The Effects of

Hypericum perforatum with *Vitex agnus-castus*

in the treatment of

Menopausal Symptoms

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Declaration

I certify that that except where due reference has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged. Approval for the project was obtained from the RMIT Human Research Ethics Committee, and ethics procedures and guidelines were followed throughout the clinical trial.

Included in the body of this thesis are four original papers, with an additional one in the appendices. All have been accepted for publication with peer-reviewed journals.

Margaret Diana van Die

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Publications and Manuscripts

The following publications and manuscripts have arisen from this thesis.

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Conference Presentations

The following conference presentations arose from the work contained in this thesis.

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2. RMIT research conference, Melbourne 2007
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1. International Conference: Evidence-based Complementary Medicine, UNE, Armidale 2009

Poster presentations

1. Southern Health Research Week, Monash Medical Centre, Melbourne 2009 (Prize: Highly Commended)
2. International Conference: Evidence-based Complementary Medicine, UNE, Armidale 2009
3. International Congress on Complementary Medicine Research, Sydney 2008
4. Southern Health Research Week, Melbourne 2008
5. Australasian Menopause Society Annual Conference, Adelaide 2007
6. Australasian Integrative Medicine Association (AIMA) conference, Maroochydore 2005

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List of Abbreviations

5-HT	5-hydroxytryptamine /serotonin
8-PN	8-prenylnaringenin
ADR	adverse drug reaction
ADRAC	Adverse Drug Reactions Advisory Committee
AMH	anti-Müllerian hormone
ANCOVA	analyses of covariance
BDI	Beck Depression Inventory
BDNF	brain derived neurotrophic factors
BHP	British Herbal Pharmacopoeia
CAM	complementary and alternative medicine
CEE	conjugated equine oestrogen
CES-D	Center for Epidemiologic Studies Depression Scale
CGI	Clinical Global Impressions
CHD	coronary heart disease
CI	confidence interval
CNS	central nervous system
CONSORT	Consolidated Standards of Reporting Trials
CRF	corticotrophin-releasing factor
CSF	cerebrospinal fluid
CVD	cardiovascular disease
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
DRI	daidzein-rich isoflavones
DRS	Depression Rating Scale
D-S	Von Zerssen Depression Self-rating scale
DSM	Diagnostic and Statistical Manual
E2	oestradiol
EM	expectation maximisation
ER	oestrogen receptor
ET	oestrogen therapy
FDA	Food and Drug Administration
FMP	final menstrual period
FSH	follicle-stimulating hormone
GABA	γ-aminobutyric acid

GAD	Generalised Anxiety Disorder
GCS	Greene Climacteric Scale
GH	growth hormone
GnRH	gonadotropin-releasing hormone
GP	general practitioner
HALT	Herbal Alternatives for Menopause
HAMA	Hamilton Anxiety scale
HAMD	Hamilton Depression scale
HDI	Hamilton Depression Inventory
HDL	high-density lipoprotein cholesterol
HDRS	Hamilton Depression Rating Scale (also HAMD)
HERS	Heart-Oestrogen Replacement Study
HF	hot flushes
HMPC	European Medicines Agency Committee on Herbal Medicinal Products
HPA	hypothalamic-pituitary-adrenal
HPLC	high-performance liquid chromatography
HRT	hormone replacement therapy
HT	hormone therapy
ICD	International Classification of Diseases
ICP/MS	inductively coupled plasma mass spectroscopy
IF	isoflavones
INH	inhibin
KI	Kupperman Index
LDL	low-density lipoprotein cholesterol
LH	luteinising hormone
MANOVA	multivariate analysis of variance
MAO	monoamine oxidase
MDD	major depressive disorder
MDWFS	mean daily weighted flushing scores
MEL	melatonin
MWMHP	Melbourne Women's Midlife Health Project
MPA	medroxyprogesterone acetate
MRS	Menopause Rating Scale
MWS	Million Women’s Study
NA	noradrenaline/norepinephrine
NIMH	National Institute of Medical Herbalists
PEPI	Postmenopausal Estrogen/Progestin Interventions (trial)

PET	positron emission tomography
PMD	premenstrual dysphoria
PMDD	premenstrual dysphoric disorder
PMS	premenstrual syndrome
PMS-A	premenstrual syndrome- anxiety sub-cluster
PMS-C	premenstrual syndrome- cravings sub-cluster
PMS-D	premenstrual syndrome- depression sub-cluster
PMS-H	premenstrual syndrome- hydration sub-cluster
PMTS	premenstrual tension syndrome
PR	placebo responder
QOL	quality of life
RCT	randomised controlled trial
REM	rapid eye movement
RR	(responder) rate ratio
SD	standard deviation
SDG	secoisolaryciresinol diglucoside
SDS	Self-rating Depression scale
SE	standard error
SEEM	selective oestrogen enzyme modulator
SERM	selective oestrogen receptor modulators
SHBG	sex hormone binding globulin
SNRI	serotonin and noradrenaline reuptake inhibitors
SPSS	Statistical Package for Social Science
SSRIs	selective serotonin reuptake inhibitors
STAI	State-Trait Anxiety Inventory
SWAN	Study of Women’s Health Across the Nation
Tc	core body temperature
TSH	thyroid-stimulating hormone
UTI	urinary tract infection
WHI	Women’s Health Initiative
WHIMS	Women's Health Initiative Memory Study
WHO	World Health Organisation

Abstract

Research into effective treatments for menopausal symptoms faces several challenges. Interpretation of the existing literature is hampered by a lack of consistency in terminology relating to the different phases of menopause. In addition, neither the endocrinology of menopause nor the mechanisms underlying hot flushes have as yet been fully elucidated. Further, there is lack of consensus regarding which symptoms are due to endocrinological changes at menopause, as distinct from the psychosocial circumstances women experience at this stage of life. Mood changes, for example, are attributed in psychosocial theory to the age-related factors such as changes in a woman's role, and the awareness of ageing. In practical terms, endocrinological events and psycho-social factors are likely to interact. It is well established that anxiety exacerbates the physiological symptoms of hot flushes, for example. Thus hormonal and psychological approaches to treatment are both relevant.

In terms of phytotherapeutic treatment, while traditional use supports a range of herbs for the symptoms of menopause, evidence supporting their use from rigorous scientific trials is limited. Moreover, in most cases, their mechanisms of action are still largely unknown. Nonetheless, use of phytotherapy for menopausal symptoms has become very popular. Interest in alternatives to hormone therapy (HT), including non-estrogenic treatments that have shown varying degrees of efficacy, has increased due to the serious health risks that have been associated with HT, despite its incontrovertible effectiveness. In terms of phytotherapeutic options, the limited research has largely focussed on the phytoestrogenic plants. However, there have been some safety concerns expressed over their long-term use. In the practice of phytotherapy according to the Anglo-American and European traditions, non-oestrogenic herbs are also employed. Two such herbs were the focus of the principal study to be reported herein, namely *Hypericum perforatum* and *Vitex agnus-castus*.

Both study herbs are also used in, and have been trialled for, the alleviation of symptoms of premenstrual syndrome (PMS), which is reported to be more severe in the perimenopausal years. Many of the so-called menopausal symptoms could be viewed as an exacerbation of PMS in the perimenopause.

Research on vasomotor symptoms during menopause is prone to a substantial response in the placebo arm, due in part to the natural history of the symptoms. There has been much

interest in increasing our understanding of the placebo response with a view to controlling it in clinical research and harnessing it in clinical practice.

The hypotheses to be tested were

1. that the combination of two herbs, *Hypericum perforatum* and *Vitex-agnus-castus*, known to have central nervous system (CNS) effects and/or efficacy in PMS or depression, would alleviate the physiological flushing symptoms and psychological symptoms of menopause and improve quality of life in late peri-menopausal and early post-menopausal women

2. that the PMS-like symptoms in the perimenopausal sub-group would be relieved by the intervention

3. that the placebo would have significant impact on menopausal and PMS-like symptoms and would be predicted by psychological factors.

The main aim of the study was to examine a specific herbal combination with CNS activity and established efficacy in PMS and/or depression in the late perimenopause and early post-menopause on

i) the physiological symptoms of flushing and sweating;
ii) the psychological symptoms of depression and anxiety; and
iii) quality of life.

Secondary aims were

i) to study the effect of this combination in PMS-like symptoms in perimenopausal women; and

ii) to examine predictors of the placebo response, and to compare these with predictors of the response to active treatment.

A double-blind, randomised controlled trial (RCT), with a 16-week treatment phase, was conducted on 100 women in the late-perimenopause and early postmenopause. For this study, the herbal combination (*Hypericum perforatum* and *Vitex agnus*-castus) was not found to be superior to placebo for any of the endpoints - daily weighted flushing scores, scores on the Greene Climacteric scale and the Hamilton Depression Inventory. However, significant improvements across the treatment phase were observed in both the placebo and active treatment groups on all of these outcome measures. No significant change was found

for either group on the Utian quality of life scale. The herbal combination was well-tolerated with no significant adverse events noted over the 16-week treatment phase.

The effects of the combination of *Hypericum perforatum* and *Vitex agnus-castus* were examined on PMS-like symptoms in the small sub-population of late-perimenopausal women. The intervention was found to be superior to placebo for total PMS-like symptoms and the sub-clusters, PMS-D (depression) and PMS-C (cravings). The active treatment group also showed significant improvements on PMS-A (anxiety) and PMS-H (hydration), although this effect was not superior to placebo. Results of a trend analyses revealed significant quadratic trends for four of the five measures in the treatment group: PMS-Total, PMS-A, PMS-C, and PMS-D, but no significant trends in the placebo group.

Predictors of the placebo response were found to include anxiety at study entry for the outcome measures of flushing, depression and overall menopausal symptoms (measured on the Greene Climacteric scale), and improvement during non-treatment run-in for depression and overall symptoms. Because no difference had been found between active and placebo groups in the RCT (above), it was hypothesised that the same predictors would similarly predict the response in the active treatment group. However, *low* anxiety was significantly associated with improvement in the active treatment group. None of the other variables found to predict the placebo response was relevant to the treatment arm. This finding is discussed with reference to the possibility that 'drug' effects and placebo effects are not necessarily additive, and that the same magnitude of effect in both arms might not necessarily imply activity via the same pathways.

Randomised controlled trials of complementary and alternative medicine (CAM) are often criticised as not reflecting actual clinical practice, which involves individualised prescribing rather than the 'one-size fits all' approach. An alternative experimental design has been proposed and trialled for use in CAM studies by Bensoussan to overcome this problem. However this design was not used in the present study as the sample size required for obtaining sufficient power would have been impractical.

Substantial placebo effects are a nuisance in RCTs. Studies of CAM may be subject to enhanced placebo effects. In addition, the assumption of additivity of treatment and placebo effects, which underlies the practice of placebo-controlled trials, has recently been challenged. Alternative paradigms are discussed.

Chapter One

A Brief History of the Treatment of Menopausal Symptoms

1.0 Introduction

The first chapter of the Literature Review examines the historical treatments of menopausal symptoms in the context of both the recognition of menopause as a syndrome, and the origins of the current practice of phytotherapy in the Anglo-American tradition. The Anglo-American tradition of phytotherapy arose from Samuel Thompson in North America and the interaction of his followers with herbalists in the UK in the nineteenth century. It is a specific form of Western herbal medicine practised in the English-speaking world, and differs from other forms of Western herbalism as practised in Europe.

1.1 A Modern Presentation?

Menopause, the cessation of menstruation, marks the end of the reproductive phase of a woman's life. Although a natural event, the transition from regular cycling to the final menstrual period (FMP) and beyond into postmenopause, is commonly accompanied by physiological and/or psychological symptoms. In addition, the incidence of certain disorders such as breast cancer, noted by Burns in 1814, has long been observed to increase in postmenopausal women.[1]

While not a condition of the modern age, the 'climacteric' or menopause transition had received relatively little attention in the published literature until the 1890's. Factors contributing to this include the shorter life expectancy in earlier times, which meant that many women did not reach menopause. At the beginning of the nineteenth century, average life expectancy was 36.5 years for a female,[2] increasing to 49 for an American female by the early 1900's.[3] Other reasons for menopausal women presenting less frequently to medical practitioners may well have included the cost of healthcare and social taboos regarding discussing such an intimate topic with male doctors.

1.2 Early references to Menopause

Nonetheless, references to menopause or the climacteric appeared in the literature as far back as Aristotle's times (384-322BC) when menstruation was observed to cease at the age of 40.[2,4] In the sixth century, Aetios of Amida wrote that "Menstruation does not cease before the thirty-fifth year, nor does it (usually) continue after the fiftieth year. Meanwhile, menstruation may in the rarest cases last as late as the sixtieth year. Fat women lose their periods very early"(p18).[5]

1.2.1 References to Associated symptoms

It was not until 1816, with the coining of the term *la Ménespausie* by the French physician CPL De Garndanne, that climacteric complaints were recognised as a syndrome with a common cause.[6] However, the association of the cessation of menstruation with other organic and emotional problems had been made in 1777 by Leake in a chapter entitled *Cessation of the Periodical Discharge in the decline of life, and the disorders arising from that critical change of* constitution. He observed that some women at this critical time of life (one meaning of term 'climacteric') were "subject to pain and giddiness of the head, hysteric

disorders, colic pains, and female weakness… The rheumatism,.. pains in the limbs and eruptions on the skin at this time frequently appear (and); also...*cancerous tumors* of the breast or womb. Women are likewise sometimes affected by low spirits and melancholy."(p90)[7]

A reference to flushings and night sweats is also made in this chapter: "Where the patient is delicate and subject to female weakness, night sweats or habitual looseness, with flushings in the face and hectic fever.."(p96).[7]

Flushes and perspiration also appear in the 1857 writings of Edward Tilt,[8] (pp4,70-1,175-9) the British physician who wrote one of the first full-length books on the 'Change of life', in which he lists a wide range of "morbid liabilities at the change of life" (pp70-2) as well as their treatments. He also describes the disorders most likely to be experienced by the different temperaments, with "..women of nervous temperament suffer(ing) ..more than all, particularly during the dodging time" (perimenopause) (p74) whereas "women with a marked biliary temperament are likely to suffer much from …..the various forms of insanity, if the nervous be associated with the bilious temperament.."(p74). Tilt's recommended treatment for reducing sweating was to "Diminish the mass of blood by taking 2 or 3 ounces from the arm at successive months"… since "The relative superabundance of blood is often the cause of the superabundant heat, and therefore of the perspirations…." (p179).[8] To relieve the "irritability of the nervous system… sedative preparations" were recommended (p179).[8]

1.3 Historical Treatments

At this time, menstruation was seen as a form of detoxification of poisons from the woman's blood[9] and the menopause as an accumulation of unexpelled toxins that could lead to the development of diseases such as cancer, gout and rheumatism and other mental and physiological symptoms. Treatment was aimed at either encouraging menstruation by the use of herbal emmenagogues, or removing these unexpelled toxins by other purification methods such as blood-letting, purging, applying leeches to the genitalia or cervix, setons (threads of horsehair or strips of linen inserted beneath the skin to provide drainage) or induction of sweating.[10]

Treatments recommended by Leake in 1777 for curing specific diseases or alleviating psychological symptoms that were associated with the menopause likewise included blood-

letting and purgatives, but also reduced intake of animal food, “a spare and simple diet consisting chiefly of vegetables, fish and spoon meats, and increased exercise”. For the delicate patient with flushings and night-sweats, ass’s milk, jellies, raw eggs and cooling fruits were recommended with “at meals…half a pint of old clear London Porter or a glass of rhenish wine”(p96).[7]

1.4 The Dawn of Hormone Replacement Therapy (HRT)

The ancient form of organotherapy, or glandular therapy, became popularised in the late 19th century with injections of ‘testicular juice’ being used for rejuvenation of men and to combat feminine debility. Ovarian therapy, the administration of crude ovaries, powdered ovaries and powdered ovarian tablets, was used for physiological and surgical menopause and, in 1896, clinical trials conducted in Germany recommended substitution with ovarian therapy as a means of alleviating menopausal symptoms.[11]

The first scientific evidence of the existence of hormones was provided in 1902, and the term ‘hormone’ adopted in 1905 (p391).[12] This replaced the earlier term ‘internal secretion’ coined by the French professor, Claude Bernard, although the *notion* of internal secretions by cells dates back to 1775 with the French physician De Bordeau.[12] (pp390-1) It was not until 1926 that oestrogenic hormones were detected by the chemists Loewe and Lange in human urine and the name ‘estrin’ was given by Parkes and Bellerby to the hormone extracted from the ovary.[13] Oestrone was isolated from the urine of pregnant women in 1929 by Butenandt and oestriol discovered in human pregnancy urine in 1930.[1] The most potent form of oestrogen in the female body, oestradiol, was isolated from sow’s ovaries in 1930 by Marrian, although it was not until 1940 that β-oestradiol was demonstrated in human pregnancy urine and the placenta.[13] In a comprehensive article on the history of HRT, Davis[13] outlines the development, promotion and challenges of hormone therapy.

Progesterone was first isolated in 1934 from corpus luteum extracts injected into rabbits. This ‘corpus luteum hormone’ was called ‘progesterone’ because it maintained gestation. In 1943 it was synthesised by Marker and colleagues from the precursor saponin, diosgenin, from Mexican wild yams (*Dioscorea composita*), that could be converted into synthetic progestin quite easily with a few chemical steps.[14] Diosgenin had previously been isolated from the rhizomes of *D. villosa* (wild yam) in 1940,[15] but because of the relatively low yield

from this species, the richer sources, tubers of *D. composita* and *D. floribunda* from Mexico and Central America came to be used by the pharmaceutical industry.[16]

The first commercially available oestrogen, 'Progynon' was developed in 1928 from animal placentas and subsequently from the urine of pregnant women.[17] A substance resembling estrin was manufactured from the urine of pregnant women and launched in the US in 1933 but faced the problem that limited raw material for production could not meet market demand.[13] The discovery of oestrogens in pregnant mare's urine in the 1930s led to the creation of Premarin (conjugated oestrogens from **Pre**gnant **mar**e's ur**in**e) and its launch onto the market in North America in 1941-2 as the first orally active oestrogen.[3] By 1975 it had become the most-dispensed drug in the United States, reportedly taken by 6 million women.[13] The preference for the combination of oestrogen with cyclic progestin therapy arose from the discovery in the 1970s of the link between unopposed oestrogen and endometrial cancers.[2] Progestins had been reported to protect against this adverse effect by counteracting the proliferative effects of oestrogens.[18]

By the 1960s, menopause had come to be viewed as a deficiency disease, a term used by the New York gynaecologist, Wilson, in his book *Feminine Forever*[19] (financed by Ayerst Laboratories). This, according to Davis and colleagues, further contributed to the success of hormone replacement by promoting its virtues in alleviating the symptoms of menopause as well as helping to maintain youth and sexuality.[13]

1.5 Plant therapies for Menopausal Symptoms

Specific references to the climacteric period do not appear in herbal texts until the mid 1800's. However, as mentioned above, treatments were historically aimed at re-instating menstruation using, among other means, herbal emmenagogues. It seems likely, therefore, that treatment of menopausal symptoms employed herbs indicated for amenorrhoea, or whose virtues included "provoking women's courses", such as *Leonurus cardiaca* (Motherwort) listed in the 17th century Culpeper's Complete Herbal (p239).[20] Its other virtues included treatment of "trembling of the heart, fainting and swooning", a possible reference to the symptoms of dizziness, heart palpitations and feeling faint that are currently associated with menopause, and "making mothers joyful" (p239).[20] Motherwort remains listed in the British Herbal Pharmacopoeia (BHP) of 1983 as indicated for amenorrhoea (p130).[21]

In 1853, Pereira refers to herbal treatment of symptoms associated with the climacteric period with *Lolium temulentum* (Bearded Darnell) which had been successfully used by Fantoni "in the case of a widow who, at the climacteric period, was affected with giddiness, headache, and epistaxis, which had resisted various other remedies."(p81)[22]

Senecio aureus (Life root) is another herb for amenorrhoea and menorrhagia referred to in a 19th century text that retains an indication for menopause-related symptoms in the current BHP (p195).[21] In 1870, Scudder's *Specific medication and Specific medicines* lists it as a "uterine tonic (for) amenorrhoea, dysmenorrhoea (and) menorrhagia"(p217);[23] in 1931, Mrs Grieve refers to its emmenagogue action(p379);[24] the 1983 BHP includes "menopausal neurosis" as one of its indications, and specific indications as "emotional and vascular instability, including the hot flush, associated with the menopause"(p195). It "may be used with *Hypericum* (also indicated for "menopausal neurosis" p115), *Avena* (for '"menopausal neurasthenia" p37), *Viburnum prunifolium* and *Pulsatilla* in menopausal disturbances"(p195).[21]

Cimicifuga racemosa, synonymous with *Actaea racemosa*, (Black cohosh), now widely employed for the treatment of hot flushes, made its way into the *Eclectic Dispensary* in 1852 as a highly regarded treatment for amenorrhoea,[25] having been adopted by the European colonists from the native Americans; the Cherokee and Iroquois people used it for "gynecopathy (diseases peculiar to women) ..and rheumatism".[26]

Other 19th century references to the 'climacteric' include herbs for uterine haemorrhage attending the menopause such as *Viburnum prunifolium* (Black haw)(p2061)[27] and *Hydrastis canadensis* (Golden seal)[27,28] for "climacteric haemorrhage"(p1028) in the 1898 *King's American Dispensatory*. Herbs in the same work that were recommended for other physiological and emotional symptoms attending the climacteric/menopause include

- *Cypripedum pubscens* (Lady's slipper) whose specific indications include "insomnia, nervous irritiability, menstrual irregularities, with despondency; tendency to dementia at climacteric"(p644)
- *Viburnum prunifolium* (Black haw) recommended by Professor Howe for the debility of the second climacteric (p2061)
- *Passiflora incarnata* (Passion flower) for various forms of "neuralgia, many reflex painful conditions incident to ..the menopause" (p1440)

- *Ignatia amara* (Ignatia) for "nervous atony, where the patient is cold, and especially when coldness of the extremities is one of the distressing features of the menopause" (p1043)
- *Cannabis indica* (Indian cannabis) for "migraine, nervous headache, facial, and other neuralgias, … attending the menopause, as well as those depending upon fatigue, (which) are relieved when nervous depression is the most marked symptom" (p425-6).[27]

Winterburn in 1882-3 listed *Sanguinaria canadensis* (Blood root) for "congestion to the head and lungs, especially at the climacteric"(p394);[29] in 1902, Potter recommended *Valeriana officinalis* (Valerian) for "hypochondriasis, especially at the climacteric period" (p147).

1.5.1 Plant remedies specifically for hot flushes

References to plant remedies for hot flushes appear in Kings Dispensatory of 1898: *Aconitum napellus* (Aconite) is recommended for "Disorders of the menopause, with alternate chills and flushes of heat, with rush of blood to the head, cardiac palpitation, dyspnoea, gastric fullness, sense of distension in the bladder, with frequent attempts to pass urine (Locke)" (p105).[27]

They can also be found in early 20th century herbal texts. For example, the Lloyd brothers' drug pamphlets of 1908 refer to *Cactus grandiflorus* (Cactus) for the flushes as well as emotional conditions: "Dr Lydia Ross has made extended and satisfactory observations concerning the influence of *Cactus* in the disorders of women. She especially advises it where there are hot flashes during the climacteric", sometimes given in conjunction or alternation with *Helleborus niger.* "The melancholia, irritability of temper, nervousness, neuralgia, hypersensitiveness, vague fears and fantasies present during the menopause are influenced by *Cactus* and *Pulsatilla*, alone or in conjunction or in alternation.(p11)"[30] This indication is echoed in 1919 by Ellingwood, who lists *Cactus* for "the hot flashes which are so disagreeable during the climacteric. The dose of specific cactus varies from a half minim to two or three minims"[*](p396).[31]

In Ellingwood's Therapeutist of 1908, it is reported that gelsiminine from *Gelsemium sempervirens* (Yellow jasmine) "will check the hot flashes that occur during or after the

[*] 1 mL= 15 minims (BHP); 1 minim = 1 drop (German Commission E)

menopause in nervous, plethoric or relaxed women.. (at a dose of).. 1/250 grain",[32] and elsewhere a single dose of "ten drops of specific gelsemium, (if necessary followed up by) 3 drop doses every hour (is) excellent for the hot flashes that are apt to occur during the menopause".[33]

Many of the above-mentioned remedies are no longer commonly used in phytotherapeutic practice that follows the Anglo-American tradition. Some have simply fallen out of popularity in favour of different herbs/plants based on greater physiological understanding, while others such as *Ignatia, Gelsemium, Aconitum, Cactus* and *Sanguinaria* are now used preferentially in homeopathic potencies, in some cases their use in material doses having been scheduled. Current phytotherapeutic treatment of menopausal symptoms additionally involves herbs with a tradition of less than 3 generations of use,[**] such as *Dioscorea villosa* (Wild yam), *Cimicifuga racemosa* (Black cohosh), *Helonias/Chamaelirium luteum* (False Unicorn root) and *Vitex agnus-castus* (Chaste-tree/berry).

1.5.2 More recent introductions

The earliest references to the treatment of menopausal complaints with *Dioscorea villosa* (Wild yam) appear after the discovery of yams as a prized source of the precursor saponin, diosgenin, for the synthesis of progestins[34] and other sex hormones.[35] The results of rodent studies published in 1992 supported diosgenin's oestrogenic activity and augmentation of oestrogenic acitivity.[36]

Chamaelirium luteum (also known as *Helonias dioica, Helonias lutea*, *Veratrum luteum;* common name False Unicorn root) is referred to by Cook in his 1869 *The Physiomedical Dispensatory*, for menorrhagia with laxity and depression, as well as restoring the menstrual flow, possible allusions to menopausal symptoms:

> "But its most prominent and valuable action is upon the uterine organs; where it scarcely has an equal in ….. menorrhagia, and similar enfeebled conditions. …(I)ts tonic influence is peculiarly efficacious in arresting too excessive menstruation and lochia, when associated with laxity and depression; That these influences over the

[**] 3 generations of use is the minimum requirement for the label '*traditional use'* according to the Therapeutic Goods Administration (TGA) in Australia.

> uterine function are due to the pure tonic action of the agent, is at once seen in the fact that it is a valuable article to restore the menstrual flow when this is absent from sheer inability of the generative organs. It is a valuable ingredient in the compounds called 'Woman's Friend (and) Female Restorative'."(p467-8)[37]

However, its role in the treatment of menopausal symptoms does not appear until the twentieth century. In 1950, Costello and Lynn reported that oestrogenic effects of *Helonias dioica* were found in animal studies.[38]

References to the use of *Vitex agnus-castus* as a sitz bath for "diseases of the uterus" can be found as far back as Hippocrates in 4th century BC and Dioscorides in AD77(p87).[39] One of the great Renaissance herbalists, Gerard, recommended it for inflammation of the uterus and as an emmenagogue.(p6)[40] Modern interest in *Vitex* has been attributed to the scientific research on its effects on the female reproductive system conducted by Dr Gerhard Madaus in Germany in 1930. The benefits of *Vitex* for menopausal complaints were reported in 1972 in a collective report on the clinical experience of five practitioners with Agnolyt® (a patent medicine extracted from dried *Vitex* berries) by Attelmann and colleagues in Germany.[41]

In Madaus' 1938 text, he wrote that *Cimicifuga/Actaea racemosa*, (Black cohosh) was recommended for climacteric symptoms, at a higher dose for somatic symptoms and a lower dose for the neurological symtpoms.[42] Remifemin®, an extract of *Cimicifuga* has been used in Germany for the relief of menopausal symptoms since the mid-1950s.[43] Positive results of uncontrolled studies with Remifemin® in menopausal women were first published in the German literature in 1957 and 1958.[44,45] Reports from clinical trials of its effectiveness in specifically relieving hot flushes are found from 1982 onwards.[46]

Hypericum perforatum (St John's wort) is recommended for "menorrhagia, hysteria, nervous affections with depression" in Kings' Dispensatory of 1898 (p1038)[27], and subsequently in the 1918 Dispensatory of the United States of America for "hysteria, mania,…, hemorrhages.. It formerly enjoyed great reputation for the cure of demoniacs."(p36)[47] In 1938, Dr Gerhard Madaus states that "in the area of the gynecology *Hypericum* (also) makes a substantial contribution…. For menopausal bleeding, it is indicated with *Viscum album*" (p1592).[42] The 1983 British Herbal Pharmacopoeia lists it as specific for

"menopausal neurosis" (p115)[21] and a study published in 1986 reported it to be of comparable efficacy to diazepam for climacteric depression.[48] Reference to its potential benefit in hot flushes appears in the published research literature in the 1990s.[49]

1.6 Summary

While references to menopause, its associated symptoms and treatments have appeared throughout history, it was only recognised as a syndrome with a common cause in 1816, when the term *'la menespausie'* was first coined. It was not until the 1890's that it began to receive more attention in the published literature, possibly due to the increasing life expectancy which meant that more women reached menopausal age. Herbal remedies specifically for hot flushes also appear in the late nineteenth century texts. However, it is possible that menopause-related symptoms were not differentiated from the same symptoms occurring at any other stage of reproductive life, and were treated accordingly with emmenagogues for amenorrhoea or herbs for menorrhagia, depression and hysteria that are listed in earlier works. As these symptoms have increasingly become the focus of published research, another difficulty has been highlighted, namely that of the inconsistency in terminology for the different menopausal phases. This remains a current issue when comparing research findings.

Chapter Two

Definitions of Menopause and its Phases

2.0 Introduction

The second chapter of the Literature Review addresses the issue of the inconsistent definitions used in the literature to describe the various phases of menopause, and defines the terms used in this thesis.

2.1 Lack of Consensus regarding Terminology

Reproductive ageing in women is a dynamic process occurring over a period of time culminating in the menopause, which is a normal "physiological event that occurs universally to all women who reach midlife".[50] Menopause, the permanent cessation of menstruation , is said to have occurred after twelve consecutive months of amenorrhoea not due to pathological or other physiological cause.[51]

While the final menstrual period (FMP) is a single event, it is preceded by a series of clinical changes occurring over period of time that follow recognisable stages, although not all women progress through the stages in a linear fashion and some skip a stage altogether.[52]

Lack of consistency in the use of terminology relating to these menopausal stages, such as pre-menopause, perimenopause, menopause and postmenopause, has dogged the literature, rendering comparisons between studies difficult. To address this problem, a scientific group for the World Health Organisation (WHO) in 1996 recommended the following definitions:

2.2 WHO Definitions

Natural menopause: the permanent cessation of menstruation resulting from loss of ovarian follicular activity. It is recognised as having occurred after 12 consecutive months of amenorrhoea for which there is no other obvious pathological or physiological cause. Menopause occurs with the FMP which is known with certainty only in retrospect a year or more after the event. An adequate biological marker for the event does not exist.

The perimenopause (or *Climacteric*): the period immediately prior to menopause (when the endocrinological, biological and clinical features of approaching menopause commence) and at least the first year after the menopause. The term *climacteric* should be abandoned to avoid confusion.

Menopausal transition: that period of time before the FMP when variability in the menstrual cycle is usually increased.

Pre-menopause: the entire reproductive period up to the FMP.

Induced menopause: the cessation of menstruation following either surgical removal of both ovaries (with or without hysterectomy) *or* iatrogenic ablation of ovarian function (for example, by chemotherapy or radiation).

Simple hysterectomy: where at least one ovary is conserved, defines a distinct group of women in whom ovarian function may persist for a variable period after surgery.

The postmenopause: dates from the FMP, regardless of whether the menopause was induced or spontaneous.

Premature menopause: should be defined as menopause that occurs at an age less than two standard deviations below the mean estimated for the reference population. In practice, the age of 40 years is frequently used as an arbitrary cut-off point, below which menopause is said to be premature.[53]

2.3 Refinements to definitions

Further refinements to these definitions have subsequently been suggested.

2.3.1 The Menopausal Transition

The menopausal transition, from regular cycling to complete cessation, is the part of the perimenopause that ends with the FMP.[54]

2.3.2 Pre-menopause

Pre-menopause is used in differently by different authors and must therefore be clarified on an individual basis. It has been used to refer to

i) the one or two years immediately prior to the FMP,
ii) the whole of the reproductive life preceding menopause or
iii) the part of the reproductive life prior to the onset of changes to the menstrual cycle pattern.[53,54]

2.3.3 The Perimenopause

The perimenopause is further divided into early and late-perimenopause although there is no consensus as to the definitions of these two phases.

2.3.4 Early perimenopause

Entry to the menopause transition, or *early perimenopause*, is often taken as the commencement of cycle irregularity[53,55-58] which precedes the FMP by an average of 3.8 years.[59] It has variously been defined by self-reported changes to the frequency and/or flow, although the reliability of self-reports is questionable given that subjective reports of irregular cycles do not necessarily track with diary entries.[60]

Examples of the variation in definitions are given below: Self-reports of

- change in cycle *frequency* but experiencing menses *in the preceding 3 months* [54]
- changes in *frequency or frequency and flow* (but not changes in flow alone) *over the preceding year* [61,62]
- 3 – 12 months' amenorrhoea [63]
- changes in *flow, flow duration or cycle length.* [64]

The Stages of Reproductive Aging Workshop [STRAW], which attempted to develop a standardised staging system, defined early menopause [Stage –2, where the FMP is the 'anchor'] as regular menstrual cycles, but a variation in length of more than 7 days different from normal. However, elsewhere it defines entry into early menopause as "variation in menstrual cycle length in a woman who has a monotropic follicle stimulating hormone (FSH) rise". [52]

Others have noted that women's *self-rated menopausal status based on their symptoms* may more reliably reflect endocrine changes than definitions based on menstrual cycle characteristics.[62,65,66] For clinical purposes, women's self-report may therefore be considered a pragmatic way of determining entry into the menopause transition[62] whilst for research purposes, Burger suggests that entry to the early perimenopause (MT1 = menopause transition 1) be defined as the *first of 3+ cycles, in any consecutive series of 10, that are outside the normal cycle range* of 23–35 days observed in reproductive-aged women (29± 6). [54,60, 67]

2.3.5 Late perimenopause

For most women experiencing a natural menopause, the time immediately preceding the FMP is characterised by cycles of increasingly variable length.[60] Definitions of *late*

perimenopause appear to be less diverse, with either a 60-day or 90-day inter-menstrual interval predominantly used as the hallmark.

An inter-menstrual interval of 60 days was associated with ovulatory failure [68] and was found to be a more consistent predictor than any other menstrual criteria of the FMP[69] within 2 years in women over the age of 45 years.[70] This is consistent with the STRAW definition of "2 or more skipped cycles and at least one inter-menstrual interval of 60 days or more". [52]

Menses within the preceding 12 months but not the preceding 3 months has commonly been used to determine late perimenopause.[51,64,71] This is consistent with findings from a longitudinal study of 250 Australian-born women over four years, that 3 –11 months of amenorrhoea had a high specificity for predicting the FMP within the next 4 years (99%).[61]

As pointed out by Burger and colleagues,[54] however, a single period of 60 or 90 days of amenorrhoea is not confined to the perimenopause and can occur during other periods of reproductive life and thus, in determining the late-perimenopause in an individual woman, previous menstrual irregularity and the existence of symptoms should also be taken into account.

2.3.6 Menopause

Menopause is generally agreed to have occurred 12 months after the FMP, which can only be determined retrospectively. This convention has arisen because in 90% of Caucasian women, the permanent cessation of menstruation is preceded by amenorrhoea for 12 months.[53] Nonetheless some researchers use 3 months[72] or 6 months of amenorrhoea to denote menopause.[73-77]

2.3.7 Postmenopause

Postmenopause: STRAW[52] suggested the division of *postmenopause* into two stages, namely early and late.

The *early postmenopause* was defined as 5 years since the FMP, during which time further dampening of ovarian hormone function continued to a permanent level, as well as accelerated bone loss. *Early postmenopause* was further divided into *segment a*, the first 12 months after the FMP, and *segment b*, the next 4 years.

Late postmenopause was defined as beginning 5 years after the FMP and ending with death.[60]

Subsequent to these recommendations, however, it is still possible to find studies on early postmenopause defining this as between five to ten years after natural menopause.[78]

2.3.8 Premature menopause

Ovarian failure can occur before the age of 40, resulting in premature menopause.

2.3.9 Artificial menopause

Artificial or *iatrogenic* menopause can follow medical or surgical interventions (See Induced under WHO definitions).

2.4 Definitions used in this thesis

In this thesis, the following definitions are used

- *pre-menopause* — prior to the onset of changes to the menstrual cycle pattern[53,54]
- *early perimenopause* — change in cycle *frequency* but experiencing menses in the preceding 3 months [54]
- *late perimenopause* — after a 90-day inter-menstrual interval until the menopause
- *menopause* — 12 months after the FMP
- *postmenopause* — from the FMP, which is only known retrospectively
- *menopause transition* — the perimenopause up to the FMP[54]
- *climacteric* — this term will not be used other than to quote from other sources, but is taken to be synonymous with the *perimenopause.*

As mentioned above, clarity and consistency in terminology is essential to permit comparisons between studies. Women recruited to the RCT to be reported herein were in the late perimenopause or postmenopause according to the above definitions, and aged between 40 – 60 years.

Chapter Three

Epidemiology

3.0 Introduction

The third chapter of the Literature Review examines the prevalence of menopause and associated morbidity.

3.1 Age at Menopause

Menopause does not occur at a specific chronological age. However, natural menopause generally occurs in women between the ages of 40 and 60 years, and predominantly between 45-55 years.[79] [80] The mean age for women of European origin is said to be 50 years 9 months [81] with median onset of the perimenopause between 45.5 and 47.5 years[81] and average duration of 3.8-5 years.[57,59,64] Most of the data on age at menopause has been derived from Caucasian women in western countries. Limited data exists for women of other racial origins (Appendix 1).

Knowledge of the age at menopause informs clinical practice as well as inclusion criteria for clinical studies. As mentioned previously, in the early 1900's the average life expectancy for an American female was 49 years.[3] As life expectancy has increased, women are not only are reaching menopause in greater numbers, but are living a greater proportion of their lives postmenopausally. Thus the incidence of menopause-related symptoms and postmenopausal health conditions has increased exponentially, necessitating research to establish appropriate treatments according to the needs of the different user groups.

3.2 Prevalence

According to the WHO population projections, the number of women aged 50 years and over is expected to reach 1,200 million by the year 2030, with 47 million new entrants each year.[82] In 1990, 25 million women worldwide reached menopause: this number is expected to double by the late 2020s.[53]

In Australia, at 30 June 2006, there were 2,771,063 women between the ages of 40 and 60 years, constituting 27% of the total female population (or 13.96% of the total population). In the 50-59 age range, there were 1,285,953 Australian women, with almost 1.5 million of the current Australian female population due to reach menopause within the next decade, using age 50 as a proxy for menopause.[83]

Statistics from the North American Menopause Society (NAMS) indicate that most American women can expect to live a third of their lives beyond menopause, given that "a woman who reaches 54 can expect to reach 84.3" years of age.[84]

3.3 Symptom Prevalence

Estimates of menopause-related symptom prevalence are widely divergent. This may be due to a number of methodological factors as well as cross-cultural differences in symptoms experience or symptom reporting. These include[53,85]

- lack of consistency in the symptoms included and instruments used
- differing definitions of menopausal stages
- lack of distinction between natural menopause and surgically-induced menopause
- different sampling methods – from the general community or menopause clinics
- inclusion criteria of age rather than menstrual characteristics as an indicator of menopausal status
- different symptoms, or symptom expression, in different cultural populations
- lack of objective measures to determine whether symptoms are related to menopause or other pathology/life event
- lack of distinction between symptom experience and experience of the symptom as problematic.

The lack of consensus regarding which symptoms can truly be regarded as part of the menopausal syndrome underlies much of the disparity in reports of their prevalence. Hot flushes and night sweats are among the symptoms that are unequivocally linked to endocrine changes associated with menopause, along with breast tenderness and vaginal dryness,[71] while the other symptoms may be accounted for by psychosocial stresses associated with the mid-life years, and the ageing process.[59]

A UK-based cross-sectional survey of 45-54 year old women from the general population reported that 84% had experienced at least one of fifteen menopause-related symptoms listed, with 40% to 45% rating their symptom(s) as problematic.[86] Findings from the USA and Australia of women who reported five or more core symptoms were respectively 35% and 22% for perimenopausal women and 32% and 36% for naturally postmenopausal women, although an underestimation of symptom prevalence in the Australian study may have resulted from the attrition rate related to women who commenced HRT due to bothersome symptoms.[71,87] Fewer than 12% of women reported having none of the symptoms in the preceding two weeks.[62,88] (Details of large cohort studies investigating menopausal symptoms are included in Appendix 2).

Symptom experience varies according to the individual and the stage of the transition.[89] Perimenopausal women have been found to experience the most symptoms, especially somatic symptoms,[62] while vasomotor symptoms of hot flushes and night sweats increase throughout the transition, becoming most prevalent in the late perimenopause and postmenopause.[90,91] The prevalence of individual symptoms is included below with a discussion of their aetiology. This will be prefaced by a general overview of the endocrinological changes associated with menopause and the menopause transition.

Chapter Four

Endocrine Characteristics of the Menopause Transition and Menopause

4.0 Introduction

The fourth chapter of the Literature Review examines the endocrinological changes at menopause. This provides the background for the discussion of symptoms that follows, and for potentially appropriate actions of targeted therapies for these symptoms.

4.1 The Normal Reproductive Cycle

During the reproductive years, normal cyclic ovarian function depends on the coordinated activity of a feedback system involving the hypothalamic-pituitary-ovarian axis (referred to as the HPO axis) with associated structural and functional changes in the target tissues, the ovaries and uterus.[92] The interaction between gonadal steroids and neurotransmitters/neuropeptides modulates ovulatory function via the synthesis and release of gonadotropin-releasing hormone (GnRH) at the hypothalamus.[93]

The endocrinological changes occurring throughout the perimenopause must be viewed in the context of the regulation of the normal menstrual cycle. A detailed outline of this has been provided in Appendix 3. In summary, episodic secretion of GnRH by the hypothalamus regulates the anterior pituitary which produces the gonadotrophins, follicle stimulating hormone (FSH) and luteinising hormone (LH), in a pulsatile manner. These, in turn, regulate ovarian function. The pulsatile frequency and amplitude are dictated by opioidergic signals and predominantly negative feedback by the ovarian steroids, oestradiol (E2) and progesterone,[94] and the FSH regulatory peptides, the inhibins (INHs).[95]

4.2 Changes at the Menopause

4.2.1 Onset of Menopause

Although recently challenged by Johnson and co-workers[96], the classical view is a woman is born with a finite primordial follicle pool. It is estimated that, at birth, the female has around a million oocytes (immature ova) that are arrested in prophase I of meiosis.[52] These are gradually lost throughout her reproductive life through ovulation or atresia, with an exponential acceleration of follicle loss after the age of 40.[94] At the time of menopause itself, few if any follicles remain within the ovary.[51] When follicle numbers reach a critical level of between 100 and 1000,[95] menstrual cycles change from regular to irregular; the changes in ovarian follicle numbers and function are also believed to be responsible for the endocrinological changes of the menopausal transition and menopause.[51]

Although exhaustion of the reservoir of ovarian follicles was once thought to be solely responsible for menopause, current evidence suggests that the age-related neuroendocrine alterations in the brain may be ultimately responsible, as the feedback system of the HPO-pacemakers becomes dysfunctional.[97,98] Changes in GnRH release and reduced responsiveness to it by cells in the anterior pituitary gland that secrete LH and/or FSH are

additionally thought to contribute to the onset of menopause.[99,100] Reduced hypothalamic–pituitary sensitivity to the effects of oestrogens on gonadotropins may also contribute to perimenopausal endocrine physiology.[101,102]

The timing of the final menstrual period (FMP) may be determined by an endometrial rather than a hormonal event,[54,103] as no single endocrinological event has been identified that coincides with the FMP; endocrinologically the late perimenopause and early postmenopause are indistinguishable.

4.2.2 Cycle Changes

Entry to the menopause transition is marked by menstrual irregularities, including changes to the flow, frequency and duration, which are estimated to precede the FMP by an average of 4-5 years.[89,104] Initially, in the late reproductive years, as the length of the follicular phase becomes shorter with the decline in ovarian function, cycle lengths also become shorter.[105,106] After entry to the menopause transition, however, with the onset of irregular cycles, anovulatory cycles begin to occur [54] and both long and short cycles become more common. As the menopause transition progresses, elongated ovulatory and anovulatory cycles become more common and normal cycles occur less frequently.[95] With the approach of the FMP, there is a tendency towards less frequent menses.[53] However, waning fertility is said to begin about 10 years prior to menopause,[107] and may be at least partly due to dysregulation of folliculogenesis, as progress of follicle growth is found to be slower in older reproductive-aged women.[108]

4.2.3 Endocrinological changes

Overall, the major endocrine changes associated with the menopausal transition are declining inhibin-B (INH-B) in the follicular phase of the cycle in conjunction with a rise in serum FSH,[109,110]followed later by declining oestradiol. There is also a decline in luteal phase progesterone levels.[111] [112] Comparing postmenopausal women with women of reproductive age, using the early follicular phase as a reference point, in postmenopausal women, FSH levels are 10-15 fold higher, (reaching their maximum 3-4 years after the FMP),[53] LH approximately 4 fold higher, oestradiol levels 90% lower and Inhibin A & B more than 90% lower.[66]

However, the menopausal transition from regular cycling to final cessation of menstruation is characterised by marked fluctuations in hormone levels.[113] The endocrinology of menopause has not yet been fully elucidated and is complicated by the irregular cycles that characterise the menopause transition; these vacillate between normal length ovulatory and anovulatory cycles, and elongated ovulatory cycles.[114] Conflicting findings from studies could reflect the different types of cycles and cycle lengths, which makes it difficult to determine the stage of the cycle and its ovulatory status.

The Individual Hormones

Follicle-Stimulating Hormone

Elevated early follicular phase FSH* has long been accepted as a hallmark of menopause[107] and a necessary feature of entry to the menopausal transition according to the STRAW definition.[52] Serum FSH levels are somewhat higher in early perimenopause compared with pre-menopause but rise markedly in the late perimenopause and postmenopause.[51]

The rise in early follicular phase FSH has been shown to be associated with a decrease in follicular phase length and menstrual cycle length in regularly cycling, late-reproductive-aged women, which may be due to earlier recruitment of follicles.[115,116,117] However, once irregular cycling has commenced, according to findings from the FREEDOM study,[116] FSH levels vary according to whether cycles are normal or the delayed-response type. Single measurements of FSH levels during the menopause transition will therefore vary according to cycle length and ovulatory status,[54] which may account for the marked variability of circulating FSH and oestradiol reported during the menopause transition by cross-sectional and longitudinal studies.[66,103,113,118,119] In elongated/delayed response cycles and anovulatory cycles, increased levels of FSH are found. With ovarian 'quiescence', when oestradiol is low, FSH levels peak .[116]

FSH is under the dual feedback control of the inhibins as well as the sex steroids, particularly oestradiol. It was previously thought that the rise in FSH throughout the menopausal transition was due to the fall in oestradiol with advancing age and the resultant

* Typical FSH reference ranges

Follicular phase	2.5 – 10.00
Midcycle peak	3.0 – 33.0
Luteal phase	1.5 – 9.0
Postmenopausal	> 23.0

removal of its suppression on FSH secretion. However, more recent studies have shown that oestradiol levels can remain unchanged or even increase throughout the menopausal transition,[66,103,120-128] [116] with elevated levels having been found in regularly cycling women up to the age of 55.[123] Hence another mechanism was implied for the early follicular phase rise in FSH.

The Inhibins

As mentioned previously, FSH is also inhibited by INH-B, which is central to the endocrinological changes at menopause. The inhibins are dimeric protein hormones made up of a common α- subunit, linked to 1 of 2 β-subunits-, β_A or β_b to give inhibins A and B respectively. There is substantial evidence to suggest that INH-A and INH-B are under differential control.[128]

Inhibin B is a product of the antral follicles and is involved in a closed loop feedback system whereby FSH stimulates secretion of oestrogens and INH-B, which together exert negative feedback on FSH. The system is maintained in a continual state of balance in the normal menstrual cycle of normal reproductive-aged women. As ovarian follicle numbers fall and/or the granulosa cells lose their ability to secrete INH-B, there is a resulting decline in INH-B levels, without any initial fall in oestradiol, and an ensuing rise in FSH levels. A clear inverse relationship has been shown between INH-B and FSH in women over the age of 40.[128] The marked fall in INH-B levels in the first half of the cycle has been found to occur in the early perimenopause, defined by self-reported changes in menstrual cycle regularity. At this time, oestradiol and INH-A levels are no different from pre-menopausal women.[129] This further supports the hypothesis that the major fall in INH-B is responsible for allowing FSH to rise while maintaining early follicular-phase oestradiol levels relatively intact. Burger and colleagues concluded that the declining concentration of INH-B levels in the first half of the cycle[129] was the earliest endocrine change marking entry into the menopausal transition.[128]

Oestrogens

The hypothesis that the fall in inhibin levels (especially INH-B) leads to the maintenance of oestrogen levels until just prior to the FMP is in contradistinction to the previously-held belief that the perimenopause is characterised by a gradual decline in oestrogen levels with rising FSH. [94] However, there is mounting evidence from studies to show that oestradiol levels

either increase or are well maintained, even in irregularly cycling women,[66,103,120-128] until about 2 years prior to the FMP,[130] although circulating FSH levels begin to rise significantly a year or two prior to the FMP.[127,131] Miro[115,116] proposed that the elevated levels of oestradiol may be due to the higher concentrations of FSH and resultant increased driving of the dominant follicle, which may lead to accelerated follicle development and occasions of multiple follicles developing at once. This suggests that, in some cycles at least, the ovary responds to the increased FSH levels by increasing oestradiol secretion.

Some studies have demonstrated marked variability with periods of hyperestrogenism consistent with erratic follicular development and multiple follicles developing at once, with elevated FSH.[103,111] Periods of unopposed oestrogen are consistent with the high incidence of dysfunctional uterine bleeding occurring during perimenopause, generally in anovulatory cycles.[54]

However conflicting results have been obtained from different studies observing oestradiol levels during the menopausal transition, including elevated levels, decreased levels or no change. In a subset of women with regular, normal length ovulatory cycles, there were no changes found in ovulatory oestradiol or progesterone levels during the entire 4–9 year period prior to the FMP.[114] It has been proposed that these conflicting findings may reflect data derived from different types of cycles or pooling of data from different types of cycles (normal length ovulatory cycles, delayed ovulatory and anovulatory cycles).[95]

Lower than normal levels of oestradiol have been found in late-perimenopausal women who had experienced 3 months' amenorrhoea[131] and in late-perimenopausal women during anovulatory cycles,[132] and in cycles with an elongated 'lag period' between the menstrual phase and onset of follicular phase.[115] As pointed out previously, these types of cycles become more common with the progression of the menopause transition. Weiss and co-workers observed 3 distinct hormonal patterns associated with anovulatory cycles: i) normal oestrogen increase with subsequent LH surge but no appreciable progesterone production, suggesting disruption to corpus luteum dysfunction; ii) oestrogen increase but not LH surge, implying failed CNS response to the oestrogen; and iii) tonically elevated LH and FSH but no increase in osteogen, which resembles the postmenopausal pattern.[102]

Ovarian refractoriness to FSH underlies elongated menstrual cycles[115] and is thought to be due to the drop to a critical number of follicles, fewer gonadotrophin receptors in the ovary

and a reduced ability to respond. This leads to the changes in menstrual flow and frequency of menstrual cycles[115] such as prolonged ovulatory cycles, resulting from delayed oestradiol secretion. In this type of cycle, dominant follicle development can lead to higher than normal levels of oestradiol but lower luteal phase levels of progesterone.[115,133] Miro and colleagues found a strong parallelism between oestrogen take-off, defined as day 1 of the sustained rise in oestradiol, and cycle length variability,[115] consistent with refractoriness of the ovary to FSH stimulation.

Oestradiol is the major secreted ovarian oestrogen in reproductive aged women. However, after menopause ovarian oestrogen secretion ceases largely or entirely and oestrone derived from peripheral conversion of androstenedione is the major form of oestrogen.[134]

Progesterone

Progesterone levels ultimately decline as the FMP approaches. Lower luteal phase progesterone levels would be expected to occur in the absence of the development of a corpus luteum.[111] With the onset of irregular cycles, decreased progesterone levels[135] and, in a randomly collected sample, fewer concentrations indicative of the normal post-ovulatory state have been observed.[130,136] Lower concentrations were especially noted in the delayed-response (D-type) cycles.[116] Some studies have found reduced excretion of progesterone's major urinary metabolite, pregnanediol glucuronide* (PdG),[103,111,119,127] although this has not been reported consistently.[114,121,137] Low progesterone is clearly associated with failure of the secretory phase; continued proliferation of the endometrium in anovulatory cycles leads to menorrhagia (heavy periods).[95]

Androgens

The major circulating androgen in women is testosterone and the major pre-androgen, dehydroepiandrosterone sulphate (DHEAS); these are produced by the ovaries and adrenals. Menopause does not appear to significantly affect the rate of decline in testosterone or DHEAS levels.[110,138] Testosterone levels in women fall by approximately 50% between the ages of 25 and 45, while DHEAS levels fall progressively with age. In the

*Cycles are classified as ovulatory or anovulatory according to blood progesterone or urinary PdG.
Blood parameters: ≥16-20 nmol per cycle = ovulatory
5-16 = luteal insufficiency
<5 = anovulatory

postmenopausal woman, the stroma of ovaries and the adrenal glands continue to produce androstenedione, which is used in peripheral aromatisation to oestrone in adipose tissue. Adrenal production of testosterone also continues,[139] and it has recently been shown that the postmenopausal ovary also makes testosterone, and continues to do so for as long as 10 years beyond the menopause.[140]

Luteinising Hormone

LH levels have been found to increase progressively with the increased frequency of delayed-response (elongated) cycles during the menopausal transition, but not to differ in normal cycles throughout the transition from those seen in regularly cycling women.[116] It appears that LH is entirely GnRH dependent.[94] LH pulses, which correspond to the pulsatile secretion of GnRH, occur approximately every 60 minutes postmenopause in the absence of negative feedback.

Anti-Müllerian hormone

Anti-Müllerian hormone (AMH), also called Müllerian inhibiting factor , Müllerian inhibiting hormone , and Müllerian inhibiting substance, has recently received attention as an indicator of reproductive ageing. AMH is a dimeric glycoprotein expressed by granulosa cells of the ovary during the reproductive years that controls the formation of primary follicles by inhibiting excessive follicular recruitment by FSH. It therefore has a role in folliculogenesis.[141] AMH is not influenced by the gonadotropic status and reflects only the follicle population.[142] Recent studies have found that AMH levels are related to onset of menopause,[143] with reductions in AMH and INH-B significantly associated with cycle irregularity, and supporting its role as a predictor of the menopausal transition, alone or in combination with INH-B.[144]

4.3 Diagnosing Menopausal stage

Endocrinologically, the late perimenopause and the early postmenopause are indistinguishable. Oestradiol levels have fallen by approximately 50% compared with earlier follicular phase levels at the time of the FMP, and FSH levels have risen to about 50% of the ultimate peak they will reach postmenopausally.[131]

As mentioned above, FSH measurements are dependent on cycle phase, cycle length and ovulatory status. As the menopause transition progresses there is an increased incidence of

elongated ovulatory cycles and anovulatory cycles while 'normal' cycles occur less often.[114,116] In view of the inconsistency between women and from cycle to cycle during the menopause transition, as well as the difficulty of determining early follicular phase in amenorrhoeic or irregularly cycling women, "single-time FSH measurements have a limited capacity to reliably predict reproductive status".[66]

The Melbourne Women's Midlife Health Project (MWMHP) found that experiencing symptoms tracks closely with hormone profiles and cycle features,[90] suggesting that symptom diaries are therefore more useful than isolated endocrine profiles in ascertaining the stage of the menopause transition.[94]

4.4 Summary

The commencement of irregular cycling marks entry to the menopause transition, after which anovulatory cycles become progressively more common. The declining antral follicle count underlies the endocrinological changes associated with reproductive ageing.[145] There is also evidence of reduced hypothalamic–pituitary sensitivity to oestrogen feedback in perimenopausal women. It is hypothesised that the fall in inhibin levels (mainly INH B), resulting from the declining antral follicle count, allows the rise in FSH, which leads to accelerated follicle development and occasions of multiple follicles developing. This causes the fluctuating or increased oestradiol secretion in perimenopausal women, until the late transition, when oestradiol levels fall markedly. After menopause, the major oestrogen is oestrone derived from peripheral conversion of androstenedione secreted by the adrenals. Progesterone levels decline with the increasing frequency of anovulatory cycles; the decline in androgen levels is associated with age but not menopause per se. The endocrine changes appear to vary between and within individuals, and substantial changes have been observed from one cycle to the next. In addition, the high degree of overlap with FSH levels in regularly cycling women makes single FSH measurements unreliable indictors of reproductive status;[146] this may be more reliably ascertained through symptom diaries.

The above has methodological implications for the study to be described in this thesis. It is also of relevance to the ensuing discussion of symptoms and their aetiology, as well as to the phytotherapeutic approach to the treatment of menopausal symptoms which will be elaborated in a later section.

Chapter Five

Menopausal Symptoms in General

5.0 Introduction

The fifth chapter of the Literature Review explores the symptoms commonly associated with menopause, their prevalence and underlying aetiology. This has relevance to the hypothesised effects of the phytomedicines tested in the RCT described in this thesis. Factors that could potentially impact on symptom experience that may be of relevance to the design and interpretation of data from the RCT are also examined.

5.1 Symptom Experience

The majority of women in the age group 45-54 years experience menopausal symptoms, although not all experience these as troublesome.[86] While endocrine changes are permanent, menopausal symptoms such as hot flushes and mood changes usually spontaneously resolve with time.[147,148] However, some symptoms, such as genital atrophy, may remain the same or worsen.[81] Quantification of menopausal symptoms is rendered difficult by their subjective nature. The high degree of overlap between the physical and psychological symptoms of menopausal oestrogen withdrawal and other common midlife conditions, such as hypothyroidism and major depression, means that symptom presentation alone is not diagnostic.[149]

Symptoms typically associated with the menopausal transition can be categorised as follows, according to standard medical texts (Table 5.1).[150,151]

TABLE 5.1 MENOPAUSE-RELATED SYMPTOMS

Symptom cluster	*Typical symptoms*
Vasomotor symptoms	hot flushes, night sweats, cold sweats/perspiration, dizziness, migraine, palpitations
Psychological and emotional symptoms	irritability, memory and concentration difficulties, depression, anxiety and nervousness, mood swings, fatigue, sleep disturbances, tearfulness, unloved feelings, loss of self-confidence, decreased libido
Changes to the reproductive cycle	menorrhagia, dysfunctional uterine bleeding, shorter and longer cycle lengths, breast tenderness, worsening premenstrual syndrome
Urogenital symptoms	atrophic vaginitis , vaginal dryness, dyspareunia, urinary (stress) incontinence, dysuria, urinary tract infections (UTIs) or other bladder dysfunction
Musculoskeletal	arthralgia, myalgia (diffuse muscle and joint pains)
Skin and other tissue changes	Mucosal thinning and drying that leads to cyclic acne, pruritis, dry eyes, sinusitis,[149] formication, dry skin, breast glandular tissue atrophy, new facial hair
Other somatic symptoms	weakness/fatigue, paraesthenia, headaches, worsening allergies[149] weight gain[152]

Menopause-rating scales such as the Kupperman Index (KI) and the Greene Climacteric Scale contain different sub-divisions such as psychological, somatic, vasomotor and sexual.[153]

5.2 Cross cultural data

It has long been observed that symptomatology varies markedly among ethnic groups, cultures, socioeconomic groups, geographic locations and climates. [52,154] The Study of Women's Health Across the Nation (SWAN) found that symptom prevalence was lower in the Japanese and Chinese populations in the US than in Caucasians, especially for the symptoms of hot flushes, vaginal dryness, sleep difficulty and urine leakage.[155] In Japanese

women, the most prevalent symptoms associated with the climacteric (‘konenki') have been found to be shoulder stiffness, memory loss, and stress.[156]

5.3 Evidence for Symptom Specificity

There is lack of consensus regarding which of the observed symptoms experienced by middle-aged women can be attributed to declining hormone levels at menopause as distinct from the process of ageing, as “the symptoms do not track closely with the menstrual cycle or endocrine changes during the menopausal transition.”[52] As mentioned previously, some research suggests only the flushing and night sweats, breast tenderness and vaginal dryness are definitely related to the endocrine changes of menopause.[157] [71] There is some evidence that the other complaints are equally common in men aged between 45 and 54 years,[158] although Dennerstein and co-workers[62] found that two groups of symptoms were significantly related to changes in menstrual status: vasomotor symptoms which increased through the menopause transition; and general somatic symptoms which were more frequent in the perimenopause. The principal way of establishing a relationship of symptoms to hypoestrogenemia is observation of any amelioration that occurs after oestrogen-based hormonal replacement. However, it has been suggested that the beneficial effect on oestrogen on many of the menopause-related symptoms found to improve in conjunction with hot flushes may be attributable to a ‘domino effect’ that is initiated by the primary relief of the hot flushes.[159]

5.4 Causes of systemic effects

Oestrogen, progesterone and androgen receptors are found in tissues throughout the body, including the brain.[160] Oestrogen receptors are found in uterus, liver, kidneys and heart (ERα), ovaries, lungs, gastrointestinal tract, bladder, hematopoetic and CNS (ERβ), with both types expressed in the breast, thyroid, adrenal, bone and some parts of the brain.[161] Additionally they occur in skin tissue, the vagina, uterus,[160] cervix, urethra, joints, muscles of the pelvic floor[162] and many other tissues, which may account for the distribution of systemic symptoms.

In the brain, oestrogen receptors are very dense in regions of the limbic system and hypothalamus, which serves as a bridge between the nervous system and endocrine systems. Oestrogens modify synthesis, release and metabolism of many neurotransmitters such as noradrenaline, dopamine, acetylcholine, serotonin and melatonin, and neuropeptides including β-endorphin, which modulate the activity of hypothalamic centres and the limbic

system.[93] The decline in sex steroids, particularly oestrogen, results in altered function of the hypothalamus and limbic system, with associated changes in thermoregulation, satiety/appetite, metabolic rate, sleep patterns, vasomotor stability,[163] blood pressure, sexuality, locomotor activity, immune response, memory and cognitive function[164] and, via the limbic system, the regulation of mood tone and psychological well-being[165,166]

Age-related decreases are observed in melatonin levels, as well as the time during which melatonin remains elevated at night.[167] Studies in women during the perimenopause reveal that the decline in melatonin precedes the FSH increase, with a decline of 41% in the age group 40 to 44 years and a further decline of 35% between ages 50-54 years and 55 to 59 years.[168] Whether this decline in melatonin secretion contributes to the development of menopause or its symptoms has not been established.[169] An association between the quality of sleep and the amount of melatonin secreted has been noted, especially in the elderly.[170]

Age-related declines are also found to occur in concentrations of noradrenaline in the hypothalamus[171] (which influences the secretion of LH, growth hormone (GH) and thyroid-stimulating hormone[172]), total brain serotonin levels,[173] β-endorphin neurones in the arcuate nucleus,[174] and γ-aminobutyric acid (GABA).[175] Age related decreases in the dopamine receptor D-2 subtype,[176] and increases in brain monamine oxidase (MAO)-B also occur.[177] In women, levels of prolactin, a precursor of noradrenaline, begin to fall around menopause.[178] This will be referred to in the subsequent sections on the specific symptoms under investigation in the studies to be described herein.

5.5 Symptoms according to Stage of the Transition

Symptom experience varies according to the individual, the stage of the transition and whether menopause is natural or surgically-induced. Hysterectomised women suffer more severe symptoms overall than women who reach menopause naturally.[50] It is during the perimenopause that most symptoms are reported.[62,90] These symptoms can be due to oestrogen excess (breast tenderness, menorrhagia, migraine, nausea, shorter cycle length)[101,111,179] or deficiency (vasomotor symptoms)[79,90] and often fluctuate, reflecting the underlying hormonal instability during these years.[179] Dysfunctional uterine bleeding is maximal during the menopausal transition due to persistent unopposed oestrogen.[128,180] Rates of psychological distress are reported to be highest during the perimenopause.[88,181-185] There is also a marked increase in disturbances of sexuality.[62,186] The other most frequently

occurring symptoms during this phase include forgetfulness, lack of energy, irritability, and weight gain, while those causing most distress include weight gain, heavy bleeding, poor concentration, leaking of urine, and feeling as though life were not worth living.[152] The incidence of hot flushes increases throughout the transition[62] with a peak generally at the time of the menopause[88] and in postmenopausal women.[187] The most common symptoms reported around the time of the menopause are musculoskeletal aches and pains, vasomotor symptoms, sleeping difficulties and fatigue, forgetfulness and nervous tension/irritability.[62,86,155,188] The incidence of symptoms in the late-perimenopause is similar to that in early postmenopausal women.[62,89]

The most prevalent symptoms are not necessarily regarded as the most problematic. A cross-sectional survey of 8,000, aged 45-54 women in Scotland found Irritability to be reported by the highest percentage, followed by sleeping problems, concentration/memory difficulties, anxiety, hot flushes, night sweats and depression. However, in terms of being the most problematic, sleep problems were rated highest, followed by concentration/memory difficulties, anxiety, irritability, night sweats, and depression.[86] Findings from this and other studies are also included in Table 5.2 below.[147]

TABLE 5.2 SYMPTOM PREVALENCE IN PERIMENOPAUSAL AND POSTMENOPAUSAL WOMEN (VASOMOTOR AND PSYCHOLOGICAL SYMPTOMS)

The Study of Women's Health Across the Cross-sectional Survey of women in US aged 40-55 years Prevalence of Symptoms in previous 2 weeks. Late-peri-/ postmenopausal[155]	*Scottish Cross-sectional Survey Prevalence of Symptoms in preceding 6 months In women aged 45-54 (% Perceiving symptom as Problematic)*[86]	*Melbourne Cross-sectional Survey Prevalence of Symptoms in previous 2 weeks in Peri-/postmenopausal women*[62]	*US (Californian) Recall Study Postmenopausal women (aged 50-89 years)*[147]
	irritability 72% (25%)		irritability 25%
difficult sleep 43.9%40.4%	sleep problems 66% (33%)	sleeping difficulties 35.35% /41.41%	insomnia 28%
forgetfulness 44.8%/42.0%	memory /concentration problems 64% (30%)	concentration difficulties 27.5% /18.31%	
	anxiety 58% (26%)	nervous tension 40.62% /40%	anxiety about looking older 11%
hot flushes 56.8%/48.8%	hot flushes 56% (22%)	hot flushes 31.51%/39.44%	hot flushes 70%
	night sweats 55%(24%)		night sweats 35%
	depression 51% (22%)	feeling down or sad-hearted 32.97%/28.73%	depression 20%
stiff/sore 54.8%/58.4%	aching/painful joints 67% (29%)	aches or stiff joints 51.55%/50.42%	

A brief outline of the symptom clusters and their aetiology will be given here. The symptoms of interest in the clinical trial reported on in this thesis, namely vasomotor and psychological symptoms will be dealt with in greater detail in the following sections.

5.6 Aetiology of Individual Symptoms/Symptom Clusters

5.6.1 Vasomotor Symptoms

Collectively, hot flushes (known as flashes in the US) and nights sweats are termed 'vasomotor symptoms' because they occur secondary to vasomotor instability.[189] However the term 'vasomotor symptoms' is sometimes used more broadly to include light-headedness/dizziness, migraine and palpitations.[190] The term 'hot flushes' will be used in this thesis to refer to hot flushes and night sweats, the latter being hot flushes with perspiration that occur during sleep.[191] The impact of hot flushes on a woman's sense of well-being and quality of life can be significant,[188] sometimes causing extreme anxiety and leading to social isolation.[152] Night sweats are thought to be at least partly responsible for disrupted sleep patterns associated with menopause[192,193] which can in turn contribute to other associated symptoms such as mood changes,[194,195] concentration difficulties and fatigue.[195]

An association between the experience of hot flushes and the overall decline of oestrogens is undisputed, and is supported by the efficacy of oestrogen administration in eliminating them.[196] However, oestrogen reduction also occurs in asymptomatic women. A complex interaction between oestrogens, progesterone and neurotransmitters may underlie the thermoregulatory changes involved in hot flushes.[197,198] This is described in more detail in the following chapter.

5.6.2 Sleep Disturbances

The factors that influence sleep disturbances have not yet been fully elucidated. While a causal connection between nocturnal hot flushes (night sweats) and sleep disturbances is not universally supported, data do exist from studies using both subjective and objective sleep assessment techniques showing an association between vasomotor symptoms and poor sleep quality, reduced sleep efficiency, longer rapid eye movement latencies and mid-sleep waking episodes and arousals.[199-202] However, not all of the waking episodes are associated with flushing,[199,201] suggesting this may not be the only factor responsible.

Conversely, not all of the nocturnal flushes are found to be associated with waking episodes or arousals. Freedman and Roehrs[202] found that in the first half of the night, hot flushes were associated with more arousals and awakenings than in the second half of the night. They attributed this to the predominance of rapid eye movement (REM) sleep in the second half of the night, suppressing thermoregulation and possibly thereby suppressing the disruptive effects of hot flushes on sleep. This may account for the finding of one study that acute sleep problems were associated with reported, but not with physiologically monitored sleep hot flushes.[203] Waking has been observed to precede nocturnal flushes and occur prior to any change in skin temperature, implying a possible central phenomenon.[200] However Freedman and Roehrs, in another study, observed arousals and awakenings to precede or follow hot flushes at approximately the same rate.[204]

An association between sleep disruption in perimenopausal women and altered mood has also been observed, with positive correlations between frequent sleep disruptions and anxiety; time spent active and negative mood; and frequency and duration of arousals with confusion.[195] This is supported by the observed age-related decrease in total brain serotonin, one of the main regulators of circadian sleep-wake cycles. [205] Deficiency of serotonin is associated with many psychological disorders as well as sleep disorders.[205] It has been suggested that anxiety and depression may be important mediators of menopausal sleep disturbances,[206] which can impact adversely on quality of life. As pointed out by Utian, inadequate and unrefreshing sleep can eventually lead to chronic sleep deficits, significantly impaired alertness and mental acuity, forgetfulness, and decreased work productivity. In the case of drenching night sweats that necessitate a change of clothes and/or bedding, the disturbance to the bed partner's sleep can also affect familial relationships.[207]

Other proposed causes of sleep disturbances in the menopause transition are nocturia,[208] hormonal changes, age-related decrease in slow-wave sleep and GH secretion,[209,210] depression,[211] stress, nocturnal myoclonus,[212] respiratory abnormalities such as sleep apnoea and hypopnoea, which are reported to increase during this time.[202,213]

5.6.3 Psychological and Emotional Symptoms

Psychological and emotional symptoms associated with menopause include irritability, inability to concentrate, depression, anxiety and nervousness, mood swings, poor short-term

memory, fatigue, tearfulness, unloved feelings, loss of self-confidence and sometimes sleep disturbances. [150,151] Controversies exist regarding whether there is an actual rise of mood symptoms during the menopause transition compared with the premenopause, as well as their aetiology. The oestrogen-withdrawal perspective holds that symptoms such as depressed mood are triggered by endocrine changes, in contrast to social circumstances frequently encountered by women aged 40 – 60 years. A biopsychosocial aetiological model has been proposed which suggests that "underlying endocrinological changes trigger emotional complaints in women who are vulnerable by virtue of developmental factors, personality, psychiatric history, known vulnerability to hormonally triggered mood changes, or current social problems."[214]

While rates of psychological distress are commonly reported to be highest during perimenopausal years,[88,181-185] some studies report that its prevalence does not differ significantly from males of the same age.[215] Methodological differences that render comparison between studies difficult include the criteria for determining reproductive status as well as mood disorders, scales for detecting and measuring depression and anxiety, and the failure to distinguish between mood symptoms and mood syndromes. The inconsistency between studies regarding inclusion of current HT users is an additional limitation. This will be discussed further in the section on psychological symptoms.

5.6.4 Memory and concentration

Forgetfulness was reported by 44.8% of late-perimenopausal and 42% of postmenopausal women in the SWAN, compared with 31% of premenopausal women. A relationship between cognitive impairment and the endocrine changes of menopause is disputed,[216,217] with evidence for the connection being mainly derived from observations following oophorectomy, where there is an abrupt decline in oestrogen levels.[218] However, spontaneous menopause appears to cause little change,[219] and it is suggested that any cognitive decline may be more related to ageing.[220] Administration of oestrogens with or without progesterone (conjugated equine oestrogens with or without medroxyprogesterone acetate) in The Women's Health Initiative Memory Study (WHIMS), did not protect against dementia or cognitive decline, but substantially increased the risk.[217] However, this finding may be explained by the characteristics of the study sample, which included postmenopausal women aged 65 years and older, who performed poorly at baseline, whereas the best evidence for a favourable effect on cognitive function is from studies on

women under 65 years of age.[221] Recent evidence from human and animal models suggests a critical time for the initiation of oestrogen in the context of memory preservation, namely at the time of menopause, or soon after ovariectomy;[218] administration a considerable time after ovariectomy had little or no beneficial effect on cognition.[222,223]

5.6.5 Changes to the Reproductive cycle

During the menopause transition changes to the menstrual flow, frequency and duration often occur, but these changes are highly variable.[52,89] One study found that 10% of women stop menstruating abruptly, while the majority experience months or years of irregular bleeding and variable cycle length before menses cease. [89] In the early perimenopause, both long and short cycles become more common, but as women approach the FMP, there is a tendency towards less frequent menses.[53] However, waning fertility is said to begin about 10 years prior to menopause.[160]

Menorrhagia can occur during ovulatory cycles due to increased oestrogen levels during the follicular and luteal phases.[95]

Although there is a peak of premenstrual syndrome (PMS) in women in their late 30's, anecdotal reports suggest that perimenopausal women also experience an aggravation of PMS-like symptoms, or at least appear to tolerate them less well.[224] It has even been proposed that mood symptoms and breast discomfort during the menopause-transition are more likely to be related to menstrual cycles than menopause, given that they improve after cessation of menstruation.[225]

5.6.6 Urogenital/Vulvovaginal symptoms

After the menopause, the vaginal mucosa atrophies, vaginal flora changes and the vaginal pH becomes less acidic.[226] [227] Vaginal atrophy can be asymptomatic or associated with pain, burning, dryness, discharge, dyspareunia and/or vaginal bleeding.[228] Changes to the thickness and secretions of the vagina are accepted as being due to declining oestrogen levels,[229-231] and can be improved by oestrogen administration.[232] However, findings are conflicting regarding incontinence and recurrent cystitis.[233,234] The beneficial effects of oestrogen in UTIs may be due to decreasing colonisation of bacterial pathogens. [232] Symptoms of incontinence, while temporally associated with menopause, may be related more to ageing and obstetric history than directly to menopause or hypo-oestrogenism.[162]

Estimates of the prevalence of vaginal dryness range from 4% to 47% depending on the stage of the transition and study population.[71,155,235,236] In perimenopause and postmenopause, data from the UK and Australian studies range between 21% to 47%.[86,71] Among sexually active postmenopausal 40 to 65 year-olds in the USA, the prevalence of vulvovaginal atrophy (vaginal dryness, itching, irritation; pain on urination; or pain or bleeding on intercourse) was 57% and female sexual dysfunction 55%.[237]

Female sexual dysfunction (the decline in sexual interest, arousal, capacity for orgasm and frequency of sexual activity) has been associated with the natural menopause transition,[50] although it is more problematic in surgical menopause with removal of the ovaries.[238] This decline may be partly attributable to ageing.[239,240] However, depression and vaginal dryness were found to be reliable predictors of low sexual desire,[241] and an association with menopausal status and oestrogen, but not testosterone levels, has been observed in some studies. [238,242] Androgen administration, in conjunction with oestrogens and progestins, or tibolone® (which has oestrogenic, progestogenic and androgenic effects) can significantly improve sexual performance, including sexual desire, sexual arousal and satisfaction[243,244] Hormonal determinants, however, have been found to be less important than psychosocial factors[238] such as previous sexual function, loss or gain of a sexual partner and feelings towards the partner.[245] Other factors affecting wellbeing such as lifestyle factors, stress, daily hassles, vasomotor symptoms, sleep, self-rated health[241] and neuroticism[246] also contribute. However, not all women experiencing low sexual function are distressed about it, and this distress declines with age.[238]

5.6.7 Musculoskeletal symptoms

Osteoarthritis and joint pain increase in prevalence from premenopausal to postmenopausal women with hormonal change implicated in their aetiology.[247,248,249] Aches and stiff joints were the most frequently reported symptom in studies of mid-life (51.7%)[62], perimenopausal (55%) and postmenopausal (58%) women.[155] Fibromyalgia commonly develops or worsens markedly during the perimenopausal period.[250,251] The experience of stiff or aching joints is not necessarily indicative of radiological osteoarthritis,[252] although this is more prevalent, severe and generalised in women than in men after the age of 50,[253] and may be linked with menopausal changes.[254] (Oestrogens may have a role in triggering the initial changes in proteoglycan in cartilage, either directly or indirectly via cytokines;[255,256] lower endogenous oestradiol levels impact on arachidonic acid metabolism, altered receptor binding and

structural proteins, such as collagens.[257]) Although HT reduces the disease progression and may prevent incident disease, evidence from RCTs is lacking.

The role of declining oestrogen production in musculoskeletal pain during the menopause transition is supported by the existence of an arthralgia syndrome both in patients taking aromatase inhibitors,[258] and at HT discontinuation,[253] as well as experimental evidence that acute aromatase inhibition affects nociception.[259] Several studies support a link between pain and oestrogens[260] and have examined their influence on cytokine production,[261] increased nitric oxide production by endothelial cells[262] and central effects via enkephalins that inhibit neurons involved in nociception.[263]

5.6.8 Skin and other tissue changes

Dry skin complaints are common among menopausal women and in severe cases can lead to intense pruritis, compromised barrier function, and secondary microbial invasion or inflammation[264] Atrophy of the dermis after the menopause is due to a decrease in the dermal skin collagen content,[265] which occurs much more rapidly in the initial postmenopausal years than in the later years with 30% of skin collagen being lost in the first 5 years after menopause.[266] HT can help to increase collagen content, dermal thickness and elasticity, possibly improve cutaneous injury repair,[267] dampen the pace of development of fine wrinkles, increase skin strength and perhaps prevent skin breakdown in elderly postmenopausal women.[264] A summary of findings relating to the effect of HT on dry skin can be found in the review by Katz and Prystowsky.[264]

High collagen content, together with hydrophilic glycosaminoglycans, is also present in intervertebral discs.[265] Changes in the collagen content occur with the ageing process and may have significant effects on the shape and biomechanics of the intervertebral disc, which could impact on vertebral body deformity and fracture. Studies on intervertebral disc height have found that hormone treated postmenopausal women have significantly greater disc heights than untreated counterparts.[265,268]

5.6.9 Weight changes and Fat Distribution

Weight gain, which can be quite marked in midlife women, has been found to be associated with ageing, rather than menopause.[269] However body fat redistribution to the abdominal

region, which occurs in association with menopause, may be attributable to the effects of oestrogen deficiency on adipocyte metabolism and fat partitioning,[270] which refers to the distribution of adipose tissue between the central and peripheral regions. Studies have found oestrogen to prevent this menopause-related increase in central fat deposition[271] and HT to be associated with less central (but not total) body fat. [272]

5.7 Predictors of Symptoms

The Melbourne Women's Midlife Health Project (MWMHP) found that predictors of experiencing fewer symptoms were increasing years of education, better self-rated health, the use of fewer non-prescription medications, the absence of chronic health conditions , a low level of interpersonal stress, the absence of premenstrual complaints, not currently smoking, exercise at least once a week, and positive attitudes to ageing and menopause,[62] consistent with an earlier study relating symptom experience with a negative attitude towards menopause.[273]

5.8 Prevalence of Morbidity in Postmenopausal Women

Rates of cardiovascular disease and stroke are four times greater in postmenopausal women compared to premenopausal women, diabetes risk and the rates of osteoporosis, immune disorders and Alzheimer's disease also increase dramatically after menopause.[149] Despite the temporal association with postmenopause, however, the relative contributions of ageing and menopause-related endocrine changes to this increased morbidity have yet to be determined. For a more detailed outline of post menopausal morbidity, refer to Appendix 4.

The symptoms constituting the main outcomes in the studies reported herein will now be considered in more depth. These are the vasomotor symptoms of hot flushes and night sweats, the mood symptoms and PMS-like symptoms.

Chapter Six

Hot Flushes/Flashes and Night Sweats

6.0 Introduction

This chapter of the Literature Review explores the aetiology of the vasomotor symptoms of hot flushes and nights sweats, symptoms under investigation in the RCT described within this thesis.

Hot flushes (referring to both hot flushes and night sweats) are considered to be the hallmark of menopause. Also referred to as 'hot flashes', they are defined as recurrent, transient episodes of a sensation of intense heat that may be accompanied by perspiration.[274] Of the symptoms typically associated with menopause, hot flushes are the most common reason cited for seeking treatment.[275]

6.1 Prevalence of Hot Flushes

The prevalence of hot flushes in women who are spontaneously menopausal varies according to country and ethnic origin. Worldwide, it is estimated that they are experienced by 50%-85% of women over 45 years.[276] In Australia, they are experienced by 83% of women experiencing the menopause transition,[90] 62% of postmenopausal women[277] and 75% overall[278] compared with 55% in UK women[279] and 68%-88% in perimenopausal women in the USA.[280,281] However, in Mayan women the prevalence is reported to be 0%[282,283] and in Chinese women, 10%.[284] A comprehensive review of international hot flush prevalence has been conducted by Freeman and Sherif.[154]

In ovariectomised women, the incidence is 90-92%.[281,285] Tamoxifen® use following breast cancer is associated with more *severe* hot flushes and night sweats, occurring in an estimated 80% and 72% respectively.[286]

6.2 Cultural differences

Lower rates of hot flush reporting occur among women from non-Western countries compared with those in Western societies[287] and women in rural areas compared with urban women, particularly in non-western countries, although the reasons for this are unknown.[154] The Study of Women's Health Across the Nation (SWAN), the largest evaluation of menopausal symptoms ever carried out, found that significantly more Afro-American and Hispanic women, and significantly fewer Chinese and Japanese women living in the USA,[288] experienced vasomotor symptoms than their Caucasian counterparts.

Japanese women are generally reported to have a low rate of hot flushes,[87] in the range of 3.0% to 22.1%,[289] although a recent study reported 40% incidence in the general population, and 70% in a menopause clinic sample.[290] The lower prevalence also applies to Japanese-American women living in Hawaii.[291] Chilliness, which also constitutes an important vasomotor symptom, has been suggested to be a more important vasomotor symptom than hot flushes and sweats in Japanese women[292] and was reported by 29.3% of participants in one study.[292] Proposed explanations for the reduced incidence of hot flushes in Japanese women include the higher phytoestrogen intake by these women, from dietary soy beans and soy products.[293,294]

Results of soy studies have been variable; in Japanese-American women, the higher intake of soy was not associated with fewer vasomotor symptoms,[291] while in other studies, soy or fermented soy product intake has been significantly negatively correlated with hot flush severity.[294,295,296] Evidence suggests that in studies reporting a statistically significant decrease in hot flush symptoms, soy products containing higher levels of genistein were used, providing more than 15 mg (calculated as aglycone equivalents) per treatment. [297]

The lack of menopausal symptoms among the Mayan women may be attributable to high yam consumption; it is not attributable to a difference in endocrinology.[282,283] Studies have provided basic evidence for the beneficial effect of yam for menopausal women.[298,299] One study found that a 30 day period of yams as the staple food improved the status of sex hormones in postmenopausal women, with significant increases serum concentrations of oestrone (26%), sex hormone binding globulin (SHBG) (9.5%), and near significant increase in oestradiol (27%)[299]. In another, ethyl acetate extracts of varieties of yam were found to activate human alpha and beta oestrogen receptors to various extents.[298]

6.3 Timing, Frequency and Duration

Hot flushes are generally found to be most prevalent in women in the late perimenopause and postmenopause, and to decrease with age.[90,91] Findings from SWAN suggest that hot flushes are more frequently reported by women in late perimenopause[155] whereas previous studies had found them to be most severe 6 months to 2 years after menopause.[188]
In more than 80% of women, they continue to occur for more than one year; in 25-50% the duration is more than 5 years[107,189,281] and in 10%. more than 10 years [281] They may even continue for several decades.[300]

The frequency of hot flushes varies from hourly to monthly.[191,300] An actual flushing episode typically lasts from one to five minutes[300] but can last up to one hour.[274] A circadian rhythm has been demonstrated with an increased frequency of hot flushes during the late afternoon and early evening.[301]

6.4 Experience of the Hot Flush

The hot flush is experienced as a sensation of internal heat in the upper body[301] and is described as a feeling of heat or burning, usually beginning at the head and neck area that may then pass, often in waves, over the entire body. It may be followed by an immediate

outbreak of sweating, most often in the face, head, neck an chest, and is frequently followed by chills.[274] Concomitant symptoms can include visible reddening of the skin, dizziness, headaches, clamminess, anxiety[300] or even panic,[302] irritability and palpitations.[303]

An aura precedes the actual flush by several seconds, which may be described as an uneasy or queasy feeling, with increased heart rate and finger blood-flow that correlate with flush intensity, reaching peak levels of 10-20 beats/min (mean of 16) and 30-fold respectively.[274]

6.4.1 Intensity

Flushing episodes can be of differing intensities, variously defined as mild, moderate or severe. While no standardised scales have been developed to measure them, typical categorisation is as follows:

Mild	flush without perspiration or clamminess	*OR*	sensation of heat
Moderate	hot flush associated with perspiration or clamminess	*OR*	sensation of heat accompanied by sweating
Severe	hot flush associated with intense perspiration that requires changing of clothing[304]	*OR*	sensation of heat accompanied by sweating, with consequences affecting daily activities[305]

6.4.2 Triggers

Triggers of individual episodes of flushing are suggested to include thermally hot food, spicy food, confined spaces, caffeine and alcohol,[300] although evidence from studies examining this relationship is limited. The Melbourne Women's Midlife Health Project (MMWHP) found no significant association between alcohol intake and hot flush rates.[277] Other triggers include stress,[306] higher ambient temperatures,[307] peripheral heating[308] and possibly conditions of fasting/low blood glucose.[309]

6.5 Risk Factors for Hot Flushes

As mentioned previously, not all women experience hot flushes. Risk factors found to be associated with experiencing these symptoms include high BMI, ethnic origin,[155] maternal history,[310] anxiety,[91] premenstrual symptoms during reproductive years,[181,277] longer perimenopause, lower education, more negative attitude toward menopause,[311] lack of exercise,[90] daily alcohol consumption (hot flushes and bothersome night sweats)[312] and smoking,[241,310] which was found in one study to increase the risk of bothersome hot flushes,

but not bothersome night sweats. [312] Higher education and a self-rating of excellent health were associated with decreased risk of night sweats, but not hot flushes.[241,312]

Low levels of circulating oestrogens[277] as well as high level of LH, and FSH prior to the menopause transition[313] have been associated with the occurrence of hot flushes, although data regarding FSH is inconsistent.[277] High levels of thyroid-stimulating hormone (TSH) were found to be related to hot flushes during the menopause transition, while postmenopausally, high levels of testosterone and DHEAS seem to protect against vasomotor symptoms.[313]

6.6 Differential Diagnoses

Hot flushes are not exclusive to menopausal women. They are reported to occur in 10% of women before the menopausal transition,[89] and also in men[88] and may indicate other disease processes that need to be excluded (Table 6.1).

TABLE 6.1 DIFFERENTIAL DIAGNOSIS OF HOT FLUSHES[276,314]

Hot Flushes Associated with Systemic Diseases	
Carcinoid syndrome	Pancreatic islet-cell tumors
Mastocytosis[315]	Renal cell carcinoma
Pheochromocytoma	Anaphylaxis[315]
Medullary thyroid carcinoma	
Neurological Flushing	
Spinal cord injury	Brain tumors
Migraine	Emotional flushing and somatic stress-related disorders (e.g., anxiety)
Parkinson's disease	
Hot Flushes Associated with Drugs/Vitamins , Alcohol (and associated tyramine or histamine) or an endogenous vasoactive mediator	
Vasodilators	Tamoxifen
Calcium channel blockers	Cyproterone acetate
Nicotinic acid	Oral triamcinolone used in psoriatic arthritis
Morphine and other opiates	Cyclosporine
Amyl nitrate and butyl nitrate	Anti-oestrogens (Selective estrogen receptor modulators--SERMs)
Selective serotonin reuptake inhibitors (SSRIs)	Luteinising hormone-releasing hormone agonists or antagonists
Cholinergic drugs	Aromatase inhibitors[316]
Bromocriptine	
Thyrotropin-releasing hormone	
Hot Flushes Associated with Eating and Food Additives	
Nitrites or sulfites	Auriculotemporal flushing after cheese, chocolate, lemon or highly spiced food
Spicy foods, red pepper or capsaicin	Dumping syndrome
Hot beverages	Gustatory flushing

6.7 Physiological signs

Thermoregulatory and cardiovascular changes have been observed to precede and accompany hot flushes. In the 60 seconds prior to the actual flush, increases have been observed to occur in mean skin temperature,[317] finger blood-flow and heart rate.[318] Skin temperature increases result from cutaneous vasodilation, and precede the small but significant elevations in core body temperature (T_c) of approximately 0.1°C that occur before most hot flushes.[301,317]

Concomitant with the hot flush, finger temperature continues to increase by an average of 3.9° C (range 0.3° – 6.6°)[274] due to peripheral vasodilation. Sweating and skin conductance responses, an index of perspiration, also increase, [198] resulting in a drop in oesophageal temperature of 0.2-0.6 degrees C.[274] in some women, a significant reduction in blood pressure can also accompany hot flushes, although this is not associated with changes in cutaneous vascular conductance.[319] Hot flushes are also accompanied by abrupt increases in plasma epinephrine (of about 150%) and concomitant decreases in noradrenaline (of about 40%), consistent with the observed cardiovascular changes such as increased pulse/heart rate.[274]

6.8 Aetiology of Hot Flushes

The pathogenesis of flushing associated with menopause has not been fully elucidated. Current evidence centres around the dysfunction of central thermoregulatory mechanisms in the hypothalamus.[198,274] Thermoregulation, or temperature homeostasis, requires co-ordination between core body temperature, the CNS (neurotransmitter activity impinging on the hypothalamus) and peripheral vasculature, and involves neuroendocrine, autonomic and somatomotor responses.[305]

Much work in the area of hot flush physiology and endocrinology has been conducted by Freedman and co-workers and is reviewed below.

6.8.1 The Role of Oestrogens

The role of oestrogens in the genesis of hot flushes is incontrovertible, since hot flushes accompany the decline of oestrogens in the vast majority of menopausal women[198] and oestrogen administration in HT virtually eliminates hot flushes.[196] Moreover, oestrogen

withdrawal rather than low levels of oestrogens, is associated with hot flushes,[320] as abrupt decline of endogenous plasma concentrations with oophorectomy in premenopausal women triggers the rapid onset of hot flushes while women with gonadal dysgenesis with low levels of circulating oestrogens do not experience hot flushes, unless exposed to oestrogen which is then withdrawn. [321] Hot flushes are also more likely to occur with withdrawal of oestrogen therapy.[322]

However declining oestrogen levels occur in symptomatic and asymptomatic women alike, and thus cannot fully account for the symptoms.[198] Furthermore, the levels of oestrogens in plasma, urine and the vagina were not found to correlate with the presence or absence of hot flushes.[323-325]

6.8.2 Luteinising Hormone

Similarly, no relationship between hot flushes and circulating levels of LH has been observed. Although most hot flushes coincide with the onset of LH pulses, with increased LH associated with most hot flushes,[274] not every increase in LH is associated with a hot flush[326] and flushing still occurs in women who have pituitary failure and thus do not secrete LH.[189] In addition, suppression of LH release with GnRH-agonists does not eliminate hot flushes,[327]nor does GnRH-stimulated LH-release *cause* hot flushes.[328] This suggest an association, but not a *casual* relationship, between hot flushes and LH-pulses.

6.8.3 Hypothalamic or Pituitary Thermoregulation?

Because removal of the anterior pituitary does not eliminate hot flushes, hypothalamic, rather than pituitary, thermoregulatory centres appear to be the site of thermoregulatory dysfunction.[321]

6.8.4 Thermoregulation

A hot flush is essentially a heat-dissipation response. The medial preoptic area of the hypothalamus is involved in thermoregulation, or the maintenance of core body temperature. When exposed to temperature changes, mechanisms of heat loss such as perspiration and vasodilation are activated to maintain core body temperature.

Core body temperature is regulated between upper thresholds for sweating and cutaneous vasodilation, and lower thresholds for shivering and vasoconstriction. Between these

thresholds is a thermoneutral zone or 'null' zone (or thermoregulatory inter-thresholds zone) within which major thermoregulatory adjustments do *not* occur. Freedman and co-workers measured the thermoneutral zone and found it to be 0.4^0C in asymptomatic women and zero in symptomatic women, suggesting that narrowing of the thermoneutral zone in symptomatic women may be involved in triggering hot flushes.[198] In a subsequent study, they found that symptomatic postmenopausal women had significantly lower sweating thresholds and higher maximum sweat rates compared with all other women.[329]

It is thought that this narrowed thermoregulatory zone is at least partly a result of elevated brain noradrenaline (norepinephrine/NA) levels, as this has been shown to narrow the thermoneutral zone in animal studies.[330] Increased levels of brain NA have been found in symptomatic women. In addition, the main metabolite of brain noradrenaline, 3-methoxy-4-hydroxy-phenylglycol [MHPG], further increases significantly between preflush and postflush measurements.[331] Considerable evidence has supported the role of α_2-adrenergic receptors in temperature regulation.[330] Central NA activity is, in turn, modulated by ovarian steroids.[332]

6.8.5 Elevations in Core Body Temperature

Within the reduced thermoneutral zone, small elevations in core body temperatures (T_c) that precede most hot flushes are thought to constitute the triggering mechanism.[198] Evidence of increased T_c preceding hot flushes was obtained by Freedman and co-workers using an ingested telemetry pill to measure T_c and a separate device measuring skin conductance level to record hot flushes.[301,317] This phenomenon is consistent with the aggravating effect of factors such as peripheral warming[333] and higher ambient temperature that raise T_c.[307,334] The T_c changes occur 15 minutes before hot flushes in up to 60% of episodes. The existence of a circadian thermodynamic rhythm is supported by the frequency of hot flushes and T_c elevations during the late afternoon to early evening. [301]

Freedman's theory of a central noradrenergic mechanism is supported by findings that

- skin temperature increases precede elevations in T_c and skin conductance responses, showing that peripheral vasoconstriction is not responsible for either event, suggesting a *central* event driving skin temperature increases, T_c elevations and metabolic rate.[331]

- the main metabolite of brain noradrenaline, MHPG, increases significantly between preflush and postflush measurements.[331] (However this represents whole-body sympathetic activation as the majority of MHPG is derived from skeletal muscle rather than brain.)[335]
- clonidine, an α_2 adrenergic agonist, reduces levels of brain noradrenaline and plasma MHPG, raises the sweating threshold and ameliorates hot flushes.[318]
- yohimbine, an α_2 adrenergic antagonist that increases brain noradrenaline, can trigger hot flushes.[331]
- oestradiol (1 mg/d 17beta-estradiol) was found to ameliorate hot flushes by widening the thermoneutral zone and thereby raising the sweating threshold.[336]
- some behavioural relaxation procedures ameliorate hot flushes, suggesting a decline in sympathetic activation may impact on the narrowing of the thermoneutral zone. As outlined above, this narrowing may be due to elevated sympathetic activity.[334]

However, this theory cannot fully account for menopausal hot flushes since increased brain noradrenaline also occurs in *asymptomatic* women of the same age.[198]

6.8.6 Other Postulated Mechanisms: Endogenous Opioids

It has been hypothesised that central noradrenergic instability associated with menopausal hot flushes could be due to the reduction of endogenous opioid activity. There is a remarkable similarity between many symptoms associated with menopause and opiate withdrawal.[337,338] The alteration in skin temperature, heart rate and LH secretion during precipitated morphine withdrawal in the rat are similar in magnitude, duration and temporal relationship to those observed during the hot flush.[338]

Furthermore, because oestrogen and testosterone can stimulate natural endorphin production, declining oestrogen levels at the time of menopause, as well as age-related androgen decline, result in reduced endogenous opioid peptide activity. Hypothalamic opioidergic activity normally has an inhibitory effect on noradrenergic neurons in the brainstem. Oestrogen withdrawal is linked with noradrenergic hyperactivity and its inappropriate activation of the medial preoptic area where thermoregulation and GnRH secretion are stimulated.[339,340] It has been suggested that in addition to triggering peripheral vasodilation, this also accounts for pulsatile LH release. Casper and Yen[320] proposed that successful therapies for hot flushes may exert their effects by increasing endogenous opioid

peptide activity with consequent inhibition of noradrenergic activity below the threshold needed to activate heat loss (Figure 6.1).

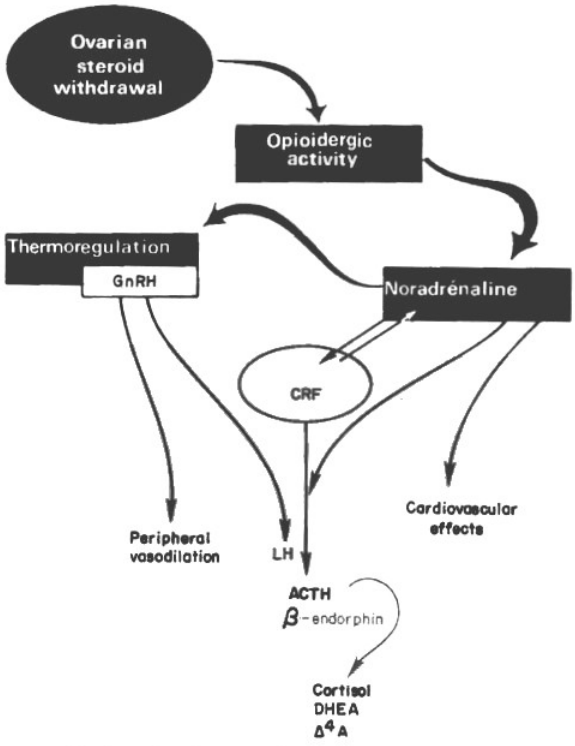


Fig. 8. A diagrammatic scheme for the proposed neuroendocrine mechanism of menopausal hot flushes. Oestrogen withdrawal induces a decrease in the inhibitory action of the hypothalamic opioidergic activity on noradrenergic neurons in the brainstem, and consequently hyperactivity of the noradrenergic inputs to the medial preoptic area, where thermoregulation and GnRH secretion are stimulated, results in peripheral vasodilation and pulsatile LH release. The increased noradrenergic activity may also account for the increased pulse rate. Activation of the CRF-POMS system may either be a component of the flush mechanism or a response to the discomfort—a central stress. *Oestrogen binding site.

FIGURE 6.1 A PROPOSED ROLE FOR ENDOGENOUS OPIOIDS IN FLUSHING

From Casper RF, Yen SS. Neuroendocrinology of menopausal flushes: an hypothesis of flush mechanism. *Clin Endocrinol (Oxf)* 1985;22(3):293-312. Reproduced with permission from Blackwell Publishing.[320]

The evidence regarding the involvement of an opioidergic system in flushing has been inconsistent. A small study in 6 postmenopausal women reported that the opioid antagonist, naloxone, acutely *blocked* subjective hot flushes. [341] This seems paradoxical as naloxone increases the magnitude and frequency of LH pulses in premenopausal women. However, it was not borne out by a subsequent study that found naloxone did not change the rate of objectively measured hot flashes, mean serum LH or FSH levels, or the frequencies or amplitudes of gonadotropin pulses.[342] Genazzani and co-workers[343] reported an increase in proopiomelanocortin-related peptides (adrenocorticotropic hormone, beta-endorphin, and

beta-lipotropin) during subjective menopausal flushes, and Tepper[344] found levels of plasma beta-endorphin levels to drop with the onset of hot flushes and then rise significantly 5 - 15 minutes post-flush. However, no casual connection between β-endorphin plasma concentrations and hot flushes has been conclusively established.

6.8.7 Dopamine

Recent evidence suggests that dopamine is an important thermoregulatory neurotransmitter, with D2 receptors involved principally with the maintenance of body temperature in euthermia.[345] Earlier research had observed the dopamine agonist bromocriptine to increase the activity of the endogenous opioid system on the thermoregulation mechanisms that regulate body temperature in postmenopausal women[346] and to be effective in alleviating hot flushes.[347] Interestingly, antidopaminergic agents were also effective, leading researchers to propose a direct dopaminergic action is in the case of bromocriptine but indirect mechanisms for antidopaminergic drugs such as stimulation of the opioid systems.[347]

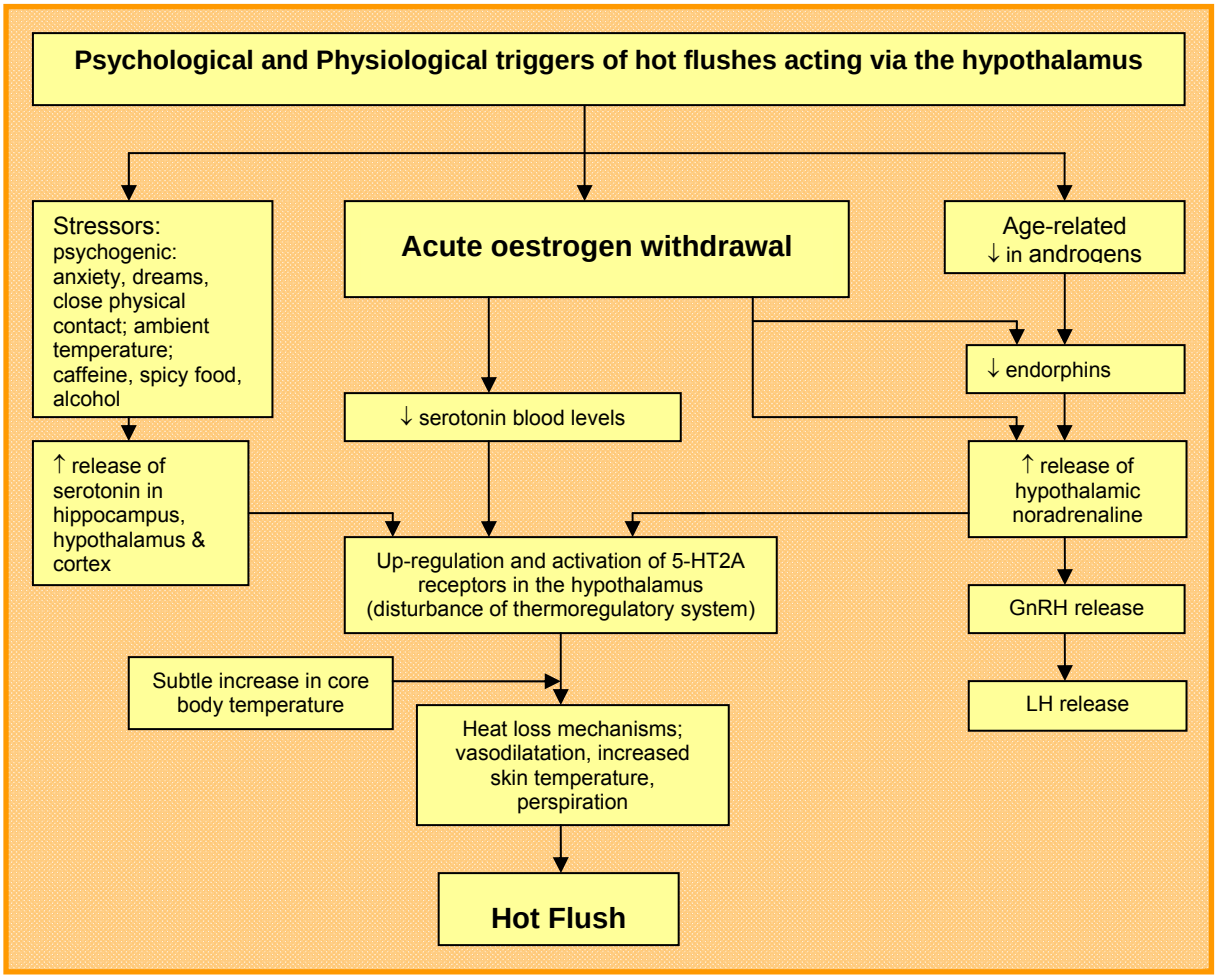
6.8.9 Serotonin

The neurotransmitter, serotonin, has also been implicated in the generation of hot flushes, through its interaction with oestrogens. Oestrogen withdrawal in menopausal women results in dramatically lowered blood serotonin levels[348,349] while treatment with oestrogen has been shown to augment the serotonergic activity.[350] Low blood oestrogen levels are correlated with upregulation of certain serotonin receptors (5-HT_{2A}) in the hypothalamus[351] that are believed to be involved in thermogenesis. Stimulation of this receptor may change the set-point temperature and thereby activate some autonomic functions to cool down the body, such as increased skin temperature and sweating, which supports an involvement of these receptors in the aetiology of hot flushes. Internal and external stimuli, including anxiety, consumption of alcohol, coffee and spicy food,[306] high ambient temperature and even dreams may activate these receptors to trigger a hot flush.[197]

The potential role of serotonin in the genesis of hot flushes has been comprehensively reviewed by Berensden, who proposed a model outlining the interaction between oestrogens, the serotonergic system and environmental and psychological stimuli.[197]

6.8.10 An Integrated Model

The following model adapted from Shanafelt[321,352] and Berensden[197] integrates the theories of several key researchers (Figure 6.2):[301,320,339,340,353]



GnRH = gonadotrophin-releasing hormone. LH = luteinising hormone 5-HT= serotonin

FIGURE 6.2 AN INTEGRATED MODEL OF PHYSIOLOGICAL AND PSYCHOLOGICAL FACTORS PROPOSED TO TRIGGER HOT FLUSHES Adapted from Berensden 2000[197] and Shanafelt[321,352] with permission.

Declining oestrogen levels are associated with noradrenergic hyperactivity, possibly mediated by decreased endogenous opioid peptide activity, [339,340] decreased blood serotonin levels and upregulation of 5-HT_{2A} receptors in the hypothalamus.[351] Activation or upregulation of this receptor as a consequence of stressors[197] or decreased plasma serotonin levels[348,349] may lower the thermoregulatory set point, and elevated NA may narrow the thermoregulatory zone, allowing subtle increases in core body temperature to trigger heat loss mechanisms.[164]

6.9 Summary

In summary, a multi-factorial aetiology has been proposed for hot flushes, including a role for known environmental and psychological triggers via their effects on neurotransmitters, specifically noradrenaline and serotonin, which interact with gonadal hormones. In terms of the mechanisms of action of the herbs trialled in this study, the potential effects of dopamine and endogenous opioids are also of interest.

Chapter Seven

Psychological Symptoms

7.0 Introduction

This chapter of the Literature Review examines the aetiology of the psychological symptoms commonly associated with the menopause transition, a secondary endpoint of the principal study to be described herein. As mentioned above, controversies currently exist regarding i) an increased prevalence of emotional symptoms associated with menopause, ii) an association between affective symptoms and the endocrinological changes that occur with menopause, and iii) effective treatment of such symptoms.

7.1 Association between mood disorders and menopause

The perimenopause has long been recognised as a time of increased susceptibility to depressive symptoms[354] with terms such as 'menopausal neurosis'[21,354] and 'involutional melancholia', (dropped from the Diagnostic and Statistical Manual (DSM) in 1980), previously used to describe the depressed mood, anxiety, irritability, nervous tension and loss of confidence commonly reported at this time. However, data from epidemiological and therapeutic studies do not consistently report an increased prevalence of mood symptoms associated with this period. Some epidemiologic studies have reported depressive or mood symptoms in up to 51% of women at this time.[62,86,88,355] This compares with a lifetime prevalence of depressive disorders of 15-25% in the US[356,357] and 6.8% for Australian females in a 30-day period.[358] Other studies, however, have failed to find any evidence of an increase in depressive symptoms associated with menopause.[89,359,360]

The discrepancy in these findings has been attributed, at least in part, to methodological differences such as the criteria used for defining reproductive status and depression, which covers psychological distress to minor or major depression,[*] as well as instruments of detection and measurement. Many studies investigating mood have employed instruments such as the Beck Depression Inventory (BDI)[361] or the Centre for Epidemiological Studies-Depression scale (CES-D)[59,355,362,363] that measure clinically meaningful minor or major depressive disorders whereas others have relied on psychological subscales of menopause rating scales that are more sensitive to less severe psychological distress. In addition, some have attempted to extrapolate these findings from depression scales to mood, despite the important distinctions between mood variability and major depression.[207] As pointed out by Klein, the diversity of emotional changes occurring at different stages of the perimenopause include both the agitated and vegetative forms of depression, general anxiety disorders, panic attacks, phobias and obsessive-compulsive disorders, possibly related to the different gonadal hormone alterations.[364] Other factors that may impact on findings regarding

[*] The American Psychiatric Association's *Diagnostic and Statistical Manual, Fourth* Edition (DSM-IV) specifies five selected core symptoms for major depression and 3 for minor depression which must persist for at least a 2-week period and be associated with clinically significant distress or impairment in social or occupational functioning, and not be caused by medications, a medical condition such as thyroid disorders, or bereavement. [See Appendix 4 for symptoms of major depressive disorder (MDD), minor depression, dysthymic disorder **(**DD) and generalised anxiety disorder (GAD).]

symptom prevalence are cultural differences and the inclusion/exclusion of current HT-users.[363]

With regard to major depressive disorder (MDD), evidence suggests that the transition to menopause is *not* associated with increased risk, particularly for women who with no past history of depression. A co-morbidity survey reported no gender-related differences in prevalence of major depressive episodes at midlife (45 to 55 years).[365] However, *non-major* depressive disorders are reported to be two to three times more common in older women than in their male counterparts.[366] It has been suggested that the terms 'subthreshold depression', 'subsyndromal depression' or 'dysphoria' are more appropriate for the minor psychological morbidity associated with the perimenopause, due to the lack of adequate symptoms or symptom severity associated with clinical depression.[80]

The Harvard Study of Moods and Cycles, a longitudinal, prospective cohort study that followed 460 premenopausal women as they entered perimenopause found that women with no lifetime history of major depression who entered the perimenopause were twice as likely to develop significant depressive symptoms as women who remained premenopausal.[367] This is consistent with previous findings that 35% of first episodes of depression in women in the UK occur during the perimenopause,[183] and 40% in the US between the ages of 40 and 60 years.[59]

This association between transition to menopause and new onset depression was found to be independent of factors such as negative attitudes, stressful events, past history of depression or severe premenstrual syndrome (PMS) or poor sleep in the SWAN, a longitudinal cohort study that enrolled 16,065 women 40 to 55 years old[185,355] and the Pennsylvania Ovarian Aging Study, an 8-year longitudinal study to identify risk factors of depressed mood during the menopausal transition.[181,368]

A number of studies have found the incidence of psychiatric symptoms during menopause to be higher in women with a history of depression or other psychiatric disorders.[362,183,367] A significant risk for first onset depression has been associated with surgical menopause[71,194,362,363] and among those who enter the transition *earlier*.[367,369] Current or prior PMS, or premenstrual dysphoria (PMD) have been found to predict as well as

accompany perimenopausal depression in some women in both retrospective and prospective studies.[62,362,369-371,372]

7.2 Psychological symptoms and Stage of transition

Menopause-clinic-based studies[22] and cross-sectional surveys[62,279] have supported the findings that it is commonly during the perimenopause, as distinct from pre- or postmenopause, that vulnerability for depression and 'psychological distress' is increased. The SWAN longitudinal study found a significant association between depressed mood and the *late* transition stage.[373] However, both the late perimenopause *and* the early postmenopause were found to be associated with an increased rate of onset of depression in a recent prospective study of 29 asymptomatic premenopausal women who were followed until 6 to 12 months after their FMP; a 14-fold increase was observed in the 24 months surrounding the FMP.[374]

In contrast, the Melbourne Women's Midlife Health project (MWMHP), a large-scale, longitudinal study recently reported that the prevalence of mood symptoms peaked *before* the expected age of natural menopause, between 35 to 40 years, and decreased with age from the middle- reproductive years.[375]

Symptoms of anxiety have similarly been found to be more prevalent in perimenopausal women and to remain that way into postmenopause, according to a population-based survey.[184] The prevalence of anxiety disorders in Australian women in the 45-54 year age-group was 15.9% in 1997[358] compared with 8.0% for males in the same age bracket and 12.1% for females in the total population over a 30-day period. Surveys of women in this same age range report 38% experienced symptoms of anxiety in the previous 2 weeks compared with 40.62% in peri-menopausal women[62] and 58% in the preceding 6 months.[86]

7.3 Aetiology: Endocrinopathy or Psychosocial?

Oestrogen deficiency has been suggested as a cause of the increased prevalence of non-major depressive disorders in midlife women but the relationship remains to be established.[354] Psychosocial theory suggests that socio-cultural and personal circumstances are more important than changing menopause status in the aetiology of psychological morbidity, [376,377] which may be caused by the vicissitudes of life.[186,378] Data from community-

based cross-sectional and longitudinal studies support the relationship of depression in this demographic to factors shown in Table 7.1 below.

TABLE 7.1 FACTORS ASSOCIATED WITH DEPRESSION IN MIDLIFE OR MENOPAUSAL WOMEN

		Study design	
		Cross-sectional	***Longitudinal/ Cohort***
Reproductive events	surgical menopause	X[59]	X[376]
	longer duration of perimenopause		X[362]
	current or prior PMS or PMD	X[369,370]	X[362,371,372]
Health	health status problems	X[59,62]	X[376,379]
	trait anxiety		X[380]
	hypochondria/disease phobia		X[381]
Stress	current stress, (especially chronic)		X[373,379,380]
	pessimism, with chronic stress		X[380]
	interpersonal stress /relationship dissatisfaction, especially marital problems or relationships with children	X[382]	X[376,379]
	multiple roles including paid work, adolescent children, ailing husbands, aging parents or parents-in-law	X[59]	
Loss	recent bereavement	X[382]	
	major life events		X[383]
	role loss	X[59]	
Negative attitude/ experience	negative attitudes towards ageing or to menopause	X[59,62]	X[273 381]
	a history of negative life events		X[367]
Demographic factors	no partner/marital status	X[369]	X[379,383]
	nulliparity	X[369]	
	lower socio-economic status	X[384]	
	ethnic origin	X[384,385]	
	lack of employment outside the home	X[386]	
Diet and Lifestyle factors	work dissatisfaction		X[383]
	smoking, current	X[369]	X[379,385]
	exercise, low		X[379,381]
	high BMI		X[373]

A variety of different rating scales were used in the above studies, with several using the CES-D.[59,273,362,367,370,376,384] The Beck Depression Inventory (BDI) was used in the study on pessimism, trait anxiety and life stress,[380] and the Hamilton Depression Rating Scale (HAMD/HDRS) in one study of the Harvard Study of Moods and Cycles.[385]

Findings regarding predictors of perimenopausal depression are not always consistent, however. This is possibly a product of differences in study design. Cross-sectional studies

have certain limitations inasmuch as they may not control for prior episodes of affective disorders, nor characterise the temporal sequence of events occurring with transition through menopause.[362] Thus, a causal connection cannot be inferred from the coexistence of these factors with depressive symptoms.

Schmidt and colleagues,[374] in a longitudinal study, found no association with the number of exit events (bereavement/divorce/family members leaving home) or personal losses, nor any other variable previously associated with the onset of perimenopausal or midlife depression. The MWMHP,[387] a 9-year annual prospective study of 438 women, failed to provide evidence for the 'empty nest' syndrome as a predictor. In fact, it found that for the majority of women, the departure of the last child from the household led to *positive* changes in women's mood state.

As pointed out by Rubinow,[354]the main predictors for depression in the menopause are the same as for other stages of life, namely prior depressive episodes[148] and poor current or past health status.[362,379,50,35] Stressful life events, history of smoking, sleep disturbances and PMD or PDD also frequently accompany depression at other stages of life.[354]

Because vasomotor symptoms and sleep disruption can co-exist with mood changes at menopause, [71,181,211,362,373,379,381,388380,387] it has been suggested that depression may be a secondary reaction to these bothersome physical symptoms. Hot flushes are hypothesised to affect sleep and thereby emotional stability and quality of life,[389] consistent with the domino theory.[159] However findings from recent studies, both longitudinal[181,368] and a community sample of 16,065 women,[185] suggest that perimenopausal depression is not an epiphenomenon to hot flushes but an independent risk factor[181,185,368] as the relationship between perimenopausal endocrine changes remained after adjusting for hot flushes.[368]

7.3.1 Oestrogen and depression

Evidence regarding an involvement of endocrine events and mood alterations is derived from

i) the relationship between the incidence of affective symptoms and stage of the MP transition

ii) studies attempting to correlate the serum levels of gonadal hormones with mood

iii) therapeutic studies investigating the effect of oestrogen administration on mood, depression and well-being in natural menopause, surgical menopause, clinically depressed and healthy women.

The involvement of endocrine events is suggested by the finding that depression is associated with the stage of the transition or entry to a different phase. The SWAN reported depressions most commonly appearing during the late-perimenopause,[355] the phase most characterised by oestradiol 'withdrawal', as hypogonadism is more prolonged than in the early perimenopause.[109,111] A large-scale cross-sectional study in Eindhoven of 8,098 women reported a high increase of depressive symptoms as women passed from pre- to perimenopause and from peri- to postmenopause.[390]

A relationship between perimenopause-related changes in oestrogen and depression remains to be established. While some studies have found no direct association between oestrogen levels and depression,[285,391,392] others report significant associations with hormone levels.[87,285,368,393] Soares and colleagues found oestradiol and FSH levels to be associated with changes in the severity of depressive symptoms in perimenopausal women.[393] Freeman and colleagues[368] found increased *variability* of oestradiol, FSH and LH to be significantly associated with high CES-D scores. A significantly increased incidence of depression as measured by the CES-D was found in the year following the last period, which coincided with a significant drop in oestradiol levels.[87] In healthy *non-depressed* women treated with oestrogen, mood covaried with circulating oestradiol levels.[394,395]

Attempts to study the effects of oestrogen therapy on mood have been hampered by the 'domino effect' – the flow-on effects from concomitant relief of hot flushes to improvements in other symptoms.[159] Nonetheless, findings from several studies of oestradiol therapy suggest its effects on depression are independent of its salutary effect on hot flushes.[361,396,389,397] No association was found between the response of the vasomotor symptoms and depressive symptoms to treatment with Premarin.[397] Similarly Schmidt and co-workers, when examining oestrogen treatment of depression in perimenopausal women with and without hot flushes, found that the presence of hot flushes did not modify the outcome.[396] This was supported by findings from a small scale clinical trial of oestrogen in peri- and postmenopausal women that the antidepressant response was not associated with concomitant improvement in menopause-related symptoms.[361]

Studies have not consistently supported a salutary effect of ostreogen therapy (ET) in mood symptoms, however. This may be attributable to the confounding factors and limitations that exist in these studies, namely, i) the intimate relationship between oestrogen-deficiency-related vasomotor symptoms, poor sleep patterns and emotional stability,[388] ii) the lack of standardised diagnostic criteria and measuring instruments for the depression syndrome, iii) the extrapolation from studies on perimenopausal depression to other menopause–related mood changes,[398] iv) different preparations of HT in different doses, v) inclusion in some studies of women with concurrent psychiatric illness[399] or malignant disease.[285]

Nonetheless oestrogen preparations, with or without progesterone or testosterone,[400] have been found to be effective over placebo in several studies. A 2001 review of the literature concerning the role of oestrogen in the treatment of mood disorders found growing evidence for oestrogen as a sole antidepressant in perimenopausal depression.[350] This is consistent with a 1997 meta-analysis[401] reporting that most studies showed HT to be effective in reducing depressed mood among menopausal women, with an overall effect size of 0.68. Findings of an RCT in major and minor depressions by Schmidt and co-workers[396] found oestradiol to significantly decrease depression rating scale scores in 80% of subjects compared with 22% of the placebo group. In an RCT of endocrinologically-confirmed perimenopausal women with major or minor depressive disorders, or dysthymic disorder, oestradiol was also reported to result in remission of symptoms regardless of type of disorder compared with placebo.[402]

In contrast, studies of HT in mood in *postmenopausal* women, such as the Women's Health Initiative (WHI) studies with combined oestrogen/progesterone,[403] have failed to find any improvement. A study of women who were 5-10 years postmenopausal with major and minor depressions, found no clinically significant effect of oestradiol (40% improvement) over placebo (44%).[404] The ineffectiveness of oestrogen in women with more *severe* depressive symptoms has also been reported,[405, 406] while *non*-depressed women, or those with mild symptoms, improved in their sense of wellbeing.[406] Acute mood-enhancing effects in asymptomatic women were also reported by Ditkoff and colleagues,[407] suggesting a different mechanism of action to traditional antidepressants.

It has been suggested that the rate of change of hormone secretion and levels may be as important as, or more important than, the absolute (low) hormone levels in provoking

affective symptoms.[408-410,411] This view receives support from findings that perimenopausal women, but not postmenopausal women with prolonged oestrogen deficiency, may respond to ET.[361,404] Additionally, mood-enhancing effects have been shown in women who have undergone surgical menopause, [395,405,412,413] where significant decrease in circulating sex hormones, with corresponding symptom experience, occurs abruptly.

While many studies have investigated depressive symptoms, few have focussed on anxiety. Anxiety symptoms were lower in women treated with ET than untreated women in an uncontrolled study,[414] and were shown to decrease in postmenopausal women during treatment with oestradiol, compared with placebo.[415]

7.4 Neuroendocrinology of Mood

7.4.1 Pathophysiology of Depression

(Refer to appendix 5 for a brief description of the monoamine transport cycle).

The underlying pathophysiology of depressive disorders is not yet fully understood. It is complicated not only by the various neurobiological and neurochemical (or neurofunctional) components involved but also by the range of depressive subtypes, which may reflect different underlying neurochemical changes. Malhi and co-workers suggest that "non-melancholic depression may be largely serotonergically driven, while melancholic and psychotic depressions have significant additional contributions from the noradrenergic and dopaminergic systems, respectively."[416] The generation and maintenance of anxiety disorders are also associated with noradrenaline and serotonergic dysfunction.[416]

Research in the past 10 years on the aetiology of depression has gone beyond the monoamine-depletion hypothesis, which was based on the observation of acute depletion of the neurotransmitters serotonin or noradrenaline/dopamine in depressed subjects[417,418] and supported by the observed activity of antidepressants that enhance the synaptic concentrations of one or more of these monoamines. However, the significant delay between the neurochemical effects of these antidepressants (which may occur within hours) and the clinical response (which may take weeks)[416] led to a shift in interest to alterations in neurotransmitter receptor levels or function, which have been observed post-mortem and in vivo.[419,418]

Dysfunction of the neurotransmitter regulation systems, especially serotonin, noradrenaline and dopamine, include a functional defect in the serotonin transporter,[418] monoaminergic underactivity, particularly involving serotonin and noradrenaline (for example, diminished noradrenergic activity in the prefrontal cortex and reduced activity of post-synaptic hypothalamic α_2 adrenoceptors), receptor density changes, such as diminished numbers of $5HT_{1A}$ and $5HT_{2A}$, changes in α_1 and α_2-adrenoceptors, (for example, increases in presynaptic α_2 adrenoceptors),[418] increased sensitivity of dopamine receptors, lower density of striatal dopamine transporters and mesolimbic dopaminergic dysfunction in anhedonia (lack of enjoyment of activities than normal give pleasure).[420]

Abnormalities have also been observed in regional blood flow and metabolism in several brain regions relevant to mood regulation[354] in post-mortem studies of patients with affective disorders.[421] Studies have also demonstrated that stress and depression can lead to neuronal atrophy and cell loss in key limbic brain regions implicated in depression, including the amygdala, prefrontal cortex, and hippocampus.[422] The synthesis of new cellular constituents such as neurotransmitter receptors and synthetic enzymes is necessary for remission of depressive symptoms[416] and it is now thought that antidepressants may act by reversing neuronal atrophy and cell loss.[422] (Enhancement of hippocampal neurogenesis by antidepressants may also explain their efficacy in hypercortisolaemia, which can cause neuronal cell death in some brain regions.[423])

Other observed changes in depressed patients, such as mechanisms involving the hypothalamic-pituitary-adrenal (HPA) axis, include:[418]

- Hypersecretion of cortisol, reflecting failure of negative feedback mechanism
- Increased activity of corticotrophin-releasing factor (CRF); increased density of CRF neurons in paraventricular nucleus of; reduced density of CRF1 receptors in frontal cortex
- Subclinical hypothyroidism; desensitised TRH receptors; defective transport of thyroid hormone
- Increased pro-inflammatory cytokines (which induce hypercortisolaemia and neurotransmitter changes) and decreased anti-inflammatory cytokines
- Neurodegenerative changes in the brain – atrophy of the hippocampus, amygdala and frontal cortex, associated with a rise in brain concentrations of glucocorticoid that

cause reduction in the synthesis of neurotrophic factors such as brain derived neurotrophic factors (BDNF), which is responsible for neuronal repair

- Disturbance in circadian rhythm
- Overactive glutamate pathways; higher cortical glutamate concentration
- Reduced concentration of GABA in cerebrospinal fluid (CSF), plasma and hippocampus
- Elevated concentrations of substance P in CSF. (Substance P modulates the stress response as well as reducing pain perception).

A neurotrophic hypothesis for the pathogenesis of depression proposes that stress decreases the expression of BDNF which, together with other factors such as down-regulation of receptor sensitivity, could contribute to the atrophy of certain limbic structures, including the hippocampus and prefrontal cortex, observed in depressed subjects.[422,424]

7.4.2 Role of Hormonal Changes at Menopause

Psychological well-being at the menopause may be affected by changes to oestrogen levels influencing neuropeptides and neurotransmitters in the brain[93] and/or the effects of oestrogen withdrawal on oestrogen receptors in the limbic system and hypothalamus.[214,425] Oestrogen deficit has been linked with changes in the cholinergic, catecholaminergic and serotonergic systems.[409] The action of oestrogen on the neurotransmitter receptors and synapses involved in all these systems is rapid.[426]

Sensitivity to change in gonadal hormones

A pre-existing sensitivity in some individuals to the change in the gonadal hormones and resultant decreases in neural transmitters, or reactions to the physiological changes associated with menopause has also been proposed.[182]

CNS activity of oestrogen that may modulate mood via effects on serotonin, noradrenaline, dopamine and opioids, include the following.

7.4.3 Neurotransmitters and Neuropeptides

Serotonin

- increasing synthesis of serotonin (5-hydroxytryptamine /5-HT)[427,428]

- increasing the rate of free tryptophan (by displacing it from its binding sites on plasma albumin) to be metabolised to 5-HT [429,430]
- reducing breakdown of 5-HT[427,431]
- modifying the concentration and availability of serotonin by stimulating degradation of monoamine oxidase (MAO, the enzyme that catabolises 5-HT)[161,429,430]
- up-regulating expression of serotonin receptors[432-434]
- increasing post-synaptic responsivity to 5-HT[435,436]
- enhancing the effect of 5-HT by increasing the affinity of certain types of 5-HT receptors for this neurotransmitter[332]

Overall oestrogen decline results in a decrease in 5-HT synthesis and activity, which is related to mood disorders.[165] Decreased serotonin 1A receptors have been observed post-mortem and in vivo in depressed subjects in radioligand imaging studies,[419] and 5-HT_{1A} receptor agonists have been shown to have anxiolytic and antidepressant properties.[417]

Noradrenaline

- increasing synthesis and
- increasing available NA by reducing breakdown by degradative MAOs[427,431]
- selectively increasing NA activity and turnover in the brain[431]
- enhancing the effect of noradrenaline by increasing the affinity of certain types of noradrenergic receptors for these neurotransmitters[332]

Noradrenaline has been suggested to play a significant role in mood and behaviour.[165] Decreased α-2 and β-adrenergic receptors have been identified post-mortem in depressed subjects.[419] In the absence of oestrogen, a decrease in activity of both of these systems would be expected. In addition, there is a dynamic interdependent relationship between the serotonergic and noradrenergic systems. Serotonergic neurons project to noradrenergic neurons and vice versa, allowing 'cross-talk' between the neurotransmitter systems. Stimulation of serotonergic neurons leads to a net *decrease* of noradrenergic transmission, while conversely, noradrenergic firing causes a net *increase* in 5-HT transmission. The stimulatory effects of NA on the serotonergic neurons may directly offset the loss of oestrogen's stimulatory effects on the serotonergic system.

Opioids

- Potentiating the activity of opiate-containing neurons[438]
- Increasing the synthesis and release of β-endorphin.

Change in β-endorphin synthesis and secretion is related to the pathogenesis of mood, behaviour and nocioceptive perception changes[439] Declining oestrogen levels result in a decrease in endogenous opioid peptide activity and its normal inhibitory effect on noradrenergic neurons in the brainstem. Thus oestrogen withdrawal is linked with noradrenergic hyperactivity.[320]

Dopamine

- directly modulating dopaminergic activity[99]
- increasing dopamine release in the hypothalamus[99]
- increasing dopamine transmission and D2 receptors[426]
- low concentrations of 17beta-oestradiol inhibit uptake via the dopamine transporter DAT[440]

In the cerebral cortex, dopamine is involved in emotional responses. In postmenopausal women, the activity of the dopaminergic system was found to be significantly lower than in premenopausal women, but was significantly increased by HT administration, with a concomitant significant decrease in psychological symptoms.[441] In contradistinction to the proposed delayed secondary effect of selective serotonin reuptake inhibitors (SSRIs), oestrogen's effect on dopamine levels and receptors is very rapid.[426]

7.4.4 Other Proposed Factors

Progesterone and Androgens

Because the menopause is often regarded as an oestrogen deficiency condition, the consequences of altered secretion of progesterone and other ovarian hormones have often been neglected.[442,443] During the perimenopause, there may be periods of serum oestrogen excess relative to serum progesterone. While oestrogens have arousing, antidepressant and potentially anxiogenic effects in excess, progesterone/allopreganolone has a potent anxiolytic effect.[444,445] Acute progesterone withdrawal can cause anxiety and seizures.[446]

Although no clear patterns of testosterone and androstenedione changes have been shown in menopausal women, androgen administration appears to improve well-being, anxiety and depressive symptoms in naturally and surgically menopausal women being treated with exogenous oestrogen. A discussion of testosterone studies in general can be found in the reviews by Alexander *et al*[447] and Arlt *et al.*[448]

It has also been suggested that the rate of change of these hormones and their ratios may be as important as their absolute levels in determining their overall effect on emotional behaviour.[364]

Dehydroepiandrosterone (DHEA)/Dehydroepiandrosterone sulphate (DHEAS)

Although menopause does not appear to significantly affect the rate of decline in DHEAS levels,[110] the drop in DHEA(S) synthesis occurring between the ages of 20 and 60 years has been associated with depressive symptoms, [449] poor subjective perception of health and life satisfaction, as well as reduced libido.[450] In addition, the alpha-reduced metabolite of androstenedione (and indirectly DHEA) is lower in menopausal women with anxiety than asymptomatic menopausal women; its serum levels correlate inversely with anxiety scores.[451]

Growth factors

Oestrogen has been shown to have reciprocal interactions with CNS growth factors. Recent reports that BDNF levels are decreased by stress and elevated by mood stabilisers and antidepressants suggest that BDNF may be of potential importance in the aetiology and treatment of depression during the perimenopause.[354]

Circadian Rhythms

The effects of ovarian hormones on circadian rhythmicity could also contribute to the development of mood disorders in predisposed women, as hormone levels fluctuate during the menopause transition and circadian rhythms become destabilised. Oestrogen treatment increases the amplitude of melatonin circadian rhythms, which lose their regularity and stability with ageing.[452]

Maintaining functional homeostasis

Another hypothesis put forward by Deecher and co-workers[453] is that, in order to maintain functional homeostasis as ovarian hormone levels become fluctuating and unpredictable, increased flexibility in neuronal responsiveness is needed. An inability to rapidly establish a new baseline of neuronal function could lead to increased susceptibility to mood disorders and diminished brain-related functions in predisposed women.[453] The lack of effectiveness of oestrogen therapy for *post*menopausal depressed mood has been interpreted as

evidence that postmenopausal brain adaptation is less dependent on and/or vulnerable to modulation by ovarian hormones.

Relationship between Premenstrual dysphoria and Perimenopausal depression

It has also been suggested that in women previously reporting a history of PMS, perimenopausal depression could represent the elimination of follicular phase-related symptom remissions, and the development of a more persistent pattern of dysphoria.[372] The overlapping symptoms of PMS and menopause is examined in the following section.

7.5 Summary

Whether or not the incidence of affective disorders increases during the perimenopause is unknown, but prevalence estimates suggest these symptoms are deserving of attention. While the exact aetiology is as yet unclear, current evidence supports interactions between steroid hormones, predominantly oestrogen, and neurotransmitters and neuropeptides as being of central importance. Evidence for conventional HT in this context is inconsistent and its use controversial. Understanding of the underlying pathophysiology of these symptoms, and of the mechanisms of phytotherapeutic interventions, assists in identifying potentially efficacious and acceptable treatments for this neglected group of women.

Chapter Eight

Premenstrual Syndrome-like symptoms during the perimenopause

8.0 Introduction

This chapter of the Literature Review examines the aetiology of premenstrual (PMS)-like symptoms in perimenopausal women. A sub-study of the RCT described in this thesis focused on the effects of the intervention on PMS-like symptoms in a late-perimenopausal subgroup. While the peak of actual PMS is found to occur in the late reproductive (premenopausal) years,[454] anecdotal reports suggest that women in the perimenopause are increasingly prone to PMS-like symptoms, or at least tolerate them less well.[224]

8.1 Co-existence of PMS and Perimenopausal Symptoms

It has been suggested that some of the perimenopausal symptoms typically attributed to menopause (such as breast discomfort and the mood symptoms of irritability, excitability, depression and poor concentration) may be more related to menstrual cyclicity than menopause.[225] Where these overlap, distinguishing PMS-symptoms from perimenopausal symptoms can be difficult. The distinction is usually made on the basis of the cyclic symptom pattern of premenstrual worsening and post-menstrual abatement in PMS.[454] However, because menstrual periods become unpredictable, perimenopausal women may be less aware of the relationship of the symptoms to the menstrual cycle.[455] Under-reporting of premenstrual dysphoria in perimenopausal women has been observed, with significant rates occurring in women who reported an absence of premenstrual symptoms.[372]

When PMS co-exists with menopausal symptoms, management with hormone therapy (HT) is difficult as progestins are found to aggravate PMS symptoms and combined HT regimes frequently induce PMS-like symptoms in susceptible women.[456] A significant need exists for effective treatments that are safe and well tolerated for these symptoms in perimenopausal women.

8.2 Definition and diagnosis

Premenstrual syndrome is defined as the repeated occurrence of behavioural and somatic symptoms severe enough to impair a woman's social and work-related functioning during the luteal phase of the menstrual cycle.[457] While definitions and inclusion criteria vary significantly among studies due to the lack of a universally accepted and implemented diagnostic tool for PMS, the key elements of a diagnosis of PMS are i) symptoms consistent with PMS; ii) occurring during the luteal phase of the menstrual cycle; iii) assessed prospectively; iv) resulting in identifiable dysfunction in social or economic performance; and v) not due to another diagnosis. Disappearance of symptoms after menstruation is the key to diagnosis. Typically women have the same set of symptoms from one cycle to the next.[458] The current World Health Organisation's International Classification of Diseases (ICD-10) description of PMS has been criticised as "vague, fail(ing) to establish the degree of impairment, list(ing) only a few specific symptoms, and ..not require(ing) prospective confirmation of symptoms to make a diagnosis".[459]

A definition developed for research purposes by the University of California requires at least 1 of 6 behavioural symptoms (depression, angry outbursts, irritability, anxiety, confusion, social withdrawal) and 1 of 4 somatic symptoms (breast tenderness, abdominal bloating, headache, swelling of extremities). The symptoms must resolve within 4 days of the onset of menses, without recurrence until at least cycle day 13. They must occur in the absence of pharmacological therapy, hormone ingestion, drug or alcohol use and result in identifiable dysfunction in social or economic performance. The diagnosis is confirmed by means of at least 2 cycles of prospective ratings.[460] It has been suggested elsewhere that the definition must include the severity of symptoms, which differentiates clinically significant pathological PMS from the physiological discomfort of normal menstrual cycle changes.[461]

Whether all symptoms occurring in the premenstrual phase should be considered as parts of a single syndrome has been questioned, as there is no evidence that all symptoms share a common pathophysiologic basis.[462] Abraham, for example, used the term 'premenstrual tension syndrome (PMTS)' in recognition that PMS represents a group of syndromes which may have different pathophysiologies,[463,464] with the common occurrence of nervous tension. More recently, it has been proposed that future directions for clinically-relevant research should include identification of specific subgroups of menstrual-related syndromes.[465]

Premenstrual dysphoric disorder (PMDD) is a more severe form of PMS with a predominance of mood symptoms, the diagnosis of which requires i) the presence of at least 5 luteal phase symptoms, at least one of which must be a mood symptom (Appendix 6); ii) 2 cycles of daily charting to confirm the timing of symptoms; and iii) evidence of functional impairment. The symptoms must not represent the exacerbation of pre-existing depression, anxiety, or a personality disorder.[466]

8.3 Aetiology of PMS

The exact aetiology of PMS remains unknown, although several biological mechanisms have been proposed suggesting that disrupted homeostasis and deficient adaptation may be core underlying mechanisms,[465] with some consideration also given to genetic propensity and environmental inputs.[467]

Recent research suggests that symptoms of PMS occur only in predisposed women who have sensitivity to the normal hormonal fluctuations that occur in the late luteal phase of the

menstrual cycle. This is supported by observations of a lack of difference in levels of gonadal steroids between women with and without PMS.[468,469]

Ovulation, and the subsequent ovulation-related production of sex steroids by the corpus luteum,[470,471] are considered a necessary pre-condition for the development of the symptoms of PMS, which disappear in anovulatory cycles when a corpus luteum is not formed,[456,472,467]and when hormonal cyclicity ends.[462]

The enhanced responsiveness to normal fluctuations in hormone concentrations[469] in PMS sufferers may be due to neurotransmitter dysfunction.[473] Several neurotransmitters are influenced by ovarian steroids, or engage in reciprocal interactions with them.

Serotonin

The serotonergic system is involved in mood, sleep, sexual activity, appetite and cognitive ability. Serotonin concentrations vary throughout the menstrual cycle, with periovulatory and premenstrual levels being higher than at the start of menstruation.[474] The involvement of serotonin in the pathophysiology of PMS is supported by studies demonstrating altered serotonin metabolism in women with PMS,[462] as well as altered postsynaptic serotonergic responsivity,[472]and serotonin *deficiency,* which may enhance sensitivity to progesterone in PMS-sufferers.[475,476] Furthermore, SSRIs are efficacious in the treatment of somatic and mood symptoms of PMS/PMDD.[462] It has been suggested that the role of serotonin may be *modulating* role rather than *causal,* as there is little evidence for luteal phase-specific serotonergic dysfunction, but there is evidence for dysregulation of serotonin control of the hypothalamic-pituitary-adrenal axis in women with PMS.[477]

Noradrenaline

In healthy women, plasma noradrenaline has a cycle with peaks at ovulation and early luteal phase. In women with PMS, lack of NA cyclicity was observed in one study, with symptoms such as abrupt mood swings, impatience, nervousness, tiredness, weakness, apathy, and headache associated with decreased noradrenergic activity at ovulation and the luteal phase, and low oestradiol levels during the late luteal phase.[478] Treatment with antidepressants predominantly affecting NA transmission, however, are found to be less effective than SSRIs for PMS.[479]

Other neurotransmitters that may have relevance to PMS include GABA[470] and dopamine.

Dopamine

Dopamine receptor agonists are possibly the most effective treatment for premenstrual mastalgia.[480] It is interesting to note that *Vitex agnus-castus* (Chaste tree), found to be effective for the treatment of premenstrual irritability, mood alteration, anger, headache, breast fullness, and other menstrual symptoms including bloating,[481,482]has dopaminergic activity.[483]

Gamma (γ)-aminobutyric acid

Low GABA activity has been reported in women with PMS and low late-luteal- phase plasma concentrations in PMDD.[484] It has been postulated that GABA's role in the pathophysiology of PMS may be due to the interaction between GABA-ergic and serotonergic neurons, consistent with the serotonin hypothesis.[462] This theory gains support from the action of SSRIs of enhancing GABA function.[485]

Progesterone

The long-held view that PMS is due to a progesterone deficiency has been challenged by the lack of efficacy of progesterone treatment. In fact, progesterone administration has been reported to aggravate PMS in women after pre-treatment with a GnRH agonist for one month to abolish hormonal cyclicity.[486] Progesterone added to HT regimens triggers PMS-like symptoms,[487] with a dose-dependent increase in depression, loss of energy, loss of libido and mastalgia.[488] This led Smith and co-workers to conclude that PMS-sufferers are hypersensitive to normal endogenous post-ovulatory levels of progesterone.[488] Other reported symptoms of progesterone intolerance are bloating, mood swings, fatigue, depression, irritability, skin disorders, weight gain and anxiety. [456]

Oestrogen

An alternative hypothesis is that PMS symptoms are triggered by the pre-ovulatory peak in oestradiol or the postovulatory peak in progesterone, or both. Although not consistent with the absence of PMS during the oestrogen-dominated follicular phase, it has been noted that luteal phase administration of oestrogen provokes or aggravates PMS-like complaints[486,489] while an oestrogen-antagonist reduced PMS mastalgia.[490]

Other Factors

Other factors potentially implicated in the aetiology of PMS include

- thyroid indices, which were found to be more variable in women with PMS than in controls;[491,492]
- changes in circadian rhythms, found to be similar to those occurring in anxiety and mood disorders, with aberrant timing of the secretion of melatonin, cortisol and prolactin.[452]

8.4 Aetiology of PMS-like symptoms in Perimenopause

According to its defining characteristics and aetiology, PMS requires the presence of ovulatory cycles, which become increasingly infrequent as the menopause transition progresses. Such symptoms experienced by late-perimenopausal women may, therefore, be more appropriately termed 'PMS-like' symptoms. The aetiology of PMS-like symptoms during this phase may differ from during the normal reproductive years. However, it should also be noted that ovulatory cycles can occur in the absence of subsequent menstruation during the perimenopause,[55] so that their relationship to cyclic hormonal fluctuations may not be apparent to the women themselves. Increased sensitivity to normal fluctuations in ovarian hormones may continue to be responsible for the symptoms during the perimenopause, as evidence supports reduced hypothalamic–pituitary sensitivity to oestrogen feedback in perimenopausal women.[101,102,372,462] Alternatively, oestrogen excess has been hypothesised to be a possible cause for these symptoms in late perimenopausal women. This is supported by the recent observation of a premenstrual dysphoria ('luteal-out-of-phase' or LOOP) phenomenon, common in the early and late menopause transitions, where it appears that in the mid-luteal phase of the cycle, a new follicle is recruited, which may go on to ovulate in the perimenstrual period at the beginning of the next cycle. This is associated with high estradiol levels peri-menstrually which could give rise to PMS-like symptoms.[493]

The increased risk of developing perimenopausal depression in PMS-sufferers has led to the suggestion that these conditions represent expressions of the same underlying disorder, namely a vulnerability to changes in ovarian steroid concentrations.[372,462] In women previously reporting a history of PMS, it has been suggested that perimenopausal *depression* could be due to the elimination of follicular phase symptom remissions of

premenstrual dysphoria, as a consequence of the shorter follicular phase, resulting in a more persistent pattern of dysphoria.[372]

8.5 Complementary and Alternative Medicine use for PMS

Complementary medicine use is high among PMS-sufferers. It was found that 91% of women attending a specialist PMS clinic in the UK had used at least one form of complementary therapy for the management of their PMS symptoms, [494] with over 40% having used St John's wort. [495] As mentioned above, the evidence for conventional therapy is inadequate and controversial[495] for the treatment of PMS, particularly when these symptoms occur in conjunction with menopausal symptoms. Conventional medical options for the treatment of menopausal symptoms will be discussed in the following section.

Chapter Nine

Pharmacological Treatment Options

9.0 Introduction

This chapter of the Literature Review explores the pharmacological treatment options for menopausal symptoms, and concerns regarding their use. As the pharmacological mechanisms of the non-oestrogenic agents may have relevance to the phytomedicines selected for the RCT conducted, these are also examined.

9.1 Hormone Therapy

Current pharmacological treatment for the relief of menopausal symptoms primarily involves hormone replacement therapy (HRT), now more commonly referred to as Hormone Therapy (HT), which aims to compensate for the declining levels of ovarian hormones, particularly oestrogen and progesterone.

Despite the well-established benefits of HT, side-effects and long-term safety concerns deter many women. Non-oestrogenic pharmacological treatments available for menopausal symptoms also carry their own limitations. The efficacy of the various pharmaceutical agents for different menopausal symptoms helps to elucidate their pharmacological mechanisms in relation to these symptoms, and thereby informs the potential role of phytotherapeutic alternatives.

9.1.1 HT Use in Australia

By 1997, 60% of Australian women 55 years and over had used HT. It has predominantly been used short-term for the relief of the vasomotor symptoms,[496] as opposed to prevention of osteoporosis or cardiovascular disease, or post hysterectomy or oophorectomy.[79] However, due to its purported protection from chronic degenerative disease, HT use for long-term indications increased throughout the 1990's[497] although this dramatically declined following publication of findings from large-scale studies, linking it with adverse events (chapter 10).

9.1.2 Types of Hormone Therapy

The main types of HT used include oestrogen monotherapy (also known as unopposed oestrogen therapy), cyclic progestin therapy alone, combined oestrogen-progesterone, or the low dose oral contraceptive pill. More recently, testosterone has also been used and *tibolone*, which combines oestrogenic, progesterogenic and androgenic effects.[79]

Conjugated equine oestrogen (CEE) prepared from **pre**gnant **mar**es' ur**in**e (hence the name Premarin is also the most widely used oestrogen preparation worldwide. (See Appendix 7 for other preparations available in Australia.)

9.1.3 Symptoms Effectively Treated with HT

HT has well-established efficacy in providing relief from hot flushes and night sweats, which are the most common of the menopausal symptoms for which women initiate treatment.[275] Its benefits in retardation of bone loss and reversal of urogenital atrophy[498] are also well established. CEE is the form of oestrogen used in the majority of studies, although it is generally considered that others forms, including oestradiol and oestriol, offer comparable benefits. However, evidence for its use in other symptoms is inconsistent and even conflicting (Table 9.1).

TABLE 9.1 SUMMARY OF FINDINGS FOR HT IN DIFFERENT MENOPAUSAL SYMPTOMS

Symptom	***Evidence***
Vasomotor symptoms	77% decrease in hot flush frequency and 87% reduction in their severity with HT (Cochrane systematic review of RCTs).[499]
Sleep	ET effective[192,193,500] via 'domino effect', and in absence of vasomotor symptoms[193] Shorter mean sleep latency, longer period of REM sleep;[501] Reduced rates of cyclic alternating patterns of sleep in conjunction with decreased frequency of nocturnal arousals and improved sleep quality.[192]
Urogenital symptoms	ET: vaginal dryness, atrophy and dyspareunia[502,503] Systemic or local for recurrent cystitis and stress incontinence (Meta-analysis);[233] Systemic HT may aggravate urinary incontinence (observational study).[234]
Loss of libido	Androgens[503] or tibolone, (Livial®) effective; Androgens conventionally given with progestogens as concerns that testosterone may induce endometrial hyperplasia. [504]
Depression	Oestradiol (17β-oestradiol): Conflicting findings: effective in perimenopausal women (RCTs);[396,402] oestrogen alone, with androgens, but less effective with progestins.[401] *O*estradiol - no effect over placebo in *postmenopausal* women;[404] WHI: oestrogen-progestin not effective.[505] Most marked effect in *surgical* menopause.[506] ET may augment response to SSRI's.[507]
Anxiety	Tibolone Over 6-months, significant improvement (small study; not RCT).[508] Little influence of oestrogen-progestin regimens.[196]
Musculoskeletal pain	Oestrogen/Progestin improved Postmenopausal Interventions (PEPI) trial,[196] Evidence inconsistent
Other: tooth loss	ET associated with a reduced risk.[509]
headaches	Inconsistent findings: alleviation, aggravation or no change with HT.[510]
Postmenopausal Health Benefits	
Fracture risk, long-term	HT reduces bone turnover, preserves bone mass and increases bone mineral density (BMD);[511] reduces fracture risk with continuous use.[505] (up to 35% in hip and vertebral fractures)[511] Continuous CEE-medroxyprogesterone acetate (MPA): 44% reduced risk of all fractures (WHI).[505,512]

Cardiovascular, Cerebrovascular disease and Cognition	Oestrogens reduce risk of vascular disease:[513] lower low-density lipoproteins (LDLs), increase high-density lipoproteins (HDLs); favourable effects on endothelial function.[513] Beneficial effects on cognition in symptomatic postmenopausal women,[514] stroke risk, dementia and Alzheimer's disease[515] now called into question by findings of large-scale studies.[505,516,517] No evidence of benefit in established vascular disease, or healthy women; may worsen outcomes in CVD and CHD.[518]

9.1.4 Advantages and Limitations of HT

Despite the incontrovertible efficacy of HT for the treatment of vasomotor symptoms of menopause,[229,519,520] many women find it unacceptable because of contraindications, associated risks, side-effects, lack of effectiveness for other symptoms or because of a natural reticence to taking drug therapy for a 'natural event'.[521] Different benefits and side-effects are associated with each form of HT and route of administration (Table 9.2). Routes of administration are discussed in Appendix 8.

9.1.5 Adverse Events Reported in Large-scale studies

The relative benefits and risks of long-term HT use have become more controversial since the publication of the findings of three large-scale studies between 2002 and 2007, linking HT use to serious adverse events such as increased risk of female reproductive cancers, venous thromboembolism, cardiovascular disease (CVD) and stroke. Cancer risks are said to increase with continued use of HT for 5 years or more. However, the risk of venous thrombosis is relevant even with short-term use, as most events occur during the first two years of HRT use.[496]

TABLE 9.2 ADVANTAGES AND LIMITATIONS OF DIFFERENT HORMONE THERAPY PREPARATIONS

Preparation	*Advantages*	*Limitations*
Oestrogen preparations	Benefit for vasomotor symptoms, retardation of bone loss and reversal of urogenital atrophy.[498]	Unopposed oestrogen increases risk of endometrial hyperplasia and endometrial cancer (so not usually prescribed for women with an intact uterus)
Combined oestrogen/progestins	Counteracts the proliferative effects of oestrogens on endometrial tissue. [497]	Short-term side-effects: withdrawal bleeding and breakthrough bleeding occur in 40-90% of users of sequential regimens. [522]
Progesterone	Megesterol acetate, safe in history of breast or uterine cancer with < 5 years continuous use[523]; decreases hot flushes approx 80% [235,321]	Concerns about increased risk of breast cancer.[524] Continuous progestins -breakthrough vaginal bleeding,[79] weight gain and bloating; may also lead to thromboembolitic events.[525] (see also appendix 9).
Tibolone	Relief of menopausal symptoms; improves libido. Improves BMD;	May lower HDL cholesterol Androgens conventionally combined with

	lower risk for breast cancer - blocks oestrogen metabolism in breast; does not increase mammo-graphic density.[79,526-528]	progestogen as concerns that testosterone may induce endometrial hyperplasia. [504]

Cardiovascular and Cerebrovascular disease

The HERS, a secondary prevention trial with combined oestrogen and progesterone for 6.8 years in women with established CVD, and an unblinded open-label follow-up (HERS II), found no benefit for either primary of secondary prevention of cardiovascular disease.[235] In fact, they observed a small increase in the risk of CVD and cardiovascular accident (stroke), venous thromboembolism and gall bladder disease.[529] Increased risks of CVD and strokes were also found in the Women's Health Initiative (WHI), a chronic disease (primary) prevention trial in older postmenopausal women, for combined oestrogen- progesterone.[512] Oestrogen alone did not increase the risk of CVD although it did increase stroke risk similarly to combined oestrogen-progesterone.[505]

Breast cancer

Administration of oestrogen monotherapy has long been known to increase the risk of oestrogen-dependent cancers. Combined oestrogen-progesterone was also found to increase breast cancer risk in the WHI, one arm of which was terminated prematurely due to risks of invasive breast cancer exceeding the stopping boundary after a mean of 5.2 years of follow-up. The breast cancers were diagnosed at a more advanced stage compared with placebo use. The percentage of women with abnormal mammograms was also substantially increased, suggesting not only that combined oestrogen plus progestin may stimulate breast cancer growth,[530,531] but also that it may hinder breast cancer diagnosis.[531]

This finding was subsequently borne out by the U.K. Million Women's Study (MWS), a longitudinal observational study of 1,084,110 women aged 50-64 years. The effect was observed for combined oestrogen-progesterone, oestrogen-only preparations (5 additional cancers per 1,000 users) and tibolone.[530] This was translated to an estimated 20,000 extra breast cancers due in UK women aged 50-64 years in the previous decade due to use of HT, of which 15,000 were associated with oestrogen-progestogen.[530]

Endometrial and Ovarian cancers

When increased oestrogen is accompanied by low progesterone, there is a potentially increased risk of endometrial hyperplasia and possibly endometrial cancer. The MWS also reported an increased risk of endometrial cancer with oestrogen and tibolone. In oestrogen-progesterone preparations, progestogens protected against the adverse effect of oestrogen on the endometrium, with a greater effect observed with longer duration of use of progestogen.[532] An increased risk of breast and endometrial cancers had previously been established with oestrogen-monotherapy.[533]

HT use was also associated with an increased ovarian cancer risk in the MWS, but not WHI, for both incident and fatal ovarian cancer. On the basis of this, it was estimated that in the UK between 1991 and 2001, there were 1,300 additional ovarian cancers and 1,000 additional deaths from ovarian cancer as a result of HT use.[534] A recent meta-analysis has confirmed the increased risk of ovarian cancers with HT use, finding a stronger association with ET than oestrogen-progestin regimens.[535]

Cognitive function

A sub-study within the WHI, the WHI memory study (WHIMS) demonstrated a lack of preventive effects for mild cognitive impairment and increased the risk for dementia when HT (oestrogen and progesterone or oestrogen-monotherapy) is commenced in postmenopausal women aged 65 years or older.[505,517,516] Greater adverse effects were noted for women with lower cognitive function at the start of treatment.[516]

Limitations of these studies

A major criticism made of the HERS and WHI, however, is that the women enrolled in these studies were not representative of the typical population in whom HT is commenced, in terms of age, reproductive status and pre-existing medical history. They were aged, on average, in their mid 60's, without menopausal symptoms, and many years past the menopause. Many had previous HT use. As such, these findings may not be applicable to symptomatic peri- or early postmenopausal women who use HT for short periods and at doses lower than those used in the trials. In addition, the WHI was not truly a primary prevention study due to the age and pre-existing medical conditions of many of those enrolled: one-third were overweight and another third obese; 40% were past smokers, 10.5% current smokers and about one-third were hypertensive.[536]

The issue of clinical significance versus statistical significance has also been raised in regards to the findings of the WHI, where the actual increase in breast cancer risk was 8 per 10,000 woman years[536] (Table 9.3).

TABLE 9.3 ABSOLUTE EXCESS RISKS PER 10 000 PERSON-YEARS ATTRIBUTABLE TO OESTROGEN PLUS PROGESTIN IN WHI[537]

Outcome	*Absolute excess risks per 10,000 person-years*
Coronary Heart Disease (CHD)	7 more
Stroke	8 more
Pulmonary embolisms	8 more
Invasive breast cancer	8 more
Colorectal cancer	6 fewer
Endometrial cancer	1 fewer
Hip fracture	5 fewer
Vertebral fracture	6 fewer

The MWS looked retrospectively at HT use and subsequent breast cancer, but not at types and regimens of HT used. As an observational study, it was therefore subject to selection bias. The large sample size would amplify any small error that occurred as a result of this bias. However, a recent study of new breast cancer events following HT in breast cancer survivors found a clinically and statistically significant increased risk,[538] consistent with findings from a meta-analysis of RCTs examining recurrence of breast cancers with HT use.[539]

The results of these studies are important and have impacted greatly on subsequent HT use and prescribing. Their impact on CAM use is discussed in the following section.

9.1.6 Contraindications to HT

Apart from unwillingness to risk or tolerate adverse events, a woman may not be eligible to use HT because of its contraindications (Table 9.4).

TABLE 9.4 CONTRAINDICATIONS TO HT[504]

Absolute contraindications
Previous oestrogen-dependent neoplasms: breast, endometrial or ovarian cancers Impaired liver function Ischaemic heart disease
Relative contraindications

Diabetes
Hypertension
Risk factors for ischaemic heart disease
Past history of thromboembolism
Significant uterine fibroids

9.2 Non-oestrogenic options

The limitations of HT have stimulated increased interest in pharmacological alternatives, especially for breast cancer survivors who are more likely to experience hot flushes and vaginal dryness due to tamoxifen® use, premature menopause from chemotherapy and/or denial of HRT.[540]

Oestrogen and progesterone strongly interact with a number of neurotransmitters. This has led to a range of non-oestrogenic treatments for hot flushes, including compounds that target various non-oestrogen components of the vasomotor response. These include agents acting via the noradrenergic or dopaminergic systems, the most promising being the anti-depressant selective serotonin reuptake inhibitors (SSRIs)[197] and serotonin and noradrenaline reuptake inhibitors (SNRIs). Other pharmacological non-oestrogenic agents include the antihypertensive, clonidine, and the anti-convulsant, gabapentin (Table 9). However, all of these, with the exception of melatonin, are associated with undesirable side-effects or safety concerns (Table 9.5).

9.3 Conclusion

Current pharmaceutical treatments for menopause-associated vasomotor symptoms include HT, which has been the mainstay for the management of these symptoms for more than 50 years, and prescription medications developed for other indications. All of these are associated with undesirable side-effects or risk of serious adverse events. The publicity surrounding findings from large-scale studies such as the WHI and the MWS substantially decreased the use of HT. Because many women now seek to avoid hormone therapy, there is a significant unmet need for additional therapies that have been validated by results from controlled clinical trials, and are safe, efficacious and well- tolerated by symptomatic menopausal women.

TABLE 9.5 PHARMACOLOGICAL ALTERNATIVES TO HT: ASSOCIATED BENEFITS AND RISKS

Substance & Definition	*Advantages*	*Evidence*	*Concerns/Limitations*
Selective oestrogen receptor modulators (SERMs) (act in a tissue-specific manner, as oestrogen receptor agonists in some tissues and as oestrogen-receptor antagonists in others)	May dissociate favourable oestrogenic effects on the bone and cardiovascular system from unfavourable stimulatory effects on the breast and endometrium.[541]	Raloxifene is safe for the endometrium and holds promise for breast cancer prevention; tamoxifen has been associated with fewer fatal myocardial events than placebo in clinical trials.[542]	Raloxifene & tamoxifen induce hot flushes
B*io-identical hormone therapy -* derivatives of plant extracts chemically modified to be exact chemical molecular copies of endogenous hormones, (typically, oestriol, oestrone, oestradiol, testosterone, progesterone, and sometimes DHEA.[543] Compounded, often on the basis of saliva tests or blood sera, by compounding chemists according to individually customised prescriptions.	Believed to provide the benefits of HT while attenuating the risks to blood lipids and vasculature, breast cancer and blood clots.[544]	Scientific evidence from well controlled studies to support these claims is lacking.	Concerns about the ratios of bio-identical oestrogens in many preparations[543] and the safety of oestriol on cardiovascular health, especially at doses used.[545] Compounded preparations not produced according to federal Good Manufacturing Practice.[546]
Non-oestrogenic Options			
Selective serotonin reuptake inhibitors (SSRIs) and Serotonin and noradrenaline reuptake inhibitors (SNRIs) (anti-depressant medication)	The effects on hot flushes are seen within a few weeks of commencement, earlier than for psychiatric symptoms, and occur regardless of co-existing depression or anxiety, suggesting a separate mechanism.[547]	Venlafaxine: dose-related effects at three doses (37.5, 75 and 115 mg/day) Paroxetine: reduced frequency x severity of 54-65% vs 19-38% for placebo.[547,548]	The highest and most effective doses caused frequent side-effects of nausea, decreased appetite, dry mouth and constipation.[540] SSRI's commonly cause nausea, anxiety, insomnia, headache, restlessness and sexual dysfunction.[189]
Clonidine (centrally-acting adrenergic agonist primarily used as an anti-hypertensive- proposed mechanism for relief of hot flushes is reduction of peripheral vascular reactivity.[549])	Of modest benefit in controlling hot flushes (1 flush per day);[549] Used in tamoxifen users.	Reduction in frequency of hot flushes of 24%-44% vs 14%-27% for placebo, or a reduction in the combined frequency x severity score of 29%-56% vs 16%-30% for placebo.[550,551] Long-term efficacy (> 8 weeks) not established. Results of RCTs inconsistent.	Side-effects: incidence of increases in the higher doses, -limits its practicality as a therapeutic agent - drowsiness, dry mouth, sleep disturbances and gastrointestinal upsets.[151]

Gabapentin (λ-amino butyric acid analogue - anticonvulsant for seizures and neuropathic pain. (Not a GABA agonist, does not inhibit GABA uptake and acts independently of GABA receptors.[321])	May increase activity of neurotransmitters in hypothalamus as a consequence of up-regulation of the gabapentin binding site from oestrogen withdrawal[552]	At 900mg/day, reductions in hot flush frequency were 44-45% vs 15-29% with placebo, no significant effect at 300mg.[552]	Side-effects: somnolence, fatigue, dizziness, rash, heart palpitations and peripheral oedema.[549]
Supplemental Dehydroepiandrosterone (DHEA) (DHEA and its sulphate ester are androgen pro-hormones produced by the adrenal glands and ovaries.)	Improved psychological well-being and the sleep pattern in clinical trials[553,554]	In *postmenopausal* women 25mg/day long-term modified circulating levels of androgens and progestins via modulation of age-related changes in adrenal function.[555] Sustained improvement in overall menopause-related symptoms, vasomotor and psychological symptoms.[556-558] No improvement over placebo on mood, memory, cognition, well-being or libido in 60 *perimenopausal* women 50mg/day. [559]	Undergoes significant first-pass metabolism, so need high doses, which increases risk of side–effects: hirsuitism, acne, increased sebum (short-term); dyslipidaemia, hepatic dysfunction (long-term).[450]
Melatonin		Significant improvement in mood and morning depression in 42-62 year-old women following evening administration.[560] 3mg improved thyroid function, returned gonadotropins towards more juvenile levels, and had some antidepressant activity in perimenopausal and postmenopausal women.[561]	

Chapter Ten

Complementary/Alternative Medicine use for Menopausal symptoms

10.0 Introduction

This brief chapter outlines the impact of the publication of results from the large-scale studies on HT use, and the prevalence of CAM use among menopausal and midlife women.

10.1 HT Avoidance

A 1997 Swdish study reported a 20% discontinuation of oestrogen from fears of cancer (9%) or other side-effects (14%), while the most common reasons cited for *refraining* from HT use were minor climacteric symptoms (27%), fear of cancer (9%) or side-effects (15%) and the opinion that the menopause is a natural process (20%). Some others gave lack of effectiveness as the reason for non-use.[521]

In 2002, in the wake of the publication of results from the Womens' Health Initiative (WHI) and the Million Women's Study (MWS), a dramatic decline in HT use ensued. The rates of discontinuation varied from country to country and for different user-groups. In the Netherlands, the MWS had a bigger impact than the WHI (Table10.1).

TABLE 10.1 HT DISCONTINUATION RATES FOLLOWING ADVERSE PUBLICITY FROM LARGE – SCALE STUDIES

Country (Study Authors)	*Years/Dates compared*	*User-group*	*Rate of Decline*
New Zealand (Lawton *et al*.)[562]	2000-2002 vs 6 months after publication of WHI	HT users from general practices	58% initially 40% still discontinued at time of survey
USA, Boston (Kelly *et al*.)[563]	The first half of 2002 to the first half of 2004	Women aged 50 years and over	57%
USA (Hersh *et al*.)[564]	January-June 2002 vs January-June 2003	Combined oestrogen/progesterone users	66%
		Oestrogen-only (Premarin) users	33%
Netherlands (Faber *et al.)*[565]	2000 vs 2003 post MWS publication	45-45 year-olds 50-54 year olds 55-59 year olds	42% 46% 37%
Italy (Parazzini *et al*.)[566]	1997-8 vs 2002-3	All current HT users	35%
Germany (Clanget *et al*.)[567]	Prior to publication vs 7 months following WHI and HERS II publication	Combined HT users Overall HT users (Aged 45-65)	41% 16%

It is unclear whether fears concerning HT use generated by publication of these large-scale studies led to an increased use of CAM for menopausal symptoms. According to the Boston study,[563] there was no correspondingly significant increase in *post*menopausal use of black cohosh (2.0%) or soy (2.0%) in 2004. However, Schonberg and Wee[568] found that 82% of women who stopped HT re-experienced some menopausal symptom, for which 48% tried at least one CAM, most commonly soy or black cohosh. Nonetheless, the rate of use of CAM is high among midlife and menopausal women.

10.2 Prevalence of Use of CAM in Midlife/Menopausal Women

Surveys of CAM use by midlife women do not always specifically focus on their use for *menopausal* symptoms. In a 2003-2004 Australian study investigating CAM use for the alleviation of menopausal symptoms in 45-65 year-old women, 53.8% were reported to have visited a CAM practitioner and/or used a CAM product during the previous year.[569] This was consistent with findings from the SWAN in 2003 that 52% of midlife women aged 48–52 used CAM, although not exclusively for menopause-related symptoms.[570] However, the prevalence of CAM use during menopausal years was found to be as high as 82.5% in women aged 47-67 years in South East Queensland in 2004. Prior to the publication of the major studies that generated fears about HT use, reports of CAM use in English-speaking countries ranged from 45% to 79% in mid-life women, and 46% to 76.1% for menopause symptoms (Table 10.2).

TABLE 10.2 PREVALENCE OF USE OF CAM IN MIDLIFE/MENOPAUSAL WOMEN

Source, Study year/s & Authors	Demographic	% CAM use	Modality/Complementary Medicine
Australia: Sydney, 2003-2004 Van der Sluijs *et al*.[569]	Women aged 45 - 65 years Menopause symptoms: menopause & general practice clinics; government agencies	53.8%	Naturopathic practitioner visits 7.2% Acupuncturist-visits 4.8% Soy 25.4% Evening primrose oil 18.4%
USA, 2003 Gold *et al*. [570]	Women aged 48 - 52 years; cross-sectional analyses of midlife women in the longitudinal Study of Women's Health Across the Nation [SWAN]	52.7%	Black cohosh 6.4% DHEA 1.3% Dong quai 2.8% Flaxseed/flaxseed oil 10.6% Ginkgo biloba 6.8% Ginseng 5.8% Glucosamine 15.9% Mexican yam/progesterone cream 2.9% MSM 3.8% SAMe 1.1% Soy supplement 16.6% St John's wort 2.2% Acupuncture 4.7% Energy healing 4.8% Tai chi 3.1% Yoga 12.8% Self-help group 5.4%
USA Published 2003 Mahady *et al*. [571]	Women aged 40-60; survey of outpatients at University of Illinois Chicago clinics using botanic dietary supplements (herbs)	79% 36.5% daily use	Soy 42% Green tea 34.7% Chamomile 20.8% Ginkgo 20.5% Ginseng 18% Echinacea 15.4% St John's wort 7.3%
USA: Washington, 2002 Newton *et al*. [572]	Menopausal aged women and menopausal symptoms; randomly sampled through primary care providers	76% of 45-65 year-old women 22% for meno-pausal symptoms	Stress management 43.1% OTC CAM 37% Chiropractic 31.6% Massage therapy 29.5% Dietary soy 22.9% Acupuncture 10.4% Naturopathy or homeopathy 9.4% Herbalist 4.6%

TABLE 10 PREVALENCE OF USE OF CAM IN MIDLIFE/MENOPAUSAL WOMEN CONT

Source, Study year/s & Authors	*Demographic*	*% CAM use*	*Modalities*
USA, 2002 Brett *et al*. [573]	Midlife women aged 45 -57 years; obtained from the 2002 National Health Interview Survey	45% within previous 12 months	Biologics (e.g. herbs) or mind-body (e.g.biofeedback) 25%, Body work (e.g.. massage and chiropractic) 15% Herbs 26.4 Relaxation 21.6 Chiropractic 9.4 Yoga/Tai chi/Qi gong 8.5 Massage 7.6 Vitamins 3.4 Homeopathy 2.7 Acupuncture 1.7 Energy healing/ Reiki 1.2 Naturopathy 0.4
Australia: South east Queensland, 2001 Gollschewski *et al*. [574]	Women aged 47-67 years postal questionnaire on menopause experience – ever used and use in previous month	82.5% (not specified ever-use or previous month)	Nutrition 67% Phytoestrogens 56% Herbal therapies 41% Evening primrose oil 34% Vitamin E 29% Ginseng 13% Unspecified CAM 25% Therapeutic techniques 83%
UK: London, 2001 Vashisht *et al*.[575]	Women aged 49-59 years; prospective questionnaire; London menopause clinic – use for menopause symptoms	66% regular users (68.5% ever used)	Phytoestrogens 1% (6.5%) Chinese herbs 1% (10%) Wild yam 3% (9.5%) Dong quai 2.5% (9.5%) Ginkgo 7% (16.5%) Black cohosh 1.5% (5%) Homeopathy 5% (19.5%) Vitamins 32.5% (50%) Acupuncture 3.5% (16.5%) Hypnosis 0.5% (6%) Spiritual Healing 4.5% (11%) Massage 0.5% (1.5%) Reflexology 0.5% (1.5%) Natural progesterone cream 4% (11.5%) Evening Primrose oil 0.5% (1.5%)
USA: Florida, Minnesota & Tennessee, 1997-8 Keenan *et al*. [576]	Women aged 45+; menopausal symptoms cross-sectional telephone survey	46% regular use	20.8% CAM only (includes chiropractic, homeopathy, naturopathy, herbalism, TCM, acupuncture, increased physical activity, dietary supplements, soy foods/beverages, mid-body techniques (e.g. biofeedback, yoga[576]) and self-help groups). 25.2% CAM + conventional therapies

These findings do not suggest that CAM use in this particular demographic has increased dramatically since the publications of results from WHI, the MWS and HERS II.

However, one limitation of comparing studies of CAM use is the different modalities included across studies; for example, some include support groups, [570,576] educational interventions, exercise, prayers for healing and dietary modification.[570] Another limitation is the different sampling methods: clinic samples of women presenting with bothersome

symptoms could be expected to generate higher figures than general population samples. Comparison is also restricted by the differing inclusion criteria and instruments for measuring menopausal symptoms and their severity.

Nonetheless, even the most conservative estimates suggest that close to half of menopausal and midlife women in Western countries use CAM. If this trend continues, it will constitute an increasingly significant demand for CAM by this user-group, given that 50 million women in the established market economies (Australia, Canada, Japan, New Zealand, USA and Western Europe) are expected to reach the menopausal age between 2000 and 2030, according to projections of the world's population by the World Bank's 1993 World Development Report (p7).[53] This is without taking into consideration the demand by women in developing nations.

Despite such widespread use, however, scientific knowledge from clinical trials regarding the effectiveness of CAM is still limited. The following sections will review the current evidence for phytotherapeutic interventions used in the practice of Western herbal medicine.

Chapter Eleven

Phytotherapeutic Interventions for Menopausal Symptoms

11.0 Introduction

This chapter contains a review of the literature relating to phytomedicines currently administered for menopause-related symptoms in the Anglo-American practice of phytotherapy. Despite the variety of phytomedicines used in the treatment of menopausal symptoms in the Western tradition, rigorous evidence from clinical trials in this context is still limited.

11.1 Phytoestrogens

The phytoestrogen-containing plants have received the most attention in the scientific arena, with relatively little research conducted on other herbs for menopause.[577] However, concerns have now been raised over the safety of long-term use of some phytoestrogens and phyto-SERMs (defined below), specifically on breast and endometrial tissue proliferation.[578,579]

'Phytoestrogens' have been defined as plant-derived diphenolic substances with a chemical structure similar to oestrogen that enables them to bind to both alpha (ERα) and beta (ERβ) oestrogen receptors.[580] Phytoestrogens might act as anti-oestrogens (oestrogen antagonists) or oestrogen agonists, depending on the levels of endogenous oestrogens or other competitive receptor ligands.[581] Thus, their oestrogen-like effects could be predominant in the oestrogen-depleted environment of postmenopause.

11.1.1 Classes of phytoestrogens

The main classes of phytoestrogens are isoflavones, coumestrol and lignans. Isoflavones such as daidzein and genistein are found in highest concentrations in soybeans and soy products. In soybean itself and most soy products, genistein and its beta glycoside, genistin, constitute about 50-55% of total isoflavone content, compared with 40-45% for daidzein/daidzin and 5-10% for glycitein/glycitin.[582] Isoflavones are also found in *Trifolium pratense* (red clover) along with coumestrol. Coumestrol also occurs in alfalfa sprouts, clover sprouts, soy sprouts and split peas.[287,583] Lignans such as secoisolariciresinol diglucosides are found in linseeds (flaxseed) and whole grains (Appendix 10, Table A10.1).[287,583]

11.1.2 Mechanisms of Action

There are several possible mechanisms by which phytoestrogens may exert their effects, including interaction with oestrogen receptors,[584,585] displacement of oestrogens from sex hormone binding globulin,[586-588] regulation of oestrogen metabolism[589,590] and inhibition of aromatase (oestrogen synthetase).[591] While the condition for phytoestrogen activity in the case of lignans is completely dependent on colonic bacteria that convert the precursor, secoisolariciresinol diglucoside, into enterodiol and enterolactone,[592] isoflavones are inherently oestrogenic and may become further activated by conversion to equol, a bacterial metabolite of daidzein in the gastrointestinal tract.[593]

11.1.3 Oestrogenic potency of phytoestrogens

The oestrogenic potency of all the xenobiotic oestrogens, however, is very low compared with that of endogenous oestrogens.[594,595,596] Genistein transactivates ERα and induces oestrogenic effects with approximately 10^3 – 10^4 less potency than oestradiol.[595,596] Similarly, for daidzein and two of its metabolites, equol and *O*-desmethylangolensin, binding affinity to ERα was found to be 10^3 – 10^4-fold lower than for the endogenous oestrogen oestradiol.[597] [S-equol has an affinity for ERβ nearly as high as that of genistein.[595]] Nonetheless, the oestrogenic potency of phytoestrogens is believed to be significant enough, especially for ERβ, that they may trigger many of the biological responses that are evoked by the physiological oestrogens.[580] After a high soy meal, serum isoflavone concentrations can reach low micromolar levels,[598,599] exceeding postmenopausal total oestrogen concentrations by approximately 10^3, although free serum genistein levels return to baseline after twenty-four hours.[600]

11.2 PhytoSERMS

Some phytoestrogens are believed to have selective oestrogen receptor modulator activity, and hence have been termed 'phytoSERM'. Like the selective oestrogen receptor modulator (SERM) drugs, they may have a selective pattern of oestrogen agonist-antagonist activity depending on the target tissue. The soy isoflavone, genistein, preferentially binds to ERβ, with a 7-30 fold greater affinity for ERβ than for ERα.[601]

11.3 ***Glycine max* L.** (Soybean, Family: Fabaceae)

11.3.1 Research on Soy for Menopausal Symptoms/Hot flushes

Evidence of benefit in hot flushes has been derived from clinical studies on soy, in the form of soy isoflavones,[602] a standardised soy extract,[603] soy protein,[76,604,605] soy foods,[606] daidzein-rich isoflavone aglycones[607] and genistein.[608,609] Genistein also significantly improved Kupperman Index (KI) scores in an open RCT, compared with baseline and with placebo, $p < 0.05$, after 12 months.[610] Another RCT with a soy isoflavone extract (total of 50 mg genistin and daidzin per day) showed a positive trend that did not reach statistical significance, $p <= 0.08$.[611] However, findings from soy studies in this context have been conflicting, with other RCTs showing improvements for the control group as well as soy flour,[612] isoflavone supplementation,[613,614] soy protein,[615,616] and soy beverage (Table 11.1).[617,618]

TABLE 11.1 RESULTS OF SOY TRIALS FOR MENOPAUSAL SYMPTOMS

Authors	*N*	*Intervention*	*Comparator*	*Study length*	*Findings*
Han *et al,*[602] 2002	80	100 mg soy isoflavones	placebo	4 months	KI scores significantly decreased from baseline $p < 0.01$, and between groups, $p < 0.01$.
Faure *et al,*[604]*2002*	75	soy IF extract (genistein/ daidzein 70 mg/day)	placebo	16 weeks	61% reduction in HFs versus 21% placebo ; Responders 65.8% vs 34.2%, $p < 0.005$
Scambia *et al,*[603] *2000*	39	standardised soy extract (SOYSELECT) 50 mg/day IF	placebo 6wks CEE added for next 6wks	12 weeks	At 6 wks, soy decreased frequency of HFs, $p < 0.01$ compared to placebo, and severity on GCS, $p < 0.001$.
Albertazzi, *et al*[76] 1998	104	soy powder 60g (IF 76 mg)	casein powder	12 weeks	HF frequency improved with soy vs casein, $p < 0/01$). 31% reduction with soy vs 31% with casein.
Washburn, *et al*[605] 1999	51	soy protein, 20 g (POs 34 mg/day)	placebo	6 weeks	Improved severity of HFs with soy vs placebo, $p < 0.001$.
Dalais, *et al*[606] 1998	52	soy or linseed diet	wheat diet	24 weeks	Reduced rate of HFs with linseed diet 451%), wheat diet (51%), but not soy.
Khaodhiar, *et al*[607] 2008	190	daidzein-rich IF, 40 or 60 mg/ day	placebo	12 weeks	At 12 weeks, HF frequency was reduced by 52% with 40-mg DRI, 51% with 60-mg DRI, and 39% with placebo, $p = 0.07$ and $p = 0.09$ vs placebo.
Crisafulli, *et al*[608] 2004	90	genistein (54 mg/day).	placebo and oestrogen/ progesterone (HT)	12 months	HFs decreased with genistein vs placebo at 3, 6 months and 12 months ($p < 0.01$). Improvement with HT greater than genistein at all times, $p < 0.05$.
D'Anna, *et al*[609] 2007	247	genistein (54 mg/d)	placebo	12 months	Genistein significantly outperformed placebo on HFs at all intervals from the first month, $p < 0.001$.
Sammartino, *et al*[610] 2003	70	genistein 36 mg/day	placebo	12 months	Improved KI score with genistein vs placebo, $p < 0.05$.
Upmalis, *et al*[611] 2000	177	soy extract (50 mg/day IF)	placebo	12 weeks	Between-group differences in HFs at week 6, $p = 0.03$, but not significant at week 12, $p = 0.08$. Significant reduction in severity of HFs with soy, $p = 0.01$
Murkies, *et al*[612] 1995	58	soy flour, 45 g/day	wheat flour 45 g/day	14 weeks	No between group differences. Reduced HFs and general symptoms in both groups, $p < 0.05$.
Secreto, *et al*[613] 2004	262	80 mg of soy IF +/- 3 mg of melatonin	placebo melatonin	12 weeks	No significant difference between groups. Median percent reductions on GCS of 39% for IF + MEL, 38% for IF alone, 26% for MEL and 38% for placebo.
Knight, *et al*[614] 2001	24	soy IF powder beverage 60 g/day (IF 134 mg/day)	casein powder	12 weeks	No between-group differences. Improved flushing frequency in both groups.
Burke, *et al*[615] 2003	241	soy drink, IF, 42 mg/day or 58 mg/day	Soy drink with IF removed	96 weeks	No between-group differences. Improved HF frequency and severity in all groups.
Van Patten, *et al*[617] 2002	157	soy beverage, IF 90 mg/day	placebo	12 weeks	No between-group differences. Improved HF frequency in both groups.
St Germain, *et al*[616] 2001	69	soy protein IF 80 mg/day or 44 mg/day	placebo (whey powder)	24 weeks	No between-group differences. Significant decline in HF frequency ($p = 0.0003$) and night sweat ($p = 0.0007$) in all groups.
Kotsopoulos,*et al*[618] 2000	94	soy supplements 118 mg IF	casein placebo	3 months	No significant between-group differences on menopausal symptoms.

IF = isoflavones DRI = Daidzein-rich isoflavones HF = hot flushes
MEL = melatonin GCS= Greene Climacteric Scale KI = Kupperman Index

11.3.2 Possible reasons for conflicting findings regarding hot flushes

It has been suggested that these contradictory findings may reflect the diverse supplement regimens studied,[601] the range of exposure measures used (urinary or serum isoflavone levels versus intake of soy products/foods/isoflavones), and/or the differing amounts of products consumed or dosages administered.[582] In addition, variations in the bioavailability of genistin may be at least partially responsible for conflicting findings. Processing has been shown to reduce the isoflavone content of some soy proteins by as much as 80%,[619] thereby affecting the oestrogenicity[620] as well as circulating (and probably also target tissue) concentrations of genistein aglycone (the biologically active form).[621] It is therefore possible that some of the isoflavone regimens tested have failed to achieve adequate free genistein concentrations in some subjects.[601] There is also considerable inter-individual variation in gut metabolism of genistein and daidzein,[593] and it has been suggested that “bacterio-typing" individuals according to their capacity to form equol may hold the clue to the effectiveness of soy protein diets in the treatment or prevention of hormone-dependent conditions.[593] As suggested by the recent study on daidzein-rich isoflavone aglycones,[607] the type of isoflavone may be the determining factor in the effectiveness of soy supplementation for alleviating hot flushes. High genistein-containing isoflavone supplements (providing more than 15 mg genistein, calculated as aglycone equivalents, per treatment) were also found to consistently report a statistically significant decrease in hot flush symptoms according to a review of 11 studies.[297] Additionally, the failure of some studies to reach significance over placebo/control, may reflect the modest benefit of soy isoflavones that is expected in relation to hot flushes.[601]

11.3.3 Other benefits of Soy in post-menopause

In addition to potential benefits on menopausal symptoms, soy has been shown to favourably affect cardiovascular risk markers such as total cholesterol, low-density lipoprotein (LDL) cholesterol,[605,622,623] diastolic blood pressure,[605] high density lipoprotein (HDL) cholesterol and triglycerides[624] and vascular endothelium.[625] Studies have also shown benefits on bone metabolism,[581,626,627] with some additional support from epidemiological studies from China and Japan for a positive relationship between bone density and soy intake.[626,628] The preventive effects of isoflavones on bone loss in early postmenopausal women were shown in a 1-year RCT to depend on an individual's equol-producing capacity.[629] Thus, dietary or supplemental soy may confer additional advantages for the postmenopausal woman at greater risk than her premenopausal counterpart of CVD and osteoporosis.

11.3.4 Soy Safety Concerns

However, because of the oestrogen-like effects exerted by isoflavones under some conditions, concerns have arisen that they may potentially promote breast and endometrial cancers.[578,579] This is discussed further below. Uterotrophic effects were shown in rats fed genistein at doses of 750 mg/kg.[630,631] In an RCT with 376 healthy postmenopausal women with an intact uterus, long-term treatment (up to 5 years) with soy phytoestrogens (150 mg of isoflavones per day) was associated with a significantly higher occurrence of endometrial hyperplasia than with placebo (3.37% vs. 0%), although no cases of malignancy were detected by endometrial histology from biopsies. However, no impact has been shown of soy isoflavones on endometrial proliferation in other clinical studies.[609,610,632,633] Also of some concern is the potential anti-thyroid/goitrogenic effect of soy, especially in an iodine-deficient environment,[634,635] and possible exacerbation of autoimmune thyroid disease with high soy intake.[636] However, this appears unlikely given the high dietary levels required.

11.3.5 Soy consumption/supplementation and breast cancer

Soy was initially investigated for its potential to *reduce* cancer risk, particularly breast cancer.[637] This is supported by epidemiological evidence showing that among Asian women, higher soy intake is associated with a lower breast cancer risk,[638-642] (nearly a one-third reduction).[639] The findings that soy isoflavones exert anti-oestrogenic effects, and may thereby be chemopreventative;[643] and a protective effect of soy against carcinogen-induced breast cancer in rodent studies[644] also add weight to this consideration.

Concerns that subsequently arose regarding a potential *adverse* impact on breast cancer risk and the growth of existing oestrogen-dependent tumours were predominantly triggered by findings from *in vitro* research, as well as rodent studies showing that isoflavones bind to and transactivate oestrogen receptors (ERs),[595,596] and induce proliferation and oestrogenic markers in MCF-7 cells (an ER positive breast cancer cell line).[578,597,645] In ovariectomised athymic mice, dietary genistein and genistin enhance the growth of human MCF-7 cell tumour xenoplants,[646,64] which appears to hinge on the activation of ERα.[601] The effect of soy protein isolates and genistein in this context is dose-dependent. [645,647] Dietary *daidzein* had only a slight but significant effect on tumour growth while equol did not stimulate the growth of oestrogen-dependent tumours in athymic mice, even though total daidzein or equol plasma levels in these mice were in the range that stimulated MCF-7 cell growth *in vitro*.[648] This may suggest that

pharmacokinetic factors attenuate the oestrogenic effects of daidzein and equol *in vivo.*[648] *Fermented* soy milk products, however, have shown growth-inhibitory effects on various human breast carcinoma cell lines, especially on MCF-7 cells in severe immune deficient mice,[649] and on induced mammary carcinogenesis in the rat.[650]

Relevance of in vitro studies and rodent models to humans

Findings from *in vitro* studies and rodent models are, however, of questionable relevance to humans. It has been pointed out that the lack of immune function in athymic mice, and the oestrogen-depleted environment do not accurately reflect the situation in either pre- or post-menopausal women.[651] Studies using parenteral doses of purified isoflavones are of limited interest, as are those using scaled oral doses 8 to 16 times higher than the typical intake from traditional Asian diets.[651] In humans, at least 95% of isoflavones are conjugated and largely inactive.[652] A higher percentage of genistein and daidzein appears in the free/aglycone form in rats.[653] In addition, the metabolite, equol, is effectively formed by gut bacteria in rodents and predominates in the serum of rodents, whereas only 30-50% of humans are equol-producers.[654] Daidzein and genistein remain the predominant isoflavones in human serum after ingestion of soy or mixed isoflavones.[593,653,654]

Biphasic effects of dose have been observed both *in vitro*[655,656] and *in vivo*.[657] *In vitro,* genistein at higher concentrations (>10 μM), inhibits growth of MCF-7 cells[656,658] (probably by oestrogen-independent mechanisms, such as modulating genes[658]) but *stimulates* growth at relatively low and physiologically-relevant concentrations[659] (<1 μM) via oestrogen-dependent mechanisms.[659,660] In standard rodent models, isolated soy protein or isoflavones were shown to *suppress* rather than stimulate growth of tumours in mice implanted with MCF-7 cells,[661] and even *enhance* the efficacy of tamoxifen.[662] Han and co-workers found that both genistein and daidzein were able to *inhibit* the proliferation-stimulating activity in MCF-7 cells in a high-oestrogen environment,[663] suggesting that the hormonal milieu may be of relevance to the *in vivo* effects. In rodent models, the timing of soy or isoflavone exposure relative to the implantation of cancer cells or the administration of carcinogens may be a critical factor in determining whether tumour development or growth is suppressed or enhanced.[657] Prepubertal administration of high–dose genistein induced a premature differentiation of breast tissue that diminished susceptibility of adult rats to carcinogen-induced breast cancer.[664]

An additional limitation with the observation of *in vitro* binding to ERα and ERβ is that ER-binding alone is a poor predictor of *in vivo* activity.[665] ER ligands often have very different/opposite effects depending on dosage and the type of tissue studied.[585]

The low nanomolar serum concentrations of *unconjugated* free genistein achieved with high-nutritional intakes of soy isoflavones are near the binding affinity of genistein for the beta receptor, but are about an order of magnitude lower than genistein's affinity for the "classical" alpha isoform of the oestrogen receptor (ERα).[601] Only about 4% of the total genistein is in free or sulphated from, while the remainder, consisting of glucuronate conjugate, is thought to have limited intracellular access.[592,666] Setchell and co-workers[667] found that unconjugated genistein constituted about 1.1-1.5% of the total plasma pool of genistein, the higher percentage being observed transiently in the first 2 hours following soy ingestion. These concentrations are far too low to inhibit tyrosine kinases or topoisomerase II, often cited as potential mediators of its physiological effects of genistein *in vitro*.[668]

Clinical studies and Epidemiological Evidence for Soy and Breast cancer

Findings from clinical studies have generally been favourable in regard to breast cancer risk, although not exclusively. In premenopausal women, soy supplements resulted in stimulation of oestrogen-sensitive markers and increased breast epithelium proliferation,[669,670] consistent with reports that low concentrations of isoflavones (isoflavone supplement 40mg per day equivalent) stimulates ER positive cells. Another study reported that after 6 months of soy protein isolate, hyperplastic epithelial cells were present in 7 of 24 completing subjects, compared with 1 at baseline.[671]

However, no increase in breast cell proliferation (a marker of potential tumour promotion) was observed following supplementation with isoflavone or isolated soy protein in four clinical studies (three with breast cancer patients[672-674] and one with healthy women[294]) in which breast biopsies were taken before and after. Two pilot studies on breast cancer survivors showed no significant effect of isoflavone supplementation on breast cell proliferation, over 2 weeks[672] and one year[674] respectively. Recently conducted one year and two-year-long studies indicated that isoflavone supplements do not affect breast density in premenopausal women.[675,676]

Case-control studies have found an inverse association between soy intake and risk for premenopausal breast cancer in Asian populations, and two such studies have found a similar association with postmenopausal breast cancer.[640,641,677-679] In one of these, high

soy intake among Asian Americans during adolescence was found to predict a lower risk for postmenopausal breast cancer, especially for those maintaining the high soy consumption into adult life.[679] Breast cancer survival is unrelated to soy food intake in epidemiological studies.[680,681]

Differential activation of ERα and ERβ?

McCarty[601] has proposed an interesting hypothesis that may account for the conflicting findings from soy studies and provide a rationale for genistein's clinical activities and safety potential. His theory is that the physiological effects of genistein are mediated by ERβ activation, and that ample activation of ERβ, but only minimal or modest activation of ERα, is achieved by physiological serum levels of free genistein achieved by a soy-rich diet. ERα and ERβ have opposing effects on cell proliferation in certain tissues, such as breast tissue, where ERα *promotes* epithelial proliferation, whilst ERβ has a restraining influence. This supports suggestions that soy isoflavones do not increase breast cancer risk, and may possibly diminish it. Their lack of uterotrophic activity may be due to ERα being the exclusive mediator of oestrogen's impact in this regard. Reported modest benefits of isoflavones on menopausal hot flushes may reflect the presence of ERβ in certain regions of the hypothalamus [682] to which soy isoflavones have access. Furthermore, the favorable influence of soy isoflavones on endothelial function in postmenopausal women and ovariectomised rats is explained because vascular endothelium expresses both ERα and ERβ, each of which has the potential to induce and activate nitric oxide synthase. The ERβ expressed in osteoblasts may mediate the reported beneficial impact of soy isoflavones on bone metabolism.

While current evidence suggests that isoflavone intake at dietary levels is unlikely to increase breast cancer risk, or oestrogen-sensitive breast cancer recurrence, most studies have been relatively short-term. Concerns exist regarding long-term safety. The possibility has been raised that, by promoting low-level activation of ERα, nutritional intakes of genistein could modestly boost cancer growth.[601] Additionally, breast cancer patients taking SERMs are advised to limit their intake of soy foods and avoid isoflavone supplementation due to concerns these may interfere with SERMS such as tamoxifen.[601,651] However, all these concerns are theoretical in nature.

11.4 ***Linum usitatissimum* L.**(Linseed/Flaxseed, Family: Linaceae)

Lignans are nonsteroidal polyphenolic substances that have been shown to bind to oestrogen receptors and exert partial agonist or antagonist action depending on the

target tissue.[683,684] They influence hepatic oestrogen metabolism and increase the synthesis of SHBG, involved in the binding and availability of sex steroids.[683]

Data from a cross-over study[685] with 25 hypercholesterolaemic menopausal women indicate that after 2 months of treatment, 40 g of crushed linseed was as effective as 0.625 mg of conjugated oestrogens in relieving mild menopausal symptoms measured on the KI, and in lowering serum levels of glucose and insulin, but produced no significant change in lipid profile. In another study, linseed supplementation (40 g/d) for 3 months was shown to improve lipid profile in postmenopausal women compared with a wheat-based regimen.[686] Elsewhere it was found to favourably but not significantly affect blood cholesterol, compared to wheat germ, after 12 months.[687]

11. 5 *Trifolium pratense* L. (Red clover, Family: Fabaceae [subfamily: Papilionaceae])

Red clover extracts are also quite popular for the treatment of menopausal symptoms,[688] and have received attention for their phytoestrogen content. A preformulated extract of red clover was found to contain the coumestan, coumestrol; isoflavones, daidzein, genistein, and their methylated precursors, formononetin, biochanin A; as well as flavonoids including naringenin. Daidzein, genistein, formononetin, biochanin A, and naringenin were oestrogenic in the alkaline phosphatase assay, and all of these, except formononetin, bound to one or both ERs. [689] In addition to SERM-activity of the phytoestrogen components, biochemical analysis shows they act as selective oestrogen enzyme modulators (SEEMs), have antioxidant activity and interact with transcription factors such as NF-kappa-β.[690]

11.5.1 Clinical trials of Red clover in hot flushes

Several RCTs have been conducted on Promensil®, an extract of red clover leaf and flower standardised for isoflavone content, administered in a 500mg tablet, containing 40mg per tablet of total isoflavones. However, results have largely been disappointing, with only one showing superiority over placebo to date. In a 12 week RCT with 30 symptomatic women aged 49-65, (26 completed), Van der Weijer and Barentsen[691] found red clover extract, Promensil® (80mg/day), to be superior to placebo for alleviating hot flushes, p = 0.015, although no significant effect was observed for overall menopausal symptoms rated on the Greene Climacteric scale. In a study by Baber and colleagues,[692] no significant difference was observed in flushing scores or Greene Climacteric scale scores (p = 0.158), in a cross-over trial with Promensil® 40mg/day and placebo for 3 months each. Fifty-one women aged 45-64 with a minimum of 3 flushes per

day were recruited; 43 completed both arms. Lack of superiority was similarly found in another 12 week RCT that randomised 37 women with 3+ hot flushes per day to either 40mg or 160 mg/day Promensil®, or placebo, although flushing frequency decreased in all arms over the 12-week period.[75] No between-group differences were observed in these studies in vaginal cytology, endometrial thickness as measured by ultrasonography, or serum hormone levels. While these studies may have been subject to lack of power due to the low numbers recruited, or lack of adequate symptom severity at baseline, a larger RCT by Tice and co-workers[693] similarly failed to support the superiority of Promensil® (82 mg total isoflavones/day) or of Rimostil® (57 mg isoflavones/day) over placebo. This RCT recruited 252 participants aged 45 to 60 years, with at least 35 hot flushes per week and followed them up for 12 weeks.

11.5.2 Other potential effects of Red clover extracts

Some evidence supports the role of red clover isoflavones in reducing bone loss induced by ovariectomy in rats, probably by reducing bone turnover via inhibition of bone resorption.[694] In an uncontrolled study involving 46 postmenopausal women, the BMD of the proximal radius and ulna rose significantly over 6 months with two doses of isoflavones extracted from red clover (Rimostil®), namely by 4.1% over 6 months with 57 mg/day (p = 0.002) and by 3.0% with 85.5 mg/day (p = 0.023) of isoflavones).[695] The effect was not dose-related. In another RCT of 205 women aged 49-65, all women lost BMD, but the group assigned to red clover-derived isoflavone supplement (providing a daily dose of 26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein for 1 year) lost significantly less.[696]

A significant increase in HDL cholesterol has been observed with administration of red clover isoflavone 40 mg/day (Promensil®) over 3 months,[75] and Rimostil® at 28.5 mg, 57 mg, and 85.5 mg over a 6-month period (15.7-28.6%),[695] although the magnitude of the response was independent of the dose used. The latter uncontrolled study also demonstrated a significant fall in serum apolipoprotein B (by 11.5-17.0%) after 6 months of treatment.[695] Also of relevance to atherosclerosis risk is the evidence from one study of improved systemic arterial compliance and elasticity with red clover supplementation in postmenopausal women.[697]

Concerns have been raised regarding the safety of use of red clover isoflavone supplements in patients with breast or endometrial cancer[698] based on data suggesting a weak oestrogenic action in the ovariectomised rat model.[699] However, a recent three-year RCT of a once-daily standardised 40 mg red clover isoflavone dietary supplement

(Promensil®, Novogen) in 401 women aged 35-70 years with a family history of breast cancer reported no adverse effect on breast density, skeletal strength or cardiovascular status.[700] No significant differences in endometrial thickness were detected between those taking red clover isoflavones and placebo, consistent with findings from a rodent model.[701]

11.6 *Cimicifuga racemosa* Nutt. (Black cohosh, Family: Ranunculaceae)

More evidence from scientific studies exists to support the efficacy of *Cimicifuga racemosa* root and rhizome in the treatment of menopausal symptoms than for any other herbal intervention used in Western phytotherapy practice.[702-718] Many studies have been conducted on an isopropanolic extract of the rhizome, Remifemin®. Studies on the combination of *Hypericum perforatum* and *C. racemosa* are included in the section on St John's wort below.

11.6.1 Chemical constituents

Chemical constituents of the roots and rhizomes of black cohosh include cycloartenol-type triterpenoids, (such as actein, 23-epi-27-deoxyactein and cimicifugoside); cinnamic acid derivatives (ferulic acid, isoferulic acid, and piscidic and fukiic acid esters).[719] There is some controversy over the presence of the isoflavone, formononetin, previously believed to be a constituent of black cohosh, as this has not consistently been detected in methanolic or aqueous methanolic extracts of the root/rhizome, and is unlikely to be present.[720,721]

11.6.2 Mechanism of action of C. racemosa

Previously regarded as a phytoestrogenic herb, more recent research has proposed an organ-dependent ER agonist-antagonist mode of action for black cohosh.[722,723] Its role as a SERM is supported by evidence from *in vitro* studies, *in vivo* studies of animals, and human epidemiologic and dietary intervention studies.[715,722,724] For example, the ethanolic *C. racemosa* extract BNO 1055 was found in a rodent model to act on the hypothalamo-pituitary unit and in the bone, but not in the uterus.[715,724] Findings from clinical studies also suggest favourable effects in the brain/hypothalamus, bone[718] and vagina,[718] in the absence of uterotrophic effects.[723,725] Lack of change in vaginal cytology measures was reported following 24-weeks administration of two different doses (39 mg and 127.3 mg) of *C. racemosa* rhizome,[708] and lack of endometrial proliferation was evident after 12 months administration of extract CR BNO 1055 to 375 postmenopausal women.[726] The Herbal Alternatives for Menopause (HALT) study found

that 12 months administration of black cohosh, used alone (160 mg/d 2.5% triterpene glycosides, 70% ethanol extract) or as part of a multibotanical product (200 mg/day), had no effects on vaginal epithelium or the endometrium.[725]]

A central activity has also been proposed for *C. racemosa* based on evidence from *in vitro* and *in vivo* studies suggesting dopaminergic activity, possibly D_2 receptor agonism,[727,728] and serotonergic activity.[729] Analysis of ligand binding data from *in vivo* rodent studies suggested that a black cohosh methanol extract acted as a partial agonist at the serotonergic 5-HT(7) receptor.[729] The reductions in hot flushes induced by administration of black cohosh may, therefore, not be due to oestrogenic properties. It has thus been suggested that black cohosh should not be regarded as a phytoestrogenic herb, nor a phyto-SERM.[730]

11.6.3 Research on black cohosh for menopausal symptoms/hot flushes

In RCTs examining its effects on hot flushes/night sweats and other menopausal symptoms such as irritability and sleep disturbances, extracts of black cohosh have shown superiority to placebo,[706,723,731,732] although one study found it only to be significantly superior for symptoms of moderate severity.[706] A RCT on 304 women administered isopropanolic extract of black cohosh rootstock 5 mg/day (equivalent to of *Cimicifuga racemosa* 40 mg daily) or placebo for 12 weeks and found a significant effect over placebo on Menopause Rating Scale (MRS) scores, $p < 0.001$, and the hot flush, $p = .007$ subscore, with a clinically-relevant effect size (0.03 to 0.05 MRS units).[731] Because this study used a validated questionnaire to measure hot flushes, rather than a daily diary record, the actual frequency and severity of hot flushes and night sweats is unknown. Equivalence to conjugated oestrogens (0.6 mg/day) was also established with 12 weeks administration of extract CR BNO1055 (Klimadynon®/Menofem 40 mg /day) for symptoms rated on the Menopause Rating Scale (MRS); both groups showed an improvement from baseline, $p = 0.05$.[723] *C. racemosa* (40 mg, 150 mg dried herb equivalent) was also equivalent to low-dose transdermal oestriol (25µg per week) over 3 months in 64 women for hot flushes, as well as anxiety and depression.[733] Both interventions significantly reduced the number of hot flushes per day ($p < 0.001$) and vasomotor symptoms ($p < 0.001$), from the first month of treatment, and anxiety ($p < 0.001$) and depression ($p < 0.001$) at the end of 3 months. However, no significant effect over placebo was found for hot flushes in the HALT Study which assigned 351 women to one of five groups to compare 4 different interventions, including black cohosh 160 mg daily, with placebo for 12 months.[734]

11.6.4 C. racemosa and Kupperman Index

On KI scores, black cohosh (extract equivalent to 160 mg/day) produced an improvement similar to three different HT interventions (oestriol 1mg/day; CE 1.25 mg/day; oestrogen-progestin 2mg + 1 mg/day respectively).[735] At all intervals measured, statistically significant reductions in symptoms were observed on the modified KI for all treatment groups, $p < 0.01$. However, this is a very low dose of a weak oestrogen that has questionable clinical effects. Doses of 39 mg/day and 127.9 mg/day resulted in a similar decrease in KI scores over 6 months (responder rate 70% and 72%, respectively) in both perimenopausal and postmenopausal women.[708] A further study in 244 Chinese women, found *C. racemosa* 40 mg crude drug/day to be equivalent to orally-administered tibolone 2.5mg/day for 3 months in reducing symptoms (including moderate to severe symptoms) measured on the KI, p (non-inferiority) = 0.002).[704] Large-scale observational studies have also shown favourable effects of black cohosh extracts on hot flushes[717,726] and KI scores.[717] However, the ethanolic black cohosh root extract, Cr 99 (daily intake 6.5 mg dried rhizome extract, corresponding to an average of 42 mg crude drug), showed no superiority to placebo in the intention-to-treat population as a whole. By contrast, in the subgroup of participants with a KI of 20 or more, significant superiority was demonstrated ($p < 0.018$). The weekly weighted flushing scores ($p < 0.052$) and the Menopause Rating Scale ($p < 0.009$) showed similar results for the women with symptoms of at least moderate severity.[706]

In premenopausal breast cancer survivors, tamoxifen induced hot flushes were significantly reduced in number and severity with co-administration of black cohosh, compared with standard care alone.[736] Almost half of the patients of the intervention group were free of hot flushes, while severe hot flushes were reported by 24.4% of patients of intervention group and 73.9% of the usual-care group ($p < 0.01$). However, in another placebo-controlled trial with *C. racemosa* in breast cancer survivors, no between group differences were observed for mean number of hot flushes, flush severity, sleep, irritability , depression headaches or palpitations.[707]

11.6.5 C. racemosa and Breast cancer risk

While oestrogenic properties have not been established with certainty for black cohosh, herbs exerting oestrogen-like effects elicit concerns regarding their effects in women with a history of, or at risk of developing, oestrogen-dependent cancers such as breast cancer. The majority of the evidence for the effects of black cohosh on breast cancer cells has come from *in vitro* research, principally using the ER+ breast cancer cell line,

MCF-7, but occasionally T-47D. Only one study found black cohosh extract to stimulate breast cancer cell lines,[737] while many others have shown either lack of stimulation[738] or inhibition of breast cancer cell growth for *C. racemosa.*[578,738-742] In some instances, it was found to kill both the ER+ MCF-7 as well as ER- MDA-MB231 cells by induction of apoptosis.[741,743] The proliferation-inhibiting effects of tamoxifen were enhanced by black cohosh.[739] In addition, some *in vitro* studies have failed to support an oestrogenic activity for *C. racemosa*,[709,738] while others have demonstrated anti-oestrogenic properties.[578,742] However the relevance of *in vitro* studies to oral dosing in humans is questionable in this context, since the putative major constituents of black cohosh, the triterpene glycosides (saponins) need to be metabolised to the active form by bacteria in the gastrointestinal tract.

In vivo research on ovariectomised rats found a lack of stimulating effects on oestrogen-dependent mammary gland for a propanolic extract of *C. racemosa*.[744] Elsewhere, no differences were detected in the incidence or onset of mammary tumors in black cohosh-treated transgenic mice (fed an adjusted dose to correlate to a human dose of 40 mg/d) versus control females.[745] This suggests no increased risk of breast cancer in healthy women with black cohosh. In contrast, black cohosh significantly increased the incidence of *lung* metastases in tumour-bearing transgenic mice compared with mice fed the isoflavone-free control diet.[745] However, the use of transgenic mice as model systems for assessing the impact of treatments on cancer risk is highly controversial.[746] Limitations include the variable mouse strains used, which can greatly influence the latency and even the type of the tumor caused by the transgenic oncoprotein, and our rudimentary understanding of the pathways that control normal mammary physiology in the mouse and human.[747]

A prospective, open, uncontrolled drug safety study found that isopropanolic extract of black cohosh (Remifemin® 40 mg/day) did not increase mammographic breast density or breast cell proliferation after 6 months of treatment in 65 postmenopausal women.[748] A recent review of the literature supported the safety of black cohosh in breast cancer patients without risk of liver disease.[749] Nonetheless, many of the human studies on black cohosh have been of relatively short duration, while treatment for menopause is rarely limited to 3 months. As such concerns have been expressed regarding the longer term safety of black cohosh with respect to hormone-dependent tissues such as the breast. Reassurance regarding safety with long term use still needs to be established in trials of at least 12 months duration.[750]

Beneficial effects on bone metabolism have been demonstrated with *C. racemosa* extracts.[718,724,751] An analyses of the black cohosh preparation CR BNO 1055 on bone turnover markers in postmenopausal women indicated a stimulation of osteoblast activity

and benefits on bone remodeling.[718] Treatment of the ovariectomised rats over a period of 3 months with the same extract showed osteoprotective effects; it significantly reduced the loss of BMD in tibia and exerted oestrogenic effects particularly in osteoblasts.[724] Findings of a further study of the isopropanolic extract of black cohosh on human osteoblasts, supported the potential of black cohosh for positive skeletal effects.[751]

11.6.6 Hepatotoxicity

Several countries, including Australia, UK, US and Canada, currently require black cohosh products to carry a warning label about the (rare) potential for liver damage. These fears were generated by published reports of liver injury that was attributed to consumption of black cohosh products. [752-755] Although many of these were found to be of dubious reliability, they prompted further investigation by authorities both in the US and Europe. The Dietary Supplement Information Expert Committee of the US Pharmacopeia's Council of Experts analysed thirty reports on the use of black cohosh products concerning liver damage. All the reports of liver damage were assigned 'possible' causality, and none was 'probable' or 'certain' causality. The clinical pharmacokinetic and animal toxicological information did not reveal unfavorable information about black cohosh.[756] The European Medicines Agency Committee on Herbal Medicinal Products (HMPC) analysed 42 cases of hepatotoxicity attributed to black cohosh collected from European National Competent Authorities.[757] Two were rated as 'probable' using the Roussel UCLAF Causality Assessment Method (RUCAM), despite the failure to identify black cohosh in the products used. Current evidence concerning liver toxicity is inconclusive[749] as a direct association between hepatotoxicity and the ingestion of *Cimicifuga* has not been demonstrated.[758]

11.7 Other phytoestrogenic herbs

11.7.1 Humulus lupulus L. *(Hops, Family Cannabinaceae)*

The strobiles of hops contain the potent phytoestrogen 8-prenylnaringenin (8-PN).[759] The ability of 8-PN to exert systemic endocrine effects in healthy postmenopausal women was demonstrated in a dose-escalation RCT, using single oral doses of 50, 250 or 750 mg 8-PN. [760] Decreases in LH serum concentrations were found after the highest dose. Methanol extracts of hops showed significant competitive binding to ER alpha and ER beta, and exhibited oestrogenic activity with cultured Ishikawa (endometrial) cells.[709] In rodent models, *H. lupulus* extracts were not found to affect uterine weight gain, but 8-PN at equivalent doses to those used in human studies did show a dose-related oestrogenic effect on the uterus, raising concerns about a potential deleterious effect in women.[701]

Convincing evidence from robust clinical trials is lacking to support the efficacy of hops extracts for reduction of hot flushes. A RCT was conducted on a standardised (on 8-PN) hop extract administered to postmenopausal women at doses corresponding to 100 µg 8-PN and 250 µg 8-PN.[761] At the lower dose, a significant reduction in the incidence of hot flushes and other menopausal symptoms (sweating, insomnia, heart palpitation, irritability) measured on the modified KI was found, compared to placebo, after 6 weeks, $p = 0.023$, but not after 12 weeks, $p = 0.086$. At the higher dose, hop extract was superior to placebo for hot flushes at 6 weeks, $p < 0.01$. Hops was one component of the morning/evening formula found to reduce vasomotor, anxiety, and depression scores on the Greene Climacteric Scale (GCS) by 50%, 56%, and 32%, respectively at the end of 8 weeks.[762] However, the lack of a control group in this study is a serious limitation. The efficacy of hop extracts in reducing menopausal hot flushes has been reported by Goetz based on treatment of patients treated using different types of non-standardised hops preparations.[763]

The effects of a topical gel containing hyaluronic acid, liposomes, vitamin E and hop extract was assessed on vaginal dryness and associated atrophic vaginitis (itching, burning, dyspareunia, vaginal inflammation/oedema and rash in 100 postmenopausal women in a multicentre, open, non-controlled design.[764] Following application of 2.5 g of gel/day for 1 week, reduced to two applications/week for 11 weeks, symptoms were significantly reduced, as assessed by a 4-point scale and presence of vaginal abrasions and disepithelialisation. An overall judgment on the acceptability of the treatment was made by the subjects, as well as an overall judgment on the efficacy and safety of the device by the investigator.

11.7.2 Trigonella foenum-graecum L. *(Fenugreek, Family: Fabaceae)*

Fenugreek has been the subject of one comparator trial with HRT that assessed the effects on vasomotor symptoms in 50 postmenopausal women.[765] Women were assigned to receive either 0.625 mg conjugated oestrogen with 10 mg medroxy progesterone acetate or 6 g fenugreek seed powder daily for 8 weeks. Significant reductions were observed in both groups after four and eight weeks of treatment. However, the HRT significantly outperformed the fenugreek.

11.7.3 Pueraria montana var. lobata (formerly *P. lobata*) Lour. *(Kudzu, Family: Fabaceae)*

Kudzu is a traditional Chinese herbal remedy for menopausal symptoms, as well as an ingredient in preparations for conditions such as osteoporosis, CHD and some hormone-dependent cancers.[766] Many different species of kudzu are used in Asia. In China, *P.*

montana (also known as *P. thunbergiana*) and *P. thomsonii* are used interchangeably. *P. mirifica* and *P. tuberosa* are also used frequently.[767] Kudzu root extract contains isoflavonoid phytoestrogen components such as daidzin, daidzein and genistein[768,769] that may be responsible for its action. Although not widely used in Australia by practitioners of Western phytotherapy, interest is growing in kudzu root for treating menopausal symptoms.[767]

No data from RCTs on vasomotor symptoms could be found for kudzu in the literature. From a non-controlled trial of 6 months duration, data were obtained for two doses of *Pueraria mirifica* (50 mg/day and 100 mg/day) for 37 pre- and postmenopausal women.[770] Significant reductions in scores, of a similar magnitude, on the modified Greene climacteric scale were seen for both groups, (decreasing from 35.6 to 26.6, 17.2 and 15.1 with 50 mg/day, while 100 mg/day resulted in declines from 32.6 to 21.0, 14.8 and 13.6 at 1-, 3- and 6-months respectively).

A pilot study of isoflavones from kudzu and red clover, along with other nutrients, on menopausal symptoms was conducted over 12 weeks on 25 menopausal women suffering from severe hot flushes and night sweats. [771] A 46% decrease in hot flushes was observed, with an improvement in subjectively-rated quality of life, $p < 0.001$. Modest improvement was observed in cardiovascular risk markers, the ratio of total cholesterol to HDL-cholesterol, and homocysteine. A statistically significant improvement was found for a proposed marker of breast cancer risk, the ratio of 2-hydroxyestrone to 16 alpha-hydroxyestrone, $p < 0.001$.

A decocted root extract of *Pueraria lobata*, equivalent to 100 mg isoflavone, was compared with HT and no treatment in 127 postmenopausal women (n = 45, n = 43 and n = 39 respectively) for a 3 month period.[766] Kudzu had no significant effect on lipid profile, FSH or LH, but did improve flexible thinking and attention span compared with no treatment, $p < 0.05$.

A herbal supplement, Avlimil® containing eleven herbal components, including kudzu was observed to stimulate MCF-7 tumor growth in a dose-dependent manner, calling into question its safety for women with oestrogen-dependent breast cancer.[772] However, no increase in uterine weight was observed in the animal model. Similarly, in healthy postmenopausal women, no endometrial proliferation or hyperplasia was reported after 24 weeks of *Pueraria mirifica* at doses of 20, 30, and 50 mg/d.[773] Beneficial effects on bone loss are suggested from studies in both human and rodent models. [773] An

oestrogen-like effect on bone turnover rate was demonstrated with *P. mirifica* for a 24-week period.[773] In the rat model, significant dose-dependent prevention of bone loss with *P. mirifica* treatment for 90 days was also observed.[774]

11.8 Saponin-containing herbs used in menopause

Other herbs that may exert oestrogenic effects on oral administration include those containing steroidal saponins such as wild yam (*Dioscorea villosa* L. Family: Dioscoreaceae), false unicorn root (*Helonias luteum (*Ker-Gawl*)/Chamelirium luteum* L. (Gray.) Family*:* Liliaceae), and tribulus (*Tribulus terrestris,* Family*:* Zygophyllaceae*),* all of which are reputed to exert benefits in the treatment of menopausal symptoms. It has been proposed that these herbs may exert their effects by binding with unoccupied receptors in the hypothalamus,[775] notably ERβ.[682] Thus, menopausal symptoms thought to be initiated via the hypothalamus may be reduced by this selective binding of the plant steroids. However, scant evidence exists from RCTs to support the roles of these herbs in this context.

11.8.1 Tribulus terrestris L. (Family: Zygophyllaceae)

In an open study, 50 menopausal women (both natural and surgically-induced) were first given placebo followed by a standardised extract of *Tribulus terrestris* leaf (dosage range of 500–750 mg/day), containing not less than 45% steroidal saponins. Predominant symptoms included hot flushes, sweating, insomnia and depression. Placebo treatment did not result in a favourable result for any symptom in any patient. After active treatment, however, 98% reported symptom improvement. No significant changes were noted in FSH, LH, prolactin, oestradiol, progesterone or testosterone.[776] This was an in-house report not published in the peer-reviewed literature.

11.8.2 Dioscorea villosa L . (Wild yam, Family: Dioscoreaceae)

Wild yam root contains steroidal saponins, with diosgenin as the aglycone.[777] An oestrogenic action of diosgenin, but no progestogenic action, has been reported following subcutaneous administration in the ovariectomised mouse.[36] An extract of a related species of yam used in TCM, *Dioscorea alata* was examined for oestrogenic activity, and found to contain compounds that activate human ERα and ERβ, supporting a beneficial effect for menopausal women.[778] *D. alata* administration also reversed ovariectomy-induced anxiety, despair behaviour and changes in neuroimmunological function in the rat cortex.[779] In a further study on *Dioscorea alata,* 24 apparently healthy postmenopausal women replaced two-thirds of their staple food with 390 g of yam in 2 of

3 meals per day for 30 days.[780] Following yam ingestion, there were significant increases in serum concentrations of oestrone (26%, p = 0.003), SHBG (9.5%, p = 0.019), and a near significant increase in oestradiol (27%, p = 0.072). Urinary concentrations of the genotoxic metabolite of oestrogen, 6alpha-hydroxyestrone, decreased significantly by 37%, $p < 0.001$. Plasma cholesterol concentration decreased significantly by 5.9%, p = 0.012. Lag time of low-density lipoprotein oxidation prolonged significantly by 5.8%, p = 0.02, and urinary isoprostane levels decreased significantly by 42%, $p < 0.001$. It was surmised that the beneficial effects on hormone status, antioxidants and lipids may potentially reduce the risk of breast cancer and cardiovascular diseases in postmenopausal women.

No evidence from RCTs on oral administration of *Dioscorea* species in menopause could be found in the literature. A negative RCT on topical administration of wild yam cream for three months in 23 healthy women found no difference between groups for hot flushes, mood, breast tenderness, libido or energy.[781] This finding is unremarkable, and the study was designed to refute a common misconception regarding wild yam as a source of progesterone. While a species of Mexican wild yam, *Dioscorea macrostachya* was employed as a rich source of the plant sterol, diosgenin, for the first large-scale production of progesterone, wild yams do not contain progesterone; nor have they been shown to promote the production of progesterone, or be converted to progesterone in the body.[782] However, diosgenin, can be converted in the laboratory to steroidal compounds including progesterone.[14]

11.8.3 Panax ginseng C.A. Mey. *(Korean ginseng, Family: Araliaceae)*

Panax ginseng is a saponin-containing adaptogenic herb with some evidence of oestrogenic activity.[783-785] Ginsenoside Rg1 has recently been shown to exert oestrogen-like actions via ligand-independent activation of ERα pathway,[784,785] failing to displace specific binding of [(3)H]17beta-estradiol from oestrogen receptors in MCF-7 whole-cell ligand binding assays.[785] An *in vitro* study found an alcoholic extract of American ginseng root (*Panax quinquefolius* L. Family: Araliaceae*)* to significantly induce the growth of MCF-7 cells by 27-fold over that of untreated control cells, raising concerns over its use in humans, pending further study.[738] The glucocorticoid-like effects of ginseng *in vivo* may be explained by the affinity for progestin, mineralocorticoid and glucocorticoid receptors demonstrated by saponins from *Panax* root extracts *in vitro*.[786]
Positive effects on well-being and psychological symptoms of menopause have been shown, and the physiological correlates examined in two studies. Over a 16-week period, administration of *Panax ginseng* (Ginsana® containing 100 mg of the

standardised extract G115, Pharmaton, SA, Lugano, Switzerland) resulted in improved quality-of-life measures, including well-being and depression, in a multicentre RCT with 384 post-menopausal women, $p < 0.05$.[787] However, it was concluded that its beneficial effects were most likely not mediated by hormone replacement-like effects, as physiological parameters such as FSH and oestradiol levels, endometrial thickness, maturity index and vaginal pH were unaffected by the treatment.

In the form of red ginseng, in which *Panax ginseng* root has been steamed before drying, oral administration of 6 g daily for 30 days was found to improve psychological scores on the Cornell Medical Index psychosomatic state and fatigability scales, $p < 0.001$, and state anxiety measured on the State-Trait Anxiety Inventory (STAI-A), $p < 0.001$, in post-menopausal women.[788] The authors concluded that the improvements appeared to be at least partly attributable to the effects of red ginseng on stress-related hormones, exhibited by a decrease in cortisol, $p < 0.05$, and the cortisol:DHEAS ratio, $p < 0.05$.

11.8.4 Glycyrrhiza glabra L. *(Licorice Family: Fabaceae)*

As an adrenal tonic, licorice has a potential role in the context of menopause. In addition, it has been found to possess oestrogenic properties. A proprietary formula was found to owe its oestrogenic property to glycyrrhiza, aletris, helonias and asclepias, but principally to the licorice.[38] *In vitro* and *in vivo* two natural compounds derived from licorice root demonstrated oestrogen-like activity on vascular tissues: glabrene, an isoflavene, behaved as a partial agonist/antagonist of oestradiol while glabridin, the major isoflavan, demonstrated only oestrogenic activity.[789] It may also have the potential to modulate bone disorders in post-menopausal women.[790] However, evidence for all the above from RCTs is lacking.

11.9 Clinical studies on other phytotherapeutic interventions

11.9.1 Angelica sinensis (Oliv.) Diels *(Dong quai, Family: Apiaceae)*

The root of *Angelica sinensis* has been introduced into Western herbal medicine from traditional Chinese medicine, in which it is used for tonifying the blood and treating female menstrual disorders (dysmenorrhoea, amenorrhea, irregular menstruation) as well as menopausal symptoms.[791] Coumarins are among the 70+ compounds isolated and identified from dong quai, although the principal biologically active components are thought to be the essential oil, Z-ligustilide, other phthalides and ferulic acid.[792] Studies of its oestrogenic activity reported only weak ER binding[709] and no oestrogen-like responses in endometrial thickness or in vaginal maturation when dong quai is used alone.[74]

Some RCTs examining phytotherapeutic formulations including dong quai on vasomotor symptoms have shown positive results.[793,794] However, an RCT evaluating the effects of dong quai as a simple (4.5g root/day standardised to 0.5mg/kg ferulic acid) found no significant differences between dong quai and placebo on symptoms evaluated by the KI and flushing diary after 24 weeks in 71 postmenopausal women.[74]

Dong quai is one component of PhytoFem®, which was shown to favourably influence symptoms of hot flushes and sleep disturbances in pre- and postmenopausal women.[793] (This study is further discussed in the section on *Vitex agnus-castus.*) However, other RCTs reported in the scientific literature have produced inconsistent results.

The herbal extract preparation, Climex®, containing *Angelica sinensis* 75 mg/day plus *Matricaria chamomilla* L. (flowers) 30 mg/day *(*German chamomile, Family: Asteraceae*)* was investigated in a placebo-controlled RCT for 12 weeks on 55 postmenopausal women complaining of hot flushes.[794] The herbal combination was found to be superior to the placebo in reducing the number and intensity of hot flushes ($p < 0.001$). A significant response was noted as early as the first month of treatment in hot flushes as well as night sweats. There was also a marked alleviation of sleep disturbances and fatigue.

A TCM formulation, Dang Gui Buxue Tang, a 1:5 combination of *Angelica sinensis* with *Astragalus membranaces* root (Family*:* Fabaceae), 3 g per day of a spray-dried aqueous extract, was trialled for 6-months in a RCT with 100 symptomatic Hong Kong Chinese women. No significant difference was observed between the herbal combination and placebo in the treatment of vasomotor symptoms in women. The frequency of mild, moderate and severe hot flushes decreased in both groups, but Dang Gui Buxue Tang was statistically superior to placebo only in the treatment of mild hot flushes.[795]

11.10 The Phytotherapeutic Approach

Because natural medicine takes the holistic approach of treating the person rather than the 'dis-ease', a wide range of other herbal actions may also be appropriate to the treatment of women with menopause-related complaints, since these vary according to the individual and the phase of the transition. During the perimenopause, for instance, a woman may seek treatment due to changes to the menstrual cycle regularity, duration or flow, exacerbation of PMS-like symptoms and/or the psychological symptoms that tend

to be more prevalent during this phase. Herbs most commonly indicated for relief of menopausal symptoms or their underlying cause are listed in Table 11.2 with some examples of herbs possessing these actions. Available evidence from RCTs for these herbs is outlined below. Rather than indefinitely 'replacing' hormones in decline, the aim of phytotherapeutic treatment is to ensure a comfortable adaptation to the fluctuating hormone levels of the perimenopause and the lower levels in the postmenopause.[775]

TABLE 11.2 OTHER ACTIONS AND PHYTOMEDICINES RELEVANT TO THE TREATMENT OF MENOPAUSE-RELATED COMPLAINTS

Herbal action	***Examples of herbs*** ***Latin binomial* (Common name)**	***Botanical family***
adaptogenic	*Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim.(Siberian ginseng)[777]	Araliaceae
	Panax ginseng C.A. Mey..(Korean ginseng)[796]	Araliaceae
	Astragalus membranaceus (Bunge)(astragalus)[777]	Fabaceae
	Withania somnifera L.(Dunal.) (ashwagandha)[797]	Solanaceae
	Asparagus racemosa Willd.(shativari)[798]	Asparagaceae
antihyperhidrotic/ reduces (night) sweats	*Salvia officinalis* L. (sage)[777]	Lamiaceae
	Astragalus membranaceus (Bunge.)[799]	Fabaceae
	Zizyphus spinosa (Bunge.) (sour Chinese date)[797]	Rhamnaceae
anxiolytic	*Piper methysticum* (Forster), (kava)[796]	Piperaceae
	Hypericum perforatum L. (St John's wort)[800-802]	Clusiaceae/Guttiferae
	Rheum rhaponticum L.. (false rhubarb)[803,804]	Polygonaceae
	Valeriana officinalis L. (valerian)[777]	Valerianaceae
	Passiflora incarnata L. (passion flower)[777]	Passifloraceae
	Zizyphus spinosa (Bunge.) (sour Chinese date)[805]	Rhamnaceae
aphrodisiac	*Asparagus racemosa* Willd. (shativari)[798]	Asparagaceae
	Tribulus terrestris L. [806]	Zygophyllaceae
astringent/ antihaemorrhagic	*Alchemilla vulgaris* L. (lady's mantle)[807]	Rosaceae
	Lamium alba L. (white deadnettle)[807]	Lamiaceae
	Trillium erectum L. (beth root)[807]	Liliaceae
	Achillea millefolium L. (yarrow)[777]	Asteraceae
	Viburnum prunifolium L. (black haw)[807]	Caprifoliaceae
hepatorestorative &/or oestrogen clearance enhancing	*Silybum marianum* L.(Gaertn) (St Mary's thistle)[796]	Asteraceae
	Cynara scolamus L. globe artichoke)[796]	Asteraceae
	Rosmarinus officinalis L. (rosemary)[777]	Lamiaceae
	Curcuma longa L. (turmeric)[796]	Zingiberaceae
hypnotic	*Humulus lupulus L.* (hops)[777]	Cannabinaceae
hypnotic and sedative	*Piper methysticum* (Forster), (kava)[796]	Piperaceae
	Valeriana officinalis L. (valerian)[777]	Valerianaceae
	Passiflora incarnata L. (passion flower)[777]	Passifloraceae
	Zizyphus spinosa (Bunge.) (sour Chinese date)[797]	Rhamnaceae
nervine tonic	*Leonurus cardiaca* L. (motherwort)[777]	Lamiaceae
	Hypericum perforatum L. (St John's wort)[796]	Clusiaceae/Guttiferae
	Turnera diffusa Urb.(damiana)[777]	Turneraceae
nootropic	*Ginkgo biloba* L.(ginkgo)[796]	Ginkgoaceae
omega-6 fatty acid deficiency corrector	*Oenothera biennis* L. (evening primrose)	Onagraceae

1.11 Antihyperhidrotic herbs

For symptomatic relief of sweating, antihyperhydrotic herbs are commonly incorporated into formulations in current Western phytotherapy practice. These include *Salvia officinalis* (sage) and the Chinese herbs, *Astragalus membranaceus* root and *Zizyphus spinosa* seed. Zizyphus is traditionally used in TCM for insomnia due to nervous exhaustion, excessive sweating and night sweats, especially when accompanied by anxiety, irritability and/or palpitations. A Chinese herbal formula mainly containing Zizyphus has been shown in clinical trials to be effective for anxiety and sleep quality.[808]

The efficacy of a plant product based on extracts of the leaves of sage and alfalfa (*Medicago sativa* L. Family: Fabaceae [subfamily Faboideae]) was investigated in the treatment of hot flushes in an open trial of 12 weeks duration with 30 menopausal women.[809] Hot flushes and night sweating completely resolved in 20 women, good improvement was observed in 4 women and a reduction in symptoms was reported in the other 6. Basal levels of oestradiol, LH, FSH, prolactin and TSH were unchanged at the end of the 3 month period. However, a slight antidopaminergic action was observed with a significant increase in prolactin and TSH response to TRH. A good safety profile was reported.[809] Sage also reduced sweat production in patients with hyperhidrosis in a number of open studies,[46] although these were not specifically in the context of menopause.

11.12 Omega-6 Fatty acid-deficiency corrector

Evening primrose oil has shown efficacy in relieving PMS-associated symptoms.[810,811] Its use for menopause-related symptoms may only be relevant where these are due to an exacerbation of PMS-like symptoms, as no support exists from RCTs for a beneficial effect in relieving hot flushes. No significant effect over placebo was observed on hot flushes with 2,000 mg evening primrose oil plus 20 mg vitamin E twice daily for 24 weeks,[812] although the small sample size (56 recruits of whom 35 completed) and the mild symptoms at baseline (3 or more flushing episodes per day) are severe limitations of this study. A significant improvement was noted in the number of *night time* flushes in women allocated to evening primrose oil plus vitamin E, $p < 0.05$. However, for the placebo group, the reduction in both daytime, $p < 0.001$, and night-time flushes, $p < 0.05$, reached significance.

11.13 Anxiety and general well-being

Several phytotherapeutic interventions have been examined for their effects on other menopausal symptoms such as the psychological symptoms. Those with positive findings are included here.

11.13.1 Piper methysticum Forster*. (Kava, Family: Piperaceae)*

Several studies have supported the beneficial effects of kava root extracts in the treatment of menopause-related anxiety.

An extract of *Piper methysticum* was found to favourably affect mood, particularly anxiety in perimenopausal women in a 3-month randomised prospective open study.[813] Calcium plus kava at doses of 100 mg/and 200 mg/day were compared with calcium supplementation alone (control). Anxiety measured on the STAI declined at 1 and 3 months significantly more than in the control group, $p < 0.001$, while significant within-group reductions were seen for depression at 3 months and climacteric scores at 1 and 3 months, $p < 0.003$. Symptoms rated on the Greene Climacteric scale declined significantly, $p < 0.001$ at 1 and 3 months.

In a 12-week RCT with 40 women with predominantly anxiety and vegetative symptoms, Kavosporal® 150 mg twice daily (equivalent to 60 mg kavapyrones daily) resulted in significant reduction in symptoms measured on the KI scores, diaries and the Anxiety State Index, $p < 0.001$ from week 4 onwards.[814] No reduction was found with placebo.

In a subsequent RCT with 20 women with menopause-related symptoms, Kava WS 1490 extract (3 x 100 mg/day) or a placebo was administered for a period of 8 weeks.[815] Overall anxiety measured on the Hamilton Anxiety scale (HAMA) significantly reduced in the kava group compared with the placebo group after only 1 week of treatment, $p < 0.001$. A beneficial effect on the neurovegetative and psychosomatic symptoms associated with menopause was suggested by improvements over the study period on depressive mood, $p < 0.01$, subjective well-being and symptoms as measured on the KI and Schneider climacteric scale, $p < 0.01$.

The combined use of HT (with progestogens, and without (ET)) and kava extract was investigated for the treatment of menopausal anxiety with 6 months of treatment with HT plus kava/placebo.[816] A significant reduction in HAMA score was observed in all four

groups of women at months 3 and 6, p <0.05, although the reduction was greater with kava extract, 55.5% for HT+ kava, and 53.3% for ET+ kava, than with HT only, 23% for HT and 25.8% for ET.

Kava was one ingredient of the morning/evening menopause formula in the uncontrolled study previously described. After the eighth week, the vasomotor, anxiety, and depression scores of GCS were reduced by 50%, 56%, and 32%, respectively[762]

11.13.2 Rheum rhaponticum L. *(False rhubarb; East Indian Rhubarb. Family: Polygonaceae)*

Heger and colleagues[804] conducted a multicenter, prospective RCT to investigate the efficacy and safety of extract ERr 731 (identical to the finished product Phytoestrol N®) of the roots of *R. rhaponticum in* hot flushes, anxiety and the overall quality of life in perimenopausal women. The tested extract contained the stilbenoid compounds rhaponticin and desoxyrhaponticin and their aglycones, rhapontigenin and desoxyrhapontigenin. The authors reported that the product, (one enteric-coated tablet (250 mg) containing 4 mg *Rheum rhaponticum* dry extract daily for 12 weeks), significantly decreased the number and severity of hot flushes, p < 0.0001, MRS II total score, p < 0.0001, and improved menopause-specific quality of life scores, p < 0.05, in perimenopausal women compared with placebo.[803,804] Anxiety (measured on the HAMA) scores also decreased significantly with ERr 731 (from 27.5+/-6.8 to 9.4+/-4.2 points) compared with placebo (from 25.1+/-6.0 to 21.6+/-8.6 points).[803] The reduction in the severity of anxiety correlated well with the reduction in number and severity of hot flushes. They also suggested that, at least in the case of breast and uterine disease, the product does not have the same level of risk associated with HT.[804]

11.14 Conclusion

While a wide range of phytotherapeutic interventions is employed in the practice of Western phytotherapy for the relief of menopause-related symptoms, convincing evidence supporting this use from RCTs is still limited. Most rigorous scientific research in this arena has focused on the phytoestrogenic options. However, treatment of menopausal complaints is often required long-term, and concerns have arisen regarding long-term safety of the plant remedies believed to exert oestrogenic activity. This highlights the need for rigorous scientific research on non-oestrogenic herbs commonly used in this context. As discussed previously, CNS-acting agents in conventional pharmaceutical medicine have demonstrated efficacy for symptoms such as flushing, depression and anxiety. In a similar fashion, CNS-acting phytomedicines with reputed

benefits in menopause could potentially exert benefits on the problematic vasomotor symptoms and psychological symptoms via the interaction between neurotransmitters and hormones. The current study thus investigated two such herbs, namely *Vitex agnus-castus* (Chaste tree) and *Hypericum perforatum* (St John's wort).

Chapter Twelve

Vitex agnus-castus
(Chaste tree/berry)

12.0 Introduction

The origin of the current practice of administering *Vitex agnus-castus* in menopause-related complaints is uncertain, but appears to be relatively recent. This chapter reviews the evidence for this application of *Vitex* from pharmacological studies and clinical research, and explores the mechanisms of potential relevance in the context of menopause.

A comprehensive review entitled *Vitex agnus-castus (Chaste-tree/berry) in the Treatment of Menopause-related Complaints,* compiled from the information presented in this literature review, has been peer-reviewed by the Journal for Alternative and Complementary Medicine. A copy of the manuscript is included in Appendix 11.

12.1 Indications for *Vitex agnus-castus*

Vitex agnus-castus belongs to the family Verbenaceae. Its English common names include 'Chaste tree', 'Chaste berry' and 'Monks' pepper'. In the Anglo-American and European practice of phytotherapy, *Vitex* fruit is most widely used for normalising the menstrual cycle, as well as for premenstrual syndrome,[796] which can occur frequently in the perimenopausal years.[224,225] In relation to female reproductive problems, it is also used in premenstrual mastalgia and fluid retention, other premenstrual aggravations such as mouth ulcers, orofacial herpes, hypermenorrhoea, polymenorrhoea, anovulatory cycles, secondary amenorrhoea, infertility and hyperprolactinaemia as well as latent hyperprolactinaemia*,[483,817] insufficient lactation and acne.[796,818]

12.2 Use of *Vitex* in Menopause

Vitex is a common ingredient of many herbal formulations marketed for relief of menopause-related symptoms (Table 12.1).

TABLE 12.1 HERBAL MENOPAUSE FORMULATIONS CONTAINING VITEX AGNUS-CASTUS ON THE MARKET

Manufacturer	*Product*
Forces of Nature	Menopause Ease (Essential oil blend – transdermal)
Fusion Health	Menopause
Gaia Herbs	Supreme Vitex/Alfalfa - *A Menopausal Corrective Formula*
Herb Farm	Healthy Menopause Tonic (Pulsatilla + Vitex Comp)
Herbs of Gold	Menopause Night Relief
Nature's Alternatives	Vitex / Black Cohosh Plus: Women's Menopause Herb Tonic MenoEze Forte; MenoEze Day Night formula; MeniThin
Naturopathica	Menopause support
Nature's Sunshine	Wild yam and chaste tree cream
Neways	MenoLife
NutraLife	Herbal Supplement for Menopause, with Black Cohosh & Vitex
Oona	Femaren
Oriental Botanicals	MenoChange Cimicifuga-Vitex Compound
Planetary Formulas	EstroTrim :Menopause Weight Control
Pretorius	Phyto-Female Complex
SuperHerb, Netanya	Estro Balance plus Vitex: Menopause Relief
Totally Natural Products	

As mentioned previously, the practice of using *Vitex* in the treatment of menopause-related symptoms appears to have originated relatively recently, following the renewed interest in the herb for female reproductive disorders generated by Gerhard Madaus in

* Latent hyperprolactinaemia is a non-physiologically stimulated prolactin release often manifest during the time of decreasing progesterone and oestradiol levels, that is frequently also accompanied by an insufficient function of the corpus luteum.

Germany in 1930.[42] However, it is possible that early references to its value for 'diseases of the uterus'[39] and as an emmenagogue to promote menstruation[40] incorporate use in menopause. In the published literature, the benefits of *Vitex* for menopausal complaints and menopausal bleeding are first mentioned in 1972 with the report of its clinical application by Warkalla and colleagues in Germany.[41] In an article entitled *Agnolyt® in the treatment of gynecological complaints,* five clinicians report on their collective experience of its employment is several thousand cases for a variety of disorders. [Agnolyt® is a patent medicine extracted from dried *Vitex* berries.]

Its use in this context appears to have now become relatively popular in the Anglo-American tradition.[777,796,807,819] A survey of 155 members of the National Institute of Medical Herbalists (NIMH) in the UK found that 86.3% prescribed *Vitex* in the treatment of menopausal symptoms such as hot flushes;[820] it is also used for HT withdrawal.[796,820] However, despite its apparent popularity in this context, no studies could be found in the literature on the oral administration of herbal extracts of *Vitex* as a sole agent for the treatment of menopausal symptoms. There were only two poorly designed studies by the same investigators examining the effects of the essential oils used in aromatherapy measured by non-validated questionnaire-based surveys (reviewed below).[821,822] The authors did, nonetheless, report the oils from both the leaf and berry to be effective in the treatment of menopausal symptoms. Evidence is less than convincing from the several RCTs[714,734,793] and one pilot observational study[823] of formulations containing *Vitex* that have appeared in the literature. (These are reviewed below). However, pharmacologically, there are some valid reasons supporting the potential utility of *Vitex* in menopause.

12.3 Phytochemistry & Pharmacology of *Vitex agnus-castus*

The part used in the Anglo-American and European traditions is the fruit. *Vitex* berries have been found to contain an essential oil, flavonoids such as casticin and iridoid glycosides including aucubin, agnoside and eurostoside.[483,796] In terms of active constituents, earlier research focussed on the volatile diterpenes[824] whereas more recent research suggests other diterpenes may have more significant activity, and are responsible for the dopaminergic activity.[483] The most active ones are relatively non-polar and probably cross the blood-brain barrier.[483]

12.3.1 Phytoestrogen content of Vitex

Findings from recent cell culture experiments indicate that *Vitex* extracts may contain phytoestrogens.[825] Using ligand binding assays, it was demonstrated that *Vitex* phytoestrogens are oestrogen-receptor (ER)ß-subtype-selective. The flavonoid apigenin was identified as the most active oestrogen-receptor-selective phytoestrogen; other isolated compounds were vitexin and penduletin.[825] However, the compounds identified are only weakly oestrogenic, not unique to this herb, and present in relatively low levels compared to other herbal and dietary sources. This finding is therefore unlikely to contribute to our understanding of the true mechanism of the action of *Vitex*.

12.3.2 Relevance of Pharmacological Actions to Menopause

Given the current understanding and hypotheses regarding the aetiology of menopausal symptoms, a number of pharmacological actions of *Vitex* may be of relevance. These include correcting relative oestrogen excess/progesterone activity, addressing latent hyperprolactinaemia associated with PMS-like symptoms experienced during the perimenopause by its dopamine-agonist activity, enhanced melatonin release, 'normalising' the menstrual cycle, and effects on opioid receptors.

Corpus luteum-like Effect

Pharmacological research in the early 1960s on guinea pigs suggested that, at normal doses, *Vitex* demonstrated a decrease of oestrogen effects and a promotion of progesterone effects, mediated through the pituitary gland: FSH secretion was decreased and LH and prolactin secretion were increased.[826] At the high dose (about 20 times normal dosage) an inhibition of all gonadotrophic hormones and growth hormone resulted.[826] It was thus said to correct a relative progesterone insufficiency via an indirect effect rather than a direct hormonal action. It was believed that the increase in LH production was responsible for effecting this shift in the ratio of oestrogen to progesterone, which gave it a corpus luteum-like effect and the ability to correct corpus luteum insufficiency.[827,828] This view was supported by the previous clinical findings of Probst and Roth in their 1954 clinical study on *Vitex*.[829]

Prolactin Inhibition

However, later *in vitro* research using rat pituitary cultures found that *Vitex agnus-castus* extract inhibited pituitary prolactin release[830] while gonadotropin secretion remained unaffected. [831] This suggested that enhancement of progesterone may be due to *Vitex*

decreasing the suppressive effect of prolactin on corpus luteal development, rather than by increasing LH. It was found to contain an active principle that binds to the D_2 receptor[831] thereby exhibiting a dopaminergic effect on the anterior pituitary and inhibiting prolactin secretion. (Dopamine inhibits prolactin secretion from the anterior pituitary.) The results of a placebo-controlled clinical study of prolactin secretion in 20 healthy male subjects suggest the effects of *Vitex* extract are dependent on the dose administered and the initial level of prolactin concentration.[832]

Latent Hyperprolactinaemia and Female reproductive Disorders

Increased prolactin levels are commonly associated with premenstrual mastalgia, corpus luteum insufficiency[833] and its associated conditions of hypermenorrhoea, polymenorrhoea, persistent anovulatory bleeding, secondary amenorrhoea, anovulatory cycles, infertility, oligomenorrhoea, menorrhagia, metorrhagia and irregular menstrual cycles.[483] Hyperprolactinaemia results in inhibition of secretion of GnRH, and decreased secretion of LH and FSH. In the ovary, this results in inhibition of progesterone secretion by the granulosa-lutein cells of the corpus luteum.[818,834] Premenstrual symptoms, particularly mastodynia, are often accompanied by *latent* hyperprolactinemia,[835,836] which can manifest during the times of decreasing progesterone and oestradiol during the late luteal phase, and can also be stimulated by stressful situations.[833,817] The mild D2 receptor agonistic properties of *Vitex agnus-castus* extracts are said to be able to inhibit latent hyperprolactinemia[837] in a similar way to synthetic D2 receptor agonist drugs used for menstrual mastalgia[483] which, however, have severe side-effects.[837] (The prolactin inhibiting effects of *Vitex* were demonstrated by a case study of a an 18 year-old girl whose symptoms of prolactinoma were found to have resolved, with a concomitant decrease in prolactin levels, after 3 months' use of *Vitex* (Agnolyt®).[838]) In light of the suggestion that many of the menopausal symptoms may represent an exacerbation of premenstrual symptoms, targeting premenstrual latent hyperprolactinemia may also be appropriate during the perimenopause.

Dopamine and Thermoregulation

Dopamine has recently been found to be an important thermoregulatory neurotransmitter, with D2 receptors involved principally with the maintenance of body temperature in euthermia.[345] Earlier research observed the dopamine agonist bromocriptine to increase the activity of the endogenous opioid system on the thermoregulation mechanisms that regulate body temperature in postmenopausal

women[346] and to be effective in alleviating hot flushes.[347] Hence, a postulated role for *Vitex* in this context is suggested.

Oestrogen directly modulates dopaminergic activity.[99] In the cerebral cortex, dopamine is involved in emotional responses. In postmenopausal women, the activity of the dopaminergic system was found to be significantly lower than in premenopausal women, but was significantly increased by HT administration, with a concomitant significant decrease in psychological symptoms.[441] The dopaminergic properties of *Vitex* may, therefore, be of relevance to the relief of the psychological symptoms associated with the menopause transition.

Melatonin

A dose-dependent increase in melatonin secretion, especially during the night, was found after administration with *Vitex* extracts 120 mg & 480 mg per day (dried herb equivalent 0.6 g and 2.4 g) in an open placebo-controlled trial. [839] Total melatonin output was approximately 60% higher than in the placebo group. This has obvious potential relevance to menopause-related sleep disturbances. However, data from a recent case report also demonstrated that melatonin was able to delay the characteristic endocrine parameters associated with menopause onset.[840] While further studies are needed to confirm this finding, it is extremely interesting in view of: i) the possible role of declining melatonin secretion (that precedes FSH increase[168]) in the development of menopause and its symptoms;[169] and ii) the effect of *Vitex* on melatonin secretion, which may be part of the rationale for using it in menopause.

Cycle Regulation

The normalising action of *Vitex* on menstrual cycles is also of potential relevance in view of i) the potential role of melatonin in delaying onset of menopause; and ii) theories regarding the possible aetiology of PMS-like symptoms during the perimenopause. For example, it has been observed that recruitment of a new follicle can occur during the mid-luteal phase (termed the 'luteal-out-of-phase' (LOOP) phenomenon). [493] This results in its subsequent ovulation at the *beginning* of the next cycle and is associated with high oestradiol levels peri-menstrually, and may account for the elimination of follicular phase remissions in the symptom of dysphoria.[372]

Endorphins/Affinity to opioid receptors

In 2000, Meier and colleagues suggest additional pharmacological actions for *Vitex agnus-castus* via opioid receptors.[841] They reported a relatively potent inhibition for opioid (mu and kappa subtypes) receptor-binding with extracts of *Vitex,* that was most pronounced in lipophilic fractions. Additionally, binding to delta opioid receptors was found to be inhibited mainly by an aqueous fraction of *Vitex. In vitro* research is of uncertain relevance to oral dosing in humans. High levels of direct exposure of test cells to the herbal extract occur in *in* vitro research, whereas pharmacokinetic factors after oral administration affect the bioavailability of the phytochemicals in human and animal models. However, it was subsequently also demonstrated in human and animal models, by Webster and co-workers, that *Vitex* acted as an agonist at the mu-opioid receptor.[842] Extracts with and without fatty acids removed showed significant affinities to the mu-opioid receptor.

These findings support the beneficial action of *Vitex* in PMS, as endorphins are shown to decrease in the late luteal phase[843,844] and are found to be associated with symptoms such as mood disorders, migraines and fluid retention.[845,846] However, they are also of potential relevance to its reputed value in treating menopausal symptoms, as the reduction of endogenous opioid activity may be responsible, at least in part, for the central noradrenergic instability associated with hot flushes.[337,338] Increasing endogenous opioid peptide activity may effect a reduction in hot flushes via inhibition of noradrenergic activity below the threshold needed to activate heat loss.[320] In an oestrogen-deficient environment, it is possible that mood enhancement may be effected by stimulating the activity of opiate-containing neurons[438] and thereby increasing the synthesis and release of β-endorphin.[439]

12.4 Clinical Studies with *Vitex agnus-castus* for Menopausal Symptoms

Despite its apparent popularity with UK herbalists, and as a component of menopause formulations, evidence from clinical trials on oral doses of *Vitex* as a sole agent in menopause is lacking, and results from the limited number of studies of formulations containing *Vitex* for the treatment of menopausal symptoms have been inconsistent.

12.4.1 Aromatherapy Studies

The steam distilled essential oil of the fruit and leaves have been investigated in two studies that reported benefits for menopausal symptoms.[821,822] The first study with 23

women contained several weaknesses, including different extracts and variable doses, different routes of administration (transdermal, inhalation, oral), lack of a standardised rating scale and failure to exclude other concomitant treatments such as HT, herbs and acupuncture. Nonetheless, the percentage of women reporting improvements with the oils ranged from 27% for memory and concentration difficulties to 80% for mood swings and depression. Interestingly, the most common 'side-effect' was resumption of regular periods.[821] The subsequent study with 52 peri- or postmenopausal women aged 38 to 73 used a 1.5% solution of the essential oil of *Vitex* aerial parts in a base cream or lotion. Participants applied 2.5 ml of the cream transdermally, once daily, 5–7 days per week for 3 months. Overall, 33% reported major improvement and 36% reported mild to moderate improvement in troublesome symptoms, with greatest improvement observed in the emotional symptoms, (16 responses), hot flushes/night sweats (15), and moderation of menstruation (12). However, these results need to be interpreted with caution due to the lack of a control group. The findings from aromatherapy studies are of uncertain relevance to administration of oral dosage forms.

12.4.2 Studies on Orally administered Formulations containing Vitex extracts

Two RCTs and one pilot study on formulations containing *Vitex* for the treatment of menopausal symptoms have been reported in the literature. Results of these studies are reported here.

Phyto-Female Complex

Vitex agnus-castus was one component of a menopause herbal formulation, *Phyto-Female Complex,* found to be significantly superior to placebo in an RCT on menopausal hot flushes and night sweats in 50 healthy perimenopausal and postmenopausal women, aged 44-65 years.[793] In the 35 who completed the study, a 73% decrease in the number of hot flushes was observed at the end of the 3-month treatment period in the active treatment (n = 19) compared with 38% in the placebo group (n = 16), p = 0.026, and the number of night sweats was reduced by 69% and 29% respectively, p = 0.027. Hot flushes ceased completely in 47% of women in the study group compared with only 19% in the placebo group. A significant benefit was also observed in terms of sleep quality. The other herbs in the formulation were *Cimicifuga racemosa* (black cohosh) root extract, 100 mg (2.5 mg triterpen glycoside, 2.5%); *Silybum marianum* (St Mary's thistle/milk thistle) herb extract, 75 mg (60 mg silymarin, 80%); *Angelica sinensis* (dong quai) root extract, 75 mg (7.5 mg ligustilides, 1%); *Trifolium pratense* (red clover) flower extract, 50 mg (4 mg isoflavone, 8%); and *Panax quinquefolium* (American ginseng) root extract, 50

mg (12.5 mg ginsenosides, 25%). The dose of *Vitex agnus-castus* fruit extract (2.5 mg vitexin, 5%) was 50 mg. Tablets were administered twice daily. The potential synergistic effects of this combination do not permit conclusions about the contributions of individual herbs. In view of the small sample size at the end of the treatment phase, these results are encouraging, although not adding specifically to the evidence for *Vitex* in this context. As discussed previously, black cohosh alone has demonstrated considerable clinical efficacy in the management of hot flushes.[704,717,723,731-733,735]

The Herbal Alternatives for Menopause study

The Herbal Alternatives for Menopause (HALT) study investigated 3 different herbal regimens compared with HT and placebo over a period of 12 months.[734] It assigned 351 peri- or postmenopausal women with 2 or more vasomotor symptoms per day to one of 5 groups: 1) *Cimicifuga racemosa* (black cohosh 160 mg daily; 2) multibotanical formulation with *Cimicifuga racemosa*, 200 mg daily, and 9 other ingredients including *Vitex agnus-castus*; 3) multibotanical plus dietary soy counselling; 4) conjugated equine oestrogen, 0.625 mg daily, with or without medroxyprogesterone acetate, 2.5 mg daily; 5) placebo. The multibotanical formulation contained the following daily doses: *Cimicifuga racemosa* (black cohosh), 200 mg; *Medicago sativa* (alfalfa) 400 mg; boron, 4 mg; *Vitex agnus-castus* (chaste tree), 200 mg; *Angelica sinensis* (dong quai), 400 mg; *Chamaelirium luteum* (false unicorn), 200 mg; *Glycyrrhiza glabra* (licorice), 200 mg; *Avena sativa* (oats), 400 mg; *Punica granatum* (pomegranate), 400 mg; *Eleutherococcus senticosus* (Siberian ginseng, standardised constituents 0.8% eleutherosides E and B), 400 mg. There was no significant difference at any of the time points measured (every 3 months) between any of the herbal interventions and placebo as measured by the Wilkund vasomotor subscale, frequency or intensity of hot flushes and night sweats. The exception was symptom intensity at 12 months, with the multibotanical plus soy intervention performing significantly worse than placebo (p = 0.016). The average difference over all the time points between herbal interventions and placebo was less than 0.55 vasomotor symptoms per day, compared with - 4.06 for HT compared with placebo. (For the multibotanical, the no significant differences were found between the study group and placebo at 3 months, p = 0.45, 6 months, p = 0.18, or 12 months, p = 0.88). While the sample size and duration of this study are definite strengths, a major limitation of this study is the recruitment of women with mild symptoms. It is recommended by the U.S. Food and Drug Administration (FDA) guidelines that seven moderate to severe hot flushes per day, or 50 to 60 per week, be the minimum requirement for menopause studies.[847]

Pilot Study

A pilot study of a formulation of 15 whole herbs suggested a potential benefit of the combination for improving moderate menopausal symptoms in women.[823] Symptoms were measured on the Modified KI, daily hot flushes severity, and overall quality of life (QoL) using the SF-36 index. The herbs were administered in 550 mg capsules, 2 capsules taken twice daily, providing a total of 2,200 mg of whole herbs per day. However, given the large number of herbs in the formulation (*Cimicifuga racemosa* - black cohosh root, *Viburnum opulus* - cramp bark, *Mitchella repens* - squaw vine, *Valeriana officinalis* - valerian root , *Polygonatum multiflorum* - King Solomon seed (also known as Solomon's seal), *Taraxacum officinalis* - dandelion root, *Vitex agnus-castus* - chaste tree berry, *Rosmarinus officinalis* - rosemary leaves, *Nigella sativa* - black seed (also known as black cumin), *Eupatorium purpureum* - queen of the meadow, *Epimedium grandiflorum* - epimedium leaf, *Ligusticum chuanxiong* - chuanxiong rhizome, *Schisandra chinensis* -schisandra berry, *Mentha piperita* - peppermint leaves, *Rubus idaeus* - red raspberry leaves), the dose of each individual herb was quite low, ranging from 80mg to 300mg per day. The dose of *Vitex* was 140 mg per day, or 6% of the total. From baseline to 3 months, a 42% decrease was observed in daily hot flushes $p = 0.0003$, and KI total symptoms score decreased by 24%, ($p = 0.0028$). In addition to the low dose of *Vitex* in the formula, the inclusion of *Cimicifuga racemosa* (black cohosh), a herb for which there is evidence of efficacy in menopause-related symptoms[717,723,731,733]means that no conclusions can be drawn about the individual contribution of *Vitex agnus-castus.* The lack of a placebo group is a major limitation. Placebo effects for vasomotor symptoms in menopause studies are substantial, with 51% being the average for studies of HT according to a 2004 meta-analysis,[499] and they are generally in the range of 30 to 41% in RCTs of medicinal herbs.[75,76,793,848,849] It is possible that the 42% reduction in vasomotor symptoms observed in this study would not be significant over placebo. The small sample size in this study also suggests this result should be interpreted with caution.

12.5 Vitex and Premenstrual Syndrome

It is possible that the practice of using *Vitex agnus-castus* for menopausal symptoms refers to its use during the perimenopause when PMS-like symptoms are anecdotally reported to be prevalent, or at least less well-tolerated.[224] Additionally, it has been proposed that some symptoms attributed to the menopause-transition such as mood changes and breast discomfort are more likely to be menstrual-cycle related, given that they improve after cessation of menstruation.[225] [Theories regarding the aetiology of

PMS-like symptoms during the perimenopause have previously been reviewed (Refer to section 8.3).] Thus, the research on *Vitex* in relation to PMS may well be relevant in this context.

12.5.1 Clinical studies of Vitex for PMS

Findings from one RCT, comparative studies and observational studies have reported a positive effect for *Vitex* in the treatment of PMS:

In a randomised, double-blind, placebo-controlled trial on *Vitex* (extract Ze 440, 60% ethanol m/m; extraction ratio 6-12:1; standardised for casticin; 20mg tablet once daily) with 170 participants over three consecutive menstrual cycles, Schellenberg[481] found *Vitex* caused significant improvement in premenstrual symptoms such as irritability, mood alteration, anger, headache, breast fullness and bloating as rated by their self assessment and changes in clinical global impression. Responder rates (considered as 50% reduction in symptoms) were 52% and 24% for active and placebo, respectively.
Lauritzen and co-workers conducted a multi-centre, randomised, double-blind comparative trial of *Vitex* (Agnolyt®) dried extract 3.5-4.2 mg per day; extraction ratio 9.58-11.5:1) and pyridoxine (Vitamin B6).[850] Results showed that premenstrual tension syndrome symptoms improved for both groups, but that the characteristic symptoms of breast tenderness, oedema, abdominal tension, headaches, constipation and depressed mood were more significantly reduced by *Vitex* than pyridoxine. In the *Vitex* group, 77.1% showed an improvement on the Clinical Global Impressions (CGI) scale. The efficacy of treatment with *Vitex* was rated as excellent by 24.5% of investigators and at least adequate by more than 80%. Assessment by patients indicated that 36.1% of the *Vitex* group felt free from complaints. The obvious limitation of this study is the absence of a placebo control group.

Findings from a placebo-controlled trial conducted in 1987 on Gerard *Agnus castus* tablets over three months were reported in a Gerard House promotional brochure.[851] Of 30 women randomised to the active treatment, 21 completed the trial. Symptoms were rated on Abraham's Menstrual Symptoms Questionnaire (MSQ). Improvements were noted in all subscales for the treatment group using a percentage reduction from the mean baseline scores in each sub-cluster to the end of 3 months: for PMS-A (anxiety sub-cluster), 52%; in PMS-H (hydration), 41% ; PMS-C (cravings), 48% PMS-D (depressive symptoms), 64%. These results were significantly superior to placebo for all sub-clusters except PMS-C where the placebo outperformed the active treatment.

In a single-blind, rater-blinded prospective study, Atmaca and colleagues compared the efficacy of 2 months' treatment with fluoxetine, an SSRI, with *Vitex* extract in 41 patients with PMDD, diagnosed according to DSM-IV.[852] The response rate was similar for both groups: fluoxetine 68.4%, and *Vitex* 57.9%. However, fluoxetine was more effective for psychological symptoms while *Vitex* was more effective for the physical symptoms.

Forty-three women completed a prospective, multicentre study that examined the efficacy of *Vitex agnus-castus* extract Ze 440 (20 mg/day) in 50 women with PMS over three menstrual cycles.[853] Thirteen patients were concurrently using oral contraceptives. There was a 42% reduction in scores on the Moos' menstrual distress questionnaire, mainly in fluid retention, negative feelings, pain and behavioural changes. Symptoms remained 20% below baseline scores 3 cycles post-cessation of treatment. The lack of a placebo arm is again a limitation to this study. The inclusion of women taking the OCP also limits the interpretation of these results.

Loch and co-workers conducted a multicentre open study to investigate a solid preparation from an extract of *Vitex* berries (Femicur® capsules; 20mg/day) on 1634 women suffering from PMS.[854] After treatment for three menstrual cycles, 93% of patients reported a decrease in the number of symptoms or even cessation of PMS complaints, including psychological and somatic symptoms. A reduced incidence of mastodynia was also observed, falling from 81% to 27% over the treatment period.

In a large, multicentre drug-monitoring study, 1592 women suffering from either PMS or menstrual disorders, predominantly corpus luteal insufficiency, were monitored for an average of six months while taking drop doses of *Vitex* tincture (mean of 43 drops per day).[827] Physicians rated the efficacy of the treatment as good or satisfactory in 90% while in the patients' assessments, 61% were good and 29% as satisfactory.

12.6 Adverse events

Serious adverse events have not been observed with *Vitex agnus-castus.* In a few poorly-documented cases, its use has been associated with persistent headaches[855] Lauritzen observed mild adverse events including gastrointestinal and lower abdominal complaints, skin manifestations (2 subjects) and transitory headache (1 subject) in a small percentage of patients (5.5%).[850]

In the RCT by Schellenberg on *Vitex agnus-castus* in PMS, mild adverse events were reported in both the treatment group (4.7%) and placebo group (4.8%).[481] Each of the following events was reported by one subject: acne, multiple abscesses, intermenstrual bleeding and urticaria, none of which caused discontinuation of the treatment.

In the comparator study with fluoxetine and *Vitex* reported by Atmaca, the most frequently reported side-effects in the *Vitex* group (*n* = 19) were nausea (*n* = 5) and headaches (*n* = 4) over the 2 month treatment phase.[852]

Loch found the most common symptoms, reported by 0.8% of participants to be acne, allergic reaction, eczema, hair loss, itching and urticaria.[854] Six patients (0.4%) reported gastrointestinal problems, such as diarrhoea, stomach pains, nausea, vomiting and abdominal distension.

Propping noted adverse effects in 2.4% of the 1,592 women monitored. [827] Nausea was reported in 7 cases, changes to the menstrual rhythm (5 cases), acne, skin rashes and headache (in 3 cases each), dyspepsia and skin reddening (2 cases each).

Berger found that the most frequently reported adverse events were those commonly reported by PMS patients regardless of participation in a trial: acne (*n* = 7), headaches (*n* = 6), spotting (*n* = 5), and gastrointestinal complaints (*n* = 5).[853] Overall, 37 events were reported by 20 participants over a total of 344 menstrual cycles. Laboratory and medical examinations showed no influence of any kind relevant to treatment.

Observation of 551 patients treated with *Vitex* by 153 gynaecologists indicated 5% of patients experienced side-effects, predominantly nausea.[856]

12.7 Contraindications

Co-administration of *Vitex agnus-castus* and progesterone drugs, the contraceptive pill[857] or HT is not recommended.[805] Due to its dopaminergic action, a possible antagonistic interaction may occur with dopamine receptor antagonists.[796] In addition, *Vitex* may aggravate pure spasmodic dysmenorrhoea not associated with PMS.[858] Although its use in lactation is sometimes cautioned against due to its prolactin-inhibiting effects, at low doses *Vitex* berry is a galactagogue. [805] It should be used in the early stages of pregnancy only if indicated.[805]

Toxicology data for *Vitex* in animals and is presented in Mills and Bone (2005: p335).[805]

12.8 Summary

The origins of the practice of administering *Vitex* in menopause are unclear. While several recent studies have suggested a benefit for formulations containing *Vitex* in the treatment of menopausal symptoms, evidence from rigorous RCTs to support its use as a sole agent in this context is lacking. Recent evidence from pharmacological studies points to possible mechanisms that could account for beneficial effects in some of the symptoms of menopause, as well as possibly influencing its onset. As the endocrinology and neuroendocrinology of menopause and its symptoms have not yet been fully elucidated, no firm conclusions can be drawn. However, the emerging pharmacological evidence supports a role for *Vitex agnus-castus* in the management of menopause-related symptoms.

Chapter Thirteen

Hypericum perforatum

(St John's wort)

13.0 Introduction

This chapter of the Literature Review examines the research on St John's wort with regard to its mechanisms of action, evidence from clinical studies of efficacy for symptoms relevant to menopause, and safety data that informed entry criteria to the current clinical trial.

13.1 Actions and Indications of *Hypericum perforatum*

St John's wort (the dried aerial parts of *Hypericum perforatum,* family Clusiaceae/Guttiferae) is best known today for its role in the treatment of mild to moderate depressive disorders, for which is has received much attention in the scientific literature.[859-872] It also has anti-viral, antiseptic, nervine and vulnerary actions.[796] Traditionally it was a highly esteemed remedy for lacerated and punctured wounds and bruises, both internally and externally.[27,47] Its many other traditional indications are largely centred around its actions on the spine and nervous system: spinal irritation and injuries, "shocks, or concussions; throbbing of the whole body without fever," (p1038)[27] "neuralgia, fibrositis and sciatica" (p115)[21] but also include "intermittent fever, dysentery, gravel, pectoral complaints, worms, and jaundice" (p36). [2] Traditional indications of potential relevance to menopause include menorrhagia and[27] hemorrhages.[47] Madaus in 1938 recommended it "For menopausal bleeding.. with *Viscum album*" (p1592).[42] Those indications involving the nervous system are also relevant, notably in *Kings' American Dispensatory* "hysteria, nervous affections with depression"[27] and subsequently in the 1918 *Dispensatory of the United States of America* "hysteria, mania…." It formerly enjoyed great reputation for the cure of demoniacs"(p36),[47] a reference to the prevailing belief at the time that psychological disturbances were the work of evil spirits. It appears in *The British Herbal Pharmacopoeia* of 1983 as specific for "menopausal neurosis" (p115).[21]

13.2 Pharmacology of *Hypericum perforatum*

13.2.1 Phytochemistry

The key active constituents of *H. perforatum* include the naphthodianthrones, hypericin and pseudohypericin, collectively referred to as total hypericins; procyanidins; phloroglucinol derivatives including hyperforin, flavonoids such as quercetin and biapigenin; and xanthones.[873] The hypericins have varying solubility in different solvents and thus, most preparations are standardised for total hypericin (hypericin plus pseudohypericin) content.[874] The exact mechanism of action is not known. Hypericin was previously thought to be the active constituent in depression[875-878] but more recent interest has focussed on hyperforin, an unstable compound, which has been shown to have antidepressant activity,[879-882,863,883] although the evidence is conflicting. The flavonoids have also received considerable attention in this context.[884,885] However, the total extract appears to be more effective than isolated constituents[886] in terms of the

antidepressant effect, which is therefore likely to involve many, if not all constituents of *H. perforatum.*[887]

13.2.2 Postulated Pharmacological Actions of Hypericum perforatum

The exact role of neurochemical mechanisms underlying *in vivo* actions of St John's wort extracts are not well defined. However, it has been demonstrated in the rodent brain that, in addition to inhibiting the uptake of the monoamine neurotransmitters serotonin, noradrenaline and dopamine, (like many other antidepressants), St John's wort extract also inhibits uptake of the amino-acid neurotransmitters, GABA and L-glutamate.[888-891] There is also evidence for β-adrenoreceptor down-regulation[892,893] and a significant up-regulation of 5-HT2-receptors in the frontal cortex.[893]

Other studies on plasma hormones and brain neurotransmitters in humans and rats after administration of *Hypericum* and its constituents, however, suggest that *Hypericum* may effect plasma hormonal changes via both serotonin and dopamine-mediated mechanisms, but do not involve noradrenaline.[894]

Schulte-Lobbert and colleagues[895] found that the potency of St John's wort products in inhibiting the uptake of serotonin depended on their hyperforin content. Further support for the antidepressant activity of hyperforin is derived from findings that it is a potent uptake inhibitor of serotonin, dopamine, noradrenaline, GABA and L-glutamate. Furthermore, the effects of two different *Hypericum* extracts in behavioral despair and learned helplessness closely correlated with their hyperforin contents.[880]

Other mechanisms that have been observed include:

- an effect on opioid systems in mesolimbic regions in the CNS in the rat brain, either by a direct or indirect mechanism[896]
- different pharmacological effects like receptor bindings[897]
- Improving resistance to stress and preventing exhaustion of the HPA system[898]
- Suppression of interleukin-6 in blood samples *ex vivo*.[899] This suppression may assist in deactivating the hypothalamic-pituitary-adrenal axis, leading to inhibition of elevated corticotrophin-releasing factor and other adrenal regulatory hormones. These changes could be linked to antidepressant activity.
- Modulation of HPA axis function;[885] influencing central neurotransmitters, thereby causing cortisol stimulation in a dose-dependent manner;[900] reducing corticosterone and cortisol in brain frontal cortex tissue, but not serum[901]

- Inhibition of MAO-A and MAO-B activity in vitro (weak)[893]
- A photosensitising effect for hypericin, since *Hypericum* treatment has lowered the amount of light necessary to obtain a clinical antidepressant effect[796]
- Inhibition of sodium-dependent uptake of catecholamines and amino acids into synaptic nerve endings, probably by mechanisms controlling the synaptic sodium concentration[902]
- Modification of specific membrane structures indifferent ways, decreasing the flexibility of fatty acids in the membrane hydrocarbon core, but fluidising the hydrophilic region of membrane phospholipids.[903]

13.2.3 Relevance of Pharmacological Actions to Menopause

The effectiveness of *Hypericum* in depression,[49,859,861,868,904,905] and potentially anxiety[48,49,800,801,880,906] and sleep[21,49,907] suggest a beneficial role for *Hypericum perforatum* in these symptoms when associated with menopause. Pharmacologically, the observed effect on opioid, and dopamine systems in mesolimbic regions in the CNS observed animal models[896] may be of relevance to hot flushes, although extrapolating results from studies in animal models to the clinical situation must be done with caution. Dopamine has an effect on thermoregulation,[345]possibly via increasing the activity of the endogenous opioid system,[346] so that dopamine agonists have been found to alleviate hot flushes.[347] The evidence for β-adrenoreceptor down-regulation[892,893] may also be relevant in this context. Elevated brain noradrenaline levels[330] have been associated with the core body temperature elevations preceding hot flushes.[198] It is possible that St John's wort, by causing down-regulation of β-adrenoreceptors[892,893] could result in inhibition of noradrenergic activity below the threshold needed to activate heat loss. Its hypothesised action of improving resistance to stress and thereby preventing exhaustion of the HPA-system[898] would be consistent with the observed effects of stress and anxiety on menopausal hot flushes.[91,197] The action of St John's wort on inhibiting GABA uptake is dissimilar to that of gabapentin. Despite its effectiveness in hot flushes,[552] gabapentin is not a GABA agonist, does not inhibit GABA uptake and acts independently of GABA receptors.[321]

13.3 Evidence from Clinical studies for the benefits of *Hypericum* in Menopause

Research from rigorous RCTs on *Hypericum perforatum* as a sole agent in menopausal complaints is lacking. However, its well-established efficacy in depression, and to a lesser extent, anxiety, suggest a role for *Hypericum* in the treatment of menopausal psychological and neurovegetative symptoms. Furthermore, selective serotonin reuptake inhibitors (SSRI's) have a potential role in the relief of hot flushes[197] and *Hypericum*, like SSRI's, may act by inhibiting the uptake of neurotransmitters,[908] thus giving it a potential role in the treatment of hot flushes.

Clinical studies on the effectiveness of *H. perforatum* have predominantly used the following preparations:

a) Lichtwer LI 160, (such as Kira® Lichtwer Pharma), containing 0.3% hypericin and an undefined amount of hyperforin
b) ZE 117 (Remotiv®, Zeller),containing 0.2% hypericin and little hyperforin
c) WS 5572, (Movana®, Pharmaton), containing 3 – 7% hyperforin.

The trials relevant to menopause are reviewed below.

13.3.1 Observational Study on a Mono-preparation

In an open, observational study of 111 women aged between 43 and 65 years, *Hypericum perforatum* Kira® preparation (Lichtwer Pharma, Berlin, Germany) was found to significantly improve the psychological (irritability, depressive moods, inner tension, anxiety disorders, disturbed sleep and concentration disorders) and psychosomatic symptoms of menopause (hot flushes, outbreaks of sweating, palpitations, dizziness, headaches. [49] In addition, a significant improvement in sexual well-being was found. The total daily dose was 405 – 675 mg extract (extraction ratio not given), standardised to 0.9mg hypericin (one tablet containing 135-225 mg St John's wort extract, standardised to 300μg hypericin, three times daily) for a period of 12 weeks. The Menopause Rating Scale (MRS) and the CGI scale were used to evaluate treatment outcomes as well as a self-designed questionnaire to assess sexuality. Participants were recruited from a general medical and psychotherapy practice and were experiencing symptoms classified as 'pre' (presumably 'peri-) and post' menopausal. One hundred and six were included in the final analysis. Total scores on the MRS fell from 63.4 ± 16.5 to 23.5 ± 25.7 (63%), or from marked intensity to slight intensity. As rated by the CGI scale, symptoms disappeared or diminished in 76.4% women by self-rating, and in 79.2% by physician

rating (p <.0.001). On the self-designed sexuality assessment scale, significant improvements were found in the feeling of attractiveness (p <.0.001) and participants' rating of the importance of sexuality (p <0.001).

The main limitation of this study is that, as an observational study, it lacked a control group. As symptoms fluctuate throughout the menopausal transition, the response in the placebo group due to natural history can be substantial, with an average of 51% observed in RCTs of HT for hot flushes.[499] However, total scores on the MRS and CGI in this study *did* fall by more than 50%. As pointed out by Linde and Knuppel,[866] non-randomised observational studies are not subject to the same restrictions as RCTs in terms of strict inclusion criteria and limited resources that can result in small sample sizes and short observation periods. Large-scale non-randomised observational studies, such as this, can provide useful data regarding long-term outcomes, prognostic factors and rare adverse events. Such studies add to the evidence base and provide the stimulus for further investigation. Another limitation is that the MRS is not used as widely as the KI or the Greene Climacteric Scale in menopause studies, which may restrict comparison between this and future studies. It could also be argued that participants' psychological symptoms were not classified according to standardised symptom-rating instruments for depression or anxiety, but in a menopausal symptoms study, rating scales for menopause may be more appropriate. In terms of reproducibility of findings, the lack of a standard dose is a limitation, as the wide range of extract contained in the daily dose meant that dosage varied for different individual participants.

13.3.2 Comparison of Hypericum with Diazepam

A 12-week drug monitoring study on a St John's wort mono-preparation, published in 1986, reported it to be of comparable efficacy to diazepam for climacteric depression.[48] On the clinician-rated CGI scale, Hyperforat® at the dose of 0.2 mg three times daily resulted in a 77.5% reduction in scores, compared with 50% for diazepam, 2 mg three times daily. On the Hamilton Anxiety Scale (HAMA), scores reduced by 72% with Hyperfort® compared with 37% for diazepam. The Self-rating Depression scale (SDS) found a 46% reduction with Hyperfort ® compared with 31% for diazepam. This study suggests beneficial effects on both anxiety and depression in this cohort. However, since diazepam is an anti-anxiety agent, its superiority in depression is hardly surprising.

13.3.3 Combinations of Hypericum with Other Plant Extracts in Menopause

Results of several studies support the benefit of the combination of *Hypericum perforatum* with *Cimicifuga racemosa* (black cohosh) in the treatment of menopausal complaints.[702,703,705,716,909]

In a double-blind, placebo-controlled RCT of 301 women experiencing menopausal complaints with a pronounced psychological component, Remifemin® plus St John's wort, a combination of an ethanolic extract of isopropanolic *C. racemosa* and *H. perforatum,* was significantly superior to placebo ($p < 0.001$).[716] The dosage regime was 2 tablets twice daily for 8 weeks, reduced to 1 tablet twice daily for following 8 weeks. Each tablet contained St John's wort extract equivalent 245-350 mg herb, standardised to 0.25 mg hypericins and black cohosh tablet extract equivalent 22.5 – 41.25 rootstock, standardised to 1 mg triterpene glycosides. Symptoms were rated on the Menopause Rating Scale and the Hamilton Depression Rating Scale (HDRS). The mean decrease in scores on the MRS from baseline to week 16 was 50% in the treatment group, compared with 19.6% in the placebo group ($p < 0.001$). The HDRS total score decreased 41.8% in the treatment group and 12.7% in the placebo group ($p < 0.001$). The inclusion criteria were based on the presence of 'climacteric' symptoms within the previous 3 months, and not according to menstrual status. One third of participants in each arm had menstruated within the previous 6 months, although no mean time or range was provided.

A randomised, double-blind, placebo-controlled clinical trial of Remifemin plus®, a combination of *Hypericum perforatum* and *Cimicifuga racemosa* (St John's wort and black cohosh) with 179 women over a period of 6 weeks found significant improvement on 'psychovegetative' menopausal symptoms such as anxiety, impaired drive, depressive mood, nervousness and irritability, as well as good tolerability and compliance.[703] Symptoms rated on the KI fell from 31.4 to 18.7 (40% reduction) in the treatment group, compared with the placebo group where the mean score fell from 30.3 to 22.3 (26% reduction). The herbal combination was found to be significantly superior to placebo, $p < 0.001$.

The same preparation of *H. perforatum* and *C. racemosa*, Remifemin plus®, had previously been the subject of post-marketing surveillance involving 812 women for 12 weeks. [909] Participants were recruited by 176 doctors. Overall improvement was reported by 90% of the women in the 'psychovegatative' symptoms of menopause such

as hot flushes and poor concentration, with 82% rating the therapeutic effect as either very good or good. An effect was demonstrated after 3 weeks' treatment.

A large-scale prospective, controlled open-label observational study of 6141 women at 1287 outpatient gynecologists in Germany investigated the effectiveness and safety of *Cimicifuga racemosa* (black cohosh) alone or in fixed combination with *Hypericum perforatum* (St John's wort) on menopausal symptoms. [910] The monotherapy was used for neurovegetative symptoms, while patients with more pronounced mood complaints were administered the combination. Treatment was selected by the participating physicians, and doses were consistent with those used in other studies. Patients were followed up for 6 months, optionally 12 months. The primary endpoint was the MRS PSYCH sub-score at month 3. The results supported the effectiveness and tolerability profiles of both therapies. For the alleviation of mood symptoms, the fixed combination of black cohosh and St John's wort was superior to black cohosh alone ($p < 0.001$). The PSYCH and HOT FLUSHES subscales showed the greatest improvements for both therapies. Improvement was maintained at 6 and 12 months for both treatments.

A multicenter RCT with 89 peri- or postmenopausal women experiencing climacteric symptoms, investigated the effects of a combination of St John's wort and black cohosh extract (Gynoplus®, Jin-Yang Pharm., Seoul, Korea, a 264 mg tablet containing 0.0364 mL extract *Cimicifuga racemosa* rhizome, equivalent to 1 mg tripterpene glycosides, and 84 mg dried extract *Hypericum perforatum* equivalent to 0.25 mg hypericine, with 80% methanol) for 12 weeks. The herbal combination was shown to be superior to placebo for hot flushes ($p = 0.021$), and overall menopausal symptoms rated on the KI ($p < 0.001$).[705]

13.4 *Hypericum perforatum* and Premenstrual Syndrome

As mentioned above, it has been suggested that some of the so-called 'menopausal' symptoms may actually represent an exacerbation of premenstrual symptoms, although not necessarily recognised as such due to the irregularity of menstrual cycles in the perimenopause and the possibility of ovulation occurring in the absence of a subsequent menstrual bleed.[55] In addition, women report an increase in PMS-like symptoms during the perimenopause. Therefore, research on *Hypericum perforatum* in relation to PMS symptoms may also be of relevance in this context.

A pilot study involving 19 women suffering at least 6 consecutive months of PMS found *Hypericum perforatum* to significantly reduce the severity of premenstrual symptoms.[911] This open, observational study of *Hypericum perforatum* (St John's wort One-A-Day *Kira*®, Lichtwer Pharma, Berlin, Germany) found significant reductions in all outcome measures after treatment for two cycles with *Hypericum* tablets standardised to 900μg hypericin per day. The mood subscale (as opposed to pain, physical and behaviour), however, showed the most improvement, and the symptoms with the greatest reductions in scores were crying, depression, confusion, feeling out of control, nervous tension, anxiety and insomnia. Scores on both the Daily Rating Scale and the HAMD were significantly reduced by the end of one cycle, $p < 0.01$. This suggests a potential role for *Hypericum perforatum* in the treatment of PMS pending further evidence from RCTs with a larger sample size.

However, data from a subsequent study did not support this initial finding. A placebo-controlled RCT with Lichtwer Pharma UK Ltd *Hypericum perforatum* was conducted on 125 normally menstruating women who experienced recurrent premenstrual symptoms.[912] The primary endpoint was anxiety-related symptoms recorded on a menstrual diary based on Abrahams' classification. The daily dose of *Hypericum* was 600 mg, standardised to contain 1,800 μg of hypericin. Although both groups improved significantly on all symptom subgroups, $p < 0.007$ for both groups, there was no difference between groups, $p \geq 0.57$ for all symptom subgroups.

A case report of premenstrual dysphoric disorder (PMDD) treated with St John's wort extract (900 mg/day) observed much improvement in symptoms for the five-month follow-up.[913]

13.5 *Hypericum* in Anxiety Disorders

As mentioned above, observational studies on menopausal symptoms suggest a beneficial role for St John's wort in anxiety.[48,49] Evidence from some RCTs on depression have supported an anxiolytic effect in these subjects.[906, 802] In addition, three case reports have been published of its successful use in moderate to marked anxiety in patients diagnosed with DSM-IV-defined Generalised Anxiety Disorder (GAD).[801] This is supported by a study of the aqueous extract of the aerial parts in mice that noted both anxiolytic effect and sedative effects.[800] The anti-anxiety activity was not due to the hyperforin content.[800] In depressive patients suffering from anxiety symptoms, St John's wort, in comparison with *fluoxetine*, was found to be particularly effective.[802] The

preparation Psychotonin® examined by Witte and co-workers also showed an anxiolytic effect in depressed subjects.[906] Some of the mechanisms of action support a benefit in anxiety, such as GABA activity and serotonin reuptake inhibition.[880]

13.6 Effects of *Hypericum* on Sleep

The British Herbal Pharmacopoeia lists *H. perforatum* as sedatve.[21] *Hypericum* extract LI 160 was found to improve sleep patterns in older participants (59.8 ± 4.8 years) in a double-blind placebo-controlled cross-over trial with 12 volunteers. [907] Analysis of slow-wave EEG activities showed it induced an increase of deep sleep during the total sleeping period, although no benefit was found for onset of the sleep, sleep duration or intermittent awakenings. Sleep disorders, as well as depressive agitation, were reduced by St John's wort in patients suffering from depression with anxiety symptoms.[802]

13.7 *Hypericum perforatum* and Depression Clinical Trials

Extracts of *H. perforatum* have been the subject of many observational, drug-monitoring studies and clinical trials.[859-872,914,915] The anti-depressant activity of many of these extracts has been established in cases of mild to moderate depression, both in observational studies,[866] placebo-controlled trials[861,867,868,904,905] and comparator studies with standard anti-depressant medication such as *fluoxetine, sertraline* (SSRI's), *amitriptyline* and *Imipramine* (tricyclic anti-depressants), and *moclobemide,* a monoamine oxidase inhibitor.[860,862,864,871,914,916-919] Meta-analyses of RCTs on St John's wort between 1996 and 2000, including up to a total of 2,291 patients, reported that extracts of *H. perforatum* were superior to placebo for the treatment of mild or moderately severe depressive disorders[920,921] (responder rate ratio 2.47; 95% CI 1.69 to 3.61[920]) (rate ratio 2.67; 95% CI 1,78 to 4.01)[921] and similarly effective as standard antidepressants (responder rate ratio 1.01; 0.87 to 1.16).[920] In a systematic review of 8 RCTs, Gaster and Holyroyd reported the absolute increased response rate with St John's wort to range from 23% - 55% higher than with placebo, but 6% to 18% lower than tricyclic antidepressants.[922] Large-scale observationals studies of patients with mild to moderate depression resport response rates between 65% and 100%.[866] Details of a selection of depression studies using a range of St John's wort extracts and including hypericin and hyperforin content, where provided, are given in Table 13.1.

TABLE 13.1 SUMMARY OF A SELECTION OF CLINICAL TRIALS AND OBSERVATIONAL STUDIES USING DIFFERENT EXTRACTS OF *HYPERICUM PERFORATUM* IN DEPRESSION

***Hypericum perforatum* extracts compared with antidepressant drugs**

Source	*Extract/ Preparation*	*Hypericin content*	*Dosage*	*Dura-tion*	*Compared with*	*Subjects*	*Outcome*
Woelk, 2000[918]	ZE 117 Remotiv Bayer Vital, Germany	1 mg/day (0.2% hypericin) 50% ethanol	500 mg/day [250 mg bd] *Imipramine* 75mg bd	6 weeks	Imipramine (tricyclic) & placebo	**Mild-moderate major** depression on HAMD-17 (14-25. Mean = 22.25) 324 outpatients from psychiatric & general medical practices	Therapeutically equivalent to *Imipramine* but better tolerated
Schrader, 2000[917]	Ze 117	2.5 mg/day= (0.5%) hypericin	500 mg/day [250mg bd]	6 weeks	Fluoxetine (Prozac-SSRI)	240 patients with **mild-moderate major** depression HAMD (21) score of 16-24	Equivalent to Fluoxetine in mild-moderate depression
Szegedi *et al*, 2005[862]	WS 5572 Hydro-alcoholic extract 3-7:1 extract ratio	1 mg – 2.5 mg =0.12%-0.28% hypericin (increased to 2 mg-5 mg in non-responders 3-6% hyperforin = 2.7-5.4 mg	900 mg [300 mg tds] initially, increased to 1,800 mg/day after 6 weeks in non-responders	6 weeks	Paroxetine (SSRI)	**Moderate to severe major** depression. HAMD-17 ≥22 and ≥ 2 on depressive mood item. 251 adults outpatients. (+ CGI, Beck & Montgomery-Asberg)	*Hypericum* at least as effective as *Paroxetine* for moderate to severe major depression and better tolerated. *Hypericum* 57% reduction *Aropax* 45% reduction Between groups, $p = 0.08$
Hypericum Depression Trial Study group, 2002[870]	LI 160 Lichtwer Pharma extract	0.12%-0.28% hypericin (1-2.5 mg, up to 2 – 5 mg/ day)	900 mg/day increased to 1200,1500 or 1800 mg prn. *Sertraline* 50 mg/day up to 75,100 or 150 mg prn	8 weeks	Sertraline (SSRI) & placebo	340 adults patients with **major** depression and HAMD-17 item of 20+	Did not support the efficacy of St John's wort in **moderately severe major** depression HAMD: *Hypericum* vs placebo, $p = 0.59$ *Sertraline* vs placebo, $p = 0.18$
Brenner, 2000[872]	LI 160 Lichtwer Pharma extract	Not specified	600 mg/day *Hypericum* or 50 mg *sertraline* Increasing to 900 mg/day & 75 mg/ day after 1 wk	7 weeks	Sertraline (SSRI)	**Mild to moderate** depression, including **major depressive** disorder, dysthymic disorder, adjustment disorder. 30 outpatients. HAMD, CGI	Severity of symptoms significantly reduced in both groups, $p < 0.01$. No significant difference between groups. ≥50% improvement in 47% *Hypericum* group and 40% *sertraline* arm

***Hypericum perforatum* extracts compared with antidepressant drugs cont**

Source	***Extract/ Preparation***	***Hypericin content***	***Dosage***	***Dura-tion***	Compared with	***Subjects***	***Outcome***
Wheatley, 1996[860]	LI 160	2.7 mg/day total hypericin equivalent = 0.3% hypericin	900 mg/day or *Amitriptyline* 75 mg	6 weeks	*Amitriptyline* (tricyclic)	149 patients with **mild to moderate major** depression DSM IV & HAMD 17-24	Comparable efficacy to *Amitriptyline* on HAMD and CGI scores
Vorbach *et al*, 1996 (1997)[914]	LI 160	5.4 mg/day = 0.3% hypericin	1800 mg/day [3 x 600 mg] or 3 x 50 mg *Imipramine*	6 weeks	*Imipramine* (tricyclic)	209 patients with recurrent **severe major** depressive disorders HAMD (17) >20	Equivalent to *Imipramine* for **severe** depression but better tolerated
Vorbach *et al*, 1993 (1994)[915,916]	LI 160 6:1 extraction ratio	2.7 mg/day = 0.3% hypericin	900 mg/day Dried herb equivalent 5,400 mg/day	6 weeks	*Imipramine* (tricyclic)	135 outpatients with HAMD-17 >20; single episode, several episodes, depressive neurosis and adjustment disorder with depressed mood	Equivalent to *Imipramine* for depression (ie comparable). Not sufficiently powered to show non-inferiority of *Hypericum*
Philipp *et al*, 1999[864]	STEI 300, Steiner Arzneimittel, Berlin	0.2%-0.3% hypericin & 2%-3% hyperforin 60% ethanol	1,050 mg/day [350 mg tds] or *Imipramine* 100mg/day	8 weeks	*Imipramine* (tricyclic) & placebo	263 patients with **moderate major** depression on ICD-10 HAMD (17) mean 22.6 & CGI & Zung	More effective than placebo and at least as effective as *Imipramine* *Hypericum* -15.4 (SD 8.1) reduction Placebo -12.1 (7.4) reduction *Imipramine* – 14.2 (7.3) reduction
Harrer *et al*, 1999[919]	LoHyp57 Dr Loges & Co, GmbH 5-7:1 extract ratio	60% ethanol	800 mg/day [200 mgx2] *Fluoxetine* 20 mg [5 mg x 2 bd]	6 weeks	*Fluoxetine* (Prozac-SSRI)	149 elderly patients, aged 60-80, with **mild-moderate major** depression acc to ICD-10. HAMD– mean 25	Equivalent to *Fluoxetine* in mild-moderate depression on HAMD: *Hypericum* reduced from 16.6 – 7.91 ***Fluoxetine*** reduced ***from 17.18 – 8.11***
Behnke *et al,* 2002[871]	Calmigen	2.7 mg/day (900 µg hypericin per tablet)	300 mg/day or *Fluoxetine* 40 mg/day	6 weeks	*Fluoxetine* (Prozac-SSRI)	70 patients (mean age 49.7) with **mild-moderate major depression** HAMD 17 item, CGI & von Zessen	Therapeutically equivalent HAMD: *Hypericum* 50% reduction, $p < 0.001$ *Fluoxetine* 58% reduction, $p < 0.001$

Hypericum perforatum extracts compared with placebo

Source	*Extract/ Preparation*	*Hypericin content*	*Dosage*	*Dura-tion*	*Compared with*	*Subjects*	*Outcome*
Kasper *et al*, 2006[867]	WS 5572	2.7 mg/day total hypericin equivalent (0.3% hypericin)	600 mg/day and 1,200 mg/ day	6 weeks	placebo	332 patients with **mild-moderate major** depression (DSM-IV); HAMD-17; CGI and Montgomery–Asberg DRS	At both doses, *H. perforatum* extracts were superior to placebo, $p < 0.001$.
Lecrubier *et al,* 2002[905]	WS 5572	2.7 mg/day total hypericin equivalent (0.3% hypericin)	900 mg/day [300 mg tds]	6 weeks	placebo	**Mild-moderate major** depression in 375 adults aged 18-65, acc to DSM-IV HAMD (17 item) 18-25 & 2+ on depressed mood item 1.	Percentage of responders (with 50%+ decrease in HAMD scores) significantly higher for WS 5570, $p < 0.05$ and Beck melancholia, $p = 0.001$
Kalb *et al*, 2001[923]	WS 5572	2.7 mg/day total hypericin equivalent (0.3% hypericin) 5% hyperforin	900 mg/day [300 mg tds]	6 weeks	placebo	72 patients with **mild-moderate major** depression (Mean 20 on HAMD 21 item scale) & CGI & von Zerssen D-S	$p<0.001$
Laakman *et al,* 1998[882]	WS 5573	4.5 or 45 mg/ day hyperforin (0.5%) or 5% hyperforin)	900 mg/day [300 mg tds]	6 weeks	2 doses of hyperforin with placebo	147 patients with **mild or moderate** depression according to DSM-IV criteria; HAMD-17; D-S	HAMD scores compared with placebo: *5%* hyperforin group, $p = 0.004$ 0.5% hyperforin group - NS
Shelton *et al,* 2001[924]	LI 160 Lichtwer Pharma GmbH, Germany	2.7 – 3.67mg/ day total hypericin equivalent	900 mg/day [300 mg tds] for 4 weeks → ↑ed to 1,200 mg/day	8 weeks	placebo	200 outpatients (mean age 42.2yrs) with **major depression** and HAMD-17 score of at least 20; HAMD, CGI & Beck	Not effective for treatment of major depression Within both groups, $p < 0.001$ Between groups, $p = 0.16$
Hängsen & Vesper, 1996[868]	LI 160	2.7 mg/day total hypericin equivalent	900 mg/day [300 mg tds]	4 weeks	placebo	102 outpatients with **mild-moderate major** depression. HAMD (16+) & D-S scale	$p < 0.001$
Sommer & Harrer, 1994[925]	LI 160 (Jarsin® 300, Lichtwer Pharma)	Not specified	900 mg/day [300 mg tds]	4 weeks	placebo	89 patients aged 20-65 years with mild depression (ICD-neurotic depression of brief depressive reaction) of short duration completed the study.	HAMD scores fell from 15.8 to 7.2 in active group, and 15.8 to 11.3 in placebo by end of week 4. Between group difference. $p < 0.01$ (After 2 weeks, $p < 0.05$) 67% responded in active grouop; 28% responded to placebo.

***Hypericum perforatum* extracts compared with placebo cont**

Source	*Extract/ Preparation*	*Hypericin content*	*Dosage*	*Dura-tion*	*Compared with*	*Subjects*	*Outcome*
Uebelhack *et al,* 2004[861]	STW *3*-VI (Laif ®)	Not specified	900 mg/day	8 weeks	placebo	Mild-moderate depression (not specified if **major**) 140 outpatients with HAMD-17 score of 20-24	*p*<0.001
Witte *et al*, 1995[906]	Psychotonin® forte Hersteller, Darmstadt	1 – 1.2 mg/ day total hypericin (0.5% hypercins)	200-240 mg/day [100-120 mg bd]	6 weeks	placebo	97 ambulant patients, 24 – 65 years old with major depression according to ICD-10 & HAMD score ≥ 16	HAMD - 79% response rate in active treatment group vs 56% in placebo group. Between groups difference, *p* = 0.019.
Volz *et al,* 2000[926]	D-0496 (Reyneke; Crinan farm)	1.03 mg/day (516 µg per capsule)	500 mg/day [250 mg bd]	6 weeks	placebo	140 participants with mild to moderate major depression according to DSM-IV, and HAMD score ≥ 21	HAMD-21 scores dropped from 21 to 12 in St John's wort group vs 20.7 to 14.3 in placebo group, *p* =0.005

Drug monitoring studies on *Hypericum perforatum* extracts

Source	*Extract/ Preparation*	*Hypericin content*	*Dosage*	*Dura-tion*	*Compared with*	*Subjects*	*Outcome*
Grube *et al,* 1999[49]	Kira ® Lichtwer Pharma, Germany	900 μg (0.9 mg) (300 μg x 3)	405-675 mg/ day [135-225 mg tds]	12 weeks	Drug monitoring study	111 women from general medical practice with '**climacteric' symptoms** – aged 43-65 Menopause Rating Scale & CGI	76.4% (patient rating) reduction in psychological & 'psychosomatic' symptoms (79.2% by physician evaluation)
Woelk *et al,l* 1994[859]	LI 160 Jarsin	2.7 mg/day (900 μg x 3)	900 mg/day [300 mg tds]	4 weeks	Drug monitoring study	3250 patients from 663 medical practices. Age range 20-90 years (mean 51). Includes **mild, moderate, severe, reactive and other.** Zerssen scale (D-S)	Severity & frequency of symptoms reduced by ≈ 50%. 80% responded positively
Holsboer-Trachsler & Vanoni, 1999[927]	LI 160 (Jarsin 300)	2.7 mg/day (900 μg x 3)	900 mg/day [300 mg tds]	6 weeks	Drug monitoring study	Mild-moderate **temporary depression** Von Zerssen depression scale	Condition improved in 75% of patients. (Somewhat slower in patients >65 yrs)
Martinez *et al*, 1993[928]	LI 160 Jarsin 300	2.7 mg hypericin	900 mg/day [300 mg tds]	4 weeks	With bright or dim light therapy for 2 hours daily	**Seasonal affective disorders** acc to DSM-III-R criteria	Significant reduction in HAMD scores for both groups, $p < 0.001$

CGI = Clinical Global Impressions
DSM = Diagnostic and Statistical Manual
DRS = Depression Rating Scale
HAMD = Hamilton Depression Rating scale
D-S = Von Zerssen Depression Self-rating scale

These include a range of RCTs comparing *Hypericum* with placebo or a comparator antidepressant, using a variety of *Hypericum* extracts, including major and non-major depressions, conducted across a time span of 15 years.

Please note: For further information, the reader is directed to the systematic reviews and meta-analysis by Linde *et al*,[865,866,929,930] published in 2005 and 2008, after compilation of these tables had been largely completed.

13.7.1 Hypericum in Severe Depression

Whilst most of the research has focused on mild to moderate depression, *H. perforatum* extract has also been compared with *imipramine* for treating recurrent severe depressive disorder in the absence of psychotic symptoms or delusions.[915] At the higher doses of 1,800 mg (6:1 extraction ratio = 10,800 mg/day), the extract LI 160 was found to be as effective as *Imipramine* in improving symptoms of severe depression, and was better tolerated.[915] More recently, Szegedi and colleagues[862] found WS 5572 extract at an initial dose of 900mg, increased to 1,800mg/day in non-responders, to be at least as effective as *paroxetine* for moderate to severe major depression (HAMD-17 entry score 22+ and 2+ on the depressive mood item; p = 0.08 between groups), and to be better tolerated. The study used a hydro-alcoholic extract containing 3-6% hyperforin, equivalent to 2.7-5.4mg, and a 3-7:1 extract ratio. Nonetheless, St John's wort is not generally recommended for the treatment of serious depression with psychotic symptoms or delusions,[915] suicidal risk or severe signs and symptoms that do not allow family or work involvement to continue.[796]

13.7.2 Hypericum perforatum and Major Depression

Some confusion was generated regarding the efficacy of *Hypericum* in *major* depression, by the 2005 publication of a meta-analysis by Linde and co-workers[929] reporting on pooled data from six large trials on major depression. It suggested only minimal benefit for St John's wort in this context, and that its beneficial effects may be confined to mild-moderate depression not meeting the criteria for *major* depression (Refer to appendix 6 for DSM criteria). However, most studies on St John's wort have been conducted on major depression (Table 13.1), and many of these have shown significant effects over placebo for various extracts of *Hypericum,*[867,868,904,905] and equivalence to standard antidepressants,[860,862,864,871,872,914,917-919] with superior tolerability.[871,917,919] (A review of some of these studies was undertaken by as a component of the current work, including comparator trials with antidepressant drugs[860,862,864,871,872,914,917-919] without/with a placebo arm,[864,870] and placebo-controlled trials. [867,868,904,905,924] Details are included in Table 13.1 and Appendix 12). A further meta-analysis by the same authors of 29 studies (of 79 identified) on major depression has very recently been published, concluding that the evidence for the St John's wort extracts tested in the trials supports its superiority to placebo, and similar effectiveness as standard antidepressants, in major depressive disorders (MDD).[930] In the 18 placebo controlled trials examined patients receiving *Hypericum* extracts were significantly more likely to be responders (RR = 1.48; 95%CI 1.23 to 1.77) and study results were highly heterogeneous (I^2 = 75%). For the 17 trials

comparing *Hypericum* extracts to standard antidepressant treatment the pooled responder rate ratio was 1.01 (95%CI 0.93 to 1.09; I^2 = 17%) based on an intention to treat approach. Based on per protocol data, the pooled responder rate ratio was 0.96 (95%CI 0.88 to 1.05; I^2 = 43%).

13.7.3 The Conflicting Findings in Depression Studies

Results have not been unanimously favourable, however. One drug comparison study by the *Hypericum* Depression Trial Study group,[870] with an additional placebo arm did not support the superiority of Lichtwer Pharma LI160 extract of *H. perforatum* over placebo for MDD (Table 13.1). However, it is noteworthy that the SSRI was not superior to placebo either. In contrast to the results of many placebo-controlled trials,[867,868,904,905] Shelton and co-workers[924] found St John's wort (LI 160) to be ineffective for major depression when administered to 200 participants at a dose of 900mg/dayfor 4 weeks, increasing to 1,200mg/day for the subsequent 4 weeks. In another study, however, the same extract at 900mg/day was found to be significantly superior to placebo when trialled for 4 weeks on 102 outpatients with mild-moderate major depression.[868]

Several possible reasons have been proposed for the discrepancy in the findings between studies. In their meta-analysis of studies on MDD, Linde and co-workers observed that trials from German-speaking countries reported more favourable findings than trials from other countries.[930] In a univariable meta-regression analyses on the selected studies of major depression, they found that over half the variance (R^2 = 0.51) in effect sizes reported could be explained by three variables: country of origin (studies from German-speaking countries showing larger effect sizes, p = 0.002), precision (more precise studies showing smaller effects, p = 0.03) and baseline values (higher values associated with smaller effect sizes, p = 0.048) were significantly associated with effects sizes.[930]

Other suggested factors for the discrepant findings relating to the antidepressant action of *H. perforatum* include:

- *the small sample size in some studies*.[924] Smaller trials tended to report larger treatment effects. A possible reason suggested for this is publication bias or lower methodological quality of smaller trials.[931] However, in studies with smaller sample sizes, there is less power to detect superiority over placebo. Despite this, St John's wort has been quite convincingly shown in a number of studies to be superior to placebo. Therefore, power was clearly not an issue. Additionally, many large-scale studies have been conducted.

- *short duration of studies*[924]*.* Once again, it may be expected that the placebo effect would be higher in studies of shorter duration, as it does not wash out before 6-weeks.[932] These studies have frequently found superiority over placebo. Linde and Knuppel[866] in a systematic review of large scale *observational* studies noted that the observation periods ranged from 4 to 6 weeks, with the exception of two studies of 52 weeks. They reported that results from both the short term and long-term studies indicate significant improvement, although the response rates tended to be slightly lower in long-term than in short-term studies.
- *diagnostically heterogeneous groups*,[924] sometimes within one study. This has in fact occurred in some studies.[872] However, as mentioned previously, there have been many studies restricting inclusion to participants with major depression, while others have focused on mild-moderate depressive symptoms in people who would not meet the criteria for major depressive disorders. The majority of studies have focused on mild-to-moderate depression but a limited number of severely depressed patients have been included.[866,914] Older trials often included patients with neurotic depression and brief depression, and may have included more patients with somatisation and atypical depressive features. Thus, as pointed out by Murck, newer trials may be excluding patient groups that are particularly responsive to *Hypericum* extracts.[933]
- *different diagnostic tools used*.[924] These include ICD-10 criteria and DSM-IV. In terms of outcome measures, the HAMD-17 and HAMD-21 have predominantly been employed.
- *lack of equivalence between preparations trialled.*[865] Among the 16 large-scale observational studies reviewed, with total of 34,804 patients (minimum of 100 participants per trial) (range 101 to 11,296), Linde and Knuppel[866] found the total number of different products to be twelve, and the daily extract dose to range from 360 to1,200mg. However, a meta-analysis of 37 double-blind, placebo–controlled RCTs on *Hypericum* found little evidence of an association between response and daily dosage and type of extract.[929] Comparison of the different extracts is confounded by several factors. The amounts of active constituents such as hypericin, hyperforin and the flavonoids can vary enormously between products. A study of *Hypericum* products on the German markets showed that a number contain only minor amounts of bioactive constituents.[934] Most studies have tested *Hypericum* products standardised for hypericin content, as this was previously thought to confer the anti-depressant activity. Relatively few specify the hyperforin content, variation in which could potentially account for the different findings. An additional limitation is that hyperforin is most unstable in solution and rapidly

decomposes at an acidic pH. Galenicals of St John's wort that are older than a few months contain no hyperforin at all.[935] The quality of *Hypericum* preparations can also differ according to the raw plant material, extraction process, ratio of raw material to extract and the solvents used. The effect of the extraction solvent on the percentages of constituents extracted is demonstrated in Table 13.2 below:

TABLE 13.2 THE PERCENTAGES OF HYPERICIN AND PSEUDOHYPERICIN OBTAINED WITH DIFFERENT EXTRACT SOLVENTS

Solvent	*Hypericin %*	*Pseudo-Hypericin %*
Methanol	75%	80%
Ethanol	34%	37%
Acetone	20%	20%
Isopropanol	10%	10%
Water	10-20%	30-40%

From Bloomfield, HH & McWilliams, P. The Hypericum Homepage. www.hypericum.com/copy.htm[936]

vi. *Several prognostic factors identified in different studies[866] may also impact on the variability of findings.* Although findings have not been uniform, these include:

- dysthymia versus major depression. Dysthymia has shown the best outcome.[866,929]
- severity of depression, with those with higher baseline HAMD scores being less responsive.[929]
- the duration of treatment. Longer duration is a negative predictor.[929]
- age. Older patients have shown slower improvement in some studies.[937]
- organic disease has negatively predicted response.[937]
- concurrent psychotropic co-medication is a negative predictor.[937]

13.7.4 Criticisms made of St John's wort depression studies

Trials of St John's wort for depression have been criticised for the failure to use standardised symptom rating instruments.[924] While this may be of relevance to observational studies, often conducted for marketing purposes, the majority of RCTs appear to have used the well-validated HAMD-17 or HAMD-21 item scales, or to a lesser extent, the BDI. Another drawback of observational studies conducted in primary care settings is that primary care physicians are not experienced in depression research.[924] However, this is unlikely to have biased findings in double-blind trials. Several older trials have been criticised because they included patients with few or mild symptoms who did not meet criteria for major depression.[929] These studies were mostly conducted on

mild-moderate depression, which is susceptible to a higher placebo response rate.[924] However, such RCTs constitute a minority of those conducted on *Hypericum.* Moreover, despite the high placebo response in these RCTs, *Hypericum* was shown to be superior to placebo.

It has been suggested that the dosages of the standard antidepressants used in comparator trials were too low, so that equivalence resulted because neither *Hypericum* nor the standard anti-depressants actually conferred substantial benefit.[865] While the lack of a placebo control group does make it impossible to definitely rule out the ineffectiveness of both interventions, it is unlikely that antidepressant drugs with established efficacy would confer *no* benefit, even at lower doses. Higher doses of antidepressants have not conclusively been shown to be more effective than lower doses.[938]

13.8 Adverse events

Adverse events associated with *Hypericum perforatum* tend to be mild, and to occur rarely. From 1968 to1998, WHO Uppsala Monitoring Centre received 57 reports of suspected adverse drug reaction associated with St John's wort, namely nervousness (5 cases), eczema, sleep disorders and paraesthesia (3 each).[939] St John's wort appears to be free of any cardiac as well as anticholinergic side-effects normally seen with antidepressant medications.[940]

In clinical trials and observational studies involving St John's wort, mild adverse events are often reported no more frequently than in placebo arm. In a review of large-scale observational studies comprising a total of 34,804 patients, the tolerability and acceptability of tested extracts was reported to be very good.[866] The percentage of patients reporting side-effects ranged between 0% - 5.9%. St John's wort extracts were associated with only mild side-effects, the most frequent being gastrointestinal symptoms. Increased sensitivity to light and skin symptoms were the second most frequent, and a variety of nervous symptoms such as agitation or restlessness were reported in a number of studies. No serious side-effects of interactions with drugs were reported.

Another systematic review of 8 studies found only 1.1% of patients discontinued *Hypericum* extract because of side-effects.[922] In placebo-controlled trials, the percentage of patients reporting at least one adverse drug reaction (ADR) with

Hypericum was 4.1% compared with 19.8% where *Hypericum* was compared with an antidepressant drug. The percentage of patients dropping out of clinical trials because of ADRs was lower for *Hypericum* than for the all other drugs.[939]

Post-marketing surveillance of 3,250 patients taking *Hypericum* extract (equivalent to 2.7mg per day of total hypericin) observed side-effects in 2.4% of patients. [859] Consistent with the findings of Linde and Knuppel mentioned above[866], the most frequently noted were minor gastrointestinal irritations (0.6%), allergic reactions such as pruritis (0.5%), tiredness (0.4%) and restlessness (0.3%).

In the drug-monitoring study of 111 menopausal women taking 900 μg hypericin/day, there were only 4 reported adverse events, namely one case of each of slight nausea, dizziness, headaches and sleep disturbances.[49]

In a comparison with imipramine, at 1050 mg/day of *Hypericum*, nausea, headache and palpitations (3.8%) occurred more frequently than for the placebo group.[864]

A review of 8 studies (total *n* = 611) found nausea, rash, fatigue, restlessness and photosensitivity to occur most frequently.[922]

Adverse events from individual case reports often involve concomitant medication or other pathologies, making it difficult to establish a causal connection with the administration of St John's wort. Cases where the link is considered probable will be included here:

- *Photosensitivity* developed in a 61-year-old woman after taking St John's Wort-extract for three years, which resulted in elevated, itching red lesions in light-exposed areas.[941]
- Four cases of *sensory nerve hypersensitivity* reactions, with increased sensitivity to heat and cold, and pain in the hands and/or feet were reported. These resolved within 1 –2 weeks of ceasing the herb.[942] It is suggested that the preparations involved were from the late-harvested herb that contained high levels of resinous constituents.[796]
- One case of *subacute toxic neuropathy* with sharp pains in sun-exposed areas (face and hands) occurred after 4 weeks' ingestion but disappeared on cessation of the *Hypericum* product.[943]
- After 2 weeks administration of *Hypericum*, a 67-year-old woman developed *adynamic ileus*, which resolved completely on discontinuation.[944]

- *Delayed emergence from anaesthesia* occurred in one case after 3 months' ingestion of up to 3 g/day.[945]
- Multiple side-effects in a 47-year-old woman, including dyspnoea, hyperventilation, palpitations, tremor, flushing, mydriasis and rhinitis, suggestive of an allergic reaction.[946]

13.9 Herb-Drug Interactions with St John's wort

Hypericum perforatum induces hepatic drug metabolism via activation of the pregnane X receptor[947,948] and thus can potentate certain enzymes of the cytochrome P450 enzyme system, notably CYP3A4.[316,948-950] Some evidence exists for induction of other cytochrome P450 isoforms such as CYP1A2[951,952] and CYP2D6[953] (modest). However, the most important in this context is CYP3A4 which is involved in the oxidative metabolism of over 50% of all conventional medications,[947] and implicated in most clinically significant drug-drug interactions.[950] St John's wort extracts therefore have the potential to induce metabolism of some co-administered medications, thus resulting in lowered serum concentration of a number of drugs, namely:

- immunosuppressants (cyclosporine)[954,955,956]
- cardiac glycosides (digoxin) for doses of St John's wort greater than 1 g/day (dried herb equivalent)[957]
- HIV medication including protease inhibitors,(indinavir),[958] the non-nucleoside reverse transcriptase inhibitor, *nevirapine*[959], and other anti-retroviral drugs[960]
- chemotherapy drugs, (irinotecan)[961]
- anti-coagulant medication such as warfarin, phenprocoumon[962,963]
- low dose oral contraceptives[962]
- theophylline (bronchodilator)[964]
- triptans (migraine medication)[965]
- midazolam (benzodiazepine)[949]
- fexofenadine (antihistamine)[966]
- the HMG-CoA reductase inhibitor simvastatin[967] and atorvastatin[968] (but pravastatin was unaffected)
- phenytoin (theoretical concern)[965]
- amitriptyline (tricyclic antidepressant).[969,970]

A comprehensive and up-to-date review of herb-drug interactions has subsequently been published by Mills and Bone.[805]

Other than hepatic and gastrointestinal enzyme induction, administration of St John's wort may also affect intestinal absorption, distribution and renal clearance of drugs such as digoxin, by activation of P-glycoprotein.[957,971,972] In conjunction with SSRIs, it may result in synergistic serotonin uptake inhibition. There have been reports of elderly patients developing 'serotonin syndrome' type symptoms with co-administration of *Hypericum* and SSRIs [973,974] This may apply to paroxetine, trazodone, sertraline, and other serotonergic agents such as nefazodone, venlafaxine[975] as well as triptans (migraine medication such as sumatriptan, naratriptans, rizatriptan and zolmitriptan).[965]

Because photosensitivity is a potential adverse reaction that has been associated with higher doses of *Hypericum,*[976] photosensitising agents (delta-aminolaevulinic acid, piroxicam, tetracycline)[i977] should be avoided in conjunction with *Hypericum.* However, studies using high dose extracts (1800mg, equivalent to 5.62 mg/day of total hypericin) over 15 days found a moderate increase in sensitivity to UVA light, but not to UVB light. Prolonged or intense exposure to UVA radiation whilst taking *Hypericum* is advised (p550).[796] It should be avoided by people with known photosensitivity.[805]

Research shows that the pharmacokinetic drug interactions are dose-related, and also depend on the preparation administered.[978] The compound most implicated in the herb-drug interactions is hyperforin, found to be responsible both for activation of P-glycoprotein[979] and the metabolic drug interactions.[978,980-983] As hyperforin is most unstable in solution and rapidly decomposes at an acidic pH,[984] tinctures and fluid extracts are low in hyperforin.[935] Thus, pharmacokinetic drug interactions should not be an issue with such preparations, nor at doses less than 2 g St John's wort herb per day or its equivalent.[935] However, hyperforin is also believed to be responsible for the antidepressant effects, although evidence is conflicting in this regard.[882,981]

Pharmacokinetic data is available;[796] toxicology data in animals is presented in Mills and Bone (2005: pp590-1).[805]

13.10 Conclusion

Although the mechanisms of action of *Vitex agnus-castus* and *Hypericum perforatum* have not yet been fully elucidated, both herbs have been subject to considerable investigation in the scientific arena. Current knowledge, based on *in vitro* and *in vivo* models, supports CNS mechanisms involving neurotransmitters and/or opioid receptors. It is hypothesised that these herbs may, therefore, exert effects on menopausal

symptoms similar to those observed with CNS-acting agents such as the anti-depressant selective serotonin reuptake inhibitors and the anticonvulsant gabapentin, which have been demonstrated to ameliorate vasomotor symptoms in menopausal women. Their proposed mechanism in this context is via the complex interaction between hormones and neurotransmitters involved in the physiology of hot flushes. Based on their known pharmacology, both *Vitex* and *Hypericum* could be expected to confer additional benefits to menopause-related psychological symptoms.

Findings from a limited number of clinical studies with potential relevance to psychological and physiological symptoms of menopause have supported the benefits of phytotherapeutic menopause formulations that include *Vitex* and/or *Hypericum*. Most of the limited, and often conflicting, evidence in this arena is for the phytoestrogen-containing plants, long-term use of which has now raised safety concerns. Evidence from rigorous research is thus needed for the benefits of non-oestrogenic phytotherapies employed in clinical practice for the amelioration of menopause-related symptoms.

The information provided regarding the indications, contraindications, herb-drug interactions and adverse events associated with these two herbs informed the selection criteria and exclusion criteria for the RCT to be reported. Before outlining the methodology of this study, however, contributing factors to the placebo effect, and its implications will be examined more closely as this has been shown to be substantial in menopause studies.

Chapter Fourteen

The Placebo Response

14.0 Introduction

The final chapter of the Literature Review examines some of the issues surrounding placebo effects that can be substantial in studies of CAM as well as of menopausal symptoms. The resultant risk of inappropriate rejection of potentially valuable treatments had led to efforts to understand this phenomenon and to control its effect in randomised, controlled trials. This includes identification of unique characteristics of placebo responders.

14.1 Magnitude of Placebo Effect in Relevant Conditions

Randomised controlled trials (RCTs) of therapy for vasomotor symptoms in menopause commonly report substantial placebo effects, averaging 51% trials in hormone treatment according to a 2004 meta-analysis,[499] and generally in the range of 30 to 41% in RCTs in trials of phytotherapy/medicinal herbs,[75,76,793,848,849] and 1% - 59% for phytoestrogen supplements.[985] This phenomenon is also observed in RCTs of treatments of depression, with placebo response rates averaging approximately 30%, ranging from 12% to beyond 50% and found to be on the increase.[929,986] This change in placebo response rates in depression studies has been observed for conventional pharmacological (7% per decade) and phytotherapeutic interventions alike, with a 1.5% per year increase in *Hypericum* trials (and a concomitant decrease in treatment response of 1.1% per year.[929] In terms of premenstrual symptoms, a 1997 meta-analysis of controlled treatment trials for premenstrual dysphoric disorder (PMDD) showed rates of placebo response ranging from 6% to 35%.[987] Earlier PMS studies indicated rates of placebo response as high as 94%.[988] In a PMS study with *Vitex agnus-castus* there was a 24% placebo responder rate (≥ 50% reduction in symptoms) or a 30% reduction in symptoms measured on a self-rating scale.[481]

It has been suggested that conditions with a strong psychological component, such as depression, anxiety and pain, all common corollaries of illness, are particularly susceptible to the placebo effect.[989] However, many studies on physical conditions such as obesity,[990] mild neurological deficits,[991] angina pectoris,[991] as well as surgical interventions for Parkinson's disease[992] and arthroscopy[993] have also reported high placebo effects.

14.2 Natural History and Regression to the Mean

The placebo response in trials is partly dependent on the condition being treated.[994] Unless an untreated control group is also included, such estimates of the magnitude of the placebo response do not account for regression to the mean or the natural history of symptoms. Natural history refers to the spontaneous variations that occur in most conditions. Regression to the mean is a statistical phenomenon that assumes people receive their initial assessment/join clinical trials when the intensity of their symptoms is greatest, and that the level of intensity is likely to be less on the subsequent assessment. (These are not generally considered to be part of the true placebo effect,[995] although Ernst[996] includes them among 'non-specific effects'). To investigate the effect of placebos beyond that of natural history, Hrobjartsson and Gotzsche conducted a meta-

analysis of RCTs that included a no-treatment control group in addition to a placebo group and concluded that placebo interventions had no clinically important effects.[997] The data from this study were subsequently reanalysed by Wampold and colleagues, who reported that , when disorders are amenable to placebos and the design is adequate to detect the effects, the placebo effect *is* robust and approaches the treatment effect.[998]

14.3 Problems associated with Substantial Placebo Effects

Although attempts to harness it in clinical practice have been referred to as 'unscientific',[999] the placebo effect can interact synergistically with the specific intervention to enhance treatment outcomes, especially where the patient's subjective viewpoint is the optimum outcome measure, (for example, quality of life) and thus may be a boon in clinical practice. However, it continues to be regarded as the bane of RCTs assessing the efficacy of potential treatments. A substantial placebo response can result in smaller effect sizes and failure to demonstrate superiority over placebo of new treatments,[1000] due to type II errors (that is, erroneous conclusions that there are no differences between the efficacy of the treatment and placebo). A high placebo response may account for the frequent failure of well-designed CAM trials to report significant effects over placebo. Similarly, the equivocal results from trials of *Trifolium pratense,* (Red Clover), in menopause symptoms,[75,691,692] may reflect different placebo responses. In addition to contributing to the rejection or discontinuation of potentially viable treatments, high placebo responses and subsequent 'negative' results of trials add time and cost to the development new treatments,[1001] which may further discourage the already-limited financial investment in research. As a result, studies attempting to understand the placebo effect are largely being directed at reducing its effect in RCTs in order to increase the power to detect drug-placebo differences.

14.4 The Meaning Response

The inclusion of placebo arms in drug studies began in 1962 when the USA FDA regulations first required drugs to be proven effective.[1002] Since then, placebo-controlled trials have come to be considered the gold standard among research designs. A placebo-controlled trial employs a sham intervention such as dummy pills that lack a specific effect – that is, an effect for which an empirically supported theory exists for its mechanism of action – for the condition being treated, in order to control for effects of the treatment setting. The placebo effect is, thus, the effect seen in patients who have received an intervention which is believed to lack a specific action for the condition under investigation. Because placebos are, by definition, inert substances, they cannot actually

cause placebo effects themselves.[995] However, their meaning can, and the term 'meaning response' or meaning-induced responses'[1003] have been proposed to embrace both the desirable responses of the placebo effect and the undesirable effects of the 'nocebo effect'.[995,1004] As pointed out by Wolf in 1950,[1005] the mechanisms of the body are capable of reacting not only to direct physical and chemical stimulation but also to symbolic stimuli, words and events which have somehow acquired special meaning for the individual.

14.5 Contributors to the Placebo Response

Hypotheses regarding factors that produce the placebo effect have focussed on i) sociocultural perspectives, emphasising the system of beliefs that the patient and practitioner bring to the treatment setting; ii) biological perspectives, emphasising the role of the endogenous opioidergic and, more recently, dopaminergic pathways,[1006] as well as a proposed genetic predisposition;[1007] and iii) clinical trial methodology and investigator effects.

14.5.1 The Socio-Cultural Perspective

The psychosocial context, also referred to as 'non-specific effects', includes individually or culturally based expectations for a treatment[1008] that are influenced by general beliefs in the culture about what constitutes good treatment as well as the individual patient's previous experience with pharmacological agents and treatment during his/her lifetime. As such, classic conditioning effects also form part of the placebo response. Other meaningful elements of the situation or context include practitioner effects such as provider-induced expectancies, credibility of the setting, therapist and treatment, positive information about the treatment or illness and support/reassurance.[1009] Practitioner certitude even affects the magnitude of the placebo effect in double-blind RCTs.[1010] The practitioner's costume, manner, style, language, mood, time available and charisma can also affect the placebo response, as can the degree of illness and even the diagnosis and prognosis.[995] The attention, time, support and reassurance that modulate the anxiety and expectations contribute to the therapeutic alliance and are believed to be potent factors, particularly (but not exclusively) in psychiatry.[1011] The pivotal role of expectancy in the placebo effect [1012] suggests that depression should be very reactive to the placebo effect, as placebos instil an expectancy for improvement, whereas at the core of depression is hopelessness, which is "an expectancy that an intolerable situation will not improve." [1013] (Indeed, from a meta-analysis of antidepressant medication, Kirsch and Sapirstein[1014] concluded that 25% of the effect was due to the true drug effect, 25% to the natural history of the condition and 50% to the expectancy effect.) The power of

expectancies to override the pharmacological effects of drug has been shown in several studies. In a recent series of RCTs for four different painful conditions using real or sham acupuncture, patients with higher expectations about acupuncture treatment experienced larger clinical improvements than patients with lower expectations, regardless of the allocation to real acupuncture or sham acupuncture.[1015] Moreover, the effects were long-lasting, still being evident at 6 month follow-up. Other studies highlighting the power of expectancies include those where participants' expectancies were contrary to the pharmacological effects of the drug,[1016,1017] and where participants were misinformed of the action of a sympathomimetic drug.[1018] Participants who expected to receive active treatment showed more significant changes in brain metabolic activity than those who expected to receive placebo, even though both groups were given an active drug.[1019] The group to which participants believed they were assigned was more powerful than the than actual assignment.[1020]

A unifying model for understanding the placebo response in clinical trials proposed by Klosterhalfen and colleagues, is based on three components: regression to the mean, Pavlovian conditioning, and signal detection theory.[1007,1021] Patients' expectations and beliefs together with classical conditioning influence the 'signal-to-noise ratio' which describes the ability of the patient or the investigator/therapist to identify a change in symptoms. [1021] This is of major importance to the validity of studies using subjective outcome measures. Regression to the mean, natural history, experimenter or subject bias and errors in measurement of reporting[995] are elsewhere excluded from the definition of the 'meaning response'.

14.5.2 The Biological Perspective

In terms of neurobiological mechanisms, both neuropharmacological studies and brain imaging investigations have found endogenous opioids to be involved in the production of placebo analgesia.[1022,1023] Inhibition of the analgesic placebo response was effected by the opioid antagonist, naloxone,[1024] and higher β-endorphin levels were found in their cerebrospinal fluid of placebo responders compared with non-responders.[1025] It has also been suggested that placebo-induced dopamine release could be a major biochemical substrate for the placebo effect not only in pain, but also in other conditions such as motor disorders and depression.[1026-1028] *In vivo* research on Parkinson's Disease, indicated that the placebo effect in Parkinson's Disease is mediated through activation of the damaged nigrostriatal dopamine system.[1006] Expectation of reward has also been shown to be at least partly mediated by the dopamine system,[1029-1032]stimulation of which may be activated by the brain opioid system.[1033] Placebo-induced dopamine release in

response to the expectation of reward (clinical benefit, in this case) has been suggested as representing a common biochemical substrate in many conditions.[1026] Opioid mechanisms are likely to be activated during the routine therapist-patient interaction.[1020]

There is evidence from studies of antidepressant medication that, while both placebo and active treatment exerted nearly equal benefits, their effects on the brain were quite different.[1034,1035] Quantitative electroencephalography revealed that placebo treatment induces changes in brain function that are distinct from those associated with antidepressant medication. Placebo responders showed a change in prefrontal cordance* that differed in direction and time course from the change in antidepressant medication responders, casting a new light on the assertion that 50% -75% of the antidepressant effect consists of placebo effect, since symptom improvement was not associated with a similar physiological alteration in the placebo responder and medication responder groups.[1036]

Brain imaging studies of placebo analgesia using positron emission tomography (PET) found that the same regions of the brain to be affected by both placebo and an opiate agonist, [1037] indicating placebo-induced psychosocial analgesia occurs via a related mechanism to the opioid-induced pharmacodynamic effect.[1020] Another study using magnetic resonance imaging indicated that placebos reduce nocioceptive transmission along the pain pathways.[1038] Further, the release of endogenous opioids might be activated in the anticipatory phase of the placebo response.[1039] Benedetti suggested that there is no certainty that painkillers administered to subjects act on the pain pathways, as they might also, or only, act on the expectation pathways (the uncertainty principle).[1033] Indeed, almost all pharmacological substances might act on the neurotransmission of the expectation pathways.[1020] [Using a classical double-blind RCT design followed by an open-hidden paradigm†* to eliminate the context effects, Benedetti and colleagues[1040] found that placebo effect accounted for the whole of the

* Cordance is a measure derived from QEEG power that has a moderately strong association with cerebral perfusion. It is calculated with a three-step algorithm that normalises power across both electrode site and frequency bands. It has been described in detail in Leuchter *et al.* 1999.[1033]

† The open-hidden paradigm involves both overt and covert administration of an active treatment. In the 'open' administration, participants are given the treatment by a practitioner or researcher in full knowledge that they are receiving it. During the 'hidden' administration, the treatment is administered via a pre-programmed infusion pump so that the participant does not know when he/she is receiving it. It thereby allows for comparison between the specific effect of the drug (hidden component) and combination of specific component and context effects (open component). The difference between open and hidden administration is the non-specific effects, which are believed to be similar to, if not the same as, placebo effects. It has been suggested that the open-hidden paradigm may offer a way to render the expectation pathways silent.[1030]

'analgesic' effect attributed to proglumide, a cholecystokinin antagonist, in the classic double-blind trial, which had shown it to be superior to placebo.]

Neural changes following a placebo procedure are not confined to pain and depression but are also present in other systems and pathological conditions, such as immune disorders[1041] and Parkinson's Disease,[1006] among others.[1042]

Although challenging an underlying assumption of placebo-controlled RCTs, it is not impossible that different mechanisms operate in the two treatment arms, with the placebo having its effect via psychological mechanisms and the active treatment, indistinguishable in appearance from the placebo, acting through pharmacological mechanisms.[998] It has been argued by Kirsch and colleagues, that it is not logically necessary for the effects of the active treatment to be additive, or composed of the two components – the placebo effect and the specific treatment effect.[1043] It is proposed that the effects may be non-additive, or only partially additive (Figure 14.1).[1043] The rationale is based partly on meta-analyses of antidepressant drugs, which find that 65% - 80% of the response to the drug is duplicated in the placebo condition, including in long-term maintenance studies.[1014,1044,1045] This suggests that if drug effects and placebo effects are additive, then the pharmacological effect of antidepressant drugs is really quite small.[1043]

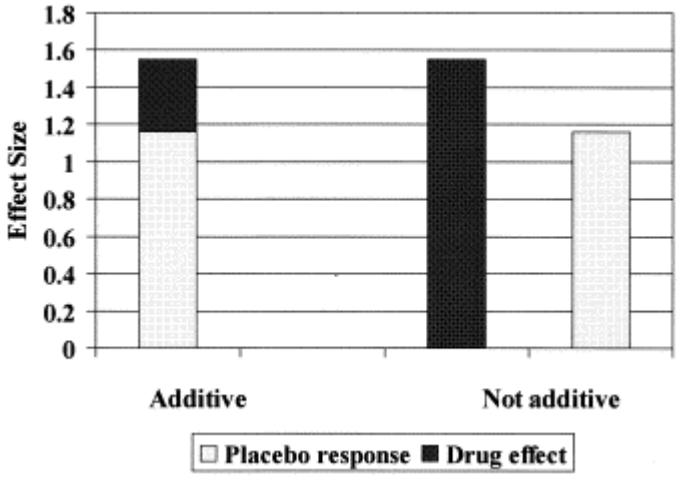


FIGURE 14.1 DRUG EFFECTS AND THE PLACEBO RESPONSE: ADDITIVE AND NONADDITIVE MODELS.

From Kirsch, I. Are drug and placebo effects in depression additive? *Biol Psychiatry* 2000;**47**(8)**:**733-5.[1043] Reproduced with permission from Elsevier.

14.5.3 Clinical trial Methodology and Investigator Effects

Aspects of clinical trial methodology that are thought to contribute to the rising placebo response include failure of trial design, investigator behaviour and the populations studied.[1000] This includes extra time being spent by the researchers with participants, increased non-specific supportive contact, reassurance, comorbid conditions that can compromise response to treatment and the recruiting of participants with disorders that have a fluctuating course or with mild disorders. Some depression studies suggest that more severely ill patients respond poorly to placebo, whereas those who are mildly ill respond equally well to antidepressants and placebo.[1046]

14.6 Predictors of the Placebo Response

Attempts to identify personality and demographic characteristics of placebo responders have yielded inconsistent results. An analysis of a range of anxiety disorders and major depressive disorder (MDD) suggests that the predictors of placebo response rate may vary according to the condition under investigation.[1046] Despite the suggestion that unique placebo characteristics do not exist, and the sometimes contradictory findings, attempts to identify predictors of placebo responders continue and have identified the following variables (Table 14.1).

Placebo rates also increase with overall treatment effect of the active agent being studied (IBS study,[1047] consistent with observations derived from a meta-analysis of 52 placebo-controlled antidepressant trials.[1048]) It is also interesting to note that the response to active treatment can be affected by the inclusion of a placebo arm, as shown in acute migraine studies which noted a significantly *lower* drug effect when a placebo group was included.[1049]

As mentioned previously, predictors of placebo effects may be condition-specific,[1046] rendering generalisability of findings uncertain.

TABLE 14.1 PREDICTORS OF THE PLACEBO RESPONSE IN STUDIES OF VARIOUS CONDITIONS

Demographic variables

- younger age (PMS study)[1050]
- aged 60+ years (depression study,[1051] but not supported by meta-analysis[986])
- male gender (peptic ulceration,[1052] functional dyspepsia,[1053] migraine,[1053,1054] depression[1051] but not consistently[986])
- European study location/population (vs USA)[1046] (anxiety disorders and MDD)
- married compared with widowed/separated/divorced or single subjects[1051]
- higher BMI (functional dyspepsia) [1053]

Affective state and symptom characteristics

14 positive response to prior therapies[1055] (depression)

15 higher (positive) patient expectations of treatment outcome[929,1015]

16 acquiescence,[1056] which could result in obliging reports

17 practitioner/investigator confidence in efficacy of therapy used[1010]

18 high levels of neuroticism, including depression and anxiety (low back pain)[1057]

19 comorbid anxiety[1058]; (*lower* baseline ratings of somatic anxiety[1059]for recurrent depressive episodes)

20 severity of symptoms/disease at baseline: (antidepressant medication: milder severity[1051,518,986]moderate severity.[1055] Social Anxiety Disorder: decreased severity.[1046])

21 chronicity of disease/symptoms. (In depression studies, [932],[1060] this has been the most consistent finding, but not universally supported.[986,1055])

22 worsening or limited change during placebo lead-in in depression; limited change in anxiety, melancholia, helplessness, and paranoia (depression studies).[1061]

23 less stable symptom pattern (functional dyspepsia)[1053]

14 suggestibility was *not* associated with healing or relief of symptoms in the patients receiving placebo (peptic ulceration).[1052]

Methodological factors

- frequency of administration of intervention (IBS study[1047] and migraine prophylaxis.[1054])
- quality of study[994]
- duration/length of the study [994,1062]
- patients' awareness that they are receiving treatment (open vs hidden paradigm.[1040] Open are more effective than hidden at eliciting 'treatment' effects.)[1003]
- unbalanced active:placebo randomisation ratios[1063] (Controversial)[1064]
- documentation of dropouts and additional treatments[994]
- prevention trial[994]
- recruitment of participants with less severe symptoms/disease (in depression studies[1046,1065] and first episodes of depression[1059])
- comparison with *active* placebo possibly due to the side-effects of the active control[1066]
- perceived assignment to treatment. (Sham acupuncture[1015] Sham surgery for Parkinson's disease;[992]
- placebo lead-in was not significantly associated with placebo response rate in a meta-analysis of depression studies.[986]

14.7 Complementary Medicine Issues

CAM research faces the challenge of demonstrating that CAM therapies have *more* than placebo effects, especially since well-designed clinical studies of CAM frequently fail to find significance over placebo. It has been proposed that CAM has an enhanced placebo effect and could be an especially successful placebo-generating health care system,[1067] possibly by its emphasis on personal responsibility, commitment and active participation in the treatment, which can facilitate adherence. Other aspects of the setting such as the therapeutic alliance, amount of time spent, enhancing patients' expectations through positive information about the treatment or illness while providing support and reassurance, all shown to significantly influence health outcomes,[1009] could also contribute to greater non-specific effects. It has been suggested that an increased anxiety among users of CAM renders them more susceptible to the placebo response.[1067] A national survey of trends in CAM use in the US from 1990-'97 reported that alternative medicine was predominantly used for chronic conditions, including back problems, anxiety, depression, and headaches,[1068] conditions described by Kaptchuk as "highly subjective symptoms lacking identifiable physiologic correlates, chronic conditions with a fluctuating course often influenced by selective attention, and affective disorders."[1067]

Conversely, it has been argued that if the placebo effect represents a conditioned response, complementary medicine would be expected to produce *lower* placebo response rates than pharmaceutical interventions since the latter have well-known large effects[1008] and a high degree of correlation between response to treatment and response to placebo has been shown.[1014]

Although the placebo response rates in studies of herbal medicine may not compare with rates seen in studies of HT for the vasomotor symptoms of menopause or antidepressant drugs, less pronounced specific treatment effects are often associated with CAM. Thus the magnitude of the context effects continues to present a challenge in CAM research.

14.8 Conclusion

Substantial placebo effects plague research on menopause, depression and CAM. This can potentially result in treatment effects being missed due to type II errors, as well as increasing the sample size required to detect a statistically significant effect. Where both arms improve significantly, an intervention found to be no better than placebo cannot be

concluded to be ineffective[999] in terms of improving the wellbeing of the participants. However, to meet the criteria of scientifically rigorous research, the specific effect must be determined, and the results must be replicable. It is therefore necessary to understand, as well as control, the placebo effect. In menopause studies, as well as depression, the natural history of the conditions may contribute substantially to the high response rate in the placebo arm. As recruits often enrol in clinical trials when their symptoms are at a peak, regression to the mean can confound studies of any condition. These threats to validity can only be distinguished from the placebo effect by the addition of an untreated arm to the design,[996,1069] although this further increases costs in terms of financial and human resources. It is thus imperative to design protocols that are both scientifically rigorous and maximise the likelihood of accurately detecting the specific treatment effect of active interventions, by controlling the context effects. To that end, different paradigms have been proposed and trialled – not exclusively for CAM research. These will be discussed further in chapter 19.

Chapter Fifteen

Methodology

15.10 Introduction

This chapter describes the aims and objectives of the study, the research design and methodology. Details of selection criteria, screening and baselines assessment are included. Information on the phytotherapeutic intervention is provided according to the elaborated CONSORT checklist. A rationale is given for the instruments used, and details of data collection, adverse events monitoring and statistical methods are outlined.

The methodology has been reported according to *The CONSORT statement for reporting Randomised Controlled Trials*[1070] and the *Elaborated CONSORT statement for reporting Randomised Controlled Trials of Herbal Interventions*.[1071]

15.1 Study Overview

The principal study was a randomised, placebo-controlled, double-blind parallel trial with a 16 week treatment phase, a 2 week non-treatment run-in and an 8 week non-treatment follow-up. Two further studies were conducted on subgroups of the same population. Three separate papers are included:

1. the RCT examined a phytotherapeutic combination for menopausal symptoms;
2. a sub-study of premenstrual symptoms was included on peri-menopausal subgroup who continued to menstruate infrequently; and
3. predictors of the placebo response were assessed from data obtained from RCT.

15.2 Study Population

Approval for the study was obtained from the Human Research Ethics Committee at Royal Melbourne Institute of Technology University. All participants gave written informed consent prior to entering the study. Baseline visits were completed in a clinic setting. Follow-up contacts were conducted by telephone.

15.2.1 Recruitment

To recruit participants, advertisements were placed in The Herald-Sun, the Leader and other local newspapers, including MX. Articles appeared in The Age newspaper, professional journals and special interest group newsletters. Two radio interviews were done with ABC 774 and a rural radio station. Fliers were displayed in waiting rooms of doctors' and allied health professionals' clinics, health food shops and The Jean Hailes Foundation for Women's Health information centre. Posters were displayed at university campuses and colleges of natural medicine, and websites at RMIT and The Jean Hailes Foundation advertised the trial. The numbers of participants recruited through each source are detailed in appendix 13.

15.2.2 Inclusion criteria

To be eligible for inclusion, women had to be aged between 40 and 60 years, which encompassed the age range within which women are likely to become peri- or postmenopausal.[57,59,64] Clinical criteria for entry to the study were based on menstrual cycle alterations and symptoms.[65] Participants were either naturally post-menopausal defined by amenorrhoea for 12 months or more, *or* late perimenopausal, defined as at least 3 months' amenorrhoea in the previous 12 months.[53,65]

Entry requirements were a minimum of five flushes (including sweating episodes) per 24 hour period (total 35 per week), consistent with baseline scores from previous positive studies,[19,25] and a minimum score of 20 on the Greene Climacteric scale, consistent with a menopause clinic sample, as distinct from the general population of menopausal women[1072] and baseline data from a comparable study.[75]

Before inclusion in the trial, medical clearance from a general medical practitioner (GP) was required after a general medical check-up, including heart rate, systolic and diastolic blood pressure and breast examination. Mammography and pap smear were performed if not conducted within the previous two years. Pregnancy was excluded at baseline. The GP was provided with a list of exclusion criteria for the trial and asked to advise whether there were any known reason why the person should not take part in the trial. (Appendix 14) Hysterectomised women were admitted if they were over the age of 53 and FSH levels were greater than 25IU/L, despite the questionable reliability, of FSH levels in determining menopausal status.[66]

Participants were advised to avoid excessive exposure to sunlight or artificial UVA light for the duration of the trial, due to the risk of developing an increase in UVA photosensitivity.[796]

15.2.3 Exclusion Criteria

Exclusion criteria were based on safety for participants, National Health and Medical Research Council (NHMRC) guidelines for involvement in clinical trials, as well as exclusion of potentially confounding factors. They therefore included concurrent major health conditions, substance abuse, concurrent menopause treatment or any medication known to interact with the study intervention, pregnancy, attempting to conceive or concurrent participation in another trial.

Concurrent Medications Excluded

Based on known herb-drug interactions (discussed in the relevant sections on the study herbs), women in any of the following were excluded:

- immunosuppressants (cyclosporine)[954,955,956]
- cardiac glycosides (digoxin) for doses of St John's wort greater than 1 g/day (dried herb equivalent)[957]
- HIV medication including protease inhibitors,(indinavir),[958] the non-nucleoside reverse transcriptase inhibitor, *nevirapine*[959], and other anti-retroviral drugs[960]

- chemotherapy drugs, (irinotecan)[961]
- anti-coagulant medication such as warfarin, phenprocoumon[962,963]
- low dose oral contraceptives[962]
- theophylline (bronchodilator)[964]
- triptans (migraine medication)[965]
- midazolam (benzodiazepine)[949]
- fexofenadine (antihistamine)[966]
- the HMG-CoA reductase inhibitor simvastatin[967] and atorvastatin[968] (but pravastatin was unaffected)
- phenytoin (theoretical concern)[965]
- amitriptyline (tricyclic antidepressant).[969,970]
- progesterone drugs, the contraceptive pill or HT[796]
- dopamine receptor antagonists.[857]

The cholesterol-lowering pro-drug *simvastatin,* was also later found to be contraindicated with *Hypericum perforatum* and was added to the exclusion criteria. (However, this did not concern any of the remaining participants.)

To eliminate any medication that could potentially confound the results, women were also excluded if taking any medications/supplements thought to affect the symptoms under observation throughout the trial. These included

- Hormone Replacement Therapy (Hormone Therapy)
- Other concomitant treatment for menopausal symptoms including phytoestrogen products, Vitamin E supplements, herbal menopause formulas
- anti-depressants, anxiolytics or hypnotics (psychotropic medication)
- herbal medicine that included *Hypericum* or *Vitex*
- anti-convulsant medication
- steroid therapy.

Based on accepted wash-out periods for different medications,[1073] participants were required to have been free of oral HRT or anti-depressant medications for four weeks (five weeks for *fluoxetine* or MAO-Inhibitors) preceding the trial. They could not have been using hormone implants or injectables for 1 year or 6 months respectively. Due to the possibility of conceiving during the perimenopause, any woman electing to discontinue oral contraceptives would be recommended other means of contraception.

As a precautionary measure, in the interests of participant safety, women with current major health conditions, derived from comparable studies,[77,304,540,550,707,1074] or any condition suspected to be aggravated by either intervention were excluded. These were

- undiagnosed vaginal bleeding (in post-menopausal women)
- history of epilepsy or seizures
- pre-existing cancer, renal or liver disease, diabetes mellitus requiring treatment, uncontrolled hypertension
- substance abuse
- bipolar disorder, severe depression, current major psychiatric disorder[796]
- history of mania
- spasmodic dysmenorrhoea not associated with premenstrual syndrome, which has been observed to be aggravated by *Vitex agnuscastus*[796]
- known photosensitivity, a potential side effect of *Hypericum perforatum*[796]
- known intolerance to *Hypericum* or *Vitex.*

Other exclusion criteria were

- medically or surgically induced menopause [due to the symptom severity[50] and lack of ovarian function]
- anyone in a dependent relationship with the principal investigator(s), such as patients and students.

In the case of unblinding, the participant was to be removed from the trial. Numbers excluded at interview and reasons for exclusion are included in appendix 15.

15.2.4 Baseline Assessment

Pre-trial symptoms were assessed at baseline using the primary and secondary outcome measures, the Greene Climacteric scale, the self-rated Hamilton Depression Inventory (HDI), Utian Quality of Life questionnaire and a daily flushing diary for the two week non-treatment run-in period. Details of these measures are provided below.

Because some studies on dietary phytoestrogens support a salutary effect of these plant constituents, especially isoflavones and lignans, on some of the outcome measures, participants were asked to maintain their baseline phytoestrogen intake throughout the trial, and were advised of the relevant foods.

15.2.5 Initial Screening Procedure

Women who expressed interest were screened by telephone to ascertain suitability for inclusion in the trial. Interested persons were only selected if they met all the criteria, which were established to determine their suitability for the research.

Telephone screening involved the investigator reading out a standard preamble to ensure all participants received the same information and were treated equally. (Appendix 16). This was piloted initially with an ineligible volunteer from the study demographic in order to ensure complete information was provided within a reasonable length of time. All calls were taken by the principal investigator for consistency. (The results of telephone screening are included in appendix 17.) This involved

1. thanking them for their enquiry
2. finding out the source of their information about the trial
3. giving an overview of what participation involved, including the herbs being trialled
4. outlining some of the inclusion criteria, without specifying the exact score so as not to encourage women to misrepresent their symptoms
5. outlining the exclusion criteria, including a detailed list of medications and health conditions to be excluded. This enabled respondents to self-select out without being required to divulge details of their personal medical history
6. the requirements of involvement in the study
7. ensuring they understood it was placebo-controlled
8. informing them that they may not benefit from their involvement in the study
9. informing them of the risks of side-effects and safety monitoring procedure
10. advising them that, if shown to be superior to placebo, the intervention would be offered free of charge for a period of 3 months to the placebo group at study termination.

If the woman was still interested and eligible, an interview time was arranged at her preferred venue (of three available) and a participant information sheet (Appendix 18 Plain language statement) sent out with directions to the interview venue. A contact number was obtained for confirming the interview time and place the day before.

15.2.6 Screening Interview

Interviews were held at Clinicare Medical Centre, North Fitzroy, RMIT city campus psychology clinic, RMIT Bundoora psychology clinic and a small percentage of rural participants were interviewed by telephone. In the case of telephone interviews, the interview consent form and Greene climacteric scale were sent out prior to the interview.

Consent to obtain background information was obtained prior to an interview (Appendix 19). All interviewees were informed that the study had the approval of the Human Ethics Committee of RMIT University, Melbourne. They were also informed that the purpose of the interview was to establish eligibility for the study, that they would be asked to provide personal information, that all information would be safeguarded by code and that they had the right to withdraw at any time from the interview.

To ensure confidentiality, an interim code number was assigned to each interviewee; the screening form was identified only by this number and contained no identifying information. Personal contact details were completed on a separate form, subsequently filed in a locked filing cabinet along with copies of consent forms and medical clearance form once obtained.

In addition to establishing eligibility for the study, the interview was used to collect pertinent information including menopausal status, health status, history of premenstrual symptoms, diet and lifestyle and current experience of menopausal symptoms (Appendix 20). Height and weight were measured and Body Mass Index calculated. For women attending the RMIT venues, where such measurement was not possible, a machine-recorded weight measurement was required, available from a variety of pharmacies.

During the interview, data were collected regarding the number and intensity of hot flushes and night sweats per 24 hour period. Interviewees also completed the Greene Climacteric scale, without being informed that a minimum score was being sought, the HDI and Utian Quality of Life Scale. The completed Greene scale was scored immediately to determine eligibility for the study.

Data from the HDI was double-entered into the computerised Hamilton Depression Inventory Scoring Program V1.0. All completed scales were scored in accordance with the appropriate scoring procedures and double checked before being entered into Statistical Package for Social Science (SPSS) Version 11 for statistical analysis.

For the HDI and Utian scale, these scores constituted baseline data. Although the HDI is not recognised as a diagnostic tool,[1075] interviewees with scores suggestive of severe depression were considered to fall within the exclusion criteria, and were consequently excluded and referred to a medical practitioner for further investigation.

All prospective participants were required to report any existing symptoms to the investigator before administration of the herbal treatment. Interested persons were reminded during the

interview that there that they may not benefit from their involvement in the study. Information regarding possible adverse effects or side effects of the herbal treatment was reiterated during the interview. Participants were also briefed thoroughly on the protocol they should follow in the event of an unwanted side-effect occurring during the treatment phase.

Options for obtaining medical clearance from a GP were also outlined during the interview. Participants were able to consult a GP of their choice. Ten GP's had been recruited in various suburbs of Melbourne to assist with the medical examination for participants who did not have a GP or chose not to consult their own GPs. Medical Clearance costs were charged to RMIT and paid for from the grant provided by Australian College of Phytotherapy.

15.3 Study Intervention

Details of the herbal intervention are provided in accordance with the Proposed Elaboration of CONSORT Checklist Item 4.[1071]

15.3.1 Herbal Product Name and Characteristics

Both the placebo and active treatment were administered in the form of tablets, identical in size, colour, coating, weight and packaging. All tablets were manufactured according to the Code of Good Manufacturing Practice[1076] by MediHerb Australia Pty Ltd. Both products are included on the Australia Register of Therapeutic goods in as Listed Medicines and are readily available over-the counter in Australia, Europe and the USA. Both were administered within standard dosage levels, as described below.

The extract was obtained from the dry herb flowering top of *Hypericum perforatum L.* (Clusiaceae/Guttiferae; St John's wort) (extraction ratio 6:1(g/ml)) and dried *Vitex agnus-castus L,* (Verbenaceae; Chaste tree/berry) fruit (extraction ratio 1:2(g/ml); the extraction solvent was 60% ethanol/water. The extract was purchased and identified and analysed by MediHerb's Quality Assurance Laboratory. Retention samples of raw materials and finished tablets are kept at MediHerb; these were validated by chemical fingerprinting against verified botanical samples maintained at the Southern Cross University Herbarium. The St John's Wort Tablets were Batch 125178 and Chaste Tree Tablets Batch 124324.

15.3.2 Dosage regimen and Quantitative description

Each St John's Wort tablet contained *Hypericum perforatum* extract equivalent to 1,800mg dry herb flowering top standardised to contain hypericins 990 mcg, 9mg hyperforin and 18 mg flavonoid glycosides. Each *Vitex agnus-castus* (Chaste Tree)

tablet contained extract equivalent to 500mg dry fruit. This tablet was not a standardised preparation. A total of three *Hypericum perforatum* (one in the morning and two later in the day) and two *Vitex agnus-castus* tablets (in the morning) or placebo were given daily for 16 weeks. The hypericin content was consistent with effective doses for mild-moderate depression used in RCTs.[915] The dosage regimen was determined by referring to previous RCTs on *Hypericum perforatum* in depression, which used a daily dose of 900mg of 6:1 extract,[915] and *Vitex agnus-castus* [839] and is consistent with current usage.[820]

15.3.3 Qualitative testing

The high-performance liquid chromatography (HPLC) chromatogram for *Hypericum perforatum* can be seen in figure 15.1. The method for performing this analysis was as follows: HPLC separation was achieved using a C18, 5μm column with dimensions (150 mm x 4.6mm) with a gradient elution program using a three solvent program B=methanol, C = 50mM phosphoric acid in deionised water and D = acetonitrile. The elution program was 0 min B=0%, C=85%, D=15%; 20 min B=10%, C=70%, D=20%; 30 min B=15%, C=10%, D=75%; 55 min B=15%, C=5%, D=80% at a flow rate of 1.0mL/min. The analysis was done by an individual with over 20 year's experience in analytical chemistry. Thin Layer Chromatography was employed for *Vitex agnus-castus*. Concentrations of heavy metals were measured by inductively coupled plasma mass spectroscopy (ICP/MS).

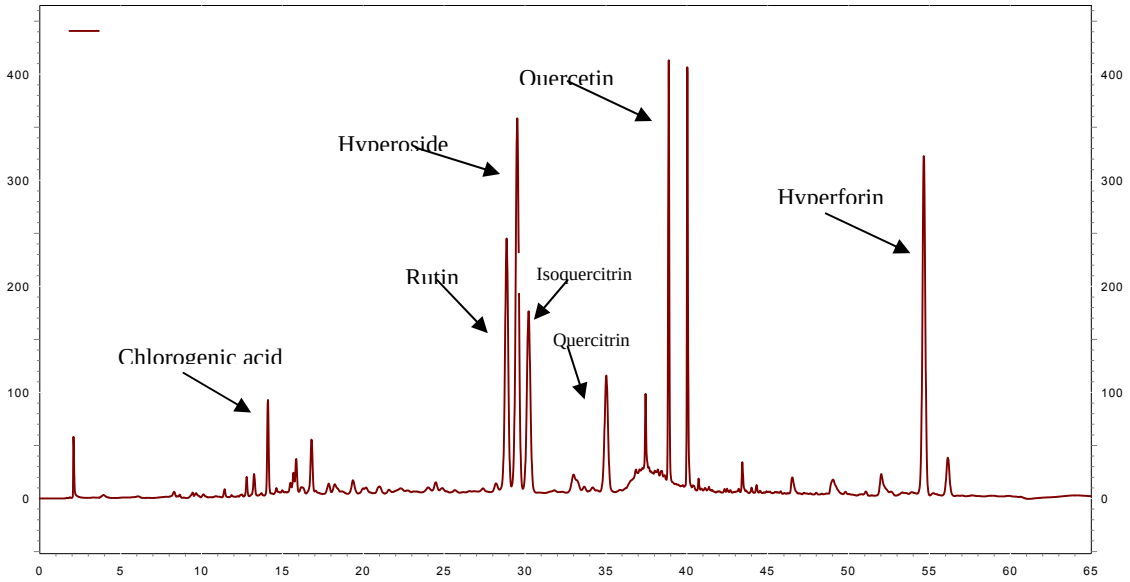


FIGURE 15.1 ST JOHN'S WORT (*HYPERICUM PERFORATUM*) HPLC CHROMATOGRAM

Hypericin was tested utilising the British Pharmacopeia UV method.[1077] The levels of flavonoid glycosides were determined using a proprietary MediHerb HPLC analytical method, (conditions above).

Herbs are chemically complex and, as with most herbal medicines, the active ingredients remain undetermined. However there are candidates such as flavonoids, hypericin and hyperforin in *Hypericum perforatum* and iridoids, diterpenes and essential oil in *Vitex agnus-castus.* All were present in the products used, which characteristically resembled the plant in terms of phytochemicals.

15.3.4 Placebo tablets

The placebo tablets contained the excipients used in the active tablets, namely modified starch, cellulose, magnesium stearate, calcium hydrogen-phosphate. These excipeints are commonly used in making pharmaceutical tablets and are known to be inert. The tablets were identical to the herbal tablets in size, colour, coating, weight and packaging. All tablets were packaged in amber glass jars with Tamper Tel plastic lids. They were labelled with code number, 'Treatment A or B' to denote *Hypericum* and *Vitex* respectively (or matching placebos), dosage instructions, time of administration, institute name (RMIT), clinical trial identification, investigator's contact number, expiry date and storage instructions.

15.3.5 Determination of Treatment and Dosage

The clinician choosing the treatment and determining the dosage had 8 years experience as a chemist and 22 years as a medical herbalist.

15.3.6 Safety and Quality data

Toxicology data are available for both herbs in animals and are presented in Mills and Bone (2005: pp335 & 590-1).[805] Pharmacokinetic data is available for *Hypericum perforatum.*[796] Safety monographs were provided by MediHerb and stability data and post-production quality control data was available for both products. There are no known adverse herb-herb interactions between these herbs.[805]

15.3.7 Assessment of Compliance

At the end of the study, all remaining tablets were returned to the study centre and manually counted to calculate percentage compliance. (Appendix 21)

15.4 Objectives

The objective of the principal study was to evaluate the effectiveness of a phytotherapeutic intervention comprising a combination of *Hypericum perforatum* (St John's wort) and *Vitex agnus-castus* (Chaste tree/berry) in the management of menopausal symptoms.

The null hypotheses were

1. That the combination of *Vitex agnus-castus* and *Hypericum perforatum* is no different to placebo in the treatment of vasomotor symptoms associated with menopause.
2. That the combination of *Vitex agnus-castus* and *Hypericum perforatum* is no different to placebo in the treatment of psychological symptoms associated with the menopausal transition.
3. That the combination of *Vitex agnus-castus* and *Hypericum perforatum* is no different to placebo in improving quality of life of women during the menopausal transition.

Based on previous research on *Hypericum* on menopausal symptoms,[49] it was also anticipated that sexual well-being may be favourably affected.

Secondary objectives were to i) assess the effect of the combination in PMS-like symptoms in perimenopausal women, and ii) to examine the role of placebo therapy in these groups. It was therefore further hypothesised that

- the PMS-like symptoms in the perimenopausal women would be relieved
- the placebo would have a significant impact on the menopausal symptoms and would be predicted by psychological factors and baseline symptom severity.

15.5 Outcome Measures

The following tests or measures were used to determine the effects of the herbal combination on the psychological and vasomotor symptoms of menopause. All scales have been well validated and widely used in previous clinical trials. (See *Instruments Used* below for further details.)

- A daily diary was used to record flushing and sweating episodes;
- The Greene Climacteric scale was used to assess menopausal symptoms at baseline and changes in these symptoms throughout the trial;

- The HDI was used to exclude any prospective participants who were suffering from severe depression. The HDI and Greene Climacteric subscales were also used to monitor changes in depression, anxiety and sleep throughout the trial;
- The 4-weekly lifestyle diary was used to monitor possible confounding variables and to monitor any side-effects and their severity;
- The Utian Quality of Life scale was used to assess general well-being and monitor any changes that occur during the trial; and
- A Menstrual Symptoms diary was used monthly with the perimenopausal group to monitor changes in premenstrual symptoms.

All were piloted on a group of seven volunteers within the demographic. Adjustments were made accordingly to typographical features and clarity of instructions and questions with respect to instruments designed for the project.

15.5.1 Timing of Data Collection

Weeks 1 –2	Run-in	2 weeks
Weeks 3 – 18	Treatment	16 weeks
Weeks 19 - 26	Non-treatment follow-up	8 weeks from completion of treatment

Contact was made with participants at interview, end of run-in (baseline) and at 4 weekly intervals throughout the treatment phase and non-treatment follow-up (Table 15.1). Initially, with the pilot group, the non-treatment follow-up period was 12 weeks, but this was subsequently altered to 8 weeks to facilitate participant recall of return of symptoms, and thus provide a more accurate picture of the washout period for the trial products.

TABLE 15.1 DETAILS OF MEASURES ADMINISTERED DURING TRIAL PHASES

	Baseline	***Treatment Phase***				***Follow-up***
Test or Measure	***Weeks 0-2***	***Week 6***	***Week 10***	***Week 14***	***Week 18***	***Week 26***
General physical examination by GP	✓	← If required →				
Utian Quality of Life Scale	✓				✓	
HDI	✓	✓	✓	✓	✓	✓
Menstrual diary (where applicable)	✓	✓	✓	✓	✓	
Greene Climacteric	✓	✓	✓	✓	✓	✓
Vasomotor Symptoms diary	✓	daily	daily	daily	daily	✓
Lifestyle & Side-effects diary	✓	✓	✓	✓	✓	✓

15.5.2 Non-treatment Run-in

A two week non-treatment run-in was commenced as soon after the interview as was convenient for each participant, except over the Christmas period when participants were asked to wait until their normal lifestyle was re-established. During the run-in period, participants were required to complete the same daily flush diary that they would complete for the 16 weeks of the treatment phase. At the end of the 2-week period, they again completed the Greene Climacteric Scale and the Lifestyle questionnaire which recorded illness/accidents, new medications/supplements as well as possible confounding factors such as stress, caffeine and alcohol intake. (Refer to sample booklets in Appendix 22).

The objectives of this were

1. to ensure the women did have the minimum required number of flushing episodes, namely 35 per week
2. to obtain more accurate baseline flushing data than could be provided at interview
3. to exclude any non compliers with completing records
4. to allow for regression to the mean
5. to control for the Hawthorne effect, an observed phenomenon of behavioural changes once people enter a study. Greene scores at the end of the run-in periods were used as baseline scores, as were the flushing counts.

Interviewees who were eligible and willing to continue with their participation at this point signed an informed consent form, witnessed by a third party, and given a copy for their own records. (Appendix 23) They were advised of the voluntary nature of their participation and that they had the right to withdraw at any time.

Documentation provided

Upon commencement of the treatment phase, participants received the tablets for the entire 16 weeks, a folder containing four 4-weekly booklets and reply paid envelopes for their return at the end of each four week period. In addition, a copy of the HDI was enclosed to enable them to complete the self-rating scale at the end of each booklet. Two self-sealing plastic bags labelled ‘Treatment A’ and ‘Treatment B’ were included for participants to return any unused tablets at the end of the treatment phase to the study centre These were then counted to assess compliance.

Participants were asked to keep dietary phytoestrogen intake stable throughout the study, although evidence from clinical trials does not consistently support the efficacy of phytoestrogens for menopausal symptoms.[75,76,604,611,612,616,1078] Participants were also asked to avoid starting any new supplements during the trial, unless on prescription, and to notify the investigators in that event. Each 4-weekly booklet contained a list of medications and conditions that were excluded and instructed participants to notify the investigators in the event that they were prescribed/diagnosed with any of these. Instructions for completion of study questionnaires were clarified with the aid of sample materials.

15.5.3 Follow up

Telephone contact was made at the end of week 1 and at the end of every 4 weeks with all participants. Case report forms were completed at each contact and a series of standard questions elicited information regarding compliance with taking tablets, counting vasomotor symptoms, illnesses/accidents, new medications, changes in phytoestrogens and stress levels. (Appendix 24)

Participants were also contacted in the non-treatment follow-up period to ascertain any potential continued benefits that had been experienced since the active treatment and to determine the length of the washout period. Participants were to be offered complementary treatment post participation for a period of 12 weeks had treatment been shown to be superior to placebo. It was intended to continue monitoring them during that period. They were asked to advise of any changes to contact details before code breaking.

Each participant was terminated from the study once the data from the non-treatment follow-up had been received and scored.

Where a participant had neglected to complete an item, she was contacted by phone if this was possible within 2 weeks of the due date of completion.

Because women were recruited over a period of 16 months, some participants' involvement ended well in advance of code-breaking and data analysis. Participants were notified at regular intervals of the progress of the trial and expected code breaking date. When the results became available, participants were invited to a meeting where the results were presented, talks were given by the principal investigator and supervisor, Professor Henry Burger, (a leading endocrinologist and researcher in the field), regarding treatment options and self-help measures, and information booklets provided

by The Jean Hailes Foundation were distributed. Women unable to attend were sent the information packs by post.

15.6 Instruments Used

Measures used throughout the treatment phase of the study were divided into four 4-weekly questionnaire booklets. (Appendix 22) Each provided contact details of the principal investigator on every page, the RMIT supervisor and safety monitor on the cover. Inside the front cover of each booklet was a list of medications not to be taken in conjunction with the herbs being trialled as well as medical conditions that would require withdrawal from the study. It also reiterated that participants were requested to maintain their current intake of phytoestrogen- containing foods, particularly soy and linseeds for the duration of the trial.

A schedule for completion of tests and records was provided, along with a statement of confidentiality and complaints procedure. Each booklet was clearly marked with the participant identification number and contained the following measures:

15.6.1 Daily Symptoms Diary

Four weekly daily symptoms diaries for participants to record the number of mild, moderate and severe flushing and sweating episodes they experienced each day and night, where night was defined as 'while in bed'. These were subjectively categorised according to the following definitions:

Mild	flush without perspiration or clamminess
Moderate	hot flush associated with perspiration or clamminess
Severe	hot flush associated with intense perspiration that requires changing of clothing[304]

These definitions were consistent with whose used in previous menopause studies and were selected to permit comparison between studies.[304,305] Measures do not exist that include quantification of intensity of sensation of heat within a hot flush and duration of flushes, so these are typically not assessed. In order to calculate the daily and weekly weighted scores, a mild flush was quantified as 1, moderate as 2 and severe as 3.

Methods of keeping count varied between participants, with some using counters or golf watches while others used notebooks or buttons.

15.6.2 The Greene Climacteric Scale

Previously the most popular menopause rating scale was the Blatt-Kupperman Index, developed in the 1950s. This has subsequently been discredited for its omission of measures of loss of libido, overlapping scores in different categories, lack of statistical justification for the weightings used, and scores being summed without being based on independent factors.[1079]

Currently, the most widely used menopause rating scale is The Greene Climacteric Scale,[153,1080,1081] a 21-item self-report inventory. It was developed in 1976 using factor analysis,[153] which analyses a large series of intercorrelated variables, establishing the relationships among them and delineating a smaller number of underlying factors. The current scale was first published in 1991, the earlier version having been modified in consideration of six subsequent factor analytic studies undertaken by different researchers.[1072] It was originally constructed on the basis of symptoms of women presenting at a Scottish menopause clinic. However examination all seven factor analytic studies found that the same three main factors emerged regardless of whether the sample was from a clinical or general population.[1080]

The 21 items on the scale are separated into four sub-domains: Items 1 – 11 address the psychological symptoms, which are further subdivided into anxiety (items 1 – 6) and depression (items 7 – 11) scores. Items 12 – 18 are somatic symptoms; items 19 – 20 are the vasomotor symptoms of hot flushes and night sweats and item 21 is a 'probe' question for sexual dysfunction. The total Greene Climacteric score is the sum of all 21 scores. Women rate each symptom according to a four-point scale: not at all (score = 0); a little (score = 1); quite a bit (score = 2); extremely (score = 3).

Content and construct validity have been well established and test-retest reliability coefficients reported for 50 women over a 2 week period. These were 0.87 for the psychological scale. 0.84 for the somatic scale and 0.83 for the vasomotor scale (Greene 1998). The scale has been widely used in previous clinical trials on menopausal symptoms.[691, 75,77]

The current study sought to recruit women with symptom severity representative of women who seek treatment. Therefore a minimum total Greene score for inclusion was based on a clinic sample, as distinct from a general population sample. Normative data using the scale is available from three sources, namely

1. the Scottish sample studied by Greene in 1991 which included 400 'menopausal and postmenopausal' women aged between 40 and 55 years drawn from the general population as well as a menopause clinic
2. a Dutch sample of 504 women from the general population aged 45 – 65 years, subdivided into pre-menopausal, perimenopausal, postmenopausal and post hysterectomy[184]
3. an Australian study of 500 premenopausal, perimenopausal and postmenopausal women aged 40 – 80 derived from the general population. As this study reported medians and used non-parametric tests, its results are not directly comparable with the two mentioned above, which reported mean values and used analysis of variance techniques.[1082]

Data available for sub-domains from the Dutch and Scottish general population samples yield good agreement (Table 15.2).

TABLE 15.2 NORMATIVE DATA FOR GREENE CLIMACTERIC SUBSCALES

Subscale	*Scottish Menopause clinic sample*[1072]	*Scottish General Population sample*[1072]	*Dutch Perimenopausal sample*[184]	*Dutch Postmenopausal sample*[184]
Psychological	12.33 (6.15)	7.42 (6.41)	7.67 (5.27)	7.44 (5.48)
Somatic	6.16 (4.25)	3.25 (3.64)	4.53 (3.76)	4.23 (3.43)
Vasomotor	4.41 (1.79)	1.79 (1.12)	2.82 (1.75)	2.67 (1.92)
Total	Not available	Not available	15.78 (9.09)	15.33 (9.01)

For *total* scores, means are only available from the Dutch sample, in which both perimenopausal and postmenopausal women scored were significantly higher than women in the pre-menopause (10.53 +/-7.36).[184]

In order to identify women suffering from severe anxiety or depression and establish need for further investigation, individual scores were checked against Greene's recommended cut-off points for severe and possibly clinical anxiety and depression.[1072] These were based on a comparison with the Hospital Anxiety and Depression Scale:

3. Clinically Anxious = Anxiety score of 10 or more
4. Clinically Depressed = Depression score of 10 or more[1072]

15.6.3 Hamilton Depression Inventory

A further secondary endpoint was depressive symptoms. In studies of menopause-related mood changes, the Center for Epidemiologic Studies Depression Scale (CES-D),

a 20-item scale that screens for depressive symptoms in the previous month,[1083] has been widely used.[59,273,362,367,370,376,384] However, in depression studies of *Hypericum perforatum* (St John's wort), as in psychiatry, the most widely used severity measures of depressive symptomatology is the clinician-rated Hamilton Depression Rating Scale (HDRS or HAMD). To permit comparison between those studies and the current study, use of the same scale was considered ideal. Because administration of the HAMD by trained clinicians was prohibited by funding limitations, the self-rated Hamilton Depression Inventory (HDI) was employed instead.

The full-scale HDI consists of 23 items, while the HDI-17 is comprised of the first 17 items of the full-scale. This 17-item HDI form corresponds in content and scoring to the standard clinician–rated 17-item HAMD, and allows comparable interpretation (p10).[1075] Six additional items (including hypersomnia, detachment, feelings of worthlessness, helplessness–pessimism, hopelessness, and difficulty making decisions) are included in the HDI-23 to enhance its content validity by including symptoms of major depressive disorder and dysthymic disorder as defined by the Diagnostic and Statistical Manual of Mental Disorders.[1084]

The HDI, emulating the clinician-rated HAM-D, evaluates multiple sub-symptoms or components of a symptom, and determines the duration and frequency of symptom occurrence, thereby providing for a more comprehensive evaluation of individual symptoms than is typically assessed by self-report depression measures. As a result of the multiple questions, the 23-item full-scale HDI includes 38 questions and the HDI–17 consists of 31 questions.[1084] the current study collected data for the complete questionnaire, but the analysis used the 17-tiem scale because it allows comparison with studies of *H. perforatum* using the HAMD.

Reliability and validity has been established for the HDI as a measure of the severity of depression and as a self-report version of the HAMD clinical interview. In addition, the HDI also demonstrated a high correlation with the Beck Depression Inventory.[1084] All versions of the HDI demonstrate high internal consistency and test–retest reliability as a self-report measure of severity of depression in adults. Extensive validity evidence includes content, criterion-related, construct, and clinical efficacy of the HDI cut-off score.[1084]

The HDI is not a diagnostic tool for depression according to the DSM-IV. However the protocol does include the Major Depression (MD) Checklist for identification of levels of symptom severity consistent with the nine primary symptoms for a diagnosis of major

depression delineated by the DSM-IV (pp8,34),[1075]namely depressed mood, loss of interest/pleasure, weight loss, insomnia/hypersomnia, psychomotor agitation/retardation, fatigue or loss of energy, worthlessness or guilt, indecisiveness and suicidal ideation/behaviour. Scores on the MD checklist was calculated at the baseline interview in order to identify any prospective participants potentially suffering from major depressive disorder so that they could be referred for further investigation. In addition, seven critical items have been identified as clinically relevant. (These are dysphoria; suicidal ideation or attempt; loss of interest/poor work performance; somatic symptoms of fatigue and muscle-aches; feelings of worthlessness; pessimism/hopelessness; and indecision.) Women with high scores (≥ 2) on three or more of these items were referred for further examination, as was anyone scoring ≥ 1 on suicidal ideation.

The HDI evaluates the severity of depressive symptoms over the previous 2 weeks.[1084] Cut-off scores for clinical severity levels on the HDI-17 scale are:[1075]

TABLE 15.3 CUT-OFF SCORES ON HDI-17 SCALE

Range of Scores	*Clinical Description*
0-9.5	Not depressed
10-14.5	Subclinical
15-19.5	Mild
20.0-24.5	Moderate
25.0-29.5	Moderate to severe
≥ 30	Severe

15.6.4 Utian Quality of Life Scale

To measure changes in quality of life and differentiate between women according to their quality of life, many studies have used the Menopause-Specific Quality of Life questionnaire (MENQOL), [1085]a validated menopause-specific instrument comprising physical, vasomotor, psychosocial and sexual domains. This scale developed in 1992 was subsequently modified in 2003 for use where certain treatment side effects could negatively impact the quality of life.[1086] However, there is considerable overlap with this scale and symptoms reported on the Greene Climacteric scale, , with 16 of the 29 items relating to physical symptoms, 3 to vasomotor, 7 to psychosocial and 3 to sexual symptoms.[1085] The impact of the symptoms on quality of life is more clearly delineated as in the Utian Quality of Life scale, which was therefore selected for use in this study.

The Utian Quality of Life scale, administered at study entry and the end of the treatment phase (end of Week 16), is a validated scale developed specifically to measure quality of life (QOL) in peri- and postmenopausal women. As distinct from a symptom inventory,

its purpose is to measure the impact of the symptoms on the sense of well-being and QOL of the woman.[1081] The four subscales - occupational, health, emotional and sexual - were derived from an exploratory factor analysis. Validity was established in reference to the Short Form-36, a widely used instrument with established reliability and validity for measuring QOL. Test-retest reliability was also established.[1081] The 23 items in the questionnaire are scaled on a 5-point Likert scale where 1 represents 'Not true of me', 3 denotes 'Moderately true of me' and 5 'Very true of me'.[1081] (Appendix 22).

15.6.5 Menstrual Symptoms Record

In order to gather data for a sub-analysis of PMS-like symptoms among perimenopausal women, the women who were still irregularly cycling were additionally required to complete, when relevant, a Premenstrual Symptoms questionnaire for the week preceding the menstrual period. The commonly used Moos' 47-item Menstrual distress Questionnaire (MDQ – 1968 and 1969) (MMDQ)[1087] containing 8 clusters, and Halbreich's 95-item (18 clusters) Premenstrual Assessment Form (PAF)[1088] were considered too extensive for the current purposes. A broad range of scales for measuring PMS has been employed, as confirmed by a 1994 review that identified 65 different PMS scales used in clinical trials for assessing entry eligibility and treatment outcomes. Of these, 18 grouped the symptoms or items into clusters or subgroups.[1089]

For the purposes of the current study, a scale containing symptom sub-clusters was sought, which also had the advantages of brevity and, consequently, acceptance by participants already completing several other measures. This would permit differential analysis of symptoms potentially having different underlying aetiologies, and comparison with sub-domains of the Greene Climacteric scale. Hence, the Menstrual Symptoms Questionnaire (MSQ) developed by Abraham[463,1090] was chosen despite its current lack of use in PMS research. Abraham used the terminology 'Premenstrual Tension Syndrome' (PMT-S); his modified questionnaire contains nineteen symptoms (having removed PMS- P for pain/ dysmenorrhoea), divided into four subgroups: PMT-A (anxiety, irritability, mood swings, nervous tension), PMT-H (weight gain, swelling of extremities, breast tenderness, abdominal bloating), PMT-C (headache, craving for sweets, increased appetite, heart pounding, fatigue and dizziness or fainting) and PMT-D (depression, forgetfulness, crying, confusion, insomnia). (Table 15.4) This categorisation arose from his recognition that PMT-S may represent a group of syndromes with different pathophysiologies.[463,464]

TABLE 15.4 ABRAHAMS MENSTRUAL SYMPTOMS QUESTIONNAIRE

PMS-A	*PMS-H*	*PMS-C*	*PMS-D*
Nervous tension Irritability Mood swings Anxiety	Weight gain Swelling of extremities Breast tenderness Abdominal bloating	Headaches Cravings for sweets Increased appetite Pounding heart Fatigue Dizziness	Depression Forgetfulness Crying Confusion Insomnia

Symptoms were graded according to the following criteria:

0 = *none*

1 = *mild –present but does not interfere with activities*

2 = *moderate – present and interferes with activities but not disabling*

3 = *severe-disabling; unable to function*

TABLE 15.5 SEVERITY RATINGS OF SCORES ON ABRAHAM'S MSQ

PMT subscale / Severity	PMT-A	PMT-H	PMT-D	PMT-C
Mild	< 5	< 5	< 6	< 7
Moderate	5 – 8	5 – 8	6 – 10	7 - 12
Severe	9 – 12	9 – 12	11 – 15	13 - 18

To qualify for PMT-S, the symptoms in at least one subgroup must be mild to absent in the mid-follicular phase and moderate to severe during the late-luteal phase Table 15.5), and the follicular phase score must be greater than the follicular phase score by at least 4 points for PMT-A and PMT-H, 5 points for PMT-C and 6 points for PMT-D. In addition, symptoms reports should be collected prospectively for at least two consecutive cycles in order to confirm the diagnosis of PMS at baseline. However, as this was primarily a menopause study, rather than a PMS study, retrospective recall was used for baseline scores.

15.6.6 Dietary and Lifestyle questionnaire

Potential confounding factors were recorded on a weekly lifestyle diary and included phytoestrogen intake recorded on a food frequency questionnaire, caffeine and alcohol consumption, smoking, formal exercise in hours per week, stressful life events recorded on a 5-point Likert scale, concurrent illnesses and medication. The total lignan and isoflavone intake was calculated according to the values reported in quantification studies.[1091,1092] Adverse events were recorded and reported 4-weekly. (Refer to sample booklet in Appendix 22).

The total isoflavone content was calculated according to the values reported in an Australian study by King and Bignell, 'Concentrations of Isoflavone phytoestrogens and their glucosides in Australian soya beans and soya foods',[1092] which were in good agreement with those listed in the USDA-Iowa State University Isoflavone Database:

- Soy milk, 1 cup/225 mls = 22.5 isoflavones
- Tofu, 50gms = 11; 100 gms = 22
- Tempeh, 100 gms = 43
- Soy beans, dry 20gms or canned, 50gms = 38mg
- Miso, 1 tablespoon/23 gms = 10
- Soy bread, 1 slice/40gms = 6
- Soy grits, 1 tablespoon/11.36 gms = 18.74gms[583,1092]

The secoisolaryciresinol diglucoside (SDG) content of linseeds varies from 0.4% to 1.2%.[1091] Therefore the median, 0.08%, was used to calculate the SDG content.[1091]
The quantity of linseeds in soy and linseed bread was calculated at 2.8gms (equivalent to 0.02gms secoisolaryciresinoldiglucosides) using information from the labels of commercially-available breads, counting the slices and weighing one slice.
Linseeds per tablespoon were taken to be 12gms, calculated by weighing a rounded tablespoon, the normal dose of linseeds. (0.1gm secoisolaryciresinoldiglucosides)

15.7 Adverse Events Monitoring

Adverse events were actively sought at each contact. In addition, participants were briefed thoroughly at interview on the protocol they should follow in the event of an unwanted side effect occurring during the participation phase. They were required to report any adverse events immediately to the investigator who was accessible by telephone 24 hours a day. Professor Helena Teede, a registered medical practitioner, would then liaise with the participant and advise her to cease therapy temporarily if required and assess whether she needed to seek GP review. If a GP visit was required, Dr Teede was to contact the GP, who would complete the Suspected Adverse Events Report Form (Appendix 25) and forward this to Dr Iggy Soosay, GP in charge of monitoring adverse events, for independent safety review. If her symptoms were not thought relevant by the safety committee, the herbs would be recommenced in conjunction with her local GP, and she would be monitored. If the symptoms recurred, the medications would be ceased. In a severe case, the participant would be instructed not to restart the medication.

If the independent safety committee were concerned, Dr Soosay would have access to a copy of the code. However, the code was only to be broken in a case where breaking it would alter management. Any such serious events were to be reported immediately to RMIT HREC, the Therapeutic Goods Administration and the Adverse Drug Reactions Advisory Committee (ADRAC). The randomisation code was readily accessible should an adverse event or serious adverse event occur. A hard copy was located in Professor Marc Cohen's office, accessible by another staff member from the Complementary Medicine Department in case of necessity.

15.8 Sample size

A power calculation was performed to estimate the required sample size. Anticipating a placebo effect of 30% for flushing symptoms[74,692,1074] based on phytotherapeutic menopause RCTs, and 30% for depression,[904] it was calculated that a sample size of 102 would permit sufficient power (0.8) for the detection of moderate effects (d = 0·5) in any outcome variables at an alpha level of 0·05.

15.9 Randomisation and Blinding

The tablets were randomised by MediHerb using a computer generated random number table, and labelled with code numbers. Women who satisfied the inclusion/exclusion criteria were randomly assigned by the principal investigator using block random sampling (blocks of four) to ensure both post and perimenopausal women were evenly assigned to each group. Within each block of four were two placebo and two active treatments. Upon entry, participants were assigned code numbers sequentially within blocks. The perimenopausal group were allocated numbers starting from the lowest, and the postmenopausal group participants started with the highest numbers and were allocated to numbers in descending order. A copy of the code, concealed in sequentially numbered, opaque, sealed envelopes, was sent to RMIT and stored in a locked filing cabinet. The code was concealed from all of the investigators and was not broken until the last participant had completed the treatment phase and data had been scored and entered into the database, when MediHerb sent a copy of the randomisation plan to the investigators.

As a further check on the validity, the veracity of the code was checked at the conclusion of the trial by Dr Kerry Penman of MediHerb Australia, using TLC assessment. The report returned can be found in appendix 26.

15.9.1 Evaluation of the Success of Blinding

Evidence for the success of blinding was obtained by participants' retrospective assessment of group allocation following the treatment phase. In order to evaluate the success of blinding of the principal investigator, prior to code breaking, the principal recorded her assessment of each participant's allocation, based on their reported symptom experience throughout the trial.

15.10 Statistical Methods

All data scoring was double checked. HDI scores were double entered by the principal investigator. All other scales were checked by a third party. After being entered into SPSS, all entries were double checked in conjunction with a voluntary research assistant. Cleaning and screening of data was conducted with the complete data file as well as those subsequently derived from it. This included checking minimum and maximum scores, range of scores, checking for outliers and further investigation of outlying scores, checking the number of valid cases and the distance of trimmed means from means.

Data were analysed using Versions 11, 15 and 16 of SPSS, with the assistance of a biostatistician. Parametric tests were used as they are more commonly reported in the literature, and are more powerful than non-parametric tests, although they do require assumption checking. Assumption tests carried out were normality, homogeneity of variance, and for analyses of covariance, linearity and homogeneity of regression slopes. However, linear modelling does not require separate assumption testing as it is incorporated into the program in SPSS. For the regression analyses, the relevant assumption tests were carried out, including Mahalanbois distance for detecting outliers, multicollinearity and singularity, normality, linearity, homescedasticity and independence of residuals. Results of assumptions testing are not included in the manuscripts but are discussed in the results section following the manuscripts accepted for publication.

Intention-to-treat analysis was used in order to preserve randomisation and thereby avoid introducing serious bias, and to represent the practical impact of the treatment.

Equality at baseline was assessed using independent *t*-tests for the continuous variables and chi square tests for the categorical variables. Contingency table analysis was also used to determine if adverse events affected both groups equally.

For the primary study of the phytotherapeutic combination on menopausal symptoms as well as the substudy on PMS-like symptoms, a mixed model, treating group as the between-subject factor and phase as the within-subject factor, was performed on the main variables. The interaction between group and phase was also examined. Where groups were not equal at baseline for a potential confounder, which covaried significantly across the treatment phase, it was included in the analysis as a covariate. Post-hoc testing using pairwise comparisons of the estimated marginal means was used for within group analysis across the trial phases. Exact details differed for each study and are reported in the relevant chapter.

Although there were multiple outcome measures in these studies, multivariate analysis of variance (MANOVA) was not used for several reasons: i) the sample size is considerably reduced as it excludes all cases without a complete data set, ii) all of the underlying assumptions, except for the assumption of linearity, were violated, iii) the dubious value of interpreting the joint distribution of outcome variables, and iv) it is considered by some to be "a poor strategy for controlling experiment-wise error rates across multiple outcome variables", when used as a follow-up to univariate *F* tests. "Bonferroni-based procedures in conjunction with univariate *F* tests are..(considered).. preferable".[1093]

Bonferroni adjustments, which are considered to be quite strict, were used for the study of premenstrual-like symptoms as both herbs had previously been trialled successfully in this condition. It was therefore considered appropriate to use a more conservative threshold. However, fundamental hypothesis testing for the exploratory menopause study did not use Bonferroni corrections, in order to avoid prematurely excluding a potentially useful therapy.

Relationships between variables were also examined, using different techniques according to the study, which included simple bivariate correlations, linear regression, hierarchical regression, multiple regression and logistic regression. As the outcome measures were continuous, rather than categorical, linear regression was preferred as this gave more power. However, results of logistic regressions analyses are also included.

As reported above, the data collected was used for three main papers. Methods relating to data collection were common to all studies. Information specific to individual studies is included in the respective chapters.

Chapter Sixteen

Hypericum perforatum with *Vitex agnus-castus* in Menopausal Symptoms

16.0 Introduction

The results are divided into four chapters, each containing a manuscript that has been submitted and/or accepted for publication by a relevant journal. Additional analyses were conducted that could not be included in the manuscripts. These are included as addenda to the respective chapters.

This chapter reports on the results of the principal study, a randomised, controlled trial of a phytotherapeutic combination for the treatment of menopausal symptoms. The manuscript has been accepted for publication and is to appear in the journal *Menopause* in January 2009, PubMed citation: Van Die MD, Burger HG, Bone KM, Cohen MM, Teede HJ. *Hypericum perforatum* with *Vitex agnus-castus* in menopausal symptoms: a randomized, controlled trial. *Menopause* 2008 Sep 10. [Epub ahead of print]

16.1 Abstract

Objective: To evaluate the effectiveness of a phytotherapeutic intervention comprising a combination of *Hypericum perforatum* (St John's wort) and *Vitex agnus-castus* (Chaste tree/berry) in the management of menopausal symptoms.

Design: A double blind, randomised, placebo controlled parallel trial was performed over 16 weeks in 100 eligible late-perimenopausal or postmenopausal women experiencing hot flushes and other menopausal symptoms. Herbal combination therapy or placebo tablets were administered twice daily. The primary end-point was flushing episodes. Secondary end-points included Greene Climacteric scale scores, Hamilton Depression Inventory scores and Utian Quality of Life Scale scores.

Results: Ninety-three completed the study. Data analysis on an intention-to-treat basis found no significant differences between the two groups for any of the endpoints. Analyses performed at interim data time-points revealed no significant differences at weeks 4, 8 or 12 for daily weighted flushes, scores on the Greene Climacteric scale or Hamilton Depression Inventory. However, significant improvements across the treatment phase were observed in both the placebo and active treatment groups for these endpoints. No significant change was found for either group on Quality of Life.

Conclusion: The herbal combination of *Hypericum perforatum and Vitex agnus-castus* was not found to be superior to placebo for the treatment of menopausal symptoms. The herbal combination was well-tolerated with no significant adverse events noted in the short term. Robust findings from quality studies such as this are important for informing the community, health-care providers and regulatory authorities.

16.2 Introduction

Despite the incontrovertible efficacy of hormone therapy (HT) for menopausal symptoms, safety concerns generated by reports from large-scale trials[1094] resulted in a significant decline in HT use[562] and increased interest in alternatives. In terms of CAM, a recent study found that 54% of Australian women transitioning through menopause use some form of treatment or product for the alleviation of menopause-related symptoms.[569]

Of the phytotherapeutic remedies, the phytoestrogen-containing plants have received the most attention in the scientific arena, with relatively little research conducted on other herbs traditionally used for menopausal symptoms.[577] However, concerns have now been raised over the safety of long-term use of some phytoestrogens, specifically on breast and endometrial tissue proliferation.[578,579] The present study, therefore, took a novel approach in that it focused on two non-oestrogenic herbs commonly prescribed for menopausal symptoms.[21,820]

Phytotherapeutic menopausal formulations are often found to include the herbs *Hypericum perforatum* (St John's wort)[21] and *Vitex agnus-castus* (Chaste-tree/berry),[820] both of which have been demonstrated to act via neurotransmitters and/or opioid receptors.[831,842,896,908] Efficacy of central nervous system (CNS)-acting agents for vasomotor symptoms has been demonstrated with pharmaceuticals such as the anti-depressant selective serotonin reuptake inhibitors and the anticonvulsant gabapentin[549] that are believed to exert their effects via the complex interaction between hormones and neurotransmitters involved in the physiology of hot flushes.[198] Various CNS mechanisms for *Hypericum perforatum* and *Vitex agnus-castus* have also been proposed based on *in vitro* and *in vivo* models.[831,839,842,896,908] For *Hypericum perforatum*, these include inhibition of the uptake of the monoamine neurotransmitters, serotonin, noradrenaline and dopamine, GABA and L-glutamate; downregulation of beta-adrenergic receptors, upregulation of serotonin 5-HT(2) receptors and the regulation of genes that control hypothalamic-pituitary-adrenal axis function.[908] It also resulted in differential modulation of the binding properties of 5-HT(1A), 5-HT(2A)- and mu-opioid receptors.[896] *Vitex agnus-castus* extracts have been shown to act as agonists at the kappa- and mu-opiate receptors,[841,842] exert a dopaminergic action mediated by D2 receptor activation[831] as well as stimulate melatonin release.[839]

In addition to its well-demonstrated effectiveness for the treatment of mild to moderate depression,[865] evidence has suggested *Hypericum perforatum* improves vasomotor, psychological and psychosomatic menopausal symptoms.[49] Several randomised controlled trials (RCTs) have demonstrated its efficacy when combined with *Cimicifuga racemosa (*black cohosh) for menopausal hot flushes and associated psychological symptoms.[702,703,705,716] *Vitex agnus-castus* was one component of a phytotherapeutic menopause formulation found to be significantly superior to placebo in an RCT on menopausal hot flushes and night sweats[793] and has also shown positive effects for premenstrual syndrome.[481]

The present study investigated the efficacy of a combination of *Hypericum perforatum* and *Vitex agnus-castus* on the physiological and psychological symptoms of menopause in late- perimenopausal and postmenopausal women in a double-blind, randomised, placebo-controlled trial.

16.3 Methods

Approval for the study was obtained from the Human Research Ethics Committee at Royal Melbourne Institute of Technology-University. All participants gave written informed consent prior to entering the study. Baseline visits were completed in a clinic setting. Follow-up contacts were conducted by telephone.

Women recruited were aged between 40 and 60 years, post-menopausal (at least 12 months' amenorrhoea) or in the late peri-menopause, defined by an intermenstrual interval of at least 3 months in the previous 12 months,[65] experiencing a minimum of five flushing/sweating episodes per 24 hours and scoring 20+ on the Greene Climacteric scale, consistent with a menopause clinic sample.[1072] Hysterectomised women were admitted if they were over the age of 53 and FSH levels were greater than 25IU/L.

Women were ineligible if using formulations that included trial herbs, pharmacological agents known to interact with either herb, or concomitant therapies for menopausal or psychological symptoms (including HT and CAM within the previous 4 weeks, hormone implants or injectables within the previous year or 6 months respectively and anti-depressant medications within the previous four to five weeks.

Women with major pre-existing illness, a history of mania or substance abuse were excluded. Other exclusion criteria were medically or surgically induced menopause,

undiagnosed vaginal bleeding (post-menopausal women), pregnancy or attempting to conceive, or concurrent participation in another clinical trial.
Pre-trial symptoms were assessed at baseline using the Greene Climacteric scale, the self-rated Hamilton Depression Inventory (HDI), Utian Quality of Life questionnaire and a daily flushing diary for the two week run-in period prior to the treatment phase.

Before inclusion in the trial, medical clearance from a GP was required after a general medical check-up, including heart rate, systolic and diastolic blood pressure and breast examination. A pap smear was performed if not conducted within the previous two years. Participants were asked to maintain their baseline phytoestrogen intake and advised of the relevant foods.

Both the placebo and active treatment were administered in the form of tablets, identical in size, colour, coating, weight and packaging. All tablets were manufactured Code of Good Manufacturing Practice by MediHerb Australia Pty Ltd. Both products are included on the Australia Register of Therapeutic goods in as Listed Medicines and were administered within standard dosage levels.

The active treatment was a combination of two herbal extracts, *Hypericum perforatum L.* (Clusiaceae/Guttiferae; St John's wort) and *Vitex agnus-castus L,* (Verbenaceae; Chaste tree/berry). Each *Hypericum perforatum* tablet contained 300 mg extract equivalent to 1,800mg dry herb flowering top standardised to contain hypericins 990 mcg, 9mg hyperforin and 18 mg flavonoid glycosides. A total of three *Hypericum perforatum* (one in the morning and two later in the day). This is consistent with dosages used in depression studies. Each *Vitex agnus-castus* tablet contained extract equivalent to dry fruit 500 mg; two tablets (in the morning) or placebo were given daily for 16 weeks. The dosage regimen was determined by referring to previous RCTs on *Hypericum perforatum* in depression[915] and *Vitex agnus-castus*[839] and is consistent with current usage.[820] Placebos contained the excipients used in the active tablets (see Appendix 1 for further details). At the end of the study, all remaining tablets were returned to the study centre and manually counted.

The primary end-point was the frequency and severity of hot flushes (including night sweats). Secondary endpoints were scores on the Greene Climacteric scale, the Hamilton Depression Inventory and the Utian Quality of Life scale.

Flushes were recorded on daily symptom diaries for the 16 week treatment phase and one week during post-treatment follow-up (week 24). Participants recorded the number and severity of flushing and sweating episodes experienced each day and night, defined as 'while in bed'. These were subjectively categorised as:

Mild	flush without perspiration or clamminess
Moderate	hot flush associated with perspiration or clamminess
Severe	hot flush associated with intense perspiration that required change of clothing[304]

The Greene Climacteric scale and the Hamilton Depression inventory were completed at 4-weekly intervals throughout the treatment period and at the 8-week follow-up. The Utian Quality of Life Scale was completed at baseline and week 16.

Potential confounding factors were recorded on a weekly lifestyle diary and included phytoestrogen intake recorded on a food frequency questionnaire, caffeine and alcohol consumption, smoking, formal exercise in hours per week, stressful life events recorded on a 5-point Likert scale, concurrent illnesses and medication. Total lignan and isoflavone intakes were calculated according to the values reported in quantification studies.[1091,1092] Adverse events were recorded and reported 4-weekly.

A power calculation was performed to estimate the required sample size. Anticipating a placebo effect of 30% for flushing symptoms[74,692,849] based on phytotherapeutic menopause RCTs, and 30% for depression,[904] it was calculated that a sample size of 102 would permit sufficient power (0·8) for the detection of moderate effects (d = 0·5) in any outcome variables at an alpha level of 0·05.

The tablets were randomised by MediHerb using a computer generated random number table, and labelled with code numbers. Women who satisfied the inclusion/exclusion criteria were randomly assigned by the principal investigator using block random sampling to ensure both post and perimenopausal women were evenly assigned to each group. Upon entry, participants were assigned code numbers sequentially within blocks. The code was concealed from all of the investigators and was not broken until the last participant had completed the treatment phase and data had been scored and entered into the database. The success of blinding was evaluated by the principal investigator and participants 'guessing' their allocation retrospectively, prior to code-breaking.

Data were analysed using Statistical Package for Social Science (SPSS) Version 11 with the assistance of a biostatistician. A mixed model, treating group as the between-subject factor and phase as the within-subject factor, was performed on the main variables (flushing, Greene scores, depression and Quality of Life). The interaction between group and phase was also examined. Isoflavone intake (mg/week) was included in the model as a co-variable where relevant as the groups were not equal at baseline (Table 16.1). Unadjusted means are included in Appendix 2. Post-hoc testing using pairwise comparisons of the estimated marginal means was used for within group analysis across the trial phases. Flushing episodes were weighted 1 for mild, 2 for moderate and 3 for severe to take into account the severity as well as the frequency. The daily weighted score was calculated by dividing the weekly weighted total by 7. Missing values from the questionnaires were imputed through expectation maximisation algorithms (EM algorithms) in SPSS.

Participants were recruited from January 2004 to May 2005 through newspapers, a radio interview, websites at RMIT and the Jean Hailes Foundation for Women's Health and fliers at community clinics. Participants were followed up at the end of a two-week non-treatment run-in, at the end of weeks 1, 4, 8, 12 and 16 of the treatment phase, and the end of the 8-week post-treatment follow-up period.

16. 4 Results

The two groups were similar at baseline on all categorical variables except nulliparity. However a Pearson's correlation found no relationship between null-parity and flushing, depression or Greene scores at baseline for either the completing or enrolled participants.

The two groups were similar at baseline on all continuous variables except isoflavone intake, mean difference = 38·3 mg/week, p = 0·04, 95% CI, (1·42 to 74·97), with the placebo group having the higher intake, and standard drinks per week, mean difference = 1·92, p = 0·035, 95% CI, (-3·71 to -0·13), with the active group consuming the greater quantity.

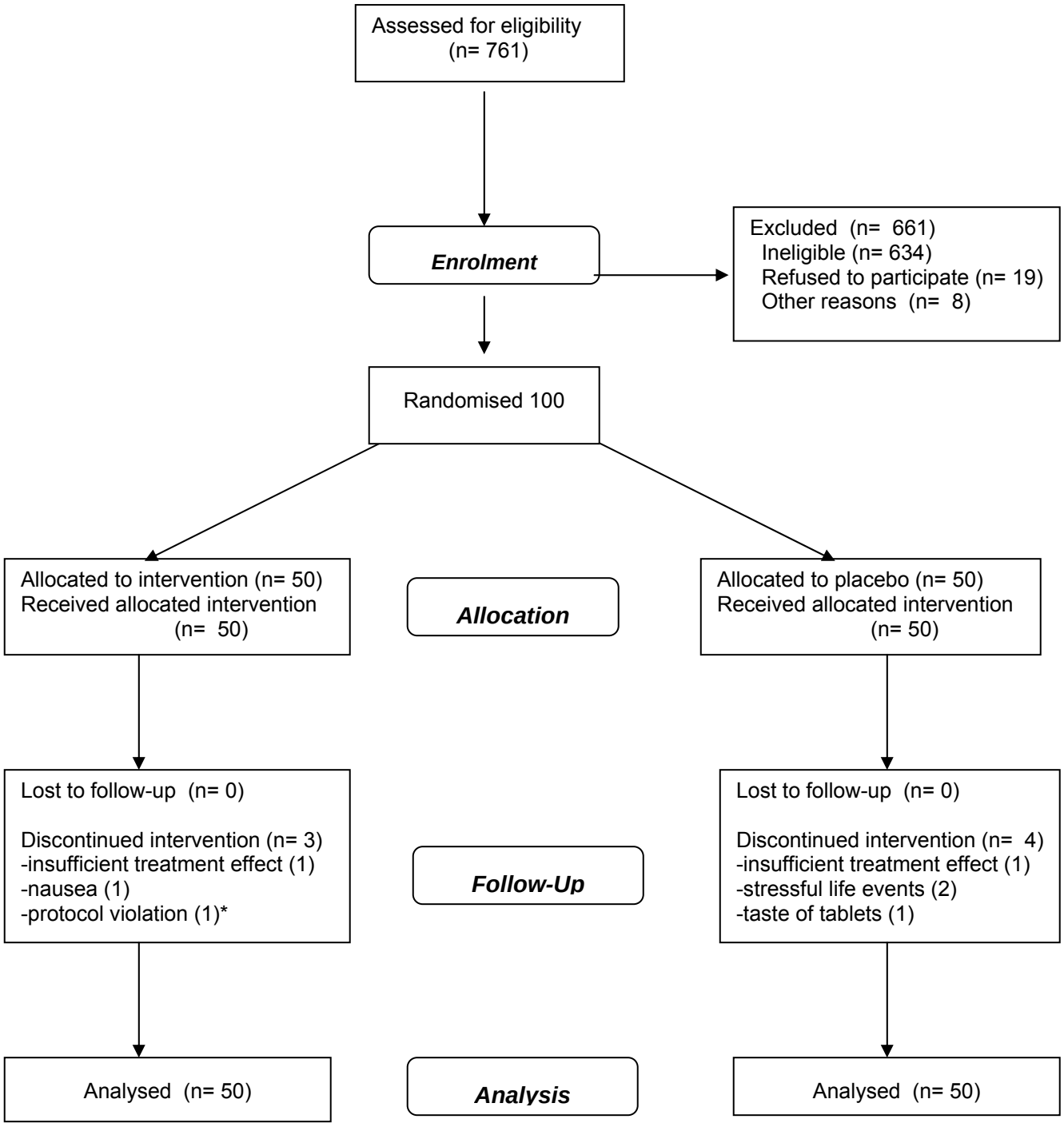
TABLE 16.1 BASELINE CHARACTERISTICS OF ENROLLED PARTICIPANTS, *N* = 100
Mean (SD)

	Placebo ***n* = 50**	***Hypericum & Vitex*** ***n* = 50**
Age at trial start (years)	52·5 (3·8)	51·9 (4·3)
Weight in kg	70·1 (13·8)	72·0 (13·1)
Height in cm	163·7 (6·1)	163·8 (6·0)
BMI	26·2 (5·0)	26·8 (4·4)
Peri-menopausal, No.	16	17
Post-menopausal, No.	24	25
Unknown (endometrial ablation), No.	10	8
Hysterectomy, No.	9	8
Two ovaries retained, No.	8	7
One ovary, No.	1	1
Months since last period	48·6 (61·9)	48·7 (67·4)
Months since onset of symptoms	57·3 (44·7)	59·2 (50·1)
Prior herbal medicine use, No. (found it effective, No.)	37 (19)	36 (16)
Prior HRT use, No. (found it effective, No.)	15 (13)	17 (12)
Vegetarian, No.	3	1
Isoflavone intake per week in mg	76·6(114·3)*	38·4(69·2)*
Lignans in linseeds, gm p/wk	0·1(0·2)	0·03(0·2)
Caffeine: cups of tea/coffee per day	4·2(2·1)	4·0(2·4)
Cigarettes per day	2·0(5·8)	1·6(4·7)
Standard alcoholic drinks per week	3·6(3·7)*	5·6(5·2)*
Satisfaction with relationships, No.	44	44
No partner, No.	11	13
Neg. attitude to menopause, No.	27	26
High stressful life events (more than average), No.	27	23
Regular stress reduction, No.	14	14
Exercise – hours per week	3·7(3·7)	3·2(2·6)
Total exercise days per week	3·9(2·6)	3·8(2·3)
Nulliparity, No.	13†	5†
Employed outside home, No.	40	43
Endpoints Unadjusted Mean (SD)		
Flushing episodes (per day)	9·42(3·31)	9·65(4·00) *n* = 49
Daily weighted score	16·35(6·69)	16·53(9 ·02)
Greene score	22·48(8·7)	22·00(6·47)
HDI-17 score	14·30(4·25)	14·76(4·42)
Utian score	77·8(12·06)	79·04(10·31)

* significant difference $p < .05$
† significant difference $p < .05$ using chi-square χ^2

Data were analysed from one hundred participants, fifty in each group, on an intention-to-treat basis. Of the 100 participants who were randomised, 47 in the active group and 46 in the placebo group completed the study (Figure 16.1).

FIGURE 16.1 PARTICIPANT FLOW



There was no significant difference between the two groups for any of the endpoints at week 16, nor at any time point measured: daily weighted flushing scores, (1·54; 95% CI, -2·23 to 5·31), *p* = 0·42; Greene Climacteric scale, (2·39; 95% CI, -0·68 to 5·45, *p* = 0·13); [Psychological subdomain 1·76, 95% CI, -0·29 to 3·81, *p* = 0·09; anxiety 0·89, 95% CI,-0·25 to 2·03, *p* = 0·13; depression 0·87; 95% CI, -0·19 to 1·93, *p* = 0·11; somatic 0·30, 95% CI, -0·70 to 1·30, *p* = 0·55; vasomotor 0·24, CI's -0·36 to 0·85, *p* = 0·43; sexual 0·34, 95% CI, -0·07 to 0·75, *p* = 0·11]; Hamilton Depression Inventory-17, (0·89; 95% CI, -1·27 to 3·04, *p* = 0·42); Utian Quality of Life scale, (3·94; 95% CI, -1·45 to 9·32, *p* = 0·15). Sub-group analysis of the clinically depressed group (HDI-17) did not change the finding.

However, significant improvements were observed for both groups for flushing, Greene Climacteric scores and depression (HDI-17) at week 16 (figure 2). The reduction in flushing scores was significant for the placebo group at $p < 0{\cdot}001$ and $p < 0{\cdot}01$ for the active group. Improvement of 50% or more in mean daily weighted flushing scores were observed in 45·6% of placebo participants and 43·5% of the active treatment group. Improvements for both groups were significant at $p < 0{\cdot}001$ on the Hamilton-Depression-Inventory and Greene scale for overall scores and all subdomains, except for the sexual domain which was not significant for the active group.

16.4.1 Ancillary analyses

Prior use of phytotherapies modified the effect, with the herb-naïve group showing a significantly greater reduction in flushing scores, -57·67% (95% CI, 40·49 to 74·86) than the prior users, -32·48% (95% CI, 19·58 to 45·39), β= -0·215, *p* = 0·041. Previous positive experience with phytotherapies predicted overall percentage improvement in depression (HDI-17) [β= -0·28, *p* = 0·007], anxiety-subscale scores [β= -0·23, *p* = 0·03] and sleep [Greene item 3; β= -0·26, *p* = 0·02] but did not differ between the active and placebo groups.

FIGURE 16.2 EFFECT OVER TIME OF INTERVENTION ON PRIMARY OUTCOME MEASURES

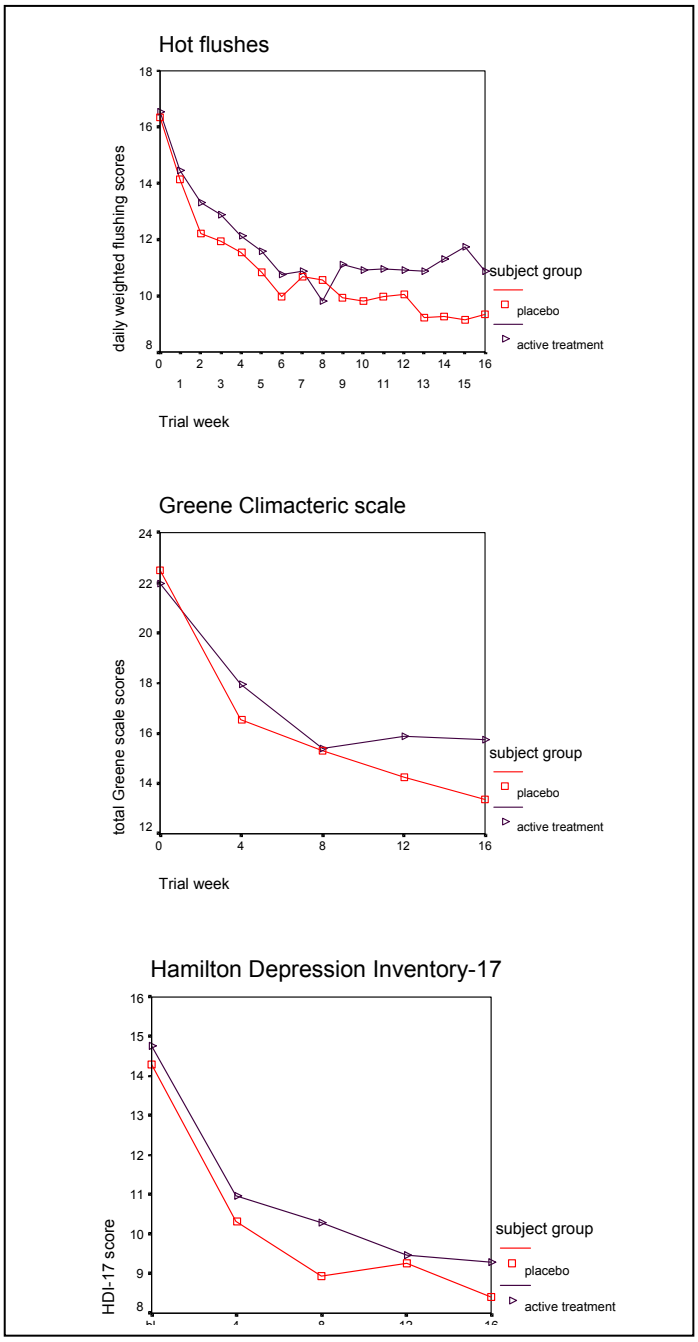


TABLE 16.2 EFFECT OF INTERVENTION ON ENDPOINTS MEAN (SE)

	Placebo			Herbal Combination			Difference between groups at week 16
	Baseline	After 16 weeks	Mean change	Baseline	After 16 weeks	Mean change	
	n = 50	*n* = 46		*n* = 50	*n* = 47		*p*-value
Flushes, daily weighted	16·35(1·30)	9·32 (1·36)	7·03(1·89)	16·53(1·32)[1]	10·86(1·34)	5·67(1·89)	0·42
95% CI	13·79,18·92	6·64,12·00	3·32,10·75	13·93,19·12	8·21,13·51	1·96,9·38	
Greene total score[2]	22·52(1·06)	13·35(1·11)	9·17(1·53)	21·98(1·06)	15·73(1·10)	6·25(1·52)	0·13
95% CI	20·43,24·61	11·17,15·52	6·16,12·19	19·89,24·06	13·58,17·89	3·25,9·24	
Greene Subscales:							
Psychological[2]	11·48(0·71)	6·73(0·74)	4·75(1·02)	11·73(0·71)	8·49(0·73)	3·24(1·02)	0·09
95% CI	10·08·12·88	5·28,8·19	2·74,6·76	10·34,13·13	7·06,9·94	1·29,5·24	
Anxiety[2]	6·36(0·41)	3·71(0·41)	2·65(0·57)	6·33(0·39)	4·60(0·41)	1·73(0·57)	0·13
95% CI	5·59,7·14	2·90,4·52	1·53,3·77	5·56,7·11	3·80,5·40	0·62,2·85	
Depression	5·12(0·37)	3·02(0·39)	2·10(0·53)	5·40(0·37)	3·89(0·38)	1·51(0·52)	0·11
95% CI	4·40,5·84	2·27,3·78	1·05,3·14	4·68,6·12	3·15,4·64	0·47,2·55	
Somatic	4·94(0·35)	2·83(0·36)	2·11(0·50)	4·64 (0·35)	3·13(0·36)	1·51(0·50)	0·55
95% CI	4·26,5·62	2·12,3·54	1·13,3·10	3·96,5·32	2·43,3·83	0·53,2·49	
Vasomotor	4·28(0·21)	2·59 (0·22)	1·69(0·30)	3·92(0·21)	2·83 (0·22)	1·09(0·30)	0·43
95% CI	3·87,4·69	2·16,3·02	1·10,2·29	3·51,4·33	2·41,3·25	0·50,1·68	
Sexual	1·72 (0·14)	1·15 (0·15)	0·57(0·21)	1·74 (0·14)	1·49 (0·15)	0·25(0·21)	0·11
95% CI	1·44,2·00	0·86,1·45	0·16,0·97	1·46,2·02	1·20,1·78	-0·15,0·65	
Sleep	1·80(0·13)	1·26(0·13)	0·54(0·18)	1·85(0·13)	1·31(0·13)	0·54(0·18)	0·59
95% CI	1·55,2·05	1·00,1·52	0·18,0·90	1·65,2·15	1·11,1·62	0·18,0·90	
Hamilton Depression	14·30(0·75)	8·40(0·78)	5·90(1·08)	14·76(0·75)	9·29(0·77)	5·47(1·07)	0·42
95% CI	12·83,15·77	6·87,9·93	3·78,8·02	13·29,16·23	7·78,10·80	3·37,7·58	
Utian Quality of Life	77·80(1·85)	77·22(1·93)	-0·58(2·67)	79·04(1·85)	81·15(1·93)	2·11(2·67)	0·15
95% CI	74·15,81·45	73·41,81·02	-5·86,4·69	75·39,82·69	77·35,84·96	-3·16,7·38	

[1] *n* = 49

[2] Covariates appeared in the model: isoflavone intake

Overall compliance with taking the tablets was excellent with a mean of 95% of all tablets taken for both groups. Of the 93/100 completing the trial, only one participant consumed less than 85% of the medications provided (77% medication taken by one placebo group participant).

The main adverse events during the trial were upper respiratory tract infections, not thought to be related to the intervention. There was no significant difference between the two groups for adverse events, chi square χ^2 (1, *N* = 100) = 1·100, *p* = 0·58.

16.5 Discussion

No significant difference was found between the herbal combination and placebo in reducing hot flushes, menopausal symptoms or depression. This trial reported a high placebo response, which was especially marked for the clinically depressed sub-group. Prior phytotherapy use was associated with a significantly *reduced* improvement in flushing compared with the phytotherapy-naïve group. Previous positive experience with phytotherapy predicted overall percentage improvement in depression (HDI-17 scores), anxiety and sleep (Greene Climacteric) but there was no significant difference between groups for this effect.

The strengths of this study include the robust study design; adequate power and excellent retention and compliance. It included a homogenous group of participants with moderately severe overall symptoms. Factors incorporated to control the Hawthorne effect and placebo response include the non-treatment run-in period, trial duration and a single contact-investigator. The intervention trialled was produced with high quality assurance levels, was well-tolerated and there was an absence of noteworthy adverse-events. Potential limitations of the trial include the lack of severity of depression and other psychological symptoms at baseline. Another is the inability to separate the effects of each individual herb in the combination. A criticism made of RCTs on fixed formulations in herbal research is that this protocol does not reflect the clinical practice of individualised prescriptions.

There have been no previous published studies of this combination of herbs, nor any randomised placebo-controlled trials (RCT's) of either herb individually in menopause. A 1999 observational study[49] on *Hypericum perforatum* in 111 women over 12 weeks found a 63% reduction in overall menopause symptoms compared with 32% in the present study. The study is not directly comparable with the present study, a robustly designed double-blind, placebo-controlled RCT. Also there was lack of consistency in nomenclature used for describing the different phases of the menopause transition and the menopausal rating scales, extracts used and the administration of a combination in the current study, rather than a single herb; the *Hypericum perforatum* extract used was the phytoequivalent of LI 160 trialled in depression studies.

The high placebo response in the current study was consistent with findings from other RCTs of hot flushes or depression.[870,1095] Our finding that women with no prior use of

phytotherapy were more likely to respond to active treatment or placebo has previously been reported in another study of herbal medicine and menopause.[849] The effect of previous positive experience with phytotherapy in predicting improvements in depression, anxiety and sleep is a novel finding.

The lack of efficacy of these non-oestrogenic herbs in comparison to the prescription non-oestrogenic *drugs*, suggests that the herbs may differ in terms of potency or mechanisms of action, since their pharmacology has not yet been fully elucidated. An antagonistic interaction between the two herbs is unlikely based on the current knowledge of their pharmacology. The failure of this study to demonstrate superiority over placebo for depression with *Hypericum perforatum* in combination with *Vitex-agnus-castus* may be due to inadequate symptom severity at baseline, with only 37 women in the clinically depressed subgroup. However, there is an important distinction between depressive syndromes detected by instruments such as the HDI, and depressive symptoms, with the latter considered to be more relevant in association with menopause.[354] The other negative findings in the current study are unlikely to be attributable to inadequate symptom severity as baseline hot flushes were as severe as baseline scores from previous positive studies,[23,28] and overall menopausal symptoms measured by the Greene climacteric scale were consistent with a menopause clinic sample.[1072]

The effect of previous positive experience with phytotherapy in predicting improvements in depression, anxiety and sleep suggests that these parameters may be more susceptible to the placebo effect, and supports the contribution of expectation to the placebo response.[1033] Much has been written on ways of controlling the placebo response in clinical trials, as well as of harnessing the true placebo effect in clinical practice to enhance treatment outcomes. Other factors potentially contributing to the placebo effect in this trial include the natural history of the symptoms, the therapeutic alliance, and experimenter as well as participant expectations of *Hypericum perforatum*, given its reputation in treating mild-moderate depression. The findings of effect modification of lack of previous experience and positive previous experience with phytotherapy for some parameters warrant further investigation. These could have implications for future research and should potentially be considered when analysing results of studies.

While an adverse interaction between the two herbs in the combination seems unlikely, any effect of the individual herbs cannot be established from this trial. Due to synergistic

effects, it is not possible to extrapolate from these findings to the effects of formulations combining a greater number of herbs, as is common in clinical practice. A recently trialled example is the formulation of six herbs including *Vitex agnus-castus* and *Cimicifuga racemosa* (Black cohosh) that showed a positive effect over placebo for menopausal symptoms.[793] Trialling phytotherapies in a way that is both scientifically rigorous and reflects clinical practice is one of the biggest challenges facing evidence-based CAM. A protocol using three arms has been designed and trialled to address this limitation and to reflect the practice of individualised prescribing.[1096]

This is the first RCT of the combination of *Hypericum perforatum* and *Vitex agnus-castus* for menopausal symptoms. The combination was well-tolerated and had a significant effect, although not superior to placebo. This was a robust study that further contributes to the growing body of scientific knowledge about complementary therapies from RCTs. Findings from such quality studies are important to inform the community, health-care providers and regulatory authorities on the role of CAM.

16.6 Manuscript Appendix 1

16.6.1 Herbal Medicine Intervention

(according to Proposed Elaboration of CONSORT Checklist Item 4)

The herbal medicine intervention was a combination of two herbal extracts, *Hypericum perforatum L*. (Clusiaceae/Guttiferae; St John's wort) and *Vitex agnus-castus L,* (Verbenaceae; Chaste tree/berry) manufactured under the Code of Good Manufacturing Practice by MediHerb Australia Pty Ltd in their Warwick manufacturing facility. These products are included on the Australian Register of Therapeutic goods in as Listed Medicines.

The extract was obtained from the dry herb flowering top of *Hypericum perforatum* (extraction ratio 6:1(g/ml)) and dried *Vitex agnus-castus* fruit (extraction ratio 1:2(g/ml); the extraction solvent was 60% Ethanol/water. The extract was purchased and identified and analysed by MediHerb Quality Assurance Laboratory. Retention samples of raw materials and finished tablets are kept at MediHerb; these were validated by chemical fingerprinting against verified botanical samples maintained at the Southern Cross University Herbarium. The St John's Wort Tablets were Batch 125178 and Chaste Tree Tablets Batch 124324. The *V. agnus-castus* tablet was not a standardised preparation. The dosage regimen for *H. perforatum* was consistent with a previous study using a daily dose of 900mg of 6:1 extract.[915]

16.6.2 Qualitative testing

The high-performance liquid chromatography (HPLC) chromatogram for *Hypericum perforatum* can be seen in figure 15.1. The method for performing this analysis was as follows: HPLC separation was achieved using a C18, 5μm column with dimensions (150 mm x 4.6mm) with a gradient elution program using a three solvent program B=methanol, C = 50mM phosphoric acid in deionised water and D = acetonitrile. The elution program was 0 min B=0%, C=85%, D=15%; 20 min B=10%, C=70%, D=20%; 30 min B=15%, C=10%, D=75%; 55 min B=15%, C=5%, D=80% at a flow rate of 1.0mL/min. The analysis was done by an individual with over 20 year's experience in analytical chemistry. Thin Layer Chromatography was employed for *Vitex agnus-castus*. Concentrations of heavy metals were measured by ICP/MS.

16.6.3 Standardisation

Hypericin was tested utilising the British Pharmacopeia UV method. The levels of flavonoid glycosides were determined using a proprietary MediHerb HPLC analytical method, conditions above.

The placebo tablets contained the excipients used in the active tablets, namely modified starch, cellulose, magnesium stearate, calcium hydrogen-phosphate. They were identical to the herbal tablets in size, colour, coating, weight and packaging. All tablets were packaged in amber glass jars with Tamper Tel plastic lids.

The clinician choosing the treatment and dosage had 8 years experience as a chemist and 22 years as a medical herbalist.

As a further check on the validity, the veracity of the code was checked at the conclusion of the trial by Dr Kerry Penman of MediHerb Australia, using TLC assessment. (Report available on request).

16.7 Manuscript Appendix 2

TABLE 16.3 UNADJUSTED SCORES FOR GREENE CLIMACTIC SCALE AND SUBSCALES Mean (SE)

	Placebo			Herbal Combination			Difference between groups at week 16
	Baseline	After 16 weeks	Mean change	Baseline	After 16 weeks	Mean change	
	n = 50	*n* = 46		*n* = 50	*n* = 47		
Greene total score	22·48(1·11)	13·33(1·11)	9·15	22·00(1·06)	15·77(1·09)	6·23	0·12
95% CI	20·40,24·56	11·16,15·50	6·15,12·16	19·92,24·08	13·62,17·91	3·24,9·23	
Greene Subscales:							
Psychological	11·54(0·71)	6·76(0·74)	4·78	11·70(0·71)	8·45(0·73)	3·25	0·11
95% CI	10·15·12·93	5·31,8·21	2·77,6·79	10·31,13·09	7·01,9·88	1·25,5·25	
Anxiety	6·42(0·40)	3·74(0·41)	2·68	6·30(0·40)	4·55(0·41)	1·75	0·16
95% CI	5·64,7·20	2·93,4·55	1·56,3·80	5·52,7·07	3·75,5·35	0·63,2·86	

16.8 Additional Statistical Analyses

Additional analyses performed that could not be included in the preceding manuscript are presented here.

16.8.1 On-treatment analysis

In the manuscript, results are reported of the intention-to-treat analysis**.** On-treatment analysis was also performed for the *completing* participants, but results did not differ from the intention-to-treat analysis.

16.8.1 Data screening

The 5% Trimmed Means and the original means were close for all endpoints at baseline for both the enrolled and completed participants. No outliers in either sample were found to have scores more than five standard deviations from the mean for any continuous variable at baseline, so the whole sample was included in each analysis.

6.8.2 Groups equal at baseline

The two groups were similar at baseline on all categorical variables except null parity for both the enrolled and completed participants. Additionally, three of the four vegetarians were in the placebo group.

However, Pearson's correlations found no relationship between null-parity and the endpoints of flushing, depression or Greene Climacteric scores at baseline for either the completing or enrolled participants. [Null parity and flushing for the completing participants: $r(N=92) = 0{\cdot}06$, $p = 0{\cdot}57$; and the enrolled participants, $r(N=99) = 0{\cdot}07$, $p = 0{\cdot}47$. Nulliparity and depression scores on the HDI-17: $r(N=93) = -0{\cdot}02$, $p = 0{\cdot}86$; $r(N=100) = 0{\cdot}01$, $p = 0{\cdot}93$; nulliparity and Greene Climacteric scale at baseline: $r(N=93) = 0{\cdot}05$, $p = 0{\cdot}62$; $r(N=100) = 0{\cdot}09$, $p = 0{\cdot}37$ Table 16.4.]

For the distribution of the vegetarians, a contingency table analysis found no significant difference between groups χ^2 (1, N = 100) = 1·04, p = 0·31, but due to the small numbers in each cell, this needs to be interpreted with caution. However, the relevance of vegetarian diet is the increased phytoestrogen content it contains; this was measured separately (Table 16.4).

TABLE 16.4 RESULTS OF CHI SQUARE TESTS FOR CATEGORICAL VARIABLES FOR COMPLETING (N = 93) AND ENROLLED (N = 100) PARTICIPANTS

Variable	χ^2 *value*	*p-value*	χ^2 *value*	*p-value*
	n = 93		*n* = 100	
menopausal status	0·53	0·77	0·27	0·87
marital status	1·40	0·24	0·22	0·64
hysterectomy	0·11	0·74	0·07	0·79
number of ovaries intact	0·00	0·99	0·00	1·00
prior herb use (*n* = 92/99)	0·10	0·75	0·00	0·95
herbs effective (*n =68/* 73)	0·18	0·67	0·31	0·58
prior HT use	0·36	0·55	0·18	0·67
HT effective (*n* = 29/32)	4·10	0·25	4·43	0·22
stress reduction	0·02	0·88	0·00	1·00
vegetarian	3·17	0·08*	1·04	0·31*
high/low isoflavone intake	0·39	0·53	0·71	0·40
attitude to menopause	0·27	0·60	0·04	0·84
relationship satisfaction	0·67	0·41	0·44	0·51
season entered trial	1·10	0·58	1·22	0·54
employed outside home	1·31	0·25	0·64	0·42
nulliparity	6·67	0·01*	4·34	0·04

Baseline categorical variables chi square and significance values *n* = 92 and *n* = 100
* At least one cell contained <5, therefore interpret with caution.

Marital status, isoflavone intake and attitude to menopause were recoded as not all cells had originally contained adequate numbers for chi square test to be reliable. Isoflavone intake was recoded as' high/low' where 'high' was 20mg+ per day, according to the findings of Guthrie and colleagues that the average isoflavone intake among Australian women was 17mg per day.[1097]

16.8.4 Assumption testing

In the manuscript, results of an intention- to- treat analysis (n = 100) were reported. Prior to this analysis, assumption testing was conducted:

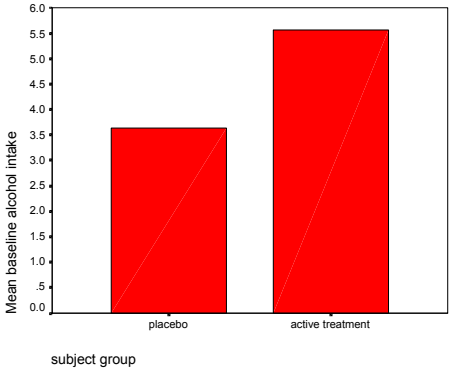
Normality testing

For enrolled participants in the active treatment group, the Shapiro-Wilk normality test showed that the assumption of normality had been violated in relation to average daily weighted flushes at baseline, $p < 0{\cdot}001$, total Greene score, $p = 0{\cdot}003$, and scores on the Hamilton Depression-17-item scale, $p = 0{\cdot}02$. For the placebo group, normality was violated for total Greene score, $p = 0{\cdot}002$. However due to the large sample size, visual inspection was considered more reliable. The normal Q-Q plots did not show significant deviation from the linear line for any of these variables.

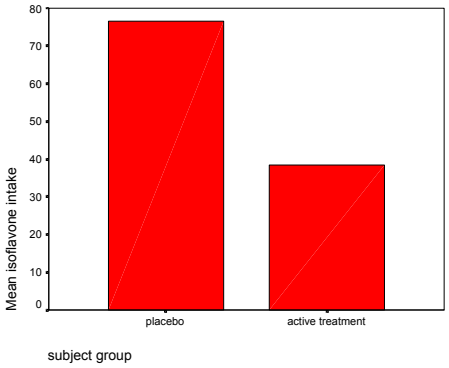
Homogeneity of Variance

For enrolled participants, the Levene test found that the assumption of homogeneity of variance was violated for isoflavone intake, p =0.02 and secoisolariciresinol diglycoside intake, p = 0.000, therefore two-tailed independent samples t-tests based on unequal variances were carried out. No significant difference between the two groups was found for secoisolariciresinol diglycoside intake, $p = 0{\cdot}05$, but a significant difference was found for isoflavone intake between the two groups at baseline $t(98) = 2{\cdot}07$, $p = 0{\cdot}035$, 95% CI (1·42 to 74·97), with the placebo group having the higher intake (Figure 16.3).

For all other continuous variables at baseline, the Levene test found that the assumption of homogeneity of variance was met, therefore two-tailed independent samples t-tests based on equal variances were carried out. No significant difference between the two groups was found for any variable except standard drinks per week $t(98) = -2{\cdot}73$, $p = 0{\cdot}035$, 95% CI (-3·71 to -0·13), with the treatment consuming the greater quantity (Figure 16.3).



Baseline standard drinks per week by group
n = 100

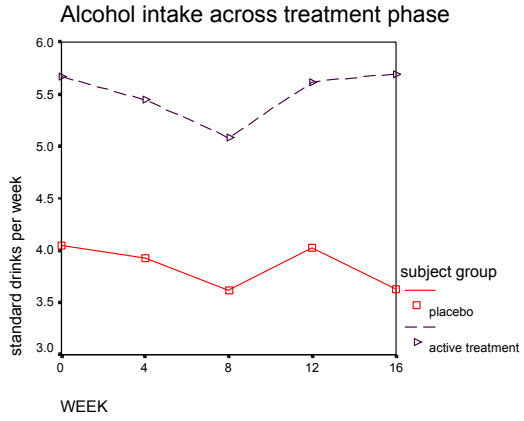


Baseline isoflavone intake by group
n = 100

FIGURE 16.3 BASELINE ALCOHOL AND ISOFLAVONE INTAKE BY GROUP

16.8.5 Time-dependent covariates

Alcohol was found to vary significantly between the two groups across time but did not covary significantly with any of the endpoints across the treatment phase (Figure 16.4). Isoflavone intake covaried significantly with total Greene Climacteric Scale scores p = 0·047, Greene psychological, p = 0·02, and Greene anxiety, p = 0·02, subscale scores, and was therefore used as a covariate in these cases, due to the baseline differences between groups. The assumption of homogeneity of slopes was met for isoflavone intake $F(1, 89) = 0{\cdot}57$, $p = 1{\cdot}04$, $\eta^2 = 0{\cdot}01$.



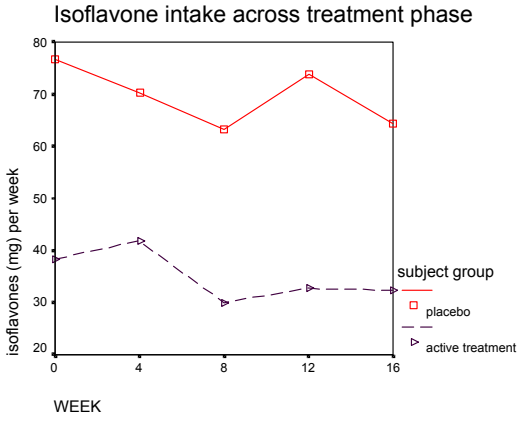


FIGURE 16.4 TIME-DEPENDENT CO-VARIATES

16.9 Additional Results

16.9.1 Effect sizes

Cohen's *d* effect sizes were calculated for the active treatment group using the means and standard deviations for baseline and week 16 scores.

Flushing, Cohen's *d* = 0.54 (medium effect size)

Greene total score, Cohen's *d* = 0.82 (large effect size)

HDI-17 scores, Cohen's *d* = 1.01 (large effect size)

For the subscales of the Greene Climacteric scale, large effect sizes were found for all subscales except the sexual subscale.

Greene psychological subscale, Cohen's *d* = 1.20

Greene anxiety subscale, Cohen's *d* = 1.01

Greene depression subscale, Cohen's *d* = 1.13

Greene somatic subscale, Cohen's *d* = 1.12

Greene vasomotor subscale, Cohen's *d* = 1.24

Greene sexual subscale, Cohen's *d* = 0.49 (medium)

16.9.2 Additional Analyses Daily weighted Flushing score

Individual weekly flushing scores are included in appendix 27.

No significant between-group difference at any time point measured

As reported in the manuscript, the difference between groups in mean daily weighted flushing scores (MDWFS) was not significant at week 16. Nor were significant between-group differences found on MDWFS at week 4 (F = 0·09, p = 0·77, η^2 = 0·00), week 8 (F = 0·11, p = 0·74, η^2 = 0·00) or week 12 (F = 0·21, p =·65, η^2 = 0·001).

Improvements across treatment phase in each group for Vasomotor Symptoms

However, significant improvements were observed for both groups from baseline to week 16 (placebo p < 0·001 and active p < 0·01). The percentage reductions in the placebo and active groups were 41% and 36% respectively. Significant improvements in both groups were also seen at weeks 8 and 12. (For the active treatment group, p = 0·001 at week 8; and at week 12, p = 0·003.) The sharpest decline, of 22%, occurred between weeks 0 and 2 (26% placebo; 19% active).

16.9.3 Additional Analyses of Greene Climacteric Scale scores

Percentage improvement on Green Climacteric scores within groups

Overall menopausal symptoms, as measured by the Greene Climacteric scale, reduced significantly in the placebo and active groups by -40% and -29% respectively. A table of scores on the Greene Climacteric subscales, including follow-up scores, is included in Appendix 28.

Significant improvement during non-treatment run-in

For the total Greene Climacteric scale scores, a significant difference between screening and baseline (after the 2-week run-in period) was found using two-tailed paired samples *t*-tests for the placebo group, $t(50) = 7{\cdot}78$, $p = 0{\cdot}001$, and for the treatment group, $t(49) = 3{\cdot}62$, $p < 0{\cdot}001$. This constituted an overall 17·6% reduction in scores: 15% for the placebo group (mean difference 4·06) and 20% for the active treatment (mean difference 5·44).

16.9.4 Additional Analyses of Hamilton Depression Inventory scores

Percentage improvement on depression for whole group Vs clinically depressed subgroup

The difference between the two groups was not significant at week 16, $p = 0{\cdot}51$. A significant reduction in scores was seen for both groups on the HDI-17, - 41% for placebo compared with -39% in the active group. Analysis of the clinically depressed subgroup did not affect the outcome, (mean change in scores was - 46% for placebo (n = 19) and -42% for active (n = 18), $p = 0{\cdot}54$ [placebo -9·2; 95% CI, 4·3 to 14·2; active -7·8; 95% CI, 2·7 to 12·9].

Percentage improvement on depression among responders

Depression scores decreased for 82% of the sample. Among these responders, there was a mean improvement of 49% for placebo and 52% in the active group.

Responder Rates for Depression

The percentage of participants experiencing a 50% or greater reduction in HDI-17 scores across the treatment phase was 32·6% of placebo and 44·7% of active treatment group for the whole sample. For those who were sub-clinically or clinically depressed at baseline (n = 81), 32·5% responded by 50% or more to placebo and 44% to active

treatment. However a chi square test found that the difference between groups was not statistically significant, χ^2 = 1·43, p = 0·23.

Significant treatment response among herb-naive group

Among the herb-naive group, a reduction in depression scores of 50% or more was observed for a significantly greater percentage of the active treatment group (67%), than the placebo group (25%), using a chi-square test, χ^2 = 4·20, p = 0·04. In the active treatment group, (n = 12), a significant improvement was observed, -51·2%, p = 0·007, while no significant reduction was seen in the placebo group, (n = 12), -31·3%, p = 0·20. It will be noted, however, that the sample size was very small.

16.9.4 Follow-up data

The effects persisted in the active treatment group but not in the placebo group (Table 16.5 and Figure 16.5). However, the difference from week 16 to the end of the non-treatment follow-up 8 weeks later was not significant for any of the endpoints using mixed model anlysis.

TABLE 16.5 FOLLOW-UP DATA COMPARED WITH BASELINE AND END OF TREATMENT PHASE

		Baseline	*Week 16*	*Follow- up*
Hot Flushes				
placebo	Mean(SE)	16·35(1·30)	9·32(1·36)‡	11·74(1·40)*
	95% CIs	13·79,18·92	6·64,12·00	9·00, 14·48
active	Mean(SE)	16·53(1·32)	10·86 (1·34)†	10·94(1·40)†
	95% CIs	13·93,19·12	8·21,13·51	8·19, 13·68
Greene Total				
placebo	Mean(SE)	22·48(1·11)	13·33(1·11)‡	15·75(1·13)‡
	95% CIs	20·40,24·56	11·16,15·50	13·53,17·96
active	Mean(SE)	22·00(1·06)	15·77(1·09)‡	16·12(1·13)‡
	95% CIs	19·92,24·08	13·62,17·91	13·91,18·33
HDI-17				
placebo	Mean(SE)	14·30(0·75)	8·40(0·79)‡	9·34(0·78)‡
	95% CIs	12·83,15·77	6·85, 9·95	7·81, 10·88
active	Mean(SE)	14·76(0·75)	9·29(0·77)‡	9·56(0·78)‡
	95% CIs	13·29,16·23	7·78,10·80	8·03, 11·10

* difference from baseline significant at $p < 0.05$
† difference from baseline significant at $p < 0.01$
‡ difference from baseline significant at $p < 0.001$

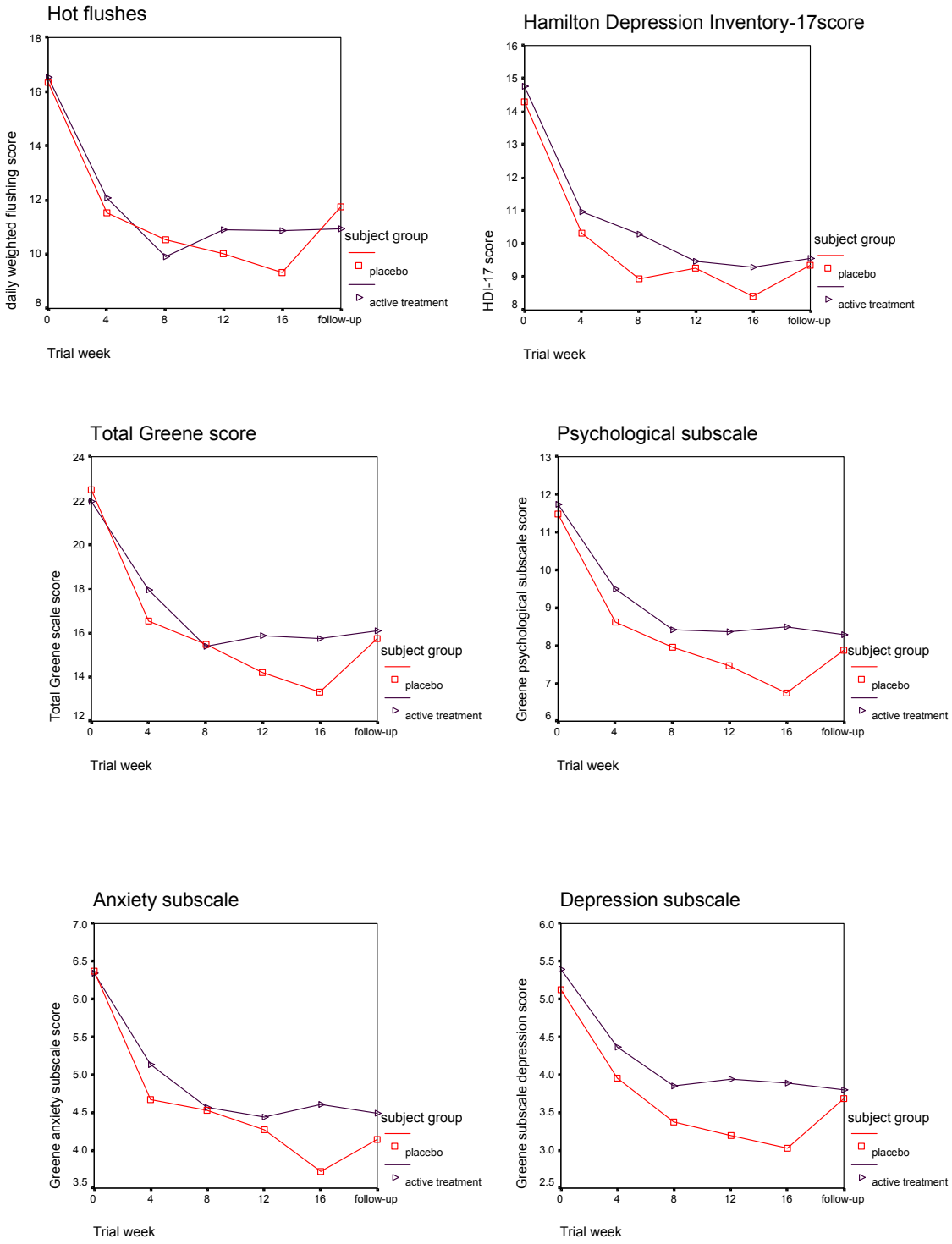


FIGURE 16.5 EFFECTS OF INTERVENTION ON ENDPOINTS, INCLUDING 8-WEEK POST-TREATMENT FOLLOW-UP

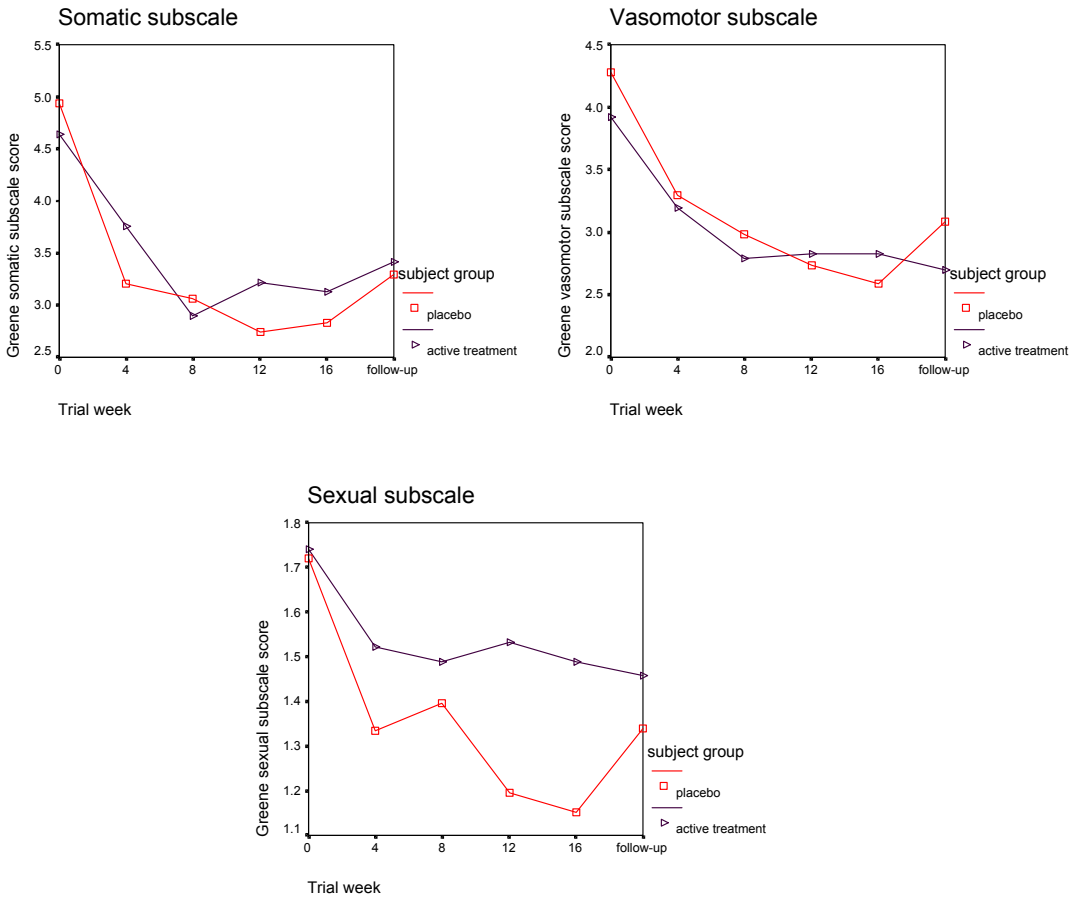


FIGURE 16.5 EFFECTS OF INTERVENTION ON ENDPOINTS, INCLUDING 8-WEEK POST-TREATMENT FOLLOW-UP CONT

16.10 Ancillary analyses

16.10.1 Menopausal status

There was no significant difference between groups according to menopausal status on any of the outcome measures. However due to the small sample size (*n* = 50) in each of the peri- and postmenopausal subgroups, these sub-population analyses were underpowered.

16.10.2 Bias

For the primary endpoints, positive previous experience with phytotherapy did not result in a significant difference between active and placebo groups according to a repeated measures analysis of the two sub-groups: previous positive experience with herbs versus none (using alcohol as a covariate).

16.10.3 Adverse Events

One participant withdrew due to an aggravation of depressive symptoms. She was referred to the safety monitor, but did not attend. No other adverse events occurred that warranted the intervention of the safety monitor. There was no significant difference between the two groups for adverse events, χ^2 (1, N = 100) = 1·10, p = 0·58. The main ones were colds and 'flu's/upper respiratory tract infections. A complete itemised list of adverse events can be found in appendix 29.

16.10.4 Allocation concealment

Unblinding did not occur. Fifty one percent of participants retrospectively guessed their allocation correctly, 50% of the placebo group and 51% of the active treatment group. (Table 16.6).

TABLE 16.6 PROSPECTIVE ASSESSMENT OF GROUP ASSIGNMENT BT PARTICIPANTS

Assessment of Allocation

Count

		active or placebo		
		placebo	active	Total
subject group	placebo	23	23	46
	active treatment	23	24	47
Total		46	47	93

In her estimations made prior to code breaking, the principal investigator was 45% correct for the placebo group and 46% for the active treatment group.

16.10.5 Accuracy of Data scoring and Data Entry

All questionnaires were double-scored; the error rate detected on checking was 0·19%. Data entry was also double-checked and the error rate found to be 0·12%.

16.11 Discussion

16.11.1 Statement of Findings

There was no difference in findings between the on-treatment analyses and intention-to-treat analyses. Analysis of data according to menopausal status showed no significant difference between groups, although the sample size in each was small. Improvements during non-treatment run-in were significant for both arms. The greatest improvement on flushing scores was observed between weeks 0 and 2 of the treatment phase. Statistically significant improvements were observed from week 8 onwards for both groups. Substantial improvements were also noted for the endpoints of overall menopausal symptoms and depression in both arms across the treatment phase. Within-group effect sizes were medium to large for these three endpoints. Although the differences were not statistically significant, higher responder rates (≥50% reduction in symptoms) were observed for the active treatment group than placebo for depression scores on the HDI-17. Among the herb-naïve group, this difference did reach statistical significance, although the sample size was very small. Any potential bias from prior positive experience with phytomedicines affected both arms equally. The follow-up data showed a washout effect for the placebo group on all the endpoints, but no washout effect was observed for the treatment group, except the somatic subscale of the Greene Climacteric Scale. However, the difference was not statistically significant at week 8 of the post-treatment follow-up. The investigator's retrospective guessing of allocation was less than 50% accurate.

16.11.12 Interpretation

As mentioned previously, the placebo response in menopause studies is largely attributable to the natural history of the symptoms, which follow a fluctuating course. Although no significant difference was found between the two subgroups according to menopausal status, the small sample size in each subgroup means that the possibility cannot be ruled out that including the perimenopausal women in the sample may have contributed to a higher placebo response. The significant improvement during non-treatment run-in may attest to impact of psychosocial factors, such as anxiety and need for support, as contributors to symptom intensity. Determination to take charge of one's own health by joining clinical research can also coincide with other concomitant lifestyle changes. The substantial initial improvement in flushing is consistent with the placebo effect. The significant improvements on the three endpoints of flushing, overall menopausal symptoms and depression, despite lack of superiority over placebo, raises

the question of what constitutes a clinically meaningful effect. This will be discussed further in chapter 19. The higher responder rates observed for depression, while not statistically significant, are consistent with previous findings on studies of St John's wort as a sole agent for depression, and suggest no negative interaction occurred between the two combined herbs in this study. The finding that this difference reached statistical significance for the herb-naïve group cannot be readily explained. It is unlikely that previous experience with phytomedicines resulted in negative expectation, as participants expecting no benefit would be unlikely to volunteer for such a study. The observation of wash-out of placebo effects, but not generally of improvements experienced in the treatment arm is interesting. While no firm conclusions can be drawn for this, it does lend support to the possibility of subtle effects of the phytomedicines being overshadowed by the substantial placebo effect in this study. Further evidence for the success of blinding is provided by the investigator's low success rate in her retrospective assessment of participant allocation.

16.12 Conclusions

The majority of participants benefited during their enrolment in the current study. However, because of the lack of statistical significance over placebo, the phytotherapeutic intervention is deemed ineffective. Whether such an improvement can justifiably be dismissed is questionable, and will be discussed further in chapter 19. Factors contributing to the substantial improvement in the placebo arm undoubtedly include an appreciable impact of natural history, but the washout effects post-treatment suggest that other factors also operated. This warrants further investigation, and gave rise to the subsequent studies reported in chapter 18.

Chapter Seventeen

Hypericum perforatum and *Vitex agnus-castus* for PMS-like symptoms in perimenopause

17.0 Introduction

The second of the results chapters reports on a sub-study of the principal randomised, controlled trial. Data were analysed from women in the late-perimenopausal subgroup who continued to experience intermittent menstruation and recorded associated premenstrual symptoms during the trial. The manuscript has been submitted as a brief communication to *The Journal of Alternative and Complementary Medicine*. [Citation: Van Die MD, Bone KM, Burger HB, Reece JE, Teede HJ. *Hypericum perforatum* and *Vitex agnus-castus* for PMS-like symptoms in perimenopause. *submitted to J Alt Comp Med Nov 2008*.] Additional analyses not incorporated into the manuscript are included as an addendum.

17.1 Abstract

The effect of a combination of *Hypericum perforatum* and *Vitex agnus-castus* on premenstrual syndrome (PMS)-like symptoms was investigated in a small sub-population (*n* = 14) of late-perimenopausal women participating in a randomised controlled trial on menopausal symptoms. Significant treatment group trends across the five phases were observed for total PMS and all subscales, all in the clinically expected direction. No significant trends were evident in the placebo group.

17.2 Introduction

It has been suggested that some of the symptoms typically attributed to menopause may be more related to premenstrual syndrome (PMS)[225] which is observed to be quite prevalent, or less well-tolerated, in perimenopausal women. However, the menstrual cyclicity of the symptoms may not always be apparent, not only because of the unpredictable nature of cycles, but also because ovulatory cycles can occur in the absence of subsequent menstruation during the perimenopause.[55] When PMS *co-exists* with menopausal symptoms, management with hormone therapy (HT) is difficult, as progestins are found to aggravate PMS symptoms and combined HT regimes may induce PMS-like symptoms in susceptible women.[456]

PMS is thought to result from sensitivity to normal hormonal fluctuations in the late luteal phase of the menstrual cycle, possibly due to neurotransmitter dysfunction.[465] As these hormonal fluctuations depend on the occurrence of ovulation, which is less frequent during the late-perimenopause, symptoms during this phase are more appropriately termed 'PMS-like'.

The herb *Vitex agnus-castus* (Chaste tree/berry) has been shown to effect significant improvement in premenstrual symptoms such as irritability, mood alteration, anger, headache, breast tenderness and bloating.[1098] It is also widely prescribed for the treatment of menopausal symptoms by UK herbalists.[1098] Various mechanisms have been proposed including prolactin inhibition, conferring benefit in latent hyperprolactinaemia, an opiate-agonist effect and stimulation of melatonin secretion.[1098]

Hypericum perforatum (St John's wort), in addition to its efficacy for mild-moderate depression,[920] has been observed to significantly reduce the severity of premenstrual

symptoms.[911] *Hypericum* extracts have been shown to influence serotonergic, noradrenergic, dopaminergic and GABA-ergic mechanisms.[908] Other potentially relevant mechanisms of action include the regulation of genes controlling hypothalamic-pituitary-adrenal axis function and opioid receptor binding activity.[908]

This study aimed to investigate the effects of a combination of *Hypericum perforatum* and *Vitex agnus-castus* on PMS-like symptoms in a small sub-population of late-perimenopausal women participating in a double-blind, placebo-controlled randomised trial on menopausal symptoms.[848]

17.3 Methods

The study protocol was approved by the Royal Melbourne Institute Technology-University Human Research Ethics Committee. All participants gave written informed consent prior to study entry.

Of one hundred volunteers recruited to the larger study, fourteen late-perimenopausal women provided baseline data for PMS-like symptoms and menstruated at least once within the last 12 weeks of the treatment phase. Exclusion criteria included concurrent major illnesses, substance abuse and concurrent treatment for menopausal or PMS-like symptoms, and any medication known to interact with the study intervention.

The daily dose of *Hypericum perforatum* was extract equivalent to 5,400mg dry herb flowering top administered via three tablets, each standardised to contain 990mcg hypericins, 9mg hyperforin and 18mg flavonoid glycosides.[848] The daily dose of *Vitex agnus-castus* was extract equivalent to 1,000mg dry fruit.[848] Tablets and matching placebos were manufactured by MediHerb Australia according to the Code of Good Manufacturing Practice, and are included as Listed Medicines on the Australian Register of Therapeutic goods. They were randomised by MediHerb using a computer-generated random number table.

Participants recorded the severity of their PMS-like symptoms at study entry and premenstrually throughout the 16-week treatment phase, on Abraham's Menstrual Symptoms Questionnaire (MSQ),[1090] consisting of 4 clusters, namely PMS-A: nervous tension, irritability, mood swings and anxiety; PMS-H: weight gain, swelling of extremities, breast tenderness and abdominal bloating; PMS-C: headaches, cravings for sweets, increased appetite, pounding heart, fatigue and dizziness; PMS-D: depression,

forgetfulness, crying, confusion and insomnia. Potentially confounding dietary and lifestyle factors and adverse events were monitored.

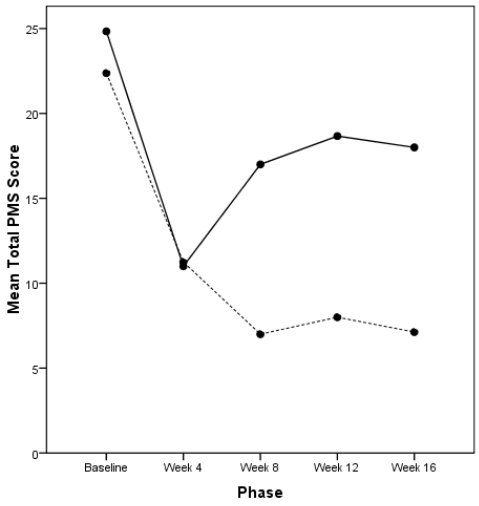
Data were analysed using Statistical Package for Social Science Version 16. Assumptions for parametric tests were met. Last observation carried forward was used to deal with missing week-16 PMS data, provided the last observation was at week 8 or later. Three sets of data analysis were conducted on the overall PMS scores and its four subscales. First, single-factor between subjects analyses of covariance (ANCOVAs) were used to evaluate the overall treatment effects with the baseline scores serving as the covariates, and the equivalent week 16 scores as the dependent variables. The treatment and placebo groups formed the between-subjects factor. Second, focussed linear mixed model single degree of freedom *F*-test comparisons, with appropriate Bonferroni corrections, were used to examine specific phase-within-group and group-within-phase differences. Finally, analyses of orthogonal polynomials (trend analyses), using a linear mixed model approach, were conducted across the five phases, separately for the treatment and placebo groups, to examine the pattern of change across the phases.

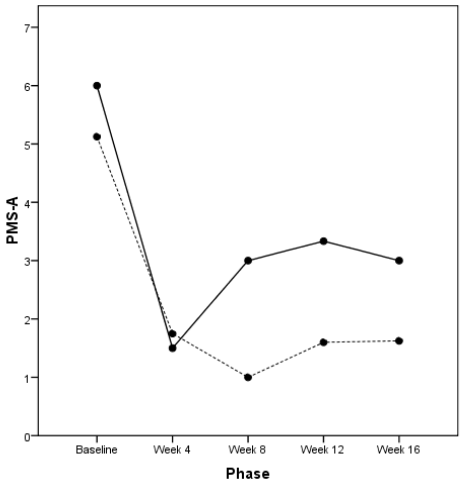
17.4 Results

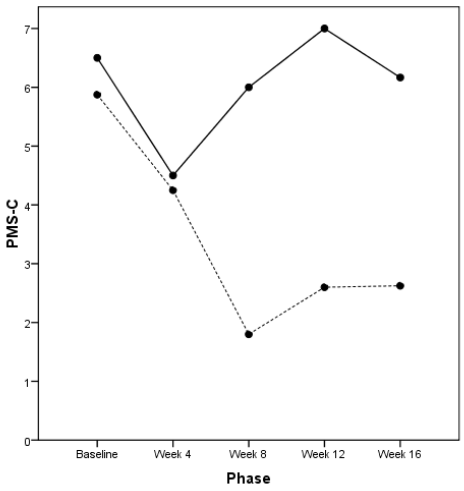
There were no significant baseline differences between the two groups in terms of sociodemographic characteristics or outcome measures. The mean age was 49 in each group. All fourteen participants in this sub-group completed the study and were included in the analyses.

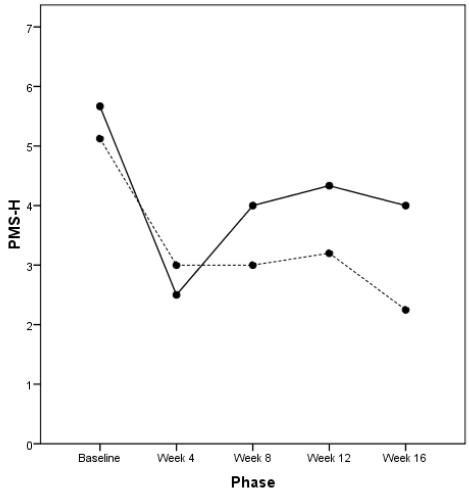
Figure 17.1 shows the pattern of change for the five measures across the five phases for the treatment and placebo groups. At a descriptive level, it is evident that there was no notable difference between the groups on any measure at either baseline or week 4, with differences emerging subsequently in the clinically expected direction. Notable group differences are evident for all measures at week 16.

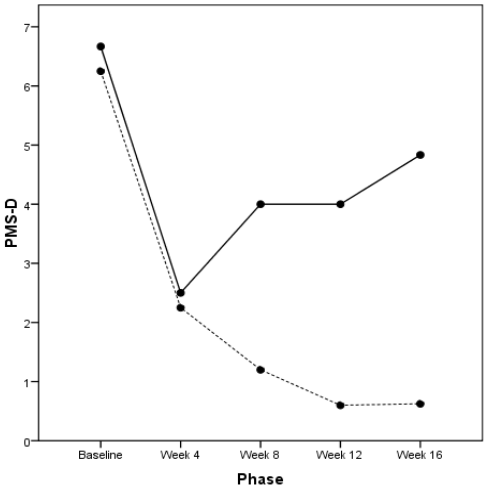
FIGURE 17.1 EFFECT OF INTERVENTION ON PMS TOTAL AND SUBCLUSTER SCORES











Legend

Active Treatment …….

Placebo _____

This interpretation was confirmed by statistical analysis. Three of the five ANCOVAs revealed significant group differences at week 16 (Table 17.2): Total-PMS, $F(1, 11) = 7{\cdot}33$, $p = 0{\cdot}02$; PMS-C, $F(1, 11) = 6{\cdot}53$, $p = 0{\cdot}03$; and PMS-D, $F(1, 11) = 11{\cdot}61$, $p = 0{\cdot}006$.

Focussed single degree of freedom tests revealed no significant differences between groups on any measure at either baseline or at week 4, but significant differences in the clinically expected direction at week 16 for PMS-Total, $F(1, 39{\cdot}81) = 4{\cdot}82$, $p = 0{\cdot}03$; PMS-C, $F(1, 35{\cdot}57) = 4{\cdot}69$, $p = 0{\cdot}04$; and PMS-D, $F(1, 39{\cdot}33) = 8{\cdot}07$, $p = 0{\cdot}007$.

The trend analyses revealed no significant trends for any test involving the placebo group, but four of the five measures in the treatment group revealed significant quadratic trends: PMS-Total, $t(18{\cdot}89) = 5{\cdot}39$, $p < 0{\cdot}001$; PMS-A, $t(19{\cdot}48) = 4{\cdot}10$, $p = 0{\cdot}001$; PMS-C, $t(18{\cdot}52) = 2{\cdot}98$, $p = 0{\cdot}008$; and PMS-D, $t(18{\cdot}95) = 3{\cdot}53$, $p = 0{\cdot}002$· PMS-H failed to show a significant quadratic trend, but did reveal a significant linear trend, $t(18{\cdot}26) = 4{\cdot}65$, $p < 0{\cdot}001$. All quadratic trends reflected the same pattern of clinical change: a steep decrease in symptoms from baseline to week 8, followed by a pattern of maintained change to week 16.

17.5 Discussion

In this sub-population of irregularly menstruating late-perimenopausal women, the combination of *Hypericum perforatum* and *Vitex agnus-castus* was superior to placebo for total PMS-like symptoms and the sub-clusters, PMS-D (depression) and PMS-C (cravings). The active treatment group also showed significant improvements on PMS-A (anxiety) and PMS-H (hydration), although this effect was not superior to placebo.

The observations from the present study support results from previous studies on the efficacy of *Vitex agnus-castus* in overall premenstrual symptoms,[1098] as well as pilot studies with *Hypericum perforatum* in PMS, particularly for depression-related symptoms.[911] However, they contrast with findings from the larger study of this combination in menopausal symptoms (measured on the Greene Climacteric scale) in late peri- and postmenopausal women,[848] and in the particular subgroup investigated here. This suggests that benefits to PMS-like symptoms do not simply reflect improvements to actual menopausal symptoms.

To our knowledge, this is the first study of this phytotherapeutic combination in PMS-like symptoms. The duration of the trial was adequate to allow for wash-out of the placebo effect, randomisation resulted in equivalent groups, and blinding was successful. Good quality assurance data were available for the phytotherapeutic intervention, for which overall tolerability and acceptability were good and compliance high.

The main limitations in this study were the small subgroup sample size and the late-perimenopausal status of the sample, with associated infrequent ovulatory menstrual cycles. As this was a sub-study of a menopause study, the diagnosis of PMS at baseline was not confirmed by 2 months of ratings, but relied on recall of symptoms, allowing for potential recall bias.

The current study suggests a potentially significant clinical application for the combination of *Hypericum perforatum* and *Vitex agnus-castus* for largely-neglected symptoms among perimenopausal women. Due to the limitations of this study, however, these findings should be interpreted with caution until replicated. This phytotherapeutic combination warrants further investigation in a dedicated study of PMS-like symptoms with a larger sample of women.

17.6 Additional Statistical Analyses

Additional analyses and results that could not be included in the brief communication in the preceding section are presented here.

17.6.1 Assumption Testing

Prior to conducting the analyses reported in the manuscript, assumption testing was carried out:

Normality testing

For enrolled participants in the active treatment group, the Shapiro-Wilk normality test showed that the assumption of normality had been violated in relation to PMS-C at baseline, p = 0·029. However visual inspection of the normal Q-Q plots did not show significant deviation from the linear line. Transformation or use of non-parametric tests was therefore not considered justified as parametric tests are quite robust to violations of normality. In addition, hypothesis tests of assumption testing are open to type II errors.

Homogeneity of Variance

The Levene test found that the assumption of homogeneity of variance was violated for total PMS, p =0.048, therefore two-tailed independent samples t-tests based on unequal variances were carried out. No significant difference between the two groups was found, p = 0·71.

17.6.1 Further Analyses

Further analyses conducted included

- ANCOVAs using week 4 data as the co-variate,
- paired samples *t*-tests for within-group changes from baseline,
- ANCOVAs on the Greene Climacteric Scale scores for this subgroup
- independent *t*-tests to compare the PMS and non-PMS subgroups of perimenopausal woman, and the postmenopausal group.
- Examination of data for one participant providing PMS data at all trial phases.

Details of adverse events and compliance are also included here.

17.7 Results

17.7.1 Baseline characteristics

The two groups in the PMS sample were equal at baseline:

TABLE 17.1 BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS N = 14
MEAN (SD)

	Placebo	***Herbal Combination***
	***n* = 6**	***n* = 8**
DEMOGRAPHICS		
Age at trial start (years)	48.8(4.1)	48.8(3.4)
Weight in kg	65.8(11.8)	66.7(11.8)
BMI	24.5(3.7)	24.7(4.1)
Caffeine: cups of tea/coffee per day	4.0(1.9)	4.1(1.0)
Standard alcoholic drinks per day	3.0(4.4)	5.3(6.5)
Stress	2.3(0.9)	2.5(0.8)
Exercise – hours per week	3.2(2.9)	3.3(3.1)
PRIMARY ENDPOINTS		
Total PMS	24.83(14.33)	22.38(6.80)
PMS-A	6.00(4.15)	5.12(2.48)
PMS-C	6.50(4.23)	5.88(3.52)
PMS-D	6.67(4.03)	6.25(3.01)
PMS-H	5.67(3.50)	5.13(2.17)

17.7.2 ANCOVA results for PMS-like symptoms at week 16

Further details of the single-factor between subjects analyses of covariance (ANCOVAs), using the baseline scores as the covariates, are given in Table 17.2.

TABLE 17.2 EFFECT OF INTERVENTION ON PMS-LIKE SYMPTOMS AT WEEK 16
COVARIATE ADJUSTED MEANS

Subscale				**Std.**	**95% Confidence**	
		N	**Mean**	**Error**	**Interval**	***p*-value**
PMS-total	placebo	6	18·39[a]	3·28	11·30, 25·76	0·02
	active	8	6·73[a]	2·84	0·48, 12·98	
PMS-A	placebo	6	3·15[a]	1·02	0·91, 5·40	0·25
	active	8	1·51[a]	0·88	-0·43, 3·44	
PMS-C	placebo	6	6·19[a]	1·06	3·86, 8·53	0·03
	active	8	2·61[a]	0·92	0·59, 4·62	
PMS-D	placebo	6	4·89[a]	0·95	2·79, 6·98	0·006
	active	8	0·59[a]	0·83	-1·23, 2·40	
PMS-H	placebo	6	3·97[a]	1·13	1·47, 6·46	0·29
	active	8	2·28[a]	0·98	0·12, 4·43	

a. Covariates appear in the model: baseline scores

17.7.3 Effects of Intervention at each phase

The effects of the intervention across the 5 phases is recorded in table 17.3 based on a mixed model, treating group as the between-subject factor and phase as the within-subject factor, and using last observation carried forward for missing data.

TABLE 17.3 EFFECT OF INTERVENTION ON PMS SUBSCALE SCORES
(MEAN +SE)

Cluster	Phase / Group	Baseline n=6, n=8	Week 4 n=2, n=4	Week 8 n=3, n=5	Week 12 n=3, n=5	Week 16 n=5, n=4
PMS-Total	Placebo	24.83(3.75)	11.00(6.49)	17.00(5.30)	18.67(5.30)	18.00(3.75)
	95% CIs	17.26, 32.41	-2.12,24.12	6.29,27.71	7.96,29.38	10.43,25.57
	Active	22.38(3.25)	11.25(4.59)	7.00(4.10)	8.00(4.10)	7.13(3.25)
	95% CIs	15.82,28.93	1.98,20.52	-1.30,15.30	-0.30,16.30	0.57,13.69
PMS-A	Placebo	6.00(1.05)	1.50(1.82)	3.00(1.49)	3.33(1.49)	3.00(1.05)
	95% CIs	3.88,8.13	-2.18,5.18	-0.008,6.01	0.33,6.34	0.87,5.13
	Active	5.13(0.91)	1.75(1.29)	1.00(1.15)	1.60(1.15)	1.63(0.91)
	95% CIs	3.28,6.97	-0.86,4.36	-1.33,3.33	-0.73,3.93	-0.22,3.47
PMS-C	Placebo	6.50(1.24)	4.50(2.15)	6.00(1.75)	7.00(1.75)	6.17(1.24)
	95% CIs	4.00,9.00	0.16,8.84	2.46,9.54	3.46,10.54	3.66,8.67
	Active	5.88(1.07)	4.25(1.52)	1.80(1.36)	2.60(1.36)	2.63(1.07)
	95% CIs	3.70,8.04	1.18,7.32	-0.94,4.54	-0.14,5.34	0.46,4.80
PMS-D	Placebo	6.67(1.12)	2.50(1.94)	4.00(1.59)	4.00(1.59)	4.83(1.12)
	95% CIs	4.40,8.93	-1.42,6.42	0.80,7.20	0.80,7.20	2.57,7.10
	Active	6.25(0.97)	2.25(1.37)	1.20(1.23)	0.60(1.23)	0.63(0.97)
	95% CIs	4.29,8.21	-0.52,5.02	-1.28,3.68	-1.88,3.08	-1.66,2.58
PMS-H	Placebo	5.67(1.17)	2.50(2.02)	4.00(1.65)	4.33(1.65)	4.00(1.17)
	95% CIs	3.31,8.02	-1.58,6.58	-1.58,6.58	1.00,7.66	1.65,6.35
	Active	5.13(1.01)	3.00(1.43)	3.00(1.28)	3.20(1.28)	2.25(1.01)
	95% CIs	3.09,7.16	0.12,5.88	0.42,5.58	0.62,5.78	0.21,4.29

Data are expressed as mean ± (std error).
Data were derived using mixed model analysis and last observation carried forward

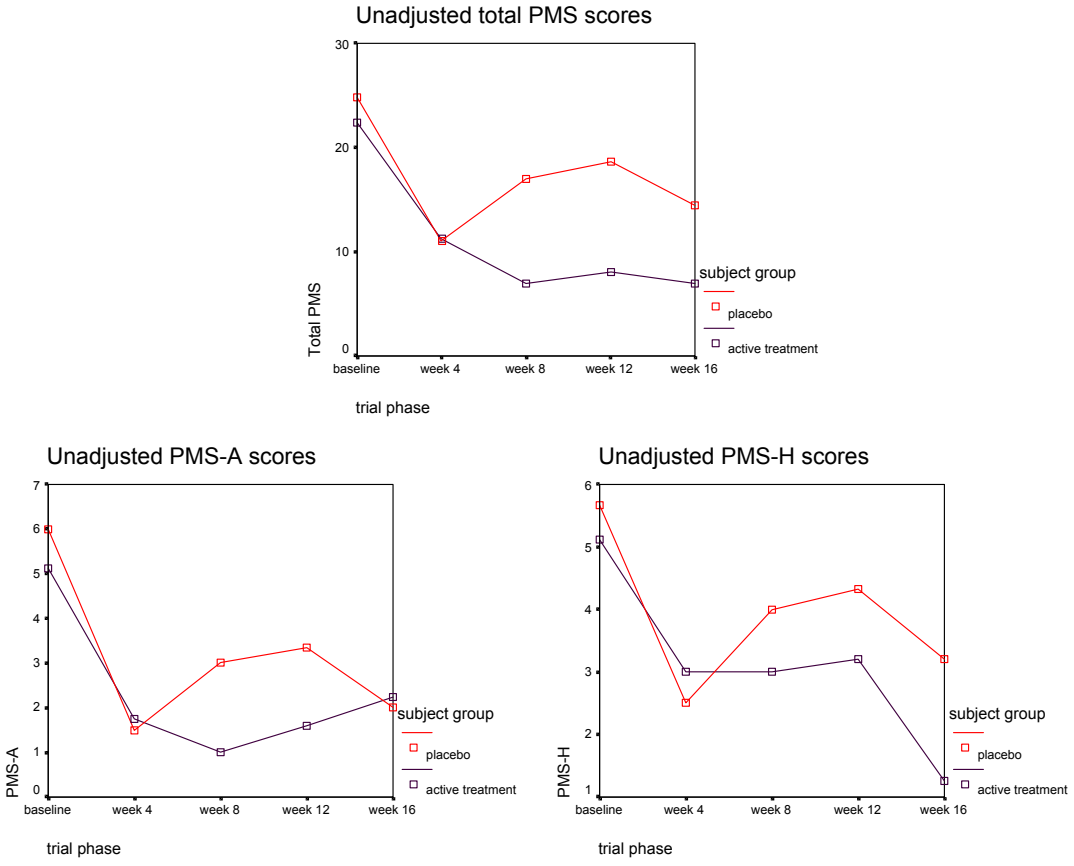
Last observation carried forward was used for missing week 16 data, provided that the last observation was reported at week 8 or later. As women reported symptoms at the end of every 4 weeks, PMS-like symptoms reported at week 4 would include those occurring in the first weeks of the trial, before any effect of the phytotherapeutic intervention would be noticed. Women who had no subsequent menstrual cycles throughout the treatment phase were therefore not included in the sample for analysis.

Raw descriptive data for the 5 trial phases, not adjusting for missing data, are shown in table 17.4 and Figure17.2.

TABLE 17.4 EFFECT OF INTERVENTION ON UNADJUSTED PMS SUBSCALE SCORES (MEAN +SE)

Cluster	Phase / Group	Baseline n=6, n=8	Week 4 n=2, n=4	Week 8 n=3, n=5	Week 12 n=3, n=5	Week 16 n=5, n=4
PMS-Total	Placebo	24.83(5.85)	11.00(8.00)	17.00(6.00)	18.67(9.56)	14.40(4.50)
	95% CIs	9.79,39.87	-90.65,112.65	-8.82,42.82	-22.48,59.81	1.90,26.90
	Active	22.38(2.41)	11.25(3.28)	7.00(1.87)	8.00(1.23)	7.00(2.12)0.
	95% Cis	16.69,28.06	0.83,21.67	1.18,12.19	4.60,11.40	25,13.75
PMS-A	Placebo	6.00(1.69)1.	1.50(1.50)	3.00(1.00)	3.33(2.40)	2.00(1.30)
	95% Cis	65,10.35	-17.56,20.56	-1.30,7.30	-7.01,13.68	-1.62,5.62
	Active	5.13(0.88)	1.75(0.63)	1.00(0.48)	1.60(0.40)	2.25(0.85)
	95% Cis	3.06,7.19	-0.25,3.75	-0.24,2.24	0.49,2.71	-0.47,4.97
PMS-C	Placebo	6.50(1.73)	4.50(1.50)	6.00(1.53)	7.00(2.65)	5.20(1.16)
	95% Cis	2.06,10.94	-14.56,23.56	-.057,12.57	-4.38,18.38	1.99,8.41
	Active	5.88(1.25)	4.25(1.97)	1.80(0.58)	2.60(0.40)	2.75(1.11)
	95% Cis	2.93,8.82	-2.03,10.53	0.18,3.42	1.49,3.71	-0.78,6.28
PMS-D	Placebo	6.67(1.65)	2.50(2.50)	4.00(1.53)	4.00(2.52)	4.00(1.38)
	95% Cis	2.43,10.90	-29.27,34.27	-2.57,10.57	-6.8314.83	0.17,7.83
	Active	6.25(1.07)	2.25(1.03)	1.20(0.74)	0.60(0.25)	0.75(0.75)
	95% Cis	3.73,8.77	-1.03,5.53	-0.84,3.24	-0.08,1.28	-1.64,3.14
PMS-H	Placebo	5.67(1.43)	2.50(2.50)	4.00(2.00)	4.33(2.33)	3.20(1.28)
	95% Cis	1.99,9.34	-29.27,34.27	-4.61,12.61	-5.71,14.37	-0.36,6.76
	Active	5.13(0.77)	3.00(0.91)	3.00(1.41)	3.20(1.16)	1.25(0.48)
	95% Cis	3.31,6.94	0.09,5.91	-0.93,6.93	-0.01,6.41	-0.27,2.77

Data are expressed as mean ± (STD ERROR)



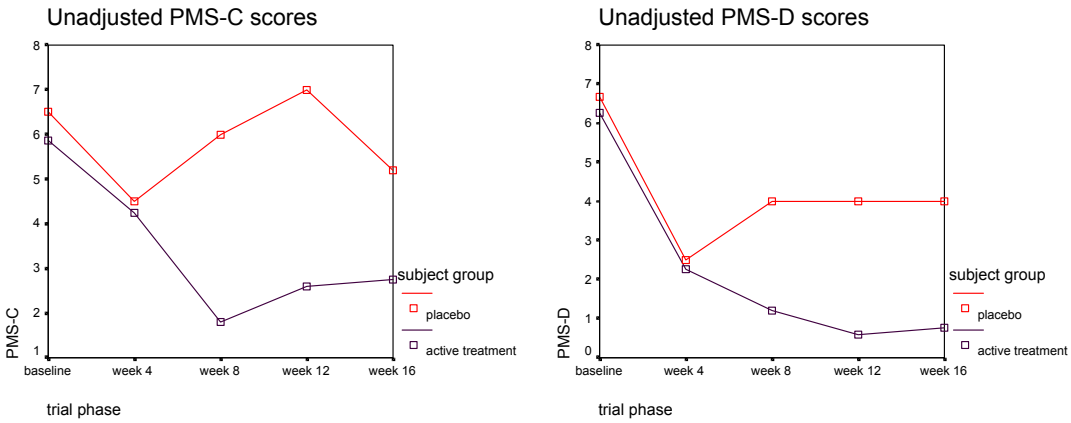


FIGURE 17.2 EFFECT OF INTERVENTION ON UNADJUSTED PMS TOTAL AND SUBCLUSTER SCORES

17.7.4 Within-group changes from baseline

As shown in the line graphs in the manuscript in the preceding section, there were significant improvements on all endpoints for the active treatment group, but not for the placebo group across the 16 week treatment phase. This finding was supported by paired-sample *t*-tests, the results of which can be seen in table 17.5.

TABLE 17.5 PMS PAIRED SAMPLES T-TESTS
(Mean + SD)

	Placebo *n* = 6			Active *n* = 8		
	baseline	**Week 16**	***p*-value**	**baseline**	**Week 16**	***p*-value**
Total PMS	24·83(14·33)	18·00(12·60)	0·53	22·38(6·80)	7·13(3·83)	< 0·001
PMS-A	6·00(4·15)	3·00(3·58)	0·35	5·12(2·48)	1·62(1·51)	0·003
PMS-C	6·50(4·23)	6·17(3·31)	0·91	5·88(3·52)	2·63(1·69)	0·01
PMS-D	6·67(4·03)	4·83(3·43)	0·52	6·25(3·01)	0·63(1·06)	0·001
PMS-H	5·67(3·50)	4·00(3·23)	0·47	5·13(2·17)	2·25(2·19)	0·002

Significance levels from paired samples *t*-tests between baseline and all data collection points for the active treatment group are included in appendix 30. Mean differences within groups from baseline to week 16 were also calculated using a mixed model analysis, and can be found in appendix 30.

17.7.5 Week 4 scores as baseline

As mentioned previously, baseline scores were based on recall and therefore potentially susceptible to recall bias. To address this, analyses of covariance (ANCOVAs) were also carried out using week 4 scores as the covariate. Only PMS-D showed a significant difference between groups, $F(1, 3) = 30{\cdot}06$, $p = 0.01$. However, by the time these symptoms were reported, the intervention had been administered for up to 4 weeks, so cannot be considered as more reliable baseline scores. Additionally, week 4 only had data for $n = 6$. Paired t-tests for weeks 4 and16 found Total PMS, $p = 0.067$, and PMS-D, $p = 0.058$, to approach significance.

17.7.6 Greene Climacteric Scale scores for the PMS subgroup

For the subgroup experiencing PMS-like symptoms, ANCOVA revealed no significant group differences at week 16 for Greene Climacteric Scale scores, $F(1, 11) = 0.44$, $p = 0{\cdot}53$, after controlling for baseline scores.

17.7.7 Perimenopausal PMS-sufferers compared with non-PMS sufferers and Post-menopausal women

Comparing the intermittently-menstruating late-perimenopausal women (all of whom reported associated PMS-like symptoms) with those no longer experiencing menstruation, and with the postmenopausal women, more severe symptoms at baseline on all endpoints were observed for the intermittently-menstruating group than the other two groups, with one exception. Loss of interest in sex was greater in the postmenopausal women. However, none of these reached statistical significance on independent *t*-tests, possibly due to the small sample size. The symptom profile of the non-menstruating, late-perimenopausal women approximated that of the post-menopausal women. Table 17.6.

TABLE 17.6 BASELINE SCORES OF POSTMENOPAUSAL PARTICIPANTS COMPARED WITH PERIMENOPAUSAL PMS-SUFFERERS NON-PMS SUFFERERS

	Post Menopause n = 49	*Late-perimenopause No menstruation n = 19*	*Late-perimenopause PMS-like symptoms n = 14*
Flushes, daily weighted	15.78(6.71)	15.96(6.98)	19.59(12.29)
Greene total	22.78(6.34)	20.47(7.25)	24.71(11.61)
Greene psychological subscale	11.98 (4.33)	10.68(4.51)	13.29(6.39)
Greene anxiety subscale	6.73(2.71)	5.74(2.40)	6.86(3.46)
Greene depression subscale	5.24(2.45)	4.95(2.76)	6.43(3.39)
Greene somatic subscale	4.86(2.65)	4.95(2.76)	5.43(5.27)
Greene vasomotor subscale	4.08(1.22)	3.89(1.29)	4.36(1.48)
Greene sexual subscale	1.86(0.98)	1.47(1.07)	1.64(1.22)
HDI-17	14.46(4.45)	14.47(4.30)	15.93 (4.27)

17.7.8 Further Details of Compliance and Adverse Events

Compliance was excellent with 96% of tablets taken by both groups. No participant was less than 85% compliant with the *Hypericum perforatum*, or less than 91% compliant overall. There were no significant adverse events and no significant difference between the two groups for these, Pearson's *R* with chi square, χ^2 (1) = 0.04, *p* = 0.85 (Table 17.7).

TABLE 17.8 ADVERSE EVENTS BY SUBJECT GROUP

ADVERSE * subject group Crosstabulation

Count

		subject group		
		placebo	active treatment	Total
ADVERSE	UTI		1	1
	accidents, injuries,	2	1	3
	anxiety	1		1
	breast tenderness	1		1
	cardiovascular	2		2
	cold/flu	3	4	7
	constipation	1		1
	cyst	1		1
	depression		2	2
	dysmenorrhoea		2	2
	fatigue	1		1
	flooding		1	1
	migraine	1		1
	other GI complaints		4	4
	other URTI	2		2
	paraesthesia		1	1
	repro		1	1
	vomiting	1		1
Total		16	17	33

In order to perform the chi square test, adverse events were recoded to form two groups, namely upper respiratory tract infections and gastro-intestinal complaints compared with other symptoms (Table 17.8).

TABLE 17.7 ADVERSE EVENTS RECODED

adverse events recoded * subject group Crosstabulation

Count

		subject group		
		placebo	active treatment	Total
adverse events recoded	URTI/GIT	7	8	15
	Other	9	9	18
Total		16	17	33

17.7.9 Examination of Data provided at all phases by one participant, N = 1

One participant provided PMS data at all five trial phases, despite having met the inclusion criteria for the menopause study of 3 consecutive months of amenorrhoea within the previous 12 months. She was on the active treatment. Visual inspection of graphs of her data suggests significant improvements on total PMS, PMS-A, PMS-D and PMS-H, that is, all endpoints except PMS-C (Figure 17.3).

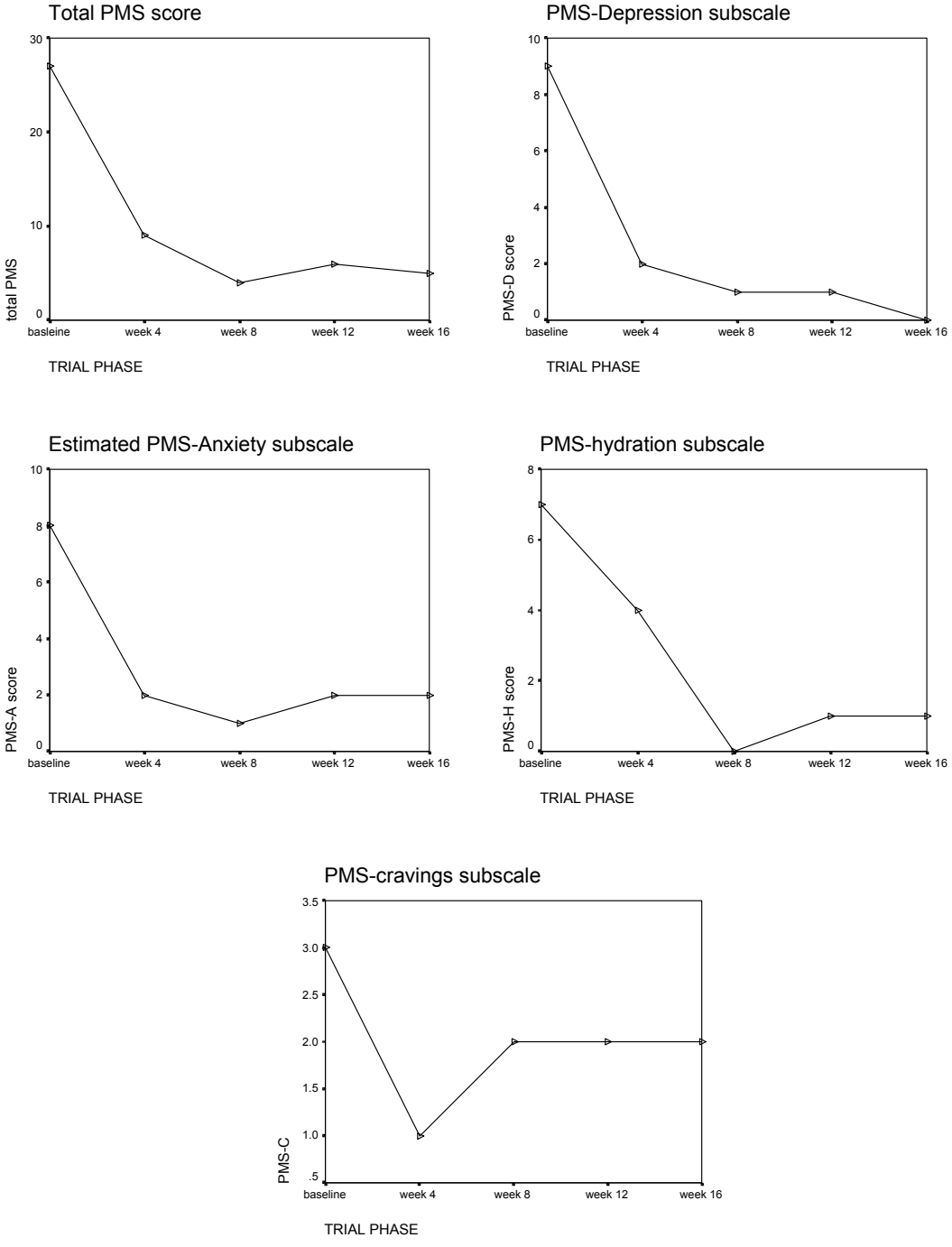


FIGURE 17.3 EFFECT OF INTERVENTION ON PMS TOTAL AND SUBCLUSTER SCORES FOR *N* = 1

Given that the baseline data were based on recall and therefore susceptible to recall bias, inspection of the difference from week 4 may be recommended, in which case, only PMS-D and PMS-H appear to show marked improvements. However, as mentioned previously, by that point the study intervention had been administered for up to 4 weeks.

17.8 Discussion

17.8.1 Principal Findings

For this participant, as for the whole sample, improvements are suggested for the phytotherapeutic combination on total PMS and all sub-clusters, although the effect for PMS-C in the case of the individual participant appears negligible. In both instances, the decline in scores from baseline to week 4 was followed by a further decline in the active treatment arm from weeks 4 to 8 on four of the scales (all except PMS-H for whole sample and PMS-C for *n* = 1). From weeks 8 to 12, there was a slight worsening of symptoms for all scales except PMS-D (and PMS-C for n = 1). Between weeks 12 and 16, scores levelled off on 3 of the five scales, except PMS-D (for *n* = 1) and PMS-H for whole sample, and consequently total PMS, (which includes the sub-cluster scores), where further slight improvements were noted. There was no significant difference on overall menopausal symptoms (measured on Greene Climacteric scale) for this particular sub-population.

17.8.2 Comparison with Other studies

These trends observed for *n* = 1 are consistent with previous studies on *Vitex agnus-castus* and *Hypericum perforatum* in PMS, where symptoms of PMS-H such as fluid retention, breast tenderness and bloating have been observed to improve, as well as irritability and depressed mood,[481,850,851,911] but not PMS-C (characterised by sweet cravings, headaches, palpitations and dizziness) in one pilot RCT on *Vitex agnus-castus* as a sole agent.[851] The observations from the individual participant supported the findings of that pilot study. For the whole PMS sample in the current study, however, PMS-H was the only sub-cluster on which significant improvements over placebo were not observed. Schellenberg, similarly, found bloating to be unaffected by treatment with *Vitex.*[481]

17.8.3 Interpretation

As mentioned previously, the decline in scores from baseline to week 4 may reflect recall bias. However, there was a further decline in intensity of symptoms in the active treatment arm from weeks 4 to 8 on four of the scales, and week 4 to week 16 on four

(three for *n* = 1). In addition, recall bias can be bi-directional. Evidence regarding recall bias has been found to result in both *under*-reporting of PMS symptoms,[1099] including dysphoria among women attending a menopause clinic,[372] and the reporting of the most *severe* PMS symptoms rather than the most frequent.[1100] The slight worsening of symptoms on some scales between weeks 8 and 12 could suggest washout of any placebo effect.

It is common that expectancy effects result in an initial placebo effect, followed by a slight worsening of symptoms in the active treatment arm and a more marked worsening in the placebo arm, which was observed for all subscales for the placebo group, and most of the subscales for the treatment arm. While the slight improvement in the placebo arm on four of the subscales between weeks 12 and 16 cannot be easily explained, the pattern seen in the treatment arm is consistent with genuine specific effects of treatment, namely a levelling off, or continued, less-marked improvement. The sustained improvement in symptoms of PMS-D to week 16 is not surprising, given the inclusion of *Hypericum perforatum* in the intervention.

As previously mentioned, the lack of a significant difference between groups at week 16 for Greene Climacteric Scale scores suggests that the finding in relation to PMS-like symptoms does not simply reflect the effect of the phytotherapeutic combination on overall menopausal symptoms in this sub-population.

In the light of the theory that some symptoms during perimenopause may be more appropriately attributed to menstruation, it is interesting to note that baseline scores on the flushing diaries, Greene Climacteric scale and HDI-17 tended to be higher in late-perimenopausal women who experienced menstruation over the subsequent 16 weeks compared with both i) those who did not, and ii) postmenopausal women. However, as none of these differences reached significance with the small samples in each of these subgroups, no firm conclusions can be drawn from this observation.

17.8.4 Strengths and Limitations

There were several limitations of this small sub-study. In addition to those already discussed, is the selection of a measurement scale that is used less commonly in research nowadays, but conferred the advantage of containing sub-clusters, and brevity, necessary for maximising compliance where participants were completing several other measures for the main study. Because PMS-like symptom reports were not collected in

the post-menstrual phase to confirm their cyclic patterns of exacerbation and remission, misclassification bias may also have occurred.

17.9 Conclusions

The observations from the individual participant, and for the subgroup as a whole, were consistent with findings from other clinical and observational studies of *Vitex agnus-castus* and *Hypericum perforatum* for PMS symptoms. There is a need for safe, effective, well-tolerated treatment for PMS-like symptoms occurring in conjunction with menopausal symptoms during the perimenopause. This is especially important as conventional medicine currently offers limited treatment options, for which the evidence is controversial. Further investigation from an RCT comparing the combination with *Hypericum perforatum* to *Vitex agnus-castus* as a sole agent would contribute useful evidence-based information with potential clinical application for healthcare providers and women in the community.

Chapter Eighteen

Predictors of Placebo Response in a Randomised, Controlled Trial of Phytotherapy in Menopause

18.0 Introduction

This chapter is divided into two sections. Both report on sub-studies of the principal randomised, controlled trial of phytomedicines in menopause. The manuscript in the first section examines predictors of the placebo response, and reports on the results of data analyses from the completing participants assigned to the placebo group. This manuscript has been submitted to the journal, *Menopause.* [Citation: Van Die MD, Teede H, Bone K, Reece J, Burger H. Predictors of Placebo Response in a Randomised, Controlled Trial of Phytotherapy in Menopause. Peer-reviewed by *Menopause* 2008; Reference will be provided when available.]

The manuscript in the second section reports on a comparison of predictors of the placebo response with predictors of the response in the treatment arm.

Additional analyses relevant to both papers are included as an addendum.

18.1 Abstract

Objectives: To evaluate predictors of the placebo response in a randomised, placebo-controlled, double-blind trial of a phytotherapeutic combination for the treatment of menopausal symptoms.

Methods: A *post hoc* analysis was conducted on data from 46 placebo participants completing the study. Variables at baseline were investigated for prediction of imporvement on any of the endpoints of flushing, depression measured on the Hamilton Depression Inventory, and menopausal symptoms measured on the Greene Climacteric scale. Hierarchical linear regression analyses were carried out on the individual endpoints, controlling for baseline scores. Multivariate linear regression analysis was also conducted on these three endpoints in combination.

Results: Anxiety at study entry predicted placebo response on all three outcome measures (flushing, $p = 0.03$, depression, $p < 0.001$, and Greene Climacteric score, $p = 0.04$) individually and in combination, $p = 0.002$, as did total Greene scores at study entry, $p = 0.005$. Improvement during non-treatment run-in predicted further improvements for depression, $p = 0.005$, menopause symptoms, $p = 0.013$, and the three combined endpoints, $p = 0.015$. Severity of scores at baseline predicted subsequent improvement on the Greene Climacteric scores only, $p = 0.009$.

Conclusions: These findings may facilitate identification of potential placebo responders in future randomised controlled trials on menopausal symptoms, and have relevance to study design in this context. Further research is required.

18.2 Introduction

In clinical practice, a substantial placebo response, if harnessed reliably, may enhance treatment outcomes, especially where the patient's subjective viewpoint is the optimum outcome measure. However, in randomised controlled trials (RCTs) designed to assess the efficacy of potential treatments, a substantial placebo effect continues to be considered a nuisance as it can result in smaller effect sizes or failure to demonstrate the superiority of new treatments or established reference drugs over placebo.[1000] This can lead to the rejection or discontinuation of potentially useful treatments due to type II errors (that is, erroneous conclusions that there are no differences between the efficacy of the treatment and placebo). In addition, it can increase sample sizes required to adequately power RCTs, adding time and cost to the development of new treatments.[1001]

The magnitude of the placebo response is found to be partly dependent on the condition,[994,1046] with depression, anxiety and pain found to be particularly susceptible to the placebo effect.[989] In studies of antidepressant medications, placebo response rates average approximately 30%, ranging from 12% to beyond 50%, and have been observed to be on the increase.[865,986] In RCTs of hormone therapy for vasomotor symptoms in menopause, the placebo response rate averages 51%.[1095] While natural history may contribute significantly to the non-specific effects in these conditions, it does not fully account for the placebo response, as many other studies such as sham surgery for Parkinson's disease[992] and arthroscopy[993] have also reported high placebo effects.

The challenge of rigorous scientific research is to accurately determine the effect of a specific intervention over and above the placebo effect, also referred to as 'non-specific effects', or 'context effects'. Attempts to increase our understanding of placebo effects have largely been directed at reducing the placebo response in RCTs to increase the likelihood of detecting specific treatment effects. A growing body of research has focused on identifying the characteristics of placebo responders. Findings from such studies have yielded inconsistent results. For example, in terms of the demographic and personality characteristics of participants, various predictors of the placebo response have been reported, such as age,[1050,1101] marital (married) status[1101] and higher BMI,[1053] chronicity of disease or symptoms,[1060] relatively short illness and a precipitating event,[1055] higher positive patient expectations of treatment outcome[865,1015] and acquiescence.[1056] Predictors of response to placebo in menopause studies have not been explored to date.

In this setting, the current study aimed to identify predictors of the placebo response in an RCT of a phytotherapeutic combination for menopausal symptoms in late-perimenopausal and postmenopausal women. Endpoints included flushing, depressive symptoms measured on the Hamilton Depression Inventory (HDI) and overall menopausal symptoms measured on the Greene climacteric scale. It was hypothesised that the placebo response would be predicted by psychological factors.

18.3 Methods

This study was a *post hoc* analysis of data from an investigation of 46 completing participants randomly assigned to the placebo group of a randomised, placebo-controlled, double-blind trial of a phytotherapeutic combination containing *Hypericum perforatum* and *Vitex agnus-castus* for the treatment of menopausal symptoms. After entry to the study, there was a two-week non-treatment run-in prior to baseline, followed by a 16-week treatment phase. The trial was approved by the Human Research Ethics Committee at Royal Melbourne Institute of Technology University.

18.3.1 Study intervention

As previously discussed,[848] women in this sub-population were assigned to the placebo intervention, which consisted of a daily dose of two placebo tablets matching the *Vitex agnus-castus* tablets administered to the active treatment group, and three placebo tablets matching the *Hypericum perforatum* tablets, administered in divided doses. The placebos contained the inert excipients used in the active tablets, namely modified starch, cellulose, magnesium stearate, calcium hydrogen-phosphate. They were identical to the herbal tablets in size, colour, coating, weight and packaging. All tablets were manufactured under the Code of Good Manufacturing Practice by MediHerb Australia Pty Ltd. Further details of the herbal intervention, according to the Elaborated Consort checklist have been provided elsewhere.[848]

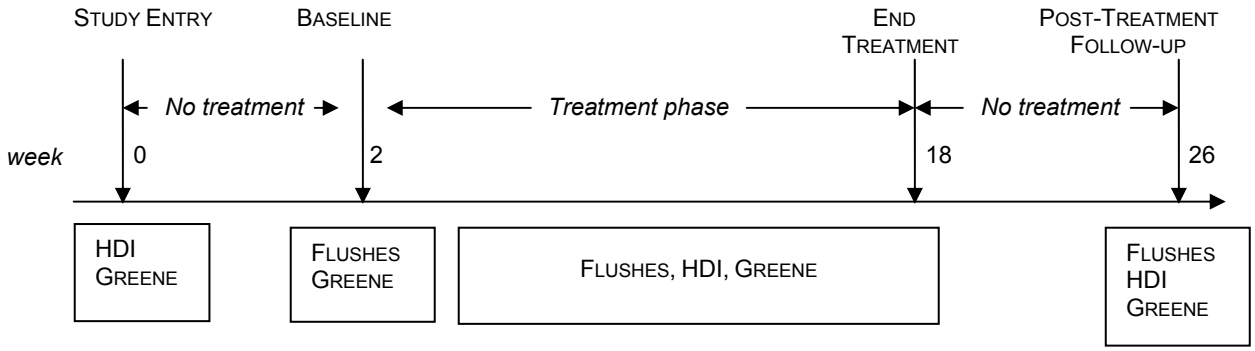
18.3.2 Participants

Fifty of the total 100 women recruited had been randomised to the placebo arm. All were late-perimenopausal or postmenopausal women, aged 40 to 60 years who had experienced a minimum of 3 months' amenorrhoea, not due to any other cause, in the previous 12 months. They were experiencing moderately severe menopause symptoms, measured by the Greene Climacteric scale, and at least 5 flushing episodes per 24 hours. Exclusion criteria included concurrent major illness, medication for any symptom under investigation within the previous 4-5 weeks if orally administered, 6 months for

injectables and 1 year for hormone implants, and current use of any medication known to interact with either study intervention. Informed consent was obtained prior to study entry. Baseline visits were conducted in a clinic setting and follow-up contact by telephone. Medical clearance was obtained from a general practitioner prior to inclusion in the trial. Participants were requested to maintain their baseline dietary phytoestrogen intake during the trial.

Baseline data were collected for age, marital status, employment, parity, menopausal status, hysterectomy, date of last menstruation, onset of symptoms, prior use of herbal medicine, trial herbs, HRT and perceived effectiveness of these, relationship satisfaction and attitude to menopause, lifestyle factors such as exercise, stress, stress reduction, smoking, intake of dietary phytoestrogens, caffeine and alcohol. Daily ambient temperature ranges were obtained from the Climate centre at the Bureau of Meteorology and recorded at baseline and the end of the treatment phase. Data were collected for the endpoints: frequency and severity of hot flushes, depressive symptoms, rated on the Hamilton Depression Inventory (HDI), and menopausal symptoms rated on the Greene Climacteric scale (Figure 18.1).

FIGURE 18.1 TIMELINE OF DATA COLLECTION



18.3.3 Statistical Analyses

In order to assess the relationship between various predictors and the placebo effect, a series of hierarchical regression analyses were carried out for each endpoint. In each instance, the baseline scores for the dependent variable were accounted for by being entered into the first step, followed by the predictor of interest. A significant R^2 for the entry of the predictor of interest was taken as an indicator of importance. A response was defined as a change in a favourable direction, that is, a decrease in severity of symptoms.

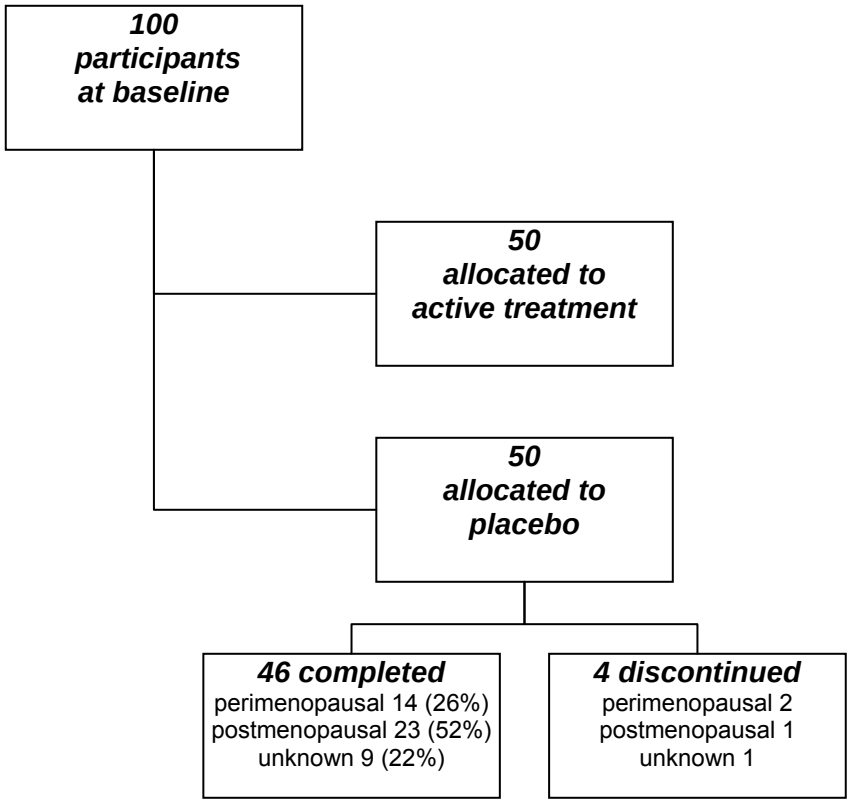
Multivariate linear regression analyses were also performed on the three outcome variables in combination in order to determine predictors of the multivariate construct formed by combining the three separate measures. The three different outcomes were combined into a single variable using MANOVA. The linear combination is created using all three outcomes.

All variables were examined using exploratory data analysis procedures and regression diagnostics to ascertain (a) the presence of data entry errors, (b) outliers, and c) whether the main variables of interest met the assumptions for parametric analysis, particularly regression modelling. Whilst there was some evidence of minor skew and outlier influence, the impact of these was not enough to justify transformation or other modification of the data or analytic approach.

18.4 Results

Of the 93 women completing the trial, 46 had been randomised to placebo (Figure 18.2).

FIGURE 18.2 PARTICIPANT FLOW



Variables found to have significant predictive ability, using hierarchical linear regression analysis and controlling for baseline scores, are shown in Table 18.1. No significant predictive ability was found for any of the other variables examined. As is conventional, a negative beta co-efficient means that more severe scores at study entry were associated with milder symptoms at week 16.

TABLE 18.1 PREDICTORS OF ENDPOINTS' WEEK 16 SCORES

Variable	Placebo Group *n* = 46		
	Flushes	***Greene Scale***	***HDI-17***
Age at trial start	NS	NS	–0.31 *
Previous phytotherapy effective	NS	NS	–0.32 *
Total *Greene* score at study entry	–0.38 †	–0.38 *	–0.47 †
Greene anxiety at study entry	–0.28 *	–0.29 *	–0.66 ‡
Improvement during non–treatment run–in	NS	–0.64 *	–0.41 †

Standardised β-coefficients (*p*-significance level) after controlling for baseline scores)
* $p < 0.05$
† $p < 0.01$
‡ $p < 0.001$

18.4.1 Anxiety at Study entry

Higher anxiety at study entry significantly predicted decreases for the placebo group on all the endpoints individually: flushes $R^2 = 0.33$, Std. β = –0.28, $p = 0.03$; HDI-17 scale $R^2 = 0.34$, Std. β = –0.66, $p < 0.001$; Greene climacteric scale $R^2 = 0.24$, Std. β = –0.29, $p = 0.04$.

More severe overall menopausal symptoms (total Greene score) at study entry significantly predicted improvements on all the endpoints individually, flushes $R^2 = 0.39$, Std. β = –0.38, $p = 0.003$; HDI-17 scale $R^2 = 0.23$, Std. β = –0.47 $p = 0.007$; Greene climacteric scale $R^2 = 0.28$ Std. β = –0.38, $p = 0.01$.

18.4.2 Change in Scores during Non-treatment Run-in

Improvement during run-in (measured on Greene scale) predicted further improvement on HDI depression scores to week 16, $R^2 = 0.24$, Std. β = –0.41, $p = 0.005$, and on Greene scores to week 16, $R^2 = 0.28$, Std. β = –0.64, $p = 0.01$.

There was *no* significant correlation between anxiety at study entry and improvement during run-in (in anxiety or total Greene score).

18.4.3 Age

For depression scores on HDI-17, older age at study entry was associated with a lower scores (milder/fewer symptoms) at end of trial, R^2 = 0.18, Std. β = –0.31, p = 0.03.

18.4.4 Previous Positive Experience

As reported previously,[848] for depression scores on HDI-17, prior positive experience with phytotherapy was associated with a greater reduction in depression, evidenced by lower scores at the end of the treatment phase, R^2 = 0.18, Std. β = –0.32, p = 0.03.

18.4.5 Multivariate Regression analyses

Controlling for baseline scores for the three major endpoints, multivariate linear regression analyses were also performed on the three outcome variables in combination. Significant predictors were: *higher anxiety at study entry,* Wilks' λ = 0.69*, F* (3,39) = 5.77, p = 0.002; *more severe overall menopausal symptoms (total Greene scores) at study entry,* Wilks' λ = 0.72, F (3,39) = 5.03, p = 0.005; *more severe baseline depression on Greene Climacteric subscale,* Wilks' λ = 0.79*, F* (3,39) = 3.44, p = 0.03; and *greater change in scores during run-in,* Wilks' λ = 0.77*, F* (3,39) = 3.92, p = 0.02.

18.4.6 Baseline severity of scores

For Greene climacteric scores, a positive correlation was found between baseline severity of scores and subsequent improvement, using Pearson's bivariate correlations, r = 0.38, p = 0.009. This was mirrored by anxiety on the Greene Climacteric subscale: severity of anxiety at baseline was significantly correlated with improvement during treatment phase on Greene anxiety, r = 0.36, p = 0.01. However, no significant correlations were found between baseline severity of symptoms and percentage improvement over trial for flushing, r = -0.05, p = 0.73, or depression (on HDI-17 scale), r = -0.001, p = 0.19.

18.5 Discussion

To our knowledge, this is the first study to examine predictors of the placebo response in the context of menopausal symptoms. We found that anxiety at study entry predicted placebo response on all of the endpoints (flushing, depression and Greene Climacteric scale scores) individually and in combination, as did total Greene scores at study entry. Improvement during non-treatment run-in predicted placebo response for depression, menopause symptoms measured on the Greene Climacteric scale, and the three

combined endpoints. Severity of scores at baseline predicted subsequent improvement measured on the Greene Climacteric scale scores only. Women with more severe baseline scores showed the greater response to placebo on the Greene Climacteric scale.

Previously identified predictors of the placebo response, predominantly from studies of analgesia and antidepressants, include positive response to prior therapies,[1055] comorbid anxiety;[1058] *lower* somatic anxiety,[1059] high level neuroticism including depression and anxiety,[1057] worsening or limited change in depression, anxiety or melancholia during placebo lead-in treatment[1061] and milder symptom severity at baseline.[518,986,1046,1055,1101] The finding from the current study that anxiety at study entry predicted the placebo response on all endpoints is consistent with findings from previous studies in the areas of pain and depression.[1057,1058] Anxiety itself is found to be particularly susceptible to the placebo effect.[989] It has been suggested that factors modulating anxiety in RCTs include the attention, time and support from investigators that contribute to the therapeutic alliance, and impact on the placebo response.[1009,1011] Support and reassurance have been shown to significantly influence health outcomes.[1009] The concomitant improvement in anxiety and other menopausal symptoms in the placebo group is consistent with the role of anxiety in triggering hot flushes in menopausal women,[91] and the salutary effect of relaxation techniques in this context.[1102,1103] The observation that more severe scores on the Greene Climacteric scale predicted placebo response may be largely attributable to the impact of the anxiety subscale.

Improvement during non-treatment run-in predicted the response in depression during the treatment phase. This is not consistent with previous observations of the placebo response in depressive symptoms,[1061] which may be due to the natural history of menopausal symptoms, which follow a fluctuating course. Alternatively, it may reflect the difference between depressive *syndromes per se* and the neurovegetative *symptoms* associated with menopause. The value of a non-treatment run-in period in identifying placebo responders is supported by this study, contrary to previous findings that such a practice makes no difference.[1000] Severity of symptoms at study entry as a predictor remains controversial. In several studies, including social anxiety disorder[1046] and depression,[518,1101] *milder* baseline symptom severity has been found to predict higher placebo response rates, although in one study of antidepressant medication, moderate symptom severity was associated with a higher placebo response.[1055] In the current study, more *severe* symptoms as rated on the Greene Climacteric scale were associated

with a greater placebo response. However, baseline severity of symptoms did not predict response to placebo for flushes or depression. This may be the result of having recruited only women with moderately severe symptoms.

The findings that improvement during non-treatment run-in and anxiety at study entry predicted the placebo response are both of importance in the design of future RCTs on menopausal symptoms. Excluding highly anxious participants from the sample could restrict the magnitude of the placebo response. However, it may result in loss of power and bias the sample, making it non-representative of the population, and thereby limit the generalisability of the findings. An alternative may be to stratify the study sample by anxiety, and to ensure recruitment of sufficient numbers of participants for adequate power. Data from highly anxious participants could be analysed separately.

The inclusion criteria of moderately-severe symptoms may have been responsible for the lack of association between symptom severity at baseline and the placebo response for flushing and depression. This supports the requirements of some bodies, such as the FDA, for inclusion of a minimum symptom severity when recruiting for clinical trials.[847]

Strengths of this study include controlling for baseline scores in the analysis by entering them as the first step of the hierarchical linear regression analyses. However, s*tudy entry* scores (prior to commencement of non-treatment run-in) were considered more relevant to investigation of the placebo response, as enrolment in a clinical trial would be expected to elicit changes in psychological measures, as well as behaviour. The inclusion of a non-treatment run-in period controlled to some extent for regression to the mean and the Hawthorne effect. However, as a non-treatment *arm* was not included, natural history cannot be distinguished from the placebo response. Because the outcome measures were subjective, obliging reports may also have biased results. An additional limitation was the small sample size, which may have resulted in important predictor variables not being detected. The improvement during non-treatment run-in was only measured on the Greene Climacteric scale, as the HDI-17 was not re-administered at the end of this period.

18.6 Conclusion

These findings have application for the designing of future studies. Randomised controlled trials of vasomotor symptoms in menopause are affected by substantial placebo effects. The observations from the current study may facilitate identification of placebo responders in menopause studies, according to high anxiety at study entry and improvement during run-in. Such identification may be used to inform strategies to limit the magnitude of the placebo response. This could be achieved by excluding such individuals (with recruitment of additional participants as necessary), stratifying according to these variables, and/or performing separate analyses of data from these participants.

The value of a non-treatment run-in is supported in this context. The findings from this study also support the recruitment of participants with moderately severe symptoms.

Controlling the magnitude of the placebo response may facilitate accurate determination of the specific effects of interventions. These findings need to be replicated in a larger study.

Chapter Eighteen (Part II)

Are we drawing the right conclusions from randomised placebo-controlled trials?

18.7 Introduction

This section contains a manuscript entitled *Are we drawing the Right Conclusions from Placebo-controlled Trials?* As no significant difference was found between groups, it was hypothesised that predictors of the response in the two arms would be the same. Predictors of the placebo and active treatment responses were compared. The findings are discussed with reference to the existing literature regarding placebo effects. This manuscript has been submitted to *BioMed Central Medical Research Methodology*, and is cited as Van Die M, Bone K, Burger H, Teede H. Are we drawing the right conclusions from randomised placebo-controlled trials? *submitted to BMC Med Res Methodol* 2008.

Additional analyses relevant to this and the previous paper are included as an addendum.

18.8 Abstract

Background: The assumptions underlying placebo controlled trials include that the placebo effect impacts on all study arms equally, and that treatment effects are additional to the placebo effect. However these assumptions have recently been challenged, and different mechanisms may potentially be operating in the placebo and treatment arms.

Objectives: To explore the nature of placebo versus pharmacological effects by comparing predictors of the placebo response with predictors of the treatment response in a randomised, placebo-controlled, double-blind trial of a phytotherapeutic combination for the treatment of menopausal symptoms. The treatment contained *Hypericum perforatum* and *Vitex agnus-castus.* No significant difference in efficacy was found between the two arms, and a substantial placebo response was observed.

Methods: A *post hoc* analysis was conducted on data from 93 participants who completed this previously published study. Variables at baseline were investigated as potential predictors of the response on any of the endpoints of flushing, menopausal symptoms measured on the Greene Climacteric scale, and depression measured on the Hamilton Depression Inventory. Hierarchical linear regression analyses were carried out on the individual variables, controlling for baseline scores, for all three endpoints and for both groups separately. Significant variables were then entered into a multiple regression model for each separate endpoint. These findings are discussed in relation to the existing literature on placebo effects.

Results: Distinct differences in predictors were observed between the placebo and active groups. None of the variables found to predict the placebo response was relevant to the treatment arm. Anxiety at study entry predicted placebo response on all three outcome measures individually. In contrast, *low* anxiety was significantly associated with improvement in the active treatment group. Improvement during non-treatment run-in predicted placebo response on depression and overall menopausal symptoms. However, this was not mirrored in the treatment group.

Conclusions: This study was a *post hoc* analysis of predictors of the placebo versus treatment response. Whilst this study does not explore neurobiological mechanisms, these observations support the hypothesis that 'drug' effects and placebo effects are not

necessarily additive. They also suggest that the same magnitude of effect in both arms may involve different mechanisms. The need for more research in the area of mechanisms of placebo versus active responses is also supported.

18.9 Introduction

The placebo-controlled trial is considered the gold standard among clinical research designs. The challenge of rigorous scientific research is to accurately determine the specific effect of an intervention over and above the placebo effect, (also referred to as 'non-specific effects', or 'context effects'). Failure to do so may result in the rejection of the intervention as ineffective as a potential treatment, as any benefits are ascribed to a placebo effect. We question this approach and suggest that inappropriate rejection of potentially viable treatments may be occurring.

The underlying assumption of placebo-controlled trials is that, for participants blinded as to their group assignment, the placebo component affects both/all arms equally, with the specific effect of the active intervention/s being additional to the placebo effect in the intervention arm/s. This has been termed the 'additivity' of effects. However, this assumption has recently been challenged. It has been argued by Kirsch and colleagues[1] that it is not a logically necessity for the effects of the active treatment to be additive, or composed of the two components – the placebo effect and the specific treatment effect (Figure 18.3).

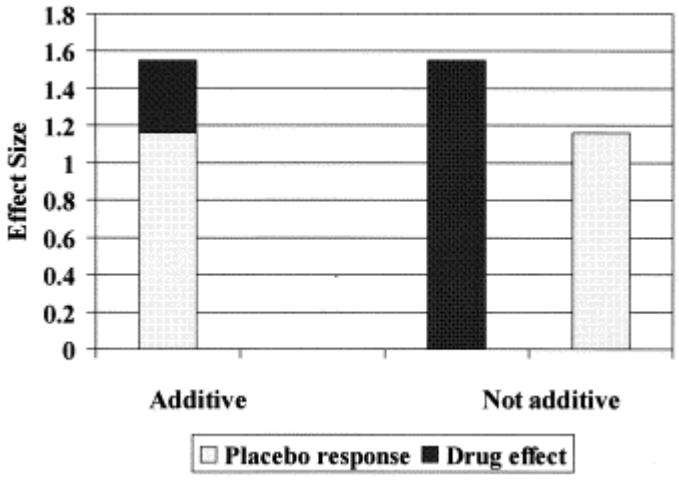


FIGURE 18.3 'DRUG EFFECTS AND THE PLACEBO RESPONSE: ADDITIVE AND NONADDITIVE MODELS.'[1043]
Reprinted by permission of Elsevier from 'Are drug and placebo effects in depression additive? by Kirsch, I. *Biological Psychiatry* **47**(8)**:**733-5. Copyright 2000 by the Society of Biological Psychiatry.

In support of their position they suggest that, if drug effects and placebo effects are additive, then the pharmacological effect of antidepressant drugs must be quite small,[1043] since meta-analyses of antidepressant drugs have found that 65% - 80% of the response to the drug is duplicated in the placebo arm, including in long-term maintenance

studies.[1014,1044,1045] They thus proposed that the effects may be non-additive, or only partially additive,[1043] suggesting different underlying mechanisms.

One obvious conclusion from this observation is that antidepressant medication *does*, in fact, exert a very small pharmacological effect. Another possible explanation that has been raised is that different neurobiological mechanisms may be operating in the two arms. In the absence of a pharmacological effect, the placebo may induce effects via psychological mechanisms, whereas the active treatment works through pharmacological mechanisms.[998] Factors identified that may be responsible for the effects produced by placebos include Pavlovian conditioning resulting from prior exposure to the therapeutic intervention, and the expectation of reward (clinical benefit, in this case).[1104]

In this setting, the current study analysed data from a previously published double-blind, placebo-controlled, RCT that had found no significant difference between the treatment and placebo on any of the endpoints, and compared predictors of the response in the placebo arm with predictors of the response to the active treatment.[848] It was hypothesised that, if the additivitiy assumption is correct, then the same variables would predict the response in both groups.

18.10 Methods

This study was a *post hoc* analysis of data from an investigation of 93 participants who completed a randomised, placebo-controlled, double-blind trial. We have previously published the outcome data on efficacy of the therapy[848] and on predictors of the placebo response in the placebo group only.[1105] Here, we extend this evaluation to examine whether these predictors are also relevant to the treatment arm, and include data from all study participants. The original RCT had investigated the effect of a phytotherapeutic combination consisting of the herbs *Hypericum perforatum* and *Vitex agnus-castus,* for menopausal symptoms in late-perimenopausal and postmenopausal women.[848] Following entry to the study, a two-week non-treatment run-in preceded the 16-week treatment phase. Endpoints included flushing, overall menopausal symptoms measured on the Greene climacteric scale and depressive symptoms measured on the Hamilton Depression Inventory (HDI), both well-validated widely available tools. The trial was approved by the Human Research Ethics Committee at Royal Melbourne Institute of Technology University.

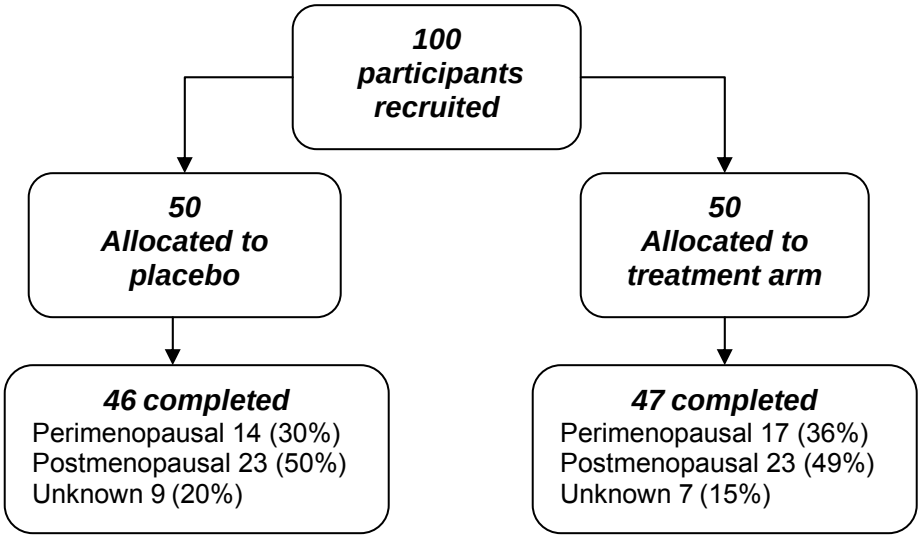
18.10.1 Study intervention

As previously described,[848] two *Vitex agnus-castus* tablets or matching placebos were administered daily, in addition to three *Hypericum perforatum* tablets or matching placebos. The placebos were identical to the herbal tablets in size, colour, coating, weight and packaging. Placebo tablets comprised the excipients used in the active tablets; these were cellulose, modified starch, magnesium stearate and calcium hydrogen-phosphate. The daily dosage of the herbs was 1,000 mg *Vitex agnus-castus*, and 5,400 mg *Hypericum perforatum*. All tablets were manufactured under the Code of Good Manufacturing Practice by MediHerb Australia Pty Ltd.

18.10.2 Participants

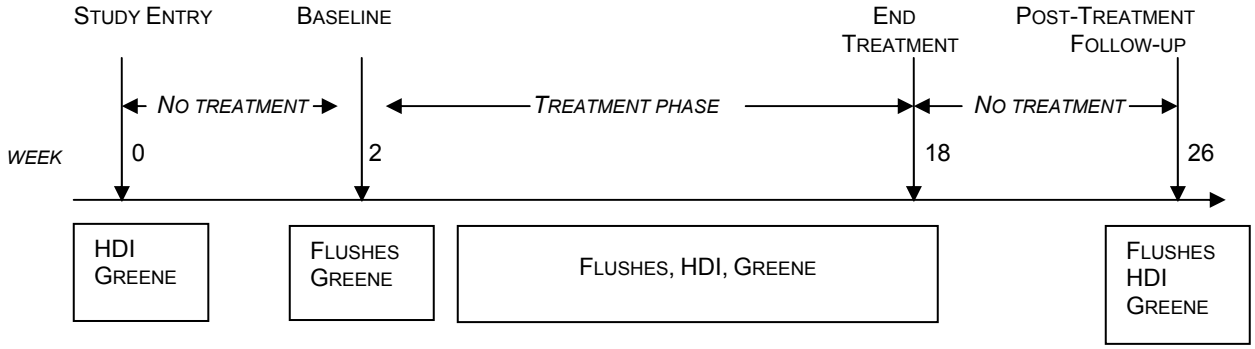
Of the 93 women completing the trial, 47 had been randomised to the active treatment group and 46 to the placebo arm (Figure 18.4). All were late-perimenopausal or postmenopausal women, aged 40 - 60 years. Details of the inclusion and exclusion criteria have been published previously.[848] Women were excluded if taking any medication known to interact with either study herb. Informed consent was obtained prior to study entry. Baseline visits were conducted in a clinic setting and follow-up contact by telephone. Medical clearance was obtained from a general practitioner prior to inclusion in the trial. Participants were requested to maintain their baseline dietary phytoestrogen intake during the trial.

FIGURE 18.4 PARTICIPANT FLOW



Baseline data were collected for a range of variables, as previously published.[848,1105] These were tested individually for their predictive ability. Measures administered at study entry, baseline and end of treatment phase are shown in Figure 18.5.

FIGURE 18.5 TIMELINE OF DATA COLLECTION[8]



18.10.3 Statistical Analyses

Data were analysed using Statistical Package for Social Science (SPSS) Version 16 with the assistance of a biostatistician. In each analysis, hierarchical regression was conducted in order to control for the baseline scores for the relevant outcome measures. Independent variables were firstly assessed individually for their ability to predict the response in each arm on the three separate outcome measures. Response was defined as change in a favourable direction. Based on the results of these hierarchical linear regression analyses, hierarchical multiple regression analyses were then conducted for each endpoint separately, in order to evaluate the predictive power of a set of relevant variables in combination. Because total Greene scale scores and Greene anxiety subscale scores were not independent, these were not entered simultaneously into a multiple regression analysis.

18.11 Results

18.11.1 Hierarchical linear regression analyses

Variables found to have significant predictive ability in the individual linear regression analyses for any of the three endpoints are presented in Table 18.2.

TABLE 18.2 PREDICTORS OF WEEK 16 ENDPOINT SCORES

	Placebo Group *n* = 46						Active Treatment Group *n* = 47					
	Flushes		***Greene Climacteric Scale***		***HDI-17***		***Flushes***		***Greene Climacteric Scale***		***HDI-17***	
	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value
Age at trial start	0.03 (0.28)	0.93	–0.26 (0.24)	0.28	–0.39 (0.17)	0.03	0.38 (0.32)	0.25	–0.21 (0.25)	0.41	0.23 (0.19)	0.24
Previous Herb Med effective	–10.9 (0.07)	0.63	–3.07 (1.84)	0.10	–3.14 (1.37)	0.03	–1.80 (3.04)	0.56	–4.46 (2.40)	0.07	–2.27 (1.88)	0.23
Attitude to menopause	–0.63 (2.18)	0.77	0.66 (1.88)	0.73	–0.24 (1.45)	0.87	–3.30 (2.97)	0.27	–4.54 (2.09)	0.04	–3.16 (1.60)	0.055
Flushes, daily weighted b/l	na		0.08 (0.14)	0.59	–0.03 (0.11)	0.74	na		0.11 (0.12)	0.36	0.12 (0.09)	0.18
Greene score, study entry	–0.53 (0.17)	0.003	–0.43 (0.16)	0.012	–0.38 (0.13)	0.007	0.23 (0.26)	0.37	0.38 (0.26)	0.15	0.36 (0.18)	0.03
Greene anxiety, study entry	–0.97 (0.44)	0.03	–0.82 (0.39)	0.04	–0.33 (0.32)	<.001	1.16 (0.47)	0.016	0.65 (0.44)	0.14	0.68 (0.31)	0.02
HDI–17 score baseline	–0.37 (0.24)	0.14	0.09 (.024)	0.72	na		0.11 (0.34)	0.75	0.25 (0.29)	0.40	na	
Change in Greene scores during non–treatment run–in	–0.02 (0.04)	0.59	–0.15 (0.06)	0.013	–0.07 (0.02)	0.005	–0.04 (0.08)	0.68	0.13 (0.08)	0.13	–0.07 (0.05)	0.15

RESULTS OF HIERARCHICAL LINEAR REGRESSION ANALYSES

Unstandardised β–coefficient (SE) after controlling for baseline scores
b/l = baseline
na: not applicable. These variables were already entered as the first step in the hierarchical linear regression analysis.

Predictors of response identified, after controlling for baseline scores, were as follows.

18.11.2 Anxiety at study entry

As described previously,[1105] anxiety at study entry significantly predicted *placebo* response on all the endpoints individually, with higher anxiety at entry associated with lower anxiety at end of treatment phase: flushes R^2 = 0.33, Std. β = –0.28, p = 0.03; Greene climacteric scale R^2 = 0.24, Std. β = –0.29, p = 0.04; HDI-17 scale R^2 = 0.34, Std. β =–0.66, p < 0.001. For the active treatment group, however, anxiety at study entry predicted *lack* of response for flushing, R^2 = 0.45, Std. β = 0.28 p = 0.02, and depression, measured on the HDI-17. R^2 = 0.28, Std. β = 0.31, p = 0.03.

None of the other predictors of the placebo response was relevant to the response in the active treatment arm (Table 1). In the active treatment group, positive attitude to menopause predicted response on Greene Climacteric scores, R^2 = 0.34, Std. β = -0.27, p = 0.04.

18.11.3 Multiple Regression Analyses

For the placebo group, controlling for baseline scores, simultaneous multiple regression analysis found week 16 Greene Climacteric scores to be predicted by the combination of anxiety at study entry and improvement during run-in on Greene Climacteric score, adjusted R^2 = 0.25, $F(3,42) = 5.91$, p = 0.002, although neither of the predictors provided significant unique predictive variance in the model (Table 18.3). Controlling for baseline scores, HDI at week 16 was predicted by the combination of anxiety at study entry, improvement on Greene score during run-in, age and previous positive experience with phytotherapy, adjusted R^2 = 0.45, $F(5,40) = 8.32$, p < 0.001 (Table 18.3).

TABLE 18.3 PREDICTORS OF ENDPOINTS FOR PLACEBO GROUP

Predictor	***Greene Climacteric Scale***		***HDI-17***	
	β (SE)	*p*-value	β (SE)	*p*-value
Age			–0.11 (0.15)	0.47
Previous Herbal Medicine effective			–2.51 (1.11)	0.03
Greene anxiety, study entry	–0.45 (0.22)	0.30	–1.03 (0.31)	0.002
Change in Greene scores during non–treatment run–in	–0.18 (0.07)	0.08	–0.05 (0.02)	0.01

RESULTS OF MULTIPLE LINEAR REGRESSION ANALYSES, CONTROLLING FOR BASELINE SCORES
Unstandardised β–coefficient (SE)

For the active treatment group, multiple regression analysis was not appropriate, due to the lack of individual predictors of the response.

18.11.4 Baseline severity of scores

As reported previously,[1105] for the placebo group, Pearson's bivariate correlations revealed positive correlations between baseline scores and subsequent percentage improvement during the treatment phase for Greene climacteric scores and the anxiety subscale scores. No relationship between severity of scores and subsequent response was found for any of the endpoints for the active treatment group.

18.12 Discussion

In the current study, there were distinct differences in predictors of outcome observed between the placebo and active groups. Anxiety at study entry predicted *placebo* response for all the endpoints. In contrast, for flushing and depression in the treatment arm, study entry anxiety significantly predicted a *lack* of response to treatment, and had no effect on the Greene Climacteric scores. None of the other variables that predicted the placebo response was relevant to the treatment response. Improvement during non-treatment run-in predicted subsequent improvement during the treatment phase on Greene Climacteric and HDI-17 depression scores for the placebo arm. This trend was not mirrored in the active treatment arm. For depression scores, older age at study entry predicted placebo response, as did prior positive experience with phytotherapy. However neither of these variables significantly impacted on outcomes in the active treatment arm. For the Greene Climacteric scale, baseline severity of symptoms was positively correlated with percentage improvement across the treatment phase in the placebo group, but not in the active treatment group.

To our knowledge, no previous studies have compared the predictors of the response in both the placebo and active arms in a study where the intervention and placebo effects were equivalent in treatment outcomes.

The variance in the predictors of placebo and active response observed in the current study may be related to different underlying mechanisms between placebo and treatment arms. Evidence of neurobiological mechanisms involved in the placebo response is derived from both neuropharmacological studies and brain imaging investigations.[1006,1035,1036,1041,1042] Much of this has focused on pain, where endogenous opioids have been found to be involved in the production of placebo analgesia.[1022,1023]

Placebo-induced dopamine release has also been suggested as a major biochemical substrate for the placebo effect, not only in pain, but also in other conditions such as motor disorders and depression.[1026-1028] Expectation of reward (clinical benefit) has been shown to be at least partly mediated by the dopaminergic system,[1029-1032] stimulation of which may be activated by the brain opioid system.[1033] The release of endogenous opioids may be activated in the anticipatory phase of the placebo response[1039] and hence during therapist-patient or investigator-participant interaction.[1020] Brain-imaging studies using quantitative electroencephalography and PET investigating the effects of antidepressant medication have found that, while both placebo and active treatment exerted nearly equal benefits, the changes in brain function that they induced were quite different.[1034,1035] This calls into question the assertion that 50% -75% of the antidepressant effect consists of placebo effect, since symptom improvement was not associated with a similar physiological alteration in the placebo and medication responder groups.[1036]

The implications of the finding that the predictors of placebo response did not predict the treatment response, despite the lack of difference in efficacy between the two groups, are intriguing. It has previously been suggested that the assumption of additivity of effects that underlies the practice of using placebos may not be a logical necessity.[1043] It is possible that psychological mechanisms may operate in the placebo arm in the absence of pharmacological effects, whereas pharmacological mechanisms are activated by effective interventions, negating the need for activation of psychological mechanisms.[998] Although it is generally accepted that there is a placebo component in the response to the active treatment when participants are blinded, this implies that the pharmacological effects of an active intervention could override this completely or partially. Essentially, the trial participants would experience either placebo or physiological intervention effects, but not both. This would render invalid the assumption that intervention effects are additive to placebo effects. It is supported by the above-mentioned neuroanatomical studies of depressed subjects, showing that placebos and active treatments have quite different effects on the brain, despite exerting similar benefits.[1035,1036] Research on analgesia has suggested that expectation pathways, rather than pain pathways, may be stimulated by placebo treatment.[1020] While no evidence exists, to our knowledge, of this phenomenon in relation to menopause studies, depression measured on the Hamilton Depression Inventory and the Greene Climacteric subscale was one of the current study endpoints. Different mechanisms operating in the two arms cannot, therefore, be ruled out.

It is interesting to note that higher anxiety at study entry was a significant predictor of the placebo response, but predicted *lack* of response to active treatment. This supports the proposition that psychological mechanisms, rather than pharmacological mechanisms, are operating in the placebo response. The observation that improvement during non-treatment run-in predicted *placebo* response on two of the three endpoints, but did not predict *treatment* response, is consistent with the proposal that activation of such mechanisms occurs during the anticipatory phase of the placebo response.[1039] The effect of investigator-participant interaction, and other context effects associated with enrolling in a clinical trial, on psychological mechanisms would be expected to occur from the point of study entry, rather than from the initiation of the intervention.[1020]

Because changes in psychological measures as well as behaviour are expected from the point of enrolment in a clinical trial, s*tudy entry* scores (prior to commencement of run-in) were considered relevant to an investigation of the placebo response. However, pharmacological effects of the intervention would only be observable from the point of administration of the intervention. For that reason, *baseline* scores were controlled for in the analysis (Figure 18.5).

A limitation in the interpretation of these findings is that there is no evidence, to our knowledge, supporting this phytotherapeutic combination is an effective treatment for menopausal symptoms. Therefore, a known pharmacological effect for this intervention in the treatment of menopausal symptoms has never been established.

This study was a *post hoc* analysis of data from an RCT and as such, was not designed to explore neurobiological mechanisms. No definite conclusions can be drawn regarding any different mechanisms of action. Other possible limitations include the relatively small sample size, the use of exclusively subjective outcome measures, and the single scale of measurement for improvement during non-treatment run-in.

18.13 Conclusions

In randomised, placebo-controlled clinical trials, greater understanding of the placebo response is needed to accurately dissect out placebo versus intervention effects. In order to conclude that a pharmacological intervention is ineffective if shown *not* to be superior to placebo, it is essential to be confident that i) the placebo has no specific effect for the condition being examined, and ii) that the effects of the placebo and active intervention are completely additive, so that subtracting the placebo effect from the

treatment effect leaves the active intervention effect. The assumption of additivitiy has been questioned, and early research on neuroanatomical and neurobiological mechanisms, primarily in the area of analgesia, suggests effects of placebo and intervention may differ. The current findings are consistent with the theory of *non*-additivity. In this setting we hypothesise that different mechanisms may be operating in treatment and placebo groups with regard to menopausal symptoms. To further test this hypothesis, comparing predictors of placebo and active responses from other studies showing non-superiority of the intervention may be useful, along with studies exploring underlying mechanisms of placebo action.

This is a very important area for future research, with potentially significant implications for the interpretation of results from placebo-controlled RCTs.

18.14 Additional Analyses

Additional analyses were performed that are not reported in the attached manuscripts. These are included here.

18.14.1 Assumption testing for the hierarchical linear regression analyses

All data were screened for common assumptions of multicollinearity and singularity, normality, linearity, homescedasticity and independence of residuals, and were checked for outliers. No problems were found with multicollinearity for any of the variables investigated in either the placebo or active treatment groups. The assumptions of normality, linearity and homoscedasticity were checked by inspection of the residual scatterplots and the normal probability plots of the regression standardised residuals, which suggested these assumptions were met for all variables examined for both the placebo and active treatment groups.

Inspection of Mahalanbois distances identified three outlying cases (two in placebo group and one in the active-treatment group) for the following analyses.

Flushes	anxiety at study entry (no. 62 –active treatment group, 14.4) total Greene score at study entry (no. 39 - placebo group, 15.07) baseline anxiety (no. 62 –active treatment group, 14.57)
Greene Climacteric	anxiety at study entry (no's 39, & 45 -placebo group, 15.2 and 13.9 respectively) total Greene score at study entry (no's 39 & 45, - placebo group 20.47 and 15.38 respectively) change in scores during lead-in (no 45 –placebo group, 14.98)
HDI-17	total Greene score at study entry (no. 39 - placebo group, 15.14)

Given the size of the data file, it is not unusual for some outliers to appear. Due to the small number of outliers in these analyses, no action was taken.[1106]

18.14.2 Multiple Regression Analyses

Simultaneous multiple regression analyses were reported in the preceding manuscript for each of the three endpoints of flushes, depression (on HDI-17) and overall menopausal symptoms (on Greene Climacteric scale) individually, including the variables found to be significant when entered alone. Bonferroni procedures were not incorporated to adjust the error rate for the number of comparisons, as the Bonferroni-corrected threshold is generally considered too strict for such cases.[1107]

The histogram and scatterplot are included below for the simultaneous multiple regression for HDI-17 scores at week 16 for the placebo group (Figure 18.6).

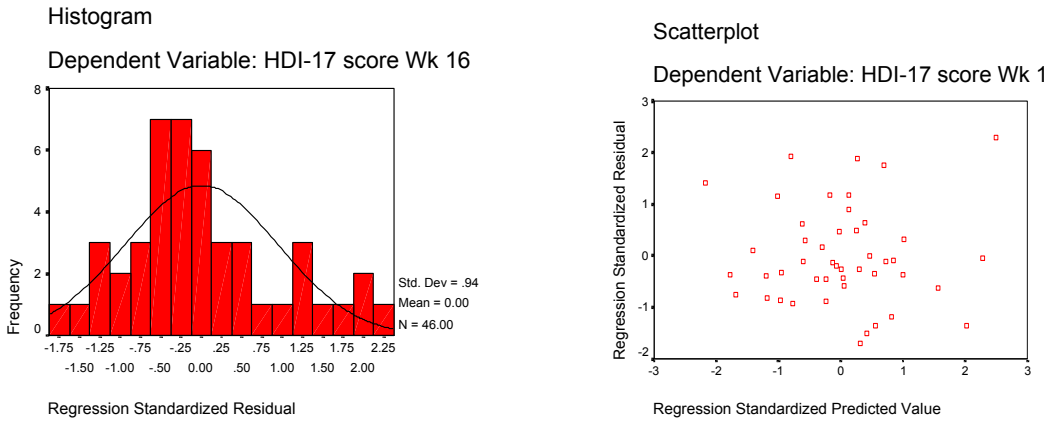


FIGURE 18.6 HISTOGRAM AND SCATTERPLOT FOR SIMULTANEOUS MULTIPLE REGRESSION ON HDI17 AT WEEK 16.

Independent variables entered into model: anxiety at study entry, change in scores during run-in, prior effectiveness of phytotherapy, age, baseline HDI-17 scores. (Whole model was significant at $p < .001$. Age did not provide significant unique predictive variance in the multiple regression models, as indicated by the *t*-tests.).

As previously reported, after controlling for baseline scores, a number of combined characteristics of placebo responders were identified for this HDI-17 at end of treatment. Although age did not provide significant unique predictive variance in the multiple regression model, it was included in the model as it was found to be a significant individual predictor in a linear regression analysis, and is a meaningful predictor. Multiple regression analyses were not appropriate due to the lack of individual predictors of the response.

18.14.3 Multivariate Regression Analyses on Combined Endpoints

In order to determine predictors of the of the multivariate construct formed by combining the three separate measures, a series of individual multivariate hierarchical linear regression analyses were also performed on the three endpoints in combination. Several individual variables were found to predict the outcome in the placebo group. These results have been reported previously. However, a limitation is that the three dependent variables were not independent, as the Greene Climacteric scale includes measures of vasomotor symptoms (flushing) and a depression subscale. For the active treatment group, multivariate regression found no significant predictors of response to treatment.

18.14.4 Logistic Regression Analyses

In the literature, results of logistic regression analyses are usually reported, comparing groups showing 50% or greater improvement with those showing less than 50% response. A 50% reduction in symptoms has become a standard endpoint,[1054] being considered indicative of a clinically significant response to placebo, or active interventions. Therefore, in conformity with this convention, data from this study were also analysed using logistic regression analyses, having collapsed the continuous variables into a categorical variable with two categories, ≥50% improvement on any endpoint versus < 50 % improvement.

Participants were considered to be placebo responders (PRs) if they had at least a 50% reduction in the relevant symptoms.

18.15 Results of Logistic Regression Analyses

18.15.1 Baseline Characteristics of Placebo responders versus Non-responders

Table 18.4 presents the baseline characteristics of the whole placebo group, the group of participants with 50% or more improvement and those with less than 50% improvement. No significant difference was found between groups using independent t-tests and chi square tests on any of the demographic variables or endpoints at baseline.

TABLE 18.4 BASELINE CHARACTERISTICS OF ENROLLED PLACEBO PARTICIPANTS N = 50

MEAN ± SD OR *n*

	Whole Placebo Group† *n* = 50	Placebo responders* *n* = 27	Placebo non-responders *n* = 19
Menopausal status: Unknown	10 (20%)	6 (22%)	3 (16%)
Perimenopausal	16 (32%)	7 (26%)	7 (37%)
Postmenopausal	24 (48%)	14 (52%)	9 (47%)
Age at trial start (years)	52·5 ± 3·8	53.0 ± 4	52.0 ± 3.7
BMI	26·2 ± 5·0	25.6 ± 4.4	26.3 ± 5.5
Months since last period	48·6 ± 61·9	47.4 ± 47.7	45.2 ± 67.9
Months since onset of symptoms	57·3 ± 44·7	62.2 ± 51.4	45.9 ± 31.6
Ambient temperature baseline (°C)	20.7 ± 4.5	20.3 ± 4.4	19.7 ± 6
Ambient temperature week 16 (°C)	19.9 ± 3.2 (*n* = 46)	19.2 ± 3.1	20.9 ± 3.1
Dietary & Lifestyle variables MEAN ± SD			
Caffeine: tea/coffee p/day (cups)	4·2 ± 2·1	4.2 ± 2.4	4.2 ± 1.6
Cigarettes per day	2·0 ± 5·8	2.2 ± 5.2	2.1 ± 7.1
Standard alcoholic drinks/wk	3·6 ± 3·7	4.1 ± 4.2	3.0 ± 2.8
Isoflavone intake per week in mg	76·6 ± 114·3	70.3 ± 119	81.5 ± 116.2
Lignans in linseeds, gm p/wk	0·1 ± 0·2	0.1 ± 0.3	0.0 ± 0.1
Exercise – hours per week	3·7 ± 3·7	3.5 ± 4.0	4.1 ± 3.6
Total exercise days per week	3·9 ± 2·6	3.7 ± 2.5	4.3 ± 2.6
Dietary & Lifestyle variables *n (%)*			
Vegetarian	3 (6%)	1 (4%)	2 (11%)
Prior herbal medicine use	37 (74%)	18 (67%)	16 (84%)
Prior positive effect of herbs (% users)	19 (51%)	12 (44%)	5 (26%)
Prior HT use	15 (30%)	6 (22%)	7 (37%)
Prior positive effect of HT (% users)	13 (87%)	5 (83%)	7 (100%)
Relationships satisfaction	44 (88%)	24 (89%)	18 (95%)
Negative attitude to menopause	27 (54%)	15 (56%)	10 (53%)
Regular stress reduction, *n*	14 (28%)	10 (37%)	4 (21%)
Nulliparity, *n*	13 (26%)	5 (19%)	7 (37%)
Employed outside home, *n*	40 (80%)	20 (74%)	16 (84%)
Endpoints MEAN ± SD			
Flushing episodes (per day)	9·4 ± 3·3	9.4 ± 3.6	9.8 ± 2.8
Flushing, daily weighted score	16·4 ± 6·7	15.8 ± 6.8	17.7 ± 6.6
Greene score baseline	22·5 ± 8·7	23.2 ± 9.5	22.7 ± 8.2
Greene anxiety baseline	6.4 ± 2.7	6.8 ± 2.9	6.2 ± 2.6
Greene depression baseline	5.1 ± 2.7	5.1 ± 2.6	5.3 ± 3.1
Greene psychological baseline	11.5 ± 4.9	11.9 ± 5.1	11.5 ± 5.1
Greene score study entry	26.5 ± 5.8	27.9 ± 6.6	25.4 ± 4.5
Greene anxiety study entry	7.6 ± 2.4	8.3 ± 2.6	7.0 ± 1.7
Greene depression study entry	6.6 ± 2.4	6.8 ± 2.8	6.2 ± 1.9
Greene psychological study entry	14.2(4.1)	15.1(4.7)	13.2(3.1)
HDI-17 score	14·3 ± 4·3	14.9 ± 14.2	13.8 ± 4.7
Utian Quality of Life scale	77·8 ± 12·.1	80.3 ± 12.5	76.5 ± 11.2

† includes participants who subsequently withdrew

* Placebo responders includes participants who showed a 50% or greater response on any of the endpoints of flushes, Greene Climacteric scores or Hamilton Depression Inventory scores

18.15.2 Percentage of Responders by Menopausal Status

The percentage of responders in either the placebo or treatment arms was not related to menopausal status (Table 18.3 above and Table 18.4).

TABLE 18.4 PERCENTAGE OF RESPONDERS BY MENOPAUSAL STATUS FOR ACTIVE TREATMENT GROUP

	Whole Active Group† *n* = 50	Responders* *n* = 26	Non-responders *n* = 21
Menopausal status: Unknown	8(16%)	5 (19%)	2 (10%)
Perimenopausal	17 (34%)	9 (35%)	8 (38%)
Postmenopausal	25 (50%)	12 (46%)	11 (52%)

18.15.3 Placebo group

Results of logistic regression analysis for the placebo group are shown here.

Anxiety at Study Entry

Anxiety at study entry predicted ≥50% improvement on flushes, $p = 0.049$, and on the Greene Climacteric scale, $p = 0.02$.

Total Greene scores at Study Entry

Total Greene scores at study entry predicted ≥50% improvement on the Greene Climacteric scale, $p = 0.003$.

Improvement during non-treatment run-in

Improvement during non-treatment run-in, measured on total Greene Climacteric Scale scores, $p = 0.02$, and the Greene anxiety subscale, $p = 0.04$, predicted improvement of ≥50% on the HDI -17depression scale during the treatment phase.

Prior Positive Experience with Phytotherapy

Prior positive experience with phytotherapy predicted improvement on HDI-17 scores for the placebo group.

Multiple Logistic Regression and Depression scores

Although not all providing unique predictive ability, the combination of anxiety at study entry, improvement on Greene score during run-in, age and previous positive experience

with phytotherapy was predictive of week 16 HDI-17 scores in the placebo group, using multiple logistic regression analysis, χ^2 (4, N = 46) = 9.74, p = 0.045.

18.15.4 Active treatment group

Logistic regression and Depression scores

Low anxiety at study entry predicted ≥50% improvement on HDI-17 scores, p = 0.02. Similarly, baseline anxiety (at end of run-in) negatively predicted HDI-17 scores at week 16, p = 0.001.

Multiple Logistic Regression and Depression scores

Multiple logistic regression analysis found the combination of anxiety at study entry, prior positive experience with phytomedicines, and age to predict week 16 HDI-17 scores, χ^2 (3, N = 46) = 8.05, p = 0.045. Lower age, lower anxiety scores and previous positive experience with phytomedicines were associated with lower scores at week 16 on the depression scale. However, age and prior positive experience failed to provide significant unique predictive variance in the logistic regression model.

18.16 Discussion

In conformity with the convention of analysing data using logistic regression analyses, data from the continuous variables in this study were collapsed into categorical variables with two categories, 50% or greater improvement on any endpoint versus less than 50 % improvement. The percentage of responders to placebo or active treatment did not differ according to menopausal status. For the placebo group, logistic regression analyses found flushing and Greene Climacteric Scores to be predicted by anxiety at study entry, consistent with findings from linear regression analyses. Unlike linear regression analysis, however, anxiety at study entry did not predict the week 16 HDI-17 scores. Total Greene Climacteric scale scores at study entry, which was shown to predict the placebo response on all three endpoints using linear regression analyses, was only relevant for the Greene Climacteric scale scores. This is consistent with the finding reported previously that more severe baseline scores were positively correlated with percentage improvement on this scale. Improvement during run-in continued to be predictive of week 16 HDI-17 scores. However, it was not predictive of improvements on Greene Climacteric scores at week 16, contrary to the findings from the linear regression analyses. The finding that improvement on HDI-17 scores for the placebo group was predicted by prior positive experience with phytotherapy was consistent with the results

of linear regression analysis. However, age was no longer a significant predictor of HDI-17 scores. The results of multiple regression analysis for the placebo group were consistent with those of multiple linear regression analysis for HDI-178 scores, although the individual variables were not predictive, with the exception of greater improvement during run-in.

For the active treatment group, the finding that low anxiety at study entry predicted improvement on HDI-17 scores was consistent with findings from the linear regression analysis, as were the lack of predictive ability of change in scores during run-in, prior positive experience with phytotherapy for depression scores. No significant predictors were identified using logistic regression analysis for flushing or Greene Climacteric scores, unlike linear regression results. Multiple logistic regression analysis found the combination of lower age, lower anxiety scores and previous positive experience with phytomedicines to predict improvement on the depression scale, although age and prior positive experience failed to provide significant unique predictive variance in the logistic regression model.

18.17 Conclusion

The results of logistic regression analyses are more conservative than those obtained from linear regression analyses due to the loss of power than results from dichotomising continuous variables. The arbitrariness of the cut-off point of 50% as representing a clinically significant improvement, in conjunction with the loss of power when using logistic regression analyses, do not support the convention of conducting logistic regression analyses on continuous variables.

Chapter Nineteen

General Discussion and Conclusions

19.0 Introduction

Relevant discussion of the work conducted has been included in the manuscript chapters in the body of this thesis. An overview is presented here, followed by the study conclusions and future directions.

19.1 Background

With increased life expectancy and population growth, growing numbers of women are now reaching menopause, a significant cause of problematic symptoms that can impact on a woman's quality of life for many years. CAM is the preferred treatment approach to the management of their symptoms by increasing numbers of midlife and menopausal women with concerns about the publicised risks or contraindications associated with the conventional treatments, and by those who prefer a natural approach for a natural event. While a wide range of phytotherapeutic interventions is promoted and administered for the management of menopause-related symptoms, evidence to support their use from rigorous scientific research is limited, with most having focussed on the phytoestrogenic options. However, treatment of symptoms is often required long-term, and safety concerns have now been raised over the long-term use of the phytoestrogen plants. Research is thus needed into other non-oestrogenic phytotherapies employed in clinical practice for the amelioration of menopause-related symptoms. Various CNS mechanisms have been proposed for the herbs selected for the current study, suggesting potential benefit for the problematic vasomotor symptoms and psychological symptoms via the interaction between neurotransmitters and hormones, in a similar fashion to the central nervous system (CNS)-acting pharmaceutical agents with demonstrated efficacy for symptom relief.

19.2 Aims and Objectives

The primary aim of the study was to investigate the effects of the combination of extracts of *Hypericum perforatum* and *Vitex agnus-castus* on the symptoms of flushing and sweating, psychological symptoms and quality of life in late-perimenopausal and early-post-menopausal women. Secondary aims were to study the effect of this combination in PMS-like symptoms in late-perimenopausal women, and to examine predictors of the placebo response, comparing these with predictors of the response to active treatment.

19.3 Principal Findings

The herbal combination (*Hypericum perforatum* and *Vitex agnus*-castus) was not found to be superior to placebo for any of the endpoints - daily weighted flushing scores, scores on the Greene Climacteric scale and the Hamilton Depression Inventory, although significant improvements across the treatment phase were observed in both the placebo and active treatment groups on all of these outcome measures. No significant

change was found for either group on the Utian quality of life scale. The herbal combination was well-tolerated with no significant adverse events noted over the 16-week treatment phase. Prior use of herbal medicine modified the effect, with the herb-naïve group showing a significantly greater response overall and to the active treatment for flushing scores than the prior users. Previous positive experience with phytotherapies predicted overall percentage improvement in depression, anxiety-subscale scores and sleep for the whole sample, but did not differ between the active and placebo groups.

On the PMS-like symptoms experienced by a small sub-population of late-perimenopausal women, the intervention was found to be superior to placebo for total PMS-like symptoms and the sub-clusters, PMS-D (depression) and PMS-C (cravings). The active treatment group also showed significant improvements on PMS-A (anxiety) and PMS-H (hydration), although this effect was not superior to placebo.

Predictors of the placebo response were found to include anxiety at study entry for the outcome measures of flushing, depression and overall menopausal symptoms (measured on the Greene Climacteric scale), and improvement during non-treatment run-in for depression and overall symptoms. In contrast, *low* anxiety was significantly associated with improvement in flushing and depression in the active treatment group. None of the other variables found to predict the placebo response was relevant to the treatment arm. Milder baseline symptom severity did not predict improvement, although more severe scores on the Green Climacteric scale at baseline were associated with a greater placebo response for this measure.

19.4 Comparison with other Studies

Comparisons between these findings and those of other researchers have been presented in the relevant manuscript chapters. These are synthesised here.

The phytotherapeutic combination used in the present study had not previously been investigated in the context of menopausal symptoms. While not directly comparable, the present study, reporting a 32% overall reduction in symptoms on the Greene Climacteric scale, did not support the findings of an observational study[18] on *Hypericum perforatum* that found a 63% reduction in overall menopause symptoms. Factors potentially contributing to the differing findings include lack of consistency in nomenclature regarding menopausal phases, the rating scales employed, ambient temperatures and extracts administered. The comparator study with diazepam outlined previously found a

77.5% reduction in scores on the clinician-rated CGI scale with *Hyperforat*® 0.6 mg daily[48], which is far in excess of the 39% reduction seen in the current study on the HDI-17. However, other studies have found significant effects over placebo, while achieving a *treatment* effect comparable to the current study's placebo effect. A RCT examining the combination of *H. perforatum* with *C. racemosa* for menopausal symptoms, found a 41.8% decrease on the HDRS total score,[716] which achieved significance over the 12.7% reduction with placebo ($p < 0.001$). The magnitude of the treatment effect achieved in the above study is comparable to the improvement in HDI-17 scores with *placebo* in the current study, namely a 41% reduction (and 46% for the clinically depressed subgroup). Similarly, in another RCT of Remifemin plus®, (a combination of *Hypericum perforatum* and *Cimicifuga racemosa),* menopausal symptoms rated on the KI fell by 40%, which was found to be significantly superior to placebo, $p < 0.001$. In the current study, the reduction in scores in the Greene Climacteric scale was 40% for the placebo group.

In comparison with the studies examining *Vitex* for menopausal symptoms, a 73% reduction in symptoms was observed with a formulation containing *Vitex,* compared with 38% in the placebo group.[793] As previously discussed, however, the study is of uncertain relevance due to the number and combination of herbs, including *Cimicifuga racemosa* (black cohosh), in the formulation. Findings from other studies on *Vitex* as a sole agent for menopause include a pilot observational study,[823] and aromatherapy studies,[821-823] which reported treatment effects similar to the placebo effect reported in the current study, namely a 42% reduction in vasomotor symptoms,[823] and 33% and 36% of women reporting major and mild improvements in symptoms respectively.[822] (The current study reported a 41% reduction in flushing symptoms).

The lack of superiority of the combination in depressive symptoms is not consistent with the many positive trials of St John's wort in this context.[920,930] The finding of effect modification of prior use of herbal medicine is consistent with findings of a previous group of CAM researchers.[849]

19.4.1 Sub-population analyses

The observations from the present study support results from previous studies on the efficacy of *Vitex agnus-castus* in overall PMS symptoms,[481,827,850,852-854] as well as pilot studies with *Hypericum perforatum* in PMS, particularly for depression-related symptoms.[911,913]

No studies could be found in the literature of predictors of the placebo response in the context of menopausal symptoms. However, the finding that anxiety at study entry predicted the placebo response supported findings from some studies of analgesics and antidepressants that report predictors to include co-morbid anxiety[1058] and high level neuroticism including depression and anxiety.[1057] However, this is not a uniform finding, with *lower* anxiety being a significant characteristic of placebo responders in one investigation of depressive disorders.[1059] The concomitant improvement in anxiety and other menopausal symptoms in the placebo group is consistent with the role of anxiety in triggering hot flushes in menopausal women,[91] and the salutary effect of relaxation techniques in this context.[1102,1103] The current study observed improvement during non-treatment run-in (on overall menopausal symptoms) to predict subsequent improvement in Greene Climacteric scale scores and HDI-17 depression scores to week 16. This contrasts with findings in relation to synthetic antidepressant medications that limited change or worsening of symptoms of depression, anxiety or melancholia during placebo lead-in treatment predicted the placebo response.[1061] These conflicting findings may represent the different conditions under investigation. The neurovegetative *symptoms* associated with menopause do not necessarily constitute clinical depressive *syndromes*, and are prone to follow a fluctuating course due to their natural history. The value of a non-treatment run-in period in identifying placebo responders is supported by this study, contrary to previous findings that such a practice makes no difference.[1] In terms of baseline symptom severity as a predictor of the placebo response, several studies have found *milder* symptoms to be predictive.[1046,1051,518] In the current study, baseline severity of symptoms did not predict response to placebo for flushes or depression, possibly due to recruitment of women with *moderately* severe symptoms. The observation that more severe scores on the Greene Climacteric scale predicted placebo response may be largely attributable to the impact of the anxiety subscale. No evidence could be found of other investigators having compared predictors of the placebo and treatment responses where superiority was not demonstrated.

19.5 Interpretation of Findings

There are several possible reasons for the lack of superiority of the phytotherapeutic combination in relation to the menopausal symptoms under investigation. The first is that this combination is, in fact, ineffective in this context, and that these herbs differ from the synthetic non-estrogenic drugs in terms of potency or mechanisms of action. As their pharmacology has not yet been fully elucidated, the latter is a possibility. An antagonistic

interaction between the two herbs is unlikely based on the current knowledge of their pharmacology. Secondly, given that the response was, nonetheless, equivalent to the treatment response observed in other RCTs demonstrating superiority over placebo, the substantial placebo effect on all the endpoints may have contributed to the negative result. Compared with pharmaceutical interventions, phytomedicines can produce less pronounced effects, which suggests that their effects may be overshadowed by substantial placebo responses, as are not uncommon in menopause or CAM research. This is supported by the observation that the effect persisted in the active treatment group at the week 26 follow-up (8 weeks post-treatment), but not in the placebo arm. The RCT design, where participants have a 50% expectation of being assigned to the active intervention, is suggested to maximise the placebo response.[1027] Thirdly, symptom severity at baseline may have been inadequate to demonstrate a substantial effect. In terms of depression, this is a distinct possibility as only 37 women were *clinically* depressed at baseline. However, there is an important distinction between depressive *syndromes* detected by instruments such as the HDI, and depressive *symptoms*, with the latter considered to be more relevant in association with menopause.[37] It is unlikely that the other negative findings were attributable to inadequate baseline symptom severity, as hot flushes were as severe as baseline scores from previous positive studies,[23,28] and overall menopausal symptoms measured by the Greene climacteric scale were consistent with a menopause clinic sample.[26] The fourth possibility is that, as suggested in chapter 14, different mechanisms are operating in the treatment and placebo arms, and that these effects are non-additive. This is discussed further below.

The effect of previous positive experience with phytotherapy in predicting improvements in depression, anxiety and sleep suggest that these parameters are more susceptible to the placebo effect, and supports the contribution of expectation to the placebo response.[38] The finding of effect modification of positive previous experience with phytotherapy in relation to depression is unremarkable, given the reputation of St John's wort in the context of mild-moderate depression. However, it highlights the questionable validity in phytotherapy research of the assumption of equipoise (indifference towards the treatment on the part of both the investigator and participant), which is central to the RCT paradigm, as both the investigator and participants are liable to be influenced by the existing body of traditional knowledge. Effect modification of *lack* of previous use of herbal medicine is not readily explained, however, but is unlikely to reflect negative bias among the prior users, as this would not support their voluntary enrolment in a CAM trial.

The findings in relation to PMS-like symptoms were derived from a very small sample and must, therefore, be interpreted with caution. Nonetheless, they suggest a potential role for the phytotherapeutic combination of *Hypericum perforatum* and *Vitex agnus-castus* in the amelioration of these symptoms in perimenopausal women, supported by the data from the individual who menstruated monthly throughout the trial (n = 1). While no firm conclusions can be drawn, this finding does tend to support the suggestion that symptoms in perimenopausal women may be more appropriately attributed to menstrual cyclicity than to menopause *per se.* Although current evidence suggests that ovulation is a pre-requisite for PMS, which is therefore relatively uncommon during late perimenopause, a different aetiology for these symptoms in this demographic is possible.

The observation that predictors of the placebo response did not predict the response in the treatment arm, despite the equivalence in the magnitude of the response in both arms, may be explained is several ways. Firstly, this analysis is based on a very small sample, which makes any conclusions tentative. Secondly, as mentioned above, it may support the suggestion that placebo and treatment effects are non-additive. It is suggested that, in the absence of pharmacological effects, the 'inert' placebo may be activating expectation pathways due to psychological mechanisms, while the active treatment elicits pharmacological effects that activate a different pathway. Thirdly, it is possible that unique placebo-responder characteristics do not exist, and that these findings occurred by chance.

19.6 Strengths of Study

Overall, the RCT was robustly designed, and conducted according to rigorous scientific practice, features lacking in much of the CAM research. The study had clearly defined endpoints with validated scales, which are commonly employed in RCTs, for the menopausal symptoms, depression and quality of life measures. It was adequately powered with excellent retention rates and compliance, and evidence for the success of blinding. The population recruited had moderately severe overall symptoms. Control of confounders was incorporated by maintenance of baseline dietary intake of phytoestrogen-containing foods, diet and lifestyle monitoring and statistical adjustment of data, where relevant, according to the between-group differences. A non-treatment run-in period was included to eliminate non-compliers, and also to account to some extent for the Hawthorne effect and behaviour modification that may potentially enhance the magnitude of the placebo response. The trial duration of 16-weeks was designed to allow for the wash-out of the placebo response. The intervention trialled was produced

with high quality assurance levels, was well-tolerated and there was an absence of noteworthy adverse-events.

19.6.1 Retention rates

Despite the intensity of involvement in the trial in terms of counting flushing episodes day and night for a total of 19 weeks and completing questionnaires on a 4-week basis, retention rates and compliance with procedures were excellent. Factors that may have contributed to these include the contact with a single investigator, regular contact with the investigator (including the initial contact after 1-week of taking tablets) without being excessively frequent, a limited number of face-to-face visits that required travel to a clinic, the choice of 3 locations for interviews and tablet distribution, follow-up contacts being conducted by telephone and weekend and after hours availability of the investigator for follow-up contact. Rapport with the investigator, an experienced practitioner of phytotherapy, may have contributed to retention rates as well as compliance. This may have been facilitated by the regular updates sent out to participants regarding the progress of the trial, as recruitment continued for 16 months. Clarity of instructions on questionnaires may have impacted on compliance. This suggests that attention to convenience for participants, the frequency of follow-up contacts and rapport with the investigator may be important to successful retention of the participant group, and compliance with procedures.

19.7 Limitations of Study

There are some limitations to this study design and methods.

The main limitations with the classical gold standard RCT for phytotherapy research are i) that substantial placebo effects may overshadow subtle effects of herbs, and thereby lead to the inappropriate rejection of potentially valuable treatments due to type II errors; and, ii) that it does not reflect the clinical practice of individualising treatment regimens in phytotherapy or the 'whole–person' approach, as distinct from 'dis-ease' treatment. An additional limitation with the current study in this regard is that it was limited to a combination of two herbs whereas formulations of a greater number of herbs are usually prescribed in clinical practice. It is obviously not possible to extrapolate these findings to formulations containing a greater number of herbs. The contribution of the two study herbs to such formulations cannot be gauged from the findings of the current study due to the synergistic effects of herbs in combination.[1108-1111] (Nor is it possible to establish any effect attributable to either of the individual herbs, although an adverse interaction

between the two herbs in the combination seems unlikely based on their known mechanisms of action.)

Because the original power calculation was done with an estimated 30% placebo response, based on other CAM trials in the literature at the time, it could be argued that this study lacked adequate power to detect any effect of the intervention over placebo. However, given that the placebo group tended to slightly outperform the active group on the endpoint of vasomotor symptoms, overall menopausal symptoms and depression, this not likely to have impacted on the findings.

This study employed only subjective measures, which are not considered to be very robust endpoints. Furthermore, the severity of symptoms recorded depended on the day the participant completes the questionnaires. However, as the intended outcome of treatment for menopausal symptoms is a woman's own subjective assessment of her quality of life, measuring change in subjectively-assessed symptoms and quality of life is clinically relevant. (This is also true of many conditions treated with phytomedicines in general, particularly given the preference for the majority of users to self-prescribe OTC remedies and to self-diagnose and self-assess.[1112,1113] In Europe, over-the-counter use of herbal remedies outstrips practitioner prescriptions probably by a factor of more than 20:1.[1112] In addition, because the selected herbs were non-oestrogenic, post-treatment hormone assays and mammography were not considered relevant. As both herbs have previously been studied reasonably extensively in other conditions, liver function tests for safety monitoring purposes were not regarded as essential, particularly in consideration of the limited available resources.

It is possible that some of the current instruments might not detect some clinically important changes in symptoms. For example, anecdotal reports from some participants suggest that the duration and intensity of heat experienced with flushing episodes were reduced, but the measures of frequency and intensity used in the trial did not reflect these changes. In relation to the depressive symptoms, as mentioned previously, it has been suggested that scales for diagnosing and measuring depression *syndromes* may not be sufficiently sensitive for measuring the depressive symptoms experienced in association with menopause. A refined version of the CES-D that attempts to achieve more diagnostic specificity has been proposed as a simple but sensitive means for diagnosing and measuring depressive disorders associated with menopause.[447] However, the current study incorporated the depression subscale of the Greene

Climacteric scale in addition to the HDI as instruments of measurement. Moreover, measurement artefacts caused by unreliability of instruments affects both groups equally.

Assessment of compliance with procedures could only be assessed by the participants' reports. While this appeared to be excellent in this study, lack of instruments to record flushes meant relying on women to record them, which introduces another element of uncertainty and potential imprecision in the data. Compliance with taking study medications was assessed by counting the tablets returned by participants and assumes all were returned. However, this reflects clinical practice, in which assessments are made in accordance with participants' reports of symptoms and disclosure of medications used.

Threats to internal validity that may have operated in this study include regression to the mean, the natural history of the symptoms, subject effects such as auto-correlation and the Hawthorne effect, experimenter effects and experimenter expectancy. Because the symptoms under investigation follow a fluctuating course, and are eventually self-limiting, the impact of natural history is undoubtedly substantial in studies of menopause-related symptoms. The principal investigator, who was the sole contact person, due to limitation of resources, is an experienced, practising phytotherapist. Experimenter expectation operated in relation the St John's wort for the depressive symptoms, although not with regard to the impact of the combination on the other endpoints. However, experimenter expectancy affects both arms equally. The reputation of St John's wort for mild-moderate depression is well-known and is likely to have resulted in participant expectations on this endpoint. Experimenter effects may have impacted inasmuch as the length of follow-up contacts was not restricted, but varied according to the needs of the individual participants. Hence, the personal contact, and experience of being listened to may have affected the outcome. Auto-correlation may have occurred as participants became more aware of triggers of their symptoms through monitoring them so closely, and modified their behaviour accordingly, for example by adjusting their caffeine or alcohol intake, or engaging in stress reduction techniques. As their behaviour modification impacted favourably on their symptoms, the motivation to avoid triggers may have declined. However, dietary and lifestyle practices were monitored through the 4-weekly questionnaires included. The decision to enrol in a clinical trial can coincide with a determination to take control of the symptoms, and hence be accompanied by other health-enhancement practices. Changes made during the trial were recorded on the Diet and Lifestyle questionnaire, and the 2-week non-treatment run-in prior to baseline

took account of any behaviour modification occurring immediately after acceptance into the study. While these are considered nuisance factors in a study aiming to determine the effects of an intervention, they are all features of clinical practice.

Other limitations with interpreting the outcomes of RCTs include the following. Clinical trials involve homogenisation of patient populations, so that only an average effect is confirmed. This means that genuine benefit for a minority of patients will be overlooked.[1114] RCTs are only externally valid for the types of patients included in the trial. Inclusion criteria may sometimes be so stringent that the results are not generalisable to the population as a whole. It has been suggested that this may particularly be relevant to studies of phytomedicines and other CAM interventions, in view of the type of conditions treated. These tend to be conditions involving a lesser degree of morbidity, lower grade of symptoms and/or more variable symptom patterns, which results in the need to recruit larger samples (a requirement not always met), and often devise artificial exclusion criteria to constrain sample variability.[1114] While enhancing internal validity, experimental control can jeopardise external validity (the 'real world' relevance of the findings, across time, populations and setting) by default.[1115]

The relevance of findings derived from RCTs to the clinical setting, as well as to certain questions being investigated by phytotherapy/CAM research, has been questioned. These issues will be explored below.

19.8 Implications

There are several implications of the present research for RCTs, phytotherapeutic/CAM research and for clinical practice.

The substantial response in the placebo arm observed in RCTs of menopause is at least partly attributable to the natural history of the symptoms. Without the inclusion of a natural history arm, which would further add to the demands of resources, it is not possible to determine the magnitude of the actual placebo *effect* (or context effects). Nonetheless, a substantial placebo effect is clearly a major nuisance in RCTs of menopausal symptoms, particularly for phytotherapeutic interventions, which may produce milder effects than synthetic pharmaceutical agents and thus be overshadowed by context effects. (This has also been observed in a study of SSRIs in mood disorders.) Alternative paradigms may be needed for examining the effects of such interventions, and conditions found to elicit substantial placebo effects.

Attempts to control the placebo response in clinical trials have included identification of the unique characteristics of placebo responders. The findings from the current study that improvement during non-treatment run-in and anxiety at study entry predicted the placebo response are both of importance in the design of future RCTs on menopausal symptoms. Excluding volunteers from the sample who have been thus identified as placebo-responders could restrict the magnitude of the placebo response. However, it may result in loss of power and bias the sample, making it non-representative of the population, and thereby limit the generalisability of the findings. An alternative may be to stratify the study sample by anxiety, and to ensure recruitment of sufficient numbers of participants for adequate power. Data from highly anxious participants could be analysed separately. The practice of a lead-in prior to baseline is supported by these findings, as is the recommendation for a minimum symptom severity for inclusion when recruiting for clinical trials.[30]

The observation that predictors of the responses in the placebo and treatment groups differed has potential implications. As discussed above, it may imply that unique placebo characteristics do not exist. However, the alternative explanation of different mechanisms operating in the two arms that are mutually exclusive, while not derived from the findings of the current study, have potentially significant implications for the interpretation of results from placebo-controlled RCTs as a whole, which rest on the assumption of additivitiy of effects. As acknowledged previously, however, this was based on data from a small sample and should therefore be interpreted cautiously. However, it suggests that this assumption underlying the RCT design warrants further investigation.

The findings of effect modification of lack of previous use of herbal medicine on flushing symptoms warrants further investigation, through separate analysis of data from prior-users. This has potential implications for the design of future studies, and should be considered when analysing results of studies. (However, recruitment of participants with no prior experience with CAM would severely restrict the available pool of participants for CAM studies.) The effect modification of positive previous experience with phytotherapy for depression suggests that the known efficacy of St John's wort in this context may have impacted here. This effect of positive expectation in enhancing treatment effects is well-established, and highlights the importance of appropriate paradigms for exploring CAM, as inappropriate negative findings have the potential to undermine clinical effects

of treatments. This finding needs to be replicated in other studies, and could have implications for future research as well as clinical practice. Factors contributing to effect modification are of relevance to the design and interpretation of findings from research.

While the findings regarding the combination of *Hypericum perforatum* and *Vitex agnus-castus* in PMS-like symptoms should be interpreted with caution until replicated, they suggest a potentially significant clinical application in symptoms that are largely-neglected in perimenopausal women. The combination warrants further investigation in a dedicated study of PMS-like symptoms with a larger sample of women. The observation that women who continued to menstruate intermittently throughout the trial, and experience associated PMS-like symptoms, had higher scores on all the outcome measures than both those who did not and the post menopausal group (except for sexual subscale) supports the suggestion that some symptoms in the perimenopause may be more related to menstrual cyclicity than menopause.

19.8.1 Clinical Implications of Findings

Despite the lack of superiority over placebo, both arms showed a statistically significant reduction in symptoms on the endpoints of flushing, overall menopausal symptoms and depression. As pointed out by Koshi,[1033] to say that a treatment is no better than placebo does not mean that it does nothing. This raises the question of what constitutes a clinically meaningful effect. It is impossible to ascertain the impact of natural history in the current study. However, the magnitude of the effect achieved may suggest the potential for a non-pharmacological intervention that could be of benefit to women. A lesser reduction in symptoms without risk of adverse events may be considered worthwhile. For the active treatment group, medium-to-large effect sizes were associated with each of the endpoints.

However, less than half of the participants experienced 50% or greater reduction in symptoms with the phytotherapeutic intervention (43·5% for hot flushes; 23·4% for overall menopausal symptoms; 44·7% for depression), which would appear to indicate a need for different, more effective alternatives, especially in view of the cost to the individual of phytomedicines, and the disruption to everyday life of many menopausal symptoms. Nonetheless, a number of participants continued to self-prescribe these two herbs after completion of the study, suggesting that at least some considered the benefits to outweigh the costs. The question of efficacy versus effectiveness is further discussed below.

The finding that anxiety predicted the response to placebo suggests that menopausal symptoms in women with high anxiety could be ameliorated by non-pharmacological interventions as a first line of treatment. This is supported by the observation that anxiety has been associated with an aggravation of menopausal symptoms, such as flushing.[27]

The phytotherapeutic combination investigated here may have potential application in the management of PMS-like symptoms experienced by perimenopausal women who are still menstruating intermittently. Targeting PMS-like symptoms in these women, rather than menopausal symptoms, may be an appropriate treatment strategy generally. However, this requires further investigation.

It appears that some women on the study benefited from factors unrelated to the possibility of taking an active intervention. Some expressed appreciation at having someone taking an interest in their symptoms. On admission to the study, some retirees expressed the need for social contact and even gratitude at the opportunity to make a useful contribution to something worthwhile, and to feel needed. Several commented retrospectively on the value of having observed their symptoms so closely in enabling them to identify triggers of their symptoms. All of these factors could contribute to an enhanced placebo response, and support the health-promoting benefits of enrolment in a clinical trial. Clinically, however, these anecdotes suggest that support, being listened to, personal contact and avoidance of trigger factors may improve outcomes. For women who are willing to engage in behaviour change and/or seek out additional sources of support, these may constitute valuable components of a multi-factorial approach to the management of their symptoms.

19.9 Directions for Future research

There are several important factors to emerge from the current study that may inform future research. These are explored below.

The need for rigorous evidence to support the practice of phytotherapy is incontrovertible, not only to substantiate safety and efficacy claims as required by regulatory bodies, but also to clear up the confusion generated by conflicting information in circulation. This is not to deny the value of traditional knowledge from hundreds or thousands of years of use. However, researching phytotherapeutic and other CAM

interventions in a way that is scientifically rigorous, that avoids inappropriate rejection of potentially valuable treatments through false negative results (Type II errors), and reflects clinical practice is one of the biggest challenges facing evidence-based CAM. While the RCT is the paradigm of choice when attempting to isolate and measure specific effects of a treatment on selected outcomes, substantial placebo effects, or 'context'-effects', may overshadow specific treatment effects and thus need to be controlled in clinical trials. However, in clinical practice, the question arises as to whether it is ethical to ignore these 'non-specific' effects, or whether they might constitute a valid component that enhances outcomes of complex CAM interventions. As pointed out by Walach and colleagues (p39),[1116] the question of specific efficacy is largely irrelevant to patients who are essentially interested in having a good chance of getting better. It has thus been suggested that the RCT paradigm may not be appropriate for examining the overall impact of potentially important complex interventions such as phytotherapy/CAM aimed at stimulating the organism's self-healing without relying on one specific effect. Alternative methods that may be more suitable for measuring the effectiveness of a 'whole system' treatment will be mentioned briefly below. However, as the objective of the current study was to assess the specific effect of a particular combination of phytomedicines, the RCT was the appropriate design.

19.9.1 Relevance of the RCT to the Clinical Setting

The role of expectation in the placebo response has been discussed in the previous chapters. In attempting to extrapolate the results of RCT to the clinic, the central assumption is that the research setting simulates the clinical setting. However, in the latter context, the patient has a 100% expectation of receiving a proven active treatment, compared with the classic 2-armed RCT where the participant has a 50% expectation of receiving the active treatment. The effect of different expectations was compared in an interesting experiment by Kirsch and Weixel involving the deceptive administration of 'coffee'.[1117] Certainty of having received an active intervention with known physiological effects (coffee) did, in fact, elicit the expected effects, despite the participants having actually received 'sham' (decaffeinated) coffee. In contrast, the "double blind" administration consistently produced placebo response curves that were approximate mirror images of those produced by deceptive administration, even on relatively independent subjective and physiological responses. In this example, a substance with well-known physiological effects was being tested, which may have impacted on the changes elicited. However, other studies have also found expectation of benefit to

improve outcomes,[1015] and even perceived assignment to be more powerful than actual assignment.[1020,1033]

In contrast, the expectation model of de la Fuente Fernandez and co-workers[1027] suggests that placebo responses are *maximised* in 2-armed RCTs investigating disorders where dopamine release plays a role. This is because such a design has an *a priori* probability of clinical benefit of 0.5, and a tonic (sustained) activation of dopamine neurons (which precedes a reward and seems to reflect uncertainty) is maximal when the probability of reward = 0.5 (29% of dopamine neurons show increases in activity). As mentioned previously, placebo-induced dopamine release in response to the expectation of reward appears to represent a common biochemical substrate in many conditions.[1026] This suggests that the placebo effect may be greater in clinical trials of such conditions than in the usual clinical practice, in which the probability of receiving an active treatment is 1,[1027] and thus raises doubts about the validity of the RCT to the clinical setting in many medical conditions. This is represented diagrammatically in figure 19.1.

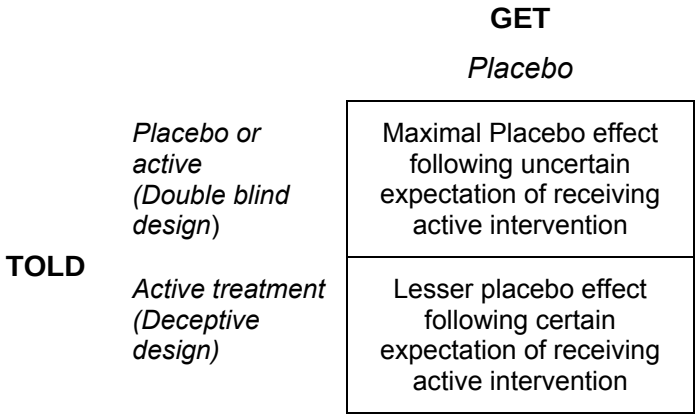


FIGURE 19.1 DOUBLE BLIND VERSUS DECEPTIVE DESIGN. Modulation of placebo effects according to expectation can be measured. The double blind design produces uncertain expectations whereas the deceptive design produces certain expectations. Adapted from Colloca *et al.* 2008.[1042]

In the open-hidden paradigm, higher treatment effects are elicited during the open than the hidden paradigm,[1033] suggesting that knowledge that one may be taking placebo detracts from the overall effects of treatment as well. Taking these findings together, it is possible that enhanced placebo effects *in conjunction with* reduced treatment effects are found in the RCT, thus artificially narrowing the margin between the placebo and treatment arms.

19.9.2 Alternative designs

Alternative designs have been proposed in order to control the confounding effects elicited by the context or setting in which the intervention is administered. The influence of expectation is especially important in this regard.

The open-hidden paradigm proposed by Benedetti, where applicable, provides an effective means of eliminating the placebo or 'context' effects, and the opportunity to gauge the magnitude of the placebo effect without using a placebo. It also overcomes some of the ethical constraints associated with inclusion of a placebo arm. However, it is not applicable to most studies of phytomedicines as whole agents, as the hidden administration requires the patient to be completely unaware that it is being given. As such, it is not relevant to interventions that are administered orally, or that require longer-term administration before a noticeable effect can be expected, as is the case with chronic conditions.

The balanced placebo design (figure 19.2) is a 2 x 2 design that involves deception, formulated by Ross and colleagues in 1962.[1118] Participants are assigned to active or placebo arms, with half in each arm being told their allocation while the other half are deceived:

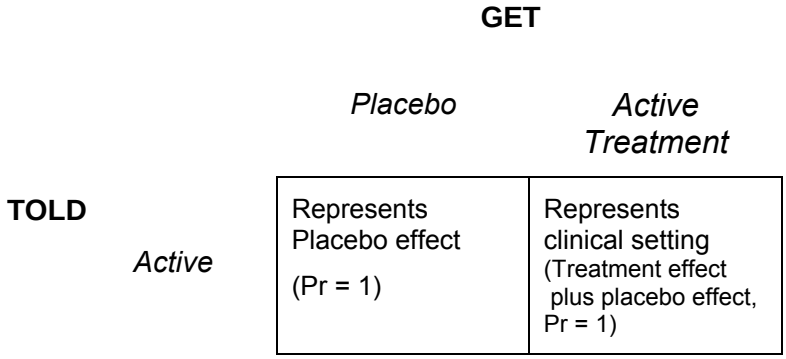
		GET	
		Placebo	*Active Treatment*
TOLD	*Placebo*	Represents Natural History	Represents Treatment Effect (Pr = 0)
	Active	Represents Placebo effect	Represents clinical setting (Treatment effect plus placebo effect, Pr = 1)

Pr = probability of receiving treatment, as perceived by the participant

FIGURE 19.2 BALANCED PLACEBO DESIGN showing what the participants are administered versus what they are told they are being administered. This allows investigators to identify the modulation of an intervention by expectation of benefit. Adapted from Colloca *et al.* 2008.[1042]

Although an ethical limitation is that this design involves deception, it does overcome the limitation of potentially maximal placebo effects caused by the perceived probability of receiving treatment being 0.5, as in the double-blind design. However, a treatment effect accompanied by the expectation of *no* benefit is totally irrelevant to the clinical setting. It also provides information on the natural history of the symptoms against which both placebo and treatment effects can be measured. Practically, however, it does add to the demands on resources in terms of sample size required, and may reduce compliance among those informed they have been assigned to placebo.

Although also entailing deception, an obvious ethical constraint, another possibility for creating equal expectations and levelling the placebo response among the whole group (with regard to expectation, at least) is an RCT where all participants are told that they are receiving the active treatment (figure 19.3). The placebo effect could then be subtracted from the response to active treatment in order to gauge the treatment effect. However, it is unlikely that any research would elicit an 'expectation' response equivalent in magnitude to that seen in the clinical setting, as research, by its very nature, involves uncertainty regarding treatment outcomes, and is thereby subject to different placebo effects from clinical practice, according to the theories outlined above.



Pr = probability of receiving treatment, as perceived by the participant

FIGURE 19.3 RCT INVOLVING DECEPTION. The differing modulation of effects according to expectation is controlled by informing all participants they are receiving the active treatment.

As mentioned previously, the RCT answers the question, "Is this intervention better than placebo?" However, a more appropriate question may be "Is this intervention better than other options available for these women who are unwilling to use HT?" This suggests that comparator studies with non-oestrogenic options or other phytomedicines may provide useful information, although a placebo arm would still be required due to the magnitude of this effect in menopause studies. The importance of examining effect sizes is also highlighted in this context.

19.9.3 Relevance of RCTs to phytotherapy/CAM approaches

There is a noticeable discrepancy between the reports of substantial clinical benefits from CAM practitioners and users [1068,1119,1120] and results of many of the published studies on CAM interventions. Even well-designed RCTs often fail to show superiority over placebo, which raises doubts about the appropriateness of this paradigm to answer questions about the often-complex treatment approaches of CAM.

Placebo-controlled trials have several underlying assumptions that have now been questioned, especially in relation to CAM research. The assumption that the results of RCTs are clinically relevant has been discussed above. As mentioned above, the assumption of equipoise, which assumes that participants and providers are indifferent, with no beliefs or preferences for a treatment, is also questionable in relation to phytomedicines, for which there exists a large body of traditional knowledge. This means that the influence of participant expectations needs to be investigated in relation to the effects that occur. Furthermore, it is assumed there is a stable effect size that is independent of the context. However, because pharmacological effects can sometimes change dramatically according to context effects such as expectation, it is suggested that psychological and pharmacological effects may be inseparable.[1115] In a 1994 review of the interaction between specific effects and context effects, Kleijen and colleagues concluded that specific and non-specific effects may sometimes be synergistic and at other times antagonistic, which suggests that "the implicit additive model of the RCT is too simple."[1121] Another assumption is that only the specific effects attributable to interventions are therapeutically valid, which leads to the conclusion drawn from RCTs that a therapeutic intervention is deemed effective only if it performs better than placebo. However, the distinction between efficacy and effectiveness has been elaborated by Walach in what he terms the 'efficacy paradox' (figure 19.4).[1115,1122] A treatment that is *efficacious* (having shown statistical superiority to placebo) can be less *effective* than another treatment that did *not* demonstrate efficacy.

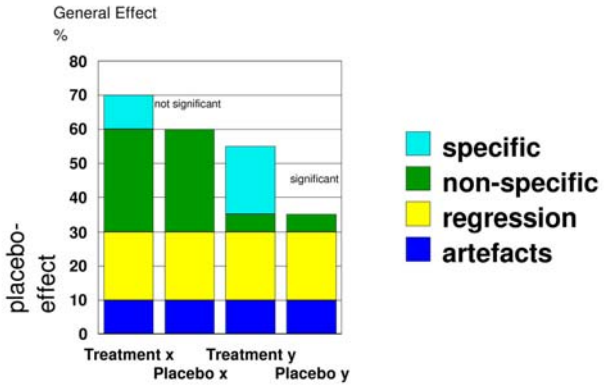


FIGURE 19.4 ILLUSTRATION OF THE EFFICACY PARADOX.

From Walach H. *et al.* Circular instead of hierarchical: methodological principles for the evaluation of complex interventions. *BMC Med Res Methodol* 2006;**6:**29. (No permission required)

This raises the question of patient outcomes. As pointed out by Jonas, "research should tell us what is useful and meaningful in patients' lives."[1123] To ascertain the clinical relevance to patients of the intervention, it is useful to elicit retrospective participant ratings of the treatment (for example, as Ineffective, Moderately effective, Good, Very good, Excellent).

The notion that only specific effects over and above placebo effects are worth demonstrating, and worth achieving has been termed the 'placebo trap'.[1122] But in relation to complex interventions as are studied in CAM research, it may not be meaningful to split into specific effects and context effects. It may be more meaningful to investigate the effectiveness of a 'whole system' treatment.[1124] In this case, research strategies need to be diversified to include multiple methods and to replicate results achieved with one method by another. Suitable protocols could include pragmatic trials, in which treatment conditions closely simulate those of the clinical setting and which could therefore accommodate individualised treatments;[1013] large outcomes studies; or pragmatically-selected comparative cohort studies in natural settings to address selection process.[1122] Where it is sufficient to document the effects as different from the natural course of the disorder, randomised comparison trials of CAM therapies against standard care or against waiting lists would be an option as they permit quantification of general therapeutic effects.[1122] Partially randomised trial designs allow patients to express a preference for the treatment allocated, and those with no preference to be randomised. The effects of receiving a treatment of choice can be measured in this case, and if the sample is sufficiently large, separate analyses can be performed for the

randomised and non-randomised groups.[1125] It is also suggested the where specific effects are likely to be small, but whole treatment effects large, due to the complex interaction of specific and non-specific effects, the RCT is inadvisable.[1115] In order to reflect the practice of individualised prescribing, a methodologically rigorous protocol including three arms has been designed and trialled by Bensoussan.[1096] In addition to the standard treatment and placebo arms, an individualised treatment arm is included, which has the advantage of allowing individualised care to be evaluated. With regard to phytomedicines, an alternative methodology that may provide valuable information is the observation of the physiological effects of treatment in healthy human subjects, rather than its direct effects on morbidity.[1114] Measuring objective parameters can be useful where these are reliable and expected to change over a reasonable course of time.

19.9.4 Recommendations

Future advances in this area will further elucidate the neuroanatomical and neurobiological mechanisms of the placebo response, and confirm or disprove the underlying assumptions of placebo-controlled trials. In order to further investigate the non-additivitiy hypothesis,[1043,1121] the possibility that different mechanisms may be operating in treatment and placebo groups with regard to menopausal symptoms, comparing predictors of placebo and active responses from other studies showing non-superiority of the intervention may be useful. (This assumes, of course, that unique placebo characteristics *do* exist.) The findings that improvement during non-treatment run-in and anxiety predicted the placebo response in the current study are of importance in the design of future RCTs on menopausal symptoms. Identification of the characteristics of placebo responders may be used to inform strategies to limit the magnitude of the placebo response. This could be achieved by excluding such individuals (without impacting on recruitment of adequate sample size), stratifying according to these variables, and/or performing separate analyses of data from these participants. Controlling the magnitude of the placebo response may facilitate accurate determination of the specific effects of interventions. These findings need to be replicated in a larger study.

Effect modification of lack of prior use of phytomedicines on flushing symptoms, and previous positive experience with phytotherapy in depression, anxiety and sleep also warrants further investigation, and should potentially be taken into account when designing and analysing data from future studies.

In future studies, the modifying effect of rapport could be monitored by including a measure of therapeutic alliance between the participant and investigator. In order to restrict the magnitude of context effects elicited by investigator effects, and hence enhance the internal validity, tablets and questionnaires might be posted out to participants, although this does restrict the external validity of the results.

Future advances in the areas of the endocrinology and neuroendocrinology of menopause and its associated symptoms, as well as PMS, will further elucidate the aetiology of the bothersome symptoms experienced throughout the menopause transition and post-menopause, including the PMS-like symptoms experienced during the perimenopause.

This may point the way to alternative targeted management options that are well-tolerated by symptomatic menopausal women. The potential benefit on PMS-like symptoms indicated for the phytotherapeutic combination investigated in the current study warrants further investigation in a RCT involving a larger sample of women, in a study dedicated to those specific symptoms. Given the evidence supporting *Vitex* in this context, inclusion of an additional arm to compare the effects of the combination with *Vitex* as a sole agent and placebo could yield potentially valuable information.

Conclusion

The current study aimed to investigate the efficacy of a combination of two non-oestrogenic medicinal herbs in the clinical management of menopausal flushing and psychological symptoms, as well as general menopausal symptoms and quality of life. Subpopulation analyses of the data investigated: i) the effects of the phytotherapeutic combination on PMS-like symptoms in a small group of irregularly menstruating perimenopausal women; and, ii) predictors of the placebo response, which were then compared with predictors of the response to active treatment.

19.10 Summary of Findings

Conclusions from the individual studies have been reported in the respective chapters describing the findings incorporated into this thesis. To summarise, the principal findings were as follows.

- The combination was not found to be superior to placebo for any of the endpoints measured. However, both groups improved significantly on the endpoints of flushing, depression and general menopausal symptoms. A substantial placebo response was observed, consistent with other RCTs on menopausal symptoms, and depression.
- Effect modification was observed for lack of prior use of phytotherapy on flushing scores. Previous positive experience with phytotherapy predicted overall percentage improvement in depression and anxiety-subscale scores for the sample as a whole.
- The placebo response in the menopausal symptoms study was predicted by anxiety at study entry for all three outcome measures. Improvement during non-treatment run-in predicted placebo response on depression and overall menopausal symptoms. Milder symptom severity at baseline was not predictive of subsequent improvement.
- The hypothesis that the same predictors would predict the effect in both arms, due to the lack of superiority of the phytotherapeutic combination over placebo, was not supported. The trend for anxiety was the opposite in the active treatment group, where *low* anxiety was significantly associated with improvement. None of the other variables found to predict the placebo response was relevant to the treatment arm.

- The combination was found to be superior to placebo for PMS-like symptoms in the small sub-population of late-perimenopausal women for whom this was relevant.

These findings have implications for future research directions and trial design in CAM, as well as clinical practice.

19.11 General Recommendations

General recommendations include the following.

- The substantial placebo effect observed in studies of menopausal symptoms renders a control or comparator group essential, as observational studies are likely to be susceptible to type I errors. However, the less pronounced effects of phytomedicines, compared with HT and other pharmaceutical options, may necessitate prohibitively large sample sizes in placebo-controlled RCTs in order to achieve adequate power. For this reason, alternative research paradigms may be more appropriate for researching phytomedicines in this context.
- In order to accurately assess the efficacy and safety of phytotherapies in a way that is scientifically rigorous and reflects clinical practice of phytotherapy, alternative research paradigms are needed. For the purposes of simulating the whole person approach, the design trialled by Bensoussan using an additional 'individualised treatment' arm may be appropriate. Studies investigating herbal *combinations*, while not permitting conclusions to be drawn about the individual contributions of the component herbs, more closely reflect clinical practice.
- The findings of effect modification of lack of previous experience on flushing, and positive previous experience with phytotherapy for depression and anxiety-subscale scores warrant further investigation. These could have implications for future research and should potentially be considered when analysing results of studies.
- Future studies of potential interventions for menopausal symptoms may use the trial findings for early identification of placebo responders, according to high anxiety at study entry and improvement during non-treatment run-in. Investigators may be able to incorporate strategies to limit the magnitude of the placebo response, and thereby facilitate accurate determination of the specific effects of the intervention/s. This could be achieved by excluding such individuals (with recruitment of additional participants as necessary), stratifying

according to these variables, and/or performing separate analyses of data from these participants. These findings need to be replicated in a larger study.

- In order to restrict the placebo response rates observed with milder baseline symptom severity, the recruitment of participants with a minimum of moderately severe symptoms is recommended in RCTs examining menopausal symptoms. This requirement is supported by the findings of the current study.
- To further test the hypothesis that different mechanisms may be operating in treatment and placebo groups with regard to menopausal symptoms, predictors of placebo and active responses could be compared from other studies that show non-superiority of the intervention over placebo. Further studies exploring underlying mechanisms of placebo action are also needed to examine this hypothesis.
- Future developments in the area will contribute to greater understanding of the endocrinology and neuroendocrinology of symptoms in the perimenopause, which is required to establish their relationship to PMS-like symptoms in the late-luteal phase.
- Further research into the effects of this phytotherapeutic intervention in a robust study of PMS and PMS-like symptoms in the perimenopause, with a larger sample size is warranted.

19.12 Overall conclusion

The RCT described throughout this thesis was a well-designed, robust study of a phytotherapeutic intervention in the management of symptoms in perimenopausal and postmenopausal women. Because many women wish to avoid therapy with synthetic pharmaceutical agents for these symptoms, there is a significant need for safe, well-tolerated, effective alternatives that have been validated by results from controlled clinical trials. The findings from the current study contribute to the growing body of available evidence regarding phytomedicines from rigorous RCTs, in addition to highlighting some areas for future research into the symptoms experienced during the perimenopause, the placebo effect and paradigms for future research into phytomedicines and other CAM modalities. In view of the demand for phytotherapeutic treatments for menopausal symptoms, this is a very important topic that is relevant to the community, health-care providers and regulatory authorities.

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APPENDICES IN SEPARATE FILE

Appendices

The Effects of *Hypericum perforatum* with *Vitex agnus-castus* in the treatment of Menopausal Symptoms

A.0 Introduction

The appendices contain additional information and analyses related to the preceding chapters, the manuscript entitled '*Vitex agnus-castus* (Chaste-tree/berry) in the Treatment of Menopause-related Complaints', submitted to the *Journal of Alternative and Complementary Medicine*, and all documentation relating to the randomised, controlled trial.

Raw data is available on request from Diana van Die at diana.vandie@rmit.edu.au

Appendix 1 Age at Menopause

A1.1 Cross-cultural Findings regarding Age at Menopause

An international WHO collaborative study reported a median age of 50 years (range 49 – 52) among 18,997 women from 11 countries (Australia, Chile, People's Republic of China, Colombia, the former German Democratic Republic, Israel, Kenya, Mexico, Nigeria, the Philippines and Thailand). Limitations of this study included selection bias, as the women were hospital controls in a multi-centre case-control study of female cancers and may therefore have been of higher socio-economic status giving them more access to hospital care. Additionally, the median age of the women in the sample was 40 years so not all had reached menopause, which was therefore estimated using a survival analysis approach.[†] [2]

On the whole, existing studies show similar findings with regard to age at menopause as data from the European studies selected for the WHO Scientific Group report. This showed that the mean age ranged from 47.5 – 49.5, with a median range of 49.6 - 51.4.[3] Countries reporting mean ages outside these ranges are marked with an asterisk*.

A somewhat lower age at menopause was observed among asymptomatic Mayan women of Mexico and the Indian women of Himachal Pradesh. However, it is possible that the inconsistent findings could be due to the methodological limitations of the research, most of which has been retrospective or cross-sectional and subject to errors of recall, biased reporting of age, as well as sampling error, such as selection bias, or to the use of inappropriate statistical techniques.

Additionally, most did not control for confounding variables that could potentially affect age at natural menopause.

[†] Survival analysis aims to follow subjects over time and observe at which point in time they experience the event of interest. It often happens that the study does not span enough time in order to observe the event for all the subjects in the study. Survival analysis therefore provides methods for estimating the distribution of survival times from a sample.[1] 1. UCLA. Academic Technology Services: www.ats.ucla.edu/stat/sas/seminars/sas_survival/default.htm.

TABLE A1 ESTIMATES OF THE AGE AT MENOPAUSE FROM SELECTED STUDIES

Country and Year of Study/ Publication	Mean or Median age at menopause (years)	Sampling Method/ age/setting
Brazil 2003[4]	51.2 (mean)	cross-sectional survey
Canary Islands 1994 [5]	48.6 (mean)	retrospective
Czechoslovkia 1975[6]	51.2 (mean)	status quo method[††]
Finland 1989[7]	51 (mean)	cross-sectional survey
*India, Himachal Pradesh 1987[8]	43.6 (mean)	unspecified
*India, Northern 2005 [9]	46.7 (mean) 48 (median)	cross-sectional survey obstetrics & gynecology clinic
Iran 2005[10]	48.3(mean) 49(median)	cross-sectional survey
Japan 1987[11]	49.3 (mean)	cross-sectional survey of 28-85 year-olds
*Kenya, West[Luhya] 1997[12]	4838 (median)	cross-sectional survey
Latin America [13]	49.4 (mean)	cross-sectional survey (health centre)
Malaysia 1994[14]	50.7 (mean)	unspecified
*Mexico (Mayan, asymptomatic) 1993[15]	44.3 (median)	cross-sectional survey
*Mexico city 1996[16]	47 (median) 46.5(mean)	cross-sectional survey (natural menopause- retrospective)
Pakistan, Lahore 2002[17]	50 (median) 49 (mean)	cross-sectional survey
Poland 2007 [18]	51.3 (median)	cross-sectional survey
Puerto Rica, 2003[19]	51.4 (median)	cross-sectional survey 30-59 year-olds
Singapore 2005[20]	49.0 mean	cross-sectional survey
South Africa [negro] 1984[21]	49.5 rural; 48.9 urban (means)	status quo method†† with probit analysis[‡]
Taiwan 1997[22]	49.5 mean	unspecified
Thai 1993[23]	49.5 (mean)	cross-sectional survey health centres
*UAE 1998[24]	48 (median) 47.3 (mean)	stratified cluster sampling
USA 1997[25]	51.5 (49.3 Afro-American)	prospective

A1.2 Factors Influencing Age at Menopause

Factors that may influence the age at menopause have been the focus of much investigation. Social and environmental factors that have been found in some studies to impact on earlier age at menopause include

- current cigarette smoking[26-31]
- increased body mass index (BMI)[26,27]

[††] The status quo method is considered to be a simple method with little bias. It provides a sample or population estimate based on the age of each woman sampled and whether or not she has reached menopause. A table is then constructed with a percentage of *Yes/No* answers at each age. From this, the median age at menopause, standard deviation and standard error are calculated using either probit or logit transformation.

[‡] Probit analysis is an alternative to logistic regression analysis for producing predicted probabilities. In this case, the age-specific prevalence of the menopausal state is transformed to a probit scale, and these probits are then plotted against age.

- current use of weight reduction diets[25]
- no prior use of oral contraceptives[31,32]
- nulliparity/fewer children[32-34 31]
- lower level of education[28,31]
- lower socio-economic status[35]
- low social participation[28]

Many of these could potentially affect the hypothalamic-pituitary-gonadal axis and its regulation of gonadotropins and sex steroid hormones.[31] This will be further elaborated in the section on the endocrinology of menopause.

Of the above factors, cigarette smoking has most consistently been associated with a 1-2 year earlier natural menopause.[26-31] Large-scale cross-sectional studies such as the Study of Women's Health Across the Nation (SWAN) and the Oslo Health Study, found that *current* smoking was significantly associated with early menopause whereas the toxic effects of past smoking on the ovary could be reversible,[30] with stopping smoking more than 10 years before menopause considerably reducing the risk.[28] While not borne out by the findings of the SWAN,[31] data from some studies suggest a possible dose-response relationship between total exposure to smoking and early menopause, [28,29] even when controlling for possible confounding variables such as parity, weight, socio-economic status, nutritional variables and lower educational attainment. [29] The Oslo study found no association with passive smoking, alcohol or coffee consumption, although such correlations had been suggested in earlier research.[36,37]

Increased BMI has also been shown to be associated with an early menopause in some studies,[26,27] although others found low relative weight to correlate. However it is also possible that lifestyle factors such as increased BMI may not only be the cause, but also the *consequence* of an early menopause, which cannot be differentiated in cross-sectional studies.

Additional factors associated with earlier menopause in the SWAN study were being separated, widowed or divorced, non-employment and history of heart disease, while Japanese ethnicity among Americans was associated with a later age. Adverse socio-economic circumstances across the life course are associated with an earlier age at menopause but this may co-vary with nutrition in childhood,[35] smoking and physical activity.

In terms of reproductive history, shorter intermenstrual intervals during the reproductive years,[38] or limited number of ovulatory cycles per lifetime[39] and irregular cycles prior to age 25[32] have been linked to earlier menopause.

Earlier onset of menopause can also follow surgical procedures such as tubal ligation or hysterectomy, surgical removal of the ovaries, and damage to the remaining follicles by radiation, chemotherapy or other endocrine-disrupting agents.[40]

A1.3 Implications of Earlier Age at Menopause

Age at natural menopause may be an important marker for subsequent morbidity and mortality. Earlier age at natural menopause has been linked with increased risk of coronary heart disease and stroke[41,42] as well as atherosclerosis and osteoporosis[43] (although the number of reproductive years is thought to be more relevant in determining osteoporosis risk than is age at menopause).[43,44] Later age at menopause has been associated with an increased risk of cancer-related mortality,[45] such as breast, endometrial and ovarian cancers.[46-51] The relationship with oestrogen-dependent cancer risk may be due to the duration of exposure to high circulating oestrogen levels as a result of cumulative number of menstrual cycles per lifetime.[52] Later age at menopause has, however, also been linked to a longer overall survival rate and increased life expectancy.[45,53,54]

Appendix 2 Cohort Studies on Symptoms in Midlife and Menopausal women

Descriptions of cohort studies on midlife and menopausal women are given in Table A2 along with location and principal investigators.

TABLE A2 DESCRIPTIONS OF COHORT STUDIES Adapted from *NLM Health Services Technology Assessment Text [electronic resource] / National Library of Medicine.* Bethesda, MD. : National Library of Medicine, 2002[55]

Cohort	*Description*	*Investigators/authors*
Copenhagen	630 women 40 years old in 1976 selected from 4 municipalities and served by one hospital in Copenhagen, Denmark. Women using postmenopausal hormone therapy or who underwent premature or surgical menopause were excluded.	Koster, Garde
Eindhoven	8,503 women recruited based on responses to an osteoporosis screening study in Eindhoven, Netherlands. Women were excluded if non-Dutch White, had hysterectomy with or without oophorectomy, had bilateral oophorectomy, are using postmenopausal hormone therapy, or were noncompliant with one or more items in the questionnaire.	Maartens, Knottenerus, Pop
Gothenburg	1,462 women 38 to 60 years old randomly selected in 1968-69 and followed for over 25 years in Gothenburg, Sweden. Measures were assessed through periodic medical exams and interviews.	Hallstrom, Rodstra, Samuelsson
The Harvard Study of Moods and Cycles	A longitudinal, prospective cohort study that followed 460 premenopausal women as they entered perimenopause	Harlow, Cohen, Otto, Soares, Spiegelman, Cramer, Wise, Otto,
Manitoba Project on Women and Their Health in the Middle Years	A cross-sectional and longitudinal cohort study that enrolled 2,500 women aged 40–59 years old from the general population of Manitoba, Canada.	Kaufert, Milsom, Gilbert, Tate
Massachusetts Women's Health Study	Cross-sectional survey 1981-2 with 8,050 women aged 45-55; and longitudinal study that enrolled 2,572 premenopausal women from Massachusetts, U.S.	Avis, Brambilla, McKinlay, , Crawford, Posner, Vass
Medical Research Council (MRC) National Survey for Health and Development	1,572 women identified from a socially stratified sample of all births in March 1943 in Britain.	Kuh, Hardy, Wadsworth, Cardazo, Rogers
Melbourne Women's Midlife Health Project	Cross-sectional and longitudinal, community-based cohort that enrolled 2,000 women 45 to 55 years old from Melbourne, Australia. Longitudinal follow up of 492 women has been ongoing since 1991. The study assesses women's health during midlife and the menopause including well being, sexuality, symptoms, and bone density and their relationship to a range of variables including hormones, age, stress, lifestyle, and other health experiences.	Dennerstein, Dudley, Burger, Lehert, Sherburn, Guthrie, Hopper, Morse, Smith

Minnesota/Tremin Trust Longitudinal Research Program on Women's Health	Longitudinal study that initially consisted of 2,350 University of Minnesota women. Between 1961-1963, a second group of 1,600 women was added, and in 1965, a third group of 1,000 native Alaskan women were added. Women in the study range from teens to mid-nineties and represent fifty states and twenty-five foreign countries.	Koch, Mansfield, Voda
National Health Examination Follow-up Study	Sample of 3,049 U.S. women 40 to 60 years old from the National Health and Nutrition Examination Survey (NHANES).	Busch, Zonderman, Costa
Ohio Midlife Women's Study	208 women 40 to 60 years old, recruited with advertisements from a community in Ohio, U.S. Cohort includes 57% Caucasian, 43% African-American women.	Glazer, Zeller, Delumba, L. Kalinyak, Hobfoll, Winchell, Hartman
Pennsylvania Ovarian Aging Study	Population-based cohort of 436 women from Philadelphia County, Pennsylvania, 35–47 years old at baseline including 50% African American, 50% Caucasian. Ongoing longitudinal study.	Freeman, Gracia, Sammel, Lin, Kapoor, Ferdousi, Liu, Nelson, Hollander
Seattle Midlife Women's Health Study	11,222 women from within Seattle, U.S. census tracts, 35 to 55 years old, including multiple ethnicities and income levels and followed for 3 years. All women had active menstrual periods at baseline and were excluded if pregnant, using postmenopausal hormones, or non-English reading/speaking.	Mitchell, Woods, Mariella, Lentz,
Study of Women's Health Across the Nation (SWAN)	U.S., community-based, multisite cross-sectional and longitudinal cohort study that enrolled 16,065 women 40 to 55 years old. Longitudinal follow up of 3,306 women has been ongoing since 1995. The goal of the study is to describe the chronology of the biological and psychosocial characteristics of the menopausal transition and its effects on health and risk factors for age-related chronic diseases.	Bromberger, Avis, Matthews, Sowers, Crawford, Gold, Kravitz, Randolf, Cordal, Schott, Brockwell, Everson-Rose, Colvin, Ory, Schocken

Appendix 3 Physiology of the Normal Menstrual Cycle

During the reproductive years, normal cyclic ovarian function depends on the coordinated activity of a feedback system involving the hypothalamic-pituitary-ovarian axis (referred to as the HPO axis) with associated structural and functional changes in the target tissues, the ovaries and uterus. Each cycle culminates in menstrual bleeding, the first day of which is accepted as a clinical reference point marking the beginning of the menstrual cycle.[56]

Central to the mechanisms of this feedback system are gonadotrophin releasing hormone (GnRH) secreted by the hypothalamus in a pulsatile fashion, modulated by neurotransmitters, including noradrenaline, serotonin, and endogenous opioids;[57] the gonadotrophins, follicle stimulating hormone (FSH) and luteinising hormone (LH) secreted by the anterior pituitary; ovarian hormones: the sex steroids oestradiol and progesterone; and the peptides- the inhibins and activins.[58,59]

The human menstrual cycle can be divided into four phases according to stages of follicular and luteal development and levels of circulating sex steroids. These are the *menstrual phase, follicular phase, ovulation* and *luteal phase.*

A3.1.1 The menstrual phase

- During the menstrual phase, 20 or so secondary (antral) follicles begin to enlarge under the influence of FSH. However folliculogenesis begins in the late luteal phase of the preceding cycle when FSH and LH levels begin to rise with the demise of the corpus luteum and resulting low levels of oestrogen, progesterone and inhibin A. GnRH from the hypothalamus stimulates the release of FSH & LH from the anterior pituitary so that levels of FSH and LH began to gradually rise 4 days prior to menses.[60] FSH and LH are released in pulses that correspond to the pulsatile secretion of GnRH. Pulsatility changes during the different phases of the menstrual cycle, increasing near ovulation and slows during the luteal phase. In the absence of negative feedback, it occurs approximately every 60 minutes.[56]

A3.1.2 The Follicular Phase

- Stimulated by FSH and LH, these follicles continue to grow and develop and to secrete oestrogens. LH stimulates theca cells of the ovarian follicle to secrete androgens

(androstenedione and testosterone) which are converted to oestrone (E_1) and 17β-oestradiol (E_2) respectively by the enzyme P450 aromatase, but preferentially oestradiol in the ovary. Secretion of aromatase is stimulated by the action of FSH on the granulosa cells of the developing Graafian (dominant) follicle.

- In the early follicular phase, granulosa cells of the antral follicles also secrete inhibin B in addition to moderate levels of E_2, which in turn inhibit the release of FSH secretion as well as LH via negative feedback on the hypothalamus, GnRH and anterior pituitary. This closed loop feedback system of FSH stimulating secretion of oestrogens and INH-B which together exert negative feedback on FSH is maintained in a continual state of balance in the normal menstrual cycle of normal reproductive aged women, and is of central importance when considering the endocrine changes that occur in later reproductive life.

- Activins are other FSH regulatory peptides found to stimulate FSH production,[61-63] although their role in the endocrinology of the normal menstrual cycle is considered by many not to be major.

A3.1.3 Late follicular phase

- As the follicular phase progresses, selection of the dominant (Graafian) follicle, a single follicle destined to ovulate, occurs by about day 6 under the influence of FSH & E_2; this continues to secrete E_2 and Inhibin A. It may also secrete small amounts of progesterone (P) one or two days prior to ovulation. The other follicles undergo atresia.

- In the late follicular phase, at day 12, high levels of E_2 (>700-800pmols for 48hrs+), possibly facilitated by P, exert positive feedback on LH (and to a lesser extent FSH) primarily via the anterior pituitary after priming with GnRH of high frequency:
  E_2 stimulates the synthesis and storage of LH but initially inhibits its secretion.
  However, the sharp rise in the levels of E_2 at day 12 triggers the discharge of LH and the resultant **LH surge** twelve hours later. This positive feedback of LH is enhanced by the pre-ovulatory rise in P, which sensitises the anterior pituitary to E_2, thus augmenting the LH surge.

The brief pre-ovulatory rise in P also suppresses E_2 secretion, together with the effect of LH on the follicle, resulting in the fall in E_2 levels prior to the LH-surge.[64]

A3.2.4 Ovulation

- The LH surge triggers ovulation and promotes formation of the corpus luteum by full luteinisation of the dominant follicle.
- "Ovulation carries away the oocyte and a cumulus of granulosa cells; the remaining theca and granulosa cells organize into a progesterone-secreting corpus luteum, which is active for about 2 weeks and then regresses."[59]

A3.1.5 Luteal Phase

- In contrast to the oestrogen dominated follicular phase, the luteal phase is characterised by progesterone dominance. The corpus luteum produces progesterone, some oestrogen, inhibin A and relaxin. Progesterone inhibits the release of LH. It also stops the positive feedback of E_2 on the hypothalamus and anterior pituitary and thereby contributes to the inhibition of FSH together with E_2 and Inhibin A. Moderate levels of E_2 inhibit the release of GnRH, FSH and LH possibly by reducing the sensitivity of the anterior pituitary to GnRH.
- In the uterus, P transforms the endometrial cells from proliferative to secretory.
- Progesterone levels reach a peak about 7-8 days post ovulation. In the absence of implantation, luteolysis occurs with a decline in E_2 and P levels during the last 4-5 days of the functional corpus luteum,(p196)[56] precipitating a menstrual bleed. Inhibin A levels also decline at his time, allowing FSH levels to begin to rise.

- **Four days prior to menses**, in the low oestrogen, progesterone and INH-A environment resulting from degeneration of the corpus luteum, GnRH from the hypothalamus stimulates the release of FSH & LH from the anterior pituitary. Levels of FSH and LH begin to gradually rise. (See beginning).

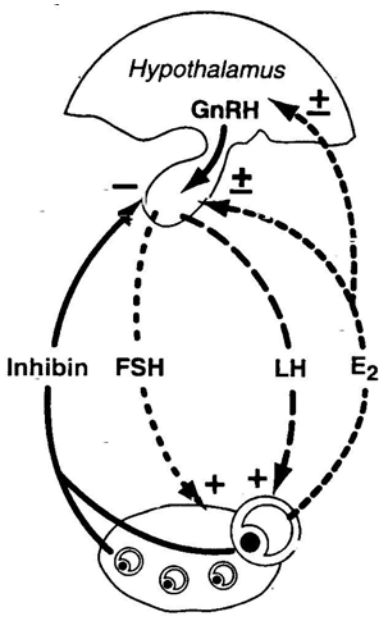


FIGURE A3.1 DIAGRAMMATIC REPRESENTATION OF THE HYPOTHALAMIC-PITUITARY-OVARIAN AXIS DURING THE FOLLICULAR PHASE. Reproduced with permission from Elsevier[65]

Under the control of noradrenaline (NA), serotonin (5-HT), corticotropin-releasing hormone (CRH) , the opioids and other neurotransmitters, hypothalamic neurones in the pre-optic and arcuate nucleus secrete GnRH into the hypophyseal portal system in a pulsatile manner. This stimulates the production and secretion of LH and FSH by the pituitary.[66] This in turn stimulates secretion of ovarian oestrogen and progesterone, which feed back at the pituitary to modulate the relative amounts of LH and FSH and at the hypothalamus to regulate GnRH. NA stimulates GnRH secretion; opiates, corticosteroids, dopamine and CRH are inhibitory.[56,59,67]

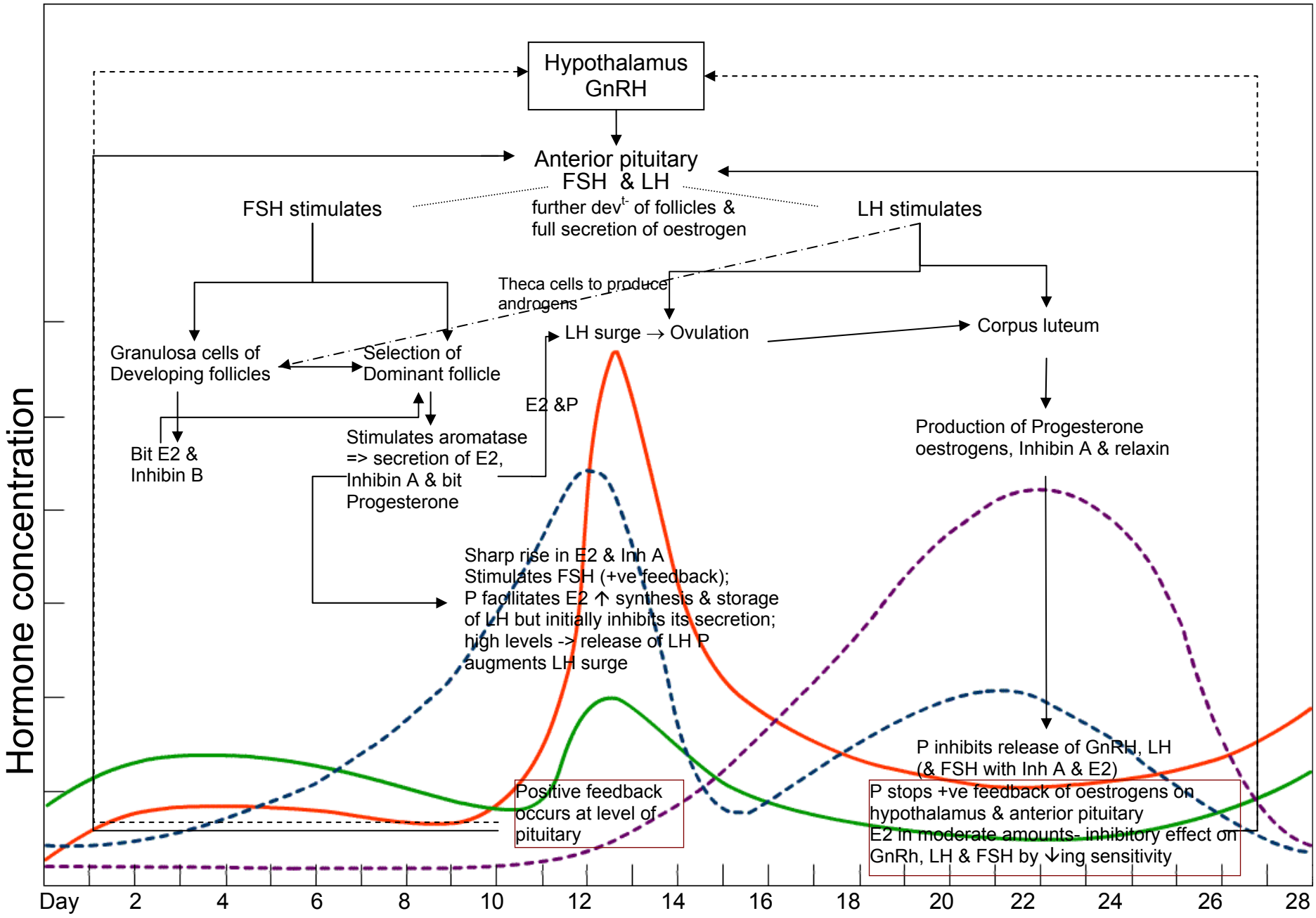
TABLE A3.1 OVARIAN HORMONES, GONADOTROPHINS & INHIBINS THROUGHOUT THE MENSTRUAL CYCLE

	Early Follicular Phase	*Late Follicular Phase*	*Early–Mid Luteal Phase*	*Late Luteal Phase*
Oestrogens	Moderate levels inhibit release of GnRH, FSH & LH via negative feedback on anterior pituitary and hypothalamus	High levels exert positive feedback on GnRH, LH & FSH (>700-800pmols for 48hrs+) via anterior *pituitary* after priming with GnRH of high frequency. Day 12 surge => 12 hours later stimulates LH surge	Moderate levels inhibit release of GnRH, FSH & LH [May do this by reducing sensitivity of anterior pituitary to GnRH].	Low levels exert no feedback on GnRH, LH or FSH => unrestrained FSH & LH [Declining levels of P & oestrogens caused by degeneration of the corpus luteum]
Progesterone		Small amounts with E2 can enhance positive feedback of LH by sensitising anterior pituitary to E2 > Discharge of LH	Suppresses LH Contributes to inhibition of FSH (with E2 & Inhibin A) [Stops positive feedback effect of oestrogens on hypothalamus & anterior pituitary] [Works with oestrogens to prepare endometrium for implantation. Transforms endometrial cells from proliferative to secretory].	Declining levels of P & oestrogens caused by degeneration of the corpus luteum causes uterine spiral arteries to constrict => cells they supply become ischaemic & die => *stratum functionalis* of endometrium sloughs off.
FSH ***Stimulates granulosa cells => aromatase***	Stimulates the initial secretion of oestrogens by growing ovarian follicle (via action of aromatase from granulosa cells on androgens)	Stimulates further development of growing follicles and the secretion of oestrogens, inhibins A & B (and possibly small amounts of P 1-2 days pre-ovulation)	Regulated by negative feedback (via hypothalamus and anterior pituitary) both by the ovarian steroids, E2 and P, and by the inhibins	4 days prior to menses, with very low levels of P & oestrogens, FSH & LH start to rise again
LH ***Stimulates theca cells => androgens***	Stimulates the further development of ovarian follicles and their full secretion of oestrogen (esp Graafian follicle)	Triggers ovulation Promotes formation of corpus luteum	Stimulates production of progesterone, oestrogens, relaxin & inhibin A by the corpus luteum	4 days prior to menses, with very low levels of P & oestrogens, FSH & LH start to rise again
Inhibins	FSH stimulates Inhibin B & activin production by granulosa cells of antral follicles. Inh B in turn inhibits FSH secretion.		Corpus luteum secretes Inhibin A	Levels of Inhibins low => allows FSH/LH secretion

TABLE A3.2 SECRETION AND INHIBITION OF SEX HORMONES, GONADOTROPHINS & INHIBINS

	Rises in response to	***Decreases as a result of***	***Post-ovulation***	***Functions***
Oestrogens	FSH => granulosa cell of developing follicles, especially dominant one	Brief rise in P pre-LH surge together with the effect of LH on the follicle.	E2 & Inhibins & relaxin secreted by corpus luteum (bit)	Selection of dominant follicle influenced by E2 & FSH
	(& LH => theca cells => androgens) [& adrenal cortex (limited amounts) throughout cycle]	(Degeneration of corpus luteum in late luteal phase)	Degeneration of corpus luteum in late luteal phase	Stimulates proliferation and repair of endometrium
Progesterone	LH	Lack of implantation => degeneration of corpus luteum (luteolysis)	Corpus luteum	Transforms endometrial cells from proliferative to secretory
	[Enhances positive feedback => contributes to FSH & LH mid-cycle peak]			Inhibits release of GnRh, LH (& FSH with E2 & Inh A)-by stopping positive feedback of oestrogen on GnRH
FSH	*During positive feedback* during E2 surge, stimulated by high levels of oestrogen (via anterior pituitary after anterior pituitary already primed by high levels of GnRH)	Suppressed by moderate levels of oestrogen, & P, E2 & Inh A & B Also by high levels except in the positive feedback phenomenon.		
LH	Synthesis: stimulated by E2 Secretion: inhibited by E2 *except* during positive feedback stage (high levels, predominantly on anterior pituitary)	Progesterone Oestrogen inhibits its *secretion* except during positive feedback stage		Stimulates further development of ovarian follicles and full secretion of E2 by dominant follicle; Triggers ovulation; Promotes formation of corpus luteum
Inhibins	Inhibin A secreted by dominant follicle Inhibin B from antral follicles (& bit from dominant one)		Inhibin A secreted by corpus luteum	

FIGURE A3.2 OVARIAN HORMONES, GONADOTROPHINS & INHIBINS THROUGHOUT THE MENSTRUAL CYCLE



Appendix 4 Postmenopausal Morbidity

A4.1.1 Effects on Bone Loss

Bone loss accelerates after a natural or surgical menopause and some studies have shown that the rate of loss is dependent on the remaining oestrogen supply,[68,69] as calcium loss from bone is related to decline in oestrogens. Numerous studies have shown that oral oestrogen and oestrogen-progestin regimens prevented bone loss in early postmenopause , when bone remodeling seems to be most pronounced, with a dose-dependent effect for oestrogen.[70] It is of interest, however, that among Mayan Indian women, there is no evidence of increased fracture even 20 years post menopause, despite showing the same endocrine changes and bone mineral density as their US counterparts.[71]

A4.1.2 CHD and CVD

Alterations in lipid profiles have been demonstrated during the perimenopause, with progressive decreases in high-density lipoproteins (HDLs) and increases in total cholesterol preceding menopause by several years.[72] However only the changes in HDLs appear to be related to the menopause per se,[73] while other changes to lipid profiles, BMI and blood pressure appear to be more related to age.[72,73] Epidemiological data show that cardiovascular disease (CVD) and coronary heart disease (CHD) are more prevalent in postmenopausal women than premenopausal women. While oestrogens have been shown to confer vasculo- and cardio-protective effects, there has been disparity among findings from observational studies and RCTs on the effects of HT on CVD and CHD. Observational studies suggest a protective role of oestrogen whereas recent RCTs report a negative effect of oral oestrogen in primary and secondary prevention of CV events.[74] It has been suggested that this may be due to the inclusion in these trials of women older than the normal age for initiating HT in clinical practice, since they are more likely to have established atherosclerosis. Oestrogen may have a primary preventive role on CHD and it may, therefore, be necessary to commence HT treatment in perimenopause.[75] A 'window of opportunity' may exist for initiation of HT; close to the time of menopause (in women less than 60 years of age) it may be cardio- and probably neuroprotective, whereas after that time it can be harmful because of established pathological changes. (Burger, 2008 Personal communication).

A4.1.3 Cognitive function

With regard to putative effects of oestrogen on cognitive skills, evidence is conflicting. Many of the actions of oestrogen suggest it should improve cognition and reduce dementia/Alzheimer's disease (AD) risk or symptoms. Among these are its effects of modulating neural activity that occurs during cognitive processing, increasing cerebral blood flow, augmenting glucose transport into the brain, and its influence on several NT systems including acetylcholine which may have relevance to memory and attention, monoamine neurotransmitters, thought to be important in cognition and mood. Oestrogenic effects on cognition are also implied by performance fluctuations during a woman's menstrual cycle. However, available evidence suggests that oestrogen does NOT have an important effect on episodic memory or other cognitive skills, and that serum oestrogen levels at midlife are unrelated to memory scores, although two studies did find oestradiol levels to be positively related to memory skills.[76]

The prevalence of AD increases with age, and is greater in women than men. Clinical evidence to date suggests that beginning HT in the presence of dementia due to AD does not improve symptoms or slow progression. In fact, findings from WHIMS raised concerns that HT elevates dementia risk within several years of initiation, although the applicability of these findings to younger women with short-term usage has been questioned. Observational studies with *younger* women indicate that HT use might reduce AD risk, but there is no evidence that initiating HT after age 63 improves memory or other cognitive abilities.[76]

A4.1.4 Glucose metabolism

It has been observed that menopause is associated with significant changes in insulin metabolism[77] and that the risk of impaired glucose tolerance increases by 6% per year after menopause,[78] although the impaired fasting glucose and increased serum insulin levels may be more related to BMI that menopause.[79] Nonetheless, middle-aged women are more affected than men and a relationship between sex steroids and insulin sensitivity is supported by the increased insulin resistance during pregnancy, the luteal phase of the menstrual cycle and in women with high androgen production (PCOS).[80] The administration of exogenous 17β-oestradiol has been observed in animal studies to play an important role in the maintenance of normal insulin sensitivity, enhancing insulin secretion in islet cells.[81] Additionally, oestrogen-treated women were found to have significantly lower levels of

insulin than women who were not taking oestrogen in a comparative study.[82] Both endogenous and exogenous oestrogens appear to have a modulating effect on insulin resistance, although the effects of exogenous oestrogens may be blunted by factors such as the first-pass effect of orally administered hormones. Results from HERS and WHI were encouraging in terms of postmenopausal HT for prevention of type 2 diabetes.[80]

A4.1.5 Immune function

Oestrogens, androgens and progestins also have important immune regulatory functions. The effects of oestrogen on immune function are complex. For example, oestradiol has been found to enhance immunoglobulin G production, down-regulate the thymus and enhance or suppress cytokine production in a dose dependent manner. Postmenopausal oestrogen replacement therapy has been associated with increased risk for systemic lupus erythematosus SLE) and oestrogen-containing oral contraceptives can exacerbate the symptoms of SLE.[74]

It is interesting to note that increased risk of osteoporosis, the metabolic syndrome and cardiovascular disease are also adverse sequelae of major depression.[83]

Appendix 5 Monamine transport cycle[84,85]

The monamine neurotransmitters, noradrenaline, serotonin and dopamine, are quite widely distributed in the mammalian brain and are involved in the regulation of mood, cognition, locomotion, sleep, appetite arousal, libido, anxiety and aggression.[85] Monoamine oxidases (MAOs) are the degradative enzymes responsible for the breakdown of these monamine neurotransmitters.

Serotonin and noradrenaline are synthesised via tryptophan hydroxylase and tyrosine respectively, and stored in synaptic vesicles. In response to action potentials, the neurotransmitters are released from the synaptic vesicles into the synaptic cleft where they exert their effects on various postsynaptic receptors.[84] Serotonin can modulate the effect of a different type of neurotransmitter on its post synaptic site. The physiological actions are terminated by the transporters which transport the neurotransmitter from the synaptic cleft, where it is either further transported into empty storage vesicles, or metabolised by monoamine oxidase. [85]

In the case of dopamine, the effects are similarly terminated by the dopamine transporter located on neuronal terminals. However in the prefrontal cortex, the transporter is absent and the action of dopamine is terminated by the noradrenaline transporter located on noradrenergic terminals.[85]

The 5-HT_{1A} auto receptors play an important regulatory role on serotonin neurotransmission via a negative feedback mechanism in response to serotonin levels in the synapse. With chronic exposure to increased serotonin levels, these receptors eventually become desensitised.[84]

The noradrenergic system is linked to an array of neurotransmitters that modulate the stress response. These include serotonin, the opioids, GABA, corticotrophin-releasing factor, dopamine and glutamate.[85]

Appendix 6 DSM Definitions

A6.1 Dysthymic disorder (DSM-IV p380)[86]

Dysthymic disorder is a form of chronic low-grade depression that is characterised by depressed mood for at least 2 years and the presence of two or more of the following symptoms:

- Chronic low-grade depression or depressed mood for at least 2 years, for most of the day, more days than not
- AND two symptoms from the following:
- poor appetite or overeating,
- insomnia or hypersomnia,
- low energy or fatigue,
- low self-esteem,
- poor concentration or difficulty making decisions,
- feelings of hopelessness

A6.2 Major Depressive Disorder

According to the Diagnostic and Statistical Manual of Mental Disorders-IV (pp356 & 411),[86] at least five of the following symptoms have been present during the same 2-week period, nearly every day, and represent a change from previous functioning. At least one of the symptoms must be either (1) depressed mood or (2) loss of interest or pleasure:

- Depressed mood most of the day, nearly every day, as indicated by self or others
- Markedly diminished interest or pleasure in all, or almost all, activities
- Significant weight loss or weight gain (5 %/mo.) or loss/gain in appetite nearly every day.
- Insomnia or hypersomnia nearly every day
- Psychomotor agitation or retardation nearly every day (as noted by others).
- Fatigue or loss of energy nearly every day
- Feelings of worthlessness or excessive or inappropriate guilt nearly every day
- Diminished ability to think or concentrate or indecisiveness nearly every day
- Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning

Symptoms are not better accounted for by a Mixed Episode, Mood Disorder Due to a General Medical Condition, a Substance-Induced Mood Disorder, or Bereavement (normal reaction to the death of a loved one)

Symptoms are not better accounted for by a Psychotic Disorder (e.g., Schizo-affective Disorder).

A6.3 Minor Depression/depressive spectrum disorder

Persistence of depressive mood symptoms for at least 2 weeks but does not meet criteria for dysthymic disorder or major depressive disorder.[87]

A6.4 Generalised Anxiety Disorder (GAD)

Diagnosis requires excessive anxiety and worry (apprehensive expectation) occurring more days than not for at least 6 months and at least six GAD symptoms from DSM-IV (pp429 & 476)[86]

- Restlessness or feeling keyed up or on edge
- Being easily fatigued
- Difficulty concentrating or mind going blank
- Irritability
- Muscle tension
- Sleep disturbance (difficulty falling or staying asleep or restless unsatisfying sleep)

Appendix 7 Hormone Therapy Preparations Available in Australia

TABLE A7 EXAMPLES OF HORMONE THERAPY PREPARATIONS AVAILABLE IN AUSTRALIA[88]

Regimen	*Type*	*Product*	*Administration*
Oestrogen only	Piperazine oestrone sulfate	Genoral	Oral
		Ogen	Oral
	Oestradiol	Zumenon	Oral
		Oestadiol implants	Subcutaneous
	Oestradiol gel	Sandrena	Transdermal
	Oestradiol cream (WA only)	Natragen	Transdermal
	Oestradiol patches	Climara,	Transdermal
		Dermestril	Transdermal
		Estraderm	Transdermal
		Femtran	Transdermal
		Menorest	Transdermal
	Oestradiol hemihydrate	Estrofem	Oral
		Aerodiol	Intranasal
	Oestradiol valerate	Progynova	Oral
	Oestriol (+ vaginal tablet, pessaries and cream)	Vagifem	Vaginal,
		Ovestin	Vaginal & Transdermal
	Conjugated equine oestrogens	Premarin	Oral
Progestogens	Medroxyprogesterone acetate	Medroxyhexal	Oral
		Provera	Oral
		Ralovera	Oral
	Dydrogesterone	Duphaston	Oral
	Norethisterone acetate	Primolut N	Oral
	Cyproterone acetate	Androcur	Oral
Combined Oestrogen/ Progestin	Oestradiol/norethisterone acetate	Kliogest[1]	Oral
		Kilovance[1]	Oral
		Trisequens[2]	Oral
	Oestradiol/norethisterone acetate patches	Estalis Sequi[2]	Transdermal
		Estalis continuous[1]	Transdermal
		Estracombi[2]	Transdermal
	Oestradiol/dydrogesterone	Femoston[2]	Oral
	Oestradiol valerate/cyproterone acetate	Climen 28[2]	Oral
		Climen[3]	Oral
	Conjugated oestrogens/ medroxyprogesterone acetate	Premia[2]	Oral
		Premia continuous[1]	Oral
		Provelle 28[1]	Oral
	Levonorgesterel	Mirena	Intrauterine
Tibolone	Metabolises to compounds with oestrogenic, progesterogenic and androgenic effects	Livial	Oral
Testosterone	Testosterone Cream (WA only)	Andro-feme)	Transdermal

1 continuous combined regimen (oestrogen and progestin daily, with no hormone-free interval. This confers avoidance of cyclical bleeding.
2 continuous-cyclic regimen (oestrogen daily, progestin 10-14 days)
3 cyclic regimen (oestrogen 11 days, oestrogen plus progestin 10 days, 7 therapy-free days during which planned bleeding occurs).

The principal oestrogen before menopause is 17β oestradiol, which is converted to oestrone in the liver and other tissues by 17β oestradiol dehydrogenase. Oestrone is further metabolised in the liver to oestrone sulphate, which constitutes the circulating oestrogen in highest concentration. Oestrone provides a reservoir for oestradiol production.[89]

Oestradiol is the most stimulating to breast tissue (causing cell division) and oestriol the least. Unlike oestrone, oestriol does not convert to oestradiol. While oestriol does offer significant bioactivity, it binds fleetingly to oestrogen receptors and is metabolised rapidly. Only 10-20% remains in circulation, and it is estimated to be only 1/10 to 1/100 as potent as oestradiol. In addition, its affinity for oestrogen receptors compared with oestradiol is only 14% for α and 21% for β-sites. Nonetheless, it appears to have the same ability to stimulate endometrial proliferation as the stronger oestrogens, despite its weaker activity at some tissue sites.[89]

Appendix 8 Benefits and Limitations of Different Routes of Administration

The non-oral routes of administration confer several advantages: i) the lower dose required, as it by-passes the first hepatic passage, results in fewer side-effects; ii) it is less likely to increase sex-hormone binding globulin (SHBG) which also binds other sex steroids such as testosterone; and iii) the high levels of circulating sulphate produced by oral oestrogen preparations are avoided. Oestrogen-sensitive tissues such as breast and endometrial tissue have a high capacity to convert oestrone sulphate to oestradiol, possibly contributing to the greater concentrations of oestrone and oestradiol in breast tissue than in serum.[88]

However, results from studies of transdermal progestins vary. One RCT reported a significant reduction in vasomotor symptoms, but no effect on bone density with 20 mg progesterone for one year in conjunction with daily multiple vitamins and 1,200 mg calcium.[90] However, a more recent study failed to detect an improvement in vasomotor symptoms (hot flashes), mood characteristics, or sexual feelings with the equivalent of 32 mg progesterone daily over a period of three months.[91]

Vaginal bleeding was reported by eight of the 43 women treated with the progesterone cream in the first study. An additional limitation of this route of administration is that is does not appear to confer the same protection of the endometrium offered by oral progestins in HT due to the insufficient serum concentrations.[45,92-94]

Appendix 9 Side-effects of Provera® and Premarin®

TABLE A9 OTHER SIDE-EFFECTS OF INDIVIDUAL INTERVENTIONS: PROVERA & PREMARIN

Provera® (Progesterone)
hypersensitivity reactions, thromboembolic disease, CNS disturbances eg nervousness, insomnia, fatigue, depression, dizziness, headaches, tremor, PMS-like depression, dermatological- urticaria, pruritis, rash, acne, hirsuitism, alopecia and sweating; GUT: irregular uterine bleeding, spotting and amenorrhoea; GIT- nausea; breast tenderness, galactorrhoea; cervix – changes in excretions and secretions; other – Cushings syndrome, weight gain, hyperpyrexia, moderate elevation of blood pressure, increases in white blood cell count and platelet counts.
Premarin® (conjugated oestrogen)
Increased risk of gall-bladder disease & breast cancer, endometrial cancer, vaginal bleeding, mastodynia, fluid retention -a problem in asthma, epilepsy, renal or cardiac dysfunction, migraine, increased blood pressure sometimes noticed, hypercalcaemia, renal insufficiency GUT: breakthrough bleeding GIT nausea and vomiting, etc, dermatological, ocular (intolerance to contact lenses, weight changes, oedema, changes to libido, aggravates porphyria, beast enlargement or secretion.

Appendix 10 Foods Sources of Phytoestrogens

TABLE A10 CLASSIFICATION AND FOOD SOURCES OF PHYTOESTROGENS

Isoflavones		***Lignans***		***Coumestans***
Soybean products	**Legumes**	**Whole grains**	**Fruits, Vegetables, Seeds**	**Bean sprouts**
Tofu Soy meal Soy grits Soy flour Soy milk	Soybeans Lentils Beans Chickpeas	Wheat Wheat germ Barley Hops Rye Oats Rice Brans	Apple Pear Cherry Carrot Fennel Onion Garlic Sunflower seed Linseed Vegetable oils (including linseed, olive)	Alfalfa Soybeans Clovers

Adapted from: Murkies AL, Wilcox G, Davis SR. Phytoestrogens. J Clin Endocrinol Metab 1998,83:297-303.[95]

Appendix 11 *Vitex agnus-castus* (Chaste-tree/berry) in the Treatment of Menopause-related Complaints

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Abstract

The origin of the current practice of administering *Vitex agnus-castus* in menopause-related complaints is uncertain, but appears to be relatively recent. Here we review the evidence for this application of *Vitex* based on evidence from pharmacological studies and clinical research. The mechanisms of potential relevance in the context of menopause are explored with reference to the current understanding of the endocrinology and neuroendocrinology of menopause and associated symptoms. We conclude that, while evidence from rigorous randomised controlled trials is lacking for the individual herb in this context, emerging pharmacological evidence supports a role for *Vitex agnus-castus* in the alleviation of menopausal symptoms and suggests that further investigation may be appropriate.

Introduction

The practice of administering *Vitex agnus-castus (*Chaste-tree/berry or Monk's pepper, family Verbenaceae) in the treatment of menopause-related complaints appears to be of relatively recent origin. In current Anglo-American and European phytotherapeutic practice, *Vitex* fruit is most widely used for female reproductive problems, finding an application in conditions such as pre-menstrual syndrome, anovulatory cycles, infertility and hyperprolactinaemia, among others.[1,2] It is said to have a normalising action on the menstrual cycle.[1,2] References to its value for 'diseases of the uterus' appear as far back as the works of Hippocrates in 4th century BC and Dioscorides in AD77.[3] Gerard, one of the great Renaissance herbalists, recommended it for inflammation of the uterus and as an emmenagogue to promote menstruation.[4]

The earliest overt reference in the literature to the application of *Vitex* in menopause-related complaints, however, does not appear until the 20th century. A 1972 publication of a collective report on the clinical experience of five practitioners with *Agnolyt*® (a patent medicine extracted from dried *Vitex* fruit) reported on its efficacy for menopausal bleeding and menopausal complaints.[5] Its use in this context appears to have now become relatively popular in the Anglo-American tradition.[6-9] A practitioner survey of 276 UK herbalists reported that 86.3% prescribed it for the treatment of perimenopausal complaints, including hot flushes.[10] It is also used in clinical practice to assist with withdrawal from Hormone Therapy (HT).[1,7,10] The fruit is a common ingredient of phytotherapeutic formulations for menopause-related complaints in several Western countries (Table 1, Appendix 1).[11-14] While evidence for *Vitex* as a sole agent in this context is lacking from RCTs, emerging pharmacological evidence, relating to its dopaminergic activity,[15-17] affinity for opioid receptors[18,19] and enhancement of melatonin secretion,[20] supports a role for *Vitex agnus-castus* in the alleviation of menopausal symptoms. This paper reviews the clinical and pharmacological evidence supporting this practice, and possible rationale for such an application.

Inconsistency of Definitions used in reference to Menopause

It is possible that the practice of administering *Vitex* for menopausal complaints is confined to those experienced during the perimenopause, rather than postmenopause.

Fluctuating or increased oestradiol secretion characterises the perimenopause[21,22] (formerly *'climacteric*', the period immediately prior to menopause up to the first year after the final menstrual period.[22]) Thus, the postulated benefits of *Vitex* in conditions where unopposed oestrogen plays a role[1] may be of relevance here. However, it is often unclear from the literature which menopausal phases, and which specific complaints[1,6-9] are referred to, due to the inconsistencies in the use of terminology relating to the menopausal stages. For example, some studies reviewed here[12-14,23] have adopted the recommended definition of natural menopause as having occurred after 12 consecutive months of amenorrhoea for which there is no other obvious pathological or physiological cause.[22] However, some others have taken 6 months of amenorrhoea as denoting entry to the postmenopause,[11] or neglected to define it at all.[10,24] Similarly, *late perimenopause* is commonly defined as menses within the preceding 12 months but not the preceding 3 months, in conjunction with the co-existence of symptoms,[25-27] after excluding other causes where there is a history of previous menstrual irregularity.[27] However, the Herbal Alternatives for Menopause (HALT) study more than one skipped menses within the previous 12 months to denote late perimenopause.[12]

Menopausal Symptoms

Symptom experience varies throughout the transition and between individuals. It is during the *perimenopause* that most symptoms are reported.[28] These can be due to oestrogen excess (breast tenderness, menorrhagia, migraine, nausea, shorter cycle length)[21,29,30] or deficiency (vasomotor symptoms,[31] breast tenderness and vaginal dryness.[32,33]) and often fluctuate, reflecting the underlying hormonal instability during these years.[30] Dysfunctional uterine bleeding is maximal during the menopausal transition due to persistent unopposed oestrogen;[34,35] low progesterone is associated with failure of the secretory phase in anovulatory cycles.[27] Rates of psychological distress are also found to peak during the perimenopause.[36-39] Anecdotal reports suggest that premenstrual syndrome (PMS)-like symptoms may be more prevalent at this time, or at least less well-tolerated than previously.[40] The incidence of hot flushes increases throughout the transition[32] with a peak generally at the time of the menopause[36] and in postmenopausal women.

Hot flushes occurring during the night have been associated with sleep disturbances.[41-45] However, some evidence suggests that not all of the nocturnal flushes result in waking episodes or arousals.[41] Conversely, other studies have found that not all of the waking

episodes are associated with flushing,[43,44] suggesting this may not be the only factor responsible for disturbed sleep. Other proposed causes of sleep disturbances include an age-related decrease in total brain serotonin, one of the main regulators of circadian sleep-wake cycles,[46] an age-related decrease in slow-wave sleep and GH secretion[47,48] and depression.[49]

Endocrine changes associated with Menopause

Before discussing the pharmacological mechanisms of *Vitex* that may be of potential relevance to the treatment of menopause-related symptoms, it will be useful to outline the current understanding of the endocrinology of menopause and the aetiology of its associated symptoms.

The endocrinology of menopause has not yet been fully elucidated, and is complicated by the irregular cycles that characterise the menopause transition; these include normal length ovulatory and anovulatory cycles, and elongated ovulatory cycles,[50] without any orderly progression from one type to another.

The previously-held belief that the perimenopause is characterised by a gradual decline in oestrogen levels with rising follicle-stimulating hormone (FSH) [51] has been challenged by current research indicating that serum oestradiol (or urinary oestrogen excretion) actually increases slowly with increasing age,[34,52-56] and declines only from about 2 years prior to final menses.[57] Research on the inhibins has helped to clarify the underlying mechanism.[58] The main action of the inhibins (ovarian dimeric glycoproteins that regulate gonadotropin release during the menstrual cycle) is to inhibit synthesis and secretion of FSH. The falling inhibin levels (especially INH-B), resulting from the declining antral follicle count as women age,[59] allows the gradual rise in FSH, which drives increased oestradiol secretion.[56,58,60] This may lead to accelerated follicle development and occasions of multiple follicles developing at once, and hence give rise, on occasion, to markedly raised oestradiol concentrations in perimenopausal women.[21,34,52] *Lower* than normal levels of oestradiol have been found in late-perimenopausal women who had experienced 3 months' amenorrhoea[60] and in late-perimenopausal women during *anovulatory* cycles, [61] and in cycles with an elongated 'lag period' between the menstrual phase and the onset of the follicular phase.[62] There is evidence of reduced hypothalamic–pituitary sensitivity to oestrogen feedback in perimenopausal women.[29,63,64]

Oestrogens modify synthesis, release and metabolism of many neurotransmitters such as noradrenaline, dopamine, acetylcholine, serotonin and melatonin, and neuropeptides including β-endorphin, which modulate the activity of hypothalamic centres and the limbic system.[65] Fluctuating levels of sex steroids, particularly oestrogen, result in altered function of the hypothalamus and limbic system, and thereby the regulation of mood, psychological well- being,[66,67] thermoregulation and vasomotor stability,[68] and many other functions.

Melatonin levels decrease significantly with age; similarly the time during which melatonin remains elevated at night decreases with age.[69] An association between the quality of sleep and the amount of melatonin secreted has been noted, especially in the elderly. [70]

Studies in women during the perimenopause reveal that the decline in melatonin precedes FSH increase during menopause.[71] Whether this decline in melatonin secretion contributes to the development of menopause or its symptoms has not been established.[72]

The Vasomotor Symptoms of Hot Flushes and Night Sweats

The term 'hot flushes' is used here to include night sweats. The aetiology of hot flushes is currently believed to involve a central noradrenergic mechanism. In symptomatic women, narrowing of the hypothalamic thermoneutral zone has been observed,[73] which is at least partly due to elevated brain noradrenaline levels.[74] Central noradrenergic activity is, in turn, modulated by ovarian steroids.[75] Within the reduced thermoneutral zone, small elevations in core body temperatures that precede most hot flushes are thought to constitute the triggering mechanism for hot flushes.[73]

The central noradrenergic instability associated with hot flushes could be due to the reduction of endogenous opioid activity[76,77] that results from declining oestrogen levels, as hypothalamic opioidergic activity normally has an inhibitory effect on noradrenergic neurons in the brainstem. Casper and Yen[45] proposed that successful therapies for hot flushes may exert their effects by increasing endogenous opioid peptide activity with consequent inhibition of noradrenergic activity below the threshold needed to activate heat loss.

Dopamine has recently been found to be an important thermoregulatory neurotransmitter, with D2 receptors involved principally with the maintenance of body temperature in euthermia.7[8] Earlier research had observed the dopamine agonist bromocriptine to increase the activity of the endogenous opioid system on the thermoregulation mechanisms that regulate body temperature in postmenopausal women[79] and to be effective in alleviating hot flushes. [80]

Oestrogen withdrawal in menopausal women also results in dramatically lowered blood serotonin levels.[81,82] Low blood oestrogen levels are correlated with upregulation of certain serotonin receptors (5-HT_{2A}) in the hypothalamus[83] that are believed to be involved in thermogenesis.

Mood changes

Findings have been inconsistent regarding an association between depressed mood and hormone levels.[84-89] However, several effects of oestrogens are of potential relevance to menopause-related mood changes (Table 2, Appendix 2). Those of interest in the context of *Vitex agnus-castus* are as follows:

i. Oestrogen potentiates the activity of opiate-containing neurons[90] and increases the synthesis and release of β-endorphin. [91]
ii. Oestrogen directly modulates dopaminergic activity,[92] increases dopamine release in the hypothalamus[87] and increases dopamine transmission and D2 receptors.[93]
iii. In postmenopausal women, the activity of the dopaminergic system was found to be significantly lower than in premenopausal women, but was significantly increased by HT administration, with a concomitant significant decrease in psychological symptoms.[94]
iv. Fluctuating ovarian hormones destabilise circadian rhythms during the perimenopause, and may contribute to the development of mood disorders in predisposed women.[95]

Perimenopausal Premenstrual Syndrome (PMS)-like symptoms

It has also been hypothesised that some symptoms attributed to the menopause-transition such as mood changes are more likely to be related to PMS, given that they improve after cessation of menstruation.[96] The PMS-like symptoms experienced during the perimenopause may differ in their aetiology from PMS during normal reproductive years,

due to increasing infrequency of ovulatory cycles as the perimenopause progresses. According to current understanding, ovulation is a prerequisite for PMS, which is believed to result from sensitivity in predisposed individuals to normal hormonal fluctuation during the late luteal phase.[97,98] It is possible that these PMS-like symptoms in the perimenopause may similarly represent an increased sensitivity to normal fluctuations in ovarian hormones.[29,63,64,99] Alternatively, oestrogen excess has been suggested as a possible cause for these symptoms in late perimenopausal women[100] Other factors potentially implicated in the aetiology of PMS include changes in circadian rhythms, found to be similar to those occurring in anxiety and mood disorders, with aberrant timing of the secretion of melatonin, cortisol and prolactin.[96]

Pharmacological Actions of Vitex with potential relevance to Menopause

Phytochemically, *Vitex* has been shown to contain essential oil, flavonoids, iridoid glycosides and dopaminergic compounds belonging to the diterpenes. Mild D2 receptor agonistic properties have been demonstrated, resulting in inhibition of latent hyperprolactinaemia, (the non-physiologically stimulated prolactin release often manifest during the time of decreasing progesterone and oestradiol levels, that is frequently also accompanied by an insufficient function of the corpus luteum).[15-17] *Vitex* has demonstrated activity as an agonist at the mu, and potentially the kappa, opioid receptor.[18,19] It has also been found to effect a dose-dependent increase in melatonin secretion.[20] These actions of *Vitex* may be of relevance to the aetiology of menopausal symptoms and are elaborated below. Findings from recent cell culture experiments indicate that *Vitex* extracts may contain phytoestrogens; the most active of which has been identified as the flavonoid, apigenin.[101] However, the compounds identified are only weakly oestrogenic, not unique to this herb, and present in relatively low levels compared to other herbal and dietary sources. We therefore suggest that this finding does not contribute to our understanding of the true mechanism of the action of *Vitex*.

Latent hyperprolactinaemia

Hyperprolactinaemia results in inhibition of secretion of gonadotropin releasing hormone (GnRH) and decreased secretion of luteinising hormone (LH) and FSH. In the ovary, this results in inhibition of progesterone secretion by the granulosa-lutein cells of the corpus luteum.[102] Premenstrual symptoms, particularly mastodynia, are often accompanied by *latent* hyperprolactinemia,[103,104] which can be stimulated by stressful situations.[15,105]

The above may have potential relevance to menopause-related symptoms in several ways: i) in light of the suggestion that many of the menopausal symptoms may represent an exacerbation of pre-menstrual symptoms, targeting premenstrual latent hyperprolactinemia may also be appropriate during the perimenopause; ii) the dopaminergic effects of *Vitex* may be of relevance to alleviating hot flushes, as did the dopamine agonist, bromocriptine.[80] Dopamine has been found to affect thermoregulation, possibly via activation/stimulation of the endogenous opioid system. The effects of *Vitex* on opioid receptors may also be of relevance in this context; iii) because lower activity of the dopaminergic system is associated with psychological symptoms,[94] the dopaminergic properties of *Vitex* may also prove beneficial in the amelioration of the emotional symptoms of menopause.

Affinity for opioid receptors

An action on mu, and potentially kappa, opioid receptors may also be of relevance to the use of *Vitex* for menopause-related symptoms such as flushes and mood symptoms.

In 2000, Meier and colleagues suggested additional pharmacological actions for *Vitex agnus-castus* via opioid receptors based on *in vitro* research.[18] They reported a relatively potent inhibition for opioid (mu and kappa subtypes) receptor-binding with extracts of *Vitex,* that was most pronounced in lipophilic fractions. Additionally, binding to delta opioid receptors was found to be inhibited mainly by an aqueous fraction of *Vitex. In vitro* research, with high levels of direct exposure of test cells to the herbal extract, is of uncertain relevance to oral dosing of herbs in humans due to the pharmacokinetic factors that affect bioavailability of the phytochemicals with oral administration. However, it was subsequently also demonstrated in human and animal models that *Vitex* acted as an agonist at the mu-opioid receptor.[19] Extracts with and without fatty acids removed showed significant affinities to the mu-opioid receptor.

These findings support the beneficial action of *Vitex* in PMS, as endorphins are known to decrease in the late luteal phase[106,107] and are found to be associated with symptoms such as mood disorders, migraines and fluid retention.[108,109] However, they are also of potential relevance to its reputed value in treating menopausal symptoms. The reduction of endogenous opioid activity may be responsible, at least in part, for the central noradrenergic instability associated with hot flushes.[76,77] Increasing endogenous opioid peptide activity may effect a reduction in hot flushes via inhibition of noradrenergic activity below the threshold needed to activate heat loss.[45] In an oestrogen-deficient environment, it is possible that mood

enhancement may be effected by stimulating the activity of opiate-containing neurons[90] and thereby increasing the synthesis and release of β-endorphin.[91]

Melatonin

The effect of *Vitex* on melatonin secretion is also of potential relevance to symptoms experienced in relation to menopause. A dose-dependent increase in melatonin secretion, especially during the night, was found after administration of *Vitex* extracts 120 mg and 480 mg per day (dried herb equivalent 0.6 g and 2.4 g) in an open placebo-controlled trial. Total melatonin output was approximately 60% higher than in the placebo group.[20] This has obvious potential relevance to menopause-related sleep disturbances. However, data from a recent case report demonstrated that melatonin was able to delay the characteristic endocrine parameters associated with menopause onset.[110] While further studies are needed to confirm this finding, it is extremely interesting in view of the possible role of declining melatonin secretion (that precedes FSH increase[71]) in the development of menopause and its symptoms,[72] and the effect of *Vitex* on melatonin secretion, which may be part of the rationale for using it in menopause.

Clinical Studies with Vitex for Menopausal Symptoms

Despite its apparent popularity with UK herbalists, and its use as a component of menopause formulations, *Vitex* as a sole agent does not appear to have been tested in oral dosage form in this context in clinical trials. The data bases searched were PubMed and Embase. Search term used were combinations of *Vitex*, chaste or agnus with *menopaus*, climacteric, flush, flash, vasomotor or *menstrua*.

The steam distilled essential oil of the fruit and leaves have been investigated in two studies[23,24] that reported benefits for menopausal symptoms. The first study, with 23 women, reported improvements following use of the oil of the leaf or fruit, with the majority of 'major improvements' related to the the leaf oil.[24] Symptoms clusters for which improvements were reported were mood, vasomotor, urogenital, sleep and dysfunctional uterine bleeding, and to a lesser extent, cognitive and sexual. Interestingly, several women reported reinstatement of regular menstruation after 3 -1 0 months of amenorrhoea, and one after 6 years without a period. However, this study contained several weaknesses, including the use of different extracts and variable doses, different routes of administration (transdermal, inhalation, oral), lack of a standardised rating scale and failure to exclude

other concomitant treatments such as HT, herbs and acupuncture. The subsequent study with 52 peri- or postmenopausal women aged 38 to 73 used a 1.5% solution of the essential oil of *Vitex* aerial parts in a base cream or lotion.[23] Participants applied 2.5 ml of the cream dermally, once daily, 5–7 days per week for 3 months. Overall, 33% reported major improvement and 36% reported mild to moderate improvement in troublesome symptoms, with greatest improvement observed in the emotional symptoms, (16 responses), hot flushes/night sweats (15), and moderation of menstruation (12). However, these results need to be interpreted with caution due to the lack of a control group. The findings from aromatherapy studies utilising the essential oil are of uncertain relevance to the administration of fruit extracts in oral dosage forms.

Three randomised controlled trials (RCTs)[11-13] and one pilot study[14] on multi-component formulations containing *Vitex* for the treatment of menopausal symptoms have been reported in the literature. A small sub-population analysis of PMS-like symptoms in perimenopausal women with the combination of *Vitex* and *Hypericum perforatum* has also been conducted.[111] Results of these studies have been inconsistent.

Vitex agnus-castus was one component of a menopause herbal formulation, *Phyto-Female Complex,* found to be significantly superior to placebo in a RCT on menopausal hot flushes and night sweats in 50 healthy peri- and postmenopausal women, aged 44-65 years.[11] In the 35 who completed the study, a 73% decrease in the number of hot flushes was observed at the end of the 3-month treatment period in the active treatment (n = 19) compared with 38% in the placebo group (n = 16), p = 0.026, and the number of night sweats was reduced by 69% and 29% respectively, p = 0.027. A significant benefit was also observed in terms of sleep quality. The other herbs in the formulation were *Cimicifuga racemosa* (black cohosh) root extract, 100 mg (2.5 mg triterpene glycosides, 2.5%); *Silybum marianum* (St Mary's thistle/milk thistle) herb extract, 75 mg (60 mg silymarin, 80%); *Angelica sinensis* (dong quai) root extract, 75 mg (7.5 mg ligustilides, 1%); *Trifolium pratense* (red clover) flower extract, 50 mg (4 mg isoflavone, 8%); and *Panax quinquefolium* (American ginseng) root extract, 50 mg (12.5 mg ginsenosides, 25%). The dose of *Vitex agnus-castus* fruit extract (2.5 mg vitexin, 5%) was 50 mg. Tablets were administered twice daily. The multicomponent make-up of this combination does not permit conclusions about the contributions of individual herbs. However, a significant contributor to the effect is likely to have been *Cimicifuga racemosa* (black cohosh), for which efficacy in menopause-related

symptoms is supported by evidence from RCTs and randomised comparison group trials.[112-115] In view of the small sample size at the end of the treatment phase, these results are encouraging, although not adding specifically to the evidence for *Vitex* in this context.

The Herbal Alternatives for Menopause (HALT) study investigated 3 different herbal regimens compared with HT and placebo over a period of 12 months.[12] Three hundred and fifty-one peri- or postmenopausal women with 2 or more vasomotor symptoms per day were assigned to one of 5 groups: 1) black cohosh 160 mg daily; 2) multibotanical with black cohosh, 200 mg daily, and 9 other ingredients including *Vitex agnus-castus*; 3) multibotanical plus dietary soy counseling (that is, advice from a clinical dietician and literature to include 2 soy food servings per day in their diet, equivalent to 12 to 20 g of soy protein); 4) conjugated equine estrogen, 0.625 mg daily, with or without medroxyprogesterone acetate, 2.5 mg daily; 5) placebo. The multibotanical contained the following daily doses: *Cimicifuga racemosa* (black cohosh), 200 mg; *Medicago sativa* (alfalfa), 400 mg; boron, 4 mg; *Vitex agnus-castus* (chaste tree), 200 mg; *Angelica sinensis* (dong quai), 400 mg; *Chamaelirium luteum* (false unicorn), 200 mg; *Glycyrrhiza glabra* (licorice), 200 mg; *Avena sativa* (oats), 400 mg; *Punica granatum* (pomegranate), 400 mg; *Eleutherococcus senticosus* (Siberian ginseng, standardized constituents 0.8% eleutherosides E and B), 400 mg. On the endpoints of the Wilkund vasomotor subscale, frequency and intensity of hot flushes and night sweats, no significant difference was found between any of the herbal interventions and placebo at any of the 3-monthly time points measured, with one exception. At 12 months, placebo significantly outperformed the multibotanical-plus-soy counseling intervention for symptom intensity (p = 0.016). The average difference over all the time points between herbal interventions and placebo was less than 0.55 vasomotor symptoms per day, compared with - 4.06 for HT compared to placebo. (For the multibotanical, no significant differences were found between the study group and placebo at 3 months, p = 0.45, 6 months, p = 0.18, or 12 months, p = 0.88). While the sample size and duration of this study are definite strengths, a major limitation is the recruitment of women with mild symptoms. It is recommended by the U.S. Food and Drug Administration (FDA) guidelines that seven moderate to severe hot flushes per day, or 50 to 60 per week, be the minimum requirement for menopause studies, with specific definitions of severity.[116]

A 16-week RCT, conducted by the authors,[111] on a combination of *Hypericum perforatum* (St. John's wort) and *Vitex agnus-castus* with 100 late-perimenopausal and postmenopausal

women found no significant effect for the herbal combination over placebo on vasomotor symptoms, p = 0·42; Greene Climacteric scores, p = 0.13; or depressed mood, p = 0·42. However, both arms showed significant improvements on all outcome measures of vasomotor symptoms, (p < 0·001 and p < 0·01 for placebo and study groups respectively), depressed mood and overall menopausal symptoms measured on the Greene Climacteric scale, (p < 0·001 for both groups). Substantial placebo effects were observed for all the endpoints: 43% for flushing and night sweats, 41% for depression measured on the Hamilton Depression Inventory and 41% for Greene Climacteric scores. The daily dosages were consistent with clinical use and other RCTs on these herbs. No conclusions can be drawn regarding the effectiveness of *Vitex agnus-castus* in isolation, as individual arms were not included. However, a negative interaction between the two herbs is unlikely based on the known pharmacological mechanisms.

A pilot study of a combination botanical containing15 herbs in 8 women suggested a potential benefit of a combination botanical for improving moderate menopausal symptoms in women.[14] The herbs were administered in 550 mg capsules, 2 capsules taken twice daily, providing a total of 2200 mg of herbs per day. However, given the large number of herbs in the formulation (*Cimicifuga racemosa* - black cohosh root, *Viburnum opulus* - cramp bark, *Mitchella repens* - squaw vine, *Valeriana officinalis* - valerian root , *Polygonatum multiflorum* - King Solomon seed , *Taraxacum officinalis* - dandelion root, *Vitex agnus-castus* - chaste tree berry, *Rosmarinus officinalis* - rosemary leaves, *Nigella sativa* - black seed, *Eupatorium purpureum* - queen of the meadow , *Epimedium grandiflorum* - epimedium leaf, *Ligusticum chuanxiong* - chuanxiong rhizome, *Schisandra chinensis* -schisandra berry, *Mentha piperita* - peppermint leaves, *Rubus idaeus* - raspberry leaves), the dose of each individual herb was quite low, ranging from 80mg to 300mg per day. The dose of *Vitex* was 140 mg per day, or 6% of the total. In addition, the administration of a multi-component preparation means that it is not possible to draw conclusions about the individual contribution of *Vitex agnus-castus.* The lack of a placebo group is a major limitation, as placebo effects with vasomotor symptoms in menopause studies are substantial, with 51% being the average for studies of HT according to a meta-analysis published in 2004,[117] and generally in the range of 30 to 41% in RCTs of medicinal herbs.[11,13,118-120] From baseline to 3 months, a 42% decrease was observed in daily hot flushes, p = 0.0003, and Kupperman Index total symptoms score decreased by 24%, (p = 0.0028). Due to substantial placebo effects found in studies of vasomotor symptoms, it is possible that the 42% reduction in vasomotor symptoms

observed in this study would not be significant over placebo. The small sample size in this study also suggests this result should be interpreted with caution.

As mentioned above, studies of multi-component formulations do not contribute to the knowledge about the individual component herbs. Herbs are chemically complex, and may contain in excess of a hundred different plant chemicals, often with synergistic actions. While studies of multi-component formulations may reflect clinical practice, the numerous potential interactions between the chemical components they contain, makes if impossible to extrapolate findings of their effects to any of the individual herbs or chemical components.

Clinical Trials on Premenstrual Syndrome (PMS) and PMS-like symptoms

It is possible that the practice of using *Vitex agnus-castus* for menopausal symptoms refers to its benefits for PMS-like symptoms reported by some women during the perimenopause. *Vitex* has been shown in placebo-controlled,[121] comparator[122,123] and observational studies[124-126] to be effective in alleviating symptoms of PMS, which may well be relevant in this context.

A small study of PMS-like symptoms by the authors has shown that these improve in late-perimenopausal women with a combination of *Vitex agnus-castus* (extract equivalent to dry fruit 1,000mg per day) and *Hypericum perforatum* (extract equivalent to 5,400 mg dry herb flowering top standardised to contain hypericins 2,970 mcg, 27 mg hyperforin and 54 mg flavonoid glycosides).[111] Improvements were observed for total PMS scores and scores on all the sub-clusters of anxiety (PMS-A), depression (PMS-D), cravings (PMS-C) and hydration (PMS-H) on Abrahams' Menstrual Symptoms Questionnaire. The herbal combination was significantly superior to placebo for total PMS, PMS-D and PMS-C. Limited conclusions can be drawn regarding the individual contributions of the herbs, which were administered in combination. An impact of *Hypericum* on the depression sub-cluster can be inferred from its established efficacy in the context of mild to moderate depression.[127] However, the findings are also consistent with those of previous research on *Vitex* for PMS in premenopausal women.[121-126]

Directions for future research

Although *Vitex* is currently used clinically and promoted as being effective in the management of menopause-related complaints, appropriate evidence to confirm its efficacy in this arena is lacking. While administering herbal formulations reflects clinical practice, RCTs of formulations do not permit the effects of the herb as a sole agent to be evaluated. Such evidence from rigorous scientific RCTs is needed to clarify the efficacy and safety of *Vitex* in this context. Because of the substantial response observed in the placebo group in menopause trials, probably largely attributable to the natural history of the symptoms under investigation, large sample sizes are required to ensure adequate power, and a control group is essential in future studies. Findings from uncontrolled studies must be interpreted with caution for the same reasons. In accordance with the recommendations of bodies such as the FDA,[116] women with adequate symptom severity should be recruited to clinical trials. The findings reported for the PMS-like symptoms, while encouraging, were from a very small sample, and need to be replicated in a larger RCT dedicated to study of these symptoms.

Conclusions

The origins of the practice of administering *Vitex* in menopause are unclear, but it appears to be widespread among some groups of herbal practitioners. While several recent studies have suggested a benefit for multi-component formulations containing *Vitex* in the treatment of menopausal symptoms, evidence from rigorous randomised controlled trials is lacking to support the use of the individual herb in this context. Recent evidence from pharmacological studies points to possible mechanisms that could account for beneficial effects in some of the symptoms of menopause, as well as possibly influencing its onset. As the endocrinology and neuroendocrinology of menopause and its symptoms have not yet been fully elucidated, firm conclusions cannot be drawn. However, in view of current understanding, the emerging pharmacological evidence supports a role for *Vitex agnus-castus* in the management of menopause-related symptoms. In particular, further research may be appropriate into its possible role in alleviating the PMS-like symptoms associated with the perimenopause.

Manuscript Appendix 1 Herbal Menopause Formulations

TABLE 1 HERBAL MENOPAUSE FORMULATIONS CONTAINING *VITEX AGNUS-CASTUS* ON THE MARKET

Manufacturer	*Product*
Forces of Nature	Menopause Ease (Essential oil blend – transdermal)
Fusion Health	Menopause
Gaia Herbs	Supreme Vitex/Alfalfa - *A Menopausal Corrective Formula*
Herb Farm	Healthy Menopause Tonic (Pulsatilla + Vitex Comp)
Herbs of Gold	Menopause Night Relief
Nature's Alternatives	Vitex / Black Cohosh Plus: Women's Menopause Herb Tonic MenoEze Forte; MenoEze Day Night formula; MenoThin
Naturopathica	Menopause support
Nature's Sunshine	Wild yam and chaste tree cream
Neways	MenoLife
NutraLife	Herbal Supplement for Menopause, with Black Cohosh & Vitex
Oona	Femaren
Oriental Botanicals	MenoChange Cimicifuga-Vitex Compound
Planetary Formulas	EstroTrim :Menopause Weight Control
Pretorius	Phyto-Female Complex
SuperHerb, Netanya	Estro Balance plus Vitex: Menopause Relief
Totally Natural Products	

Manuscript Appendix 2 Hormones and Menopause-related Mood Changes

TABLE 2 OTHER PROPOSED ROLES FOR HORMONES IN THE AETIOLOGY OF MENOPAUSE-RELATED MOOD CHANGES

- increased *variability* of oestradiol,[128] follicle-stimulating hormone, and luteinising hormone[88]
- the *rate of change* of hormone secretion and levels[129-30]
- changes to oestrogen levels influencing *neuropeptides and neurotransmitters* (cholinergic, catecholaminergic and serotonergic[130]) in the limbic system and hypothalamus.[65]
- periods of *elevated oestrogens*, or excess relative to serum progesterone during the perimenopause. Oestrogens are potentially anxiogenic in excess, while progesterone/allopreganolone has a potent anxiolytic effect.[131]
- a pre-existing *sensitivity* in some individuals *to the change* in the gonadal hormones and resultant decreases in neural transmitters[38] such as noradrenaline.[132]
- in women previously reporting a *history of premenstrual syndrome*, perimenopausal depression could represent the elimination of follicular phase-related symptom remissions, and the development of a more persistent pattern of dysphoria.[99]
- Oestrogen has reciprocal interactions with CNS growth factors. *Brain-derived neurotropic factor* (BDNF) levels may be of potential importance in the aetiology and treatment of depression during the perimenopause.[133]

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Appendix 12 *Hypericum* in Major Depression

As mentioned in the body of the text, most studies on St John's wort have been conducted on major depression (Table 13.1). Some studies have included a range of depressive disorders.[96,97] Rarely, only the minimum HAM-D score for inclusion is reported,[98] which does not enable one to determine whether the study focused on *major* depression. This must be established with reference to some of the key items on the scale. [The defining characteristics of major depression and other depressive disorders can be found in Appendix 6.]

A12.1.1 Hypericum compared with standard antidepressants and placebo in Major depression

In terms of the trials comparing *Hypericum* extracts with standard antidepressants, most are restricted to patients with major depression [96,99-106] and the results have largely been positive. When compared to the SSRI *fluoxetine* in major depression, *H. perforatum* has been found to be at least equivalent in efficacy, but superior in terms of overall incidence of side-effects.[99,100,103] Compared to 75mg twice daily of the tricyclic antidepressant *imipramine, Hypericum* extract at the dose of 250 mg twice daily for 6 weeks was found to be therapeutically equivalent for mild to moderate major depression, but better tolerated.[106] Tolerability of *H. perforatum* extract was significantly better than older antidepressants and slightly better than SSRIs.[99,102,107]

A12.1.2 Hypericum compared with standard antidepressants and placebo in Major depression

Drug comparison studies that included an additional placebo arm are limited in number and have produced conflicting results. As mentioned previously, the *Hypericum* Depression Trial Study group[108] compared St John's wort with the SSRI *sertraline* and placebo, and in 340 patients with *major* depression (HAMD-17 score of 20+) and reported no effect for St John's wort Lichtwer Pharma LI160 extract of *H. perforatum* administered to at a dose of 900 mg per day, increasing to 1,200 or 1,500 or 1,800 mg/day as required. However, no effect over placebo was reported for the SSRI either. In contrast, Phillip and co-workers[102] found *Hypericum* to be more effective than placebo and at least as effective as 100mg *imipramine* daily in the treatment of moderate major depression in 263 patients with a mean

baseline HAMD-17 score of 22.6. The average dose of *H. perforatum* STEI 300 administered was 350mg, three times daily.[102] Both RCTs were of 8 weeks duration.

A12.1.3 Hypericum compared with Placebo in Major Depression

As mentioned in the body of the text, placebo-controlled RCTs conducted on St John's wort for major depression have generally been favourable.[109-112] In addition to those already discussed, several RCTs with the extract WS 5572 in mild to moderate major depression have observed significant effects over placebo over 6 weeks duration. These include studies by Lecrubier and colleagues[111] with 375 participants, and WS 5572 at a dose of 900mg/day, consistent with the 1996 study by Kalb and co-workers[110] on 72 patients. Kasper and colleagues subsequently trialled *the same extract* in 332 patients and found it to be superior to placebo at two different doses, 600mg/day and 1,200 mg/day.[109] Further details of these studies can be found in Table 13.1.

Appendix 13 Recruitment

Participants were sourced through

- 45 newspaper articles/advertisements
- 3 advertisements in professional journals/special interest group newsletters
- 16 talks at Universities, Colleges of Natural medicine, Women's groups
- 2 radio interviews
- fliers in waiting rooms of doctors/allied health professionals' clinics, health food shops, The Jean Hailes Foundation, Women's Health information centre
- posters at university campuses and colleges of natural medicine
- websites at RMIT and The Jean Hailes Foundation.

The numbers of enrolled participants recruited from each source is outlined in table A13.1 below.

TABLE A13.1 RECRUITS DERIVED FROM EACH SOURCE

Source	*Number recruited*	*Source*	*Number recruited*
Age newspaper	**15**	**Leader newspapers**	**24**
Herald-Sun newspaper	**11**	• Monash (3)	
Radio interview ABC 774	**7**	• Progress (5)	
Colleague in Sydney	**7**	• Stonnington (3)	
RMIT global email	**8**	• Diamond Valley (2)	
Word of mouth	**6**	• Preston (2)	
		• Whitehorse (2)	
Other local papers:	**15**	• Bayside (1)	
• Geelong Advertiser (6)		• Bayswater (1)	
• Emerald Hill Times (2)		• Chelsea (1)	
• Balwyn (1)		• Mornington (1)	
• Cobram Courier (1)		• Mt Waverly (1)	
• Daylesford Advocat (1)		• Mulgrave (1)	
• Melbourne Times (1)		• Whittelsea (1)	
• Whittlelsea Star (1)			
• Unspecified (2)		**Fliers**	**2**
		Talk	**1**
Zest magazine	**3**	**Unknown**	**3**
MX	**1**		

Additional sources of information and enquiries that did not result in any recruits are shown in table A13.2.

TABLE A13.2 ADDITIONAL SOURCES OF ENQUIRIES YIELDING NO PARTICIPANTS

Source	*Source*
Other Local Papers: • Alexandria & Eildon Standard • Bendigo Advertiser • Euroa Gazette • Melbourne Weekly • Moreland Community News • Mt Waverley Gazette • Portland local paper • Unspecified Queensland newspaper **Special Interest Groups:** • Life newsletter • NHAA journal • ATMS journal	**Leader Newspapers:** • Brunswick- Moreland • Dandenong • Darebin • Keysborough • Knox • Maroondah • Northcote • Port Phillip • Prospect • Richmond • Yarra **Radio Interview:** 3GG radio

Appendix 14 MEDICAL PRACTITIONER CLEARANCE FORM

RMIT UNIVERSITY School of Health Sciences

Complementary Medicine

Project Title: The effect of a Herbal Combination on Menopausal Symptomatology

Names of Investigators:

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I _____
(name of medical practitioner)

have examined _____
(name of participant)

I have also seen the attached summary of the study and understand that participants may be given herbal medicine preparations.
In my opinion, there is no medical problem present or current medication being taken that suggests this woman should not participate.

Medical Practitioner's Signature:

_____

Qualifications: _____

Address: _____

_____

Telephone: _____

Date: _____

If you need further information, please contact Diana van Die as above.

Needs blood test to determine if she is post or peri-menopausal. Yes □ No □

Inclusion criteria include: *Please tick to confirm that these have been performed*

□ recent breast examination
□ pap smear within past 2 years
□ measurements of blood pressure & pulse
□ a general physical check up

Exclusion Criteria *Please indicate whether any of these apply*

Women on the following medications will be excluded:

□ HRT or concomitant treatment for menopausal symptoms that cannot be discontinued for the duration of the trial. [This includes phyto-oestrogen and Vitamin E supplements.]
□ Oral contraceptives, or injected or sub-dermal
□ Herbal medicine that includes *Hypericum* or *Vitex*
□ Anti-depressants: SSRI's and related drugs (citalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, nefazodon
□ anxiolytics or hypnotics (psychotropic medication)
□ Digoxin
□ Clonidine
□ HIV medication (including protease inhibitors & non-nucleoside reverse transcriptase inhibitors)
□ Anti-coagulant medication such as Warfarin, Phenprocoumon
□ Theophylline
□ Immune-suppressant medication: eg Cyclosporin/tacrolimus
□ Triptans (sumatriptan, naratriptan, rizatriptan, zolmitriptan)
□ Anti-convulsant medication: carbamazepine, phenobarbitone, phenytoin
□ Dopamine receptor antagonists (Largactil, Modecate, Clozapine, Haloperidol)
□ Steroids (eg Cortisone, Prednisolone)

Also excluded are women with the following: *Please indicate if any of these apply*

□ medically or surgically induced menopause
□ spasmodic dysmenorrhoea not associated with PMS
□ undiagnosed vaginal bleeding (in post-menopausal women)
□ known photosensitivity
□ known intolerance to *Hypericum* or *Vitex*
□ history of epilepsy or seizures
□ pre-existing cancer
□ renal or liver disease
□ major heart disease
□ diabetes mellitus requiring treatment
□ uncontrolled hypertension
□ bipolar disorder
□ severe depression
□ current major psychiatric disorder, history of mania
□ substance abuse
□ pregnancy or attempting to conceive

To the best of my knowledge, none of the above exclusion criteria applies to me.

_____ _____
(signature) (date)

Appendix 15 Interviewees

Participants were interviewed at Clinicare Medical Centre, North Fitzroy (134), RMIT city campus psychology clinic (40), RMIT Bundoora psychology clinic (16) and a number of rural participants were interviewed by telephone (17). Of the 207 interviewed, 148 were eligible. However, 26 of those failed to meet run-in requirements and 19 declined consent. The remaining 103 were randomised. Reasons given for lack of consent or ineligibility can be seen in tables A15.1 and A15.2.

TABLE A15.1 REASONS FOR INELIGIBILITY AMONG WOMEN INTERVIEWED

Failed to meet inclusion criteria:	
• Greene score < 20	44
• Age > 60	1
• No ovaries	1
• Not 3 months' amenorrhoea	2
• Insufficient vasomotor symptoms	3
Exclusion criteria:	
• Concomitant contraindicated medication	1
• Participating in another trial	1
Co-morbidity:	6
• Severe depression (2)	
• Half a kidney (1)	
• Excess alcohol consumption (1)	
• BMI 53 (1)	
• Flushing pattern atypical of menopause (difficult to quantify) (1)	
Did not complete Run-in requirements:	
• Flushing symptoms abated during run-in to less than 5 per 24 hours	21
• Failed to get Medical clearance from GP	2
• Started HRT during run-in	3
Total	**85**

TABLE A15.2 REASONS FOR LACK OF CONSENT AMONG WOMEN INTERVIEWED

• Lifestyle too stressful/busy to permit compliance with procedures	7
• Counting flushes too depressing	1
• Undisclosed	5
• Lost to follow-up	2
• Unwilling to have pap smear (required within past 2 years)	1
• Symptoms too severe	1
• Fear of herbs	1
• Decided to recommence OTC supplements	1
Total	**19**

Appendix 16 Procedure for Telephone Screening

(Takes 10 minutes)

- Thank caller for enquiry
- Enquire about her source of Information about trial?
- Enquire whether she has already read brochure?
- Explain Objectives of research: 2 herbs (St John's wort and Chaste tree), 2 groups of symptoms (flushing and psychological) , 2 sub-groups (late-peri and post menopause)
- **"We are looking for** women aged 40-60 with flushing/or both types of symptoms, who still have at least 1 ovary and naturally menopausal (not brought on by surgery, chemotherapy or radiotherapy); not pregnant or attempting to conceive and not currently in another trial."

Exclusion criteria

Include, for example, other medication for symptoms: HRT, OCP, anti-depressant or anti-anxiety medication; phytoestrogens, Vitamin E for flushing; Vitex of Hypericum *unless willing to go off for trial period.*

- Intolerance of Vitex or Hypericum; photosensitivity

"Shall I go on?"

- **Herbs-** give information about known effects.
- **"You will be asked to** take tablets twice daily for 16 weeks and keep daily and weekly diaries (5 -10 mins max). Follow-up 8 weeks later.
- Explain 'Placebo controlled'. Placebo group will be offered treatment free of charge for 3 months at end of trial if found to be effective
- Chance of ADR's => Doctor in charge of monitoring.

Contraindicated with herbs are:

- HIV mdx or immune-suppressant mdx;
- Digoxin;
- anti-coagulant medication such as Warfarin, Phenprocoumon;
- . Theophylline for asthma
- Triptans (migraine medication)

- Medication for epilepsy or seizures
- Dopamine receptor antagonists (Largactil, Modecate, Clozapine, Haloperidol)
- Steroids (eg Cortisone, Prednisolone)

Also excluded are women with

- A history of epilepsy or seizures
- current cancer, renal or liver disease, diabetes mellitus requiring treatment, uncontrolled hypertension
- bipolar disorder, major psychiatric disorder, history of mania
- substance abuse

“Shall I continue, or are you excluded?”

If still eligible and interested,

- get details to send Plain Language Statement
- arrange interview place and time.
- ask them to bring list of known medications.
- Get phone number to confirm appointment.
- Ask to bring PLS and any questions.

“**Any questions?”**

Appendix 17 Respondents

Of the 761 respondents, 8 calls could not be returned, 546 were excluded at telephone screening and the remaining 207 were interviewed. Reasons for exclusion are contained in table17.

TABLE A17 REASONS FOR INELIGIBILITY AMONG WOMEN SCREENED BY TELEPHONE

Did not meet inclusion criteria:	
• Insufficient vasomotor symptoms	160
• Not 3 months' amenorrhoea	64
• No ovaries	18
• Outside acceptable age range	8
• Interstate	45
• Male	2
• Only vasomotor symptoms	1
	298
Exclusion criteria:	
• Concomitant contraindicated medication For menopausal symptoms 101 (HRT 58; Natural 43) For other medical conditions 29	130
• Co-morbidity (inadmissible medical condition)	23
• Participating in another trial	4
	455
Other:	
• Fear of side-effects	6
• Unwillingness to risk allocation to placebo group	12
• Personal situation incompatible: stressful/ traumatic/ busy	19
• Undisclosed	34
• Failed to appear for Interview	15
• Discouraged by naturopathic practitioner	1
• Wanted Chinese herbs	1
• Trial quota already reached	1
• Only seeking information	2
	546

Appendix 18 PLAIN LANGUAGE STATEMENT

School of Health Sciences (Complementary Medicine)

Project Title: **The effects of a Herbal Combination on Menopausal Symptomatology**

About the Study

The aim of this study is to test a combination of two commonly used herbs in the treatment of menopausal symptoms in 40-60 year old women. It is estimated that at least 84% of women experience one or more menopausal symptoms with 40% to 45% rating their symptom(s) as problematic. Typical symptoms include depressed mood, anxiety and nervousness, mood swings, irritability, memory and concentration difficulties, fatigue, sleep disturbances, night sweats, hot flushes, sweating, dizziness, migraine, palpitations, vaginal dryness, bladder dysfunction, decreased libido and dry skin as well as changes to the regularity and flow of menstrual cycles.

The objective of this study is to investigate the effectiveness of a herbal treatment in providing relief from menopausal symptoms, especially mood changes and hot flushes.

The study is being undertaken by Diana van Die in the School of Health Sciences (Complementary Medicine), RMIT as part of a Higher Degree. The supervisor for the study is Professor Marc Cohen. RMIT has in place public liability insurance in connection with this research. The herbal tablets to be used in the trial have been prepared by MediHerb Pty Ltd, a major manufacturer of practitioner herbal products in Australia. The study is being funded by RMIT.

About the Treatment: St. John's wort and Chaste tree

St. John's wort (*Hypericum perforatum)* is probably best known nowadays for the relief of depression and much research has shown it to be as effective as anti-depressant medication for the treatment of mild to moderate depression. Traditionally, however, it was used specifically for the treatment of menopausal symptoms to relieve anxiety, irritability, nervous tension and insomnia. A recent research study found it effective in reducing the psychological symptoms of menopause as well as hot flushes.

Chaste tree (*Vitex agnus castus*), also traditionally used in menopause, is still widely used by medical herbalists today for this. Most research, however, has been on its effectiveness in relieving the symptoms of pre-menstrual syndrome, which are commonly experienced in the years leading up to menopause (peri-menopausal years).

The combination of these two herbs is commonly prescribed in clinical herbal practice, although no reports of clinical trials on this specific combination were found. In the current study, these herbs will be given in tablet form. The St. John's wort tablets contain Hypericum extract equivalent to 1.8g dry fruit each, standardised to contain hypericin 0.99mg each; the Vitex tablets contain extract equivalent to 500mg dry fruit each. If this combination is found to be effective in reducing menopausal symptoms, it may offer an important alternative to HRT.

Safety and Side-effects

As with any form of medication, there is a chance of side effects occurring. Both herbs are on the List of approved substances by the Therapeutic Goods Administration in Australia and are readily available over-the counter. They are suitable for long-term use when used within the recommended dosage range.

Mild side effects have been found with St. John's wort, although these are rare (2.4%-4.1%). Recorded adverse events occurring in a small minority of people include minor gastro-intestinal irritations (0.6%), slight nausea, rash (0.5%), fatigue (0.4%), restlessness (0.3%) and photosensitivity. Photosensitivity may occur with prolonged exposure to full sun or artificial UVA radiation (sunlamps, solariums). You are therefore advised to avoid using solariums while on this trial, and to avoid exposing your skin to the sun without sun block.
Less common are dizziness, headaches, sleep disturbances, paraesthesia (numbness and tingling) (3 cases) and palpitations.

There have been no reports of serious adverse effects with Chaste tree. In a few cases (approximately 5%), its use has been associated with headaches, gastrointestinal complaints, nausea and acne. There has been one report each of abscesses, bleeding between periods and nettle rash.

Should any information become available that may affect your willingness to continue participating in the trial, you will be notified as soon as possible.

Adverse reactions

If an adverse reaction *does* occur while you are participating in this study, we advise that these steps be taken:

- Temporarily discontinue the tablets
- Contact Professor Marc Cohen or Diana van Die
- Dr Helena Teede will speak with you and advise you how to proceed.

What you will have to do

An initial interview will be conducted to assess your eligibility for the study. You will be asked about you current health status, current menopausal symptoms and their severity, current medication, including natural supplements, and past medical history. It is important that you provide this information as fully and accurately as possible to avoid putting yourself at risk as both herbs are contraindicated in certain medical conditions and both are known to interact with certain drugs. Anyone with drug or alcohol addictions will not be eligible to participate. If you are eligible for the study, you will be asked to sign a consent form, given to you at the initial interview.

You will then be randomly allocated to one of two groups: the placebo group or the treatment group. There will be weekly questionnaires to fill out (which should take no more than 5 - 15 minutes), and you will be required to take three tablets on rising and two tablets later in the day. There is no guarantee that you will derive any alleviation of menopausal symptoms from participation in this study. However, at the end of the trial, those participants in the placebo group will be offered the treatment for a standard treatment period of 3 months if it is found to be effective.

If you become pregnant during the trial, you must notify the investigators immediately. Your participation in the trial will cease. Your participation on the trial must also cease if you commence taking any of the following drugs: *Digoxin*, HIV medication, *Warfarin*, *Phenprocoumon*, antidepressants, oral contraceptives, *Theophylline, Cyclosporin*, immune-suppressant medication, Triptans (migraine medication such as *Imigran*) or dopamine receptor antagonists. You must notify the investigators immediately if you are prescribed any of the above medication.

Summary of Treatment Program:

You will be required to continue the treatment for 16 weeks. However, the total period of your participation will be 20 weeks with a follow-up questionnaire at week 32.

Weeks 1 –2 Baseline	General physical check-up by GP; diet & health questionnaire, menopausal symptoms scale, depression scale & quality of life scale. Daily flushing record is to be kept during run-in.
Weeks 3 –18 Treatment	Menopausal symptoms scale, depression scale and lifestyle diary are required to be completed monthly. A flushing diary is to be completed daily. A menstrual symptoms diary will be completed every 4 weeks where relevant.
Weeks 18-20 Post-treatment	Dietary questionnaire & Quality of Life scale to be completed in week 18.
3 months after treatment Follow-up	Menopausal symptoms scale, flushing count, depression and lifestyle questionnaire are required to be completed. Telephone contact will be made to determine your current symptoms and discuss any issues that may have arisen since your involvement with the study.

Confidentiality

All data such as questionnaires collected from the research will be kept confidential and secured in a locked filing cabinet in the School of Health Sciences (Complementary Medicine) at RMIT. No identifying information will be included in data; only participant code numbers will be used. Only the investigators will have access to confidential information. Your participation in this study is voluntary and you may withdraw from the trial at any time. If you have any further enquiries about this study, please feel free to contact us at any time.

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Assoc Prof Kerry Bone, Consultant, Founder and Director of Research & Development, MediHerb, Associate Professor of University of New England

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. Telephone (03) 9925 1745. Details of the complaints procedure are also available from the above address.

Appendix 19 Initial Interview Consent Form

RMIT University School of Health Sciences

Complementary Medicine

Initial Interview Consent Form

Project Title: The Effects of a Herbal Combination on Menopausal Symptomatology

Names of Investigators:
Diana van Die (Investigator)
Tel: (03) 9925 7596
Email: s9403478@student.rmit.edu.au

Professor Marc Cohen (Supervisor)
Tel: (03) 9925 7440
Email: marc.cohen@rmit.edu.au

I agree to take part in the initial interview for the above study.

I understand that I will be asked to provide personal information. I am aware that this interview is for the purpose of establishing whether or not I am eligible to participate in the above study, and that I may not be invited to participate.

I have been informed that all information I provide during this interview will be treated as confidential. It will be safeguarded by code and only disclosed in a de-identified form.

I am aware that I may withdraw from this interview at any time.

Participant's Consent:

Name: _____ Date: _____
(Participant)

Name: _____ Date: _____
(Witness to signature)

Name: _____ Date: _____
(Consented by)

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. Telephone (03) 9925 1745. Details of the complaints procedure are also available from the above address.

Appendix 20 Screening and Recruitment Forms

Appendix 21 Drug Accountability Form

Appendix 22 Sample Questionnaire booklet

Appendix 23 Informed consent Form

RMIT HUMAN RESEARCH ETHICS COMMITTEE

Prescribed Consent Form For Persons Participating In Research Projects Involving Tests and/or Medical Procedures

SCHOOL OF **HEALTH SCIENCES**

AREA COMPLEMENTARY MEDICINE

Name of participant:

Project Title: The effects of a Herbal Combination on Menopausal Symptomatology

Name(s) of investigators: (1) Diana van Die Phone: 9925 7596

(2) Professor Marc Cohen Phone: 9925 7440

1. I have received a statement explaining the procedures involved in this project.

2. I consent to participate in the above project, the particulars of which - including details of procedures – have been explained to me.

3. I authorise the investigator or his or her assistant to use with me the tests or procedures referred to in 1 above.

4. I acknowledge that:

   (a) The possible effects of the tests or procedures have been explained to me to my satisfaction.
   (b) I have been informed that I am free to withdraw from the project at any time and to withdraw any unprocessed data previously supplied (unless follow-up is needed for safety).
   (c) The project is for the purpose of research. It may not be of direct benefit to me.
   (d) The privacy of the personal information I provide will be safeguarded and only disclosed where I have consented to the disclosure or as required by law.
   (e) The security of the research data is assured during and after completion of the study. The data collected during the study may be published, and a report of the project outcomes will be provided to RMIT University. Any information which will identify me will not be used.

Participant's Consent

Name: _____ Date: _____

(Participant)

Name: _____ Date: _____

(Witness to signature)

Name: _____ Date: _____

(Consented by)

Participants should be given a photocopy of this consent form after it has been signed.

Any complaints about your participation in this project may be directed to the Secretary, RMIT Human Research Ethics Committee, University Secretariat, RMIT, GPO Box 2476V, Melbourne, 3001. The telephone number is (03) 9925 1745.
Details of the complaints procedure are available from the above address.

Appendix 24 Case report forms

The following appendix contains Case Report Forms completed for each participant throughout the trial.

These include

- Follow-up forms for contact at the end of weeks 1,4, 8,12 and 16
- hospitalisation form
- Follow-up record at 8 weeks post completion
- study termination record form.

Appendix 25 Suspected Adverse Events Report Form

Appendix 26 Thin Layer Chromatography (TLC) assessment to check veracity of code

"Tablets were crushed and extracted using ultrasound 91 tab/5mL methanol. Samples were transferred to disposable glass tubes, centrifuged and transferred again to small plastic effendorf tubes and centrifuged again using the microfuge. Samples were then suitable for application using the Camag semi-automatic spotter.

A luteolin-7glycoside standard was prepared. Two Mediherb liquid samples , St John's Wort (Batch no 125178) and Chaste tree (Batch no124324) were also spotted onto plate. The plate was the standard Merck TLC silica gel 60 F354 plate used for in-house TLC analysis at Mediherb.

Samples were spotted left to right on plate as detailed in table below and shown in the photograph below.
Chromatogram development was by using the solvent mix as outlined in the Camag application note for Chaste tree flavonoids.
(THF-Toluene-Formic Acid-Water / 16:8:2:1).
Visualisation was by derivatisation using natural product reagent/PEG.

FIGURE A26 TLC FINGERPRINTS FOR ST JOHN'S WORT AND CHASTE TREE SAMPLES

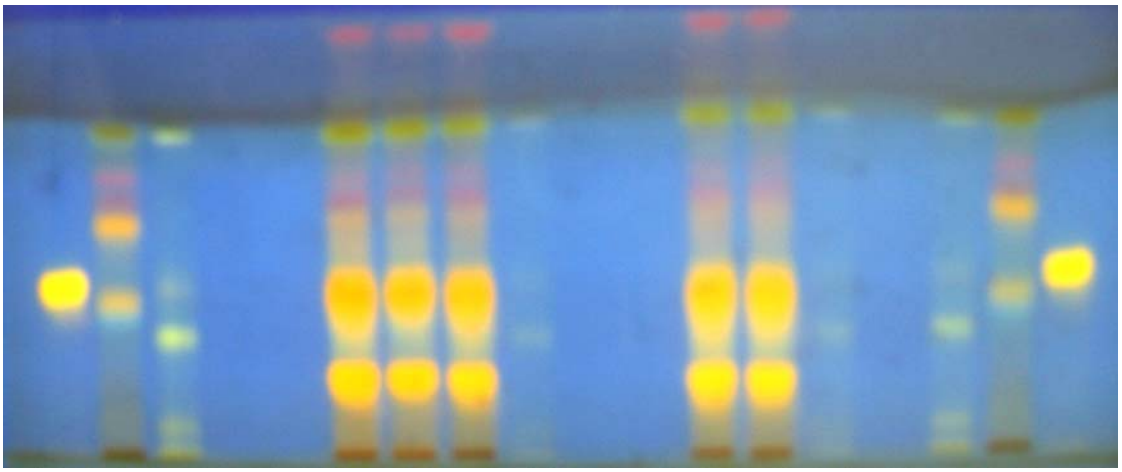

TABLE A26 RESULTS OF VISUAL AND TLC ASSESSMENT WITH RETURNED TABLETS

Position #	*Sample*	*Herb identity in sample*	*Prior Visual Assessment result*	*Do the Different Analyses Match*
1	Luteolin-7-gly	-	-	-
2	M/H SJW Liquid	-	-	-
3	M/H Chaste tree Liquid	-	-	-
4	11A	Placebo	Placebo	Match
5	11B	Placebo	Placebo	Match
6	38A	SJW	SJW	Match
7	72A	SJW	SJW	Match
8	92A	SJW	SJW	Match
9	92B	Chaste Tree	Chaste Tree	Match
10	99A	Placebo	Placebo	Match
11	99B	Placebo	Placebo	Match
12	101A	SJW	SJW	Match
13	103A	SJW	SJW	Match
14	103B	Chaste tree	Chaste Tree	Match
15	67B	Placebo	Placebo	Match
16	M/H Chaste tree Liquid	-	-	-
17	M/H SJW Liquid	-	-	-
18	Luteolin-7-gly	-	-	-

Comments and Observations:

"The match of both the visual and TLC results to each other and also to the information supplied in the email confirms there is no mix-up of tablets for the ones analysed here. As noted in the visual assessment report analysis of several of the requested tablet samples was not possible since none were returned for those particular numbers."

Appendix 27 Unadjusted flushing scores

TABLE A27 UNADJUSTED MEAN DAILY WEIGHTED FLUSHING SCORES BY STUDY WEEK

Hot Flushes

Dependent Variable: average daily weighted score

		PHASE																
subject group		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
placebo	Mean	16.35	14.13	12.23	11.95	11.52	10.84	9.97	10.67	10.55	9.94	9.82	9.96	10.03	9.21	9.28	9.13	9.32
	Std. Error	1.33	1.34	1.35	1.35	1.35	1.34	1.31	1.42	1.37	1.38	1.38	1.40	1.40	1.38	1.38	1.38	1.38
	95% Confidenc Interval	13.75	11.49	9.57	9.29	8.87	8.21	7.40	7.89	7.86	7.23	7.10	7.21	7.29	6.50	6.56	6.42	6.60
		18.96	16.76	14.89	14.61	14.18	13.47	12.55	13.44	13.24	12.66	12.53	12.70	12.78	11.93	11.99	11.85	12.03
active treatme	Mean	16.53	14.45	13.30	12.90	12.13	11.58	10.77	10.89	9.82	11.13	10.90	10.94	10.91	10.88	11.29	11.73	10.86
	Std. Error	1.34	1.37	1.40	1.40	1.40	1.35	1.34	1.42	1.38	1.37	1.37	1.37	1.37	1.37	1.37	1.37	1.37
	95% Confidenc Interval	13.90	11.77	10.56	10.15	9.39	8.92	8.14	8.11	7.10	8.44	8.21	8.25	8.23	8.20	8.61	9.04	8.17
		19.16	17.14	16.04	15.64	14.88	14.24	13.40	13.67	12.53	13.81	13.58	13.63	13.60	13.57	13.98	14.41	13.55

Generated using Linear Mixed model analysis

Appendix 28 Greene Climacteric Subscale scores

TABLE A28 GREENE CLIMACTERIC SUBSCALE SCORES (INCLUDING FOLLOW-UP)

Mean (SE) 95% Confidence Intervals, Lower bound and Upper bound

Subscale	Phase / Group		baseline	Week 4	Week 8	Week 12	Week 16	Follow-up
Psychological subscale[1]	placebo	mean	11·48	8·63*	7·95**	7·47**	6·73**	7·88**
		Std error	0·71	0·73	0·74	0·75	0·75	0·75
		95% - CI	10·07 – 12·89	7·19 – 10·06	6·50 – 9·41	6·00 – 8·94	5·27 – 8·20	6·41 – 9·35
	active	mean	11·73	9·51*	8·42**	8·37**	8·50**	8·29**
		Std error	0·72	0·75	0·74	0·74	0·74	0·76
		95% - CI	10·33 – 13·15	8·04 – 10·98	6·96 – 9·88	6·92 – 9·83	7·05 – 9·96	6·81 – 9·78
Anxiety subscale[1]	placebo	mean	6·36	4·67*	4·53**	4·28**	3·71**	4·14**
		Std error	0·40	0·41	0·41	0·42	0·42	0·42
		95% - CI	5·58 – 7·15	3·87 – 5·47	3·72 – 5·33	3·46 – 5·09	2·90 – 4·53	3·33 – 4·96
	active	mean	6·33	5·14*	4·57**	4·43**	4·61†	4·49**
		Std error	0·40	0·42	0·41	0·41	0·41	0·42
		95% - CI	5·56 – 7·12	4·32 – 5·95	3·76 – 5·38	3·63 – 5·24	3·80 – 5·41	3·67 – 5·32
Depression subscale	placebo	mean	5·12	3·96*	3·38**	3·20**	3·02**	3·68**
		Std error	0·36	0·37	0·37	0·38	0·38	0·39
		95% - CI	4·39 – 5·85	3·24 – 4·70	2·63 – 4·12	2·44 – 3·96	2·26 – 3·78	2·93 – 4·43
	active	mean	5·40	4·37*	3·85**	3·94**	3·89**	3·80**
		Std error	0·37	0·39	0·38	0·38	0·38	0·39
		95% - CI	4·67 – 6·13	3·61 – 5·13	3·10 – 4·60	3·18 – 4·69	3·14 – 4·45	3·04 – 4·56
Somatic subscale	placebo	mean	4·94	3·21**	3·06**	2·74**	2·83**	3·30*
		Std error	0·35	0·36	0·36	0·37	0·37	0·36
		95% - CI	4·25 – 5·63	2·51 – 3·91	2·36 – 3·76	2·02 – 3·46	2·11 – 3·54	2·59 – 4·01
	active	mean	4·64	3·76*	2·89**	3·21**	3·13**	3·41**
		Std error	0·35	0·36	0·36	0·36	0·36	0·37
		95% - CI	3·95 – 5·33	3·05 – 4·48	2·19 – 3·60	2·50 – 3·92	2·42 – 3·84	2·70 – 4·13
Vasomotor subscale	placebo	mean	4·28	3·29**	2·98**	2·74**	2·59**	3·09**
		Std error	0·21	0·22	0·22	0·22	0·22	0·22
		95% - CI	3·86 – 4·70	2·87 – 3·72	2·56 – 3·40	2·31 – 3·17	2·15 – 3·02	2·66 – 3·51
	active	mean	3·92	3·20*	2·79**	2·83**	2·83**	2·70**
		Std error	0·21	0·22	0·22	0·22	0·22	0·22
		95% - CI	3·50 – 4·34	2·76 – 3·63	2·36 – 3·22	2·40 – 3·26	2·40 – 3·26	2·26 – 3·13
Sexual subscale	placebo	mean	1·72	1·33*	1·40	1·20*	1·15**	1·34
		Std error	0·14	0·15	0·15	0·15	0·15	0·15
		95% - CI	1·44 – 2·00	1·05 – 1·62	1·11 – 1·68	0·90 – 1·49	0·86 – 1·44	1·05 – 1·63
	active	mean	1·74	1·52	1·49	1·53	1·49	1·46
		Std error	0·14	0·15	0·15	0·15	0·15	0·15
		95% - CI	1·62 – 2·02	1·23 – 1·81	1·20 – 1·78	1·24 – 1·82	1·20 – 1·78	1·16 – 1·75

1. Covariates appear in the model: baseline phytoestrogen intake

*significant from baseline at $p < 0.05$ ** significant from baseline at $p < 0.01$

Appendix 29 Total adverse events

TABLE A29

Total adverse events during trial by subject grouping

Count

	subject group		
	placebo	active treatment	Total
MS pains, strains +	6[1]	13[7]	19
UTI	1	3[1]	4
accidents, injuries,	5	5[2]	10
allergy	1[1]	1	2
anxiety*	1		1
breast tenderness*	2		2
breathlessness		1	1
cardiovascular	6[1]		6
cold sore*		3	3
cold/flu	18[3]	23[5]	41
constipation*	3	2	5
cyst	2[1]	2[1]	4
dental complaints	1[1]	4[3]	5
depression		3[1]	3
dizziness*	2[1]	1	3
dry eyes	1		1
dysmenorrhoea*		2	2
fatigue	1	1[1]	2
headache*	4[1]	7[2]	11
migraine*	1	2	3
nausea*	3[1]	4	7
nosebleed		1	1
other GI complaints*	14[4]	13[3]	27
other RTI	10	4[2]	14
other infection	3[2]		3
palpitations*	1	2	3
paraesthesia*	1	2	3
period		2	2
reproductive		2[1]	2
skin complaints*	11[1]	3	14
stomach upset/cramp*	5	2	7
vomiting	1	1	2
weakness		1	1
weight gain	3[1]		3
Total	107	110	217

[]. denotes nubmer of cases rated as severe

Adverse events reported in previous trials of *Hypericum* or *Vitex* are indicted with an asterisk.

Appendix 30 PMS-subscale Scores

TABLE A30.1 SIGNIFICANCE LEVELS FROM PAIRED SAMPLES *T*-TESTS BETWEEN BASELINE AND ALL DATA COLLECTION POINTS FOR ACTIVE TREATMENT GROUP

Week / Cluster	Week 4 *p*-value	Week 8 *p*-value	Week 12 *p*-value	Week 16 *p*-value
PMS-Total	0.007	0.003	0.006	0.000
PMS-A	0.02	0.04	0.04	0.003
PMS-C	0.04	0.03	0.12	0.01
PMS-D	0.03	0.01	0.006	0.001
PMS-H	0.22	0.08	0.07	0.002

TABLE A30.2 MEAN DIFFERENCES FROM BASELINE TO WEEK 16 FOR PLACEBO AND ACTIVE ARMS

	Placebo *n*= 6			Active *n* =8		
	Mean Diff (SE)	95% CIs	*p*-value	Mean Diff (SE)	95% CIs	*p*-value
Total PMS	6.83(5.30)	-3.88, 17.54	0.205	15.25(4.59)	5.98,24.53	0.002
PMS-A	3.00(1.49)	0.008,6.01	0.051	3.50(1.29)	0.90,6.11	0.010
PMS-C	0.33(1.75)	-3.21, 3.87	0.850	3.25(1.52)	0.18, 6.32	0.038
PMS-D	1.83 (1.58)	-1.37,5.03	0.254	5.63 (1.37)	2.85,8.40	0.000
PMS-H	1.67(1.65)	-1.66,5.00	0.318	2.88(1.43)	-.008,5.76	0.051

Mean (SE)
Calculated using last observation carried forward and mixed model analysis.

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