.5.f Sample Preparation and Cold Distillation

Clean and dry Thunberg tubes were used for the cold distillation of plasma and urine samples. Grease was carefully applied to the stopper which was then turned in the cone to spread the grease. 1 ml of sample was carefully pipetted into the bulb, following which the tube was restoppered to obtain an airtight seal. Each Thunberg tube was carefully evacuated under low vacuum to remove dissolved gases. The tubes were then placed in the deep freeze for 3 hours. Freezing the sample at this stage prevented boiling in vacuo and thus stopped the sample bubbling over into the distillate reservoir. When the samples were frozen solid, further evacuation under high vacuum was performed. Each tube was then carefully placed in liquid nitrogen in a Dewar flask by dipping the base of the tubes in and out of the liquid nitrogen. This gradual process of cooling reduced the thermal shock to the tube. When quite cold the Thunberg tube was inserted with the arm of the tube resting on the top of the Dewar flask. When distillation was complete the tubes were taken out of liquid

nitrogen and placed on a rack for about 30 minutes to thaw and obtain room temperature.

7 low background glass scintillation vials were labelled thus:-

Background

Standard

Preplasma

4 hour plasma

Pre urine

4 hour urine

Standard

For the counting of aqueous (i.e. these) samples a scintillation fluid containing diphenyl-oxazole (5gm), 1 litre toluene and 500 ml Triton -X was used. 10 ml of this mixture was pipetted into each of the above vials. Then 0.5 ml of distilled water was added to each vial, apart from the background vial, to which 1 ml was added. To the standard vial 0.5 ml from the standard (^{3}HHO) flask was added. To the sample vials 0.5 ml of each of the appropriate sample distillates was added. After mixing, the vials were counted in a ß Counter, each one being counted for 300 seconds for 5 cycles.

5.4 Calculations

5.4.1 Calculations for TBW

1) Total counts ingested =

2x(Mean standard counts – background) x standard volume (ml) x 5 = A

2) Total counts lost in urine =

2 x (4 hr urine counts – Pre urine counts) x (0 – 4 hr) urine volume = B

3) 4 hr plasma activity = 2 x (4 hr plasma counts – pre plasma counts) = C

4) Total counts retained at 4 hours = [A – B]

5) TBW = Total counts retained ÷ 4 hr plasma activity = [A – B] ÷ C

6) TBW was calculated as above for each counting cycle and the mean of 5 counting cycles used.

5.4.2 Calculations for ExNa

5.4.2.a For the first test

1) Total counts ingested = standard counts/ml x standard volume (ml) = "A"

2) Counts lost in urine =

0–24 hr urine counts/ml x 0–24 hr urine volume (ml) = "B"

3) Counts retained = "A" – "B"

4) Distribution volume = ["A" – "B"] ÷ 24 hr plasma counts/ml = V (ml)

5) Exchangeable Na (mmol) =

[V x 24 hr plasma stable sodium concentration (mmol/L)] ÷ 1000

5.4.2.b For the second test

The principle is exactly as for the first test. However some ^{22}Na from the first test is still present in the body (i.e. in blood and urine as far as we are concerned) both at the start of, and during, the second test. The differences from the first test then are how we use the pre dose urine to find the lost counts and how we use the pre dose plasma to obtain the "true" plasma counts. For these we considered four variants:–

1) "Lost" counts: As for the first test, multiplying the pre urine counts by the 24 hour urine volume, then subtracting these counts from the total 24 hour urine counts.

2) "Lost" counts: Using the pre dose urine sample, derive counts/mmol stable sodium. We know the total urine stable sodium excreted in 24 hours, hence we can derive counts appearing in urine from previous test. The difference between this and total counts in urine must be "lost" from the second test.

3) "True" 24 hour plasma counts: Exactly as for the first test, subtracting pre plasma counts from 24 hour plasma counts.

4) "True" 24 hour plasma counts: We know the 24 hour plasma counts from the first test, the pre plasma counts from the second test and the time interval between the two. Assuming a simple, single exponential excretory pattern we could calculate a predicted value for the counts in the 24 hour plasma (2nd test). The difference between this and the actual counts would be the true plasma counts.

These four variants allow four possible derivations of ExNa. For consistency we have used a combination of variants 2) and 4). One suspects that previous workers may not have adopted such an approach. It is of interest to note that when all four methods are used the maximum difference in the results usually spans less than 5% of their mean.

5.4.3 Calculation for PV and ECFV

The set of samples (5.3.5) were counted twice. Firstly on the evening after the test for 1 Ksec per sample obtaining counts in the ^{55}Cr and ^{99m}Tc

photopeaks. Elapsed counting time was also recorded with these. Secondly about 14 days later, for 3 Ksec per sample, obtaining counts in the ^{22}Na and ^{51}Cr photopeaks. Two sets of counts were advisable since:–

1) The ^{99m}Tc HSA provided a high count (typically 100 Kcounts per 1 Ksecs). Its short half life however precluded delaying counting further.
2) The comparatively low count rates for ^{51}Cr and ^{22}Na required a longer counting time to acquire good counting statistics.
3) Any high energy ^{99}Mo, as a contaminant for ^{99m}Tc generator, would have a chance to decay away.

The two sets of counts were viewed together and processed thus:–

1) Background counts in each channel were subtracted from all counts in that channel to give background corrected counts (now simply referred to as counts).
2) Using the ^{22}Na standard counts a crossover factor was derived and the counts in the ^{51}Cr channels and the ^{99m}Tc channels were corrected for crossover from the ^{22}Na in the samples.
3) Using the ^{51}Cr standard counts a crossover factor was derived and the counts in the ^{99m}Tc channel were corrected for cross over from the ^{51}Cr in the samples.
4) The doubly corrected ^{99m}Tc counts were corrected for decay using the elapsed time.
5) Thus were derived the "true" counts for ^{51}Cr and ^{99m}Tc.

The energy spectra of ^{99m}Tc, ^{51}Cr and ^{22}Na are illustrated in Figure 5.1.

Calculations for ECFV

1) The ^{51}Cr standard counts and the corrected ^{51}Cr counts for the 1, 2, 3, and 4 hour samples were used.

2) A graph was drawn on semilog paper, with sample time on the X axis (linear) and sample counts on the Y axis (log).

3) A line of best fit through the 2, 3, and 4 hour points was drawn. The 1 hour point lying above the drawn line was taken as some measure of quality assurance of the injections.

4) The intercept of the line with the Y axis (theoretical count rate at zero time) was noted (P_o). Using this figure a value for $T_{½}$ (hours) was derived by inspection.

5) Knowing the counts/ml of the ^{51}Cr standard, the standard volume, the weight of dose dispensed into the standard and the weight of the dose injected into the patient, the total counts injected into patient was calculated (C).

6) The distribution volume was given by $(C \div P_o) \div 1000 = V$ litres. This is generally accepted as equivalent to ECFV.

7) GFR was calculated from:–

$[0.693 \times V \times 1000] \div [T_{½} \times 60] = GFR$ (ml/min)

Calculations for Plasma Volume

1) The ^{99m}Tc standard counts and the fully corrected ^{99m}Tc counts for the 10, 20, 30 60 and 120 minute samples were used.

2) A graph was drawn on semilog paper with sample time on the X axis (linear) and samples count on the Y axis (log).

3) Two lines of best fit were drawn – one through the first 3 points, the other through the last 3 points (Figure 5.2).

4) The two intercepts (P_{oH} and P_{oL}) with the Y axis were noted.

5) It was assumed that chemical impurities in the preparation may have contributed to the failure to fit all points on one line. The lower P_o value (P_{oL}) was assumed to be the more correct, however two separate calculations were done (as below) to derive two PV's.

6) Knowing the counts/ml of the ^{99m}Tc standard, the standard volume, the weight of dose dispensed into the standard and the weight of dose injected into the patient the total counts injected into the patients (C) was calculated.

7) The distribution volume (= plasma volume) was given by

$C \div [P_o \times 1000] = PV$ (litres)

5.5 Accuracy of Technique

The overall accuracy is limited by a) radioactive measurements, b) chemical analyses, c) biological variations.

Many factors interact to contribute to the total error of the measurement and the deviation of that measurement from the true result. Typically we would encounter:–

1) volumetric, pipetting and weighing errors,

2) the statistical errors of counting,

3) the correction errors from counting multiple isotopes,

4) the individuality of equilibration times,

5) the marginality of body compartments,

6) chemical and radioisotope impurities in preparations,

7) injection and sampling errors,

8) errors of correction applied for previous tests.

The accuracy is usually +/- 3–4 %. The coefficient of variation of repeated measurements of TBW has been reported as 2.3, ECFV as 4.1, and ExNa as 5.9 (Haxhe, 1971).

5.6 Method of Data Analysis

The data were analysed by the following methods;

a) Paired and unpaired non-parametric tests between the follicular and luteal phases of the menstrual cycle and between the patients and controls respectively (Table 5.6, 5.7). The non-parametric paired test used was Wilcoxon Rank Sum test, and the non-parametric unpaired test was Mann Whitney U test.

b) Nonparametric testing for possible correlation between various parameters measured. The test used was Spearman's correlation test (Table 5.8 a-f).

c) Graphical display of data to analyse patterns of changes and to look for deviation of trends in an attempt to analyse individual variations (Fig 5.3a and Fig 5.3b).

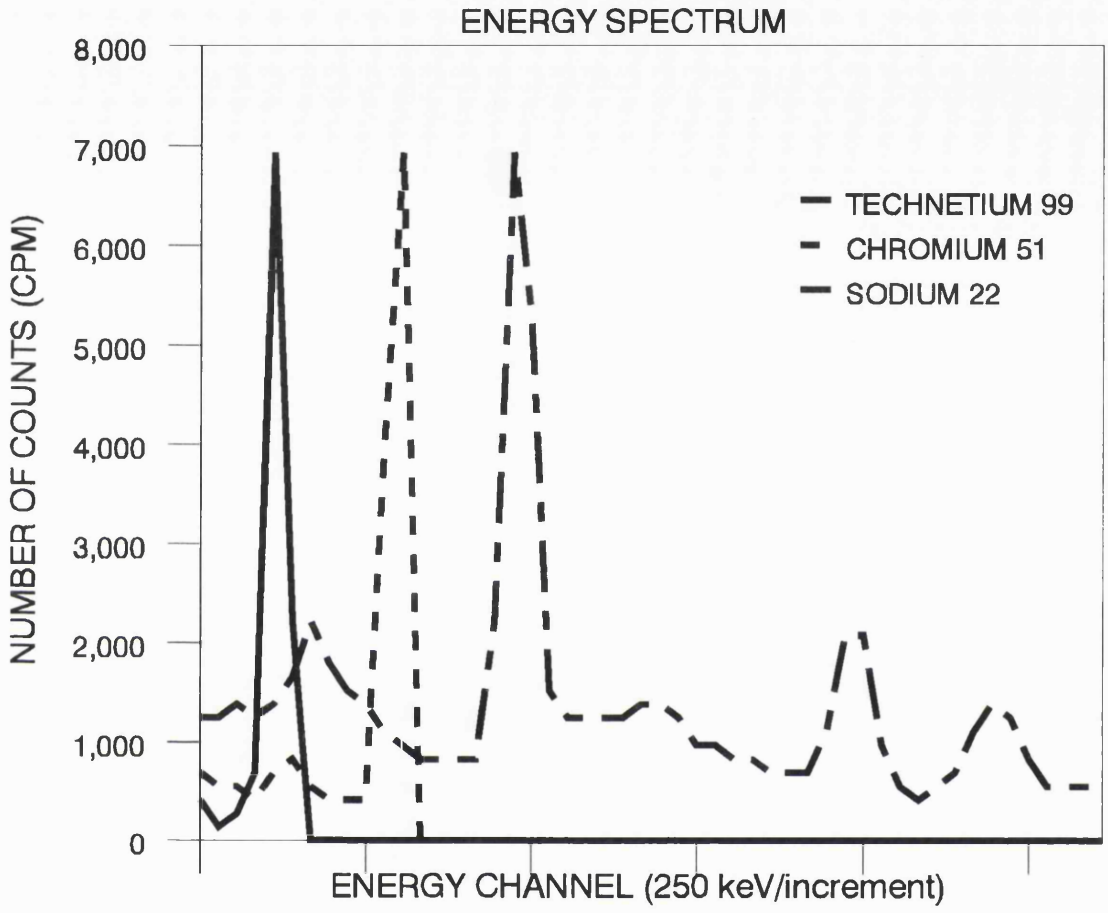
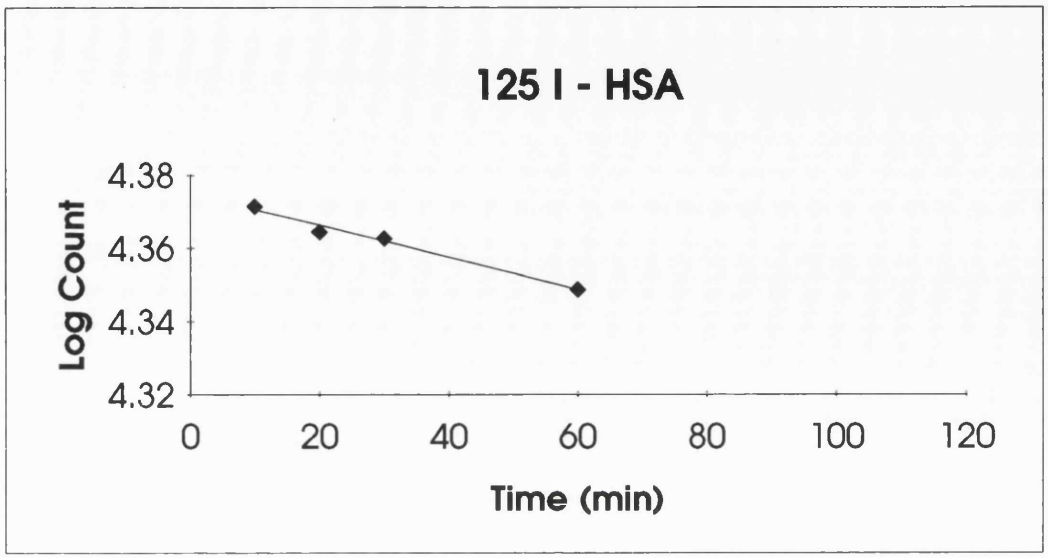


Figure 5.1

Plot of energy spectra for ^{99m}Technetium, ^{51}Chromium and ^{22}Sodium.



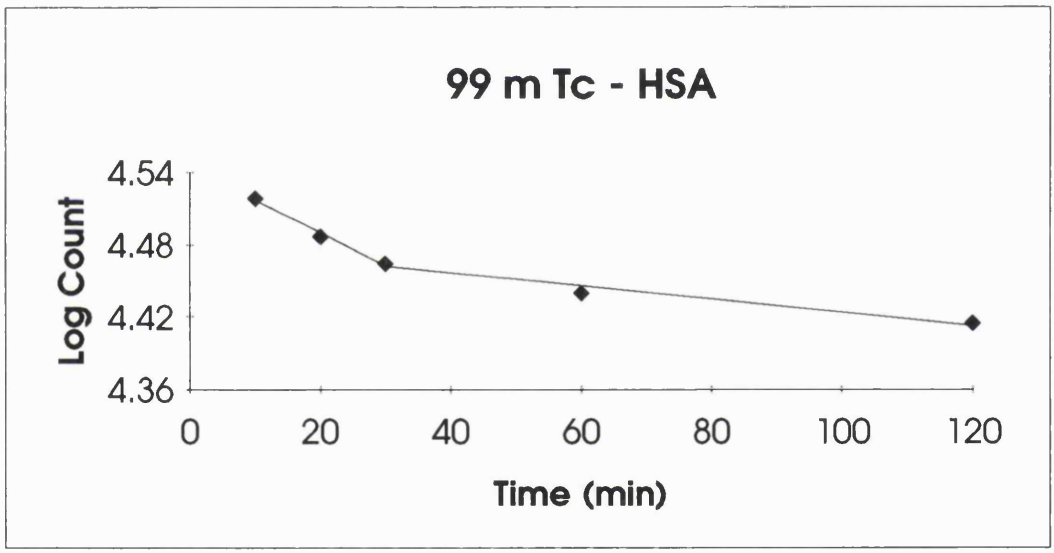


Figure 5.2

Graph representing sample time on X axis (linear) and sample counts on the Y axis (Log). The upper graph represents typical data with ^{125}I-HSA (not used in the study in order to lessen radiation dose). The lower graph illustrates the more complex situation typically found with ^{99m}Tc HSA. Two alternative line are shown: one drawn through the first 3 data points, the other through the last 3. The intercept of the first of these lines with the Y axis (Time=0)is used to give an intercept value P_{0H} used to calculate one estimate of plasma volume. The intercept of the second line gives an alternative intercept value P_{0L}.

5.7 Result

The patients and controls were of comparable age, weight, height and parity (Table 5.4)

5.7.1 Total Body Water (TBW)

The TBW slightly decreased in the patients from the follicular to the luteal phase of the menstrual cycle, whereas in the controls there was a slight increase. Neither change was statistically significant (Fig 5.4).

5.7.2 Plasma Volume (PV)

The PV results (whether the high or low reading were taken) did not change significantly between the two phases of the menstrual cycle or between patients and controls. Taking the readings from up to the first hour the PV was found to very slightly increase whilst taking the readings up to 4 hours the PV slightly decreased, but these differences were so minor that this did not imply any significance. However it was more likely that the PV result obtained from the 4 hour readings was more reliable as equilibrium would be more probable at 4 hours (Fig 5.5)

5.7.3 Extracellular Fluid Volume (ECFV)

The ECFV increased in the patients while decreasing in the controls during the menstrual cycle. This was not statistically significant (Fig 5.6)

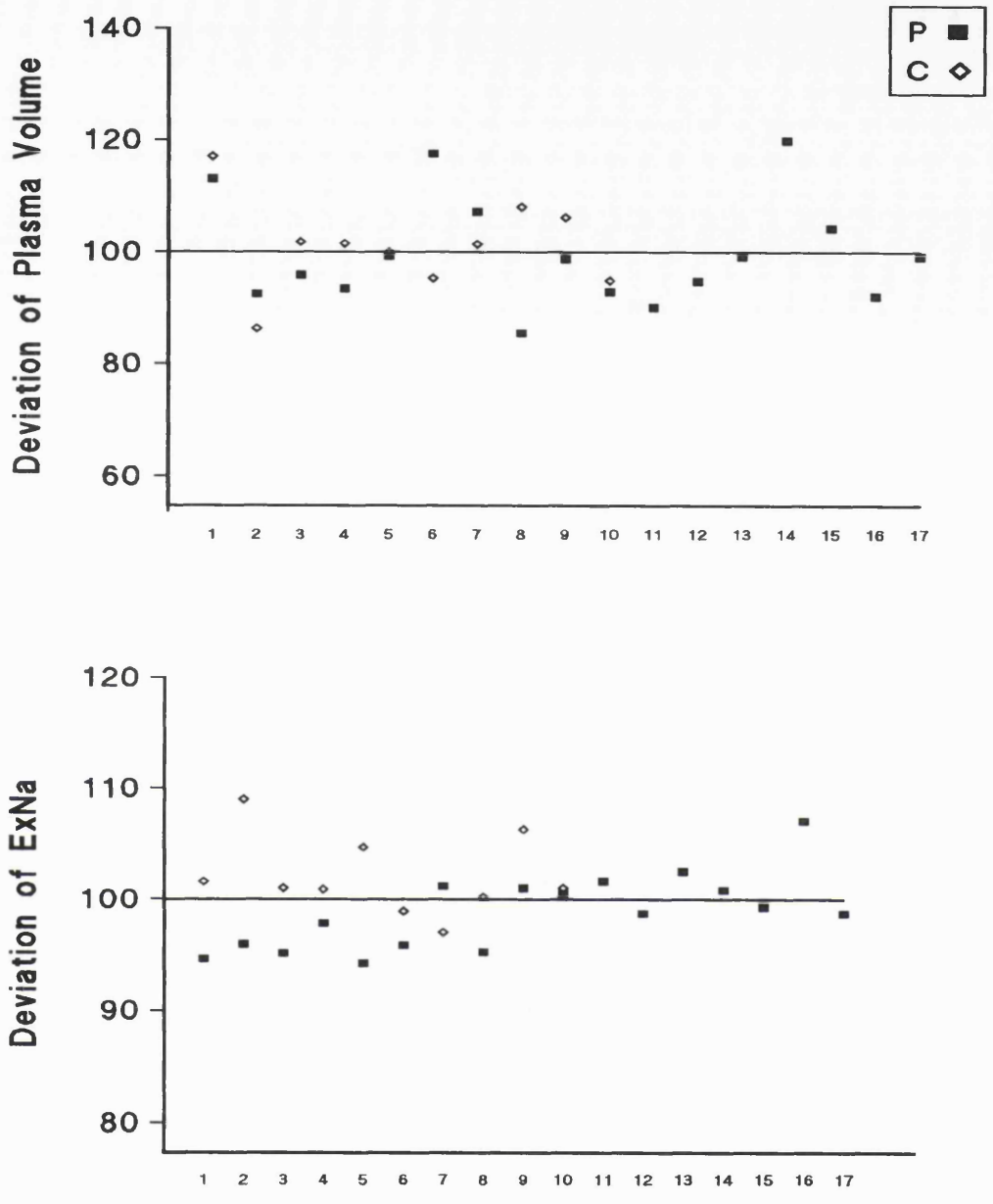


Figure 5.3a

Follicular to luteal deviation from an arbitrary baseline 0f 100 of Plasma volume and total body exchangeable sodium (ExNa) in 17 patients and 10 controls.

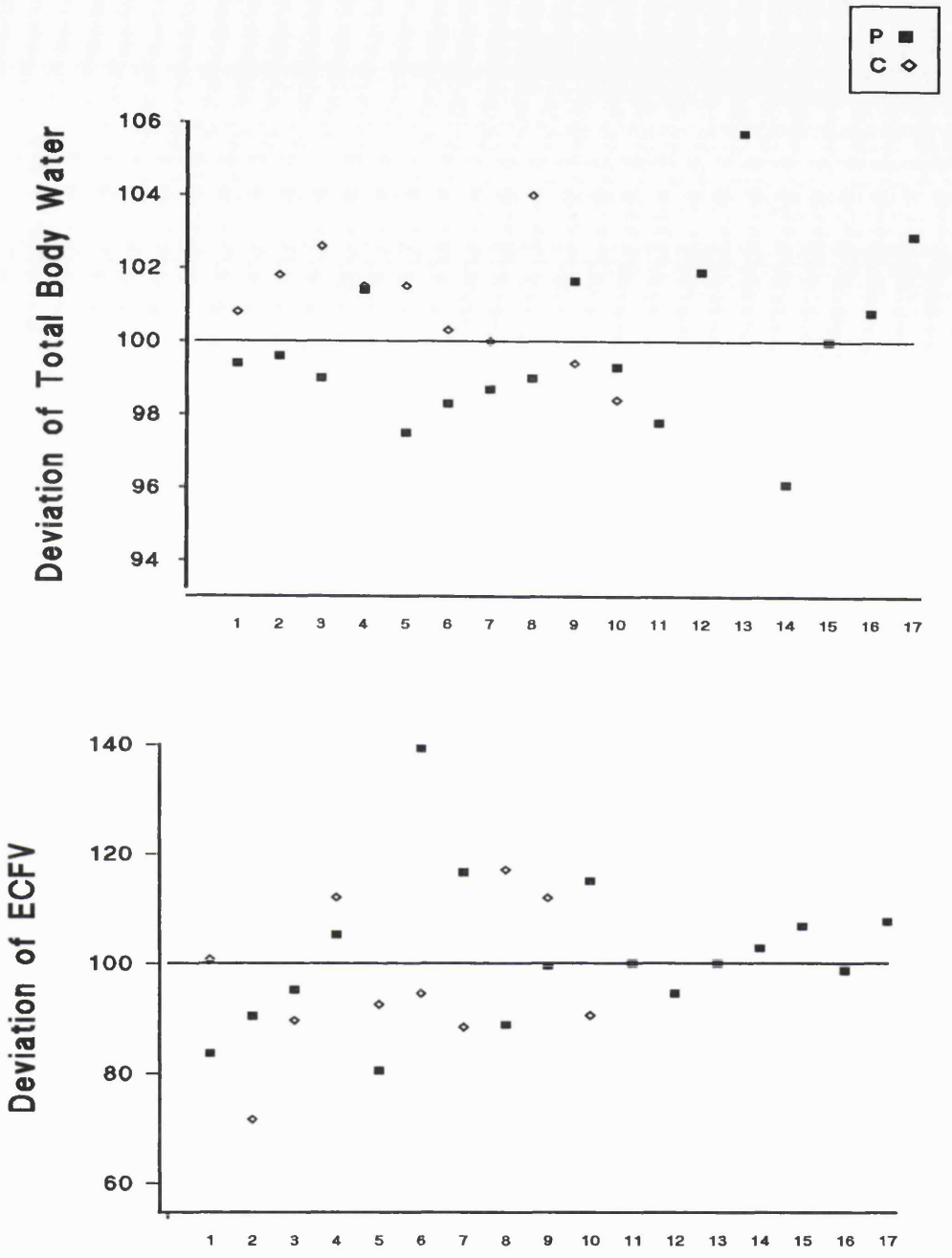


Figure 5.3b

Follicular to luteal deviation from an arbitrary baseline 0f 100 of Total Body Water, Extracellular fluid volume (ECFV) in 17 patients and 10 control.

Table 5.6

Median values of parameters measured in 17 patients and 10 controls.

	Patients				Controls			
	F	L	p value	95% CI	F	L	p Value	95% CI
Weight	62.0	62.8	0.85	-0.7500, 0.3500	67.00	67.10	0.28	−1.2000, 0.2000
TBW	32.7	32.5	0.87	-0.3000, 0.3600	33.11	33.45	0.09	-0.007, 0.004
PV(low)	2.770	2.760	0.44	-0.1150, 0.1650	2.720	2.750	0.55	−0.2000, 0.1450
PV(High)	3.03	3.0	0.813	-0.1100, 0.1700	3.010	2.980	0.85	−0.1550, 0.1610
ExNa	2394	2352	0.201	-19.50, 71.50	2390	2384	0.08	−129.5, 9.500
ECFV	14.000	14.040	0.932	-0.9000, 0.9750	14.450	14.200	0.72	−0.7500, 2.100
Sodium space	17.1	17.2	0.394	-0.2000, 0.4500	17.25	17.2	0.314	-0.7500, 0.2000
Plasma								
Urea	4.5	4.9	0.35	-0.6000, 0.2000	3.9	3.65	0.32	−0.5500, 0.8000
Sodium	141	140	0.24	-0.006, 1.5	140	141	0.44	−3.000, 1.000
Potassium	4.0	3.9	0.53	−0.1500, 0.4500	4.0	3.8	0.63	−0.300, 0.500
Progesterone	2.2	40.1	<0.0001	−46.65, −30.00	2.0	34.0	<0.006	−50.50, −20.35
Oestrogen	173	430	<0.0001	−341.5, −154.0	323	333	0.83	−239.5, 22.5
FSH	4.8	2.5	<0.0001	1.8, 3.0	3.9	3.15	0.18	−0.200, 1.95
LH	6.8	3.3	0.006	1.35, 4.4	4.7	5.8	1.000	−3.150, 3.250
Urine								
GFR	108.0	110.0	0.07	−11.000, 0.5000	104.0	110.5	0.2	−12.000, 3.000
Volume	1450	1260	0.036	15.000, 350.0	1130	1580.0	0.15	−1165.0, 55.00
Creatinine	7.5	10.1	0.003	−3.800, −0.700	8.25	7.8	0.12	−0.500, 5.25
Urea	213	268	0.001	−82.00, −17.00	196.0	198	0.41	−71.5, 118.0
Osmolality	500	585	0.058	−183.00, 1.000	529	492	0.04	0.00, 283.50
Potassium	52	53	0.14	−29.5, 4.500	54.5	45.0	0.5	−52.50, 39.0
Total Sodium	87.4	83.0	0.11	−4.350, 41.700	91.8	122.4	0.3	−51.65, 21.65
Sodium conc	83.0	70.0	0.65	−8.5, 17.5	70.5	62.0	0.5	−10.0, 21.0
TBW/Wt	0.5205	0.5203	0.705	-5.0-03E, 0.006	0.5051	0.5057	0.476	-1.1E-02,0.003
ExNa/Wt	38.63	38.59	0.08	-0.075, 1.08	36.03	38.45	0.154	-1.440, 0.2178
PVLow/Wt	0.0446	0.0449	0.538	-1.8E-03, 0.002	0.0422	0.0426	0.838	-2.3E-03, 0.002
ECFV/Wt	0.2204	0.227	1.0	-0.0145, 0.015	0.208	0.207	0.610	-0.0102, 0.0283

Table 5.7

Comparison of the median follicular (F) and luteal (L) values of patients and controls by using Mann Whitney Rank Test. 95% CI = 95% Confidence Interval. The median values are shown in Table 5.6

	F vs F		L vs L	
	p Value	95% CI	p value	95% CI
Weight	0.113	−11.70, 0.80	0.080	−12.40,1.6
TBW	0.407	−2.900, 1.20	0.291	−3.20, 0.899
PV (Low)	0.880	−0.1699, 0.250	0.407	−0.2301, 0.1599
ExNa	0.820	−169, 153.9	0.482	−276.0, 89.9
PV (High)	0.706	−0.23, 0.17	0.860	−0.300, 0.200
ECFV	0.633	−1.900, 0.701	0.980	−1.101, 1.00
Sodium space	0.687	-1.299, 0.901	0.482	-2.000, 0.7000
Plasma				
Urea	0.059	−0.001, 1.801	0.01	0.201, 2.500
Sodium	0.546	−1.00, 2.00	0.580	−3.00, 1.000
Potassium	0.537	−0.2999, 0.4999	0.466	−0.0999, 0.3998
Progesterone	0.315	0.00, 0.400	0.451	−11.01, 18.99
Oestradiol	0.074	−332.9, 22.9	0.053	0.0, 255.1
FSH	0.011	0.302, 2.099	0.46	−1.6, 0.70
LH	0.17	−0.399, 2.800	0.07	−3.501, 0.501
Urine				
GFR	0.980	−9.00, 11.00	0.880	−7.00, 9.00
Volume	0.860	−434.8, 680.1	0.003	−1079.9, -110.2
Creatinine	0.821	−3.400, 1.902	0.005	1.298, 6.298
Urea	0.280	−140.0, 59.0	0.451	−46.0, 117.0
Osmolality	0.563	−260.0,107.9	0.047	9.1, 278.0
Potassium	0.598	−36.0, 13.02	0.08	−0.99, 42.87
Total Sodium	0.860	−33.1, 41.28	0.029	−54.8, −4.71
Sodium conc.	0.530	−21.01, 29.98	0.366	−12.01, 22.99
TBW/Wt	0.167	-0.015, 0.068	0.238	-0.019, 0.066
ExNa/Wt	0.047	0.051, 5.647	0.238	-1.33, 5.08
PVLow/Wt	0.138	-0.001, 0.007	0.303	-0.001, 0.007
ECFV/Wt	0.379	-0.016, 0.035	0.059	-0.001, 0.034

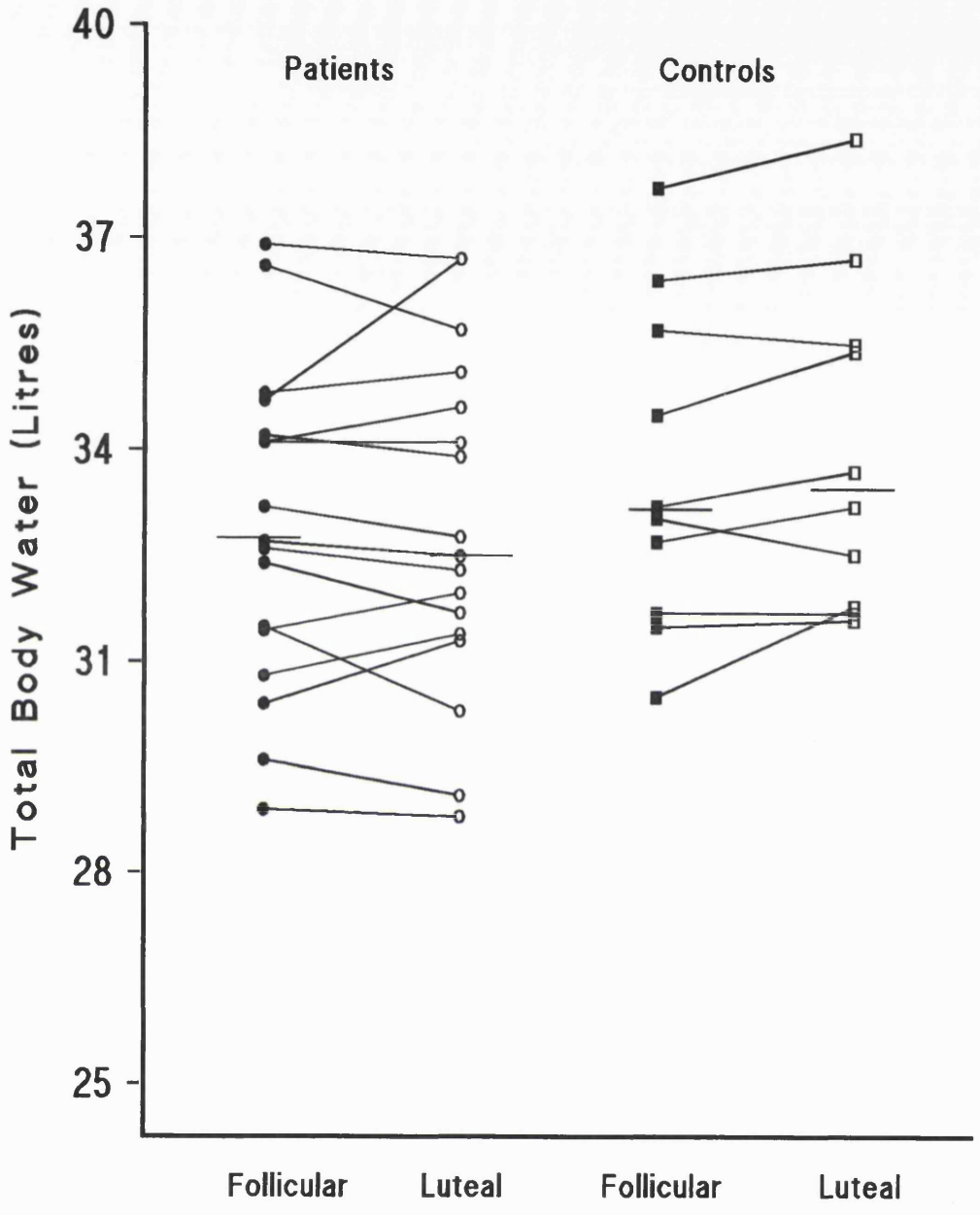


Figure 5.4

Total body water in 17 patients and 10 controls during the follicular and luteal phases of the menstrual cycle. No significant differences were shown from the follicular to luteal phases and between patients and controls. Bars denote median values.

5.7.4 Total Body Exchangeable Sodium

ExNa decreased from follicular to luteal phase in 10 out of the 17 patients but increased in 8 out of 10 controls. The patient control difference in follicular to luteal change was statistically significant (Fig 5.7).

5.7.5 Weight

The median weight of the patients was 62.0 kg in the follicular phase with a slight increase in the luteal phase to 62.8 kg which was not statistically significant. The controls were heavier weighing 67.0 kg and similarly very slight gain in weight was noted in the luteal phase (67.1kg) (statistically insignificant) (Fig 5.8).

5.7.6 Plasma Urea, Sodium & Potassium

The plasma urea increased in the luteal phase in the patients while decreased in the controls. The plasma Na and K both fell insignificantly in the patients during the menstrual cycle. In the controls similarly K concentrations were lower but the Na concentration increased during the menstrual cycle. All these changes were statistically insignificant (Table 5.6).

5.7.7 Glomerular Filtration Rate (GFR)

The GFR increased in both groups but no significance was noted.

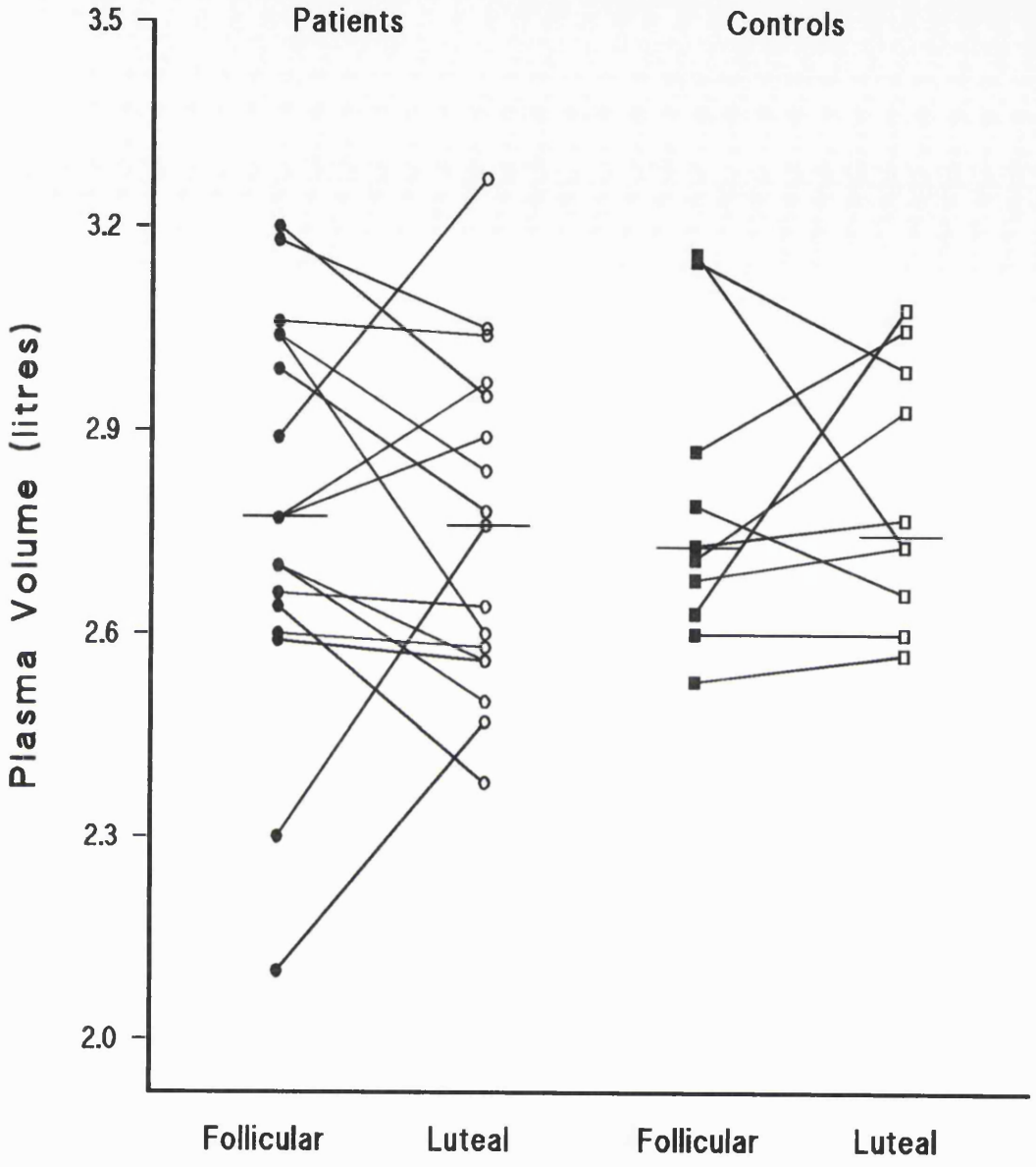


Figure 5.5

Plasma volume in 17 patients and 10 controls during the follicular and luteal phases of the menstrual cycle. No significant differences were shown from the follicular to luteal phases and between patients and controls. Bars denote median values.

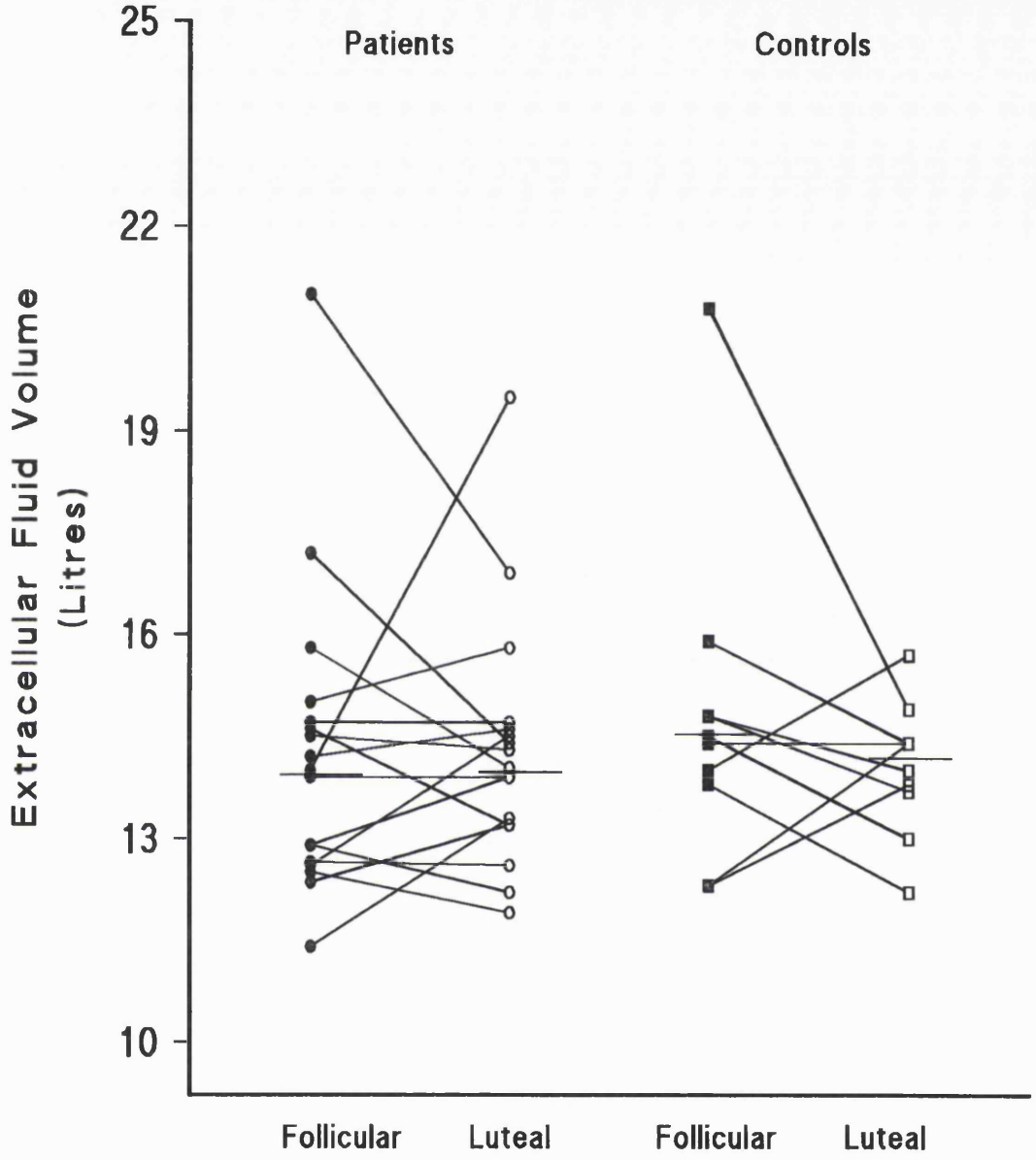


Figure 5.6

Extracellular fluid volume in 17 patients and 10 controls during the follicular and luteal phases of the menstrual cycle. No significant differences were shown from the follicular to luteal phases and between patients and controls. Bars denote median values.

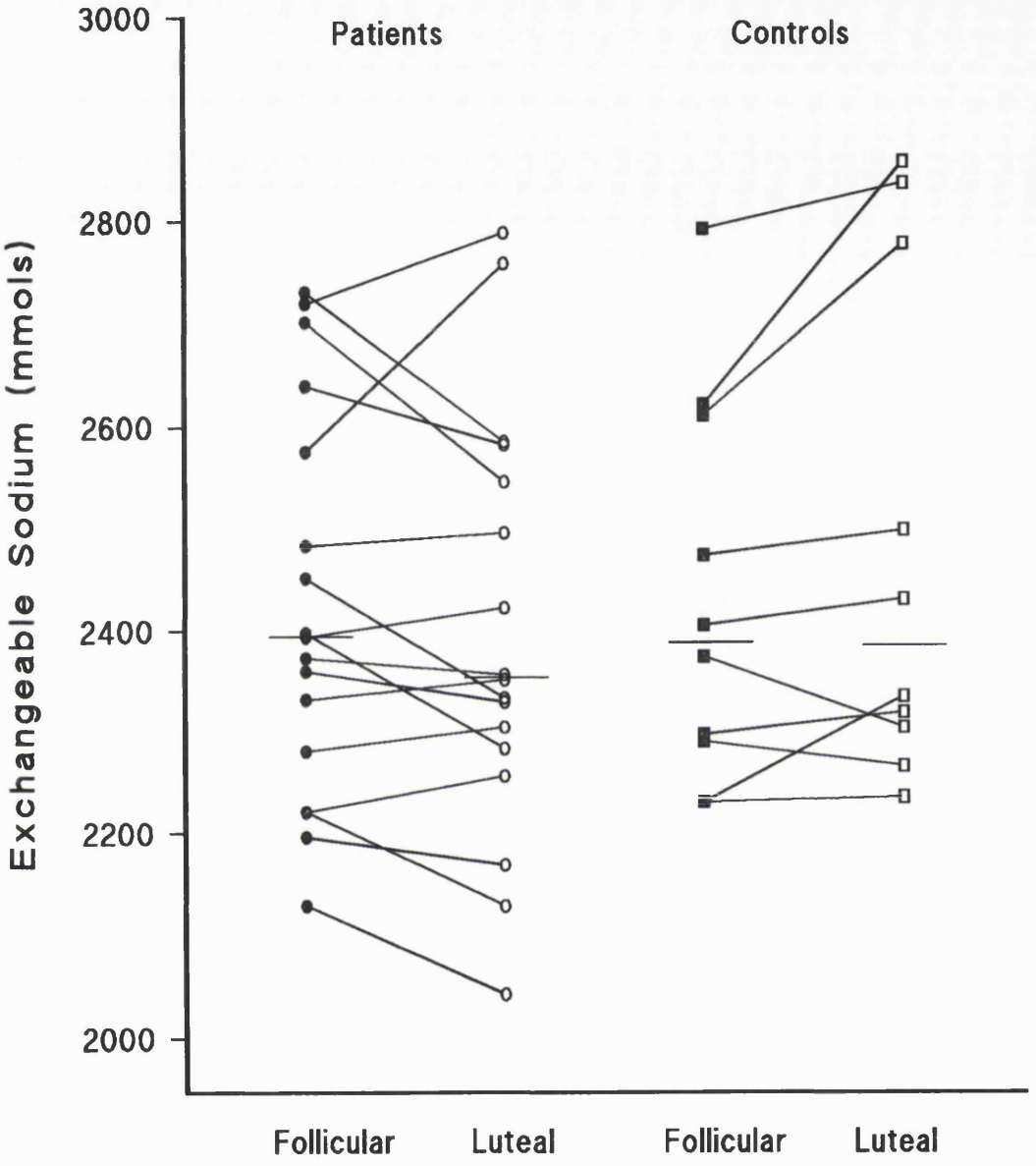


Figure 5.7

Total body exchangeable sodium in 17 patients and 10 controls during the follicular and luteal phases of the menstrual cycle. In both groups a decrease occured from the follicular to the luteal phase. This decrease when compared between patients and controls was significant (p value =0.037,95% CI 2.0, 150). Bars denote median values.

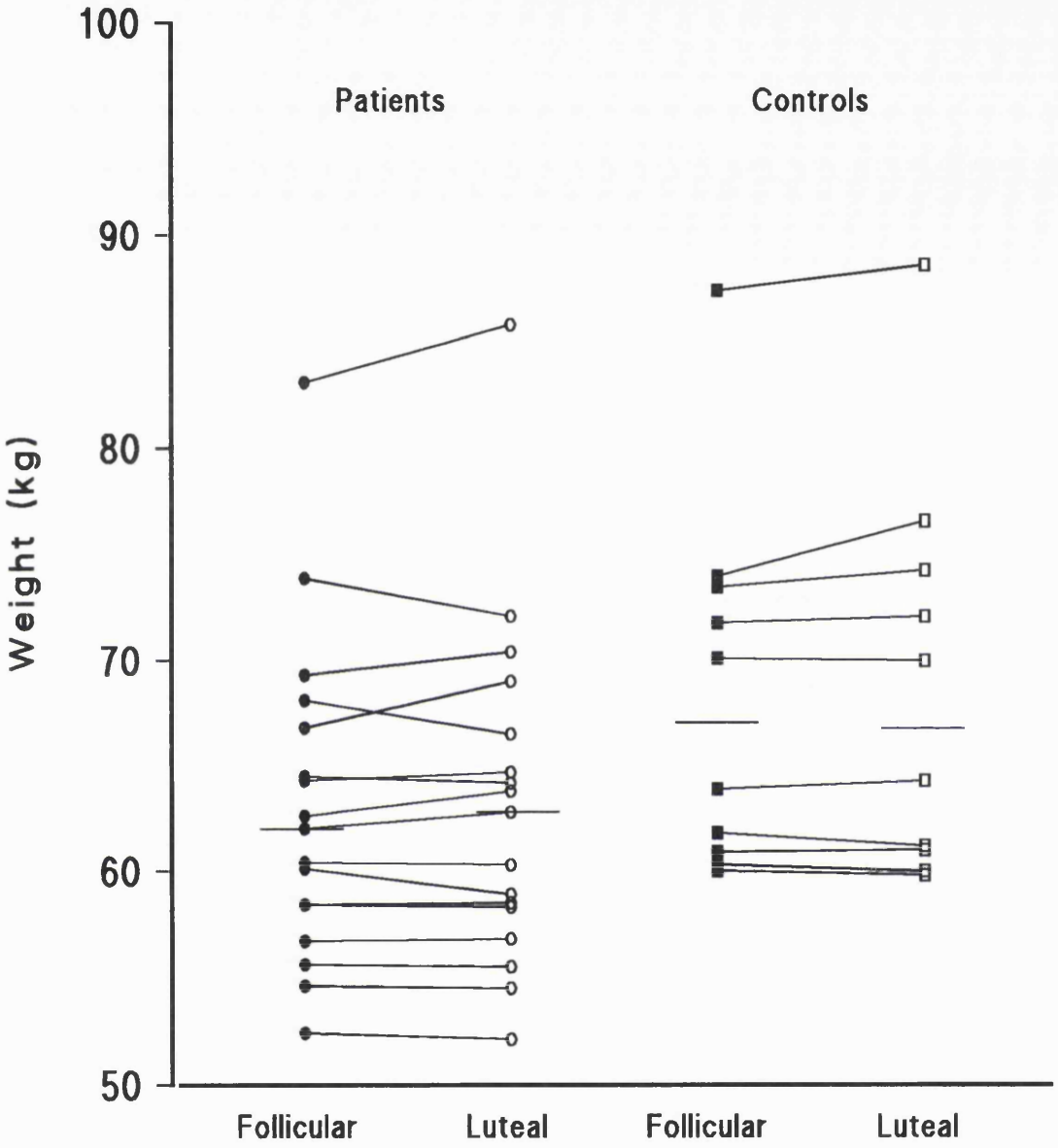


Figure 5.8

Weight in 17 patients and 10 controls during the follicular and luteal phases of the menstrual cycle. No significant differences were shown from the follicular to luteal phases and between patients and controls. Bars denote median values.

Table 5.8.a

Spearman's Rank Correlation

Factors		N	DF	Spearmans RC	p value
*TBW	Wt	54	52	0.6896	<0.001
TBW F-L	Wt F-L	27	25	0.2437	0.221
TBW F-L	Wt F-L Control	10	8	0.1121	0.758
TBW F-L	Wt F-L Patient	17	15	0.3450	0.175
*TBW	PV-low	54	52	0.5712	<0.001
TBW F-L	PVlow F-L	27	25	-0.0366	0.856
TBW	Pvlow F-L Control	10	8	0.2091	0.571
TBW	PVlow F-L Patient	17	15	-0.1771	0.497
*TBW	ECFV	54	52	0.3681	0.006
TBW F-L	ECFV F-L	27	25	0.0357	0.860
TBW F-L	ECFV F-L Control	10	8	0.1030	0.777
TBW F-L	ECFV F-L Patient	17	15	0.0288	0.913
*TBW	ExNa	54	52	0.8622	<0.001
TBW F-L	ExNa F-L	27	25	0.3020	0.126
TBW F-L	ExNa F-L Control	10	8	0.0091	0.980
TBW F-L	EXNa F-L Patient	17	15	0.2708	0.293
TBW L	Blo Mx	27	25	0.1779	0.866
TBW L	Bre Mx	27	25	-0.0688	0.733
TBW L	Dep Mx	27	25	-0.1177	0.559
TBW F-L	Blo D	27	25	0.1877	0.348
TBW F-L	Bre D	27	25	-0.0015	0.990
TBW F-L	Dep D	27	25	0.2224	0.260

Table 5.8.b

Spearman's Rank Correlation

Factors		N	DF	Spearman's RC	p value
*ExNa	Wt	54	52	0.6067	<0.001
*ExNa F-L	Wt F-L	27	25	0.5791	0.002
*ExNa F-L	Wt F-L Control	10	8	0.6364	0.048
*ExNa F-L	Wt F-L Patient	17	15	0.5460	0.023
*ExNa	ECFV	54	52	0.4233	0.001
ExNa F-L	ECFV F-L	27	25	0.1677	0.403
ExNa F-L	ECFV F-L Control	10	8	-0.0939	0.796
ExNa F-L	ECFV F-L Patient	17	15	0.4332	0.082
*ExNa	PV	54	52	0.6017	<0.001
ExNa F-L	PV F-L	27	25	-0.0403	0.842
ExNa F-L	PV F-L Control	10	8	-0.1303	0.720
ExNa F-L	PV F-L Patient	27	25	-0.1452	0.578
ExNa F-L	Blo D	27	25	0.2337	0.241
ExNa F-L	Blo D Control	10	8	-0.0182	0.960
ExNa F-L	Blo D Patient	17	15	-0.171	0.510
ExNa F-L	Bre D	27	25	0.1213	0.547
ExNa F-L	Bre D Control	10	8	-0.0909	0.803
ExNa F-L	Bre D Patient	17	15	-0.2911	0.256
ExNa F-L	Dep D	27	25	-0.0180	0.929
ExNa F-L	Dep D Control	10	8	0.1394	0.701
ExNa F-L	Dep D Patient	17	15	-0.1195	0.648

Table 5.8.c

Spearman's Rank Correlation

Factors			N	DF	Spearman's RC	p value
*Wt	ECFV		54	52	0.4972	<0.001
Wt F-L	ECFV F-L		27	25	0.0621	0.758
Wt F-L	ECFV F-L	Control	10	8	0.1909	0.597
Wt F-L	ECFV F-L	Patient	17	15	0.0868	0.743
Wt	PV		54	52	0.3638	0.007
Wt F-L	PV F-L		27	25	0.0067	0.980
Wt F-L	PV F-L	Control	10	8	0.0394	0.914
Wt F-L	PV F-L	Patient	17	15	0.0067	0.980
Wt F-L	Blo D		27	25	-0.0740	0.714
Wt F-L	Blo D	Control	10	8	0.3212	0.365
*Wt F-L	Blo D	Patient	17	15	-0.4902	0.046
Wt F	Blo Mn		27	25	0.5136	0.006
Wt F	Blo Mn	Control	10	8	0.4667	0.174
Wt F	Blo Mn	Patient	17	15	0.3407	0.183
Wt F-L	Bre D		27	25	-0.2761	0.163
Wt F-L	Bre D	Control	10	8	-0.2848	0.425
*Wt F-L	Bre D	Patient	17	15	-0.6703	0.003
Wt F	Bre Mn		27	25	-0.0513	0.799
Wt F	Bre Mn	Control	10	8	0.3182	0.370
Wt F	Bre Mn	Patient	17	15	0.1771	0.497
*PV	ECFV		54	52	0.3356	0.023
PV F-L	ECFV F-L		27	25	0.3683	0.059
*PV F-L	ECFV F-L	Control	10	8	0.6727	0.033
PV F-L	ECFV F-L	Patient	17	15	0.2623	0.309
PV F-L	Blo D		27	25	0.2682	0.176
PV F-L	Blo D	Control	10	8	0.3424	0.333
PV F-L	Blo D	Patient	17	15	0.0411	0.876
PV F-L	Bre D		27	25	0.0803	0.691
PV F-L	Bre D	Control	10	8	0.0030	0.993
PV F-L	Bre D	Patient	17	15	-0.2163	0.404

Table 5.8.d

Spearman's Rank Correlation

Factors			N	DF	Spearman's RC	p value
ECFV F-L	Blo D		27	25	-0.0401	0.842
ECFV F-L	Blo D	Control	10	8	0.2455	0.494
ECFV F-L	Blo D	Patient	17	15	-0.0129	0.961
ECFV F-L	Blo Mn		27	25	0.3771	0.052
ECFV F-L	Bre D		27	25	-0.1770	0.377
ECFV F-L	Bre D	Control	10	8	0.3485	0.324
ECFV F-L	Bre D	Patient	17	15	-0.2586	0.316
ECFV F-L	Dep D		27	25	-0.1368	0.509
ECFV F-L	Dep D	Control	10	8	-0.1788	0.621
ECFV F-L	Dep D	Patient	17	15	0.1814	0.486
TUNa	ExNa		54	52	0.5107	<0.001
TUNa	ExNa F-L	Control	10	8	-0.3394	0.180
TUNa	ExNa F-L	Patient	17	15	0.4859	0.048
TUNa	Wt		54	52	0.5107	<0.033
TUNa	Wt F-L	Control	10	8	-0.5030	0.158
TUNa	Wt F-L	Patient	17	15	0.5674	0.018
TUNa	TBW		54	52	0.4794	<0.001
TUNa	TBW F-L	Control	10	8	0.3061	0.390
TUNa	TBW F-L	Patient	17	15	0.2886	0.261
TUNa	ECFV		54	52	0.2097	0.128
TUNa	ECFV F-L	Control	10	8	-0.0515	0.888
TUNa	ECFV F-L	Patient	17	15	0.3836	0.129
TUNa	PV		54	52	0.6079	<0.001
TUNa	PV F-L	Control	10	8	0.3303	0.351
TUNa	PV F-L	Patient	17	15	0.4835	0.049
TUNa	Blo D		27	25	0.2271	0.255
TUNa	Blo D	Control	10	8	0.3212	0.365
TUNa	Blo D	Patient	17	15	-0.3413	0.180
TUNa	Bre D		27	25	0.0336	0.868
TUNa	Bre D	Control	10	8	0.2121	0.556
TUNa	Bre D	Patient	17	15	-0.5988	0.011
TUNa	Dep D		27	25	0.3098	0.110
TUNa	Dep D	Control	10	8	0.1636	0.651
TUNa	Dep D	Patient	17	15	0.1801	0.489

Table 5.8.e

Spearman's Rank Correlation

Factors		N	DF	Spearman's RC	p value
UCreatinine	TBW	54	52	-0.0451	0.746
UCreatinine	TBW F–L	27	25	-0.2039	0.308
UCreatinine	TBW F-L Control	10	8	-0.2818	0.430
UCreatinine	TBW F-L Patient	17	15	0.2114	0.415
UCreatinine	Wt	54	52	-0.1155	0.406
UCreatinine	Wt F–L	27	25	-0.1803	0.368
Ucreatinine	Wt F–L Control	10	8	0.0667	0.855
UCreatinine	Wt F–L Patient	17	15	-0.1605	0.538
UVolume	TBW	54	52	0.5304	<0.001
UVolume	TBW F–L	27	25	0.4014	0.038
UVolume	TBW F–L Control	10	8	0.5394	0.108
UVolume	TBW F–L Patient	17	15	0.1789	0.492
UVolume	Wt	54	52	0.5304	<0.001
UVolume	Wt F–L	27	25	-0.1784	0.374
UVolume	Wt F–L Control	10	8	-0.3470	0.256
UVolume	Wt F–L Patient	17	15	-0.2384	0.357
UVolume	ExNa	54	52	0.5411	<0.001
UVolume	ExNa F–L	27	25	-0.0749	0.710
UVolume	ExNa F–L Control	10	8	-0.4669	0.010
UVolume	ExNa F–L Patient	17	15	-0.0956	0.715
UVolume	PV	54	52	0.6020	<0.001
UVolume	PV F–L	27	25	0.3782	0.052
UVolume	PV F–L Control	10	8	0.2636	0.462
UVolume	PV F–L Patient	17	15	0.3254	0.203
UVolume	Blo D	27	25	0.3336	0.089
UVolume	Blo D Control	10	8	-0.1424	0.695
UVolume	Blo D Patient	17	15	-0.2377	0.358
UVolume	UOsm	54	52	-0.4919	<0.001
UVolume	UOsm F–L	27	25	–0.5247	0.005
UVolume	UOsm F-L Control	10	8	–0.3667	0.297
UVolume	UOsm F-L Patient	17	15	–0.3278	0.199
UOsm	TBW	54	52	–0.0465	0.739
UOsm	TBW F–L	27	25	–0.0601	0.766
UOsm	TBW F–L Control	10	8	–0.1121	0.758
UOsm	TBW F–L Patient	17	15	0.1587	0.543
Irr Mx	E2 F-L	27	25	0.2402	0.227
Irr Mx	E2 F-L Control	10	8	-0.0667	0.855
Irr Mx	E2 F-L Patient	17	15	-0.0637	0.804
Irr D	E2 F-L	27	25	0.2326	0.243
Irr D	E2 F-L Control	10	8	0.3091	0.385
Irr D	E2 F-L Patient	17	15	-0.3897	0.122

Table 5.8.f
Spearman's Rank Correlation

Factors		N	DF	Spearman's RC	pvalue
ExNa	UOsm	54	52	-0.0936	0.501
ExNa	UOsm F–L	27	25	–0.2065	0.301
ExNa	UOsm F–L Control	10	8	0.2364	0.511
ExNa	UOsm F-L Patient	17	15	–0.0754	0.774
ExNa	E/P	54	52	–0.0548	0.694
ExNa	E/P F–L	27	25	–0.2668	0.179
ExNa	E/P F-L Control	10	8	–0.1030	0.777
ExNa	E/P F–L Patient	17	15	-0.2721	0.291
ExNa	E2	54	52	–0.2341	0.240
ExNa	E2 F–L	27	25	–0.2814	0.155
ExNa	E2 F–L Control	10	8	–0.2485	0.489
ExNa	E2 F–L Patient	17	15	–0.0760	0.772
E2 F–L	Blo D	27	25	–0.2218	0.266
E2 F–L	Blo D Control	10	8	–0.0788	0.829
E2 F–L	Blo D Patient	17	15	0.3358	0.489
E2	Bre D	27	25	–0.0728	0.718
E2	Bre D Control	10	8	–0.0909	0.803
E2	Bre D Patient	17	15	0.4675	0.058
E2	Dep D	27	25	–0.3347	0.088
E2	Dep D Control	10	8	–0.8182	0.004
E2	Dep D Patient	17	15	0.4308	0.084
E/P F–L	Dep D	27	25	–0.1189	0.555
E/P F–L	Dep D Control	10	8	–0.6970	0.025
E/P F–L	Dep D Patient	17	15	0.4087	0.103
E/P F–L	Blo D	27	25	–0.1534	0.444
E/P F–L	Blo D Control	10	8	–0.2364	0.511
E/P F–L	Blo D Patient	17	15	0.1985	0.445
E/P F–L	Bre D	27	25	–0.1009	0.617
E/P F-L	Bre D Control	10	8	–0.3818	0.276
E/P F–L	Bre D Patient	17	15	0.3727	0.290

5.7.8 Urinary Volume

The urinary volume significantly decreased from the follicular to the luteal phase in the patients (Fig 5.9). In the controls the trend was an increase in the total urinary volume from the follicular to the luteal phase of the menstrual cycle (statistically insignificant).

5.7.9 Urinary Osmolality

The osmolality significantly decreased in the controls ($p=0.041$ C.I. 10, 283.50) whereas a possible trend towards an increase was noted amongst the patients from the follicular to the luteal phase ($0.01 > p > 0.05$) (Fig 5.10).

5.7.10 Urinary Creatinine, Urea

The urinary creatinine and urea concentration both significantly increased in the patients from the follicular to the luteal phase (Fig 5.11, 5.12)

5.7.11 Urinary Sodium & Potassium

The urinary Na concentration decreased whereas the K concentration increased in the patients. In the control group the K concentration dropped along with the Na concentration during the menstrual cycle. These were statistically not significant. The total urinary sodium decreased during the menstrual cycle amongst the patients whilst increased in the controls (statistically insignificant). The total urinary sodium in the luteal phases when compared were significantly different ($p<0.03$ C.I. –54.8, –4.7).

The total urinary sodium was significantly correlated to ExNa, weight, TBW and PV. The change from follicular to luteal phases of ExNa, weight, PV also correlated significantly to the change in total urinary sodium, but only amongst the patient group. Breast symptoms (Delta scores) was the only symptom which significantly negatively correlated to total urinary sodium loss.

5.7.12 Hormones

The oestradiol concentration in the patients increased significantly in the luteal phase ($p<0.0001$ CI = 341.5, 159) (Fig 5.13). Progesterone concentrations were not significantly different between patients and controls (Fig 5.16). However it is important to note that the above hormonal concentrations were determined only once during the follicular and luteal phases of the menstrual cycle and therefore may not be truly representative. The FSH ($p<0.0001$, CI = 1.8, 3.1)(Fig 5.14) and LH ($p < 0.006$ CI = 1.35, 4.4) (Fig 5.15) levels decreased significantly in the luteal phase in the patient group. The changes in the controls were not significantly different. Of note is that 2 patients and 1 control had high FSH & LH concentrations in the follicular phase. However their luteal phase levels were within the normal range. Both FSH and LH were significantly negatively correlated to oestrogen concentrations (Table 5.8). There was no correlation noted between the oestradiol values and TBW, ECFV, PV or ExNa (Table 5.8). The TBW, ExNa and ECFV were all significantly positively correlated to weight (Table 5.8) when all patients and controls, both follicular and luteal values were taken. The difference between follicular and luteal phase weights was negatively significantly correlated to the difference in bloatedness and breast symptom scores in the patients.

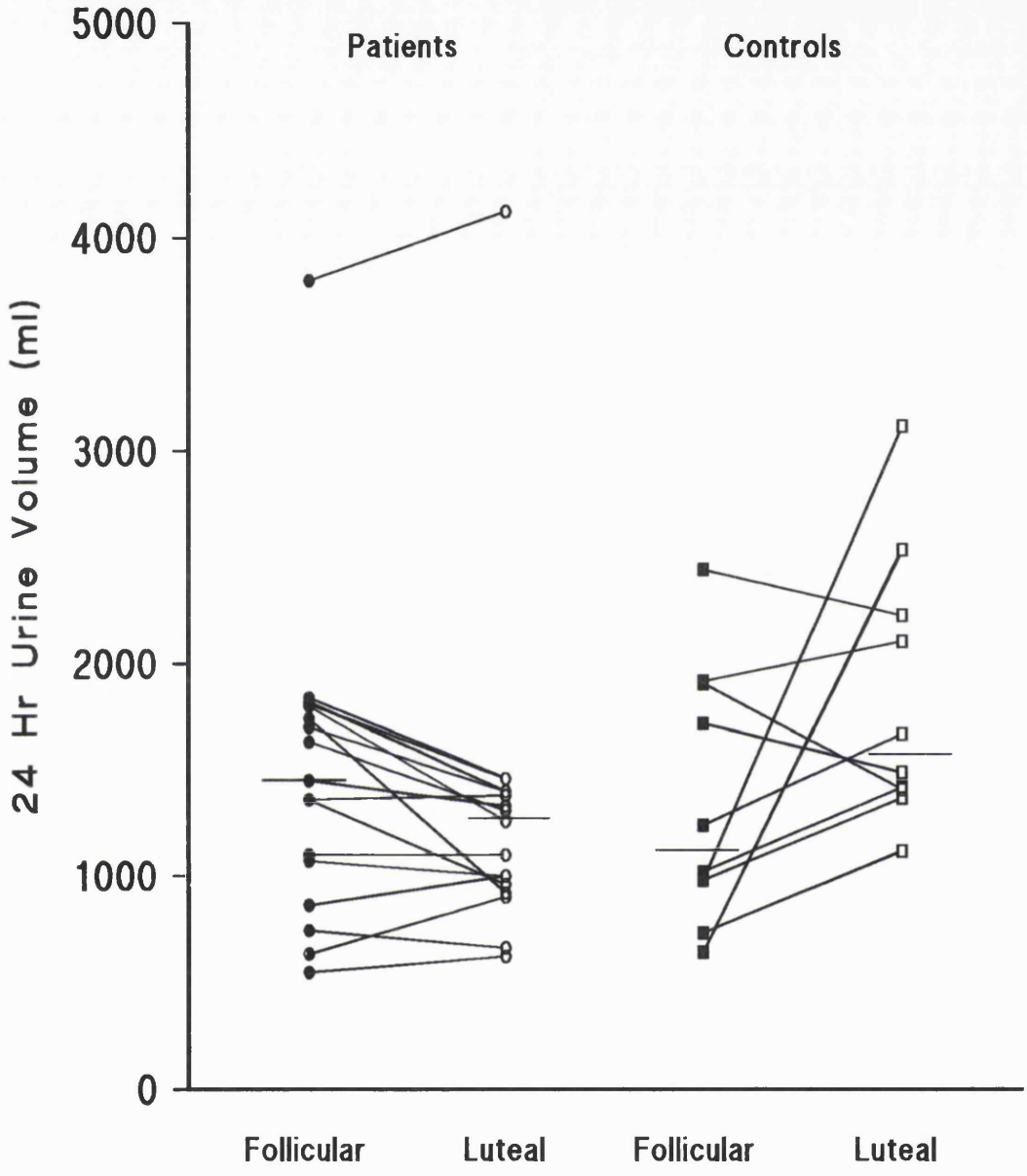


Figure 5.9

Urine volume in 17 patients and 10 controls during the follicular and luteal phases of the menstrual cycle. The urine volume was significantly reduced in the luteal phase in the patients when compared to the follicular phase ($p = 0.036$). Bars denote median values.

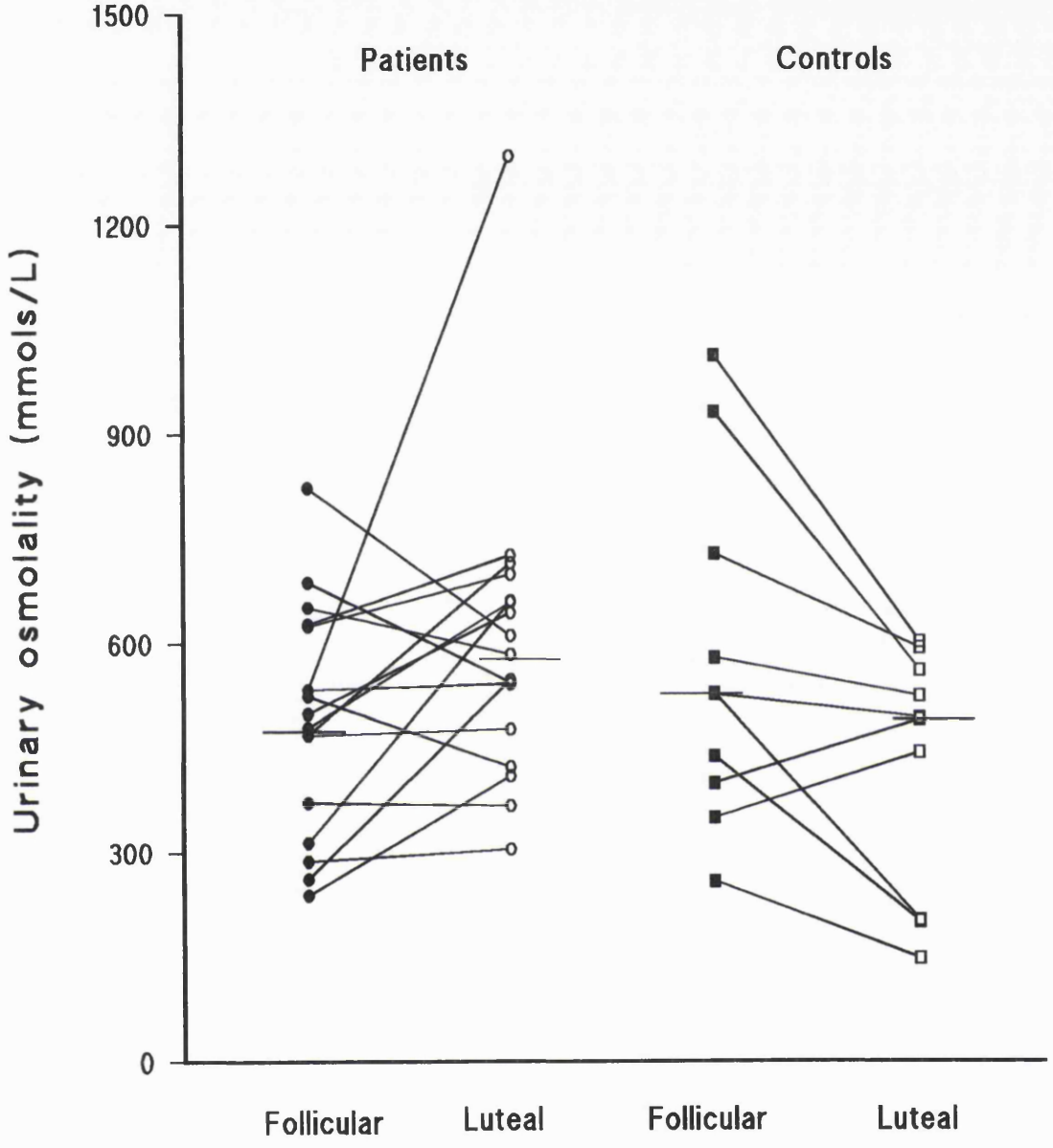


Figure 5.10

Urine osmolality in 17 patients and 10 controls during the menstrual cycle. The osmolality was significantly reduced in the luteal phase in the controls (p =0.04). In the patients the osmolality increased in the luteal phase but did not achieve significance (p=0.058). Bars denote median values.

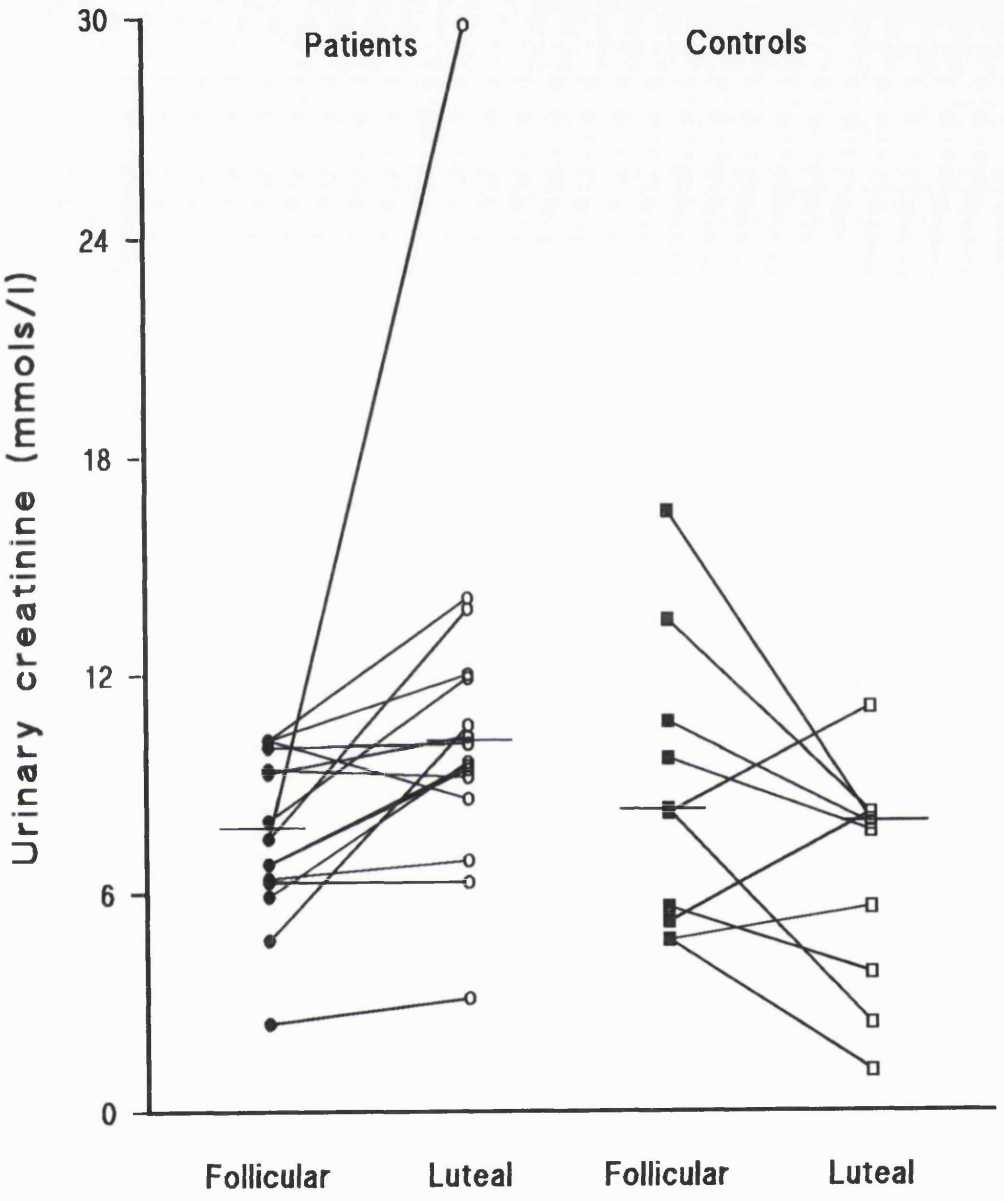


Figure 5.11

Urinary creatinine in 17 patients and 10 controls during the menstrual cycle. The creatinine levels in the patients were significantly higher in the luteal phase when compared to the follicular phase ($p = 0.003$). Bars denote median values.

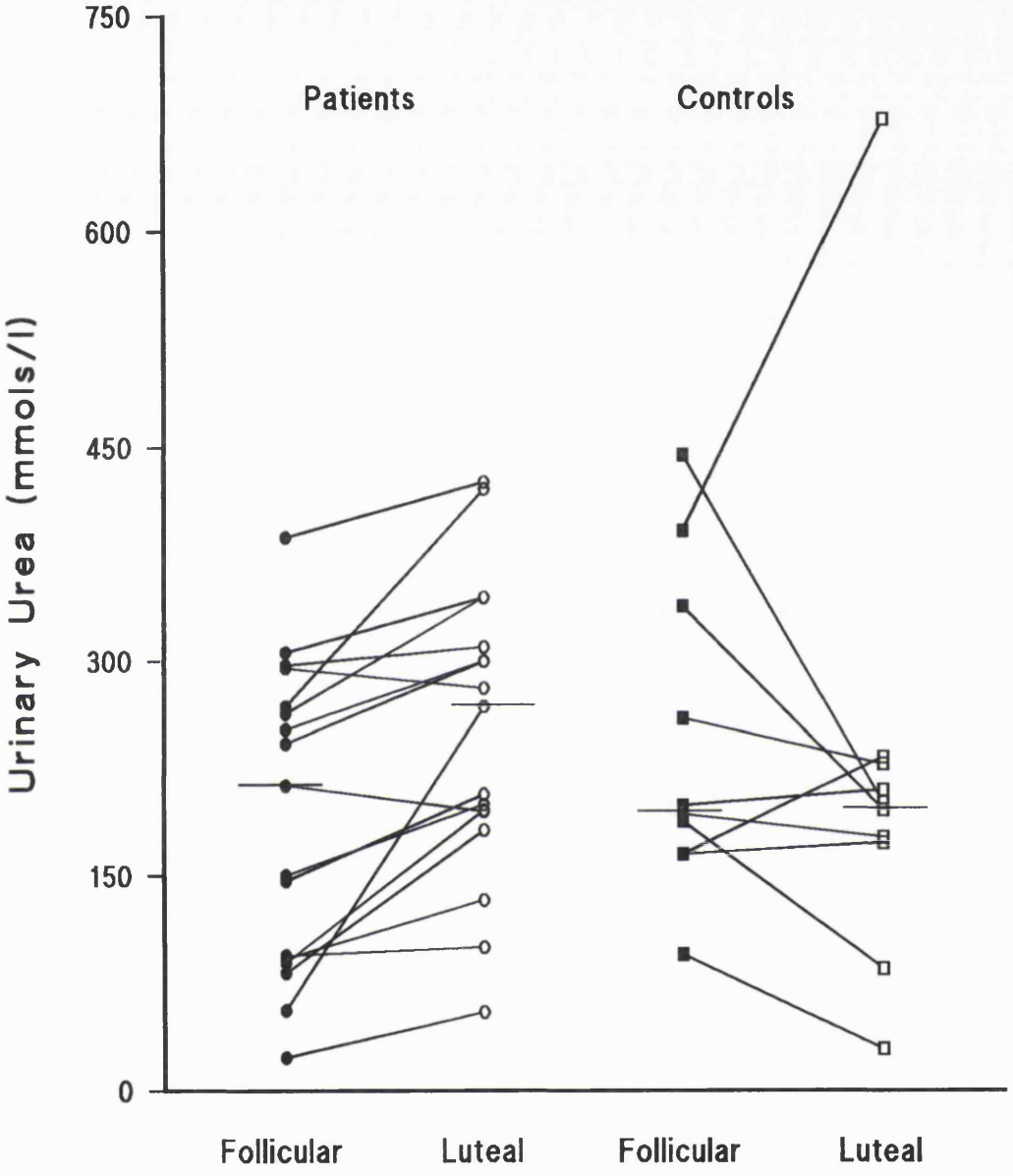


Figure 5.12

Urinary urea in 17 patients and 10 controls during the menstrual cycle. The urea concentrations were significantly higher in the luteal phase in the patients ($p = 0.001$). Bars denote median values.

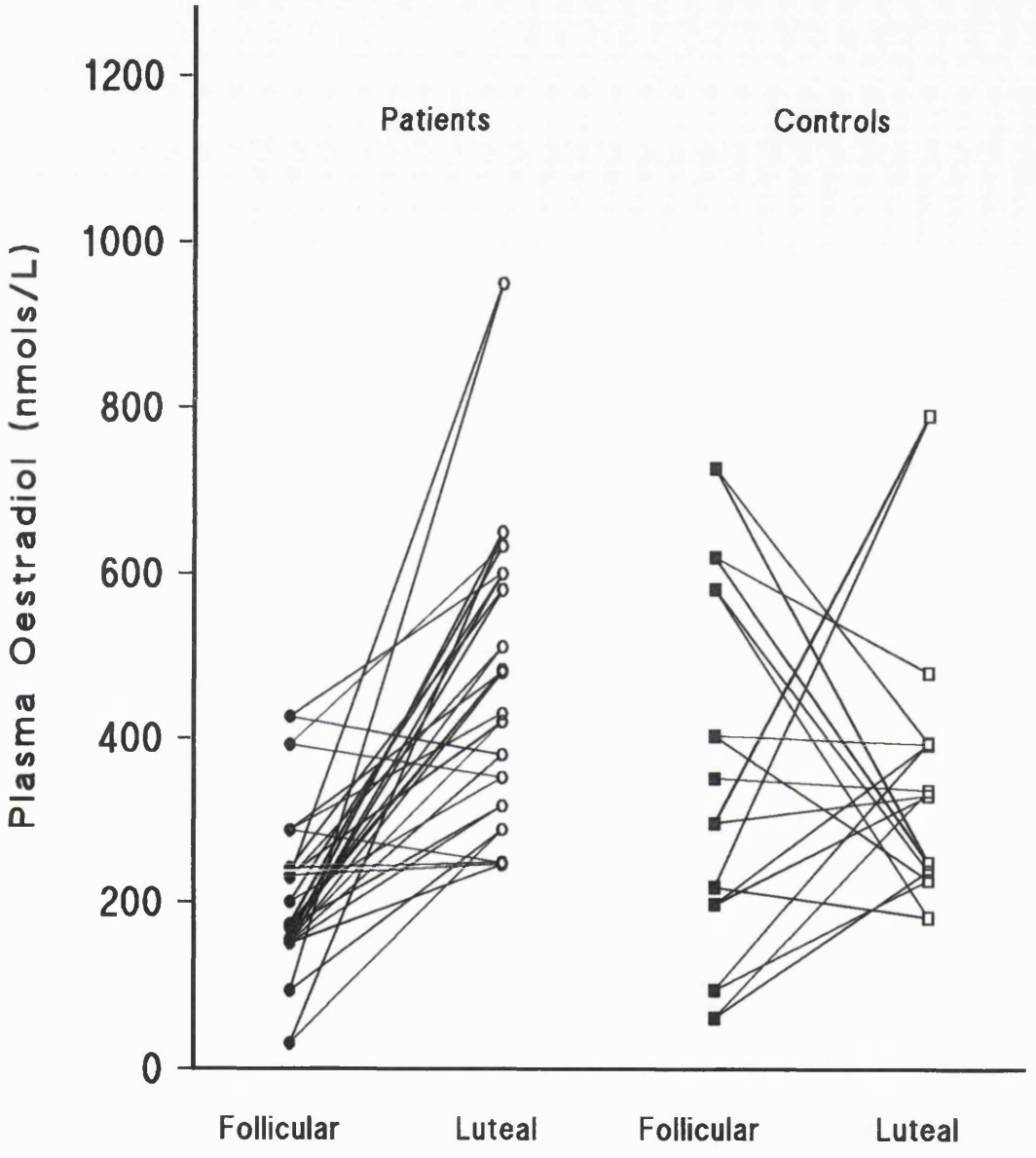


Figure 5.13 Plasma oestradiol concentrations during the menstrual cycle in 17 patients and 10 contols. The oestradiol concentrations were significantly higher in the luteal phase in the patients ($p < 0.0001$). However sampling for oestadiol levels were only performed onc3e in each phase. Bars denote median values.

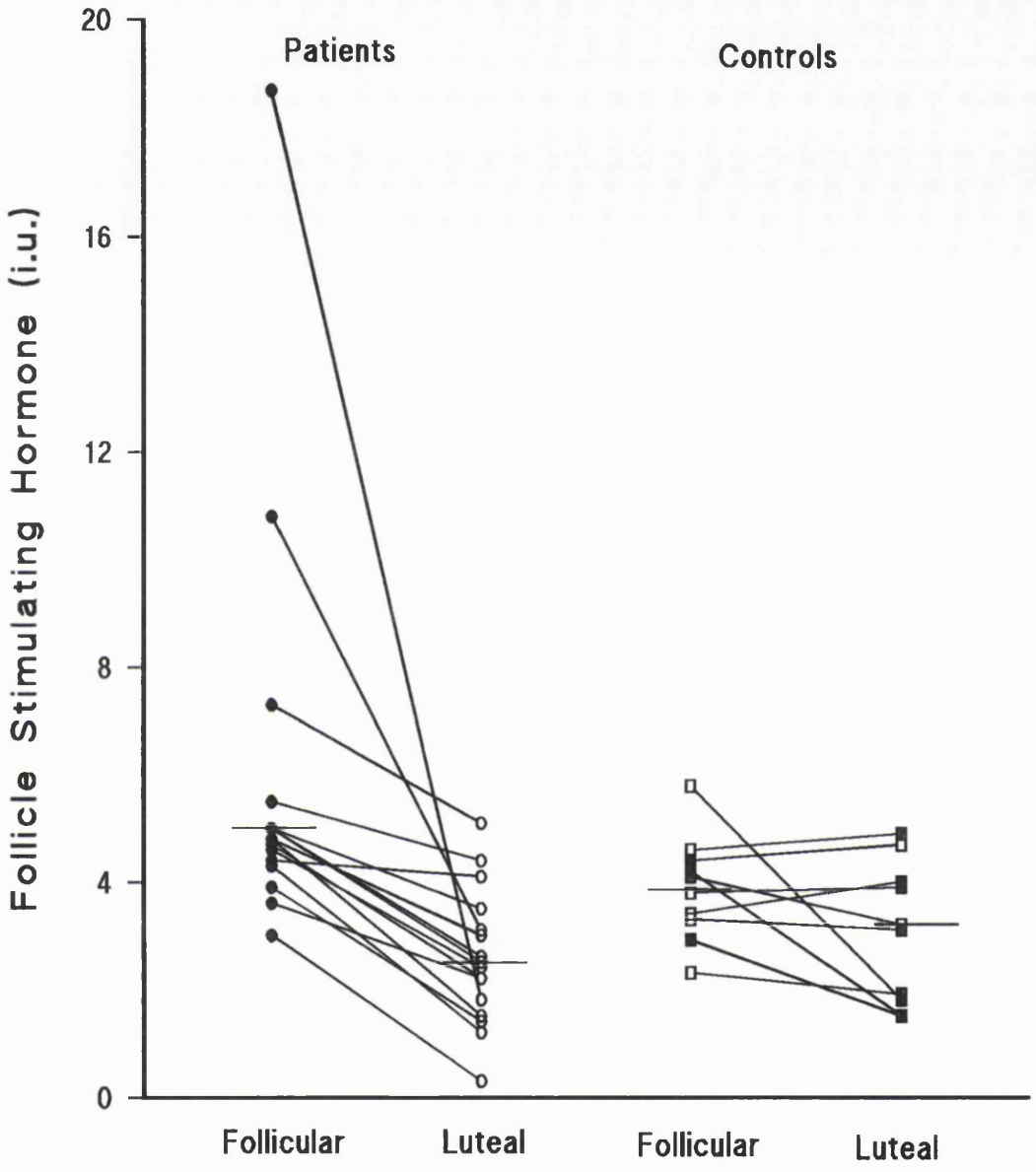


Figure 5.14

Plasma follicle stimulating hormone (FSH) in 17 patients and 10 controls during the menstrual cycle. The FSH concentrations were significantly lower in the patients in the luteal phase when compared to the follicular phase ($p < 0.0001$). Bars denote median values.

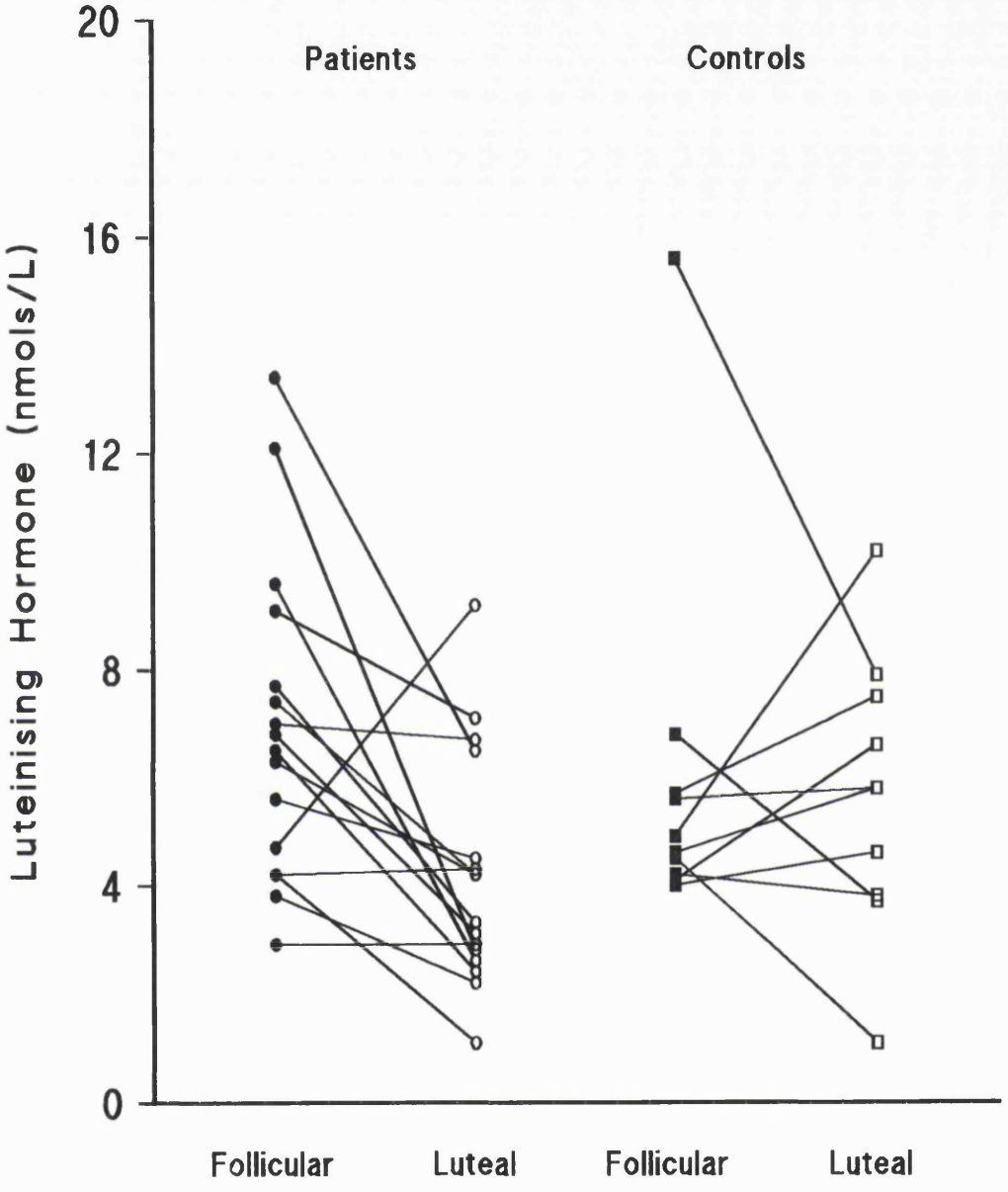


Figure 5.15

Plasma luteinising hormone (LH) during the menstrual cycle in 17 patients and 10 controls. The LH concentrations were significantly lower in the patients in the luteal phase when compared to the follicular phase ($p = 0.006$). 2 patients and 1 control had high LH levels in the follicular phase. This may be due to their being perimenopausal. However their luteal levels were within the normal range. The bars denote median values.

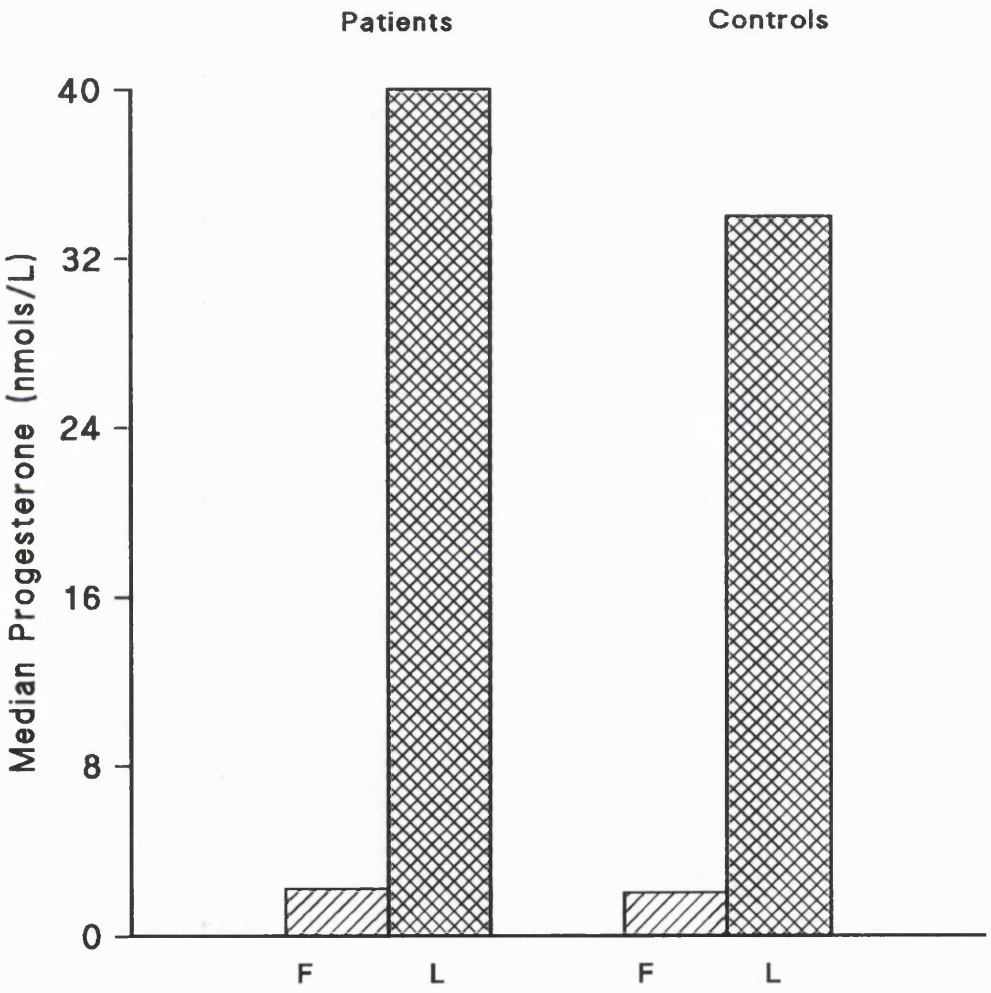


Figure 5.16

Plasma progesterone concentrations during the menstrual cycle in 17 patients and 10 controls. There was no significant difference in the progesterone concentration in the patients and controls follicular (F) and luteal (L) phases were compared.

5.8 Discussion

The results obtained from the experiments conducted above have mostly shown insignificant differences between the patient and the control groups, and the follicular and the luteal phases of the menstrual cycle. No specific patterns of interrelationship could be detected between the fluid compartments measured (Fig 5.3).

The TBW did not show any significant changes but a trend was noted in the patients demonstrating a decrease from the follicular to the luteal phase; this was not significant. This is similar to those findings of Herzberg (1971). The weight did not reflect parallel trends. In only eight patients and three controls did TBW and weight reflect similar changes when each result was looked at individually. The difference between follicular and luteal phase weights were correlated negatively to bloatedness and breast symptom delta scores. This finding was contrary to that expected. Taking the median values a trend towards a decrease in TBW was noted in the patients as compared to a gain in the controls. It is interesting to note that increase in TBW has been demonstrated by some workers in patients recovering from depressive disorders (Coppen, 1963, Hullin et al, 1967, Mangoni et al, 1967). In conjunction with this an increase in intracellular water has also been noted in depressed patients being treated with lithium who have shown an improvement in symptoms (Mangoni et al, 1970). Urinary sodium output has also been noted to fall in depressed patients when compared to manic patients (Hullin et al, 1967) suggesting a decrease in total body sodium.

It is interesting to note that in our group of patients the total sodium excretion and the urinary concentration of sodium were both decreased in the luteal phase. In the luteal phase it would be expected to have increased sodium loss in view of progesterone being secreted. However sodium balance is achieved through a number of complex mechanisms and the final outcome is the combined effect of these mechanisms. As the oestrogen concentration increased significantly in the luteal phase in patients ($p<0.0001$, CI=159.0, 341.5), its sodium retaining effect could account for the lowered sodium concentration. In the controls in the luteal phase the urinary sodium concentration was lower than the follicular phase, although the *total* sodium excreted was much higher in the luteal phase. The oestrogen concentration was significantly raised in the luteal phase in the patients but this was not significantly different from that of controls. Also the oestrogen progesterone ratios were not significantly different when patients were compared to controls. It has been shown that oestrogen can increase membrane excitability and it has been suggested that this may account for the increased agitation or irritability in PMS patients. Backstrom et al (1974) have demonstrated significantly higher oestrogen levels on days 5-2 before menstruation and the oestrogen to progesterone ratios being higher on days 6-3 before the onset of menstruation. In our study although only one measurement was taken in each phase of the menstrual cycle, which of course has significant limitations, the significantly higher oestrogen level was clearly demonstrated in the luteal phase in the patient group as compared to the follicular phase whereas no significant difference was noted amongst the controls. However in our group of patients and controls no significant

correlation could be detected between irritability scores and oestradiol concentrations either absolute values or follicular–to–luteal changes.

The slight decrease in PV in patients with an increase in the ECFV and vice versa in the controls may reflect a shift in the body compartments. The median ExNa fell in both groups but more markedly in the patients in the luteal phase, though these were not statistically significant. When the follicular–to–luteal change in ExNa was compared to that of controls a significance was detected ($p = 0.037$, 95% CI= 2.0, 150) This result is contrary to that shown by Herzberg (1971), who demonstrated no difference in the ExNa. Of particular interest was the demonstration of a decrease in the sodium pump activity and increase in the percentage cell water in the PMS group in the luteal phase, in a study being carried out simultaneously by Ozin (1992). Also the decrease in the urinary sodium concentration in the patients may be a reflection in the lower ExNa demonstrated in the luteal phase and may be an attempt to conserve sodium. In the controls on the other hand the total urinary loss of sodium is greater in the luteal phase, which may reflect the higher ExNa noted in the controls in the luteal phase. Klein & Carey (1957) did not show any alteration in the ExNa in 20 normal subjects throughout the menstrual cycle and of note did not demonstrate any correlation with urinary sodium loss. In our premenstrual syndrome patients the urinary volume was significantly lower associated with a higher osmolality in the luteal phase (Table 5.6 & Fig 5.10). This may be an effect of a reduced TBW and PV in the luteal phase in patients. These findings were in contrast to the findings in that of controls in whom an increase in the urinary volume and decrease in urinary osmolality was noted in the luteal phase

associated with an increase in the TBW and PV in the controls. The increased osmolality of urine in the patients could be related to the higher loss of creatinine and urea in the luteal phase, this was significantly increased in the patients premenstrually (Table 5.6, & Fig 5.11, 5.12). Why the patients should produce less volume of more concentrated urine and the controls more dilute urine is of interest, but not explained.

These findings are more likely to be a reflection of events as opposed to the cause of symptoms. The findings in the controls of an increased loss of sodium and increased volume of urine in the luteal phase is that which is an expected finding with progesterone secretion occurring in the luteal phase (Parboosingh et al,1974). However the findings in the patients were contrary to those expected. The TBW was not increased, the ExNa fell and PV decreased with a slight increase in the ECFV. Although the findings did not achieve statistical significance, what was of importance was that all these trends were the opposite to that noted in the controls.

Increased progesterone concentration in symptomatic women has been demonstrated (O'Brien et al, 1980). This would lead to increased natriuresis and thus aldosterone concentrations would increase in response to this increased progesterone secretion. However aldosterone concentrations were not found to be increased in the symptomatic group by O'Brien et al (1980). The decreased urinary concentration of sodium is contrary to what was expected with the increase in progesterone concentration in the luteal phase in the patients especially as this was higher than the controls, who on the other hand put out more total sodium. This decreased loss in the luteal phase in the patients may be an attempt to

conserve sodium as ExNa was found to be lower in the luteal phase. The sodium loss per litre of urine was however higher in the patients in the luteal phase when compared to the controls. This could again be explained by the higher progesterone levels in the patient when compared to the controls.

Analysis of symptom scoring has consistently demonstrated very high luteal phase scores in the PMS patients. Of special interest to us was the bloatedness scores and its correlation to weight, TBW, ECFV, ExNa and PV. Apart from a significant negative correlation of bloatedness delta scores to delta weight no significance was demonstrated. This is represented in Fig 5.17 where the median (range) deviation from follicular to luteal phases are scored on an arbitrary baseline of 100.

5.8.1 Do Compartmental Volume Changes Relate to Hormone Changes ?

Very significant changes in plasma oestradiol concentrations from follicular to luteal phase were detected in patients but not controls (Table 5.6). By contrast, compartmental volumes showed no change indicating significance (Fig 5.4–5.7) save only for the patient/control total body exchangeable sodium difference (Fig 5.7, Table 5.9). Nevertheless it seemed important to check whether any subtle shift in fluid volumes might relate to hormonal changes and might not have been detected by the preceding analysis. Excess oestradiol and progesterone concentrations have been demonstrated previously and have been correlated to adverse mood symptoms (Hammarback et al,1989).

However, the significant changes in the concentrations of these hormones in the study made it important to analyse their association with body fluid compartments. Further analysis was restricted to measurements concerned with exchangeable sodium or sodium space in relationship to measurements relating to the balance between progesterone and oestradiol in the luteal phase of the cycle. This was to test the hypothesis that an "oestrogen–progesterone" imbalance might influence compartmental volumes. Results were generally statistically insignificant except that follicular to luteal changes in the ratio of ECFV to sodium space showed a significant correlation with two different measurements of luteal "oestrogen–progesterone" balance (Table 5.10). The more significant of the two relationships is illustrated in Fig 5.18. However the oestrogen and progesterone levels were determined only once in the follicular and luteal phases of the menstrual cycle and may not be truly representative. These results should be regarded as speculative because they result from retrospective searching of a limited data set to see if any significant relationships could be detected. They do however provide indicators for possible future studies. They also imply that if there are subtle shifts in body fluid compartments during the menstrual cycle these may well relate to the balance or imbalance between progesterone and oestrogen.

5.9 Summary

TBW, ECFV, PV and ExNa were determined simultaneously in 17 patients and 10 controls by radio-isotope dilution methods. Various biochemical and physical properties of urine were also measured. The changes in TBW, ECFV and PV were not significant.

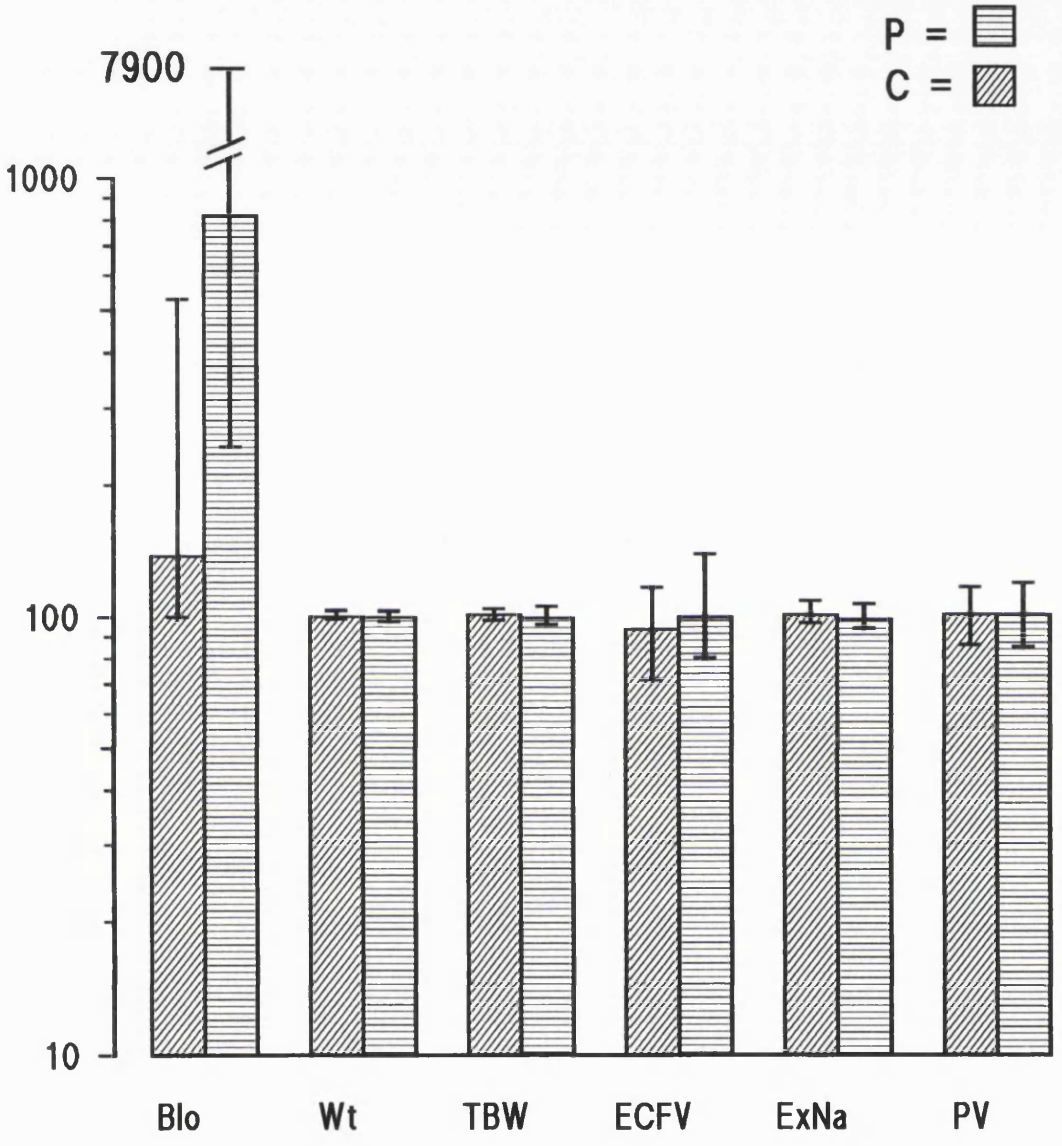


Figure 5.17

Median (Range) deviation from an arbitrary baseline of 100, of the follicular to luteal bloatedness (blo), weight (wt), total body water (TBW), extracellular fluid volume (ECFV), exchangeable sodium (ExNa) and plasma volume (PV) in 17 patients (P) and 10 controls (C).

Table 5.9

Comparison of the changes (median values) from the follicular to the luteal phases between the patients and controls using the Mann Whitney Rank U test.

	p Value	95% CI
Weight	0.651	–0.500, 1.100
TBW	0.13	–0.1000, 0.9201
PV Low	0.31	–0.1599, 0.2501
ExNa	0.037	2.0, 150.0
PV High	0.80	–0.2300, 0.2099
ECFV	0.46	–2.0000, 1.299
Sodium Space	0.258	-2.00, 0.900
Plasma		
Urea	0.14	–1.100, 0.100
Sodium	0.19	0.00, 3.001
Potassium	0.92	–0.4003, 0.4999
Progesterone	0.451	–20.7, 11.0
Oestradiol	0.059	–486.1, 3.1
FSH	0.008	0.599, 2.801
LH	0.039	0.1.2, 5.299
Urine		
GFR	1.00	–7.00, 9.00
Volume	0.012	115.1, 819.9
Creatinine	0.005	–7.902, –1.868
Urea	0.04	–156.0, –2.000
Osmolality	0.006	–403.0, -68.0
Potassium	0.167	–49.02, 10.02
Total Sodium	0.102	–5.81, 74.11
Sodium Conc.	0.94	–23.01, 17.99

Table 5.10

Correlation of follicular–to–luteal change in ECFV/Sodium space ratio to a) oestradiol/progesterone ratio (luteal) and b) oestradiol–10* X progesterone concentration (luteal).

Correlation	Oestradiol–Progesterone Ratio	Oestradiol–10 X Progesterone
Non–parametric	$r_s = -0.4352$	$r_s = -0.4300$
	$p < 0.03$	$p < 0.03$
Parametric	$r = -0.50$	$r = -0.60$
	$p < 0.01$	$p < 0.001$

* 10 factor based on observation that control luteal oestradiol levels were typically of the order of 10 x progesterone levels.

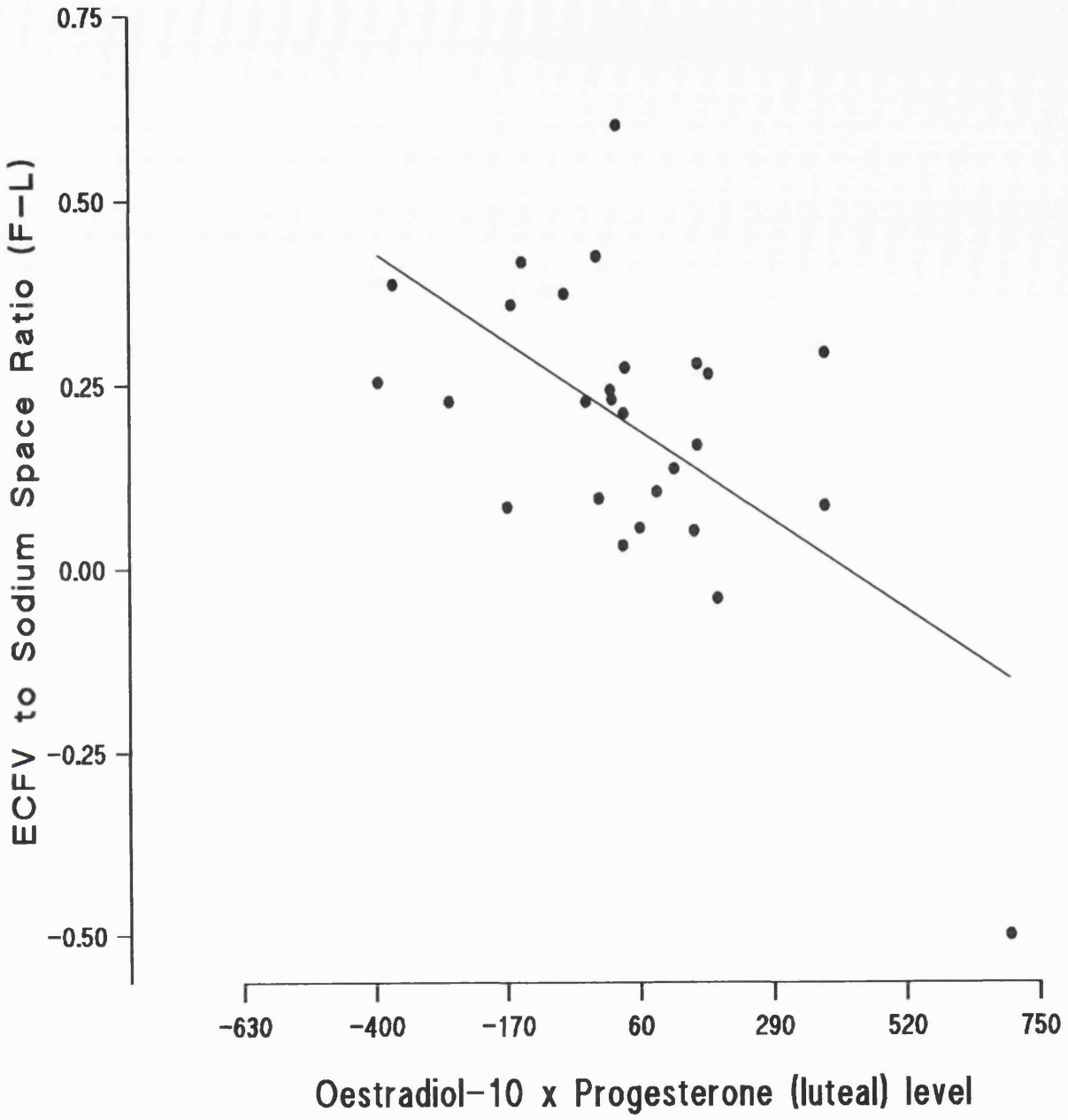


Figure 5.18

Correlation of follicular–to–luteal change of Extracellular fluid volume to sodium space ratio with (Oestradiol–10 x Progesterone) luteal levels, used as an index to oestradiol–progesterone balance ($p < 0.001$).

However it is important to note that the oestrogen and progesterone levels were determined only once during the follicular and luteal phases of the menstrual cycle and therefore may not be truly representative.

A decrease occurred in the ExNa from the follicular to the luteal phase of the menstrual cycle in both patients and controls. This decrease was significantly different when patients were compared to controls.

The patients also produced significantly more concentrated urine and of less volume in the luteal phase when compared to controls. Significantly higher oestrogen concentrations were found in the patients during the luteal phase. An oestrogen/progesterone "imbalance" may be correlated in some way to the follicular to luteal changes in the ratio of ECFV/sodium space.

CHAPTER 6

GENERAL CONCLUSION

The aims of this thesis (Chapter 1, page 60) were

1. To establish a simple effective method to diagnose Premenstrual Syndrome (PMS) based on previously validated tools.
2. To study atrial natriuretic peptide (ANP) in the normal menstrual cycle and in PMS and, if changes were demonstrated, to administer exogenous ovarian hormones in an attempt to mimic these alterations.
3. To study vascular permeability changes in the normal menstrual cycle and in PMS and, if changes occured, to study the effect of exogenous ovarian hormones on vascular permeability.
4. To detect any changes in total body water, total body exchangeable sodium, extracellular fluid volume and plasma volume in the normal menstrual cycle and in PMS. To determine whether these changes correlated with either somatic or psychological symptoms. If major changes were detected to administer cyclical ovarian hormones in an attempt to mimic these changes.
5. To determine whether there is likely causal relationship between symptoms of PMS and the compartmental fluid, electrolyte and hormonal changes.

In the studies described in this thesis all asymptomatic and PMS patients were screened for psychiatric disorder. The General Health Questionnaire was used as this has been widely employed and well validated in the general

population for the screening of psychiatric ill health (Goldberg & Hillier, 1979). From our studies it became apparent that in the luteal phase of the menstrual cycle the PMS patients all scored above the threshold for diagnosing psychiatric disease. This was not surprising as there was an overlap of questions with that of the Moos' Menstrual Distress Questionnaire (Moos, 1969b). This clearly suggests that any assessment of psychiatric disease in women should be performed at specifically designated phases of the menstrual cycle in order to obtain an accurate assessment.

In the studies described in this thesis a simple method was described to diagnose PMS using 3 point moving averages of daily prospective scoring of six symptoms on a linear Visual Analogue Scale which was validated against existing more complex techniques (Moos, 1969b).

Atrial natriuretic peptide, a natriuretic and diuretic hormone which could be altered in PMS, was measured. Changes in this hormone could either mediate or reflect fluid and electrolyte or compartmental shifts, although such changes would appear unlikely to be the sole aetiological factor. These ideas led to the work described in Chapter 3. A finding to be stressed is that the concentration of ANP was significantly lower in PMS patients *throughout* the cycle (Hussain et al, 1990). This was totally unexpected as the theory that PMS patients retained sodium did not appear to be plausible (Greenhill & Freed, 1941). What was even more surprising was that the PMS patients demonstrated a significant *decrease* in the ANP concentrations in the luteal phase when compared to the follicular phase of the menstrual cycle. The controls did not demonstrate any such change during the menstrual cycle. These findings were contrary to those expected and raised the

question: Is there fluid retention in PMS patients resulting in a dilutional effect and therefore lowered ANP concentration? Or do PMS patients have a fundamentally lower concentration of sodium which therefore results in reduced ANP secretion.

It was felt that administration of gonadal hormones (oestrogen and cyclical progestogen) might demonstrate such changes more precisely. Post menopausal women were therefore recruited and given cyclical hormone therapy. ANP concentrations were measured in the appropriate phases. Of note was that during oestrogen administration a similar *significant decrease* in ANP concentration occurred. Progestogen therapy did not produce any significant change. Oestrogen is known to cause water retention and therefore could have accounted for the dilutional effect (Skinner et al, 1969). It is difficult to know the true answer as menopausal patients may have different characteristics in homeostasic mechanisms due to their initial hypooestrogenism or because of the unknown effects of aging.

Alternatively it has been suggested that some of the effects of ANP may be due to alterations in vascular permeability (Trippodo et al, 1987). Thus the next logical area to investigate was thought to be vascular permeability, in the normal menstrual cycle and in PMS.

The vascular permeability was measured as the 0–10 minute albumin change on application of 80mm of Hg cuff pressure on the upper arm during the different phases of the menstrual cycle. In the PMS patients the 0–10 minute change was significantly greater in the luteal than in the follicular phase, which was opposite to that in the controls; the change was greater in the follicular phase. The increase in the 0–10 minute albumin concentration in the patients in the

luteal phase could be accounted for by an increase in the vascular permeability to water (Wong et al, 1972). This raised the possibility that increased movement of fluid into the extravascular space could have occurred in the luteal phase in the patients. It has been suggested in the past that an extravascular shift of fluid may occur in the luteal phase in PMS (Andersch et al, 1978a). Again it was felt that exogenous administration of hormones (oestrogen/progestogen) might help to determine the possible mechanism of altered vascular permeability. However hormone administration did not produce any significant change. This may, of course be due to a decreased receptor sensitivity to hormones in the menopausal patients.

Although it was not possible to come to definite conclusions from the above study, a significant alteration of vascular permeability during the menstrual cycle and in PMS was demonstrated. It was therefore logical to proceed to design a study where the fluid-electrolyte compartments could be measured simultaneously in premenopausal women with and without PMS.

In the past there have been isolated attempts at studying fluid and electrolyte changes in the menstrual cycle and PMS. Andersch et al (1978a) studied total body water and found no significant difference between PMS patients and controls. Herzberg (1971) demonstrated a significant *decrease* in the total body water in a group of nuns but no control subjects were studied. Metabolic balance studies have also been undertaken by various workers (Bruce & Russell, 1962, Janowsky et al, 1973). The results demonstrated, under rigid and restricted conditions, that weight change, sodium and electrolytes varied imprecisely with symptoms. Janowsky et al (1973) suggested that there was an

increase in urinary potassium to sodium ratio and drew the indirect conclusion that there may be aldosterone excess and sodium and water retention in the late luteal phase. Bruce and Russell (1962) noted very little weight gain when studying women in a metabolic unit with premenstrual symptoms under rigid dietary restrictions. Preece et al (1975) have also studied total body water in women suffering from PMS particularly premenstrual mastalgia but did not find any significant difference during the menstrual cycle.

The simultaneous measurement of total body water, extracellular fluid volume, plasma volume and total body exchangeable sodium has not been previously performed. Radio-isotope dilution techniques were utilised for these determinations as described in Chapter 5. The gonadal hormones oestrogen and progesterone on precisely the same days were also determined and detailed urinary electrolyte studies were performed.

Total body water, extracellular fluid volume and plasma volume did not show significant differences between patients and controls or between follicular and luteal phases within either group. There was also no significant weight change during the menstrual cycle for either patients or controls despite significant increases in scores for bloatedness. However, the difference between the follicular and luteal phase weights was significantly negatively correlated to the difference in bloatedness and breast symptom scores. The complete cycle of experiments would have included determination of the fluid and electrolyte changes following exogenous administration of oestrogen and progestogen. However the results demonstrating an absence of major fluid and electrolyte shifts made these further

experiments unnecessary particularly as the administration of radio-active isotopes could no longer be justified in the light of the first part of the study.

Importantly total body exchangeable sodium *decreased* from follicular to luteal phase in *both* groups. This change through the cycle was significantly greater in the *patients* when compared to controls ($p < 0.03$).

The urinary osmolality, urea and creatinine loss was significantly greater in the patients than controls i.e. more concentrated and smaller volumes of urine were being excreted by patients in the luteal phase. This appeared to correspond with the trend noted in the exchangeable sodium differences during the menstrual cycle. There appears to be a mechanism in play which attempts to conserve sodium in the patients during the luteal phase. Whether the noted changes reflect an attempt to conserve sodium in the patients as a response to a decrease in total exchangeable sodium during the menstrual cycle, or an effect of some other pathophysiological mechanism is not known. Of particular interest is that other recent research has demonstrated the sodium/potassium pump activity to be decreased in the luteal phase of the cycle associated with an increase in the percentage cell water in the PMS patients (Ozin, 1992, Ozin et al, 1992).

The significant differences that were demonstrated in this study appear to be associated with total exchangeable sodium. An oestrogen/progesterone "imbalance" may in some way be related to the alteration in the exchangeable sodium. Further statistical analysis was therefore performed correlating the follicular to luteal changes in the ratio of ECFV/sodium space to that of a derived value reflecting an index of oestrogen/progesterone balance. This was found to be highly significant (Table 5.10, Fig 5.18).

However it is important to note that any conclusions relating to oestrogen and progesterone may be limited as their concentrations were only determined once during each of the follicular and luteal phases of the menstrual cycle.

The results presented in this thesis demonstrate that many longheld assumptions relating to the fluid and electrolyte shifts during the menstrual cycle in patients with PMS are clearly unsupported. Moreover, atrial natriuretic peptide and total body exchangeable sodium change significantly in the opposite direction to those anticipated. Neither the physical nor the psychological symptoms of premenstrual syndrome can be the consequence of sodium and water retention and thus the pathogenesis must be more complex than this.

Recent work in parallel with this study has shown that there was an increase in the percentage cell water in the luteal phase in PMS patients which may play a significant role (Ozin et al, 1992). Data from this thesis suggest that future work should pay particular attention to the possibility that oestrogen/progesterone "balance" may influence sodium homeostasis.

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