Effects of Kiwi-Fruit Seaweed Extract on the metabolism of female reproductive hormones

A Thesis Presented for a Doctor of Philosophy Degree

By

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May, 2014

Declaration

I, Lulu Fu, declare that the PhD thesis entitled *Effects of Kiwi-Fruit Seaweed Extract on the metabolism of female reproductive hormones* to contain no more than 100,000 words in length, exclusive of tables, figures, appendices, reference and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signature **Date 30th May, 2014**

Acknowledgments

I am most grateful to my principal supervisor, Professor Hong Xu, for her constant support, guidance and encouragement throughout the entire course of this study, and for her professional advice in the preparation of the clinical trial and the drafting of this thesis. Words are not enough to express my utmost respect and appreciation for Professor Hong Xu.

During my PhD study, another person I would like to thank is Associate Professor Jack Antonas, my second supervisor who assisted with the human research ethic application at the initial stage of my study and my thesis editing at the final stage of my study

My sincere thanks also extend to Ms Julia Tracker, Mrs Lena Berias, Dr Deanne Skelly, Dr Mark Scarr, Dr Claude Bemkemoun, and Ms Ann Marie Angebrandt, who have all supported me to complete my PhD. My thanks and appreciation also extends to the staff at Victoria University, who supported me during this journey. All those mentioned had given me generous assistance and encouragement and without them, it would be extremely difficult to pass all the research works.

Finally, I would like to express my sincere gratitude to my parents and to my son, for their continuous support and encouragement. Without their support, I would have found it difficult to complete my PhD study.

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ABSTRACT

Hormonal fluctuations are known to affect a female's quality of life during the different stages of their lifespan. In middle-aged women, hormonal fluctuations are especially known to impact on their mental and physical health. During the peri-menopausal years, a woman's health can deteriorate and unfortunately may eventuate in severe diseases.

The perspective on conventional and Chinese medicine is that there are interventions available for hormone-related diseases. Within the conventional medical field, little is known about the interventions available within Chinese medicine (CM) practice and even less is known of the efficiency of Chinese Medicine interventions, even within the Chinese medicine practice. Conventional Medicine and Chinese medicine both rely on diagnosis as a pre-requisite to prescribing intervention. However, the main difference between the two medical fields is that diagnosis in Chinese medicine relies on patterns of dysfunction of the Chinese medicine organ systems, rather than any underlying causes explained in terms of a pathophysiological malfunction.

The aim of this research was to investigate the effects of a Chinese food formula, Kiwi-Fruit Seaweed Extract (KFE), on the regulation of the biomarker 2-hydroxyoestrone:16α-hydroxyoestrone, relating to thermography changes and magnetic field changes in the woman's breast. The magnetic field changes which indicated by the pulsed electromagnetic field test overlaid the Liver Meridian, Kidney Meridian and other Meridians in the trunk area as described from the Chinese medicine perspective. This study also examined the general improvement and emotional impact of KFE on women with Liver Qi Stagnation and Liver Kidney Yin Deficiency.

In addition, this study has investigated the relationship of the female reproductive hormonal metabolism changes with Chinese medicine diagnosis in Liver Qi Stagnation (LQS) and Liver Kidney Yin Deficiency, thermography changes and the magnetic field changes. When these research methods were used together, the evidence found was able to indicate towards an early development of breast diseases in its preclinical form, which may be of interest to Conventional Medicine for defining preclinical breast changes in breast cancer.

In a double-blind, placebo-controlled clinical trial, KFE was provided for Australian peri-menopausal women for 4 weeks intervention. Assessments were conducted at baseline and every 10 days. The results of the pre-trial assessments have demonstrated Liver Qi Stagnation and Liver Kidney Yin Deficiency are the main patterns, 73% and 58% respectively for the applicants of peri-menopausal women (n = 56). Kiwi-Fruit Seaweed Extract can regulate the female reproductive hormone metabolism and resolve the LQS pattern ($p < 0.05$, $t = 6.19$). In the KFE group of participants (n = 18), the pre-trial (13.90 ± 4.35) and post-trial results (5.10 ± 4.88) of LQS pattern indicated the lessening of LQS symptoms. The ratio of 2-hydroxyoestrone:16α-hydroxyoestrone was elevated by the intervention of KFE. The pre-trial (0.91 ± 0.20) and the post-trial (1.42 ± 0.34) of the biomarker test results in the KFE group (n = 18) indicated a significant improvement in female reproductive hormone metabolism ($p < 0.05$, $t = 2.109$). The improvement of health condition was also demonstrated in the magnetic test results and a regulation of the thermography temperature. In addition, the general wellbeing of the individual participants had improved in the KFE group, which was demonstrated by the self-rated health questionnaire (SF36).

This study indicated that the patterns of disharmony within the group of middle aged Australian women, who participated in the research, reflected the Chinese Medicine theory that Liver Qi Stagnation and Liver Kidney Yin Deficiency were the main patterns for this group. The biomarkers used in this study were both reliable and sensitive for investigating the hormone imbalance. The selected Chinese therapeutic food, Chinese Kiwi-fruit Seaweed Extract could benefit those concerned with peri-

menopausal symptoms or diseases associated with hormone disorders and treat the patterns of disharmony. The pulsed electromagnetic field test was an effective diagnostic tool for the detection of acupuncture meridian disorder. The stagnation of the meridian was related to the magnetic field change. Chinese Kiwi-fruit Seaweed Extract could regulate the function of the acupuncture meridians which have been proved by the pulsed electromagnetic tests. These approaches can be easily applied as early preventative and diagnostic strategies that integrated both Chinese Medicine and contemporary therapies.

Publications

Fu, LL. and Xu, H. (2012). Acupuncture meridians, pulsed electromagnetic field, thermography and Chinese food therapy. *Evidence-based Complementary and Alternative Medicine (eCAM).* Submitted for publication

Fu, LL. and Xu, H. A preliminary study of the effectiveness of Chinese therapeutic food on regulating female reproductive hormones. *Integrative Medicine Insight*. 2011; 6: 7-12. (In English)

Fu, L and Xu, H. Traditional Chinese medicine patterns of disharmony and preclinical changes in breast. Australasian Acupuncture & Chinese Medicine Annual Conference. May 24-28, 2009. Melbourne, Australia

Fu, LL., Xu, H. and Antonas, J. Integrated Chinese medicine strategies in the prevention of breast cancer. 3rd International Congress on Complementary Medicine Research 2008. Sydney, 29-31 March 2008

Definitions and Abbreviations – Biomedicine

16α–OHE	16α–hydroxyoestrone is an oestrogen agonist and stimulate the cell proliferation and differentiation (Lotinun, 2001, Bentz, 2005)
2 OHE	2-hydroxyoestrone has anti-oestrogenic activity, antiproliferation on mammary cells (Bentz, 2005)
36-Item Short-Form Health Survey	Short form 36 was most popular to use for self assessment in the conventional medical research. The 36 items of the questionnaire was used to determine dimensions of physical, mental and social functioning, vitality and general health which was associated with SRH (Hanmer, 2009).
Apoptosis	A dying cell caused by the activation of the intracellular enzymes such as capsases. Capsases could destroy DNA, cytoskeleton and caused the cell falling into a quick death (Marieb, 2001).
Binucleate lymphocyte cells	The cell of lymphatic system briefly had two nuclei in the telophase and cytokinesis at the end of mitosis (Marieb, 2001)
Breast Thermography	Thermography is a digital infrared thermal imaging, which detects the skin temperature in the breast area affected by the metabolic activity and vascular circulation in both pre-cancerous tissue and the area surrounding developing breast cancer (Amri, 2011)
cAMP	Cyclic adenosine monophosphate acts as a second messenger and is activated after an adrenergic receptor function stimulated. Increasing cAMP leads the cell activity to elevate (Galbraith, 2004)
Dimethylnitrosamine	Dimethylnitrosamine ($C_2H_6N_2O$) found in tobacco smoke

	and certain foods which are known to be a potent carcinogen. Abbreviation is DMN or DMNA
Dyslipidaemia	Dyslipidaemia was an elevation of plasma cholesterol and or triglyceri that contributes to the development of atherosclerosis. It was caused by primary (genetic) or secondary (other disease) (Beers, 2004).
Estradiol	Is one of the three of oestrogen in the blood and urine, with oestrone and estriol (Faupel-Badger, 2010)
Isoflavones	Isoflavones are a class of phytoestrogen (Kok, 2004)
Limbic-hypothalamic-pituitary-adrenal axis	An endocrine system which includes a group of brain structure hippocampus, gyrus fornicates, amygdala and pituitary gland, and a structure located above the kidney organ- the adrenal glands (Marieb, 2001).
Low frequency PEMF	Low frequency PEMF is a Pulse Electric Magnetic Field (Yung, 2005) with the low frequencywhich is less than 1000 Hz. The strength of PEMF is between 200uT (2 Gauss) to 400uT (4 Gauss) (Naomi, 2006, Thomas, 2007). Extremely low frequency magnetic field was at 50 to 60 Hz (Leman, 2001)
Mann-Whitney U-test	Mann-Whitney U test is often viewed as the non prametric equivalent of student's t test, which is used to determine if a difference exists between two groups.
Mitochondrial Oxidative stress	Mitochondrion is one of the small cytoplasmic organelles inside the cells. It contained enzymes of the tricarboxylic acid cycle used for fatty acid oxidation and producing ATP for the cell energy. When the function of mitochondria reduced, reactive oxygen species (ROS) increased and implicated in an array of inflammatory disease and tumour growth such as breast cancer (Sotgia, 2011).
Phytoestrogen	Oestrogens in plant foods is called phytoestrogen, which are known to improve well-being and reduce primenopausal

	discomfort (Kok, 2004)
TGA	Therapeutic Goods Admonistration is an Australia's regulatory authority for therapeutic goods
TSQ quantum™	TSQ quantum ™ is a themo- electron- technique for the mass spectrometry and chromatography
COAT score	Comprehensive arthritis test score is comprised of four sub-scales: pain, stiffness, difficulty with physical activity and overall symptom severity (Myers, 2010)
BRCA1, BRCA 2 and P53	Germline mutations in the tumor suppressor genes BRCA1 and BRCA2 predispose individuals to breast and ovarian cancers. P53 promoters determin whether the function of BRCA1 in the SWI/SNF complex is to direct chromatin remodeling to sites of DNA damage, allowing repair proteins to function, and/or whether this complex is essential for activation of genes critical to the DNA damage response pathway. BRCA1 and BRCA2 may also regulate transcription of genes involved in other cellular functions. Chromosomal instability as a result of BRCA1 or BRCA2 deficiency may be the pathogenic basis for breast tumor formation (Welcsh & King 2001).
IGF-1	Insulin-like growth factor -1 is a risk factor for breast cancer in postmenopausal women, which can be decreased by soy and seaweed intaken (Teas & Irhimeh, 2011)

Definitions and Abbreviations – Traditional Chinese Medicine

(WHO international standard terminologies on traditional medicine in the western pacific region, 2007)

Blood	*The red fluid circulating through the blood vessels, and nourishing and moistening the whole body*
Essence	*The fundamental substance that builds up the physical structure and maintains body function. The reproductive essence stored in the kidney*
Kidney	*A pair of organs located in the lumbar region, which store vital essence, promote growth, development, reproduction, and urinary function, and also have a direct effect on the condition of the bone and marrow, activities of the brain, hearing and inspiratory function of the respiratory system*
Kidney Qi	*Essential qi of the kidney, the physical substarata and dynamic force of the functional activities of the kidney*
Liver	*The organ located in the right hypochondrium below the diaphragm, which stores blood, facilitates the coursing of qi, and is closely related to the function of the sinews and eyes*
Liver Kidney Yin Deficiency	*A pattern attributed to insufficiency of yin fluid of the liver and the kidney with harassment of endogenous heat, marked by dizziness, blurred vision, tinnitus, forgetfulness, insomnia and dream-disturbed sleep, hypochondriac pain, aching lumber (lower back pain) and poor muscle tone in legs, flushed cheeks, heat sensation in the chest, palms and*

	soles, night sweating, nocturnal emission in men and scant menstruation in women, reddened tongue and scanty coating, rapid fine pulse
Liver Qi Stagnation	*Constrained liver qi and disharmony of liver qi could lead to functional disturbances of the liver, manifested as irritability, distension and pain of the chest, hypochondrium and lower abdomen distending pain of the breast and abnormal menstruation.*
Meridian	*A system of conduits through which qi and blood circulate, connecting the bowels, viscera, extremities, superficial organs and tissues, making the body on organic whole, the same as channels and networks, Meridians or channels in short*
Postnatal essence	*Called acquired essence as well, the essential substance acquired from the food after digestion and absorption, and used to maintain the vital activities and metabolism of the body*
Qi	*The basic element that constitutes the cosmos and through its movements, changes and transformations, produces everything in the world, including the human body and life activities. In the field of medicine, qi refers both to the refined nutritive substance that flows within the human body as well as to its functional activities*
Tian Qui	*Heavenly tenth was upon which development of the reproductive organs and maintenance of reproductive function depends, derived from the kidney essence when it is abundant, also called sex stimulating essence and menstruation in female*
Yin	*In Chinese philosophy, the feminine, latent and passive principle such as dark, cold, wetness, passivity, disintegration of the two opposing cosmic forces into which*

	creative energy divides and whose fusion in physical matter brings the phenomenal world into being. Yin opposites to Yang
Hot sensation in the Five Centres	*The heat in the palms of hands, soles of feet and anterior of the chest, accompanied by uneasiness or restlessness, also called Five Centre Heat.*
Ren Meridian	*Ren Meridian is one of the eight extraordinary meridians in acupuncture meridian. It is also name conception vessel, which starts in the lower abdomen, exits at the GV1 and ends at CV24. Another branch travels internally from the pelvic cavity and ascends the spine to the throat, also called controlling vessel.*
Chong Meridian	*Chong Meridian is also named Chong main and Thoroughfare vessel, which is one of the eight extraordinary meridians. It starts in the lower abdomen, exits at the perineum and comes into confluence with Kidney Meridian and then runs upward along the two sides of the abdomen to the chest.*
Kidney Yin Deficiency	*A pathological change characterized by deficiency of yin to nourish the kidney, leading to deficiency –fire or deficiency -heat*
The First Classic of Chinese Medicine-Plain Question	*Plain Question is the book of CM theory, also name Suwen (素問) which translate to Basic Question or Plain Question which covers the theoretical foundation of Chinese Medicine and diagnostic methods.* (*Long et al 1998)*

CHAPTER 1

INTRODUCTION

1.1. Female reproductive hormone changes and their impact on middle aged women

Middle age is the period beyond young adulthood, prior to the onset of old age. Middle aged adults normally show visible signs of ageing such as loss of skin elasticity and the greying of hair. Physical fitness also usually wanes, with a reduction in aerobic performance and a decrease in the maximum heart rate. According to Beers and his colleagues (2006), the ovarian reserve begins to decline due to falling oestrogen levels at thirty years of age or even earlier, and decreases rapidly after 40 years of age. An advanced maternal age sharply increases the risk of miscarriage and birth defects. Beers also states that natural fertility ends when women go through menopause, during their late forties to mid-fifties. Around this time, a decline in ovarian reserves can be an independent predictor of infertility (Beers et al, 2006; Collier & Longmore, 2003).

During the peri-menopausal period, the transition symptoms vary and they include an irregular menstrual cycle, hot flushes, breast problems, night sweats, depression and vaginal dryness (Robling & Matthews, 2002; Cherrington & Lewis, 2003). The incidence of malignant breast diseases is elevated and may be associated with hormonal fluctuations. However, women who have suffered from hormonal receptor positive tumours will most likely be affected by menopausal symptoms. The quality of daily life for a middle aged woman is affected by these hormonal conditions, with many women suffering from a depressed mood or peri-menopausal symptoms. Women who have an early diagnosis of breast diseases can benefit from early intervention, which can result in a significant improvement in the quality of their daily life.

Most hormone levels decrease but some remain normal and a few hormone levels even increase during this period. The hormone levels which decline include GH, melatonin, dehydroepiandrosterone, pregnenolone, oestrogen and testosterone**.** The hormones which remain at normal levels include TSH, ACTH, thyroxine, cortisol, 1,25 dihydroxycholecalciferol and insulin. Hormones that increase include ACTH

which respondsd to corticotrophin releasing hormone, follicle stimulating hormone, gonadotropins, parathyroid hormone, norepinephrine, choecystokinin, vasoactive intestinal peptide and arginine vasopressin, and atrial natriuretic factor (Beers et al, 2006).

Beers and his colleagues (2006) state that age related diseases in women can be closely related to hormonal disorders such as an increase of gonadotropins. These can result from an increase in the follicle stimulation hormone and luteinizing hormone, which are the major components in gonadotropins. The increase of gonadotropins in middle aged women is due to a reduction of oestrogen and progesterone. To reduce problems associated with an imbalance of hormones, Hormone Replacement Therapy (HRT) is usually used to replace oestrogen and progesterone. The benefit of using HRT for menopausal women is to reduce severe menopausal symptoms (Collier & Longmore, 2003). Treatment of menopausal symptoms can reduce the effects of the following conditions: hot flushes, night sweats and vaginal dryness. HRT can also be used to reduce the risk of osteoporosis and colorectal cancer (Beers et al, 2006). The disadvantage of using HRT for postmenopausal women is the increased risk of breast cancer after taking HRT for more than 3 years (Beers et al, 2006; Graf & Geller, 2003). In addition to taking oral contraceptive pills, undertaking HRT slightly increases the risk of breast cancer occurring. The risk is higher in women who took oral contraceptive pills before 20 years of age (Beers et al, 2006). To potentially reduce the risk, an alternative treatment was to use phytoestrogen to replace oestrogen. In spite of the adverse effect of HRT as a treatment for breast cancer, the benefit of using phytoestrogen is to reduce the risk of side effects such as ischemic stroke, pulmonary embolism, dementia and coronary artery disease (Xu, 2006).

Interventions for hormonal related diseases are available from both conventional and Chinese medical perspectives. However, within the conventional medical world, little is known of the interventions used in Chinese Medicine (CM) practice, and even less is known of the efficacy of the Chinese Medicine interventions used.

In this investigation, the use of Kiwi-Fruit Seaweed Extract (KFE) applied under Chinese Medicine theory was considered for reducing the impact of hormone disorders, and it can also be used for the prevention of breast diseases, such as breast cancer. A number of scientific methodologies were adapted in order to test the effectiveness of Chinese Medicine practice when using KFE. KFE (a registered food formula/product) has been used in China for over 10 years. Its therapeutic effects and safety have been well examined by laboratory and human studies.

1.2 Chinese Medicine views on female reproductive hormone change

The First Classic of Chinese Medicine, Plain Question, states that a girl at 14 years of age showed signs that the Kidney was mature, the Ren Meridian was free flowing, the Chong Meridian was exuberant and the Tain Qui (menstrual water and blood) had arrived (Long et al, 1998). Every seven years a woman experiences changes with her biological health. Females around 49 years of age show the Kidney Qi to be debilitated and the Tain Qui exhausted, which lead to the Chong and Ren vessels being malnourished, resulting in the ending of the menstrual cycle. Due to the natural process of ageing, the reproduction cycle begins to decline around 35 to 40 years of age. The decline of the reproduction cycle is affected by the decrease of Blood production, which is required for menstruation to continue. The decrease in the reproductive cycle is affected by the reduction of Postnatal or Acquired Essence. Postnatal Essence is an important source of the remaining Prenatal Essence. The less amount Postnatal Essence, the more the body consumes Prenatal Essence (Xu, 2005). Essence is a fundamental substance that builds up the physical structure and maintains body function. The Postnatal Essence is the acquired essence from food after digestion and absorption which is used to maintain the vital activities and metabolism of the body. Once the Postnatal Essence is depleted, the body activity and function has to use the Prenatal Essence, such as consuming flesh. The Prenatal Essence is also closely related with aging, which is the innate essence, as an original substance responsible for the construction of the body and generation of offspring (WHO, 2007).

In traditional Chinese Medicine, the *True Transmission of Medical Theory* (Yi Xue Zheng Chuan, 1515) states, "The menses are a transformation of Kidney-Yin, when this is deficient, menstrual Blood dries up. The deficiency of Kidney-Essence could also take the form of Kidney-Yang deficiency; this leads to the formation of cold symptoms which obstructs the Uterus and causes no period" (Maciocia, 2004). The Liver and Kidney are the main organs controlling the menstrual period and are also associated with diseases occurring after menopause, such as breast cancers (Xu, 2005). In summarising the aetiology of breast cancer, Dr Dan Xi Zhu states that a woman who is worried and depressed will suffer from an accumulation of Stagnation. Stagnation is a cause of Spleen qi deficiency, with the Liver qi rebelling horizontally and the Stagnation eventually turning in to nodules (Long et al, 1998).

1.3 Hormone fluctuations and the effects of Kiwi-fruit Seaweed Extract

In Chinese Medicine, it is believed that the human lifespan goes through different stages, including the onset of hormonal fluctuations which can affect a female's quality of life. This is particularly marked in middle-age women, where hormonal fluctuations can impact on their mental and physical health (Xu, 2006). The Chinese food formula KFE contains a high level of isoflavones such as phytoestrogen. The herbal formula enhances the bone density in menopausal women which was supported by the findings of Xu's research (Xu, 2002). KFE can nourish Yin and regulate Qi which is then able to treat those aforementioned symptoms, thus preventing unwanted changes in hormonal levels and improving the quality of life for women (Xu, 2006).

In summary, both Chinese and Conventional Medicine theories have similar views regarding the health of middle aged women. They both contend that hormonal change and the impact of age related diseases increases dramatically after the age of 40. As such, life style habits and adopting a healthy food regime becomes an important factor for middle age women. A preventative medical strategy is imperative for this age cohort. Dietary habits can contribute to the risk of, or the

prevention of breast cancer developing. However, conclusive evidence about the effect of a particular diet was not enough biomedical research to identify which food product could increase the incidence of breast cancer (Beers et al, 2006). This illustrates that further research needs to be undertaken to investigate the correlation between dietary habits and the diseases associated with female reproductive hormonal disorders, such as breast cancer.

1.4 Investigating the effectiveness of using Kiwi-fruit Seaweed Extract (KFE) in Chinese Medicine theory and scientific methods

This research conducted a Chinese medical assessment where an investigation was undertaken in to the effects of KFE, and the signs and symptoms of Chinese Medicine patterns of disharmony such as Liver Qi Stagnation and Liver Kidney Yin Deficiency. The pattern of Chinese Medicine diagnosis was reviewed in this research. If the two main patterns mentioned above had major effects and influenced the health of the middle age women's group, the progress of the two patterns can be assessed as an important key issue for improving middle aged women's general health and the reduction of the impact of hormonal disharmony on this group. Current evidence indicates that no research had been undertaken in countries such as the USA and Australia to investigate KFE and its effects on the metabolism of the female reproductive hormone, such as oestrogen, by using bio-markers.

The aim of this research is to investigate the effects of a Chinese food therapy formula such as Kiwi-fruit Seaweed Extract (KFE) to treat middle age women who experience peri-menopausal symptoms and hormone disorders. The investigation aims to investigate this therapy in the prevention of hormonal related diseases such as breast disease. This study examines the general health effects and emotional impact KFE has on women with Liver Qi Stagnation and Liver Kidney Yin Deficiency. There was also a need to explore the effects of Chinese Medicine modality on the regulation of the biomarker 2-hydroxyoestrone:16α-hydroxyoestrone, in relation to thermography changes and magnetic field changes

in a woman's breasts, as the magnetic field changes overlap the Liver Meridian, as understood from the perspective of Chinese Medicine.

The trial studies the effect of KFE on the signs and symptoms of Chinese Medicine patterns of disharmony - Liver Qi Stagnation and Liver Kidney Yin Deficiency through a Chinese Medicine assessment. The effect of KFE on physical and psychological well-being is examined in the Short Form 36 Health Survey (SF-36).

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

The literature review in this chapter focuses on the following areas: Chinese Medicine intervention regimes, Chinese Kiwi-fruit extract and its effect on hormone regulation, Chinese Medicine differential diagnosis, and disease patterns in women of peri-menopausal age.

Comparing Chinese Medicine theory to conventional Western medicine techniques consists of the following procedures: Laboratory biomarker tests in relation to hormone imbalance, acupuncture meridian changes in the magnetic tests of Pulsed Electromagnetic Field, diagnosis in Liver Qi Stagnation and Liver Kidney Yin Deficiency comparison to participants' self assessment (SF-36), and the use of breast thermography to show temperature changes related to Chinese Medicine pattern and meridian changes.

2.2. Female reproductive hormone disorders and breast disease

During the peri-menopausal period, estradiol levels are high in the follicular phase. The inappropriately high estrodiol levels over follicular phases were strongly supported by evidence based studies (Prior, 1998). Prior to that 1998 study , the previous data indicated that oestrogen production fluctuated during the peri-menopausal period. When the oestrogen level elevated, the significant clinical manifestations included alteration of menstrual intervals, heavy flow, increased dysmenorrhea, breast tenderness, nodularity, emotional stress, weight gain and cyclic hot flushes. These conditions were reported by Reyes and Winter (1977), and Abe et al (1983), who also reported that the oestrogen level was imbalanced and was high in the follicular phase for peri-menopausal women.

The mean of estrodiol level in pre-menopausal or peri-menopausal women was 273pmol/L in the follicular phase compared to 173 pmol/L estrodiol level of post-menopausal women who ceased their menstrual cycle within 3 months. Urinary hormone measurements were used and focused on the incidence on ovulation disturbances during three consecutive cycles in women aged between 40 to 55

years of age. The duration of peri-menopause can average around four years. Medical research supports that anovulation occurred for around 50% of women who begin a peri-menopausal stage in general (Beers et al, 2006). The withdrawal or decrease of oestrogen can lead to Vasomotor Symptoms (VMS). The higher the initial oestrogen level and the faster decreasing oestrogen, the greater the VMS reaction created (Prior, 1998). Hormonal elevation and disturbance in middle aged women can be an important trigger point for breast disease. It can have a similar effect to long term use of oral contraceptive pills and postmenopausal hormone therapy which are also risk factors for breast cancer (Beers et al, 2006).

The onset of breast disease in females can have an impact on their reproductive life span and quality of life. The SF-36 questionnaire is a self-assessment, and used for evaluation of the participant's quality of life during the research. SF-36 questionnaire is widely adapted by medical and psychological studies. (Collier & Longmore, 2003). In the Robling & Matthews study (2002), the SF-36 questionnaire had responses from 848 participants, who had a mean age of 45 years. In this group, 41% of participants reported symptoms of breast lumps and 65% reported breast pain. The research found that breast problems are more prevalent in middle age women. For any woman, the loss of a breast and undergoing breast cancer intervention is a traumatic event. Even though breast reconstruction surgery can have positive benefits for a female's psychosocial and sexual well-being, post surgery can still have a negative impact on their self-esteem (Dian & Schwenn, 2007). The findings of Robling and Dian encouraged scientists to explore the risk factors linked with breast disease. The most important risk factor is the hormonal disturbance in middle aged women. Female reproductive hormones have an important role in hormonal related breast cancer growth (Thomas et al, 2005; Horia & Watkins, 2005). Estradiol (E2) has proven to be a stimulator for cell proliferation by increasing the expression of genes that regulated cell growth and cell cycle progression, 16α–hydroxyoestrone (16α–OHE) is more potent than E2 (Lewis & Thomas, 2001). Ten non-cancer female patients and thirty three female breast cancer patients had their female reproductive hormones tested. The three dominant oxidative biotransforations of estradiol were examined, namely E2,

16α–OHE, 2-OHE. The results indicated 16α–hydroxyoestrone increased and 2 hydroxyoestrone was reduced, and were the most significant of the changes in estradiol metabolism in breast cancer (Schneider & Kinne, 1982).

2.2.1. The effects of female reproductive hormone metabolism on breast diseases

The finding that breast cancer can be associated with changes in female reproductive hormones is concerning. Therefore the use of hormone replacement therapy should be approached with caution due to the relationship between hormones and cancer. Applying this theory, treatments such as the oestrogen receptor blocker tamoxifen have been developed and widely used (Longmore & Wilkinson, 2007). Breast cancer oestrogen receptor (ER) status is useful in predicting benefits from endocrine therapy (Beers et al, 2006). The oestrogen receptors are HER2, HER3 and HER4, which are associated with the epidermal growth factors in breast cancer cells (Shadeo & Lam, 2006). Further research has found that genetic polymorphism is related to oestrogen metabolism. The important genetic polymorphism, CYP1B1 codon from 453A to G variant allele which affected the breast cancer risk, regulated the levels of urinary 2-OHE and 16α–OHE. CYP1B1 variant allele increased the enzyme activity in the 4 hydroxylation pathway which decreased 2-OHE and 16α–OHE ratio (Greenlee & Chen, 2007). In 2010, Ronco also stated that there is "good" and "bad" oestrogen present in the human body. For example the "good" oestrogen was 2 hydroxyoestrone and the "bad" oestrogen was 16α– and 4α– hydroxyoestrones. The elevation of 16α– and 4α– hydroxyoestrones levels can induce the onset of malignant breast diseases. However the increase of 2 hydroxyoestrone level in malignant breast diseases can be reduced (Ronco & Eduardo, 2010).

Oestrogen is metabolised in the liver through two mutually exclusive pathways yielding metabolites with different biological activities; the low oestrogenic 2-hydroxyoestrone (2-OHE) and the highly oestrogenic 16α–hydroxyoestrone (16α–OHE) (Kishida & Beppu, 2000; Bentz & Schneider, 2005). The predominant

oestrogen produced by the ovaries was 17β–oestradiol. In the mammary cells there are enzymes to convert oestradiol to oestrone, which can subsequently be hydroxylated at positions 2 or 16α. 2-OHE is considered a weak oestrogen due to its rapid methylation, rapid clearance rate, a weak binding affinity for oestrogen receptor and having an anti-proliferative effect on mammary cells. The 16α–OHE demonstrated oestrogenic properties through covalent bonding with the oestrogen receptor and the stimulation of mammary cell proliferation. The ratio of 2-OHE: 16α–OHE changed without relating to the total oestrogen levels in the female body (Atkinson et al, 2004). A lower ratio of 2-OHE: 16α–OHE may indicate an increased likelihood of breast cancer developing (Bentz & Schneider, 2005,).

2.2.2. The ratio of 2 OHE:16α– OHE

The ratio of the "good" oestrogen to the "bad" oestrogen is important because the ratio relates to the risk of breast cancer (Sepkovic & Bradlow 2009; Ronco & Eduardo, 2010). A high level of 16α-OHE stimulated the ER-positive breast cancer growth and a high ratio of 2-OHE:16α–OHE was associated with reducing the incidence of breast cancer (Ursin & London, 1997). The ratio of 2-OHE:16α–OHE was also used as a biomarker for breast cancer and intraepithelial neoplasia in clinical trials (Kishida & Beppu 2000; Lord & Bongiovanni, 2002; Thompson & Reilly , 2003; Tou & Thompson,1999; Xu, 2004; Lukaczer et al, 2005; Naik et al, 2006).

16α-OHE is a potent oestrogen because it regulates cell cycle in MCF-7 breast cancer cells. The level of DNA synthesis increased eight fold in the cells treated with 16α-OHE (Lewis & Thomas, 2001). 16α-OHE significantly enhanced DNA synthesis in MCF-7 cells and caused an accumulation of cells in the S phase of the cell cycle. The induction of cell cycle progression by 16α-OHE was associated with increasing cyclin D1 and cyclin A, Cdk2 activation and hyperphosphorylation of retinoblastoma (pRB), and included a fifteen fold increase ERE-driven luciferase gene, which induced ER-mediated gene transcription (Lewis & Thomas,

2001). The gene of pRB which remained in hypophosphorylation was a tumour suppressor that inhibited progression through the G1 cell cycle (Harbour & Dean, 2000).

The 2-OHE:16α-OHE ratio is produced by specific pathways of oestrogen metabolism which can play a role in the aetiology of breast cancer (Kabat et al, 2006). The 2-OHE:16α-OHE ratio and other oestrogenic hormones, such asoestrone(E1), 17 beta-oestradiol (E2) and oestriol (E3), can be tested in urinary samples (Ursin & London, 1997). A recent study suggested that the ratio of 2-OHE:16α-OHE metabolites can be a biomarker for oestrogen metabolism associated with the development of breast cancer. The reduction of the ratio can be affected by risk factors such as sedentary lifestyle, ethnic heritage, obesity, high fat intake, human papilloma virus infection, dimethylbenzathracene, polycyclic aromatic amines and a high intake of omega 6 fatty acids (Ronco & Eduardo 2010). The ratio reduction can also be influenced by detrimental lifestyle habits such as, smoking, drinking, a poor diet and being overworked (Michnovicz & Naganuma, 1988; Bentz & Schneider, 2005). Ronco & Eduardo (2010) stated that the potential preventive factors for breast cancer are physical exercise, muscular build-up, a slender body, a dietary intake consisting of fish, cruciferous vegetables and a high intake of omega 3 fatty acids and insoflavonoid products.

Due to the benefits of oestrogen receptor blockers for breast cancer intervention, researchers had investigated oestrogen specific pathways which are involved in oestrogen metabolism and playd a role in the aetiology of breast cancer (Bentz & Schneider, 2005; Thompson & Reilly, 2003). Using a biomarker, 2-OHE:16α-OHE ratio, a prospective study was based on a population of 5104 women, aged above 35 years. The mean of 2-OHE:16α-OHE ratio was reported at 1.6:1 in 42 postmenopausal patients of the sample group and of 1.7:1 for the 139 control subjects in 5104 women. The mean of the ratio in the breast cancer patients of the cohort study was reported at 0.71:1. The results of the study demonstrate that the metabolite ratios in the highest levels are related to a lower risk of breast cancer

incidence. (Meilahn et al, 1998). Another prospective study based on a large population of 10,786 women assessed the association of the urinary oestrogen metabolites 2-hydroxyoestrone (2-OHE) and 16α-hydroxyoestrone (16α-OHE) and their ratio in the risk factors of breast cancer. Among the group of premenopausal women, a higher 2-OHE:16α-OHE ratio correlated with a reduced risk of breast cancer (Muti & Bradlow, 2000).

Schneider's study showed that 2-OHE was a weak acting oestrogen, non-genotoxic and has an antioestrogen effect (Schneider & Kinne, 1982). In Lewis' research (Lewis & Thomas, 2001), 16α−OHE was found to be as a potent oestrogen which was capable of accelerating cell cycle kinetics and stimulating the expression of cell cycle regulatory proteins. Lewis also stated that 16α–OHE is a potent stimulator of DNA synthesis in ER-positive breast cancer cells (Lewis & Thomas, 2001).

In view of the above, a high level of 16α-OHE stimulated the ER-positive breast cancer growth and a high ratio of 2-OHE:16α–OHE was an important factor in reducing theincidence of breast cancer. Additionally the 2-OHE:16α–OHE ratio was used as a biomarker for intraepithelial neoplasia in a clinical trial (Naik et al, 2006). This ratio was produced through specific pathways of oestrogen metabolism which played a role in the aetiology of oestrogen receptor positive breast tumours (Kabat et al, 2006).

2.2.3. The methodology for testing the ratio of 2-OHE:16α–OHE and the participants selection

Most of the urinary oestrogen studies for female hormones selected chromatography or enzyme linked immunoassay (ELISA). Oestrone (E1) and oestradiol (E2) can be metabolized into 2-OHE and 16α-OHE. In 1996, oestrogen metabolism was analysed using a cylinder chromatography to extract oestrogen with ethyl acetate: hexane solvent. ELISA supplied by Immunacare was an

available test for measuring oestrogen by the relevant solid antibody. In Faupel-Badger's study, both methods were compared for measurement of urinary oestrogen (Faupel-Badger & Fuhrman, 2010).

The study population consisted of 264 pre-menopausal women in a luteal phase, 98 pre-menopausal in a non-luteal phase and 168 post-menopausal females. The age of participants ranged from 22 to 54 years. The ELISA results and the chromatography results were significantly different. The results of ELISA were 3 times higher than the concentrations measured by the chromatography in the pre-menopausal group. In the post-menopausal group the 2-OHE was 6 times higher and the 16 α–OHE was 12 times higher than the chromatography result. The important factor was that the ratio of 2-OHE: 16 α–OHE was not significantly different across the sub groups, regardless of whether it was determined by ELISA or chromatography. However, the ELISA measurements of urinary oestrogen metabolites matched well with the chromatography measurement in pre-menopausal women (Faupel-Badger & Fuhrman, 2010).

Urine samples were collected over a 12 or a 24 hour period. Another method used in the collection of urine samples was at spot urinations such as the morning void. The urine sample collection method corresponded to the methodology indicated by Faupel-Badger & Fuhrman (2003) and Falk & Fears (2005). The urine samples were stored on ice or in a refrigerator during the overnight collection. The following day the samples were mixed after they were decanted and liquefied. The mixture of the urine samples was stored in a long term -70^{o}C freezer (Faupel-Badger & Fuhrman, 2010). The research with chromatography used the duplicate urine samples, but ELISA used the triplicate urine samples. Only 0.5ml of urine samples were used for the test. The double blind control research included pre-menopausal women in the luteal phase, pre-menopausal women in the non-luteal phase and the post-menopausal women's group. Respectively the urine ratio in ELISA tests was 1.4-1.6, 1.2-1.5 and 1.2-1.4 (Faupel-Badger & Fuhrman, 2010). In the Faupel-Badger research, the results provided the range of the ratio of 2-

OHE:16 α–OHE in a healthy female urine sample. Westerlind's research collected three types of urine samples over 24 hours, during a spot urine sample or a morning void. The samples were stored on ice and transported to -80^{o}C for long term storage. The results indicated that the first morning void urine sample was even to the sample of a 24 hour collection and the ratio of 2OHE:16 alpha OHE was constant throughout the 24 hour period (Westerlind et al, 1999). Im & Vogal's research (2009), used the spot urine sample after collection and added 400mg of ascorbic acid which was then stored in a -20^{o}C freezer. The triplicate urine samples were tested by ELISA and the result demonstrated the median value of the ratio was 2.13 in post-menopausal women. The mean of the ratio results of women with breast cancer was 1.15; and the ratio dropped further to 0.97 for women with a high risk of breast cancer (Im & Vogal, 2009). Based on the work of Im (2009), Westerlind (1999) and Faupel-Badger (2010), morning urine voids and ELISA tests were selected in this study.

In the aforementioned research by Atkinson, Bentz and Falk; the participants included criteria which had established similarities to their studies. Most of the researchers selected the inclusive criteria of age for middle aged women and further considered their menopausal status. Most participants were peri-menopausal and at early stages of menopause. Other conditions were considered as genetic markers, risk factors of breast cancer and the demographic effect (Atkinson & Skor, 2003; Bentz & Schneider, 2005; Falk & Fears, 2005).

The Faupel-Badger and Bentz research found that most of the other concerns for the excluded criteria were the long term use of contraceptive pills or the intervention of hormone replacement therapy. Other excluded criteria concerns were body mass index, an oophorectomy operation and severe medical conditions such as liver and kidney diseases. Only a few researchers considered food restriction, race and lifestyle in the excluded criteria (Faupel-Badger & Fuhrman, 2010, Bentz & Schneider, 2005). The above methodologies in previous published research guided the experimental methodology designed for this research.

2.2.4. Comparing the ratio of 2-OHE:16α–OHE with urinary sample, plasma sample and breast tissue

The Schneider & Kinne study (1982) found that the discrepancy of the three oestrogen hormones (17β–estradiol, 2-OHE and 16α– OHE) were reported and 16α–OHE was noted as presenting a higher risk of breast cancer. The immuno method was used to measure the three hormonal levels in the urinary samples and the blood samples. The evidence suggests that oestrogens had a positive etiologic effect for breast diseases and also illustrated that the blood sample results were connected to the results of the urinary sample (Schneider & Kinne, 1982).

In Bradlow's research (2005), the 2-OHE:16α–OHE ratio had been tested in urine and plasma for comparison. The results showed that the two different source samples were linked. The investigation explored different factors which could cause the 2-OHE:16α–OHE ratio variation such as different ethnic groups, occupation status, caffeine consumption and the different days of the menstrual cycle. The standard range was established for the urinary 2-OHE:16α–OHE ratio. The methodology used was a competitive solid phase enzyme immunoassay in urine samples. The same method was designed for the measurement of plasma samples but the incubation was changed to 16 hours at 4^{o}C (Bradlow & Jernstrom, 2005).

There are a few studies involving phytoestrogen interventions which use the biomarker test for detecting the oestrogen level in urine (Schneider & Kinne 1982; Ursin & London, 1997; Bradlow & Jernstrom, 2005). In a randomized control study, twenty participants' urine samples and blood samples were tested after using phytoestrogen extracted from soy products over one month. The plasma phytoestrogen concentration was significantly elevated, which corresponded to the urinary sample (Nettleton & Greany, 2004). Phytoestrogen had been tested for a cancer-preventive effect which may shift away from the genotoxic metabolites towards the production of inactive metabolites (Kishida & Beppu, 2000). In the research method, the urinary sample was collected and tested by the solid phase

enzyme immunoassay-ESTRAMET^{TM}. The effect of soy consumption on steroid reproductive hormones in females had been investigated and it was suggested that soy products could reduce the risk of breast cancer (Lu & Anderson, 1996; Kishida & Beppu, 2000; Muti & Bradlow, 2000; Nettleton & Greany, 2005).

Oestrogen and oestrogen metabolites target the tissue and the cell receptors. The researchers explored the relations between the alternations of the oestrogen level in the urine sample to the breast tissue. The comparison of oestrogens and oestrogen metabolites in human breast tissue and urine samples were conducted by Taioli & Im, 2010. The methodology used a quantitative liquid chromatography-mass spectrometry. An Agilent 1200 series nanoflow liquid chromatography system was coupled to a TSQ^{TM} quantum Ultra triple quadruple mass spectrometer. For the urine sample, a morning void was obtained and preserved by an addition of 400mg ascorbic acid and frozen at -20^{o}C after collection. The breast samples with CYP1B1 genotype were collected from nine participants. Unconjugated oestrogen samples were extracted from human tissue samples using dichloromethane. Afterward, 8 microliters of sample extracted from the oestrogen sample was injected into the chromatographic column and the mobile phase was a flow rate of 4 μl per minute. The final results were compared with the standard control of oestrogen and a scan under selected reaction monitoring by the mass spectrometer. The results illustrated that the sum of the oestrogen levels (oestrone and 17β−estradiol, as "bad oestrogens" relating to a high incidence of breast cancer) was higher in breast tissue than in urine, and that 2-OHE:16α−OHE was lower in urine simultaneously (Taioli & Im, 2010). In addition, the CYP1B1 variant allele could increase enzyme activity, specifically the 4 hydroxylation pathway, therefore decreasing the ratio of 2-OHE:16α−OHE (Greenlee & Chen, 2007). Greenlee & Chen stated that it was important to explore specific genetic polymorphisms, which were able to alter the hormonal levels and metabolites. All of the above researches indicated the lower ratio of 2-OHE:16α−OHE which relate to a higher level of 17β−estradiol and the risk of breast cancer.

Different types of oestrogens showed different effects. 17β−estradiol was able to increase both uterine wet weight and epithelial cell height. Compared to 17β−estradiol, 16α−OHE and 2-OHE had a much lower affinity for the oestrogen receptors and a greater concentration was present in the incubation of reproductive organ tissue (Lotinun et al, 2001). However, Greenlee & Chen (2007) stated that some studies found that 16α−OHE could stimulate mammory tissue proliferation which could become tumours (see Section 2.2.2). The aforementioned findings demonstrate that the plasma sample concentrations of oestrogen metabolites are closely related to the variation in urinary samples (see Section 2.2.4).

2.2.5. The summary of the participants recruitment criteria and the test for the ratio of 2-OHE:16α−OHE

The 2-OHE:16α−OHE ratio from the urinary sample can be a biomarker test for the female reproductive metabolism disorder, which relates to a risk of breast cancer. The lower the ratio, the higher the risk was of breast cancer occurring. For minimal errors to occur with the hormonal test, the recruitment procedures for the participants' age was limited from 20 to 50 years by Naik, 2006; Lukaczer, 2005; Xu, 2004; Thompson, 2003; Lu, 1996. It included the following criteria: for participants to have a close relative in the family that had suffered from breast cancer around 40 years of age, for participants to have not been diagnosed with any breast cancer disease, for participants to not be pregnant, breast feeding or overweight (less than 90kg), for participants to be non-smokers and have had no general anaesthesia within the previous 3 months, or taken any hormonal medication in 6 months. It was also important that the participants menstrual bleeding occurred in the previous 6 weeks or that they had an irregular menstrual cycle (Naik et al, 2006; Lukaczer & Darland, 2005; Xu, 2004; Thompson & Reilly, 2003; Lu & Anderson, 1996). The importance of breast cancer classification was that different breast cancers lead to different intervention results and prognosis in both Chinese Medicine and Conventional Medicine. The clinical manifestations are affected by age and different heritages (Kroman et al, 2000). All those recruitment

criteria have been carefully concerned the effectiveness of hormonal metabolism in the different age groups and daily life. It demonstrated the recruitment criteria which could affect to the results of this study.

Ursin's research proved that there were five different types of estradiol biotransforations detected within the human body. Ursin conducted research involving seventy participants who had a strong family background of breast cancer, and twenty seven participants with no family history of breast cancer. The five oestrogens detected were tested, namely 2-OHE, 16α–OHE, oestrone (E1), oestradiol (E2) and oestriol (E3). The result in Ursin's research indicated that for teenage girls who had a family history of breast cancer, their oestrogen levels E1, E2 and E3 were higher than the negative breast cancer family history group. In peri-menopausal women with a family history of breast cancer, their urine samples were associated with a higher 16α–OHE level or a lower ratio of 2-OHE:16α–OHE. The ratios of 2-OHE:16α–OHE below 2.0 had been suggested as an index of a high risk of breast cancer occurring (Ursin & London, 1997).

The results of the participants first morning void had proven to be no different from the results of the 24 hour urine collections in the solid enzyme immunoassay of 2-OHE:16α–OHE ESTRAMET test (Falk & Fears, 2005; Westerlind et al, 1999). E1, E2 and E3 were tested by a high-performance liquid chromatography with radioimmunoassay (Faupel-Badger & Fuhrman, 2010). The ratio of 2-OHE:16α–OHE was tested by the solid enzyme immunoassay and its result was similar to the result of a high-performance liquid chromatography (Faupel-Badger & Fuhrman, 2010).

2.3. Chinese Medicine and Conventional Medicine applied to peri menopausal women

In Conventional Medicine, hormonal fluctuations are known to affect a person's quality of life during different stages of their lifespan. The physiology of hot flushes is associated with reduced reproductive hormonal levels that affect the

thermoregulatory system in the body, resulting in bodily sensations of heat. During the pre-menopausal period, chemotherapy intervention can induce the onset of acute menopausal symptoms as a result of toxicity to the ovary through commonly used chemotherapy drugs such as doxorubicin, cyclophosphamide, methotrexate or fluorouracil. Taking tamoxifen, which is an anti-oestrogen drug, can enhance the symptoms of hot flushes. Breast cancer patients who were taking Hormone Replacement Therapy (HRT) reported that their symptoms of hot flushes were less severe and frequent (Graf & Geller, 2003).

In Chinese Medicine, herbal formulae containing phytoestrogen can be used to treat breast cancer patients and hormone related diseases. The herbal formula - Si Wu Tang which contains high phytoestrogen is an increased antioxidant response element (ARE) activity in MCF7 and HEK293 cancer cells. The formula contains phytoestrogenic properties and regulates the expression of hormone dependent genes-oncosuppressor BRCA1 of breast cancer. For example, resveratrol regulates gene expression via the oestrogen receptor pathway and also to the undetermined pathway (Le Corre & Fustier, 2004). Further exploration of this formula included laboratory experiment conducted by Niu and his colleagues (2007), where biomedicine evidence was arrived at. Si Wu Tang, which is a herbal formula of Chinese Medcine, contains six chemical components, namely ecdysterone, saffcomin A, tanshinone I, tanshinone IIA, psoralen and isopsoralen. Tanshinone I and tanshinone IIA showed inhibitory effects on the proliferation phase of the MCF7 breast cancer cell and that the inhibitory effects were blocked by ICI182-780, which was an oestrogen antagonist (Niu et al, 2007). Ecdysterone, saffcomin A, psoralen and isopsoralen demonstrated the stimulative effects on the cell proliferative cycle from G1 to S (Niu et al, 2007). Those researches indicated Chinese Medicine intervention in hormonal treatment had biomedicine evidence.

2.3.1 Breast disease diagnosis in Chinese Medicine

Chinese Medicine practitioners rely on diagnostic patterns as a pre-requisite to intervention. The diagnosis in Chinese Medicine relies on patterns of dysfunction

of the organs' systems rather than any underlying causes explained in terms of pathophysiological malfunction. According to Borud's research, it was reported that 50% of their total menopausal participants were diagnosed with Kidney Yin deficiency. In addition, 19% of the participants were diagnosed with Liver Qi Stagnation (Borud & White, 2009). Liver Qi Stagnation and Liver Kidney Yin Deficiency were recognized as the two main patterns in peri-menopausal and menopausal women (Xu, 2006; Fu & Xu, 2011).

The *Yellow Emperor's Classic of Internal Medicine* states the clinical description of breast cancer as a breast lump which related to Liver Qi Stagnation (Ni, 1995). The prognosis for survival was thought to be up to ten years after diagnosis, whichalso means the patient can suffer from the process of metastasis and die within ten years after diagnosis (Wu, 1993). Breast cancer, known as Ru Yan in Chinese Medicine, was thought to be the product of a long accumulation of risk factors which originated from poor nutrition, being overworked, emotional alternation, unsuitable life style choices, environmental pollutants and exposure, a history of infectious diseases, genetics, ageing and hormonal metabolism changes (Tagliaferri & Cohen, 2002; Maciocia, 2004).

Chinese Medicine intervention relies on identifying the pattern of Chinese Medicine diagnosis theory. The patterns in relation to breast lumps include Liver Qi Stagnation; Liver Kidney Yin Deficiency; Spleen Yang Deficiency with Phlegm; Disharmony of Directing and Penetrating Vessels; and Stagnant Liver Qi turned into Fire and Toxic heat (Maciocia, 2004). The early stages of breast cancer can relate to Liver Qi Stagnation (Maciocia, 2004; Xu, 2006) and Liver Kidney Yin Deficiency (Xu, 2006; Fu & Xu, 2011). The patterns commonly appearing during the postmenopausal period were Kidney Yin deficiency with Empty Heat and Liver Qi Stagnation (Borud & White, 2009).

In terms of Chinese Medicine, female hormonal related diseases and breast diseases are related to the Liver Meridian (Xu, 2006). In addition to the Liver Meridian, the

upper lateral part of the breast can also relate to the Heart, Pericardium, Spleen and Stomach Meridians (Deadman et al, 2001). In menstrual disorders, Liver Meridian and Kidney Meridian functions were important factors (Maciocia, 2004). During the Fourteenth century, Zhu Dan Xi (1281-1358) summarised the aetiology of breast cancer, stating that a woman who was worried and depressed would suffer from an accumulation of Stagnation (Maciocia, 2004). Stagnation can be a cause of Spleen Qi deficiency, the Liver Qi rebelling horizontally and the Stagnation turning in to nodules (Long et al, 1998; Maciocia, 2000). The Chinese Medicine practitioner Chen Bai Ming indicated the development of breast cancer as: the beginning was of a small accumulation like a turtle egg which was not red, nor painful. After several months it grew larger, and then the lump broke up like a ripe pomegranate and it burst through as though a deep hole (Maciocia, 2004). This was due to the 'Liver and Spleen being affected by anger, Qi and Blood were exhausted and it was called Ru Yan'. Another Chinese Medicine practitioner, Fu Qin Zhu, stated that a breast carbuncle was due to toxic heat in the Stomach and Gall Bladder with Stagnation of Qi and Blood which caused pain, a rash and swelling and was easy to treat. However, the resulting small nodules caused no pain, there was no appearance of a swollen rash and it was difficult to treat (Maciocia, 2004). The Stagnation of Qi in women was very often secondary, as it could be the result of the consequences of a deficiency of the Liver and Kidneys being affected by the Directing and Penetrating Vessels function declining. Being over worked could also induce the weakening of the Liver and Kidney Yin, which was the root of Liver Qi (Maciocia, 2004; Maciocia, 1989).

Comparisons between modern diagnostic methods (eg: bio-marker tests) and Chinese Medicine diagnostic methods also indicate that Liver Qi Stagnation and Liver & Kidney Yin deficiency were the two main patterns of pre-clinical changes in the breast (Xu, 2006). The Liver Qi Stagnation pattern was most likely the main diagnosis pattern in the pre-operation group of breast cancer patients (Ling et al, 2006). If the findings of Yin deficiency are correct, it is reasonable to argue that Yin nourishment was necessary, irrespective of the presence of Liver Qi Stagnation. The purpose of further diagnosing breast cancer in the different

Chinese Medicine patterns can be significant for early intervention, early diagnosis and an improvement in the patient's medical prognosis.

In Chinese Medicine, Qi Stagnation and Blood Stasis can be a result of emotional stress such as anger, irritation, frustration, resentment, worry or anxiety. Although 'anger' is always mentioned as the emotion that may lead to Stagnation of Liver Qi, other emotions such as worry and anxiety, or even sadness, may affect the Liver. Long term Stagnation of Liver Qi in women easily leads to stasis of Blood (Machacia, 2004). Liver Qi Stagnation can alternatively aggravate the Qi and Blood Stagnation and produce lumps in the breast area. Chen Shi Gong (1617) stated that depression injured the Liver and this consequently affected the Spleen. An obstruction developed in the Heart and the channel Qi stagnated and generated nodules, as observed by Zhu Dan Xi (Machacia, 2004).

2.3.2. The risk factors of breast diseases and Liver Qi Stagnation

In Chinese Medicine theory an inappropriate diet with an excessive consumption of dairy foods, fatty foods and sweets can lead to Phlegm which can form a lump in the breast over time. As previously discussed, a female who is over worked or experiencing feelings of extreme tiredness has increased risk factors which can weaken the Liver and Kidney Yin. Liver Yin was the root of Liver Qi. When Liver Yin depleted, the harmonizing of Yin and Yang in the Liver became imbalanced and Liver Qi Stagnation occurred. The Stagnation of Liver Qi was nearly always the initial stage of breast lumps forming (Machacia, 2004).

Emotional problems were the most significant cause for Stagnant Qi in the Liver Meridian. If the Qi Stagnation is present for a long period of time, it can cause a Heat or Fire and Toxic Heat in the breast area. Chen Shi Gong (1617) stated that depression injured the Liver, pensiveness affected the Spleen, negative energy accumulation could develop in to Heat as the temperature had elevated, so that the Meridian Qi stagnated and generated nodules (Maciaca, 2004). Menopause affected the Directing and Penetrating Meridian and caused a relative imbalance

between the top and bottom parts of the body. The Penetrating Meridian influenced the Uterus and Breast. Penetrating Meridian Qi rebelling can also lead to lumps growing in these two organs (Maciaca, 2004).

2.3.3. The Liver Qi Stagnation pattern in Chinese Medicine

There are internal relationships between the Liver and Kidney: the Liver is often over powered by the Spleen, and Spleen disease in its late stages usually exacerbates Kidney problems (Long et al, 1998). Liver Qi Stagnation refers to the syndrome in which the Liver fails to conduct and disperse Qi and as a result the Qi cannot flow smoothly. As a consequence the Liver Qi transversals invaded the Qi of the Spleen and Stomach (Maciaca, 1989). The clinical manifestations of Liver Qi Stagnation included depression and fullness in the hypochondria area, a feeling of obstruction in the throat, dreaminess, fear, distending pain in the breasts, irregular menstruation, a bloated feeling in the lower abdomen and a taut pulse (Long et al, 1998).

2.3.4. The Liver Kidney Yin Deficiency pattern in Chinese Medicine

Liver Kidney Yin Deficiency leads to a reduction in the Essence, Blood and body Fluid. Kidneys belong to the water element and store unique Essence from other viscera. The Kidney Ministerial Fire is the root of other organs. Most of the Kidney diseases are due to a lack of Essence and belong to deficiency symptoms, where the essence can be depleted by congenital weakness, ageing and injury. Kidney Yin deficiency symptoms can be caused by congenital deficiencies, indulgence in sexual activities and emotional stress which can then transform into Fire, febrile disease and chronic diseases (Long et al, 1998). The symptoms caused by declining Kidney Functions can be reduced and prevented through Chinese Medicine (Long et al, 1998). A Chinese Medicine intervention to nourish Kidney Yin can use acupuncture, herbs and food therapy. Acupuncture points for Kidney Yin are Ren 4 (Guanyuan), Kid 6 (Zhaohai), Kid 3(Taixi), Kid 13 (Qixue), Kid 10 (Yingu), Kid 9 Zhubin and SP 4 (Sanyinjiao). The herbal formulae for Kidney Yin are Liu Wei Di Huang Wan, San Jia Fu Mai Tang, Zuo Gui Wan, Qing Hao Bie

Jia Tang, Qing Jing San, Gui Shao Di Huang Tang and Yang Jing Zhong Yu Tang (Maciocia, 2004). Chinese food therapy, Kiwifruit and seaweed extract can improve Kidney Yin deficiency and increase bone density in middle aged women (Xu, 2005).

Liver Kidney Yin Deficiency is manifested through symptoms of heat, such as red in the zygomatic area. Clinical manifestations included vertigo, tinnitus, poor memory, insomnia, dry throat, cheek rash, night sweating, vexation, hot sensation in the Five Centres (Five Center Heat); pain in the back region, lumbago and aches in the knees, seminal emission in males or scant menses in females; while the tongue was red with little coating and the pulse was faint and rapid (Long et al, 1998). The key diagnostic point was the coexistence of hypochondriac pain, lumbago and the Yin deficiency syndrome.

2.3.5 Impact on female health

The disturbing facts about female reproductive hormones are that they have an important role in hormonal related breast cancer growth (Thomas et al, 2005; Horia & Watkins, 2004). Estradiol (E2) has been proven to be a stimulator for cell proliferation by increasing the expression of genes that regulated cell growth and cell cycle progression and alternate genetic polymorphism. The research outcome was that 16α−OHE was more potent than E2 (Lewis & Thomas, 2001; Greenlee & Chan, 2007).

Under the current conventional medical system screening tests, most breast cancers are diagnosed at the early stage of cancer developing, with the average age of diagnosis being 50 years (Vallejos & Gomez 2010). The early stage was diagnosed by the classic clinical tumour node and metastatic (TNM) information (Ravaioli & Bagli, 1998). To further reduce the incidence, one of the preventive strategies is to rectify the risk factors which affect the hormone disorders.

2.3.5.1. The prevention of hormone related breast disease in conventional medicine

2.3.5.1.1. Genetic Polymorphism linked to breast disease

In conventional medicine, it is important to identify the tumour type for understanding the heterogeneity in a clinical response and for developing new effective therapies (Teschendorff & Sergio, 2010). Breast cancer covers a group of heterogeneous diseases with different clinical, biological and molecular characteristics. For example, the differentiation of the genetic mutation can help the clinical diagnosis, as to differentiate between mutations such as BRCA1, BRCA 2 and P53 (refer to page XXV). The morphological types are separated by ductal and lobular neoplasia (Russo & Russo, 1999). The current immunohistochemical evaluation is used for identifying oestrogen receptors (ER), progesterone receptors (PgR) and human epidermal growth factor receptor 2 (HER2). Using immunohistochemical methods, breast tumour phenotypes were classified into 4 subtypes: Luminal A, Luminal B, Basal and HER2/neu (see Table 2.1). Most cases of of those aged under 50 years were Luminal B (59.5%) (Vallejos & Gomez, 2010). HER2/neu was more frequent in the group aged 50 to 69 years and had the worst prognosis (Vallejos & Gomez, 2010). HER2/neu was the protein produced by the c-erbB-2 oncogene, which is a transmembrane tyrosine kinase to stimulate cell growth. The c-erbB expression was an important predictor of resistance to tamoxifen in node negative (Carlonagno et al, 1996) and metastatic patients (Leitzel et al, 1995)**.**

Table 2.1: The relation between breast tumour phenotypes and receptors (Russo & Russo, 1999; Vallejos & Gomez, 2010; Carlonagno et al, 1996; Leitzel et al, 1995**)**

Breast Tumour Phenotypes	**Luminal A**	**Luminal B**	**Basal**	**HER2/neu**
Receptors	ER+ and/or PgR+, HER2-	BR+ and/or PgR+, HER2+	ER-, PgR-, HER2-	ER-, PgR-, HER+

Targeted therapy is designed to exploit specific molecular characteristics of the tumour and a monoclonal antibody was able to target the HER2 receptor, not only for an early breast cancer but also a metastatic case. The intervention result may be improved by using specific molecule drugs (Blank et al, 2010).

In ER negative basal and HER2 breast cancer, gene expression modules reflecting T-cel helper-1 and T-cell helper-2 mediated immune responses and played antagonistic roles, which are major risk factors for distant metastasis. In ER positive breast cancer, the higher c-myc proto-oncogene protein (MYC) and Ha-Ras1 proto-oncogene protein (HRAS) activity presented a significantly worse prognosis (Teschendorff & Sergio, 2010). This molecular pathway research had explained that the breast cancer patients with the same histopathological features had shown variations of the clinical outcome.Teschendorff's and co-workers' research further developed and verified the large mRNA expression data sets of ER positive and ER negative tumours over a common set of 6265 genes. ER receptor subtypes are ERβ and ERα. The ratio of ERβ and ERα can be used for the prediction of the prognosis of breast cancer. A higher ERβ is a relative benign breast cancer. Resveratrol and phytoestrogen inhibited ERα and associated P13K activity; Genistein and daidzein activated AKT in ERα positive T47D breast cell lines and induced the cancer cell apoptosis. In the human breast cancer cell line, MCF7 cell line, resveratrol inhibited the DNA binding activity of NFkB and AP-1. In a high concentration of resveratrol, the MCF7 cells inhibited the proliferation and induced cell death (Vanden & Dijsselbloem, 2006; Mense et al, 2008). In MDA-MB-468, an ER negative breast cancer cell line, resveratrol inhibited cell proliferation at all concentrations lower than 10nM (Mense et al, 2008).

2.3.5.1.2. Lifestyle is an important factor for breast disease

Aecologic observations indicate that incidences of breast cancer are much lower in Asian women. They consume significantly higher amounts of phytoestrogen than Western women. However, after migrating to western countries, it has been discovered that second and third generation descendants of Asian women have

breast cancer risks similar to those of women in their new country. Those results suggest that lifestyle is also an important factor and not only genetic factors to explain the lower breast cancer risk observed in Asian women (Mense et al, 2008).

As Longmore observed, the only good medicine was preventive medicine (Longmore & Wilkinson, 2007). It was possible to reduce the incidence of breast cancer by discovering the risk factors which trigger the development of breast cancer. Primary prevention should aim to lower the incidence through a reduction in the exposure to risk factors and through an increase in exposure to protective factors (Ronco & De, 2010). Prophylactic mastectomy required surgery and was followed with reconstruction and a possible decrease in the quality of life for the female (Beers et al, 2006). Tamoxifen as chemoprevention was used for a short period of time because it could cause endometrial cancer, stroke and pulmonary embolism. Chemoprevention was successful in only 50 per cent of patients (Ozanne & Wittengerg, 2010). It is therefore necessary to develop a better method of preventing breast cancer than prophylactic mastectomy and Tamoxifen intervention.

2.3.5.1.3. Effects of stress in breast disease

Stress and emotional change were recognized as risk factors for the risk of developing cancer. The discovery and identification of risk factors would further improve the prevention of breast cancer, which could effectively reduce the intervention costs and benefit the wellbeing of middle age women (Ozanne & Wittengerg, 2010). A stressful life style can reduce a person's immune system function which can be an important risk factor for cancer developing (Wang et al, 2010). Stress is also a risk factor for hormonal disturbance and the development of tumours.

Toda reported scheduled stress tests performed on female mice over 10 days. After increasing stress level, it increased the endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (NOS) expression in vaginal tissue. Endothelial nitric oxide synthase increased in the artery related to oestrogen replacement,

which was higher than the control group. These researches also indicated that chronic stress induced vascular oxidative stress by activating the angiotensin II AT_1 receptor signalling pathway (Toda & Toda, 2011). The research findings demonstrated that the reproductive hormones were influenced by the psychological stress level.

Galen (AD 200) stated that melancholic women were more susceptible to swellings of their breasts than sanguine women. Psychological stress can affect the Limbic-hypothalamic-pituitary-adrenal axis (HPA) and down-regulate various parts of the cellular immune response, such as reduction of neutrophil phagocytosis, NK cell counts and T cells. A randomised clinical control trial was conducted over one year and the results were judged as remarkable and clinically relevant. The results demonstrated that psychological intervention could prolong the period of survival for breast cancer patients (Reiche & Nunes, 2004).

In addition, psychological stress can also influence hepatic blood flow by inducing vasospasm and centrilobular hypoxia, which lead to liver damage (Vere et al, 2009). Psychological stress caused the Type 1 helper T lymphocytes to be suppressed and responded to cytokines production, tumour necrosis factor−α (TNF−α), interleukin-1 (IL-1) and interleukin-6 (IL-6), which reduce the abilities of the immune system to combat diseases. In vitro, carcinoma was associated with high concentrations of TNF−α. Stress levels were shown in several studies to directly influence TNF−α, IL-1 and IL-6. Psychosocial stress was also linked to increased DNA damage, alterations in DNA repair and inhibition of apoptosis. Type 1 personality and psychosocial stress were positively linked to the severity of carcinoma and chronic disease such as liver cirrhosis (Vere et al, 2009). Psychological stress can also increase the susceptibility of vascular damage and endothelial dysfunction such as vasodilation or constriction. Ghiadoni & Donald, (2000) indicated the vasodilation continued after the stress test, which was not simply explained by the current knowledge as the reaction of the sympathetic system.

Mitochondrial oxidative stress caused tumour progression and metastasis. In breast cancer patients, antioxidants are taken to reduce reoccurrence of symptoms and the risk of mortality. A powerful antioxidant, N acetylcysteine, has anti-tumour properties which were been recommended for melanoma chemo-prevention (Sotgia & Martinez-Outschoorn, 2011).

2.3.5.1.4. Hormone related factors linked to breast disease

The risk factors of breast cancer are listed as: Women who have a first degree relative that suffered from breast cancer, with a history of in situ or invasive breast cancer, and who had early menarche or late menopause; Women who had a late in life first pregnancy, a history of fibrocystic changes or benign lesions, who took long term oral contraceptives or postmenopausal hormone replacement therapy and who were identified as obese postmenopausal; women who had a history of exposure to radiation therapy before the age of 30 years; and those with an inappropriate diet are all at a heightened risk of breast cancer disease (Beers et al, 2006). A family history based on certain demonstrated gene mutations accounts for between 5 to 15 % of incidences. Menarche time, nulliparity, a first delivery in later years, short term breast feeding time and a late start to menopause were major factors and contribute to 25% of the incidences of breast cancer. Environmental factors were considered the most important risks, which corresponded to a 60 to 70% of incidence of breast cancer. The remaining risk factors included excess weight and obese metabolic factors, such as insulin resistance, low serum vitamin D level, dyslipidaemia and diabetes (Ronco & De, 2010). Of the environmental factors, dietary factors were reported as the most important factor, contributing 40% in risk factors for breast cancer (Xu, 2006). The risk factors also included food intake, as nutrition affects hormonal and metabolic modulation. For example, South America is a developing region but it shares some characteristics with other developed regions; such as consumption of high levels of red meat and cooking under direct heat. In such cases, the continent has shown a high occurrence of a disease typical of developed countries, such as a top ranking of regions for breast cancer incidence (Ronco & De, 2010). Genistein one of the isoflavone in soy

products can reduce ER-levels in the mammary gland and reduce persistent up-regulation of BRCA1 in the mammary gland, as found in the research of both animal and human populations (Cabanes & Wang, 2004)

From a nutritional perspective, cancers are mostly associated with an excessive consumption of meat with high calories, or a diet lacking in fish, vegetables and fruits. Fats and refined sugars are also risk factors for breast cancer (Hems, 1978). Deficiency of selenium also causes breast cancer. The lower the selenium level, the higher the risk of breast cancer. Selenium can reduce the mortality rate in breast cancer patients (Rayman, 2005). In addition, a low level of physical activity can also strongly affect hormonal production (Hanf & Gonder, 2005). Some of the known risk factors are smoking and alcohol intake, and the use of or exposure to chemical sprays such as pesticides. Responsive strategies for cancer prevention are to increase the intake of Vitamin C, Vitamin D, flavonoids, Omega 3 and PUFAs (Ronco & De, 2010). Dietary changes can lead to a long lasting up-regulation of BRCA1 mRNA in rat mammary glands, suggesting an increase in DNA repair capacity (Cabanes & Wang, 2004). The condition of hypoxia and malnutrition can induce endoplasmic reticulum stress (ER stress). The primary function of ER is to assist synthesized protein refold into native conformation in cytoplasm. When ER stress is present, LCN 2, a neutrophil gelatinase associated lipocalin is up-regulated and it leads to increased tumour progression in breast cancer (Wang & Hua, 2008, Mahadevan & Rodvold, 2011). KFE, which contains rich flavonoids and Vitamin C, is a valuable product for the prevention of breast cancer (Xu, 2006; Franceschi & Parpinel, 1998). Human monitoring studies showed that a regular kiwi-fruit intake can alter antioxidant effects and DNA repair systems (Collin & Harrington, 2003; Skinner & Loh, 2011). A recent research demonstrates the indicator of inhibitory effects on DNA oxidation, 8-hydroxy-2’-deoxyguanosine (8-OHdG) is decreased by the intake of kiwi fruit. The antioxidant effects of kiwi fruit in the human body may prevent the diseases caused by oxidative stress (Iwasawa et al, 2011).

Table 2.2: Conventional medical viewpoints on risk factors linked to breast disease

Researcher	Risk factors linked to breast diseases
Leitzel et al, 1995; Carlonagno et al, 1996; Russo & Russo, 1999; Teschendorff & Sergio, 2010; Vallejos & Gomez, 2010	Genetic polymorphism linked to breast disease
Cabanes & Wang, 2004; Vanden & Dijsselbloem, 2006; Mense et al, 2008	Genistein, Daidzein and Resveratrol inhibited breast cancer cell proliferation for all concentrations lower than 10nM and induced the cancer cell apoptosis
Mense et al, 2008; Ronco & De, 2010	Risk factors and protective factors for prevention of breast diseases
Ghiadoni & Donald, 2000; Reiche & Nunes, 2004; Vere et al, 2009; Ozanne & Wittengerg, 2010; Toda & Toda, 2011	Stress and life style factors linked to breast diseases
Beers et al, 2006; Xu, 2006	Hormone related factors linked to breast disease
Hems, 1978; Cabanes & Wang, 2004; Rayman, 2005; Xu, 2006; Ronco & De, 2010; Collin & Harrington, 2003; Skinner & Loh, 2011; Iwasawa et al, 2011	Environmental factors, dietary factors linked to breast diseases

2.3.5.2. The prevention of hormone related breast disease in Chinese Medicine

Chinese Medicine and Conventional Medicine consider breast disease as a disease associated with multiple risk factors. The main risks of developing breast disease are closely related to age, life style choices and family genetics. The identification methods for the different types of breast cancer are an important strategy to improve diagnosis and prognosis. Medical researchers have endeavoured to identify the multi risk factors for breast cancer in both medical fields. In Chinese Medicine terminology, Liver and Kidney functions are related to ageing and genetic changes. Long and co-workers reported that the ratio of cAMP to cGMP

was lowered due to Liver Kidney Yin Deficiency (Long et al, 1998). In both medical systems, it is recognised that hormonal changes can be strongly influenced by lifestyle, environment, diet, emotional changes, age and family history. It is important to identify the detection methods which are able to improve the early diagnosis of breast cancer. For breast cancer diagnosis, using the early diagnosis methods and clarifying the early symptomatic patterns in Chinese Medicine and Conventional Medicine are important to ongoing research.

The findings of Conventional Medicine research indicate that stress is a known factor in causing damage to the liver organ, vascular epithelia tissue, hormonal balance, nervous synapse connection and immune function (Toda & Toda, 2011, Sotgia & Martinez-Outschoorn, 2011, Vere et al, 2009). Stress can be a crucial factor in triggering Liver Qi Stagnation in Chinese Medicine diagnosis (Long et al, 1998; Maciacia, 2004; Fu and Xu, 2011). As such, there are commonly held views between Chinese Medicine and Conventional Medicine. However, some of the known differences between Chinese Medicine and Conventional Medicine are the terminologies, theories and diagnosis methods. This may increase the difficulties associated with communication between the two medical fields, which may lead to two different methods of intervention. In terms of the known risk factors, both medical fields have comparable ideas which can lead to similar prevention methods being used.

2.4. Kiwi-Fruit Seaweed Extract

Chinese herbal formulae, food therapy and acupuncture have long been used to effectively regulate endocrinal disorders in Chinese clinical practice (Maciocia, 2004; Tou & Thompson, 1999; Wolfe & Flaws, 1998). One Chinese food therapy of interest is the Chinese food formula, Kiwi-Fruit Seaweed Extract (KFE), which is a safe food therapy that has been examined and investigated by many researchers and practitioners over a decade.

2.4.1. Kiwi-Fruit Research

Kiwi-fruit is named Mi-hou-tau in Chinese Medicine and is also known as the Chinese gooseberry. Kiwi-fruit belongs to the botanical family Actinidiaceae, and its pharmaceutical name is Actinidia chinensis. Kiwi-fruit is a fruit native to eastern Asia. It is rich in vitamin C, vitamin E, vitamin K, folate, antithetic acid, niacin, lutein, zeaxanthin, arytenoids, falconoid, calcium, iron, manganese, selenium, zinc, copper, potassium and magnesium, fibre, omega-3 fatty acid, amino acids and also a high level of isoflavone (Collins et al, 2001; Edenharder, 2002; Yong, 1997; Xu, 2002) (Table 2.2).

The intake of isoflavone extract can reduce the 16α–hydroxyoestrone but may not correspond to the 2 hydroxyoestrone which is related to the risk of breast cancer (Atkinson & Skor, 2003 Cabanes & Wang, 2004; Xu, 2002; Xu, 2004; Bradlow et al, 2006). Kiwi-fruit itself is a potential therapeutic product for preventing cancer as indicated by many studies (Nebe, 2006; Motohashi & Shirataki, 2002; Horn-Ross et al, 2001; Collins et al, 2001; Song, 1984).

Table 2.3: The effects of using kiwifruit in current research

Researcher	Ingredients	Effect
Collins et al 2001 Yong, 1997	Vitamin C, vitamin E, vitamin K, folate, antithetic acid, niacin, lutein, zeaxanthin, arytenoids, falconoid, calcium, iron, manganese, selenium, zinc, copper, potassium and magnesium, fibre, omega-3 fatty acid, amino acids	Kiwifruit protects against oxidative DNA damage in human cells and in vitro the ingredients in kiwifruit such asVitamin E, C, A may relate to reduce the risk factor of cancer
Edenharden, 2002	Isoflavone	Isoflavone was used against genotoxicity by beverages, fruits vegetables, herbs and flavonoids
Atkinson & Skor, 2003	Isoflavone	The intake of isoflavone extract can reduce can reduce the 16α–hydroxyoestrone but may not correspond to the 2 hydroxyoestrone
Xu, 2002 Xu, 2004 Bradlow et al, 2006	Kiwifruit extract	KFE has been used to increase the bio-marker 2-OHE:16α-OHE ratio and to improve the quality of life for women suffering from endocrinal disorders
Nebe, 2006 Motohashi, 2002 Horn-Ross, 2001	Kiwifruit	Kiwifruit is used in the prevention of cancer

2.4.2. Seaweed Research

Seaweed (Hai-zao) in Chinese Medicine is prescribed for patients who suffer from phlegm nodules and heat. Seaweed can also be used as a diuretic function for oedema intervention and pain relief (Bensky & Gamble, 1986). The pharmaceutical name for seaweed, which belongs to the Sargassum family, is Herba Sargassii. Since ancient times, seaweed has been an important ingredient in Asian cuisine. Seaweed varieties are found to be rich in polysaccharides, minerals, vitamins and dietary fibre. Antioxidant and anti-mutagenic effects of dietary seaweeds have been

reported in vitro and in vivo studies (Teas & Irhimeh, 2011; Blanc & Hauchard, 2011; Yang & Nam 2010; Saker & Fike, 2004). Extracts of seaweed have identified as having antioxidant, anti-tumour and chemoprotective qualities.

In Teas & Irhimeh study, 30 participants were involved in a double-blinded, randomized, placebo-controlled crossover clinical trial. They consumed 5g of seaweed or placebo per day for 7 weeks. After crossover of another 7 days, the results illustrated that the use of aglycone, an isoflavone, significantly reduced IGF-1 by about 40% (P =0.01). The role of IGF-1 signalled a pathway into carcinogenesis, with the blockage of the pathway beneficial to cancer patients (Teas & Irhimeh, 2011).

Phenols in seaweed helped neutralize free radicals linked to the development of degenerative disease and conditions which include cancer, cardiovascular disease, cognitive impairment, immune dysfunction, cataracts, macular degeneration, asthma and diabetes (Blanc & Hauchard, 2011).

In Chinese Medicine a dosage of 4.5 to 15g per day of seaweed is recommended as a treatment for the heat and phlegm nodules that appear in the body. Chinese Medicine practitioners also prescribe seaweed as an ingredient to be used for the treatment of goiter and scrofula. Seaweed when combined with other herbs can be considered for the treatment of leg oedema, which is caused by a difficulty in passing urine (Bensky & Gamble , 1993). When applying a very large dosage of seaweed (0.75g per kilogram body weight), this can assist with the reduction of blood pressure (Bensky & Gamble, 1986). In osteoarthritis research, the phase I and II of the clinical trial were completed and seaweed was proven to be safe in the COAT score and full blood test. No toxicity issues were observed over the hemopoietic, hepatic and renal system study period. Myer's research found that if the daily dosage was increased to 1200mg per kilogram of a rat's body weight, the clotting time was significantly prolonged. Furthermore, a polyphenol rich fraction of Fucus was also shown to lack acute toxic effects (Myers et al, 2010).

Table 2.4: The effects of using seaweed in current research

Researcher	Ingredients	Effect
Teas & Irhimeh , 2011	polysaccharides, minerals, vitamins and dietary fibre extract from seaweed and soy combination	Serum IGF-1 concentrations change with soy and seaweed supplements in healthy postmenopausal American women. Seaweed can reduce the negative impact of insulin-like growth factor 1 (IGF-1) which is associated with an increased risk of postmenopausal breast cancer
Yang & Nam 2010	Iodine, polysaccharide and porphyran	All three components of extracts of seaweed act as antioxidants and can induce apoptosis in human breast cancer cell lines and gastric cancer cell lines.
Blanc et al, 2011	Phenols in brown seaweed	Radical-scavenging capacity of phenol fractions in the brown seaweed ascophyllum nodosum
Saker & Fike, 2004	Brown seaweed	Brown seaweed (TascoTM) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. Increased phagocytic capability of monocytes, improve the lifespans of erythrocytes and leucocytes because of its antioxidant function
Jimenez-Colmenero & Cofrades, 2010	Konjac gel, bioavailable carbohydrate, protein content, high fibre and minerals in seaweed products	By reducing the fat intake in their foods, it can have a positive impact on the number of people that suffer from high cholesterol. Known effects of seaweeds in humans are reducing the problems associated with constipation and decreasing the plasma levels of glucose and insulin.
Bensky & Gamble, , 1986, Bensky & Gamble, 1993	Alginic acid, mannitol, potassium, iodine, laminine and sargassan. Phenolic compounds	To treat goiter, hypothyroidism and hyperthyroidism Painful hernia, symptoms of Yin deficiency with copious sputum, Phenolic compounds found in seaweed were an important substance for antioxidant activity, anticoagulant action and stop bleeding, hypertension intervention and the control of high cholesterol. Inhibit funghi growth, benefit immune enhancement and increase the lipid hydroperoxide metabolites.
Xu, 2006	KFE	Nourishes Yin and regulates Qi which is able to reduce symptoms of and prevent unwanted changes in hormonal levels and improve the quality of life for middle age women. Improve the effects of middle age female hormonal disorders and benefit patients by releasing Liver Qi Stagnation

An excessive intake of seaweed can also result in iodine toxicity. Excessive iodine ingestion can cause hyperthyroidism (Beers et al, 2006). Evergy qantity of 5 gram seaweed intake per day showed no toxic and anti-nutritional components in the seaweed detection test (Carvalho et al, 2008). In a trial by Myers, the daily intake of seaweed extract for rats was 3g per day, which included 75% fucoidan. There was no significant prolonged thromboplastin time and clinical toxicity (Myers et al, 2010).

2.4.3. Chinese Kiwi-fruit and food therapy

In southern China, kiwi-fruit is found growing in the wild along the dangerous narrow glens by the Yangtze River, also known as Chang Jiang. Since ancient times, people of that area have appreciated the delicious flavour of kiwi-fruit. It has been referred to in literature since 1400 A.D. There have also been many references to cancer-like diseases in China since the oldest recorded reference by Nei Jing, which was recorded in 720 B.C. It was recorded by Zhou-Litain-Guan during the in the 6th Century B.C., noting that kiwi-fruit was used as a medicine that helped in therapy for eight cancer patients. Jin-Shu (700 B.C.), Zhu-Bing–Yuan–Hou-Lun (610 A.D.) and Sanyin Fang (1174 A.D.) also recorded an intervention method for cancer treatment. Many cancers (Xu, H.E. 2004; Motohashi & Sharataki, 2002), specifically digestive system cancers, liver cancers and mammary gland cancers have been treated with a kiwi-fruit prescription. As mentioned by Motohashi & Sharataki, in Liu's (1994) research, kiwi-fruit extract showed a 95% inhibition in Ames' test on cancer growth. In Jin-Long's study (1986), kiwi-fruit extract inhibited the growth of a cultured human hepatoma cell (BEL-7404) (Motohashi & Sharataki, 2002).

KFE contains isoflavones (phytoestrogen). Their functions are different to oestrogen (Horn-Ross et al, 2001) and the isoflavones do not cause the side effects attributed to hormone replacement therapy (HRT). A recent cohort study found that HRT, which combined oestrogen with progestin, was associated with an increased risk of breast cancer, stroke, venous thromboembolic disease and heart disease

(Rossouw et al, 2002). Hormonal replacement therapy (HRT) can not be used in all situations for women with breast cancer, or for those at risk of breast cancer (Chen, 2002). Natural medicines and exercise regimes are increasingly accepted as an essential part of a well balanced and healthy life style. A phytoestrogenic product such as soy isoflavonoid is recognised as being valuable for bone health, and without any associated risk factors of breast cancer (Xu, 2005). The rich phytoestrogen supplement of Kiwi-fruit Seeweed Extract can also benefit bone density, reduce symptoms of menopause and increase 2OHE:16α–OHE, which is closely related to breast cancer incidence (Xu, 2006). Isoflavones can increase the ratio of 2OHE:16α–OHE and reduce the risk of breast cancer (Kishida & Beppu, 2000).

Phytoestrogen intake can benefit female hormone metabolism in pre-menopausal women (Dailey & Neal, 2003). Phytoestrogen supplements, which can relieve the most troubling symptoms of menopause such as hot flushes and palpitations, have been subject to clinical trials conducted using an isoflavonoid combination. The pilot trial suggested that the isoflavonoid nutritional supplement can relieve these symptoms, as well as conferring some cardio protective benefits (Lukaczer & Darland, 2005). In Dailey & Neal's study (2003), 397 female participants between the ages of 40 to 55 years were involved in research over six month duration. Two medical centres were in metropolitan areas, four medical centres were in suburban areas and two medical centres were in rural areas. 63% of the participants were from a Caucasian background and 57.9% of the participants had a college education. Four herbals were selected, namely phytoestrogen, St Johns Wort, Ginkgo and Ginseng. The most stated self-assessed medical conditions and their associated percentages were hypertension (26.7%), menopause (15.9%), muscle and joint pain (14.8%), thyroid disease (11.6%), allergies (11.1%) and depression (9.7%.). The symptoms included four limbs numbness, depression, sleep disorder, dizziness, heart palpitations, headache, hot flushes, irritability, itching, joint or muscle pain, decline of libido, memory deterioration, nervousness, night sweats, pain during intercourse, vaginal dryness and fatigue. The symptoms improved for the participants in the intervention group. These were compared with non-herbal

users on demographic characteristics such as menstrual status, menopause symptoms, current medications and medical conditions. The results found that only phytoestrogen significantly relieved hot flushes and night sweats as compared with non-herbal users. It also compared participants who used St John's Wort, Ginkgo biloba and Ginseng. The outcomes implied that the phytoestrogen could have a beneficial effect on female reproductive hormone metabolism (Dailey & Neal, 2003).

Foods containing isoflavones and other phytoestrogen have potential therapeutic benefits for cancer, such as breast and prostate cancer (Lephart et al, 2004; Horn-Ross et al, 2001; Ferguson & Philpott, 2004). Consumption of the high Isoflavones reduces the chemical induced rodent mammary cancer significantly. Isoflavones implicates as an important dietary factors in cancer prevention (Messina & Barnes, 1991). Isoflavones were also important in treating cardiovascular diseases and osteoporosis (Lephart et al, 2004). A phytoestrogen rich diet was shown to improve the general health and to limit the growth of lung cancer (Schabath et al, 2005). Isoflavones were also shown to protect the skin of rats exposed to solar stimulated UV and radiation exposure. Phytoestrogens were also anti-inflammatory, immune protective and anti-carcinogenic (Reeves et al, 2005, Moon et al, 2006).

Kiwi-Fruit Seaweed Extract (KFE) was investigated in a series of laboratory, animal and human studies; and found to have anti-mutagenic, anti-carcinogenic, anti-oxidant, anti-atherogenic and anti-toxicity effects, as well as assisting in DNA repair and stimulating the immune system (Xu, 2004). In anti-carcinogenesis animal tests, KFE was fed to rats that were treated with diethylnitrosamine. The pathological changes subsided with the administration of KFE. The effect of KFE was more marked than the use of selenium compound and sodium selenate (Xu, 2004). Selenium was used as a comparison compound because selenium was confirmed to have some cancer prevention effects (Rayman, 2005). In a previous pilot study, six menopausal women were administered 100ml of KFE for 2 days. Their urine sample ratio of 2OHE:16α–OHE, used as a biomarker for breast cancer (Naik et al, 2006; Lukaczer & Darland, 2005; Lu & Anderson, 1996; Xu, 1999),

was elevated by the intake of KFE (Xu, 2004) (see Table 2.4). After a two day period of KFE use, 16α-OHE had significantly decreased. As such, KFE can be used to improve the quality of life of women suffering from endocrine disorders (Xu, 2004; Fu & Xu, 2011).

Table 2.5: Chinese kiwi-fruit formula affecting the urinary 2-OHE and 16α-OHE in menopausal women (Xu, 2004)

Menopausal women	**Age (years)**	**2-OHE (ng/ml)**			**16α-OHE (ng/ml)**		
		Pre supply	Post supply	**P**	Pre supply	Post supply	**P**
n=6	52± 4.24	5.51± 5.08	6.82± 3.76	0.21	2.86± 1.88	1.54± 1.02	0.02

In a previous clinical trial (Xu, 1999), 16 participants who were diagnosed with breast cancer and had just completed chemotherapy, were given a daily KFE drink (equal to 20g of KFE) over a 7 day period. The results indicated the rate of binucleate lymphocyte cells with micronuclei in these 16 participants had significantly decreased. This experiment indicated a recovery of the chromosome change in the breast tissue (Xu, 1999). The results of the clinical trials indicated strong antioxidant effects and stimulation of DNA repair systems by the regular consumption of kiwi-fruit, which were not solely attributed to Vitamin C (Collin & Harrington, 2003; Skinner & Loh, 2011). Phytoestrogen was proven to act against the active free radical oxygen by carotenes, cryptoxanthins, polyphenols and flavonoids. The absorption of free radical oxygen was inhibited in the small intestine by dietary fibres or by the regulation on efflux and influx of ions in the mineral path way of the cell membranes. These findings support the use of KFE in the prevention of tumours (Motohashi & Kawase, 1999). Dietary mutagens such as the compound of charcoal red meat, N-nitroso compounds, smoked food and fungal toxins can trigger the onset of cancer. Therefore a diet of anti-mutagens is crucial for the prevention of cancer. Antioxidants are suggested to scavenge free radicals and prevent their interaction with the cellular DNA. Antioxidants within kiwi-fruit

are recognised as having a positive effect on the DNA repair enzymes (Ferguson & Philpott, 2004).

Although there is no reported evidence of any severe side effects caused by an intake of kiwi-fruit over the last 20 years, there has been an increase in reports of minor allergic reactions, resulting in kiwi-fruit being recognized as a food allergen. Most patients with a kiwi-fruit allergy reported symptoms that were localized to the oral mucosa but some severe systemic reactions were reported, particularly in young children. Kiwi-fruit is not listed as a major allergen in the United Kingdom but people susceptible to food allergies should be cautious (Lucas & Nieuwenhuizen, 2007). Lucas also states that five allergens in kiwi-fruit were proteins and the proteins were broken down by pepsin. If pH gastric increases from 1.5 to 2.5, the reduction of pepsin function could affect the breakdown of kiwi-fruit allergens (Lucas & Cochrane, 2008). So far eleven kiwi-fruit allergens have been found. The diagnosis of kiwi-fruit allergies was detected by the serum IgE and IgG but the methodology was not significantly sensitive (Bublin & Dennstedy, 2010). In this research applicants who had known food allergies were excluded. Adverse effects during the intervention were monitored, however none were found.

Table 2.6: Chinese Medicine's view of Kiwi-Fruit Seaweed Extract (compiled from Xu, 2004; Bensky & Gamble, 1986)

Chinese herbal name	Mi-hou-tau (Kiwi-fruit)	Hai-zao (Seaweed)
Pharmaceutical name	Actinidia chinensis fructus	Sargassii Herba
Properties	Sweet and sour	Bitter, salty and cold
Meridians entered	Liver and Kidney	Kidney, Liver, Lung and Stomach
Action and indication	Clear Qi Stagnation in Liver Meridians Calm the Shen and benefit the digestive system	Clears heat and reduces phlegm nodules Promotes urination and reduces oedema Releasing pain associated with hernia disorder
Caution and contraindications	Food allergic reaction	Contraindicated in cases with cold from deficiency of the Spleen and Stomach Incompatible with Gan-cao

2.4.4. The management of food allergies

Participants with any known food allergies were excluded; however extreme care was taken to monitor any allergic symptoms that appeared. Symptoms and signs vary by allergen, mechanism and the age of the patient. With regard to kiwi-fruit allergy symptoms, most were located in the oral mucosa area. The systemic reaction of allergic reactions can be GI symptoms, asthma, rhinitis and dermatitis. Others symptoms can include migraine, cheilitis, aphthae, pylorospasm, spastic constipation, pruritus and eczema. In severe cases, the symptoms of allergic reactions can include explosive urticaria, angioedema and anaphylaxis (Beers et al, 2006).

2.5. Thermography and breast diseases

2.5.1. Overview

Thermography is a painless procedure when applied to the breast area with no contact to the body and no use of radiation. Thermography is a non-invasive imaging technique using infrared light to detect temperature changes in the body. Thermography can indicate any breast pathology changes; which includes cancer, fibrocytic disease, infectious breast and vascular alternation in the breast. The signal is amplified and recorded by a computer program. The rise in the temperature is due to the tumour needing an increase in the local blood supply, with the angiogenesis factors secreted by the local tumour cells. Thermography is particularly useful for women under 50 years of age where mammography testing is less effective. Thermography has a role in the diagnosis of breast cancer and other breast disorders, assisting with the early detection and monitoring of abnormal physiology, and the establishment of risk factors for the development or existence of cancer.

According to available literature, no research has been conducted to investigate thermography changes compared with Chinese Medicine diagnosis pattern changes and magnetic tests. No research has explored the correlation between thermography results and the use of a KFE intervention.

2.5.2. The background of breast thermography

In 1956 a clinical trial of breast thermography was first conducted (Kennedy & Lee, 2009). Since 1970, thermography has been widely used for breast cancer research and the prognosis follow-up. The elevation of temperature conditions in breast cancer was first reported by Lawson and Gaston (Amri et al, 2011; Ng & Ung, 2001). The breast area temperature had varied due to hormonal changes over a normal menstrual cycle. The abnormality of breast thermography images demonstrated a poor prognosis for breast cancer (Head et al, 1993; Gautherie, 1980; Gautherie, 1987). Furthermore, the degree of the temperature change in thermograms reflected the effectiveness of chemotherapy (Kurihara et al, 1993)

(see Figure 2.1). Thermography was shown to have a high false-positive and false negative rate. It is unable to directly support the spatial localization for surgery. Needle biopsy and mammography still play an important role for the diagnosis of breast cancer, in spite of the benefits of thermography (Greenblatt, 1982; Amalric & Gautherie, 1981).

Thermovascular changes associated with in situ and minimal breast cancers were investigated in a four year prospective study (Gautherie, 1987). There were 6,459 thermograms completed by the Washington Clinical Thermography Centre, which provided a data set of participants with a family history of having a high predisposition to developing breast cancer (Nyirjesy et al, 1986). Several American and European medical centres reported that thermographic imaging had an increase of heat emissions by 71-94% of breast carcinomas (Nyirjesy et al, 1986).

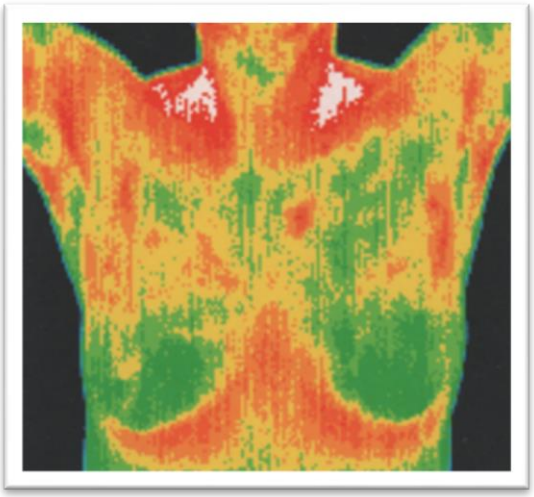


Figure 2.1: Thermography image for breast temperature

In 1980 the results of Gautherie's research indicated that thermography had the ability to detect breast cancer. Thermography had a true positive rate of between 84% to 95% and a false positive rate of 6% to 13% (Keyserlingk & Ahlgren, 2000). Some research reported that breast thermography could detect any changes in the

breast which had occurred up to 10 years earlier. (Golab-Lipinska, 2004; Gautherie, 1980; Gautherie, 1987; Keyserlingk, 1997; Ahlgren et al, 1998; Belliveau et al, 1998). However, a high rate of false positives had been identified as a weak point of thermography which caused a decline in its use (Kennedy & Lee, 2009).

Thermography has continually improved, along with exploration of its use in diagnosis of other human diseases. In 2004, Ng suggested the possibility of extending numerical modelling and computerized image analysis in the breast area, which became an early detection tool for breast cancer (Ng, 2004). In addition to early detection, another benefit of thermography was that examinations can be repeated at short intervals without any known hazard to the patient (Golab-Lipinska, 2004). The 3D images of thermographic temperature distribution have improved by using computerized analysis, numeric analysis, bio-statistical analysis with normal distribution and papametric factoranalysis (Lin & Yang , 2009).

Thermography can detect relatively deep levels of temperature change in tissue of the human anatomy. For example, diseases associated with a vascular pathological change, which is able to elevate tissue temperature of thermographic images including coronary diseases and tumours. Verheye (2004) stated that 68% of myocardial infarction is related to the small uncompleted, obstructive and haemodynamically insignificant atherosclerotic plaques that appeared to be responsible for sudden death, due to acute vessel closure and subsequent myocardial infarction. Current standard technology such as coronary angiography was not able to predict the likelihood of rupture of such plaques, as it was also unable to visualise the plaques with reasonable specificity. Temperature heterogeneity had been associated with inflammation and high risk plaque. Verheye and colleague's (2004) attempted to find a new method of thermography to detect the risk of plaque in the coronary system.

Thermography when compared with mammography was found to detect breast cancer at an earlier stage. Since 1960, mammography has become a regular breast scanning test (Kennedy & Lee, 2009) and the risk of death from breast cancer has

reduced by approximately 30% in women over 50 years of age. However, mammography was found to have a few weaknesses, such as not being well suited for women with a high density of breast tissue, in particular younger females, those with breast implants, fibrocystic breasts or females on hormone replacement therapy (Fletcher & Elmore, 2003; Hoekstra, 2001). These conditions can possibly reduce the accuracy of a mammography test. It was found that a mammography test would cause minimal pain during the test and any applied pressure may increase the risk of a rupture of the encapsulation of a cancerous tumour. The radiation exposure of mammography conferred a slightly increased risk of causing radiation induced breast cancer. The small amount of ionizing radiation used in a mammography screening was thought to be dangerous for women with BRCA1/2 genetic mutations as a family history of breast cancer. It also found that proliferation of these mutated cells under the influence of oestrogen increased by 10% (Hoekstra, 2001; Kennedy & Lee, 2009). Mammography was reported to have high false positive readings (Lin & Yang, 2009). Taking this information into consideration, thermography is therefore also widely used for breast examination.

2.5.3. How does thermography identify breast disease?

Thermography detects the subtle physiologic changes which relate to change in the local temperature. The first image provides the baseline of the thermographic signature. The serial scans were able to provide the thermal pattern in a client. Relying on the pattern, an abnormal alteration is identified clearly (Amri et al, 2011). Thermography showed significant indicators several months before any of the clinical signs of inflammatory breast disease appeared, such as skin discoloration, swelling and pain. Inflammatory breast diseases and changes most commonly seen in younger women cannot be detected by mammography, as the high density breast tissue in younger women was shown to be less beneficial with mammography testing. This outome had affected the early diagnosis of breast cancer in the younger female age group. In terms of breast cancer, early detection provided the best hope of survival. Thermography combined with mammography

became a powerful screening test for breast diseases, which could achieve 95% sensitivity in the early stages (Keyserlingk & Ahlgren 2000).

Thermography is able to detect temperature changes in the breast area. Temperature variations were affected by hormonal changes in females. The temperature was also altered by the menstrual cycle and the different days of the period cycle (Ng, 2001). Furthermore, the breast thermogram can be influenced by temperature changes which are also affected by any emotional stress that may have occurred (Ng, 2001). This evidence explains why Liver Qi Stagnation can initiate breast diseases. A lengthy period of accumulated minor factors exerts their effects for many years and slowly erodes the body's ability to nurture, protect and regulate itself. These effects include environmental pollutants, chemical exposure, a history of infectious diseases, availability of food, nutrient levels in the food, dietary habits, mental and emotional attitudes, balance between work and rest, sexual activity, inherited physiological makeup, water balance and metabolism (Tagliaferri & Cohen, 2002). These risk factors can affect the Liver Qi and the Liver Blood Stagnation as they are closely related to emotional stress (Maciocia, 2004). The Liver Meridian was a major responder to the menstrual cycle, which also correlated with the Penetrating Vessel and Directing Vessel. Stress can block the free flow of Liver Qi, thus affecting the proper movement of blood for a normal period to occur (Maciocia, 2000).

Breast thermography had the ability to detect a pre-cancerous state of the breast tissue or signs of cancer at an extremely early stage (Gautherie, 1980; Keyserlingk, 1997; Ahlgren et al, 1998; Belliveau et al, 1998). The reason thermography is able to detect and monitor the earliest changes in tissue was due to the temperature variations and new blood vessel formations in breast cancer. From a pathological viewpoint, the increased temperature is related to angiogenesis stimulated secretion by the cancer cells. The local temperature is raised due to the chemical material which is produced by tumour cells. This sign can be detected several years earlier than the tumour forming as a lump (Lee & Yang, 2010). In a benign disease, the

temperature also increases due to the blood flow being elevated in the local area, such as due to inflammation or a benign tumour (Fan & Fan, 1988).

Thermography image testing can be used detect an increase in the temperature within the local breast area through the growth of tumour capillaries. In cancer cell proliferation, the growth of the cancer depletes an enormous amount of oxygen and glucose. To obtain enough nutrition, tumour cells secrete angiogenesis factors which will stimulate the forming of tumour capillaries (Verheye & De Myer, 2004). The use of Digital Infrared Imaging was based on the principle that metabolic activity and vascular circulation in both pre-cancerous tissue and the area surrounding a developing breast cancer is almost always higher than in normal breast tissue, which is related to the neoangiogenesis reaction. This method of breast cancer detection is able to identify a preclinical change in the breast at an earlier stage than mammography by up to ten years (Nyirjesy et al, 1986). The tumour size had a less important role in controlling changes of the temperature area, compared to the depth of the tumour (Amri et al, 2011).

In order for cancer to grow, cancer should develop blood vessels to deliver the necessary nutrients and oxygen to support their growth. These blood vessels develop in a process of pathologic angiogenesis, and they provide the cancer tumour with a dedicated blood supply (Kennedy & Lee, 2009). Nitric oxide was another vasodilatory substance produced by tumour cells. The inflammation, wound healing and associated infectious diseases all lead to vasodilation and recruitment of the variety of cells and blood in the process (Anbar et al, 2000). Thermography was able to detect the abnormalities in all of the above.

2.5.4. Methodology development in thermography

The current breast screening methods for breast disease are Doppler ultrasound, mammography and magnetic resonance imaging (MRI). Thermography is a relatively new procedure introduced for breast disease screening. The theory is to establish thermography as a detection of vascular change and temperature elevation which indicates disease in the breast area. With thermography it is recommended

that serial tests are conducted. For example, the first initial test is important to identify the normal breast pattern, which is seen to be beneficial at a later stage for the detection of any abnormal breast changes. This process can potentially identify inflammation or neoplastic changes. In breast cancer the phenomenon is indicated in thermography testing as the skin temperature elevated between 1 to 3^{o}C. The new vascular feed, the metabolic activity and the discreet area of hypothermia stayed displaced around the surrounding hyperthermia. These images indicated the pre-cancerous tissue and the area surrounding a developing breast cancer (Amri et al 2010; Tan & Ng, 2009). For the accurate thermo mapping of the breast, recent advances in infrared camera technology combined with a computerized image processing system have constantly been improved. The thermal breast image can be a valid adjunct tool to overall breast pathologic diagnosis (Amri et al, 2010).

Ng's study (2004) stated that the combination of mammogram and thermography testing increased the detection of any breast changes by up to 93%. When all of the following three methods - clinical examination, mammogram and thermography were used - it increased the detection of any breast changes by up to 98%. Thermography is only a test of physiology where a mammogram is a test of anatomy. It is hoped that thermography, along with numerical modelling will become an adjunct testing tool (Ng, 2004).

Thermographic images can be affected by the following influences; alcohol consumption, physical exertion and a change in room temperature which had to remain between 18^{o}C to 22^{o}C. To minimise these influences, the result will show in the alternation of the skin surface temperature with a variance between 1.27^{o}C +/- 0.37^{o}C, due to the growth of the tumour (Amri et al, 2011). A sophisticated formula and calculation was developed by Amri and colleagues, which applied the Pennes equation and Kirchoff's law while also using the lattice Boltzmann method and the finite element method.

According to the Hoekstra thermology signs, the diagnostic criteria were asymmetric and hyperthermic vascular patterns found in the breast area. The focal

patterns of temperature were elevated with a 2.5°C difference from other areas of the same breast. The asymmetric and atypical complexity of vascular patterns can indicate the neoplasm area. The asymmetric and diffuse hyperthermia was at least 2°C higher in the periareolar area or the entire breast. The localized heat had an abnormal physical contour and an uneven edge (Hoekstra, 2003). Nyirjesy and colleague's (1986) concluded that thermography separates five grades and the lower three grades demonstrated a benign situation. If the fourth and fifth grades remained the same with no change in improvement then malignant pathology is possible. The focus of the thermography researchers was to use the equipment of thermography to interpret the image criteria such as thermal capacity, average temperature, maximum and minimum temperature, methods used to analyse the difference of temperature, temperature standard deviation, temperature skews and steep temperature (Lee & Yang, 2010; Ng, 2004).

The criteria for thermography are constantly developing. Most practitioners apply the following criteria (Kennedy & Lee, 2009)

- Anarchic or complex vascular features
- Hyperthermic focal patterns greater than 1 to 3^{o}C difference
- Asymmetric and abnormal complexity of a vascular pattern
- Asymmetric and abnormal physical contour of more than one quadrant of a breast
- Any combination of these thermology signs

2.5.5. The benefits of thermography

Thermography cannot replace mammography but it may increase the accuracy of mammography testing. The accuracy of breast mass detection was 81.82% with a single use of mammography (Fan & Fan, 1988). Mammogram detection rates were found to be 83% for Grade 2, 68% for Grade 3, and 55% for Grade 4. Mammogram detection was affected by the breast density. The higher the breast density, the lower the successful detection rate (Kennedy & Lee, 2009). If mammography testing can be combined with Thermography, the accuracy of breast cancer

detection can be increased to 95.5% (Fan & Fan, 1988; Kennedy & Lee, 2009).Mammography has been the gold standard for detecting breast cancer since 1960 (Kennedy & Lee, 2009). Mammography is also used to identify the anatomical structures of breast abnormality. Mammography is not seen to be suitable for younger women due to the high density of their breast tissue. It is also difficult to diagnose breast cancer for those with breast implants, fibrocystic breasts and those taking hormonal replacement therapy (HRT) (Fletcher & Elmore, 2003, Hoekstra, 2001). Mammography is limited in that it only detects cancer when it appears as a white patchy image on the x-ray. This weak point of mammography testing can affect the early detection and diagnosis for more aggressive forms of cancer (Wright & Mueller, 1995; Agnese, 2005).

Thermography is not affected by the high density of breast tissue, therefore it is recommended for breast scan testing in younger aged women. Ng investigated women aged between 21 to 45 years (Ng & Ung, 2001). Thermography is able to detect a pathologic state of the breast up to 10 years before a cancerous tumour can be detected by any other procedure, which makes it highly suitable for early detection of breast tumours (Ng, 2004; Lin & Yang, 2009). Thermography can indicate any temperature changes within the breast area. This relates to oestrogen mediated vasodilation and by the increased local production of nitric oxide (Kennedy & Lee, 2009).

2.5.6. Further improvement of Thermography

Since 1982, infrared imaging has been used for breast cancer detection after being approved by the FDA for use in the USA (Moore, 2001). Unfortunately at an early stage of its development and due to unsatisfactory results, it was reported as a weak point for thermography. It has a widely variable and subjective interpretation among image readers and a high false positive rate and false negative rate was reported (Kennedy & Lee, 2009). False negative results were micro-calcifications, which suggested that infrared imaging may not be able to detect these abnormalities as well as mammography testing does (Parisky & Sardi, 2003).

The standards of breast imaging have been studied and the contour shape of the breast was reviewed by clinicians. The comparison of breast imaging was used and it determined how it could vary due to individual breast shapes. As a result, it was difficult for a breast shape image to be matched and used as a standard mapping guide. Therefore, a 3D anatomical image was not set up as a standard diagnosis method. The diagnosis criteria are continually improving by computerised techniques. Thermography as a thermal picture of the skin is unable to localize a small lesion in deep tissue or to generate a 3D image of a tumour. Therefore this weakness of thermography is not able to support a surgical biopsy procedure (Head et al, 1993).

There has been no previous study of hot and cold spot mapping used in infrared thermography diagnosis. The biostatistical technique was proposed as the next stage of abnormal region identification which could set up the future statistical standards of hot and cold spot mapping. Anatomical organ matching templates can provide additional information for clinical diagnosis (Lee & Yang, 2010).

2.5.7. Thermography test and Chinese Medicine diagnosis

Explaining thermography change and Liver Qi Stagnation pattern in Chinese Medicine is not a topic that has been widely developed. The emotional stress related to the Liver Qi Stagnation pattern may affect the temperature in the breast area without any cancer mass. In Chinese Medicine, emotional problems such as aetiology contribute to the onset of breast disease, and are listed as a priority in the book, *Obstetrics and Gynaecology in Chinese Medicine* (Maciocia, 2004). In this work, a study was designed to observe and compare Liver Qi Stagnation and Liver Kidney Yin Deficiency and thermal image tests.Its objective was to explore if emotional stress was related to the Liver Qi Stagnation pattern, and whether it could have affected the temperature of the local section within the breast area without any cancer mass. In Chinese Medicine, most acupuncture meridians accumulated in the upper lateral quarter area of the breast. Any obstruction of Qi

and Blood flow in acupuncture meridians can form lumps in the breast, if the breast area was in a relative deficient condition (Deadman, et al 2001).

2.6. Magnetic test - Pulsed Electromagnetic Field Test (PEMF)

2.6.1. Overview

The relationship between Chinese food therapy-KFE, pulsed electromagnetic field (PEMF) tests and Chinese Medicine diagnosis have been investigated in this study. A series of studies demonstrate the integrative abilities of hormone regulation and subsequent preventative strategies with the use of Chinese Medicine (CM) in the prevention of breast cancer and balancing hormones. The ratio of the bio-markers' variation can possibly affect the Chinese Medicine pattern change which is related to the acupuncture meridian change. The acupuncture system is very complex; with meridians and acupuncture points invisible to anatomical research. The electrical resistance of acupuncture points were indicated in the research of Nakatani (1956). The magnetic network was recognised as being similar to the acupuncture meridian in the study of electromagnetic models (Omura, 1986 Yung, 2005).

Previous research explored the possibility of the selected Kiwi-Fruit Seaweed Extract benefiting women with hormone imbalance or hormone related diseases (Xu, 2006). This study aims to identify the likelihood of Kiwi-Fruit Seaweed Extract affecting the pathological changes in the acupuncture meridians.

2.6.2. The background of Magnetic test: Pulsed Electromagnetic Field Test (PEMF)

Electromagnetic fields are present everywhere in our environment but are invisible to the human eye. One of the main characteristics which define an electromagnetic field is its frequency or its corresponding wavelength. Fields of different frequencies interact with the body in different ways (WHO, 2011).

Humans have an electromagnetic energy field, as the human body is an aerial that can transmit and receive energy (William, 2002). In Chinese Medicine, when a disorder is diagnosed; the magnetic field can be altered by *Qi and Blood* Stagnation. According to Chinese Medicine history, magnetic methods have been used in Chinese Medicine practices since 200 BC, which discribed in *The Yellow Emperor's Canon of Internal Medicine*. Magnetic fields have been reported as being used to treat headache and arthritis (Chou et al, 2001, Smith et al, 2004).

In 1850, Lente reported that passing direct currents in the fracture gap could enhance healing. Electromagnetic treatment methods re-emerged from 1950 to 1960 in the USA and Japan. In 1979, the use of PEMF was approved as a medical device by the USA Food and Drug Administration (Vincent & Andrasik, 2007). Following the ruling, PEMF was formally included in Complementary and Alternative Medicine in the USA. In 1982, the utility of PEMF for bone healing was confirmed for non-union bone fractures. PEMF combined with bone grafting could achieve a high successful rate for non-union bone fractures and was proven in 45 patient cases (Bassett et al, 1982).

Magnetic methods used in Chinese Medicine were also heading in a new direction, moving beyond magnetic stone therapy which was applied on the skin‘s surface at acupoints. Chinese Medicine practitioners were interested in comparing the effects of magnetic intervention with an acupuncture needling technique. In the early stages of the study of electromagnetic methods, magnetic networks was recognised as being similar to the acupuncture meridians (Omura, 1986). Later studies indicated that the acupuncture meridians were related to the electromagnetic methods of transmission lines (Yung, 2005). The magnetic test used a polarized light to possibly detect a defect at the cellular level (Nalbandian et al, 1995; Gianni et al, 2006). In this study polarized light was used to detect the magnetic field change near and along the acupuncture meridian.

PEMF has been used to treat diseases which relate to blood flow disorders and pathological changes which could be improved by increasing blood circulation.

PEMF has demonstrated a successful result in the intervention of Alzheimer's disease (Sandyk, 1994), bone fracture (Bassett et al, 1982; Marcer, 1984), osteoarthritis (Borsalino et al, 1988), depression (Sandyk, 1991) and the reduction of pain (Shupak et al, 2006). Most intervention periods were 15 to 30 minutes long with a low magnetic frequency impulse of 5 to 50 HZ. The intervention times range from 10 to 20 exposures of PEMF. Magnetic tests can also be used for detecting malaria infection, with a better sensitivity than the thin blood film test (Nalbandian et al, 1995), and it was also used for measuring the inhibition of cerebellar activity (Daskalakis & Christensen, 2005).

Currently, researchers focus on using low frequency pulse electromagnetic field studies. The researchers try to investigate which human diseases can be detected by PEMF intervention. The investigation for the anatomy physiology change of PEMF was conducted in a laboratory at cells level. Magnetic study selects the Ultra-long Electromagnetic Wave technique, which is TW-1 supplied by Health Link Food & Equipment Pty. The electric wave is 6 million metres of alternating magnetic line of force and alternate current 50 Hz. This was explored by Professor T. Fujiyama in 1932. A rise in temperature of the human body may be produced by the machineand can increase the local capillary circulation (Health Link Food & Equipment Pty).

2.6.3. Methodology for magnetic studies

PEMF magnetic test is based on the different electrical resistance in the acupoint area. In Nakatani's research (1956), the electrical resistence in the acupuncture points were 5 to 50 KΩ but the other skin areas were between 0.3 to 3 MΩ (Wolfson, 2003). The test of the electromagnetic field is not only affected by the exogenous electromagnetic field but also by the intrinsic hyperpolarizability of the internal cells molecular change (Watkins & Kuzyk, 2009, Chen et al, 2011). The exogenous electromagnetic field can affect the intrinsic polarizability, which was elevated by the exogenous electromagnetic field to a certain limit. The limit was restricted by the intrinsic energy level. If the intrinsic energy level was reduced or

degenerated, the exogenous electromagnetic field could not be varied by the geometry of a molecule or intrinsic hyperpolarizability (Watkins & Kuzyk, 2009). Electromagnetic field resonance was used for detecting the biological reactions of the acupuncture point stimulations. Research was conducted by Dr Omura; with his results exploring the meridian and organ biological function in the human body (Chen & Zheng, 2011). The Pericardium Meridian corresponded to the adrenal gland; while the Triple Burner Meridian related to tests in males and the ovaries in females and all meridians were linked with the cerebral cortex. It is reasonable to consider that the representative areas of the organs in the cerebral cortex were able to be stimulated by the intervention of acupuncture meridians (Chen & Zheng, 2011).

Adverse effects may result, such as an exacerbation of hot flushes and skin irritation due to the adhesives that hold the magnets to the body, which may be caused by the tape which holds the magnetic mass (Colbert & Cleaver, 2008). PEMF tests did not use the exact same method, but the skin reaction and hot flushes were observed over this research period.

This study also investigates the relationship between the 2-OHE:16α–OHE ratio and the magnetic field changes in the breast and trunk area, which was related to the Liver Meridian. The magnetic field changes were measured by a non-invasive medical device, which had been approved by TGA and was supplied on the market for 10 years.

2.6.4. Acupuncture meridians

In this study, magnetic tests were used to identify the presence of Liver Qi Stagnation and Liver Meridian obstruction. If Liver Qi Stagnation or Liver Meridian obstruction occurred, the flash of polarized light could be changed in the affected Liver Meridian area. The diagnostic method had to be combined with the regional symptoms due to the structural connections with the Liver Meridians and its various branches. The symptoms included inguinal hernia and pruritus.

Gynaecology in Chinese Medicine, lower abdominal pain and irregular menstruation suggests Liver Meridian Stagnation. Enuresis and renal stone diseases were also considered. Stomach symptoms can be caused by Liver Organ function disharmony including pain, vomiting and diarrhoea. Gall bladder and Liver symptoms were hypochondriac pain, pressure on the chest, a bitter taste in the mouth, depression, mood swings and jaundice. Lungs affected by the Liver disorder showed symptoms of loud coughing and pain in the hypochondriac. In the chest area, there were symptoms related to the Liver Meridian such as rib pain, lymphadenopathy, swelling and pain in the breast. Du Meridian related to the Liver Meridian symptoms and was taken into consideration in the diagnosis, such as headaches, special vertex headaches, central nervous system signs of tremor and dizziness. Others symptoms were facial paralysis, trigeminal neuralgia, temple headache, visual disorders and pain around the eyes, dysphagia, globus hysterics and goitre (Long et al, 1998; Pirog 1996). In light of the aforementioned symptoms and signs, Liver Qi Stagnation in the Liver Meridian can be diagnosed with a positive result of the magnetic test.

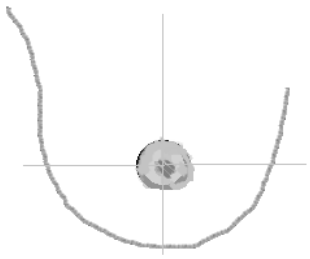
In this study, polarized light was used to detect the magnetic field change near and along the acupuncture meridian. In Chinese Medicine, all of the twelve primary meridians passed the trunk area and spread over to the peripheral extremities. The Yang Meridians traversed to the outer surface of the arm or leg and travelled to the head and the back, despite the Stomach Meridians that were running to the lateral side of the Kidney Meridians, and at the anterior of the trunk. All Yin Meridians traverse or bypass the breast area and the special superior lateral side of the breast. These Meridians each correspond to different organ (Zang Fu) systems. Meridians harmonise the whole body's function when Qi and Blood circulate well, and they can also reflect the direct trauma and the Zang Fu function. Pathogens and diseases can make visible changes on the related meridians (Tang et al, 1999). The acupuncture meridians are important transportation pathways, as any blockage of the meridians is able to cause disease and disharmony in the body.

A menstrual period and breast disease are closely related to Liver Qi and Blood. Liver Qi is an important factor in a woman's physiology and menstrual cycle in Chinese Medicine. Liver Qi and Liver Blood are the Yang and Yin part of the Liver. Yin needs to root and embrace Yang; Liver blood deficiency can cause secondary Liver Qi Stagnation. Liver Qi stagnation can cause irregular periods, dysmenorrhoea and pre-menstrual syndrome. Liver Qi Stagnation also triggers breast discomfort, pain and growth of lumps. Pre-menstrual breast tenderness is a very common symptom for Liver Qi Stagnation. Liver Kidney Yin Deficiency in females is most commonly caused by overwork or exposed to prolonged stressful conditions without adequate rest. Liver Kidney Yin Deficiency is the most common cause of pari menopause symptom in modern societies (Maciocia, 2004). Kidney and Liver Yin share the same resources. Kidney and Liver Yin deficiency can lead to secondary Stagnation of Liver Qi. This occurs when the deficient Yin fails to work as the root of the Yang. The aetiology of an unhealthy diet can also cause Liver Qi Stagnation, such as over consumption of greasy food, raw cold food and dairy products. Emotional strain was also present, leading to Stagnation of Liver Qi. The Stagnation Qi can obstruct the transforming fluids and enhance the opportunity for phlegm formation. When the phlegm formation occurs, the Qi Stagnation is aggravated and vice versa (Maciocia, 2004).

In acupuncture meridians, Liver Meridian was an important factor in the breast area and also linked to other meridians. The major meridians that passed through the breast area are shown in detail in the following diagram. (Figure 2.2)

Breast diseases occur most frequently in the upper lateral quadrant area. In Chinese Medicine teaching, the acupuncture meridians pass the upper lateral quadrant of the breast more than any other areas of the breast. In the aetiology of Chinese Medicine, there was a higher risk of Qi Stagnation in the acupuncture meridian in the upper lateral quadrant. Liver Meridian is the most concerning meridian for breast diseases and female hormonal diseases.

Upper Lateral Quadrant	**Upper Medial Quadrant**
Foot Taiyin Spleen Meridian	Foot Shaoyin Kidney Meridian
Hand Jueyin Pericardium Meridian	Foot Yangming Stomach Meridian
Foot Jueyin Liver Meridian	Foot Jueyin Liver Branch
Hand Taiyin Lung Meridian	
Hand Shaoyin Heart Meridian	Hand Shaoyin Heart Branch



Inferior Lateral Quadrant	**Inferior Medial Quadrant**
Foot Taiyin Spleen Meridian	Foot Shaoyin Kidney Meridian
Foot Jueyin Liver Meridian	Foot Yangming Stomach Meridian

Figure 2.2: Diagram of acupuncture meridians in four quadrant areas within the right breast

2.6.4.1. Liver Meridian

The origin point of the Liver Meridian starts from the lateral aspect of the dorsum of the big toe at the Dadun point (Liv 1). It runs along the foot to the anterior of the medial malleolus. The meridian continually ascends to the interceptive point of Sanyin Jiao, which is the Spleen Meridian. After intersecting with the Spleen Meridian, the Liver Meridian rises from the anterior media of the tibia than extend to the posterior of the Spleen Meridian at the knee. The uprising continues to the pubic region via Yinlian (Liv 11) and Jimai (Liv 12) where it encircles the genital area. The Liver Meridian continually ascends to the lower abdomen to meet the conception vessel at Qugu, Zhongji and Guanyuan. The meridian curves around the stomach before entering the Liver and also connecting with the Gall Bladder. The meridian crosses to the diaphragm and spreads into the costal and hypochondriac region via Zhangmen (Liv 13) and Qimen (Liv 14). The Liver Meridian rises upwards continuously to reach the posterior of the throat and passes through the nasophrynx and links with the tissues surrounding the eyes. Finally the meridian

meets the vertex to intersect the Governing vessel at Baihui (Du 20) which is the highest point, and connects with all of the Yin Meridians (Deadman et al, 2001; Pirog, 1996). When Liver Qi Stagnation occurs, the Stagnation affects the other yin organ meridians. The disharmony condition triggers diseases in the different systems of the human body. A branch of the Liver Meridian veers off from the eye, descends down the cheek and circles the inner region of the lips. Another branch comes out of the liver, penetrates through the diaphragm and enters the lung (Pirog, 1996). MostYin Meridians are best understood in terms of the function of the viscus which they control. The Liver Meridian is an exception to this rule and its exterior pathway forms a veritable road map of pathological indications. For example, the occurrence of Lumbago with the inability to bend at the waist might be a symptom related to the Liver Meridian domain over the sinews, which is mentioned in Chapter 10 of Ling Shu (Pirog, 1996).

During the Fourteenth century, Dr Dan-Xi Zhu summarized the aetiology of breast cancer, arguing that a woman who was worried and depressed can suffer from an accumulation of Stagnation (Maciocia, 2004). The Stagnation can be the cause of Liver Qi rebelling horizontally with the Stagnation ending in nodules (Niu, 1996). Female hormonal related diseases and breast diseases are related to the Liver and Kidney Meridians (Fu and Xu, 2011).

Along the Liver Meridians, the depth of the meridian demomstrates that it is running differently. The meridian starts from the big toe and runs to the lateral part of the trunk. Table 2.4 shows a list and the order of the acupuncture points, outlining how deep the meridian is as it passes through the whole of the body. Taking into consideration the depth of the meridian, it can affect the test result. The PEMF test can potentially be more accurate where the meridian was shallow as it ran through the trunk area.

Table 2.7: Pathways of the Liver Meridian (Deadman et al, 2001)

Acupoint	Name	Depth from the skin
Liver 1	Dadun	0.1 or 0.2 cun
Liver 2	Xingjian	0.5 to 0.8 cun
Liver 3	Taichong	0.5 to 1.5 cun
Liver 4	Zhongfeng	0.3 to 0.5 cun
Liver 5	Ligou	0.5 to 1 cun
Liver 6	Zhongdu	0.5 to 1 cun
Liver 7	Xiguan	1 to 2 cun
Liver 8	Ququan	1 to 1.5 cun
Liver 9	Yinbao	1 to 2 cun
Liver 10	Zuwoli	0.5 to 1.5 cun
Liver 11	Yinlian	0.5 cun to 1.5 cun
Liver 12	Jimai	0.5 to 1 cun
Liver 13	Zhangmen	0.5 to 1 cun
Liver 14	Qimen	0.5 to 1 cun

The depth of the Liver Meridians affected the magnetic test. The meridians in the trunk area detected by the magnetic test device were more clearly outlined than the lower limbs which only detected the shallow area and the acupoints. The hypothesis was that if the shallow area can easily be detected by the magnetic test, this might then also be indicated by the thermography. In this research, the scanning test focused on the trunk area.

2.6.4.2. Kidney Meridian

The Kidney Meridian begins beneath the little toe and crosses to the sole of the foot to Kid 1, which is located between the second and third metatarsal bone, approximately one third of the distance between the base of the second toe and the heel. From Kid 1 to Kid 2 acupuncture points, the Kidney Meridian passes the anterior and inferior to the navicular tuberosity then travels past the posterior to the medial malleolus, which arrives at Kid 3. After Kid 3, the meridian descends through to the heel and then ascends to below the medial malleolus at the Kid 6 accupoint. From Kid 6, the meridian ascends along the medial aspect of the leg then intersects with the spleen meridian at Sp 6 (Sanyinjiao). The Kidney Meridian rises up to the medial side of the popliteal fossa at Kid 10 (Yingu). The meridian

continues to ascend to the coccyx and meets the Governing vessel, Du Meridian at Du 1. The primary Kidney Meridian threads its way through the spine, entering the Kidney, connecting with the Bladder and intersecting with the Conception vessel, Ren Meridian at Ren 3 (Zhongji), Ren 4 (Guanyuan) and Ren 7 (Yinjiao) and continues to rise at the bilateral side of Ren Meridian. The primary Kidney Meridian stops at Kid 27 (Shufu), which is located at the first intercostal space, away from the midline 2 cun. One branch of the Kidney begins at the Kidney, travelling to the Liver and passes the diaphragm, where it enteres the Lung after which the meridian ascends along the throat to terminate at the root of the tongue. Another branch of the Kidney Meridian spreads into the Lung, then joins with the Heart and disperses in the chest to link with the Pericardium Meridian and Conception vessel (Ren 17) (Deadman et al, 2001; Pirog, 1996).

The Kidney Meridian is associated with the Bones and the Lumbar Vertebrae, due to an internal branch that travels up to the lower spine. This accounts for the most common application of this meridian to be used in modern clinical practice, as intervention for lower back pain. Kidney Meridians are linked with the Urinary bladder, Liver, Lungs and Heart. An internal branch connects with the throat and the root of the tongue, thus explaining how the dry pharynx and sore throat can be caused by the Kidney Yin deficiency pattern. Knee pain is also related to the Kidney deficiency pattern as the clinical practise diagnosis (Pirog, 1996).

The Kidney Meridian represents the deep level of energy in the body. Some of the symtoms and signs derived from the Kidney Meridian can indicate that the patient was suffering a severe illness or perhaps dying, which was described by Ling Shu:
“The patient experiences hunger with no desire to eat, complexion black as charcoal, spitting of blood, panting with a hun-huh sound, an urge to rise up when seated, eyes so blurred that they cannot see. When the Qi is insufficient, it results in fear. The heart is frightened and distressed, like a man about to be arrested. This is vacuity in the bones ”.

Table 2.8 : Pathways of the Kidney Meridian (Deadman et al, 2001)

Acupoint	Name	Deep from the skin
Kid 1	Yongquan	0.5 to 1 cun
Kid 2	Rangu	0.5 to 1 cun
Kid 3	Taixi	0.5 to 1 cun
Kid 4	Dazhont	0.5 cun
Kid 5	Shuiquan	0.3 to 0.5 cun
Kid 6	Zhaohai	0.3 to 0.5 cun
Kid 7	Fuliu	0.5 to 1 cun
Kid 8	Jiaoxin	0.5 to 1 cun
Kid 9	Zhubin	1 to 1.5 cun
Kid 10	Yingu	1 to 1.5 cun
Kid 11	Henggu	0.5 to 1 cun
Kid 12	Dahe	0.5 to 1 cun
Kid 13	Qixue	0.5 to 1 cun
Kid 14	Siman	1 to 1.5 cun
Kid 15	Zhongzhu	1 to 1.5 cun
Kid 16	Huangshu	1 to 1.5 cun
Kid 17	Shangqu	1 to 1.5 cun
Kid 18	Shiguan	1 to 1.5 cun
Kid 19	Yindu	0.5 to 1 cun
Kid 20	Futonggu	1 to 1.5 cun
Kid 21	Youmen	0.5 to 1 cun
Kid 22	Bulang	0.5 cun
Kid 23	Shenfeng	0.5 cun
Kid 24	Lingxu	0.5 cun
Kid 25	Shencang	0.5 cun
Kid 26	Yuzhong	0.5 cun
Kid 27	Shufu	0.5 cun

In another paragraph of Ling Shu, severe Kidney Yin deficiency had been described as;

"There is heat in the mouth, a dry tongue, swollen throat, a sensation of qi rising in the body, sore dry throat, vexation in the heart, heart pain, jaundice, diarrhea, pain in spinal column and the medial posterior region of the thighs where the Meridian passes, fatigue and somnolence, heat and pain in the sole of the foot " (Pirog, 1996).

According to Chinese Medicine theory, unhealthy lifestyle choices and sociocultural stress do make a difference to symptoms during menopause and peri-menopause. Lifestyle choices lead to Liver Qi Stagnation, tumours, depression and menopausal symptoms (Wolfe & Flaws, 1998; Xu, 2005). The vital essence stored in the Kidneys is the basis of reproduction, growth and development, formation of marrow, nourishment of bones and functioning of the brain. In Chinese Medicine, it is believed that half of Yin is depleted by the age of 40. Since the Kidneys are the root of all Yin and Yang in the body, kidney vacuity, whether Yin or Yang, will give rise to vacuity and insufficiency in other hollow organs viscera (Long et al, 1998; Xu, 2005).

In view of the above two meridians, the meridians are running at a relatively shallow point in the trunk area. Both meridians are important to female hormonal disease and breast diseases. The pathway of the meridians indicates that those meridians connect with all other Yin Meridians, the Governor vessel and the Conception vessel. In this research, the effectiveness of food formula KFE is explored as a method of improving Liver and Kidney Meridian function and general well being. In addition, changes to thermographic and PEMF test results were also investigated.

2.7. Short Form 36 (SF-36) questionnaire

The Short Form 36 questionnaire is a popular choice to use as a self assessment in conventional medical research. In this research a sophisticated self assessment method for examining the general well-being of participants was adopted.

SF-36 is able to provide details of the participants‘ daily condition. SF-36 was scored by the participants and was valid when combined with a patient-generated index of their quality of life. The scores of SF-36 were reproduced and quantifiable, relating to the participants‘ clinical state (Collier & Longmore, 2003). The standard form for version 1, SF-36, was published in 1990 and the standard form was developed for a medical outcome study (Hanmer, 2009).

The Short Form 36 (SF-36) questionnaire is most frequently used as a Self Rated Health (SRH) method. The 36 items of the questionnaire were used to determine dimensions of physical, mental and social functioning, vitaity and general health which associated with SRH. All subscales of the SF-36 were independently associated with SRH and the physical functioning was more strongly associated with SRH than the mental health and the social functioning (Mavaddat & Kinmonth, 2010; Baumann et al, 2009; Lee & Simpson, 2009). All dimension scores represented the mean value of each item. The values were obtained when the number of missing values was no more than half of the total, otherwise the score was declared missing. The dimension scores were standardised from 0 to 10 (Baumann et al, 2009).

SF-36 was used for detecting the impact of menopausal symptoms in the participant after systemic breast cancer intervention which done by Dorjgochoo and his colleagues (2010). The results indicated that the lower scores of SF-36 were in the group of women who had been suffered from menopausal symptoms or the failure of the ovarian function. In Dorjgochoo research, the menopausal symptoms of the participants were affected by the intervention of immunotherapy or tamoxifen for breast cancer treatment. The research of Dorjgochoo and his colleagues indicated the SF 36 score was affected by female reproductive hormonal disturbance indirectly. Participants who suffered from hormonal receptor positive tumours would most likely be affected with menopausal symptoms. The participants with a depressed mood were more likely to have lower scores for the mental health scale and for the physical health scale in the SF-36 results (Dorjgochoo et al, 2010). Hot flushes, such as Vasomotor symptoms, were the most prevalent menopause related symptom significantly decreasing the quality of life. Hot flushes were especially problematic in breast cancer survivors because they were often diagnosed around the time of natural menopause or, because acute menopause occured as a result of a chemotherapy intervention (Graf et al, 2003). Fatigue was one of the most common and vexing symptoms for participants who had a cancer diagnosis. A range between 40% to 90% of those patients complained of fatigue. In such cases, fatigue gained attention as an important symptom in

oncology reseach as well as in clinical practice. SF-36 was a general measure of energy and fatigue (Brown et al, 2011).

The SF-36 questionnaire was a significant and accurate method to detect the early stages of depression, as the investigation of depression conducted by Kristjansdottir and his colleagues (2011). He used SF-36 questionnnaire to investigate the general well being of youth that suffered from depression. The SF-36 was selected for measuring the KFE intervention outcomes in our research; because it is used to evaluate general well-being, fatigue and depression related symptoms. In my research the participants self assessment results are compared to the Chinese Medicine practioniers diagnosis results; with the intention to highlight any concerns of related observations. In Chinese Medicine, symptoms of depression and fatigue as emotional changes were closely related to the Liver Qi Stagnation. This was the first attempt to compare the psychological research methods to Chinese Medicine diagnosis within Australia (WHO, 2004).

This research focuses on the effects of Kiwi-Fruit Seaweed Extract (KFE), and investigates how KFE may reduce the symptom of the two Chinese Medicine diagnostic patterns found in peri-menopausal women. Those diagnostic patterns are Liver Qi Stagnation and Liver Kidney Yin Deficiency. Furthermore, investigations were conducted to explore the biological effects on the Chinese Medicine pattern, and to study the effects of KFE on the regulation of the biomarker 2-hydroxyoestrone:16α-hydroxyoestrone, in relation to thermography changes, magnetic field changes and changes to general wellbeing.

CHAPTER 3

RESEARCH METHODOLOGIES

3.1. Overview

The following sections introduce the methodologies used for this project, the criteria of the participants' recruitment and the related ethics approval. The detailed methodologies are described under the sequence of the hormone biomarker test, PEMF test, Chinese Medicine diagnosis patterns, thermography and the methods of the SF-36 questionnaire. The methodologies were selected to achieve the aims of the research. The research followed the principle of a double blind, placebo-controlled clinical trial.

The study was approved by the related research committees and ethics approval (HRETH 07/207) was gained from the Human Research Ethics Committee of Victoria University, Australia. This study aimed to investigate the following:

a: The effect of a Chinese food therapy, Kiwi-fruit Seaweed Extract (KFE), on the metabolism of female reproductive hormones in middle aged women by using a urinary bio-marker test.

b: The potential effects of KFE on acupuncture meridians and changes in breast thermograms.

c: The effect of KFE on the signs and symptoms of Chinese Medicine (CM) patterns of disharmony - Liver Qi Stagnation and Liver Kidney Yin Deficiency by using a CM assessment.

d: The effect of KFE on physical and psychological well-being as investigated by using the Short Form 36 Health Survey (SF-36).

The recruitment process was via an advertisement in a newspaper (the *Herald Sun*), on local community boards (Sport Centres and Shopping Centres in Moonee Ponds, Highpoint, Essendon, Keilor East) and an internal email newsletter

circulated at Victoria University. All participants received a project explanation document and a consent form to complete.

The age cohort of the participants was between 40 and 55 years, due to this being the main age range group reporting hormonal disturbances (Prior, 1998). The liver organ is very important for oestrogen metabolism (Ng & Ung, 2001); therefore participants with liver diseases were also excluded from the research. The urinary sample was selected for testing the ratio of 2OHE:16α–OHE, therefore participants with kidney diseases were excluded from the research as were pregnant females. Menopausal women who had experienced more than two years of amenorrhea were also excluded from the research. Women with amenorrhea were not included because their endocrine system was abnormal. Any oestrogen hormones from an external source would affect the level of hormonal concentration. Women undertaking hormonal replacement therapy, the contraceptive pill and those taking tamoxifen were also excluded, due to the contents of the oestrogen receptor agonists or antagonists.

Table 3.1: The criteria for the participant selection

Applicant inclusion	Applicant exclusion
• Female, Melbourne resident • Aged from 40 to 55 years • Chinese Medicine diagnostic pattern should be matched to Liver Qi Stagnation and Liver Kidney Yin Deficiency as the main pattern of disharmony, which may combine with Liver Heart blood deficiency; Liver Stomach disharmony and Liver Spleen disharmony. • Participants with the above patterns were recruited	• Pregnant or breast feeding • Positive mammogram or ultrasound diagnosis of breast cancer • Undertaking hormone therapy or taking any form of contraceptive pills. • Taking tamoxifen or undergoing chemotherapy after surgical intervention breast cancer • Suffering from liver and/or kidney disease • Currently post-menopausal or experiencing amenorrhoea • Food allergies or a history of intolerance to Kiwi-fruit, Seaweed, Lactose or Tea • Suffering from a major chronic disorder and taking medication

Fifty six female candidates expressed interest in participating in the research. The potential candidates were interviewed with an initial introduction and an initial Chinese Medicine diagnosis. The participants selected were aged between 40 and 50 years and they were required to live in Melbourne during the research. They were required to commit for a two month period to participate in the research.

Thirty-six middle aged peri-menopausal women were randomly assigned into two groups. The KFE group took KFE and the control group took a placebo. The group assignments were kept confidential from the participants and the data collectors.

3.2. Information given to participants

All of the participants were individually interviewed. The guidelines for the applicants' inclusion and exclusion are outlined in Table 3.1. Once the candidates were selected, more detailed information was given to explain the aims and detailed procedures of the research. The participants were required to drink KFE or a placebo twice a day, and to take a morning urine collection on the consultation day. A container (a urinary collection kit) was provided to the participants to bring their urine sample on the consultation day. During the research, they were required to attend five sessions of Chinese Medicine diagnostic assessment and three sessions of PEMF magnetic tests on acupuncture meridian. They also arranged to attend two non-invasive breast thermography image tests at the beginning and at the end of the research. They were asked to complete a self-assessment questionnaire on their general health at the beginning and at the end of the research.

Table 3.2: Procedure of urine collection

Items	Usage
A urine sample container	For collecting the urine
A large container	Ice and salt was placed in the large container
A swab	To clean the genital area before collecting the urine
A pair of gloves	For the purpose of hygiene
Instruction	Mid-stream urine was collected
	After collection of urine
	Ice and salt was placed in the large container provided
	To place the sample container in the large container
	To keep the urine sample cold and delivery to the laboratory

3.3. Participants informed of the tests procedures

The following procedures for the bio markers, thermography and PEMF magnetic test were outlined in the three tables below. All of the information was explained by the researcher prior to the consent form being signed by the participants.

Table 3.3: The PEMF magnetic test procedure

Procedure of PEMF meridian detection	
Step 1	The participants lie down in a supine position with the abduction of both arms
Step 2	A scan with polarized light was used to detect any changes in the magnetic flux
Step 3	The polarized light instrument skimmed the trunk area and marked dotes
Step 4	The results were recorded on an anatomical map

Table 3.4: Thermography

Procedure of thermography	
Step 1	The participants sat at the front of the thermography camera
Step 2	The participants exposed their breast to the thermography camera
Step 3	Five images were taken in the chest area (1 frontal, 2 oblique, 2 lateral)
Step 4	A computer program generated an image and temperature mean and standard deviation
Step 5	If the thermography result was positive, it was recommended that they see a GP

The record included a depiction of any defective areas found in two dimensions, length and width. The area on the body tested was restricted to the trunk area,

which is the Chinese Medicine meridian area. The measurement was performed at the beginning and end of the study.

In addition, risk factors associated with the study had been discussed with participants and means of resolving these outlined. Although a rare occurance, a participant maybe allergic to kiwi-fruit or seaweed products; resulting in an allergic reaction. Participants with a known history of allergies to kiwi-fruit or seaweed were excluded. If they demonstrated any allergic reaction (e.g. itchy skin) after taking the products, they were advised to report to the researcher and to immediately stop taking KFE. A visit to their GP was recommended for further investigation. Those participating in this study attended a fortnightly one hour Chinese Medicine diagnostic consultation with the researchers. This accumulated to a total of five clinic visits and two thermographic scans over a 2 month period, where each participant was required to complete all of the data collection.

3.4. Equipment and methods

3.4.1. Biomarker assay for oestrogen metabolism

The biomarker assay used competitive, solid phase enzyme immunoassay as an assay for the female reproductive hormones on the urinary ratio 2-hydroxyoestrone (2– OHE) and 16α– hydroxyoestrone (16a-OHE). The urinary samples were collected every 10 days (Figure 3.1).

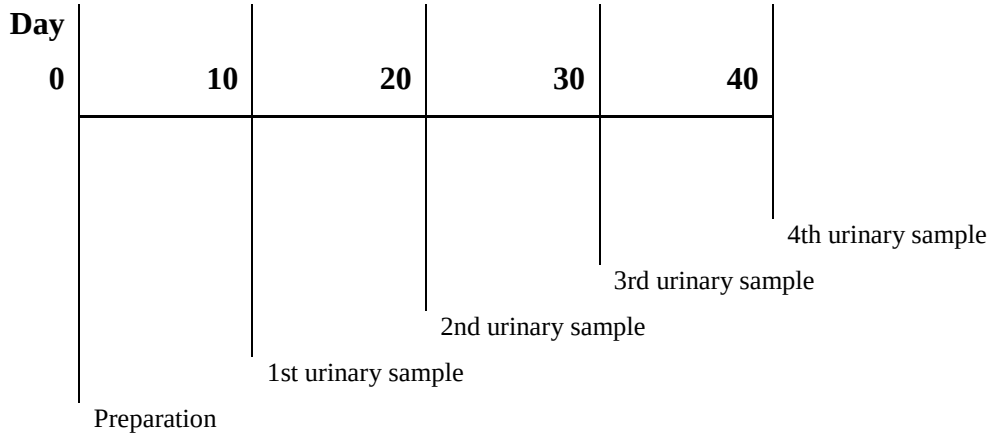


Figure 3.1: Urinary sample collection timetable

In the assay, binding of the antigen-enzyme conjugate by the antibody was inhibited by free antigen. The antibody was captured on the solid phase and the antigen, the oestrogen metabolite was labelled with the enzyme. Since a restricted number of antibody binding sites were available, the enzyme activity was lower after it bonded with the solid phase in the presence of the free antigen. When enzyme substrate was added to the washed solid phase, the enzyme product concentration was inversely proportional to the concentration of the free antigen. In the assay, monoclonal antibodies to oestrogen metabolites were captured directly into the solid phase. The oestrogen metabolites were then conjugated to alkaline phosphatase enzyme.

According to the two studies performed by Westerlind, which compared a 24 hour urine collection with a first morning void, the results were not significantly different in the ratio of 2 OHE:16α–OHE between the 24 hour urine collection and the first morning void (Westerlind et al, 1999). In this research, the morning void midstream urine was collected for the 2-OHE:16α–OHE assay (Figure 3.1).

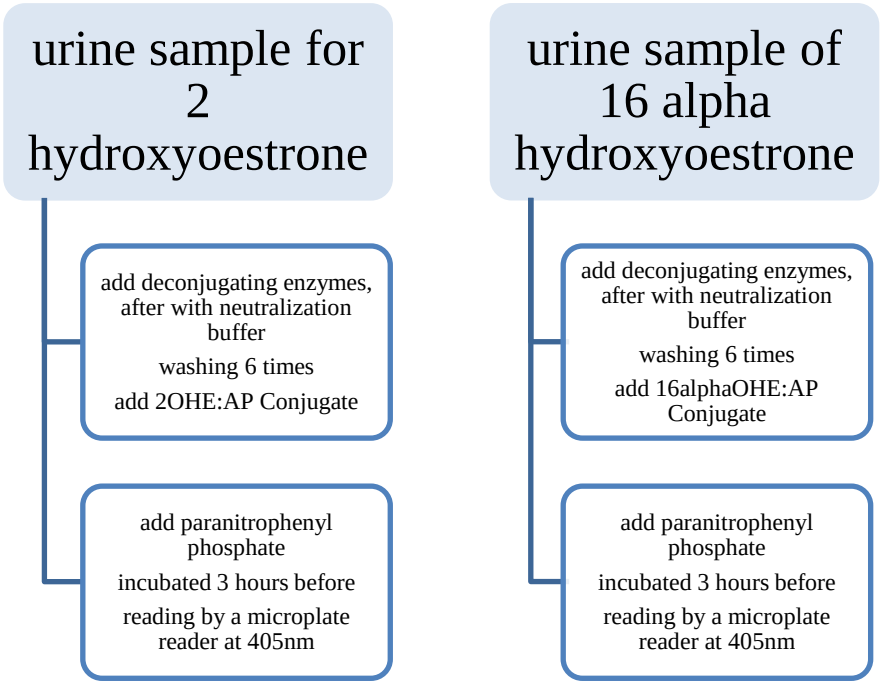


Figure 3.2: Procedure of the biomarker urine assay

A urinary collection kit which included containers, a swab and a pair of gloves was given to the participants. They used the provided kit for collecting their morning midstream urine on the day they visited the researcher. Upon delivery, the urine sample was kept fresh by being placed in an ice container under -10^{o}C. After the urine sample was delivered, it was immediately stored in a -80^{o}C freezer for long term storage until the assays were performed.

Before the urine assays were performed, the urine samples were left under a constant room temperature of 22^{o}C until they defrosted. All urine samples were placed in Eppendof tubes for centrifuging. Centrifuge (Avanti TM30 centrifuge, Backman Coulter) was under 4^{o}C, 8000RPM for 8 minutes. The clear top urine samples were diluted to 1:4 (1 part of urine in 3 part of dilutent in the kit provided) and 10μl of urine was transferred to the micro tubes which were ready for the assay.

Urinary oestrogen metabolites were analysed using an enzyme immunoassay 2/16 kit (ESTRAMET, ImmunaCare, and Bethlehem, PA, USA) according to the manufacturer's instructions. For the preparation of the test, all components of the 2/16 kit were brought to room temperature for 1 hour. The standard solution with a different concentration and 10μl urine samples were transferred to each microtube in triplicates. Afterwards, 190μl of deconjugating enzyme solution was added into each microtube. The samples were incubated for 2 hours under room temperature. After incubation, a 200μl neutralization buffer was aliquoted into the microtubes then the microtubes were placed in vortex for a few seconds. The samples became a 1:40 diluted solution and the sample preparations were completed.

EIA micro-plates were washed by TBS solution (pH7.4, 0.05% Tween 20) six times; the samples in the microtubes, 75μl, were allocated into each cell of the EIA plates. Each sample was triplicated in the EIA micro-plates. Prepared 2OHE1 alkaline phosphatase enzyme conjugate solution, 75μl, which mixed with 2 OHE1 diluted conjugate buffer, was added into each well of 2 OHE EIA plate. 16OHE1

alkaline phosphatase was added into 16OHE1 conjugate diluent buffer then placed in vortex for a few minutes. The prepared 16OHE1 alkaline phosphatase solution was allocated 75μl into 16α–OHE EIA plate. The plates were sealed by placing an adhesive coated plate sealer on top of the plates. The sample plates were incubated at room temperature after a gentle rotation and mixing.

After 3 hours of the incubation, the plates were washed by using TBS solution six times. 100μl AP substrate was poured into each well of the plate. The plates were rotated on the rotator set at 4-5 rps for 2 minutes, and then the plates were ready for an end point reading after 20 minutes by microplay reader.

The 96 well micro-plate was placed in the reading equipment, Palarstar Galaxy, Fluo32 (BMG Labtech), using absorbance as the operating instructions. 2–OHE was found in the urine as 3–glucuronide and 16α–OHE in the form of 3, 16 α–glucorionide. Prior to recognition by monoclonal antibodies, glucuronic acid and sulphate were removed by incubation with the deconjugating enzymes, β–glucuronidase and arylsulphatase, respectively. A calibration curve was derived from the six standards supplied by the kit, from 0.625 to 15ng/ml. The reading results of the urine samples were compared with the standard and the results was obtained as μg per ml. Finally, the ratio was the 2-OHE results divided by the corresponding 16α–OHE results.

3.4.2. PEMF meridian detection

A pulsed electromagnetic field (PEMF) test was performed pre-trial and post-trial at the trunk area, where the pathway of acupuncture meridians was distributed. In this research, each test lasted less than 10 minutes (5 to 8 minutes). It is reasonable to set up a short period of exposure to the PEMF as the reference of most intervention periods were 15 to 30 minutes long with a low magnetic frequency impulse of 5 to 50 HZ; (Bassett et al, 1982, Marcer, 1984). A low frequency of PEMF (50 Hz) was used to go through all the meridians on anterior, posterior and bilateral aspects of the trunk area. No adverse effects were reported by any of the participants during

this research. The intervention times were proven safe, even with 10 to 20 exposures of PEMF. This was demonstrated when PEMF was applied on the bone fracture (Bassett et al, 1982, Marcer, 1984), osteoarthritis (Borsalino et al, 1988), depression (Sandyk, 1991) and the reduction of pain (Shupak et al, 2006).

3.4.2.1. PEMF equipment and methods

The Ultra-long Electromagnetic Wave instrument (TW-1) was supplied by Health Link Food & Equipment Pty Ltd, Melbourne. The magnetic field changes were measured by TW-1, a non-invasive medical device which had been approved by the TGA and supplied to the market for 10 years. This test, using a polarized light, can detect a defect at the cellular level (Gianni, 2006). A polarized light was used for detecting changes in the magnetic flux. When the magnetic flux was blocked, the polarized light was dimmed.The results were recorded on a map where there was an anatomical drawing of the human trunk. These included the depiction of any defective areas found in the two dimensions (length and width). A 0-10 scale was used to measure the degree of the blockage, "0" represented unblocked and "10" represented fully blocked. In normal conditions, all systems in the same intrinsic hyperpolarizability share the same energy ratio and dominated transition moment. The exogenous eletromagnetic field was detectable, except for the blockage by some conditions such as noise of various intensities, and intrinsic hyperpoliarizability, which was limited by the defect area (Gianni, 2006; Watkins, 2009) In this research, the tested area on the body was restricted to between the mid-clavicular line and mid-axillary line, from the iliac crease to the clavicula and the axillary line at the trunk area, which matches the Chinese Medicine Liver Meridian area (see Figure 3.2).

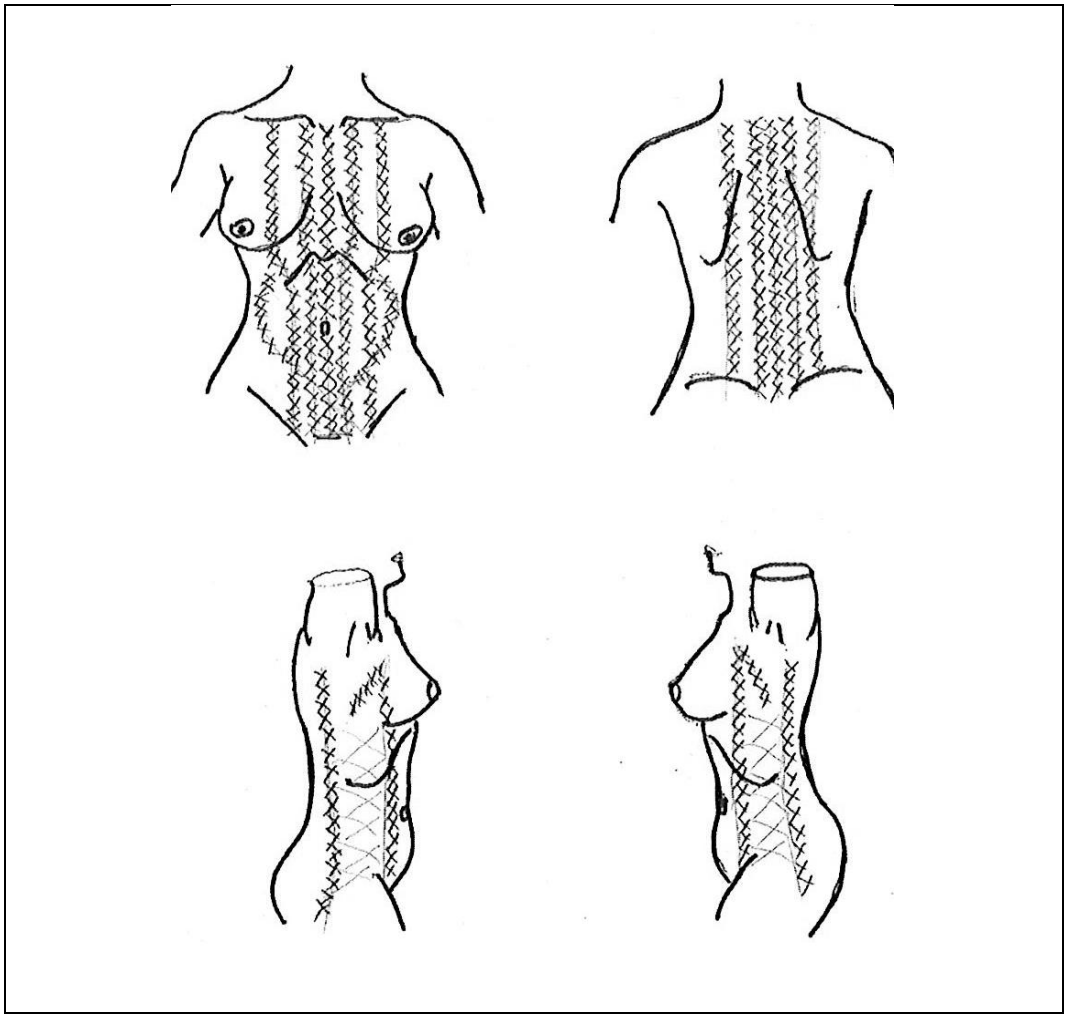


Figure 3.3: PEMF transmission lines of magnetic flux

3.4.2.2. The detailed steps of magnetic flux detection by PEMF

A technician followed a precise procedure described in the following steps:

- Explained the test purpose and procedure to the participant individually. Obtained consent from the participants to record the dots on their bodies
- Placed the patient in a supine position with the arm at 90 degree abduction.
- Scanned the trunk area with the polarized light.
- Record the magnetic flux changes on the surface of the skin with a marker using dots. The marker will show any defective areas found along the liver meridain area.

- Remove the marks on the skin, cleaning with ethanol swabs or a wet cotton ball.

The training program for PEMF was provided by the Health Link Food and Equipment Pty Ltd. A technician performed the magnetic test and the Chinese Medicine practitioner recorded the results.

3.4.3. Chinese Medicine diagnosis

Chinese Medicine diagnostic methods cover observation, auscultation, olfaction and palpation, which were applied to the participant during examination by the CM practitioner using their visual sense, auditognosis, osphresis and tactile sensation. The diagnostic information was recorded, which included the participants describing their feelings and the symptoms and signs in CM diagnostic theory. Chinese Medicine diagnostic methods followed the standard procedure (Long et al, 1998).

The main complaint given by the participants, which was causing concern and discomfort, was documented during each interview. Other Chinese Medicine diagnostic methods included the Ten Diagnostic Questions, pulse diagnosis and tongue diagnosis.

The Ten Diagnostic Questions were an important method of investigation, which covered the participants' symptoms, life style and disease history. The 10 questions summarised the experiences of clinical practices from Zhan Jingyue (Ming Dynasty). The questions include cold and fever, sweating status, the problems of head and limbs, urinary condition and defecation, dietary condition, chest problems, deafness or reduced hearing, thirst condition, the cause and colour of Yin and Yang balance, odour and vitality (Long et al, 1998). The menstrual period and child birthing history was an important part of the patient's history taken during this research. Past history regarding diseases and operations were also taken into account.

The Cun-kou Pulse (the area on the wrist over the radial artery) diagnosis was chosen. The pulse taken was followed by the divided methods, as Cun, Guan and Chi, which had been described by Wang Shuhe (Jin Dynasty). The nature of the pulse was differentiated during the Chinese Medicine consultation as the 28 Pulse. The 28 Pulse included a floating, deep, slow, rapid, weak, strong, slippery, uneven, long, short, surge, faint, tight, moderate, taut, hollow, tympanic, soggy, firm, feeble, scattered, thread, hidden, bouncing, running, knotted, regular intermittent and swift pulse (Long et al, 1998).

Tongue diagnosis implied the internal viscera and Meridian changes. The tongue observation included the tongue shape, coating, tongue colour and the locality change (Long et al, 1998). Tongue diagnosis could indicate Zang and Fu viscera functions and reflect disease in different aspects, such as the Eight Principle Syndrome Differentiation.

Both Liver Qi Stagnation and Liver Kidney Yin Deficiency patterns have seven main symptoms and three signs (Long et al, 1998). The score was set up 0 to 10 for each occurred symptom and zero for no occurred symptom. The scores for the signs were the same as the symptoms (see Table 3.5).

Table 3.5: The score of Chinese Medicine Patterns

Liver Qi Stagnation

unhappiness	anger or mood swings	abdominal bloating	stress	vivid dreams
0 - 10	0 - 10	0 - 10	0 - 10	0 – 10

menstrual irregularity	swollen tongue	bitter taste	wiry pulse	lower energy
0 - 10	0 - 10	0 - 10	0 - 10	0 – 10

Liver Kidney Yin Deficiency

lower back pain	tinnitus or hearing deterioration	hot flushes	knee weakness	night sweats
0 - 10	0 - 10	0 - 10	0 - 10	0 – 10

LQS = Liver Qi Stagnation; LKYD = Liver Kidney Yin Deficiency

Total score = 20 (LQS = 10; LKYD = 10)

The researcher who was a registered Chinese Medicine practitioner recorded the Chinese Medicine assessment.

3.4.4. Thermography

In this study, if the first results of thermography testing indicated a positive pathogenic change, the participant was referred to a GP. If a mammogram result was negative of any pathogenic change, the participant was able to remain in the research group where they were observed and a free consultation was offered. However, if the mammogram result was positive, a referral letter would be sent by the GP to an oncologist for a consultation, with a request to undertake an emergency intervention. This would mean that the participant would be excluded from the research group.

Thermography was conducted by a qualified thermographist, who had been trained at the American College of Clinical Thermology. The digital infrared camera with

computer software was supplied by Wellbeing Diagnostic Clinic, Melbourne. The breast thermography reading depended on the colour changing on the breast image, correlated to the alteration of the temperature in the breast area. The colour and temperature relationship was stated as per Table 3.6 (Blum & Farrier, 2003). Using Blum's colour scheme, the score was applied to analysse temperature elevation in the breast area (see Table 3.6).

Table 3.6: Score methods of Blum & Farrier's Thermography Criteria (Blum & Farrier, 2003)

Colour	T^{o}C	Score
White	38.5	10
Red/White	38.2	9
Red	37.9	8
Orange/Red	37.6	7
Orange	37.3	6
Yellow/Orange	37	5
Yellow	36.7	4
Green/Yellow	36.4	3
Green	35	2
Blue/Green	34.5	1
Blue	34	0

The criteria for thermography are continuing to develop. Most practitioners applied the following criteria when viewing the thermographic images; anarchic or complex vascular features; hyperthermic focal patterns that are greater than 1 to 3^{o}C in difference; asymmetric and abnormal complexity of a vascular pattern; asymmetric and abnormal physical contours of more than one quadrant of the breast area as well as other combinations of these thermography signs (Kennedy et al, 2009). In Table 3.7, the score for the signage of thermography was based on the Kennedy criteria. The score for each the criteria in the Kennedy method was from 0 or 1, the total score for both breasts were 10 (see Table 3.7).

Table 3.7: Score methods for the Kennedy's Thermography criteria

Score methods for thermography		
Items	**Left breast**	**Right breast**
Anarchic or complex vascular features	1	1
Hyperthermic focal patterns greater than 1 to 3^{o}C in difference	1	1
Asymmetric and abnormal complexity of a vascular pattern	1	1
Asymmetric and abnormal physical contours of more than one quadrant of the breast area	1	1
Any combination of these thermology signs	1	1

Note: 0 = not abnormal; 1 = abnormal sign;

Total score of abnormal thermography for both breasts= 10

3.4.5. SF-36 questionnaire

Another assessment of the KFE effect was the Short-Form 36-Item Health Survey (SF-36). This reviewed the participants' quality of life and its impact on their self-estimation of their own health component summary score. At the baseline and final visit, each of participants completed two copies of the SF-36 form which was used for an additional evaluation, regardless of their quality of life improving or not.

As discussed in the Literature Review in Chapter 2, SF-36 is a self estimation questionnaire which covers the participants' physical, mental and social functioning, vitality and general health. All the subscales of the SF-36 were independently associated with the Self Rated Health (SRH) method and reflected the holistic picture for the participants' general well being. All dimension scores represented by the mean of the item values were obtained. If the number of missing

values was more than half of the total, the score was declared missing and the data could not be used for the research. Dimension scores were standardized from 0 to 10 (Baumann, 2009). The mean of each subscale would be added to obtain the average of the mean, which was the final score for SF-36. Statistical methods were ANOVA, *t*-test which was used for the comparison between the SF-36 results to PEMF and the biomarker results of 2-OHE: 16α–OHE test.

3.5. Kiwi-Fruit Seaweed Extract and placebo

The participants took a total of 20g of powder per day, with the powder being either KFE or a placebo. This was taken in 10g dosage per twice a day and mixed with 100ml of warm water. The powder was administered one hour before breakfast and dinner over a one month period.

The selected Chinese therapeutic food was a wild resourced Chinese kiwi-fruit extract, Hong En No. 1 (also known as Hong En Health Drink) in a powder form and was provided by Professor Houen Xu of Beijing University (register number for health food, Wei Shi Jian Zi [1998] No. 331, P. R. China Health Ministry). Kiwi-fruit (Actinidia chinensis) and Seaweed (Herba Sargassii) extract (per 100g) contain energy 980KJ, protein 36g, fat 10g, carbohydrate 45g, sodium 30mg, potassium 20mg, fruit acids > 0.38g, zinc 0.08-0.3mg, 3-OH Isoflavones 400mg. The placebo was made at the food laboratory at Victoria University, and contained a powder of vitamin C, lactose and red tea. The colour and taste was similar to KFE and packed into the same bottle as the KFE container. A practitioner who was familiar with this type of clinical trial was invited as the third party to label the intervention and placebo containers. The powder was assigned to match the participants‘ codes and groups (i.e. A1, A2). Names were kept confidential. The practitioner was asked not to reveal relevant information to either researchers or participants until the final data analysis was completed. The participants were advised to report any adverse signs or symptoms to the Chinese Medicine practitioner.

In this study the participants should not have had a history of allergy to kiwi-fruit. The participants were informed of the possible allergic reactions caused by kiwi-fruit and if an allergic reaction occurred, the participant would be referred to a GP or to a hospital for further intervention. The initial procedure asked that the participants inform the researcher if they had any history of food allergies. This was followed up with an exclusion of the candidate/s with known allergies to participate in the research. However, if allergy symptoms appeared during the study, the researchers referred the participant for further medical intervention. and any participant with an allergic reaction was withdrawn from the research project.

A previous clinical trial (Xu, 1999) with 16 participants, who were diagnosed with breast cancer and had just completed chemotherapy, were given a KFE drink (equal to 20g of KFE powder) daily over a 7 day period. The results indicated that the rate of binucleate lymphocyte cells with micronuclei in these 16 participants had significantly decreased. This indicated the recovery of a chromosome change in the breast tissue (Xu, 1999). Therefore, the forty days of intervention period was considered a sufficient time for this trial.

3.6. Statistical analysis

All of the results were expressed as Mean ± Standard deviation. Experimental results of biomarker tests represented triplicate determinations for each group, intervention and control group. Samples were compared by *t*-test and a one-way ANOVA with comparisons based on Fisher's least square differences was used to determine significance at $P < 0.05$. Analyses were performed using SSPS software, version 11.0.

In a previous pilot study, six menopausal women were administered 100ml of KFE (=20g KFE powder per day) for 2 days. Their urine sample ratio of 2-OHE:16α–OHE, as a biomarker of breast cancer (Naik, 2006; Lukaczer, 2005; Lu, 2000; Xu, 1999) was elevated by the intake of KFE (Xu, 2004) (see Table 3.8). After a two day periodusing KFE, 16α-OHE had decreased significantly which

indicated thatfurther study was necessary. The sample size was decided by power analysis and the original sample size was 40 subjects to achieve 98% confidence. The power analysis was based on Xu's research, as shown in Table 2.2 (Xu, 2004). At the end of this research the sample size was 36 subjects and the power analysis result was at 88% confidence. The techniques which were used in this study were able to assist with the further development of the Chinese Medicine research.

CHAPTER 4

RESULTS

4.1. Overview

In this trial, the results of the biomarker urinary test for the ratio of 2−OHE: 16α−OHE were compared with the results of the magnetic tests, Chinese Medicine diagnosis patterns, thermography results and the results of self-assessment of general well-being.

Fifty six middle aged women expressed interest in participating in this research (see Table 4.1 and Figure 4.2). Thirty nine females were recruited as participants for this study at Victoria University according to the criteria approved by the Ethics Committee. Thirty six female Melbourne residents aged from 40 to 55 years completed the required two month study period. Of the 56 applicants, there were 73% that demonstrated Liver Qi Stagnation and 58% demonstrated Liver Kidney Yin Deficiency (see Table 4.1).

At the end of the research, there were eighteen participants in each group. The participants were aged 48.4 ± 3.97 in the KFE group and 48.5 ± 5.15 in the control group. There was no significant difference in the age between the two groups ($P > 0.05$) (see Figure 4.1).

Table 4.1: The number of applicants and Chinese Medicine patterns

Total applicants	LQS ****	LKYD ****	Enrolled participants	Completed research participants
56	41	33*	39	36**
	73%	58%	69%	64%***

Note: n = 56 (applicants)

* Number of applicants suffering from the CM patterns

** Number of the participants who completed the research

*** Percentage of the 56 applicants

**** LQS=Liver Qi Stagnation; LKYD= Liver Kidney Yin Deficiency

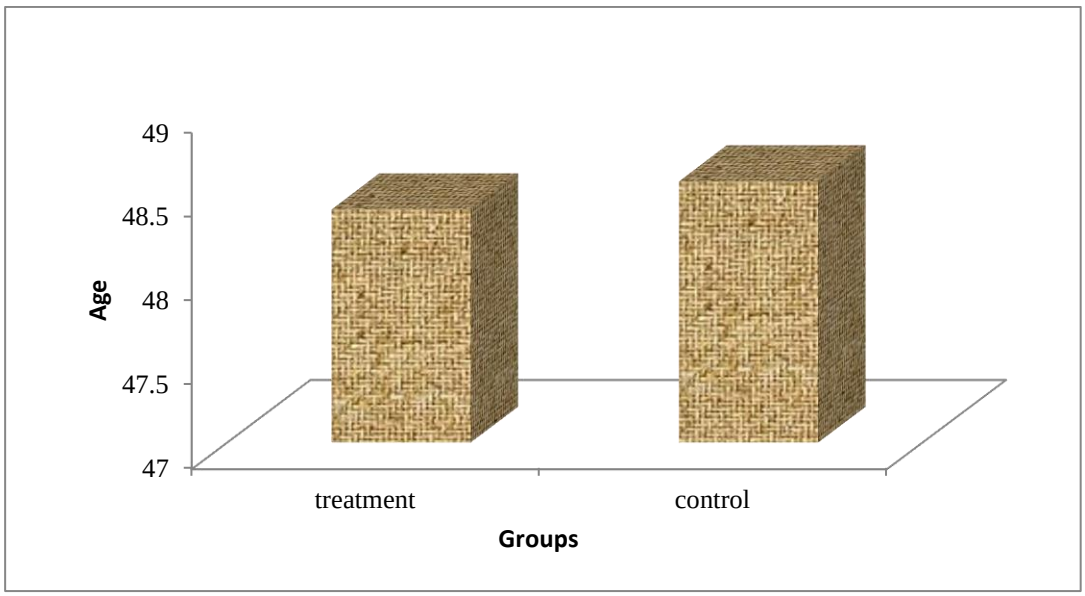


Figure 4.1: The comparison of the participants age between the KFE group and the control group

*Notes: P = 0.81; n = 36 (participants)

A total of 39 participants were reduced to 36 in the first ten days. The KFE group and the control group became 18 participants evenly (see Figure 4.2). No allergic incidents occurred during or after the study. There were eleven participants that completed pre and post thermography tests. Biomarker tests, Chinese Medicine diagnosis, magnetic tests and SF-36 questionnaires were completed by all of the 36 participants.

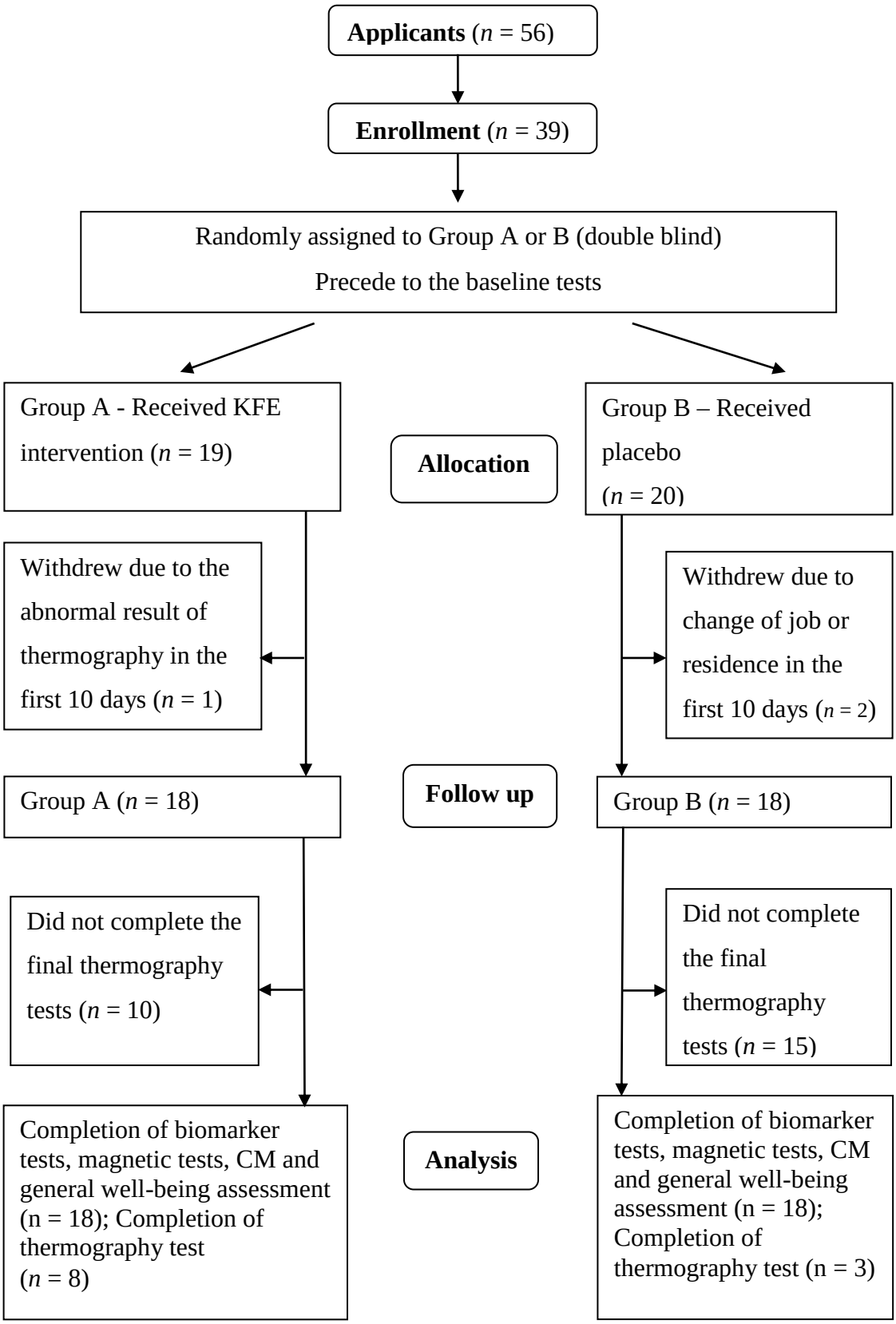


Figure 4.2: The flow chart of the participants during the research

4.2. Biomarker test result

The 2–OHE:16α–OHE was calculated by dividing the reading results of 2–OHE by that of 16 α–OHE from Palarstar Galaxy, Fluio32. The KFE group and the control group results were demonstrated in Table 4.2 and Table 4.3. The pre-trial results between the KFE group and the control group were not statistically significant. In the KFE group, the comparison of the pre-trial result to the post-trial results demonstrated significant improvement after administering KFE, $P < 0.05$. In the control group, the pre-trial and post-trial result saw no significant statistical change. The pre-trial and post-trial results in both groups are also illustrated in Figure 4.3.

Table 4.2: The pre-trial and the post-trial results of the urinary biomarker ratio in the KFE intervention and control groups

Groups	n	Pre-trial biomarker ratio	Post-trial biomarker ratio
Control	18	1.10 ± 0.32	1.01 ± 0.32***
Intervention*	18	0.91 ± 0.20**	1.42 ± 0.34

Notes: *Pre and post comparison in KFE group, P <0.05 (= 0.001), t = -6.2;

** Pre-trial comparison between KFE and control groups, P >0.05, t = 0.8

***Post-trial comparison between KFE and control groups,

P <0.05 (= 0.0035), t = 3.4¶

Table 4.3: Effects of Chinese Kiwi-Fruit Seaweed Extract on the biomarker results of peri-menopausal women

	Urinary test for 2OHE/16a-OHE					**Urinary test for 2OHE/16a-OHE**			
Intervention group					control group				
ID	1st sample	2nd sample	3rd sample	4th sample	**ID**	1st sample	2nd sample	3rd sample	4th sample
1	0.79	1.14	1.24	1.24	**1**	1.34	0.93	0.61	1.00
2	1.14	1.81	1.19	1.38	**2**	1.17	0.98	1.31	0.83
3	1.11	1.32	1.20	1.50	**3**	1.43	1.12	1.87	1.44
4	1.32	1.17	1.19	1.31	**4**	1.31	0.97	1.95	0.79
5	0.83	1.10	1.21	1.25	**5**	1.49	0.96	1.09	0.99
6	0.60	0.74	0.85	1.51	**6**	1.01	1.96	1.49	1.01
7	0.89	1.35	1.34	1.14	**7**	0.83	1.08	0.60	1.92
8	0.74	0.99	0.79	1.54	**8**	1.16	1.08	1.13	1.16
9	0.56	0.68	0.67	1.10	**9**	1.79	1.63	0.90	0.58
10	0.81	1.41	1.96	2.12	**10**	1.21	1.48	0.89	1.22
11	0.98	1.04	1.15	1.56	**11**	1.25	0.95	1.05	1.05
12	0.91	0.98	1.10	0.91	**12**	1.12	1.30	1.21	0.99
13	0.95	0.96	1.07	1.41	**13**	0.85	1.00	0.91	0.71
14	0.95	1.01	1.29	1.58	**14**	1.00	1.89	2.20	0.79
15	0.94	1.00	1.17	1.54	**15**	0.57	0.94	0.67	0.88
16	1.02	1.75	1.63	2.05	**16**	0.57	0.69	0.63	0.61
17	1.17	1.61	1.96	1.68	**17**	0.69	1.28	0.94	0.94
18	0.72	0.91	0.77	0.80	**18**	1.04	1.35	1.31	1.35
Mean	0.91*	1.16	1.21	1.42**	**Mean**	1.10***	1.20	1.15	1.01****
SD	0.20	0.32	0.36	0.34	**SD**	0.32	0.35	0.47	0.32

Note: *KFE group pre- trial 0.91 ± 0.20;

** KFE group post- trial 1.42 ± 0.34

***Control group pre-trial 1.10 ± 0.32;

****Control group post-trial 1.01 ± 0.32

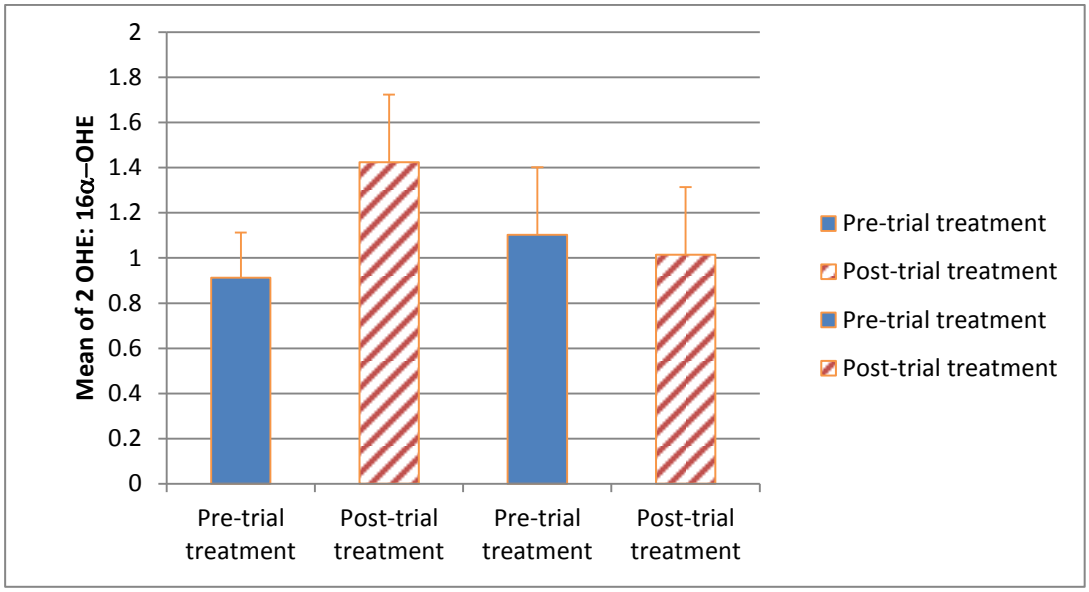


Figure 4.3: Comparison of the results of pre-trial and post-trial biomarker tests between the KFE group and the control group

Notes: *Pre and post comparison in KFE group, P <0.0001, t = -6.2;

Pre and post comparison in control group, P = >0.05, t = 0.8

** Pre-trial comparison between KFE and control groups, P >0.05, t = -1.9

***Post-trial comparison between KFE and control groups, P <0.05, t = 3.4

The biomarker results of 4 urinary samples were analysed for the KFE group and the control group. The pre-trial and post-trial results of the control group were not shown to be statistically significant. In contrast, the results of the KFE group in which the participants had been administrated KFE, were indicated the mean of 2 OHE:16α–OHE elevated (see Table 4.2 and Figure 4.4).

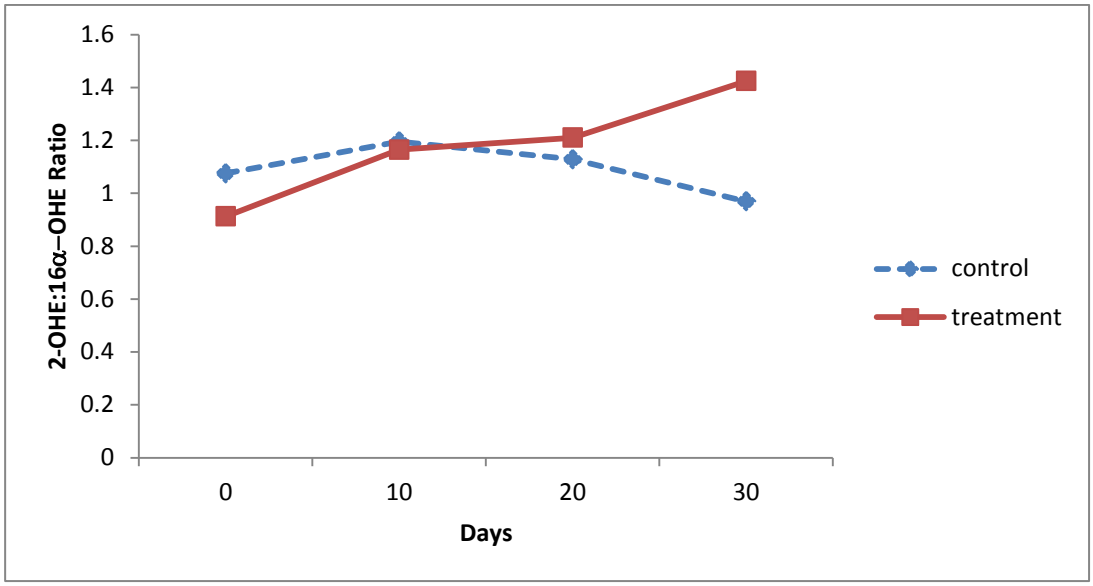


Figure 4.4: The comparison of the biomarker results between the KFE group and the control group

4.3. PEMF – Magnetic test result

A comparison was undertaken for the PEMF magnetic test results between the KFE group who took Chinese Kiwi-Fruit Seaweed Extract (KFE) and the PEMF magnetic test results of the control group who took a placebo. Before consuming the KFE, a pre-trial PEMF test was conducted and the results of the initial test indicated that the meridians of both the KFE group and the control group were blocked at similar score results, as there was no significant difference between the pre-trial comparisons in the two groups ($P > 0.05$).

In the pre-trial results of the PEMF magnetic test, the participants had different areas of the electromagnetic field change in the upper lateral quarter area of their breast: 26% of them had Liver Meridian stasis, 32% had Spleen Meridian stasis and 20% of the participants had Kidney Meridian stasis. The rest of the Meridians in the trunk area such as the Stomach, Gall Bladder, and Bladder Meridians were less than 11%; which were 4%, 7%, 10% meridian stasis reflected by the electromagnetic field change respectively. Their Liver Meridian and Spleen Meridian in the breast and flank areas were most affected. In pre-trial results, the

acupuncture meridians and the pulsed electromagnetic field changes are closely related to the pre-trail CM diagnostic patterns (see Table 4.1, Table 4.4, Figure 4.5 and Figure 4.6). In comparison with the results of the PEMF magnetic test with CM diagnostic patterns, the results of the CM diagnostic pattern showed that 73% had strong symptoms of Liver Qi Stagnation pattern, while 58% had a Liver and Kidney deficiency pattern. This indicates that Liver Qi Stagnation and Liver-Kidney Yin deficiency are the main patterns and that these percentages were higher than the percentage of other acupuncture meridians. The Spleen Qi deficiency is also an important pattern for peri-menopausal women but the symptoms were not significant in these research groups.

Table 4.4: The pre-trial results of the Meridian stasis in PEMF test

Liver	Kidney	Spleen	Stomach	Gall Bladder	Bladder
26%	20%	32%	4%	7%	10%

Note: n = 36

% = individual meridian blockage/ total sum of meridian blockage

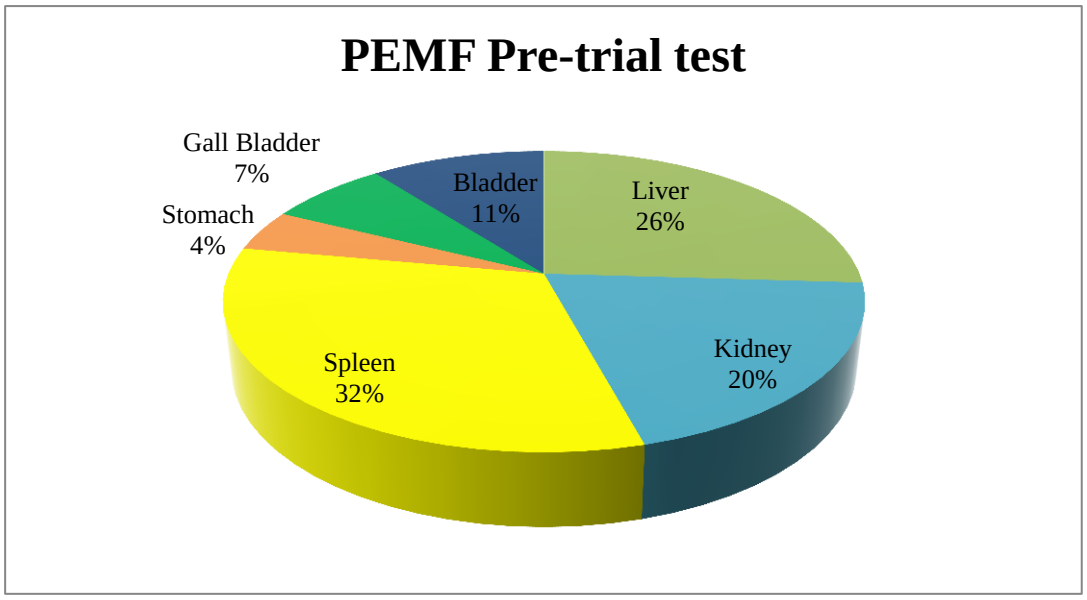


Figure 4.5: Pre-trial PEMF results – stasis in Meridians in thirty-six participants

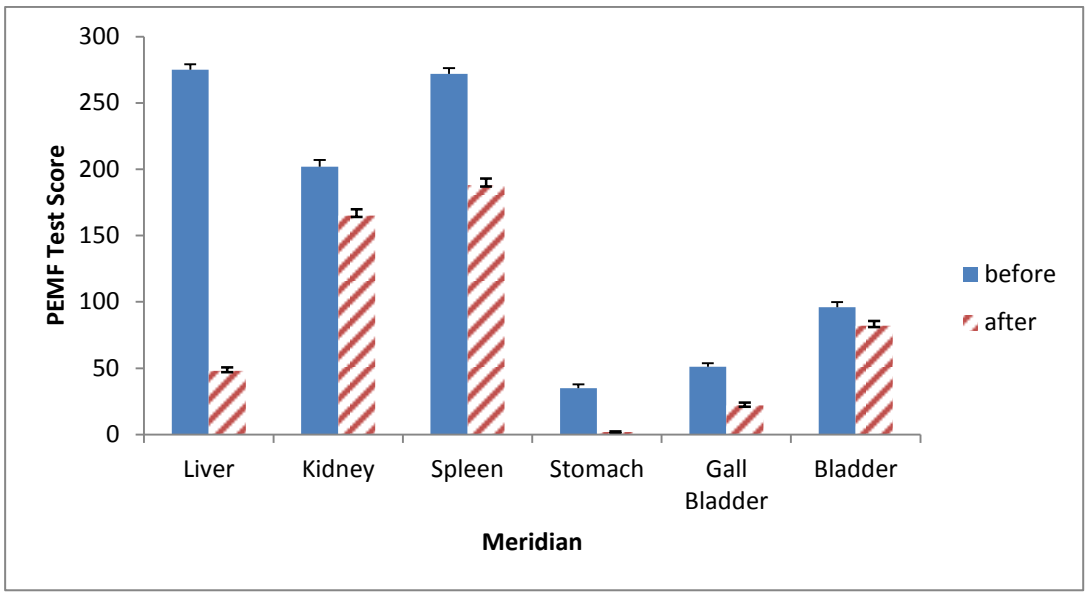


Figure 4.6: Pre-trial and Post-trial meridian stasis of PEMF tests in the KFE group

(n=18, full score of each pair of meridians in the group = 360)

The total score of the magnetic flux blockage in the acupuncture meridians of the KFE group is demonstrated in the y axis. 0= nil; 10= magnetic flux blocked in the whole meridians in trunk area (see Section 3.4.2.1). The full score of the magnetic flux blocked were 360 for a pair of meridians in 18 participants of KFE group, such as a pair of Liver Meridians or a pair of Kidney Meridians (see Table 4.5).

After the intervention, the results of the PEMF tests were significantly different between the KFE group and the control group. The data and the statistical results were different between the pre and post intervention on the Liver Meridian (see Table 4.5 and Table 4.6). This demonstrates that KFE can improve a Liver Meridian's function (see Table 4.7). It also indicated a reduction in the Kidney Meridian blockage. Conversely, the results of the pre and post intervention comparison on the Liver Meridian saw a slight change, but the Kidney Meridian indicated no change observed in the control group (see Table 4.8).

Table 4.5: Pre-trial and post-trial PEMF results comparison between the KFE group and the control group of peri-menopausal women (refer Appendix 4 Table 4)

	PEMF	Liver Meridian	Kidney Meridian
Control group	Pre-trial	6.11 ± 4.94	4.89 ± 4.86
	Post-trial	4.67 ± 4.95	4.89 ± 5.00
KFE group	Pre-trial	7.64 ± 4.18	5.61 ± 4.99
	Post-trial	1.33 ± 2.68	4.58 ± 4.88

The results of Table 4.6 indicate the pre-trial PEMF magnetic test results in the KFE group and the control group. The higher score in PEMF magnetic test results indicate more blockages existing in the tested areas. In this study, the polarized light was used to detect the magnetic field change near and along the acupuncture meridian. A highest score of 10 was given when the polarized light was dim over the whole lengh of the meridian in the trunk area. The opposite was a score of 0 indicating the polarized light was bright over the whole lengh of the meridian. The Liver and Kidney Meridians in the breast and trunk areas were mostly affected by the Qi and Blood Stagnation in the participants. The results also demonstrated that in those two meridians the blockages were reduced after using KFE. The average of the test results on both the left and right sides of the Liver and Kidney Meridians, in both of the intervention and the control group were analyzed (see Table 4.7 and Table 4.8).

Table 4.6: Pre-trial base line comparison between the age and PEMF test results (Mean ± SD) between the KFE group and control group of peri-menopausal women

Groups	**n**	**Age (years)**	**Liver Meridian PEMF test**	**Kidney Meridian PEMF test**
Control	18	48.50 ± 5.15	6.11 ± 4.94	4.89 ± 4.86
KFE	18	48.38 ± 3.97	7.64 ± 4.18	5.61 ± 4.99

Notes: The pre-trial results of PEMF magnetic test between KFE group and control group for Liver Meridian was P > 0.05, t= 1.4;
Kidney Meridian were P > 0.05, t= 0.8

Table 4.6 demonstrated that the initial test of PEMF for the KFE group and the control group did not show a substantial difference. After four weeks of intervention, the control group (see Table 4.7) did not indicate an improvement in the Liver Meridian (P > 0.05, t = 1.8). The participants in the KFE group presented a notable change, as the stagnation of the Liver Meridian was released (P < 0.0001, t = 8.864). In the Kidney Meridian, the PEMF magnetic test results of the KFE group were improved and were much more obvious than in the control group (see Table 4.8).

Table 4.7: Comparison of the pre-trial and the post-trial results of the PEMF tests in Liver Meridian

Groups	n	Pre-trial Liver Meridian PEMF test results	Post-trial Liver Meridian PEMF test results
Control	18	6.11 ± 4.94	4.67 ± 4.95***
KFE *	18	7.64 ± 4.18	1.33 ± 2.68**

Notes: *Participants take Chinese kiwi-fruit extract (Hong En No. 1) 10g X 2/day.
**Pre and post comparison in KFE group, P <0.0001, t= 8.86;
***Post-trial comparison between KFE and control groups, P <0.05, t=-3.2
control group pre-trial and post-trial comparison, P > 0.05, t= 1.8¶

Table 4.8: Comparison of the pre-trial and the post-trial results of PEMF tests in Kidney Meridian (Mean ± SD)

Groups	n	Pre-trial Kidney Meridian PEMF test	Post-trial Kidney Meridian PEMF test
Control	18	4.89 ± 4.86	4.89 ± 5.00***
KFE	18	5.61 ± 4.98*	4.25 ± 4.95**

Notes: * Compare the pre-trial result of Kidney Meridian in the KFE group with the control group, P > 0.05, t=0.8

** Compare the KFE group result, pre-trial and post-trial results of the Kidney Meridian, P >0.05, t=1.9

***Compare the pre-trial and post-trial results of Kidney Meridian in control group, P > 0.05, t=0.0

The results of the pre and post intervention comparison of the Kidney Meridian indicated a reduction of the Meridian blockage in the KFE group, while there was no change observed in the control group. The pre-trial and post-trial results in the KFE group did not see a significant change, P>0.05. However, the Liver Meridian blockage improved more than the Kidney Meridian as demonstrated in Figure 4.6. The function of the Liver Meridian and Kidney Meridian was improved by the use of KFE and also associated with changes in the biomarker 2-OHE:16α-OHE (Fu & Xu, 2011).

4.3.1. The comparison results between Liver and Kidney Meridians

The test results on both the left and right sides of the Liver and Kidney Meridians, in both of the intervention and the control groups were analyzed using *t-test* by SPSS 19. The KFE group results improved more significantly than the control group. The results of the pre and post intervention comparison on the Kidney Meridian indicated a reduction in the meridian blockage while there was no change observed in the control group. However, the Liver Meridian blockage improved more than the Kidney Meridian as demonstrated in Table 4.9 and Figure 4.8.

Table 4.9: Comparison of the pre-trial and post-trial PEMF results between Kidney Meridian and Liver Meridian in KFE group

KFE group	n	Pre-trial PEMF test results	Post-trial PEMF test results
Kidney Meridian Blockage	18	5.6 ± 4.98	4.25 ± 4.95**
Liver Meridian Blockage	18	7.63± 4.18	1.33 ± 2.68***

Notes: **Kidney Meridian pre-trial and post-trial PEMF results P >0.05, t= 1.9

***Liver Meridian pre-trial and post-trial PEMF results comparison was P<0.05, t=8.86

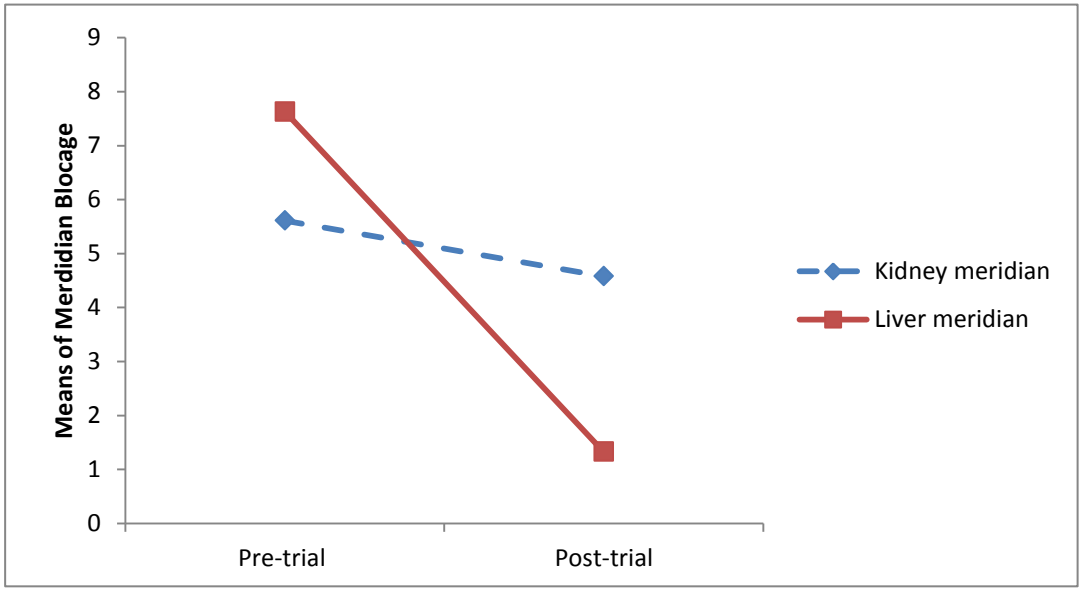


Figure 4.7: The pre-trial and post-trial PEMF magnetic test results between Liver Meridian and Kidney Meridian in the KFE group

4.3.2. The comparison results between the PEMF test and biomarker test

The following Table 4.10 indicated the magnetic and biomarker tests results both improved after KFE intervention. The PEMF test results demonstrated the Liver Meridian's blockage had reduced which is associated with an improvement in the

biomarker results at the end of KFE intervention. When the Liver Meridian stagnation showed reduced results of PEMF magnetic test, the ratio of the biomarker has risen in correlation, -0.47 (see Table 4.10).

Table 4.10: Comparison between Liver Meridian magnetic test and the biomarker urinary test results in the KFE group

Test	n	Pre-trial results	Post-trial results
Biomarker	18	0.91 ± 0.20	1.42 ± 0.34*
Liver Meridian Blockage	18	7.63± 4.18	1.33 ± 2.68**

Notes: * Comparison between biomarker pre-trial results and post-trial results $p < 0.05$, $t = 2.03$

**Comparison between Liver Meridian magnetic test pre-trial and post-trial results, $P < 0.05$. ($P = 0.000$), $t = 2.03$

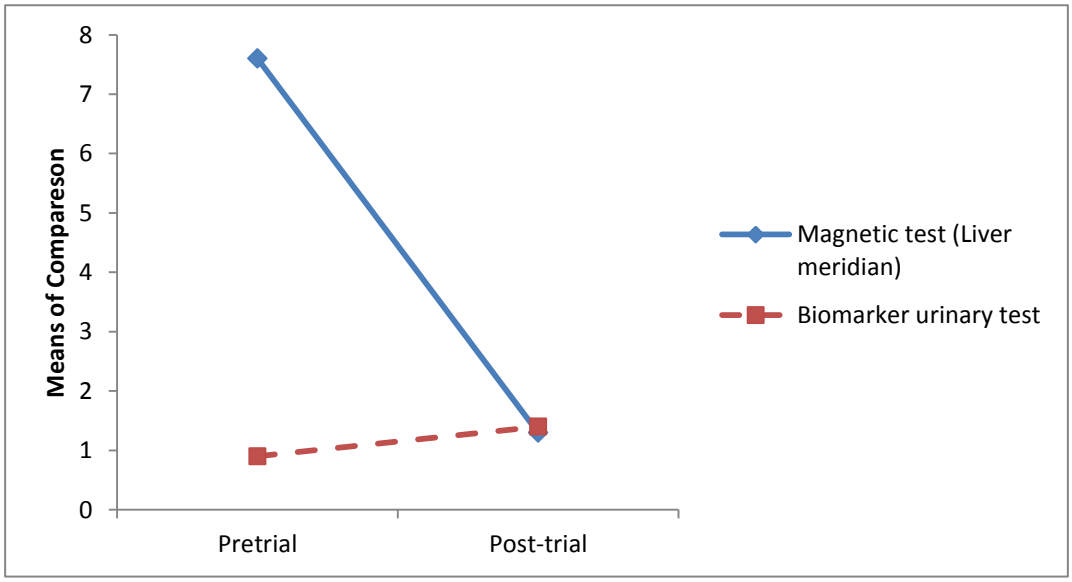


Figure 4.8: Comparison of the Liver Meridian PEMF result and the biomarker urinary test in the KFE group

4.3.3. Summary of the magnetic test results

A pre-trial base-line comparison of PEMF test results (mean ± SD) between the intervention and the control group of peri-menopausal women was not significantly different (P= 0.11) (see Table 4.6). After one month of intervention, the results for both meridians demonstrated that the Liver Meridian was significantly unblocked (Tables 4.7 to 4.9), whereas the control group had no improvement (see Table 4.7 and Table 4.8). In the Kidney Meridian, the results did not show the same degree of change as the Liver Meridian. In the KFE group, an improvement of the Kidney Meridian was indicated but the improvement was not statistically significant, P = 0.06, t=2.03 (see Table 4.8 and Table 4.9).

In this trial, the changes in the biomarker urinary test for the ratio of 2 OHE:16α–OHE matched the changes of the magnetic test results. When the Liver Meridian's condition improved, the Liver Qi Stagnation recovered and the urinary test ratio of 2OHE:16a-OHE was elevated. When comparing this outcome to the control group, the results for the ratio of 2 OHE:16α–OHE in the KFE group showed a substantial improvement.

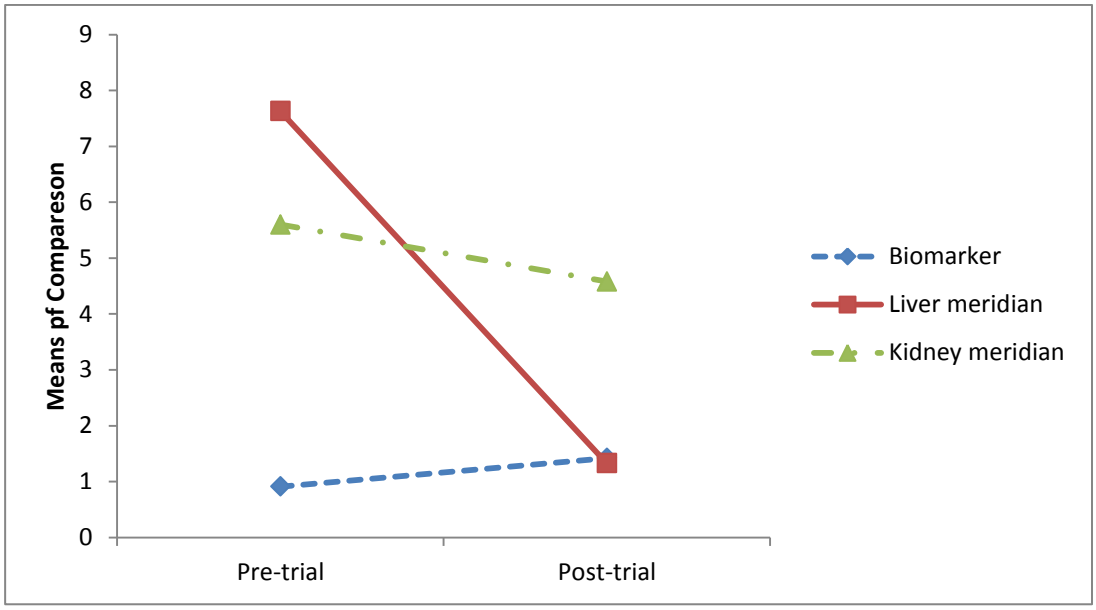


Figure 4.9: Comparison between the biomarker and the PEMF meridian results in the KFE group

4.4. Chinese Medicine diagnosis related to hormone disorders

4.4.1. Liver Qi Stagnation symptoms

The symptoms of Liver Qi Stagnation collected during this trial included unhappiness, mood swings, abdominal distension or discomfort, tiredness, vivid dreams, menstrual irregularities and a bitter taste in the mouth. The signs of Liver Qi Stagnation included a swollen tongue, a thin white tongue coating and a wiry pulse. The KFE group saw a significant improvement in these symptoms. However the symptoms and signs within the control group did not show a significant improvement (see Tables 4.11 and Table 4.12). Table 4.12 indicated the *t* test results of the participants in the control group and the KFE group. The symptoms of irregular menstrual period are very difficult to measure in peri-menopausal women.

As discussed in Chapter 3, subsection 3.4.3, both Liver Qi Stagnation and Liver Kidney Yin Deficiency patterns have seven main symptoms and three signs (Long, 1998). A score was set up as 10 for each occurring symptom or signs, 0 for no symptom or signs (see Table 3.5). In the KFE group before administering KFE,

there were 17 out of 18 participants who reported symptoms of unhappiness. After intervention, the symptoms of unhappiness in the KFE group were significantly reduced to 5 out of 18 participants. In contrast, the control group results were not significantly improved after the research period. Other symptoms in the KFE group were improved but to different levels. The signs of tongue coating in the KFE group did not change significantly by the end of the research but another sign, the pulse, showed significant improvement. The Liver Qi Stagnation in the KFE group and the control group was significantly different in the post-trial results. The improvement of the KFE group was statistically significant in the CM diagnostic patterns (see Table 4.12).

Table 4.11: Pre-trial and Post-trial comparison of the Liver Qi Stagnation Pattern

Liver Qi Stagnation symptoms	**KFE group**			**Control group**		
	pre-trial	**post-trial**	**P**	**pre-trial**	**post-trial**	**P**
Unhappiness	7.2±1.4	4.0±1.3	<0.0001	6.8±1.8	6.2±1.8	0.11
Anger or mood swings	8.2±1.0	4.6±1.8	<0.0001	7.8±1.1	7.5±1.0	0.33
Abdominal bloating	5.4±1.9	0.7±1.2	<0.0001	3.7±2.2	3.8±2.2	0.33
Stress	7.9±1.3	4.8±2.1	<0.0001	7.7±1.2	7.4±1.8	0.39
Vivid dreams	4.9±2.4	2.1±2.1	<0.0001	5.2±2.4	5.5±1.9	0.30
Irregular menstrual cycle	7.2±2.6	5.6±3.3	0.038	4.2±4.1	3.9±4.0	0.53
Swollen tongue	8.7±1.7	4.3±2.2	<0.0001	8.4±0.8	8.4±0.9	0.58
Lower energy	6.6±2.0	3.1±2.0	<0.0001	6.8±2.5	6.2±2.3	0.35
Wiry pulse	6.5±2.5	2.6±2.6	<0.0001	6.2±2.9	6.0±2.9	0.33
Bitter taste	2.4±3.3	0.5±0.2	0.008	2.2±3.7	2.5±3.6	0.40

Note: *n= 36 (KFE group =18; Placebo group= 18)

** Post-trial symptoms were worse than the pre-trial results

Table 4.12: Comparison of the pre-trial and the post-trial results of Liver Qi Stagnation (Mean±SD) in the KFE group and the control group

Groups	n	Pre-trial Liver Qi stagnation	Post-trial Liver Qi stagnation
Control	18	5.92 ± 0.75	5.74 ± 0.82**
Intervention *	18	6.50 ± 0.95	3.19 ± 0.98***

Notes: *Participants take Chinese kiwi-fruit extract (Hong En No. 1) 10g X 2/days
Pre-trial results comparison between the intervention and control group, P >0.05, t = 2.11
** Pre-trial and post-trial results comparison in the control group, P >0.05 (=1), t = 2.03
*** Pre-trial and post-trial results comparison in the KFE group, P >0.05 (=0.06), t = 2.03

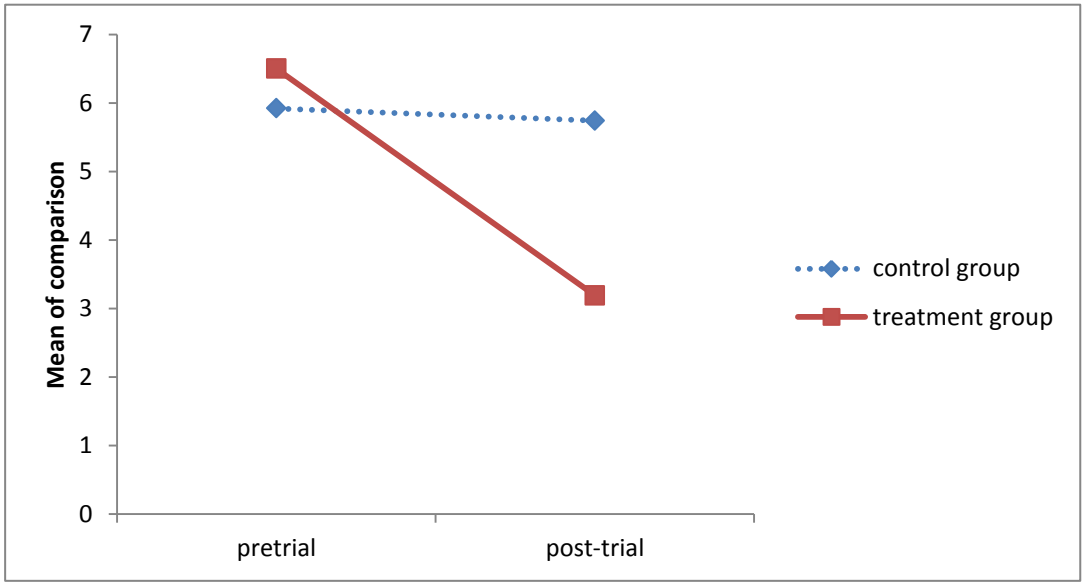


Figure 4.10: Comparison of the level of Liver Qi Stagnation between the KFE group and the control group

4.4.2 Comparison the results of the biomarker test and Chinese Medicine diagnosis pattern: Liver Qi Stagnation

A comparison of the KFE group results with the control group results showed that the biomarker urinary test outcome was related to Liver Qi Stagnation (LQS), correlation= -0.62. When the pattern of LQS was released or subsided, the urinary result demonstrated that 2OHE was raised and the ratio increased. Comparing the results of the LQS pattern and biomarker test for the 36 participants, the results also indicated $P < 0.05$. The KFE group results revealed that the effect of the urine test was determined by the increase in the ratio. The control group did not present significant results. These findings demonstrate that Liver Qi Stagnation can be related to a hormonal metabolism imbalance (see Table 4.13 and Table 4.14).

Table 4.13: Pre-trial and post-trial results between the biomarker and the Liver Qi Stagnation pattern in the KFE group

Test	n	Pre-trial results	Post-trial results
Biomarker	18	0.91 ± 0.20	1.42 ± 0.34*
Liver Qi Stagnation	18	6.50 ± 0.95	3.19 ± 0.98**

Notes:*Comparison of pre-trial and post-trial results in KFE group between biomarker, P<0.05 (=0.000), t= -6.21

**LQS results of the pre-trail and post-trial was $P < 0.05$, t=1.83. Refer to Table 4.12

The results of biomarker ratio and the score of Liver Qi Stagnation (LQS) indicated a reversed relationship (see Table 4.14). When the Liver Qi Stagnation symptoms are in recovery and the LQS score is reduced, the biomarker ratio of the post-trial test was elevated when compared with the pretrial results.

Table 4.14: Effects of Chinese Kiwi-Fruit Seaweed Extract on biomarker 2-OHE: 16α-OHE and Liver Qi Stagnation

Group	Urinary test for 2OHE/16α–OHE				Liver Qi Stagnation	
	1st sample	2nd sample	3rd sample	4th sample	pre-trial	post-trial
Control						
1	1.34	0.93	0.61	1.00	6.3	5.6
2	1.17	0.98	1.31	0.83	5.8	5.8
3	1.43	1.12	1.87	1.44	6.2	6.2
4	1.31	0.97	1.95	0.79	6.2	6.0
5	1.49	0.96	1.09	0.99	6.2	5.3
6	1.01	1.96	1.49	1.01	5.9	5.9
7	0.83	1.08	0.60	1.92	4.6	4.9
8	1.16	1.08	1.13	1.16	4.7	4.7
9	1.79	1.63	0.90	0.58	5.8	6.1
10	1.21	1.48	0.89	1.22	7.2	7.1
11	1.25	0.95	1.05	1.05	6.2	5.5
12	1.12	1.30	1.21	0.99	4.3	3.5
13	0.85	1.00	0.91	0.71	6.2	6
14	1.00	1.89	2.20	0.79	6.4	6.5
15	0.57	0.94	0.67	0.88	6.3	6.4
16	0.57	0.69	0.63	0.61	6.1	6.1
17	0.69	1.28	0.94	0.94	6.8	6.5
18	1.04	1.35	1.31	1.35	5.3	5.3
Mean ±SD	1.10 ± 0.32			1.01 ± 0.32	5.92 ± 0.75	5.74 ± 0.82
Intervention						
1	0.79	1.14	1.24	1.24	7.8	1.6
2	1.14	1.81	1.19	1.38	6.3	3.4
3	1.11	1.32	1.20	1.50	7.6	3.5
4	1.32	1.17	1.19	1.31	6.3	4.1
5	0.83	1.10	1.21	1.25	6.4	3.3
6	0.60	0.74	0.85	1.51	7.0	2.9
7	0.89	1.35	1.34	1.14	6.7	5.4
8	0.74	0.99	0.79	1.54	8.3	2.7
9	0.56	0.68	0.67	1.10	4.9	2.3
10	0.81	1.41	1.96	2.12	6.5	3.3
11	0.98	1.04	1.15	1.56	5.6	1.7
12	0.91	0.98	1.10	0.91	5.8	1.9
13	0.95	0.96	1.07	1.41	6.5	4.2
14	0.95	1.01	1.29	1.58	6.2	3.7
15	0.94	1.00	1.17	1.54	6.2	3.5
16	1.02	1.75	1.63	2.05	6.3	3.9
17	1.17	1.61	1.96	1.68	4.8	2.3
18	0.72	0.91	0.77	0.80	7.9	3.8
Mean ±SD	0.91 ± 0.20			1.42 ± 0.34	6.50 ± 0.95	3.19 ± 0.98

4.4.3. Liver Kidney Yin Deficiency symptoms

The collected symptoms for the Liver Kidney Yin Deficiency include lower back pain, knee weakness, hot flushes, tinnitus or hearing impairment, night sweats, scant irregular menses and a dry throat. The signs of Liver Kidney Yin Deficiency are red tongue, less coating, thready and rapid pulse. The score method for this was introduced in Chapter 3, which was the same as the LQS pattern scoring method.

The pre-trial results between the KFE group and the control group were not statistically significant ($P > 0.05$). The symptoms and signs of Liver Kidney Yin Deficiency showed a substantial improvement at the end of the KFE intervention ($P < 0.05$), but no improvement in the control group ($P > 0.05$). The mean of each individual symptom showed an improvement in the KFE group, such as lower back pain and a weakness in the knees (see Table 4.15). Other symptoms showing improvement were hearing deterioration or tinnitus and night sweats which was closely related to the vascular-nervous system regulation. The *t* test results of the group symptoms are demonstrated in Table 4.16.

Table 4.15: Pre-trial and post-trial results in Liver Kidney Yin Deficiency symptoms in the KFE group and the control group

Liver and Kidney Yin deficiency symptoms	KFE group pre-trial	KFE group post-trial	P	Control group pre-trial	Control group post-trial	P
Lower back pain	6.2 ± 2.9	1.6 ± 2.6	<0.0001	5.6 ± 2.5	5.2 ± 2.5	>0.05
Knee weakness	2.4 ± 2.9	0.6 ± 1.5	<0.05	3.4 ± 3.0	3.4 ± 3.0	>0.05
Hot flushes	3.4 ± 3.7	0.7 ± 2.1	<0.05	4.2 ± 3.9	4.2 ± 3.9	>0.05
Tinnitus, hearing impairment	2.8 ± 3.5	0.7 ± 1.7	<0.05	1.9 ± 3.0	2.0 ± 3.0	>0.05
Night sweats	2.7 ± 3.2	0.7 ± 1.6	<0.05	0.9 ± 2.4	0.9 ± 2.4	>0.05
Scant menses	5.1 ± 3.8	3.2 ± 3.7	>0.05 (=0.06)	3.3 ± 4.2	3.3 ± 4.2	>0.05
Red tongue	6.7 ± 2.6	2.8 ± 2.9	<0.05	7.3 ± 2.3	6.8 ± 2.8	>0.05
Less coating	3.5 ± 2.3	1.8 ± 2.2	<0.05	3.9 ± 2.5	4.4 ± 2.1	>0.05
Thready, rapid pulse	6.2 ± 2.6	5.1 ± 2.8	>0.05 (=0.06)	4.5 ± 3.0	5.1 ± 3.1	>0.05
Dry throat	2.7 ± 3.2	1.2 ± 2.6	>0.05 (=0.06)	3.9 ± 3.9	3.8 ± 3.7	>0.05

Notes: *n = 36 (KFE group n = 18; control group n = 18)

Table 4.16 Comparison between the pre-trial and the post-trial results of liver Kidney Yin Deficiency symptoms in the KFE group and the control groups

Groups	n	Pre-trial Liver Kidney Yin deficiency	Post-trial Liver Kidney Yin deficiency
Control	18	3.91 ± 1.47	3.91 ± 1.52***
KFE *	18	4.19 ± 1.42	1.85 ± 1.38**

Notes: * Comparison between the pre-trial results of the intervention and control group P = >0.05, t=0.69

** Comparison between the pre-trial and post-trial results of the KFE group P <0.0001 t=5.98

***Comparison to the pre-trial result and post-trial result of the control group P >0.05, t=- 0.11

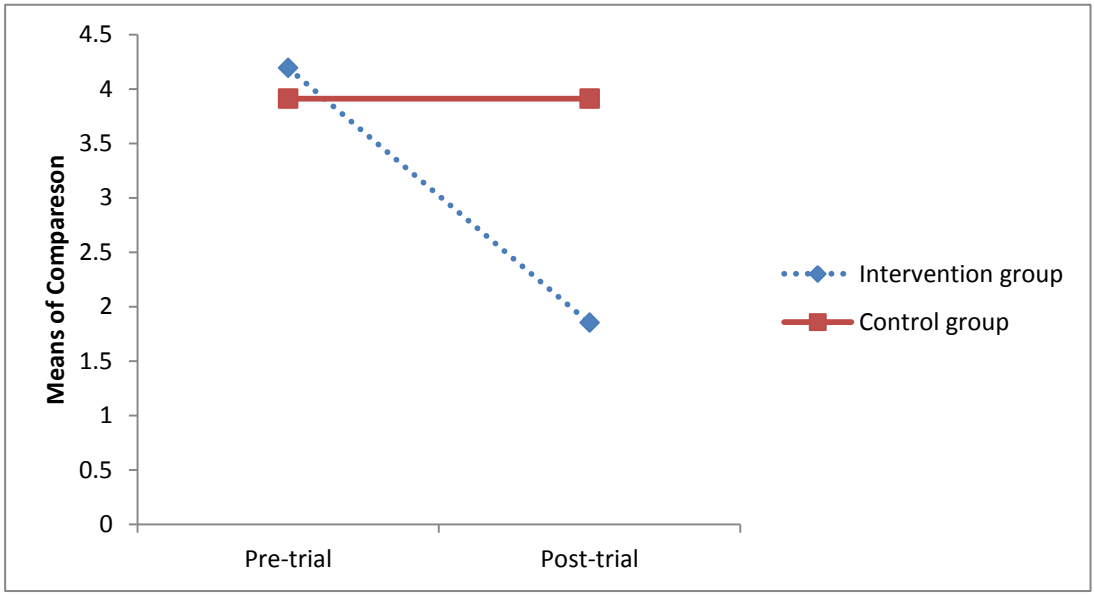


Figure 4.11: The comparison of the Liver Kidney Yin Deficiency in the KFE group and the control group

In Table 4.17 and Table 4.18, the means of CM diagnosis pattern in the KFE group and the control group were compared with the results of PEMF magnetic test. The

results of KFE group were significantly improved in Liver Meridian and Liver Qi Stagnation pattern. In comparison between pre-trial and post-trial results of PEMF Kidney meridian in KFE group, the result was not significant, P>0.05, t=1.90. In control group results, it was not significantly changed, either PEMF magnetic tests or CM diagnostic patterns.

Table 4.17: Comparison between CM diagnosis pattern and PEMF magnetic tests in KFE group

KFE group	n	Pre-trial	Post-trial
Liver Kidney Yin Deficiency	18	4.19 ± 1.41	1.85 ± 1.37
Liver Qi Stagnation	18	6.50 ± 0.95	3.19 ± 0.98
PEMF Liver meridian	18	7.63 ± 4.18	1.33 ± 2.68
PEMF Kidney meridian	18	5.61 ± 4.98	4.25 ± 4.95

Note: Comparison between pre-trial and post-trial results of Liver Kidney Yin Deficiency P<0.0001, t=5.98

Comparison between pre-trial and post-trial results of Liver Qi Stagnation P<0.0001, t=11.42

Comparison between pre-trial and post-trial results of PEMF Liver meridian P<0.0001, t=8.86

Comparison between pre-trial and post-trial results of PEMF Kidney meridian P>0.05, t=1.90

Table 4.18: Comparison between CM diagnosis patterns and PEMF magnetic tests in control group

Control group	**n**	Pre-trial	Post-trial
Liver Kidney Yin Deficiency	18	3.90 ± 1.47	3.91 ± 1.52
Liver Qi Stagnation	18	5.92 ± 0.75	5.74 ± 0.82
PEMF Liver meridian	18	6.11 ± 4.94	4.67 ± 4.94
PEMF Kidney meridian	18	4.89 ± 4.86	4.89 ± 5.00

Note: Comparison between pre-trial and post-trial results of Liver Kidney Yin Deficiency P>0.05, t=-0.11

Comparison between pre-trial and post-trial results of Liver Qi Stagnation P>0.05, t=1.99

Comparison between pre-trial and post-trial results of PEMF Liver meridian P>0.05, t=1.81

Comparison between pre-trial and post-trial results of PEMF Kidney meridian P>0.05, t=0.00

4.4.4. Chinese Medicine pulse and tongue signs

4.4.4.1. Pulse signs

After a one month intervention, the signs of pulse and tongue diagnosis in Chinese Medicine were improved except for the white coating which is exists in the Liver Qi Stagnation pattern and also in the normal tongue (see Table 4.19). The pulse and tongue signs saw improvement along with the Liver Qi Stagnation and Liver Kidney Yin Deficiency symptoms' recovery. The statistical results for the KFE group and the control group were not significant in the pre-trial score, $P > 0.05$. The pre-trial and post-trial results comparison in the KFE group were statistically significant, $P < 0.05$. In the control group, the pre-trial and post-trial results comparisons were not significant, $P > 0.05$ (see Table 4.20).

Table 4.19 demonstrates that the pulse results in the KFE group were significantly improved, but the results in the control group showed no improvement. The symptoms of both groups were different after intervention. As shown in Table 2 in Appendix the raw data was analysed by statistical methods, t test, and the static results as shown in Table 4.20.

Table 4.19: Pulse and Tongue diagnosis changes in the intervention and control group

	Control pretrial	Group post-trail	% recovery	Intervention pretrial	Group post-trial	% recovery
Swollen tongue	15	14	6.67	17	5	70.59
White coating	15	17	-13.33	17	17	0.00
Wiry pulse	15	16	-6.67	11	2	81.82
Red tongue	13	13	0.00	16	6	62.50
Less coating	3	4	-33.33	4	1	75.00
Thready, rapid pulse	3	3	0.00	6	3	50.00

Table 4.20: Comparison of the pre-trial and the post-trial results of pulse and tongue signs

Groups	**n**	**Pre-trial signs of pulse and tongue**	**Post-trial signs of pulse and tongue**
Control	18	10.67± 5.99	11.16± 6.11***
Intervention *	18	11.83± 5.77	5.67± 5.85**

Notes: *Participants take Chinese kiwi-fruit extract (Hong En No. 1) 10g X 2/days

**Pre and post comparison in the KFE group, P = 0.02, t = 2.57;

Pre-trial comparison between the intervention and control group, P = 0.33; t=1.08

***Post-trial comparison between the intervention and control group, P = 0.05; t=-2.43

In terms of pulse diagnosis, the signs of the pulse were improved after using KFE in that group. A comparison of the pre-trial and post-trial results in the KFE group indicates a significant statistical change in the pulse and tongue sign, P <0.05 (= 0.02). In the control group, the pre-trial and post-trial results did not change significantly (see Table 4.19 and Table 4.20).

4.4.4.2. Tongue diagnosis

At the beginning of the study, some participants' tongues showed swelling, a big tongue with teeth marks and a tongue with a red edge and a white coating. In the KFE group, the participants' tongue changed significantly compared with those in the control group. These results are demonstrated in Table 4.19.

4.4.5. Summary of the Chinese Medicine assessment results

The majority of the participants in the KFE group reported an improvement in their general well-being after taking KFE. The details included a decrease in hot flushes, mood swings, tiredness, abdominal distension and anger. However, these changes were not reported by the control group. There were no adverse effects noted by these participants.

Tables 4.11 and 4.12 have demonstrated the improvement of the Liver Qi Stagnation symptoms in pre-trial and post-trial.results Table 4.15 and 4.16 showed the recovery of the Liver Kidney Yin Deficiency symptoms before and after the trial. In the statistics analysis, the improvement of Liver Qi Stagnation (LQS) was stronger than the improvement of Liver and Kidney deficiency patterns (LKYD). Comparing the results of the KFE group to the control group, Liver Qi Stagnation

and Liver Kidney Yin Deficiency results in the KFE group had statistically significantly improved but not in the control group.

The results strongly indicate that the Chinese Medicine diagnosis patterns for Liver Qi Stagnation are related to female reproductive hormone metabolism. The changes of the pulse and tongue also demonstrate that the consumption of KFE can affect the pulse and tongue results positively and relate to the improvement of the Chinese Medicine diagnosis pattern.

4.5. Thermography results

Chinese Medicine theory states that after a long period of time, deficient heat accumulates as a result of Liver Qi Stagnation. This theory was reinforced by the results of breast thermography changes. In this research, the standards set for the thermography results applied to the following criteria which were illustrated by Kennedy and his colleague, 2009 (see Table 4.21).

The score demonstrated that the majority of the participants' maximum temperature of the breast was at least $1^{o}C$ above the average temperature of the same breasts. Signs of specific vascular patterns did not exist in the non-cancerous breast. The participant who was excluded from the research due to a suspicious cancer thermography image and was then referred to a GP and specialist, saw her breast result score 5 as the temperature had risen and asymmetric vesicular patterns appeared in the image of the thermography report. In reviewing the results of the thermography in the research, any participant who suffered from breast cancer would be scored at least 5 according to Kennedy and his colleague criteria (2009). The score for peri-menopausal female breast temperature, which indicated the difference between the maximum and the average temperature, was no more than a score of 2 in Kennedy and his colleague criteria (2009).

The score of the control group was not significantly different to the score of the KFE group in the initial image tests using Kennedy and his colleague (2009)

criteria (see Table 21). The mean of the breast temperature was 30.8^{o}C in the KFE group before the trial (see Table 4.22) and the temperature of the intitial thermography tests between the control group and KFE group were also not statistic significant (see Table 22). The results in Table 4.34 demonstrated that there was a statistically significant comparison between the results of the Chinese Medicine diagnosis as Liver Qi Stagnation and the temperature changes of thermography image in the pre-trial and post-trial tests. The Chinese Medicine diagnosis pattern Liver Qi Stagnation improved after the trial and, was most likely related to the regulation of the maximum focal temperature in the same breast. The high temperature area was decreased, with the gap between the maximum temperature and the mean of the breast temperature reduced (see Figure 4.13).

The pre-trial temperature value of thermography (maximum temperature minus the averege temperature in the same breast) compared with the results of Kennedy et al's thermography criteria, is another method to analyse thermography results. The P value is >0.05, which indicated that the pre-trial results of thermography are not statistically significant between the KFE group and the control group in both analysis methods.

The comparisons between the mean values of the thermography tests were not statistically significant in the pre-trial result between the KFE group and the control group (see Table 4.21). The mean values were indicated by the thermography readings for the left and right breast temperatures, which were recorded in Table 4.21. There was no statistical significance and P > 0.05. The majority (95%) of the results of the thermography mean in the participants were between 28.7 and 33^{o}C. One participant was excluded from the research because of suspicion of breast cancer as a result of the thermogram report. The mean of the participant's thermogram report was 34.4^{o}C and SD 0.42in addition to a special image of vascular pattern change if breast cancer existed.

Table 4.21: Applying Kennedy and his colleague (2009) criteria of thermography to the pre-trial results

Placebo group	score	KFE group	score
1	1	**1**	0
2	2	**2**	2
3	1	**3**	2
4	0	**4**	2
5	2	**5**	1
6	0	**6**	2
7	0	**7**	1
8	1	**8**	2
9	0	**9**	2
10	1	**10**	2
11	1	**11**	2
12	2	**12**	1
13	1	**13**	1
14	2	**14**	2
15	1	**15**	2
16	2	**16**	2
17	2	**17**	1
18	2	**18**	1
Mean	1.17± 0.78		1.56± 0.61

Note: Comparison between the intervention and control group; P >0.05; t= 1.51

Placebo group pre-trial thermography score (mean ± SD) = 1.17 ± 0.78

KFE group pre-trial thermography score (mean ± SD) = 1.56 ± 0.61;

Table 4.22: Comparison of the pre-trial temperature value of thermography between the KFE group and the control group

Groups	n	Pre-trial thermography
Control	18	31.21 ± 1.04
Intervention	18	30.81± 2.08

Note: P = >0.05 (0.34); t= 0.94

Table 4.23: Chinese Medicine diagnosis pattern and breast thermography

Participants ID KFE group	Liver Qi Stagnation Score	Liver and Kidney Yin deficiency Score	Left breast Temperature (^{0}C) (Mean ± SD)	Right breast Temperature (^{0}C) (Mean ± SD)
1	7.8	3.1	**33.20±0.22**	**33.20±0.23**
2	6.3	4.6	**32.00±0.47**	**31.60±0.90**
3	7.6	3.9	**25.70±0.94**	**27.20±1.27**
4	6.3	4.5	**27.30±0.77**	**28.30±0.52**
5	6.4	5.2	**31.50±0.32**	**32.10±0.30**
6	7	5.6	**31.00±0.53**	**31.00±0.53**
7	6.7	2.2	**31.20±0.40**	**31.00±0.35**
8	8.3	4.9	**31.30±0.46**	**31.10±0.51**
9	4.9	5.4	**27.20±0.94**	**28.40±0.51**
10	6.5	5.3	**30.30±0.43**	**30.20±0.51**
11	5.6	3.6	**31.30±0.44**	**31.20±0.62**
12	5.8	2.6	**31.60±0.26**	**31.90±0.32**
13	6.5	6.2	**30.60±0.32**	**31.40±0.31**
14	6.2	5.5	**30.70±0.37**	**31.50±0.29**
15	6.2	4.8	**33.30±0.50**	**33.80±0.35**
16	6.3	3.8	**27.40±0.45**	**28.30±0.45**
17	4.8	3.6	**31.50±0.75**	**32.20±0.45**
18	7.9	0.6	**34.10±0.24**	**33.70±0.31**
control group				
1	6.3	4.3	**30.90±0.32**	**31.10±0.42**
2	5.8	5.4	**30.30±0.34**	**29.90±0.42**
3	6.2	5	**31.60±0.46**	**30.70±0.54**
4	6.2	3.1	**31.70±0.31**	**31.90±0.29**
5	6.2	5	**31.60±0.34**	**32.20±0.27**
6	5.9	5.7	**30.80±0.29**	**31.00±0.23**
7	4.6	1.8	**32.50±0.25**	**32.30±0.30**
8	4.7	4.8	**29.80±0.40**	**29.80±0.47**
9	5.8	4.7	**31.80±0.36**	**31.90±0.30**
10	7.2	4.4	**30.80±0.45**	**30.20±0.39**
11	6.2	3.7	**29.70±0.26**	**30.30±0.26**
12	4.3	3	**29.60±0.42**	**29.30±0.41**
13	6.2	1.3	**31.60±0.46**	**30.70±0.54**
14	6.4	2.1	**30.60±0.29**	**30.70±0,49**
15	6.3	3.4	**31.00±0.32**	**31.40±0.26**
16	6.1	4.7	**32.40±0.27**	**33.00±0.29**
17	6.8	6.2	**33.00±0.46**	**33.20±0.35**
18	5.3	1.7	**32.00±0.45**	**32.10±0.37**

In this research, an analysis of the thermography results was applied directly to the colour scheme, using the Blum & Farrier's method (2003), which is a colour scheme to monitor the temperature change in the breast area. The results demonstrated that the temperature of the upper lateral quadrant area of the breast was higher than the mean temperature of the same breast (see Table 4.24).

Table 4.24: Blum & Farrier, (2003) colour scheme relating to temperature changes in thermography

Colour	Temperature Celsius
White	Above 38.5^{o}C
Red	38 to 38.4^{o}C
Orange	37 to 37.7^{o}C
Yellow	36.7 to 37^{o}C
Green	34 to 36.5^{o}C
Blue	<34^{o}C

The analysis of the KFE group results in Table 4.25 showed that the lateral upper area had the highest temperature when compared to the remaining area of the same breast. The second highest temperature area was the medial upper area, with the following higher temperature area being the lateral lower area. The lowest temperature area in the breast was the medial lower area. The means of the temperature results for the thirty-six participants in the KFE group are shown in Table 4.25 and Figure 4.12.

Table 4.25: Higher temperature (> 36.7°C) location in the Left and Right breast of 36 participants' pre-trial results (Blum & Farrier's, 2003)

Lateral superior	Lateral inferior	Areola nipple	Medial superior	Medial inferior
51	21	11	40	18
36%	15%	8%	28%	13%

Note: total n = 72 breasts (36 participants)

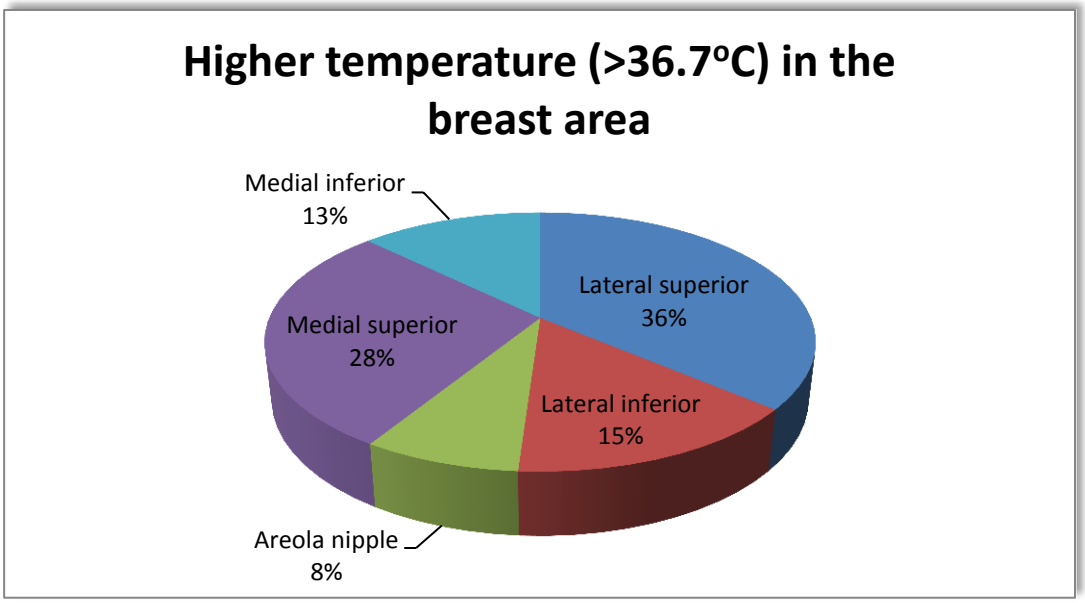


Figure 4.12: The lateral superior area of the breast which had a higher occurrence of thermography change

The use of Blum & Farrier's (2003) colour scheme saw the breast temperature valued and recorded in Table 4.26. The mean was accomplished by t-test and was compared with the pre-trial and post-trial results in the intervention and control group. There were eleven cases which completed two thermography tests (see Table 4.26 and Table 4.27).

Table 4.26: The pre-trial results of the eleven cases of thermography changes (Blum & Farrier, 2003)

	Left breast				Right breast			
ID	Upper lateral	Upper medial	Lower lateral	Lower medial	Upper lateral	Upper medial	Lower lateral	Lower medial
3	6	5	0.5	0	6	5	0.5	0
7	3	5	2	1.5	7	6.5	3	3
8	6	2.5	6	1	6	2	3	1
10	5	3.5	2	2	6.5	3.5	2	2
12	3.5	2	2	2	4.5	2	2	2
14	4.5	3.5	2	1	5.5	3.5	2	2
15	5.5	4.5	2.5	3	7.5	5.5	3.5	3
18	5	5.5	4.5	3.5	4.5	4.5	4.5	3
23**	3.5	2	2	2	6.5	5.5	3.5	2
29**	5	3	3	2.5	7.5	5.5	7	5
30**	5.5	2	2	2	3	2	2	2

Note: ** Three participants in control group

The scoring method was applied according to Blum & Farrier's (2003) colour scheme.

Table 4.27: Pre-trial thermography of eight participants in the KFE group: The mean of Blum & Farrier's score in the left and right breasts

KFE groups	n	Lateral	Medial
upper	8	5.37 ± 1.20	4.00 ± 1.40**
lower	8	2.46± 1.28	1.87 ± 1.07***

Note: n=8

Table 4.28: Post-trial results of thermography for eleven participants

	Left breast				Right breast			
ID	Upper lateral	Upper medial	Lower lateral	Lower medial	Upper lateral	Upper medial	Lower lateral	Lower medial
3	6	5	1.5	1.5	6	4.5	1.5	1
7	3	3	2	2	7	5	3	4.5
8	4.5	5	3	1	6.5	5	5	1
10	4.5	4	3.5	3	5.5	5.5	2	5.5
12	2.5	1.5	1.5	2	4	2	2	1.5
14	4.5	4	3	2	5	3	3	2
15	5	2.5	2	2	5.5	3	2	3
18	4.5	5	5	4.5	3.5	4.5	4.5	3
23**	6.5	6	2	2.5	7	7	5.5	3.5
29**	5	3	3	2.5	7	3	5.5	2.5
30**	5.5	2.5	3	2	5	2.5	2	2

Note: ** Three participants in control group

The scoring method was applied according to Blum & Farrier's (2003) colour scheme.

Table 4.29: Post-trial thermography of eight participants in the KFE group: The higher temperature in the breast area (Blum & Farrier, 2003)

KFE groups	n	Lateral	Medial
Upper	8	4.87 ± 1.28	3.90 ± 1.24
Lower	8	2.84± 1.33	2.46 ± 1.35

Note: the t tests have been processed after the reading of Blum & Farrier's colour scheme

The post-trial results in the KFE group are shown in Tables 4.28 and 4.29. The results indicate that the temperature became more harmonized in the breast after KFE intervention. The higher temperature in the lateral upper and the medial upper area was reduced. Simultaneously, the lower temperature in the inferior lateral and

the inferior medial areas were elevated slightly within the normal range in the KFE group. The temperature regulation in the breast area did not occur in the control group (see Figure 4.13 and Figure 4.14). This may indicate Qi and Blood circulation regulation as a result of the effect of KFE according to Chinese Medicine.

Table 4.30: Pre-trial and Post-trial thermography (mean) of eight participants in the KFE group (Blum & Farrier, 2003)

	Superior Lateral	Superior Medial	Inferior Lateral	Inferior Medial
Pretrial	5.37	4.00	2.46	1.87
Post-trial	4.87*	3.90**	2.84***	2.46****

Note: n=16 (8 participants)

* P= <0.05 t=2.82 *** P= >0.05 t=-1.07

** P= >0.05 t=0.24 **** P= <0.05 t=-2.25

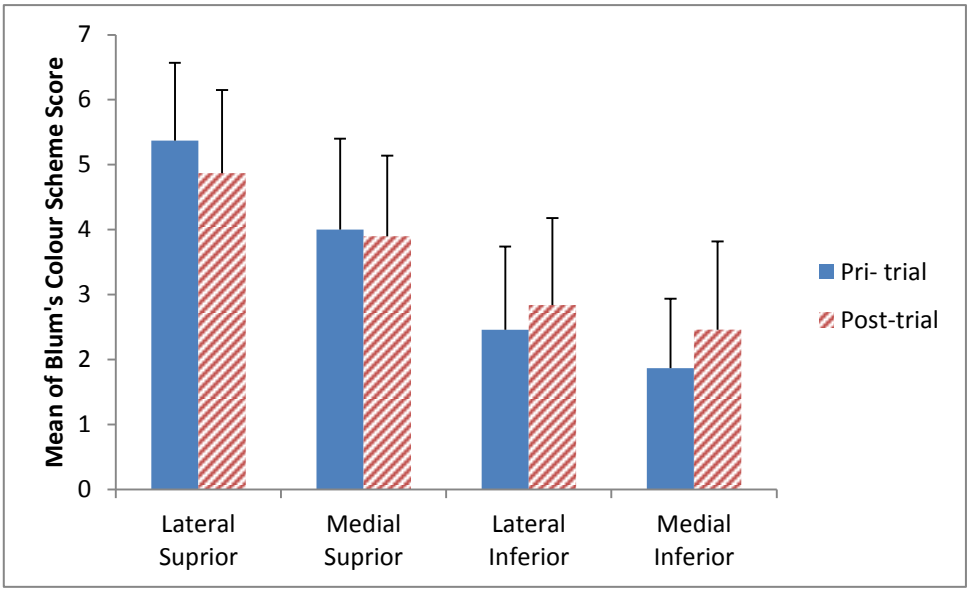


Figure 4.13: Comparison between the pre-trial and post-trial results of the breast thermography in the KFE group

Table 4.31: Pre-trial and Post-trial thermography (mean) of three participants in the control group (Blum & Farrier, 2003)

	Superior Lateral	Superior Medial	Inferior Lateral	Inferior Medial
Pre trial	5.17	3.33	3.25	2.58
Post-trial	6.00*	4.00**	3.50***	2.50****

Note: n=3

The scoring method was according to Blum & Farrier (2003) colour scheme.

* P= >0.05 t=1.49

** P= >0.05 t=0.77

*** P= >0.05 t=0.52

**** P= >0.05 t=-0.15

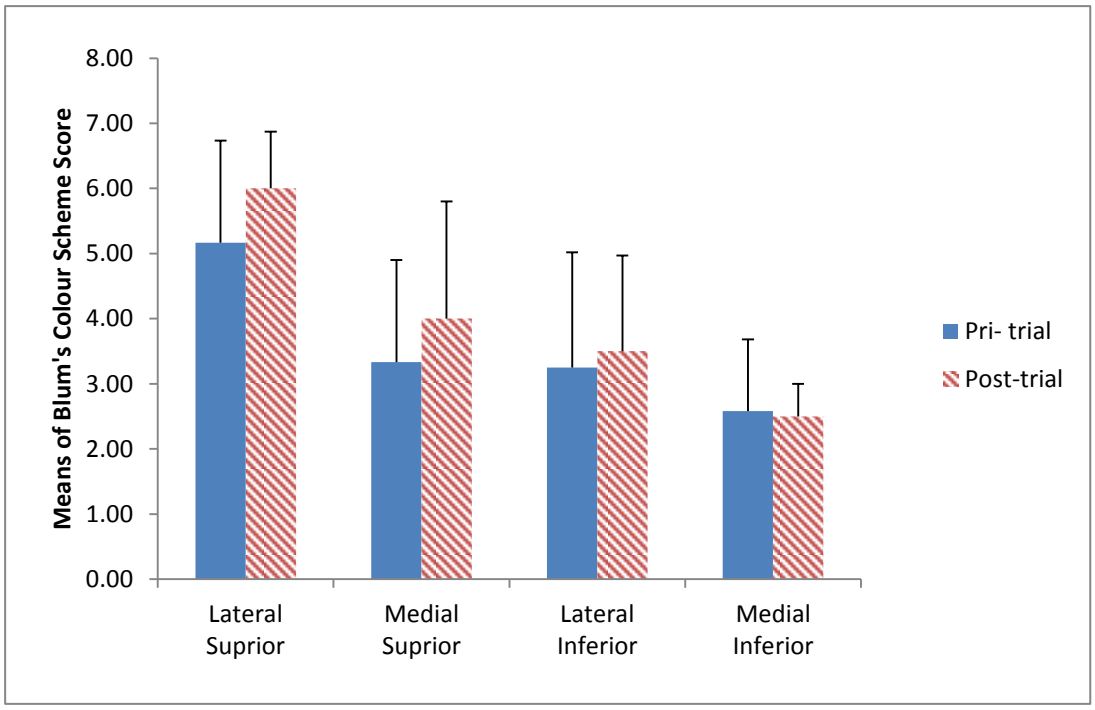


Figure 4.14: Comparison between the pre-trial and post-trial results of the breast thermography in the control group

Table 4.32: The pre-trial and post-trial results of the eleven cases of thermography changes

Breast	Pre-trial left maximum-average	Post-trial left maximum-average	Pre-trial temperature minus post-trial temperature	Pre-trial right maximum-average	Post-trial right maximum-average	Pre-trial temperature minus post-trial temperature
KFE group	2.7	2.2	0.50	2.6	2.1	0.50
	0.9	1	-0.10	1	1.5	-0.50
	1.4	1.1	0.30	1.9	1.5	0.40
	1	1.3	-0.30	1.6	1.1	0.50
	0.7	0.8	-0.10	1.1	1	0.10
	1.4	0.9	0.50	1.2	0.7	0.50
	1.1	0.7	0.40	1.1	1.1	0.00
	0.6	0.8	-0.20	1	0.8	0.20
Total decrease Temperature			1.00			1.70
Control group	1.3	1.3	0.00	1	1	0.00
	1.1	1.4	-0.30	0.7	1.1	-0.40
	1	0.7	0.30	1.1	1	0.10
Total decrease temperature			0.00			-0.30

In Table 4.32, the figures are the computerized results of thermographic image which were obtained by the maxmum temperature minus the average temperature in the same breast. The results of the eleven participants in Table 4.32 were also indicated the similar outcome by the Blum & Farrier's colour scheme. The results of KFE group are statistic significant but not the control group (Refer to Table 4.30 and Table 4.31).

Table 4.33: Liver Qi Stagnation patterns and the maximum temperature of breast thermography

	Participants ID	Pre-trial Liver Qi Stagnation	Post-trial Liver Qi Stagnation	Pre-trial left breast Maximum average	Thermo-graphy right breast Maximum-average	Post-trial left breast Maximum-average	Thermo-graphy right breast Maximum-average
Control	23	6.2	5.3	1.3	1	1.3	1
group	29	6.2	5.5	1.1	0.7	1.4	1.1
	30	4.3	3.5	1	1.1	0.7	1
Mean±SD		5.6± 1.09	4.8±1.1	1.03± 0.19		1.08 ± 0.25	
Intervention	3	7.6	3.5	2.7	2.6	2.2	2.1
group	7	6.7	5.4	0.9	1	1	1.5
	8	8.3	2.7	1.4	1.9	1.1	1.5
	10	6.5	3.3	1	1.6	1.3	1.1
	12	5.8	1.9	0.7	1.1	0.8	1
	14	6.2	3.7	1.4	1.2	0.9	0.7
	15	6.2	3.5	1.1	1.1	0.7	1.1
	18	7.9	3.8	0.6	1	0.8	0.8
Mean±SD		6.50 ± 0.95	3.19 ± 0.98	1.33± 0.61		1.16 ± 0.45	

Table 4.34: Comparison between Liver Qi Stagnation and thermography in the KFE group

	n	Pre-trial result	Post-trial result
LQS	8	6.50 ± 0.95	3.19 ± 0.98
Thermography	8	1.33± 0.61	1.16 ± 0.45

Note: Comparing the result of LQS and thermography in the 8 participants left and right breasts, P = <0.0001

The symptoms of Chinese Medicine diagnostic pattern, Liver Qi Stagnation, subsided after the KFE intervention. The maximum temperature of the thermography were also decreased slightly (Table 4.34).

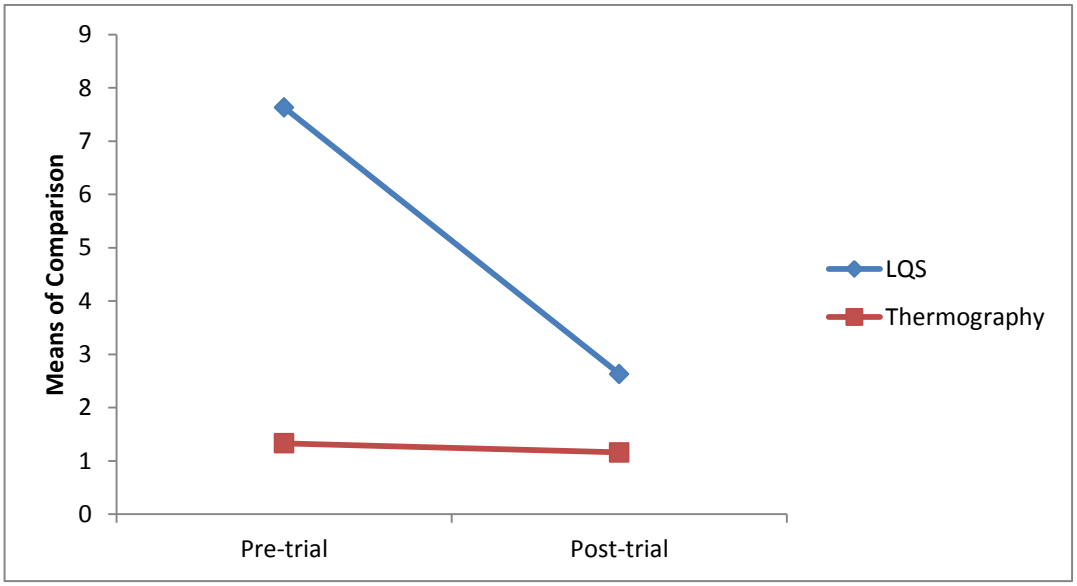


Figure 4.15: Comparison of the pre-trial and post-trial results of Liver Qi Stagnation and the temperature (means) in the KFE group

Table 4.35: Comparison between pre-trial and post-trial thermography results in the KFE group and control groups

Groups	n	Pre-trial temperature of thermography	Post-trial temperature of thermography
Control	3	1.03± 0.19	1.08 ± 0.25***
Intervention *	8	1.33± 0.61	1.16 ± 0.45 **

Note: Compared pre-trial result and post-trial result in KFE group, P < 0.05**; t=2.03

Compared pre-trial result and post-trial result in control group, P > 0.05***; t=-0.47

Before taking KFE or the placebo, the maximum temperature of the breast thermography results minus the mean temperature of the breast thermography results are demonstrated in Table 4.22 and the results of the temperature differences were analysed by t-test. The mean of the t-test indicated in Table 4.22 showed no statistical significance between the two groups. Comparing the pre-trial results of thermography to the post-trial results in Table 4.35, the maximum temperature had reduced in the KFE group with a statistically significant change ($P = 0.03$). In the control group, the maximum temperature remained the same (see Table 4.35). However, the sample size was small and further investigation would be needed.

The results of the Liver Qi Stagnation and the thermography changes demonstrated that both were $P < 0.05$(see Table 4.34). When the Liver Qi Stagnation reduced and the participants' general wellbeing improved, the maximum temperature of breast thermography decreased and the gap between the maximum temperature and the average temperature were reduced. The LKYD pattern had a significant improvement in the symptoms and matched to the maximum temperature change of the thermography results in the KFE group (see Table 4.37)

Table 4.36: Pre-trial and post-trial results between Liver Kidney Yin Deficiency pattern and the thermography maximum temperature in the KFE group and control groups

	Participants ID	Pre-trial LKYD pattern	Post-trial LKYD pattern	Pre-trial left breast Maximum-average	Thermography right breast Maximum-average	Post-trial left breast Maximum-average	Thermography right breast Maximum-average
Control	23**	5	4.9	1.3	1	1.3	1
group	29**	3.7	4.2	1.1	0.7	1.4	1.1
	30**	3	2.9	1	1.1	0.7	1
Mean± SD		3.90± 1.01	4.00± 1.0	1.03± 0.19		1.08 ± 0.25	
Intervention	3	3.9	2.4	2.7	2.6	2.2	2.1
group	7	2.2	4.2	0.9	1	1	1.5
	8	4.9	2.3	1.4	1.9	1.1	1.5
	10	5.3	1.5	1	1.6	1.3	1.1
	12	2.6	0.6	0.7	1.1	0.8	1
	14	5.5	1.8	1.4	1.2	0.9	0.7
	15	4.8	1	1.1	1.1	0.7	1.1
	18	0.6	0.6	0.6	1	0.8	0.8
Mean± SD		3.08± 1.75	1.43± 1.1	1.33± 0.61		1.16 ± 0.45	

Note: In the KFE group pre-trial thermography mean and SD 1.33± 0.61; Post-trial thermography mean and SD 1.16 ± 0.45; P <0.05 (P =0.03)

In the control group, pre-trial thermography mean and SD 1.03± 0.19. Post-trial thermography mean and SD 1.08 ± 0.25; P > 0.05 (0.66)

Table 4.37: Pre-trial and post-trial results between Liver Kidney Yin Deficiency pattern and thermography maximum temperatures in the KFE group

KFE groups	n	Pre-trial	Post-trial
Thermography	8	1.33± 0.61	1.16 ± 0.45*
LKYD	8	3.08± 1.75	1.43± 1.19**

Note: Thermography pre-trial and post-trial results, P < 0.05; t= 2.03

LKYD pre-trial and post-trial results, P < 0.05; t= 7.27

Table 4.38: Pre-trial and post-trial results between Liver Kidney Yin Deficiency pattern and the thermography maximum temperature in the control group

Control Groups	n	Pre-trial	Post-trial
Thermography	3	1.03± 0.19	1.08 ± 0.25**
LKYD	3	3.90± 1.01	4.00± 1.01***

Note: Thermography in 3 control participants pre-trial and post-trial results, P >0.05**

LKYD in 3 control participants, pre-trial and post-trial results is P>0.05, t=-0.5***

Results of the Liver Kidney Yin Deficiency were compared with the breast thermography maximum temperature changes (see Table 4.37). There were no statistically significant changes in the thermography and LKYD results of the control group. The P value in the KFE group was < 0.05 and the control group was > 0.05 (see Table 4.38).

4.6. Self Rated Health (SRH) method for general health, physical and social abilities

In Chapter 2, SF-36 was described as the most frequently used Self Rated Health (SRH) method. As the methodology in Chapter 3 states, SF-36 is a self assessment questionnaire completed by the participants. The average of the total means of all subscales reflected the overall picture of the participants‘ general well being under their self assessment. The pre-trial and post-trial results of SF-36 test showed an improvement in the KFE group but not in the control group (see Table 4.39 and Table 40; Figure 4.16 and Figure 17).

Table 4.39: The pre-trial and post-trial SF36 score in the KFE group

	General condition	Physical condition	Emotional change	Social life	Pain score	Energy and emotions
Pre-trial	8.21 ± 1.15	6.94 ± 1.55	6.93 ± 1.46	7.00 ± 2.65	7.78 ± 1.73	6.38 ± 1.39
Post-trial	8.59 ± 0.94	7.50 ± 1.29	7.59 ± 1.28	7.94 ± 1.89	7.61 ± 1.80	6.88 ± 1.19

Note: $P < 0.05$ for emotional, energy and emotions

$P > 0.05$ for general, physical and social

$P = 1$ for pain

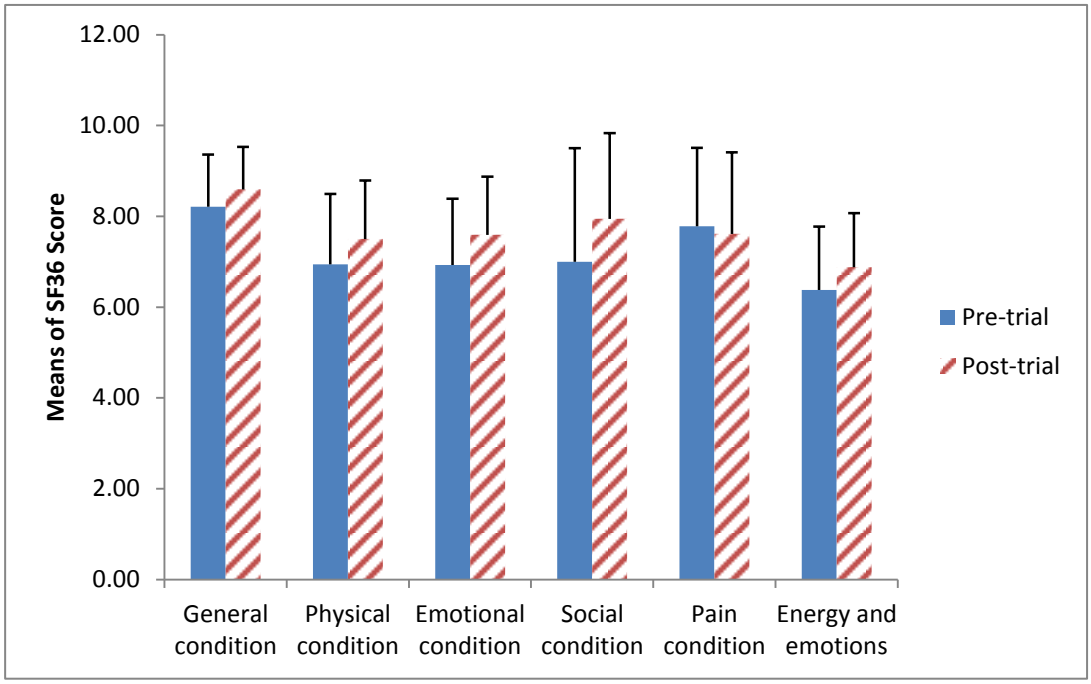


Figure 4.16: Comparison between pre-trial and post-trial SF-36 results in the KFE group

Table 4.40: The pre-trial and post-trial SF36 results in the control group

	General condition	Physical condition	Emotional change	Social life	Pain score	Energy and emotions
Pre-trial	7.81 ±1.25	7.33 ±1.64	7.07 ±1.76	7.67 ±2.22	7.00 ±1.97	6.71 ±1.71
Post-trial	7.66 ±1.25	7.42 ±1.30	7.03 ±1.44	7.50 ±1.82	7.33 ±1.61	6.56 ±1.60

Note: P > 0.05 for all items

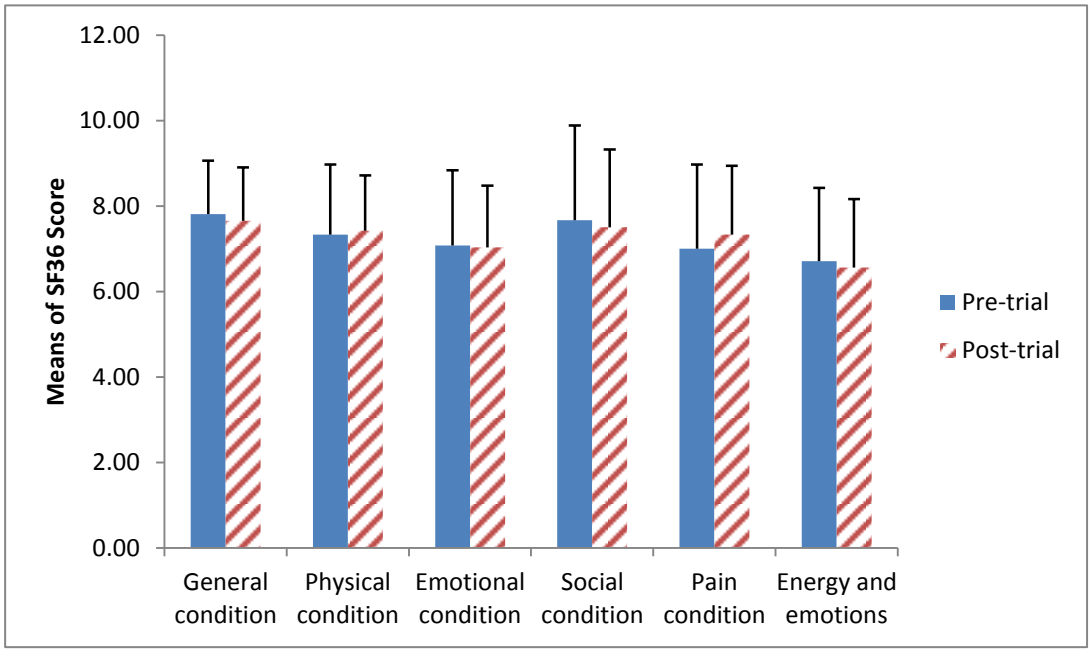


Figure 4.17: Comparison between pre-trial and post-trial SF-36 results in the control group

The comparison of the pre-trial and post-trial SF-36 results indicated a general improvement in the KFE group but not in the control group (see Tables 4.39 and Table 4.40). Emotional and energy levels improved to a level of statistical

significance in the KFE group after KFE intervention. Pain, as one of the somatic conditions, did not change in the KFE group.

Compared with the pre-trial condition of Liver Qi Stagnation (LQS) and Liver Kidney Yin Deficiency (LKYD) patterns, the KFE group also demonstrated the alternation in the post-trial results of LQS and LKYD. In the placebo group, the results of the pre-trial and post-trial had not changed significantly. The initial data demonstrated in Table 3 in appendix and the statistical results of LQS pattern and SF-36 in the KFE group was compared in Table 4.43. The pre-trial and post-trial results of LKYD and SF-36 in the KFE group were substantially statistically different, $P < 0.0001$ (see Table 4.44).

4.6.1 Comparison of general well-being and Chinese Medicine patterns

In the SF-36 assessment, the results of the KFE group indicated that the general well-being result had improved and this matched the pattern of the Liver Qi Stagnation (LQS) subsiding (see Table 4.43). The KFE group results in Figure 4.19 demonstrated the inverse direction of both lines. The inverse relationship of the two lines in Figure 4.19 indicated the improvement of the LQS. The control group result showed no signs of a significant improvement and the pattern of Liver Qi Stagnation had not subsided as Figure 4.18 demonstrates.

The comparison of the general well-being result with the Liver Kidney Yin Deficiency results, Figure 4.20, demonstrates that the inverse relationship was not profound in the KFE group. The result in the control group showed no significant change, as indicated in Figure 4.20.

Table 4.42 showed the pre-trial and post-trial results between SF-36 and LQS were significant in the KFE group ($P < 0.05$). The results were also demonstrated in Figure 4.18. The inverse direction indicated an improvement of the general wellbeing of the participants, as shown in the self-assessed SF-36 results. When compared to the SF-36 results, the Liver Qi Stagnation symptoms diagnosed by a

CM practitioner had improved as well. LQS symptoms subsiding in the diagnosis by the CM practitioner were confirmed by participants' self-assessment, the SF-36 results.

Table 4.41: Effects of Chinese Kiwi-Fruit Seaweed Extract on general well-being results (Mean ± SD) in the KFE group and control groups

Groups	n	Pre-trial SF-36	Post-trial SF-36
Control	18	7.26 ± 1.38	7.25 ± 1.12***
Intervention *	18	7.21± 1.26	7.68 ± 1.12**

Note: KFE group P = 0.013, t = -2.74**Control group P > 0.05 (0.95), t = 0.06***

Table 4.42: Comparison of the pre-trial and post-trial results (Mean ± SD) between general well-being (SF-36) and Liver Qi Stagnation in the control group

Control Groups	n	Pre-trial result	Post-trial result
SF-36	18	7.26 ± 1.38	7.25 ± 1.12**
LQS	18	5.92 ± 0.75	5.74 ± 0.82***

Note: Comparison between general well-being and LQS, P > 0.05 in the pre-trial and post-trial results

Comparison between general well-being pre-trial and post-trial result in the control group, the P > 0.05 (= 0.95) **

Comparison between LQS pattern pre-trial and post-trial result in the control group, the P > 0.05, t = 1.99***

Referring to Table 4.42 and Figure 4.18, the post-trial results of the SF-36 in the control group was not significant. The differences in results were not significant between general well-being and the LQS pattern in the control group.

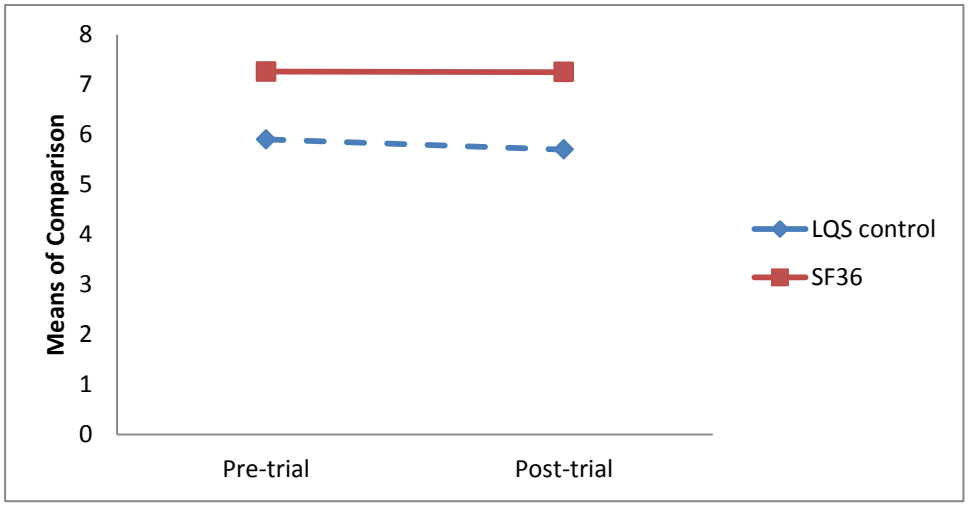


Figure 4.18: The SF-36 general well-being result and Liver Qi Stagnation changes in the control group

Note: Total score in the control group was in the Y axis

Another set of results of Chinese Medicine diagnosis patterns, Liver Kidney Yin Deficiency (LKYD), was compared to the SF-36 results. After the participants took KFE , the LKYD pattern in the KFE group was significantly improved, while the SF-36 methods demonstrated that the general well-being of the participants was increased ($P < 0.05$). This is shown in Table 4.44 (see Figure 4.20).

Astatistical analysis of SF-36 compared with the LQS pattern in Chinese Medicine diagnosis was a weak inverse correlation (- 0.26). In the KFE group, the LQS pattern had improved as well as the general well-being of the participants' self-rating. These improvements were statistically significant, $P < 0.05$ in both one tail and two tail tests (See Table 4.43). The results in the control group were not significant compared to the results in the KFE group, $P > 0.05$ in both one tail and two tail tests and the correlation result was a weaker inverse correlation (- 0.19) (see Table 4.41 and Table 4.42). Furthermore, the Liver Kidney Yin Deficiency patterns had shown an improvement but the result was not as substantial as the LQS patterns after a one month intervention (see Table 4.12 and Table 4.16). The results for SF-36 compared with LKYD were outlined in Table 4.44.

In the control group results, there was no statistical significance (P > 0.05) in the comparison of SF-36 and Liver Kidney Yin Deficiency (see Table 4.45). There are lesser changes in Figure 4.21 of the control group than the KFE group (Figure 4.20).

Table 4.43: Comparison of the pre-trial and post-trial results (Mean ± SD) between SF-36 and Liver Qi Stagnation in the KFE group

KFE groups	n	Pre-trial result	Post-trial result
SF-36	18	7.21± 1.26	7.68 ± 1.12*
LQS	18	6.50 ± 0.95	3.19 ± 0.98**

Note: Comparison between SF-36 pre-trial and post-trial result in KFE group, the P < 0.05 (= 0.013) , t=-2.74*

Comparison between LQS pattern pre-trial and post-trial result in KFE group, P < 0.0001, t = 9.92**

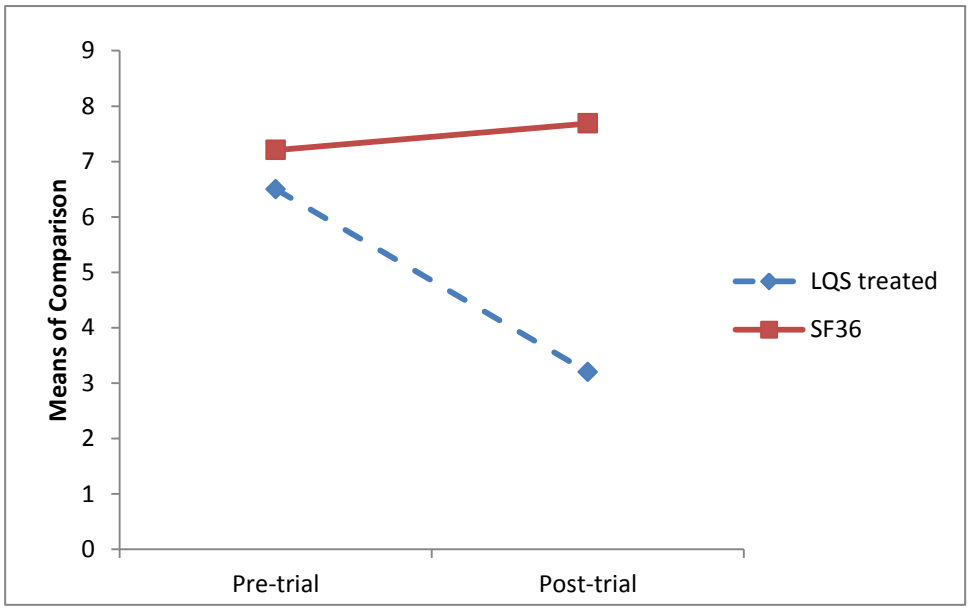


Figure 4.19: The improvement of the SF-36 result and Liver Qi Stagnation in the KFE group

Table 4.44: Comparison between the pre-trial and post-trial results (Mean ± SD) of SF-36 and Liver Kidney Yin Deficiency pattern in the KFE group

KFE groups	n	Pre-trial result	Post-trial result
SF-36	18	7.21± 1.26	7.68 ± 1.12**
LKYD	18	4.19 ± 1.42	1.85 ± 1.38***

Note: Comparison between SF-36 pre-trial and post-trial result in the KFE group, the P < 0.05 (= 0.013) , t=-2.74**

The pre-trial and post-trial results of LKYD in the KFE group P < 0.0001, t= 5.98***

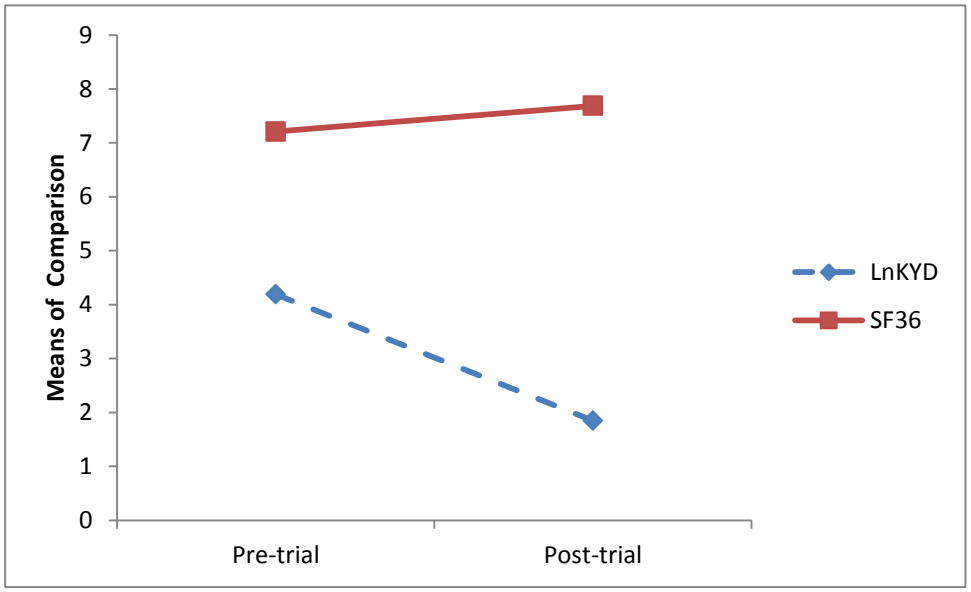


Figure 4.20: The SF-36 result and the Liver Kidney Yin Deficiency pattern changes in the KFE group

Table 4.45: Comparison between the pre-trial and post-trial results (Mean ± SD) of SF-36 and Liver Kidney Yin Deficiency pattern in the control group

Control Groups	n	Pre-trial result	Post-trial result
SF-36	18	7.26 ± 1.38	7.25 ± 1.12**
LKYD	18	3.91 ± 1.47	3.91 ± 1.52***

Note: SF-36 and LKYD results were both P >0.05 in control group

The pre-trial and post-trial of SF-36 results were P =0.94, t=0.06**

Comparing with LKYD pre-trial and post-trial result, P =0.57, t=-0.11***

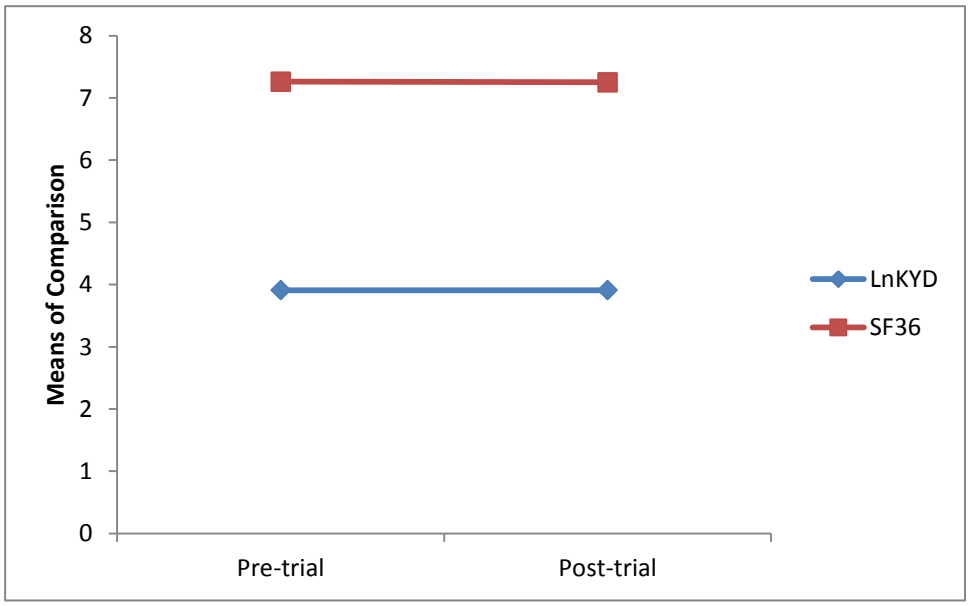


Figure 4.21: The SF-36 results and Liver Kidney Yin Deficiency pattern changes in the Control group

4.6.2. Comparison of somatic and vasomotor symptoms in the self-rated questionnaire between the intervention and control groups

Graf (2003) states that somatic symptoms and vasomotor symptoms were most often experienced by peri-menopausal women. In this study, the results found that somatic symptoms occured in most of the participants (n=36), with pain being the most frequent symptom before intervention with 35 out of 36. The pre-trial somatic

symptoms and vasomotor symptoms did not show satistical significance (P = 0.89,> 0.05) in the intervention and control groups . The pre-trial results for the total participants indicated that 97.22% of the participants had suffered from somatic symptoms and 55.56% of participants complained of vasomotor symptoms (see Table 4.46). After intervention, the somatic symptoms of the KFE group were improved and five participants reported that their pain levels had reduced (P = 0.01, <0.05).

In pre-trial results, vasomotor symptoms occurred less than the somatic symptoms (see Table 4.46). At the begining of the research, both groups demonstrated vasomotor symptoms, with 8 participants in the KFE group and 7 participants in the control group (each group n=18) reporting that they had suffered from hot flushes. After the intervention, there were 6 participants in the KFE group who reported that the vasomotor symptoms had subsided (P < 0.05). In the control group, it was reported that none of the participants improved their vasomotor symptoms (P = 0.94, > 0.05) (see Table 4.46 and Table 4.47).

Table 4.46: Pre-trial and post-trial somatic and vasomotor symptoms in total participants

	Pre-trial		**Post-trial**	
		%		**%**
Somatic symptoms	35	97	31	86
Vasomotor symptoms	20	55	10	27

Note: total n = 36

Table 4.47: Pain and hot flushes in both the intervention and control groups

	Pre-trial somatic symptom pain		Post-trial somatic symptoms pain		Pre-trial Vasomotor symptoms Hot flush		Post-trial Vasomotor symptoms Hot flush	
	Yes	No	Yes	No	Yes	No	Yes	No
KFE group	17	1	13	5	8	10	2	16
Control group	18	0	18	0	7	11	7	11

Note: total n = 36; Pre-trial results in the intervention and control groups P > 0.05 (0.89)

Pre-trial and post-trial results of the control group, P > 0.05 (0.94)

Pre-trial and post-trial results of the KFE group, P < 0.05 (0.01)

4.7. Results summary

The results of this study testing the effectiveness of KFE on the metabolism of the reproductive hormonal changes in middle aged females indicated that KFE can affect the female reproductive hormone metabolism in vivo. A risk-factor modification of the KFE intervention could increase the ratio of 2-hydroxyoestrone:16α-hydroxyoestrone, regulate the focal temperature in the breast area and resolve the acupuncture meridian blockages. At the conclusion of the trial, the SF-36 results demonstrated an improvement in general well-being within the KFE group after a one month intervention.

This study demonstrates that the Chinese Medicine diagnosis patterns which have the same trend to the urinary biomarker changes achieved 88% of the confidence of the power analysis. The study also examined the effects of KFE on general improvement and emotional change in women with Liver Qi Stagnation and Liver Kidney Yin Deficiency. The study explores the Chinese Medicine diagnosis patterns linked with biochemistry and physiology changes in vivo. The magnetic field changes were not only overlaid upon the Liver Meridian as viewed from a

Chinese Medicine perspective, but also related to the improvement of the Liver Qi Stagnation pattern.

CHAPTER 5

DISCUSSION

In order to determine the effects of KFE on the female hormonal metabolism and Chinese Medicine diagnostic patterns, this study has utilised multi-disciplinary research methods involving 36 participants. Methods included a urinary biomarker test, Chinese Medicine diagnosis, PEMF magnetic test, thermography, and an SF-36 questionnaire. The results have objectively indicated improvements in Chinese Medicine patterns and Acupuncture meridians after taking KFE. These improvements are related to changes in the biomarker-oestrogen metabolism and magnetic field, and thermographic and SF-36 results.

5.1 Participants

As most of the 36 participants attended their assessments after working hours and on the weekend due to their busy lifestyles, the distances needed to travel to the thermography testing locations and then on to their consultations and urinary tests, were arduous. Furthermore, as testing laboratories are not open on weekends, the samples needed to be frozen prior to being taken to the laboratory for testing. Other difficulties that were noted during the recruitment process were that some of the middle aged participants were unable to devote the recommended time for the two month study period. A more significant result may have been gained if the number of participants had been higher. For example, in the thermography results, only eleven participants were able to complete the pre-trial and post-trial tests.

The high occurrence of Liver Qi stagnation (73%) in this group of participants may indicate that the peri-menopausal age group experienced mood swings and stress which were related to their busy life style and as such, were factors contributing to their inability to fully participate in the study. In the practise of Chinese Medicine, these emotional fluctuations and hormonal disturbance symptoms are closely related with Liver Qi Stagnation (Long et al, 1998). To release Liver Qi stagnation symptoms, the holistic Chinese Medicine approach is beneficial in the prevention and intervention of hormone imbalance related conditions and minimising the emotional changes and impact of stress.

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5.2. The placebo

In the placebo effect, there is a correlation reaction with the limbic system and nervous reactions which release neural substances such as endorphins, dopamine and serotonin (Beers et al, 2006, Chou et al, 2007). Symptoms of the control group may vary when an inactive substance mimicking the medication is taken by participants, causing them to feel better or worse. In the double blind placebo control of this study, the majority of participants felt a little better. These results were then compared with the clinical results of the intervention group. In the control group, the placebo substances could influence participants' symptoms by up to 50% and show that a "good feeling" mimics the intervention results (Beers et al, 2006).

In contrast to the strong placebo effect reported by Beers and his co-workers (2006) the control group results in this study indicated that the placebo effect was not significant. The comparison between the pre-trial and the post-trial results in the control group indicates that the placebo effect was very low. Contrary to the placebo effect, the results of the intervention in this study were scientifically effective.

5.3. The biomarker test

The biomarker test was selected to examine the KFE effect on the hormonal metabolism in order to investigate the relation between Liver Qi stagnation patterns after KFE usage and changes in the female reproductive hormone metabolism. After the study, this study concluded that KFE could elevate the "good" oestrogen (see Section 2.2.1 and Section 2.2.2) and also decrease Liver Qi stagnation to regulate the female reproductive hormone.

In conducting this research related to female reproductive hormones, there are three important issues to be addressed concerning the biomarker urinary test 2-OHE:16α–OHE, using ELSIA method (Lord et al, 2002). The first issue put forward by Lord and co-workers was how collecting urine samples at different

times during the menstrual cycle may affect the result. The second question was how the stages of the menstrual cycle differ according to age. The final issue pertained to the timing of the urine collection and its effect on test results. In this research, the use of Lord's questions are justified in the literature review and considered in the methodology of the research (indicated in section 2.2.3 and section 3.2). Participants in this research were not undergoing hormonal treatment and the peri-menopausal menstrual status of participants was not significantly different. The times of the menstrual period cycle in the peri-menopausal women were different because of irregular periods; however the intervention group results were generally more significant than the control group results, which related to the sample size. Lord's three questions assisted in narrowing down the occurrence of error in this research research to yield biomarker test results that were within the range of the previous published papers listed in the literature review. In this research, the methodology has been concerned with the hormone fluctuation in the menstrual cycle and also in the daily influence. Morning voids were selected in line with previous published papers (indicated in section 2.2.3) demonstrating that morning voids yield the same results as 24 hour urinary collections. Most of the baseline results were lower than the normal range of 2-OHE:16α–OHE ratio (Table 4.3, page 95) which is from 1.2 to 1.6 (Faupel-Badger & Fuhrman, 2010). Following from this research using KFE intervention, the ratio of the intervention group increased and closed to 1.6, having achieved the ratio at the highest limit. As indicated in Chapter 2, this ratio elevation shows KFE to have benefits for the treatment of hormonal disorders and associated diseases.

Isoflavones exhibit specific biological responses due to interactions with oestrogen receptors, inhibiting steroidogenic enzymes and interfering with the oestrogen binding. Isoflavones have been shown to increase 2-OHE in the blood and reduce 17b-estradiol which related to an induction of CYP1A1. Isoflavones may have effects on the prevention of breast cancer (Lord et al, 2002). As stated in the literature review, kiwifruit contains rich isoflavones. The results of this research demonstrate that KFE can change the oestrogen metabolism and cause the elevation of the urinary ratio of 2-OHE: 16α–OHE.

These results posed a question for the researchers as to how KFE could elevate 2-OHE: 16 α–OHE. As discussed in Chapter 2, kiwifruit contains rich phytoestrogen such as isoflavones and resveratrol. Phytoestrogen binds to the oestrogen receptor α and β and acts as an anti-atherosclerosis, anti-oxidant, anti-inflammatory, anti platelet, a cancer chemotherapeutic agent and vasorelaxant (Hirotoa & Tadokoro, 2011). Hirotoa showed phytoestrogen acts on hepatocytes (Hirotoa & Tadokoro, 2011). Except for the phytoestrogen effects in the liver, it is hypothetical to assume that phytoestrogen may act on the kidney capillary endothelia cells which could increase the excretion of 2-OHE and 16α–OHE.

In Schneider and co-workers study (1982), 17β–oestradiol, 2-OHE and 16α– OHE were found in the urinary samples which were closely related to the three hormone levels in the blood samples by the same immune measure method. The results of Schneider and coworkers research indicated the ratio of 2-OHE: 16α– OHE in urine accurately reflected the plasma ratio changes. The result of Schneider and co-workers study may demonstrate the phytoestrogen most likely to affect the oestrogen metabolism in liver and not in the kidney. However, there is no evidence to show that the kidney cells increase the ratio of 2-OHE and 16α– OHE after using phytoestrogen.

Another study explored phytoestrogen effects on the function of the liver (Cline & Wood 2009). Genistein, a phytoestrogen, acts on the oestrogen metabolism in the liver and also binds and acts on the α and β oestrogen receptors in the breast tissue. The high α–oestrogen receptors can increase the risk of breast cancer occurring so Genistein was able to depress the proliferation of the oestrogen receptor positive breast cancer cell. The progress of the depression was affected by the antagonism and dosage. Genistein, as an antioxidant, was also an inhibitor of DNA topoisomerase, which was an integral part in controlling the differentiation of the breast cancer cell in vitro (Cline & Wood, 2009). Phytoestrogen, Genistein, also improved liver function and ameliorated the damage of the non-alcoholic fatty liver. Phytoestrogen also activated the antioxidant profile; decreased interleukin-6

(IL-6) and tumour necrosis factor–α (TNF–α) concentration. The benefit of decreasing tumour necrosis factor–α (TNF–α) and interleukin-6 (IL-6) could improve the immune system which was the monitoring function for the prevention of cancer (Mohamed-Salih & Nallasamy, 2009; Vere et al, 2009). In breast cancer, the down regulation of the α–oestrogen receptor is important to inhibit the oestrogen receptor action, which is related to the liver receptor hormolog-1 as an oestrogen regulating gene. Liver receptor homolog-1 (LRH-1) is a key regulator of oestrogen responses and regulates the oestrogen receptor expression in breast cancer cells (Thiruchelvam et al, 2011).

The effects of KFE can change the oestrogen metabolism which was most likely to be via the primary metabolic detoxification of the liver. The metabolic detoxification can reduce the accumulation of steroids and hormones such as altered exogenous and endogenous chemicals and hormones, which can cause adverse effects to the human body such as the development of breast tumours (Kishida & Beppu, 2000; McCance & Juether, 2006). The human liver represents a major site for biotransformation, conjugation and catabolism of sex steroids, being featured by the presence of key steroid enzymes, oestrogen sulfotransferase. The formation of 16α–OHE in the human liver can increase carcinogenesis (Campisi et al, 2009). 16α–OHE is an active derivative oestrogen with a high affinity for binding with the oestrogen receptor which triggers the formation of DNA adducts in human breast cancer cells. In the HepG2 human liver cells culture, 16α–hydroxyestrone increased the free active oestrogen levels up to 20 times more than the normal range, which escalated the risk of breast cancer (Campisi et al, 2009). The oestrogen receptor α is present in the majority of breast cancer cells and the inhibition of oestrogen receptor α is a key part of breast cancer intervention (Thiruchelvam et al, 2011). In such cases, the elevation of the ratio2-OHE: 16 α–OHE by KFE may alternate the oestrogen metabolic pathway in the liver and may assist in the prevention of breast tumours.

The plasma ratio of 2-OHE: 16α– OHE correlates with the urinary level of the ratio and also the higher 2-OHE: 16α– OHE in blood circulation and the higher 2-OHE: 16α– OHE in tissue (Bradlow & Jernstrom, 2006). The similar ratio of plasma 2-OHE: 16α– OHE and the ratio of urinary secretion indicate that the kidney is not the main organ to change the ratio (Bradlow & Jernstrom, 2006). An animal study was conducted to prove that the kidney has mineral metabolic function for 2-OHE and 16 α–OHE (Zhu et al, 1994). In Zhu and colleague's (1994) study, the results of the animal samples indicated that most of 16 α–OHE metabolism was in hepatic microsomes but there was only a minor occurrence in renal microsomes. Lotinun and colleagues (2001) reported that 17β-estradiol reacted with 17b-dehydrogenoase and metabolized to estrone, which is primarily hydroxylated in liver at either C-2 to form 2-OHE or C-16alpha to form 16α–OHE.

Furthermore, another study was undertaken to compare breast tissue and urine samples, which proved that the ratio of 2-OHE: 16α–OHE was similar in both areas. The result provided the evidence used for the urine sample to estimate the level of the ratio in the breast tissue (Taioli & Im, 2010). It is reasonable to state that the hypothesis level of 2-OHE and 16α– OHE in the glomeruli are similar to the concentration of the 2-OHE: 16α–OHE in the blood circulation. The urinary 2-OHE: 16α–OHE could be slightly higher than in the plasma level because of the minor level of metabolism of oestrogen in the kidneys (Zhu et al, 1994). This will not substantially change the 2-OHE: 16α–OHE without KFE intervention as observed in this research study.

In summary, oestrogen is primarily metabolised in the liver through two mutually exclusive pathways yielding metabolites with different biological activities; the low oestrogenic 2-hydroxyestrone (2-OHE) and the highly oestrogenic 16α-hydroxyestrone (16α-OHE) (Kishida et al, 2000; Bentz et al, 2005; McCance et al, 2006). Phytoestrogen can increase 2-OHE and relatively reduce 16α-OHE (Lord et al, 2002; Campisi et al, 2009; Mohamed-Salih & Nallasamy, 2009).

The pre-trial results in the urinary biomarker test were not significantly different between the two groups. It can confidently be stated that the baseline of both groups were similar. The post-trial results indicated the ratio of 2OHE:16α–OHE was substantially elevated in the urinary sample of the KFE group but not in the control group. The selected urinary biomarkers 2OHE and 16α–OHE were both reliable and sensitive for investigating the hormone imbalance. This test was convenient and non-invasive to the participants. All participants completed the required urinary tests and cooperated well in collecting the urine samples. Generally speaking, a higher ratio of 2 hydroxyestrone to 16a-hydroxyestrone at baseline was associated with a reduced risk of breast cancer (Muti & Bradlow, 2000).

5.4. PEMF and the thermography tests

In PEMF Spleen Meridian test results, the blockage of the meridian was significant in the pre-trial results (32%), which improved after the KFE intervention in the test group. Spleen Meridian was one of the significant stagnant meridians in the pre-trial result. This was caused by the Spleen Meridian blockage being affected by Liver Qi Stagnation, as there is a close relation between Spleen and Liver functions in Chinese Medicine theory. This explains how unblocking of the Spleen Meridian may be related to the KFE releasing Liver Qi Stagnation. It may also explain how Spleen Deficiency symptoms in peri-menopausal women in this research were not significant; such as distending hypochondriac pain, poor appetite, abdominal distension and loose stool motion. The participants in our research were most likely enjoying their food with no loose stool motions but tiredness and gain weight were common. These conditions may be a mild suppression of the Spleen function and the tiredness was a result of an interaction of Spleen deficiency and Liver excess (Maciocia, 2000).

In the post-trial result, the Liver Meridian’s condition had substantially improved with the other meridians all showing signs of different improvement. The results of the PEMF test were matched to the changes of the Chinese Medicine diagnosis

patterns (Table 4.7, refer page 100 and Table 4.8, refer page 101)**.** The Liver Meridian stagnation was reduced and closely linked to the diagnosis pattern of Liver Qi Stagnation recovery in Chinese Medicine. The PEMF results also indicated that the Liver & Kidney Yin deficiency pattern was the second most affected pattern after intervention with KFE.

Acupuncture meridian theory is an important root of Chinese Medicine (Chen & Zheng, 2011). In this research, the results supported Chen and his colleague's view that the PEMF polarization changes in the acupuncture meridian were closely related to the variations of Chinese Medicine diagnosis patterns. Chen and his colleague indicated that the meridian related pattern differentiation identifies with the symptoms and signs, which are linked to disorders associated with the meridian pathway. In this research, the results supported the meridian related pattern differentiation, which may be a useful assessment tool as a key to enhancing therapeutic effects and diagnosis accuracy.

Comparing the PEMF results with Chinese Medicine diagnosis patterns in this research, the initial PEMF magnetic test indicated that the Liver Meridians and Kidney Meridians had the highest meridian blockages. The patterns of Chinese Medicine diagnosis are dominated by Liver and Kidney Symptoms. The results also demonstrated that Spleen Meridians had higher meridian blockages, but there were no common Spleen deficiency symptoms in this Chinese Medicine diagnosis. Therefore it is reasonable to use this to explain the dimming of the polarized light on the Spleen Meridian in the trunk area during the PEMF magnetic test. This was affected by the Liver Meridian blockage, as the Liver Meridians are closely located and crossed to the Spleen Meridian in the trunk area. The Spleen Meridian stasis was partially released as the Liver Meridian stasis was partially resolved after the KFE intervention. This outcome can be interpreted in light of Long and his colleague's (1998) finding that Liver (Wood) Qi Stagnation can affect the Spleen (Earth), as the excessive Wood element can disturb (Ke) the Earth element.

The scan results of the thermography were also matched to the PEMF test in this research. The PEMF results also demonstrated that the upper lateral and medial areas were the most common locations for blockages which affected the Qi and Blood flow in the meridian pathway. The thermography results demonstrated that the superior lateral quadrant has a higher occurrence of temperature elevation, which was over 36.7^{o}C. This research also found that the majority of the middle aged female participants experienced a temperature increase caused by different types of inflammation around their breast area. This may have been caused by the participants wearing an unsuitable bra and sinusitis affecting the temperature within the facial and neck areas and extending to the auxiliary lymphatic nodes. The auxiliary lymphatic nodes' increase in temperature was caused by muscular pain around the shoulder area which then affected the breast area.

Thermography scans are important to identify the temperature increase area in the breast, which can assist in the early diagnosis of breast diseases. The increase in temperature can be caused by a local inflammation or tumours. In Conventional Medical research, inflammation and cancer have a very close relationship. The evendence for this is found in the inflammatory elements elevated in the breast cancer area, such as cyclooxygenesis 2 (COX-2). Further evidence is found through the use of anti-inflammatory medicine - Aspirin was evident as an inverse correlation for the reduction of breast cancer with positive hormone receptors and also breast cancers with negative hormone receptors (McCance & Juether, 2006). The increased breast temperature can also result from the tumour metabolism which was increased by pro-inflammatory prostaglandin E2 and angiogenesis which was produced by the breast cancer cells (McCance & Jeuther, 2006). In addition, the breast tumours more frequently occur in the superior lateral quadrant of the breast area (Sickles, 1986). In perimenopausal women, the pre-trial results of this research indicated that the temperature increasing in the breasts was higher in the superior lateral quadrant area, when compared with other areas of the same breast. This phenomenon may be affected by hormonal disorders, as mammary tissue is sensitive to oestrogen fluctuation (see Section 2.2.4).

The results of this research demonstrate that the breast focal temperature had increased by 1–2^{o}C in the peri-menopausal participants' breast. This result remained within the normal range according to Hoekstra's (2001) research. The abnormal thermology signs of tumour growth should appear as asymmetric and hyperthermic vascular patterns with at least 2.5°C difference within the surrounding breast tissue. The localized heat area can have an abnormal physical contour and signs of an uneven edge (Hoekstra, 2001). Furthermore, to improve the thermography analysis methods, a few sophisticated formulas and calculations were developed by Amri and colleagues, such as the Pennes Equation and Kirchoff's Law, as well as the Lattice Boltzmann method and the Finite Element method. The fixed room temperature was conditioned to remain between 18-22^{o}C. When exposed to this room temperature, the rise of the breast focal temperature was due to the high metabolic rate of the tumour and the perfusion rate of blood. The presence of the skin surface temperature, varied between 1.27^{o}C +/- 0.37^{o}C, and was caused by the growth of the tumour (Amri et al, 2011).

There were two kinds of criteria for the interpretation of thermography images: graphic criteria and thermal criteria. The graphic criteria included vascular patterns with the temperature increasing. The thermal criteria included the temperature elevating in vascular or non-vascular surfaces (Ng & Ung, 2001). In this research, Kennedy's criteria and Blum & Farrier's colour scheme were used to interpret the research results of thermography. Kennedy's graphic criteria included few patterns. Blum & Farrier's colour scheme was based on thermal criteria. The results demonstrated the normal range of Kennedy's criteria was 0 to 2 with the change of the patterns. There was one participant who was excluded in the first ten days due to her score showing 5 with a thermography diagnosis. The final result from her specialist was a diagnosis of breast cancer. The Kennedy criteria were more suitable for identifying cancer or inflammation patterns from participants showing breast temperature elevation in pre-clinical conditions. The Blum & Farrier colour scheme was suitable when comparing the temperature alternation in the breast area. It may not be suitable for use in identifying cancer and inflammation in participants with a lower body temperature which matches Chinese Medicine Yang deficiency

patterns. Comparing the maximum temperature after subtracting the mean of the temperature in the same breast, the maximum temperature was reduced by the intervention of KFE and also correlated with the decrease of the Blum & Farrier colour score.

The results of this trial indicated that the Chinese Medicine patterns were associated with thermographic changes. The superior lateral quadrant area of the breast has a higher occurrence of tumour and inflammation. This area also has more acupuncture meridians passing by it. Middle age women may have a higher incidence of blocked meridians caused by the stagnation of Qi and Blood, and Liver Kidney Yin Deficiency. KFE intervention demonstrated its effectiveness on improving the acupuncture meridians pathway, which can relate to improvement of Qi and Blood circulation in the meridians. (Refer to Table 4.30, refer page 140; Table 4.6, refer page 100; Table 4.7, refer page 100; Table 4.8, refer page 101 and Figures 4.6, refer page 98).

Table 5.1: List of Acupuncture meridians passing the upper lateral and upper media quadrant area of the breast (Refer to Page 97)

Upper Lateral Quadrant	Upper Media Quadrant
Foot Taiyin Spleen Meridian	Foot Shaoyin Kidney Meridian
Hand Jueyin Pericardium Meridian	Foot Yangming Stomach Meridian
Foot Jueyin Liver Meridian	Foot Jueyin Liver Branch
Hand Taiyin Lung Meridian	Hand Shaoyin Heart Branch
Hand Shaoyin Heart Meridian	

5.5. Relationship between the different tests and assessments

In this trial, the results of the urinary test for the ratio of 2 OHE:16α–OHE matched the results of the PEMF magnetic tests and Chinese Medicine diagnosis patterns, Liver Qi Stagnation and Liver Kidney Yin Deficiency (Table 4.10 page

101, and Table 4.17 page 111). When the Liver Meridian condition improved, the Liver Qi Stagnation (LQS) recovered and the urinary test ratio of 2OHE:16α–OHE elevated. When comparing this outcome to the control group, the results for the ratio of 2 OHE:16α–OHE showed a substantial change. The Liver Qi Stagnation symptoms were released after the participants ingested KFE and the PEMF magnetic test indicated that the Liver Meridian blockage had been reduced.

In Chinese Medicine, a Liver Qi Stagnation pattern may relate to pathophysiological changes in Conventional Medical research. In Conventional Medicine, the liver function involves the oestrogen metabolism. In Vere's research (2009), the liver function may be affected by psychological stress, which is able to influence hepatic blood flow by inducing vasospasm and centrilobular hypoxia, which therefore can lead to liver problems. Psychological stress caused the Type 1 helper T lymphocytes to be suppressed and respond to cytokines production, tumour necrosis factor–α (TNF–α), interleukin-1 (IL-1) and interleukin-6 (IL-6) which decreased the immune system. In vitro, carcinoma was associated with high concentrations of TNF–α. Stress, as outlined in several studies (see Section 2.3.5.1) directly influenced TNF–α, IL-1 and IL-6. Psychosocial stress was also linked to increased DNA damage, alterations in DNA repair and inhibition of apoptosis. Type 1 personality and psychosocial stress were positively linked to the severity of carcinoma and chronic disease such as liver cirrhosis (Vere et al, 2009). Mental stress had an impact on hepatic inflammatory responses and could also induce excess glucocorticoid appearing, which stimulated the over production of Reactive Oxygen Species (ROS). ROS can increase Nitric Oxide (NO) bioavailability and therefore cause the impairment of endothelial function. The endothelial function impairment triggered by ROS elevation may lead to various epithelial cancers (Sotgia & Martinez-Outschoorn, 2011, Toda & Toda, 2011). In light of this, the emotional changes affected the Liver Qi stagnation diagnosis in Chinese Medicine, which may relate to the stress level leading to a change of the liver function in human anatomical physiology in Conventional Medicine.

Conventional medical researchers are aware of psychological stress effects on liver function. This may include changes to the metabolism of female reproductive hormones. Oestrogen is metabolised in the liver through two mutually exclusive pathways yielding metabolites with different biological activities; the lower oestrogenic 2-hydroxyoestrone (2-OHE) and the higher oestrogenic 16α–hydroxyoestrone (16α–OHE) (Kishida & Beppu, 2000; Bentz & Schneider, 2005). The 16α–OHE demonstrated oestrogenic properties through covalent bonding with the oestrogen receptor and stimulation of mammary cell proliferation (Bentz & Schneider, 2005). KFE which contains rich flavonoids and Vitamin C, may be a valuable ingredient in efforts towards the prevention of breast cancer (Xu, 2006; Franceschi & Parpinel, 1998), due to its antioxidant effects and DNA repair actions (Collins et al, 2003, Skinner & Loh, 2011). This can be a valid argument for use of KFE in the prevention of breast cancer and to also resolve the Liver Qi Stagnation pattern. In another clinical trial, sixteen participants diagnosed with breast cancer and who had just completed chemotherapy, were administered KFE for seven days. The results indicated that the rate of binucleated lymphocytes with micronuclei in these participants had significantly decreased (Xu 1999).

The result in the Liver Kidney Yin Deficiency (LKYD) pattern supports the argument that KFE can improve the Yin deficiency pattern. The relationship between LKYD and the biomarker test saw a similar trend to the LQS patterns: a reversed relationship. When the biomarker results increased as the 2-OHE elevated, the LKYD score was reduced and the symptoms and signs subsided. At the end of this research, the symptoms of Liver Kidney Yin Deficiency had improved through KFE treatment but the result was not as significant as Liver Qi Stagnation. The reason for this may be that LKYD had occurred as a result of of ageing which can be exacerbated at middle age and onwards. Recovery of LKYD can take a longer time based on degenerative conditions improving Liver Meridian blockages can be resolved in a shorter period of time because Liver Qi Stagnation is released and the participants were much happier after the KFE intervention. According to both Chinese Medicine theory and research, it is possible to improve the Kidney Meridian through KFE intake because the meridian was related to the organ

function. In an earlier study (Fu and Xu, 2011), the Kidney function was able to improve by using KFE as indicated by the biomarker 2-OHE:16α-OHE change. This theory was also supported by the previous study (Xu, 2005).

Chinese Medicine diagnostic methods indicate that Liver Qi Stagnation and Liver Kidney Yin Deficiency may be the two main patterns of pre-clinical changes in this group of participants. The changes of the disharmony patterns of the Chinese Medicine diagnosis methods may be useful in the Conventional medical assessment of hormone fluctuation and pre-clinical breast diseases. The use of KFE may reduce the suffering of female hormone imbalance or hormone related diseases (Xu, 2006). KFE was strongly recommended to be used for the regulation of Liver Qi, the nourishment of Yin and the diminishing of the stagnation in Liver Meridian and Kidney Meridian. The biomarker result of the KFE intervention may indicate the benefits for decreasing the female reproductive hormonal disorder. These results support the finding of a connection between the meridian function and female reproductive hormones. Acupuncture meridians relating to the organs of endocrine systems are discussed by Omura (1987), such as a triple burner meridian which is related to the function of ovaries and the adrenal glands in females.

5.6. The use of PEMF magnetic tests to detect acupuncture meridians

The diagnosis of Liver Qi Stagnation and Liver Kidney Yin Deficiency should have an anatomic-physiology foundation. The PEMF magnetic test provided the opportunity for this research to identify the meridian function related to the diagnosis patterns.

In the research, the running of Chong Mai was clearly separated from the Ren Meridian. The PEMF test could clearly differentiate the Stagnation in the Chong Meridian (Penetrating Meridian) from the Ren Meridian. These two acupuncture meridians were very close. The functions and symptoms of both meridians were to overlap each other, thus being difficult to identify as to which meridian had a

disorder. Both of the meridian lines are along the middle of the abdominal area, with the Ren Meridian running on the midline of the trunk area and Chong Meridians running to the bi lateral side of the Ren Meridian. The functions of both the Ren Meridian and Chong Meridians are associated with digestive and gynaecological disorders (Pirog, 1996). Further study of PEMF tests is necessary because Chong Meridians were overlapping with the Kidney Meridians as two dimensional images in this research but, in a logical hypothesis, the distribution of the meridians should be in three dimensional images, which would help to separate Chong Meridians from Kidney Meridians. It is important to develop PEMF testing within acupuncture practice as it may improve the detection of the fine definition of acupuncture points on the meridians. It could also increase the accuracy of the Chinese Medicine diagnosis and intervention. The polarized light combined with the computer generated graphics may improve the PEMF performance by showing fine detail of the mapping. In this research, the Liver Meridian and Kidney Meridian were detected by the polarized light but other extraordinary meridians were difficult to observe in the trunk area. The reason for this phenomenon is due to overlapping or closed meridians in 2D mapping, such as the Chong Meridian being located close to the Kidney Meridian; and the Yin Wei Meridian and Yang Wei Meridian being overlapped by the Spleen Meridian and Gall Bladder Meridian respectively (Deadman et al, 2001).

The pathway of a magnetic field had overlapped the location of the Liver Meridians on the trunk area. This study explored the possibility of a correspondence between the pathway of the magnetic field to the Liver Meridians and the related Chinese Medicine diagnosis patterns – Liver Qi Stagnation and Liver Kidney Yin Deficiency. This study also examined the effectiveness of KFE which may affect the magnetic field on the Liver Meridians and the regulation of the hormonal metabolism for middle age women.

In terms of Chinese Medicine theory, the acupuncture meridian function was closely related to the named organ function and the Liver Meridian function was linked to the Liver organ function. The meridian could transfer Qi and Blood,

which was able to defend the Zangfu and body. Liver Qi Stagnation can lead to distension and pain in any portion of the Liver Meridian such as breast pain (Deadman et al, 2001). The findings on the acupuncture meridian function and the magnetic field changes are reported in Chapter 4, 4.2 (refer page 92). The outcomes indicate that the Liver Qi Stagnation symptoms had improved and therefore had responded to the magnetic pathway blockage being resolved. As Watkins & Kuzyk (2009) and Chen et al (2011) indicate, the PEMF test is not affected by a change of exogenous electromagnetic field and the PEMF test is more related to defects in or changes in internal cells. According to Chinese Medicine theory, it is reasonable to consider that the dim area of polarized light was the lower energy area where the Qi and Blood were blocked; as indicated in Chapter 2, 2.6 (refer page 53).

PEMF magnetic tests in this research are a detective method to observe the polarization light changes in the human trunk area whichrelate to the magnetic field alternation and are also closely related to the acupuncture meridian Qi and Blood flowing. In this research, thermography was applied to test the trunk and thus ultimately visualise the meridian blockage in the breast area. The results of thermographic imaging did not show the blockage of acupuncture meridians. The hypothesis is that the blockage of the acupuncture meridian did not demonstrate much change with the temperature, when compared with the surrounding tissue. At least, it was not detected by the current thermography equipment, which may lead to the conclusion that the equipment did not display a high definition with its reading.

Overdhkin and Lee (2001) attempted to explore the blockages of meridians which can affect the Qi and Blood circulation in Chinese Medicine theory. The study found that an immunodeficiency syndrome can affect the temperature of the acupuncture points and cause the temperature to change to hyper-thermatic or hypo-thermatic conditions. The dynamic changes of temperature distribution over the acupuncture points were demonstrated in the thermography result on a patient's chest in a case of immunodeficiency syndrome (Figure 5.1 (1). After acupuncture intervention, the results showed the temperature distribution changed

from hyper-thermic or hypo-thermic to an isothermic temperature, which is a similar temperature to that of the surrounding tissue (Overdhkin & Lee 2001). The results of Overdhkin and Lee's research led to a conclusion that thermovisual analysis can be used as a proper medical intervention technique which can also offer an objective monitoring method for the acupuncture results (Figure 5.1 (1). Overdhkin and Lee also found that acupuncture technique can change the temperature in the local area and harmonize the temperature in the chest area after intervention (Overdhkin & Lee 2001).

Another result of Zhang's research was an indication that acupuncture points can be observed by thermogram. The results demonstrated that the acupuncture intervention can be guided by the thermogram, which increased the effectiveness of the intervention in a clinical trial (Zhang, 2007). In Zhang's research 180 participants were randomised and assigned to either the KFE group or the control group. The KFE group used thermography to guide the acupoint selection. Using a thermogram to identify the left and right side temperature of facial acupoints, the researcher selected the acupoints with a different temperature between the two sides of the facial area with the temperature gap being 0.8^{o}C. In the control group, the acupuncture intervention was performed on the standard menu of the general conventional acupuncture, such as ST8, UB2, GB14, TE23, EXHN4, EXHN5, ST2, SI18, ST7, LI20, LI19, GV26, ST4, ST6, CV24, TE17, and GB20; as well as added peripheral points such as LI4, ST36, LIV3 and SP6. The outcomes from both groups showed the KFE group at 90% and the control group at 77.5% respectively (Zhang, 2007). In Agarwall-Kozlowski and his colleague's research, the thermographic results indicated that the temperature had significantly changed due to the insertion needle in the acupuncture points but not in the sham acupuncture points (Agarwal-Kozlowski et al, 2009). However, the accuracy of the acupuncture meridians was not visualized in Overdhkin, Zhang and Agarwal-Kozlowski and their colleague's research.

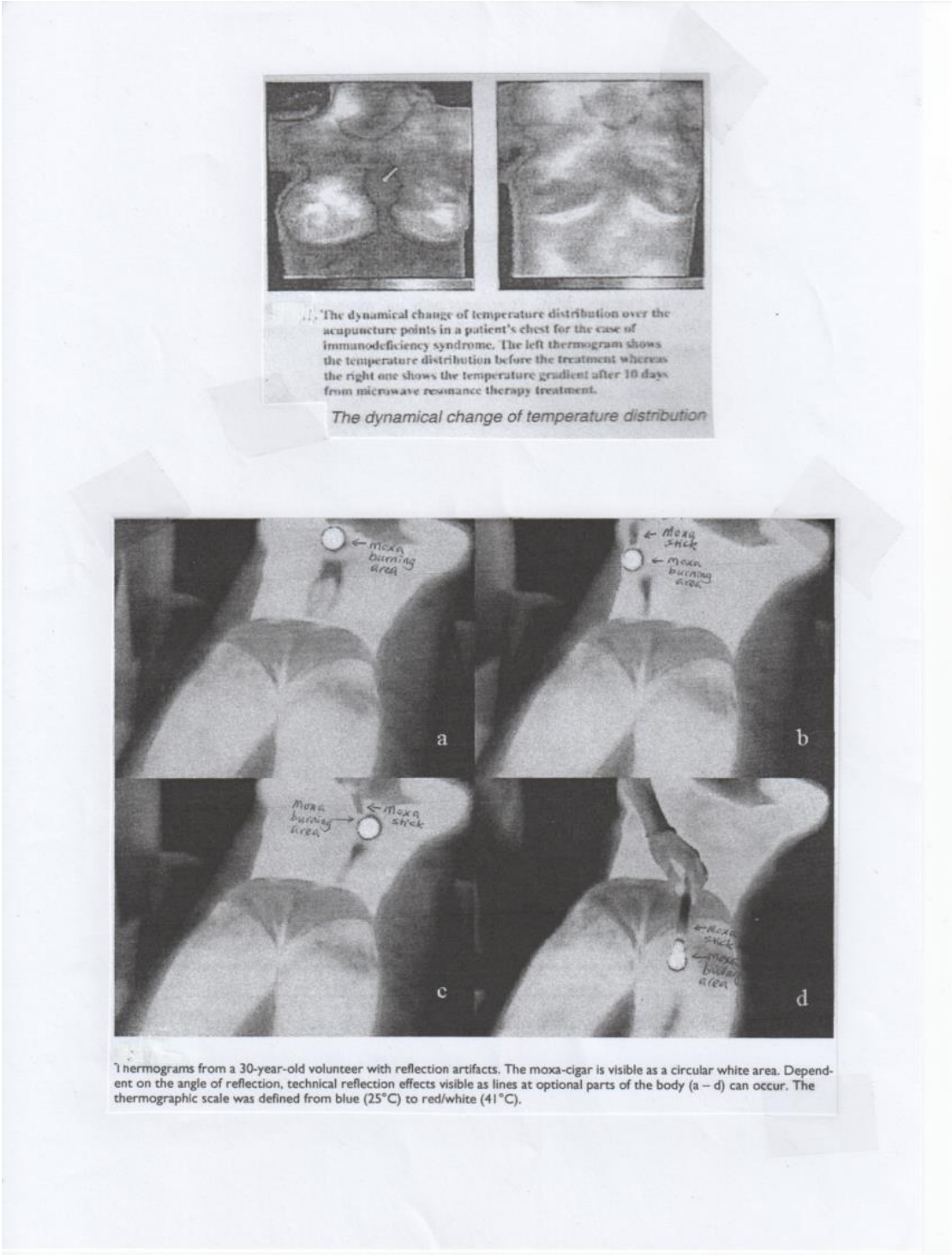

Figure 5.1.

(1) Thermography detected the chest temperature change in pre-trial and post-trial images (Overdhkin & Lee, 2001).

(2) Images a), b), c) and d) demonstrated the moxa-stick alternated the temperature change on the skin (Litsher, 2005).

For observations of the acupuncture meridian, Litsher (2005) warmed the volunteer's skin by using a moxa stick. The results indicated an artificial heat produced by the moxa which could change the temperature on the skin and was similar to the meridian Qi flow direction; even though the author believed the result was "not" on the meridian (Figure 5.1 (2). As demonstrated in Figure 5.1 (2), although the artificial heat affected an area of the skin, it was assumed that the surrounding area would also be warm, but the image has not demonstrated this hypothesis (Litsher, 2005). Furthermore, the artificial heat has not indicated that this heat affected the skin dermatomes map of the sensory nerve. These results may explain the possibility of an energy change in the acupuncture meridian, which include the Jing and Luo meridians. The results of Litsher's research demonstrated the Qi and Blood changed closely along the meridian and also followed the meridian energy flow direction which continued a distance of around 10cm in length. If the methodology of artificial heat changed to a warm needle technique, it may indicate that the meridian was clearer where the acupuncture needle inserted into the acupuncture point and a moxa-cone was applied to the needle.

Litsher's research has been criticised by Chen and Lv who argue that the meridian research should be restricted to a living body because the observation involves the reaction of the body's function, and thus Litsher's research method was not sound (Chen & Lv, 2011). The following observation was made by Professor John Longhurst, *"Sometimes thermograms have been thought to actually show the pathway of a weakly luminescent meridian, but such demonstrations have been inconsistent between meridians. Furthermore, there is no physical proof that such oscillations occur, that they change with disease or that they actually represent meridians."* This statement argues that there was not enough evidence to visualise the acupuncture meridians in the current scientific research of thermography (Chen & Lv, 2011). The statement may also lead to a hypothesis: thermograms can observepathological changes on a meridian when the temperature is elevated.

According to the above reference papers, the thermographic results have a certain relationship with the acupuncture meridian; however there was no direct visible meridian blockage in the thermographic images used in this research.

In Conventional medical research, chest thermography is used to investigate metabolic activity and vascular circulation, which demonstrates that a change in temperature is always higher in tumours than in a normal breast area (Acharya et al, 2012, Boquete et al, 2012). The results indicate that a temperature increase in a mammary region can be associated with a high risk of tumours in that area (Boquete et al, 2012). Thermography as an infrared image can focus on finding thermal evidence that suggests the presence of an early stage tumour which cannot be detected physically, or suggest a precancerous stage in the breast through minute variations in normal blood vessel activity (Acharya et al, 2012). It illustrates that the improvement of the Qi and Blood flow in the acupuncture meridian can adjust the temperature in the breast area and to benefit the prevention of breast tumours (Overdhkin & Lee, 2001) and that the movement of Qi in the acupuncture meridian was related to the blood movement in the vessel.

In the post-trial results of this research, the lateral area of the breast remained at a higher temperature than the medial area, however the temperature was reduced in the superior lateral breast area. The temperature of the breast area was regulated, with the lower temperature slightly elevated and the higher temperature reduced (Table 4.30; refer page 125). The superior lateral area of the breast has more acupuncture meridians to pass through. This area becomes a central pathway which may be more easily affected by a meridian disorder occurring in the superior lateral area. The breast temperature became more harmonized in the KFE group. This phenomenon may relate to the intake of KFE, which may affect female reproductive hormone metabolism and the Qi and Blood flow of Liver and Kidney meridian in this study.

Additionally, the PEMF magnetic test results demonstrate that the Liver Meridian and Spleen Meridian had the highest blockage pathway in the breast, with these

meridians running at the lateral area of the breast area (Table 4.4, page 97). Compared to Conventional medical research conducted from January 1976 to February 1984, where 300 cases were reviewed and retrograded, non palpable breast cancer was examined with mammography. The results found that the majority of the non-palpable tumours arose from the superior lateral quadrant area of the breast, which had a 52% incidence of breast tumour in the total participants. 5% incidences of breast tumours were located at the inferior medial quadrant. The remaining incidences of breast tumours were evenly divided into three areas; the superior media quadrant, the inferior lateral quadrant, and the retro areolar region (Sickles, 1986). The higher temperature location of the participant's breast thermography in this study was in the superior lateral quadrant area, which was the same location of the pathological change discovered by Sickles. Even though all participants in this study were not diagnosed with breast cancer, the temperature changes demonstrated the same area as the incidence area of breast cancer, which may indicate a pre pathological change as Acharya and colleagues stated (2012) (Figure 5.2).

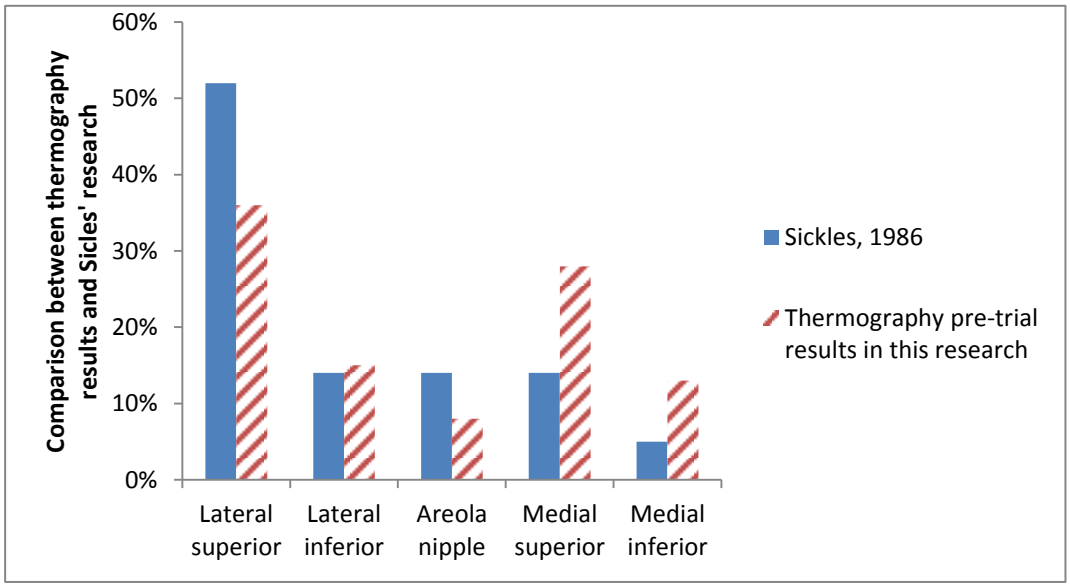

Figure 5.2: Comparison between pre-trial thermography outcomes in the breast with Sickles' findings

The lower medial areas of the breast showed an elevated temperature after KFE intervention. In participants with ID numbers three and eight, the pre-test temperature results were lower (<34^{o}C) than the post-test results. ID number 3 indicated that the lower temperature of the breast could imply that the local blood circulation was too low to support the circulation in normal breast tissue. (Page number 127) In such cases, the mutation cells can be increased under hypoxic and haemostasis conditions. The regulation of the temperature above 34^{o}C can be beneficial to the health of the female breast. PEMF magnetic test results of participant ID number 3 originally dominated the Liver Qi Stagnation pattern. After the KFE intervention, the Liver Qi Stagnation was reduced but the Kidney Meridian and Spleen Meridian result dominated the PEMF magnetic test results. The Liver Qi Stagnation in women is very often secondary to the deficiency of Liver and Kidney (Maciocia, 2004). As such this appears to be why the Liver Qi Stagnation resolved and the deficiency symptom appeared. In participant ID number 8, the post-test temperature in the right axillary area had elevated above 38^{o}C. The cause of this may be linked to a mild illness such as a fever and pain. A mild infectious change may also be a risk factor for breast diseases.

The Chinese Medicine diagnosis pattern and the Liver Qi Stagnation changed after the KFE intervention, and were most likely related to the regulation of the maximum focus temperature in the same breast. The high temperature area was decreased and the gap between the maximum temperature and the mean of the breast temperature was reduced. The result of the improvement in Liver Qi Stagnation and the changes in temperature in thermography are demonstrated to be a corresponding relationship.

In this trial, the Liver Qi Stagnation may be a condition of pre-clinical breast diseases. The symptoms of Liver Qi Stagnation were investigated along with the related factors through the PEMF magnetic test, the thermography changes and the biomarker test. The results of this study suggest that the Liver Qi Stagnation and Liver Kidney Yin Deficiency are the most frequent Chinese Medicine patterns seen in middle age women. In Chinese Medicine theory, both external and internal

factors can contribute to diseases including breast cancer. External factors include Wind and Cold attack, extremely toxic-heat pathogenic Qi in Chinese Medicine (Yu et al, 1998, Tagliaferri et al 2002, Lin et al 2006), which is similar to the theorem of Conventional Medicine which articulates an oncogenic virus invasion (Alibek & Kakpenova 2013; Butel, 2000; Becker et al, 2009). In addition, another external factor was the cause of environmental contamination such as the use of organochlorin pesticides, which had been investigated and proven to trigger the development of breast cancer (Khanjani et al, 2006). In terms of any internal factors, emotional disorders such as behaviour disturbances were found to be associated with endocrine system disorder (Reus, 1986). An endocrine system disorder may also indicate a heightened risk of breast cancer (Xu & Xu, 2006).

In summary, both Chinese Medicine and Conventional Medicine approaches identify the liver as an important organ and the first to be affected by anger, depression and stress (Fu & Xu 2011, Vere et al, 2009, Reiche et al 2004). The Liver organ function in Chinese Medicine is closely related to the Liver Meridian function. As the Liver Meridians pass the reproductive organs and the female breast areas, the Liver Meridian can affect female reproductive hormone disorder diseases in Chinese Medicine theory.

5.7. Chinese Medicine patterns and SF-36 results

As previously stated in Chapter 2, females aged between 35 and 40 years commence a decline in their reproductive function. At around 49 years of age, the Kidney Qi is debilitated and the Tain Qui is exhausted, which leads to the Chong and Ren vessels being malnourished and results in the cessation of the menstruation cycle. The results of this study supported the arguments of Long et al (1998), stating that the lifestyle of middle age women usually experiencing stress and being overworked may well cause the Qi and Blood disturbance which can relate to a hormonal imbalance (Maciocia, 2004, Xu, 2006). This is aligned with the Chinese Medicine Liver Qi Stagnation and Liver Kidney Yin Deficiency, and

therefore possibly can pose a higher risk of related diseases such as breast cancer (Maciocia, 2004).

In this research, the Chinese Medicine food formula KFE had an effect of improving the Liver Qi Stagnation pattern and Liver Kidney Yin Deficiency pattern, which was related to the improvement of the participants' general well-being. In general, lower scores of SF-36 were present in participants with serious medical and psychiatric conditions (McHorney & Ware, 1993). At the end of the research, the KFE group results of the SF-36 score were increased. The results of SF-36 in the KFE group indicated the improvement of the condition in their health.

Borud et al (2009) states that most of the Chinese Medicine diagnostic patterns seen in post-menopausal women were Kidney Yin Deficiency and Liver Qi Stagnation, which matched the outcomes of this research. An initial baseline test of Borud and his colleague's research (2009) found that the self-reported health result was generally "good" as it totalled 78% from the acupuncture KFE group and 74% from the control group. In contrast with the self rated score of good general health, 98%of the total number of participants also simultaneously reported that they had suffered from vasomotor symptoms and 50% of the participants suffered from a sleep problem. Somatic symptoms explored in Borud and his colleague's research was found in 48% of the KFE group and 55% in the control group. The occurrence of vasomotor symptoms was more prevalent than any other disorder, which is likely to be a result of the participants being post-menopausal women (Borud et al, 2009).

The results of this study were different to Borud and his colleague's research in that these findings showed a higher percentage of participants with somatic symptoms and a lower percentage of vasomotor symptoms. The Liver Qi Stagnation and Kidney and Liver Yin deficiency were most likely diagnosed but the symptoms of Liver Qi Stagnation dominated. In this research the peri-menopausal participants were selected, and in the general self-rated SF-36 questionnaire, most of the participants reported "good" in general health (94%), which was a higher score

than in Borud and colleague's research. Somatic symptoms such as pain were reported by 97% of the participants and vasomotor symptoms were reported by 41% of the participants before intervention in the control group. This difference between Borud and his colleague's research and this study, is that the participating group were going through the peri-menopausal stage rather than the post-menopausal. Their hormonal levels may be different and their stress levels could also be different in this aspect which affects their physical condition.

The somatic symptoms in the KFE group improved with five of the participants reporting a reduction in pain individually. The reason for this can be explained by the acupuncture meridian blockage being reduced and thus it was able to improve the Qi and Blood circulation in the body. The levels of participants reporting pain in the KFE group results did not change after KFE intervention, however asomotor symptoms appeared to improve after the KFE intervention. The reason for this improvement may relate to the phytoestrogen effect in the hippocampus area and binding with ERβ, which is supported by research conducted by Zhao & Mao (2011) that dissected hippocampi of mice brains increased certain proteins after being fed ERβ selective phytoestrogenic formulation. KFE contains higher phytoestrogen- insoflavonoid, which may affect the the hippocampus and binding with ERβ.

The hippocampus is a part of the Papez circuit with amygdala, parahippocampal gyrus, frnix, mammillary body, hypothalamus, thalamus and cingulate gyrus. The hippocampus controls the primary behaviour response, emotional expression and consolidated memory (McCance & Juether, 2006). The hippocampus is not only involved in the function of changing the temporary memory to permanent memory and also controls vasomotor regulation. Hot flushes are a mechanism for dissipating heat through vaso dilatation and perspiration in response to the thermoregulatory centres in the hypothermic and hippocampus area (Maciocia 2004; Zhao & Mao, 2011). Vasomotor symptoms such as hot flushes occurring are probably due to fluctuating pulses of the secretion follicle stimulating hormones

from the pituitary gland. The vasomotor symptoms were more severe during the perimenpausal and menopausal period. This was due to pressure placed on the endocrine system to provoke ovulation from increasingly unresponsive follicles (Maciocia, 2004). In Chinese Medicine theory, the vasomotor symptom is related to Kidney and Liver Yin deficiency (Maciocia, 2004). It is therefore entirely reasonable to state that KFE can improve Liver Kidney Yin deficiency, which may also affect the function of the hippocampus or hypothalamus incecreasing vasomotor symptoms.

CHAPTER 6

CONCLUSION

In this study, the positive effects of the selected Chinese food formula - Chinese Kiwi-Fruit and Seaweed Extract (KFE) on the regulation of the female reproductive hormone metabolism was examined by monitoring biomarker 2-hydroxyoestrone:16α-hydroxyoestrone tests. The research explored the relationship between biomarker alternation and the changes in thermography and magnetic fields in the participant's breasts. The study also showed that KFE released the two main Chinese Medicine diagnostic patterns – Liver Qi stagnation and Liver Kidney Yin Deficiency - in peri-menopausal women. In Chinese Medicine, the diagnosis patterns of Liver Qi stagnation and Liver Kidney Yin Deficiency illustrated the similar symptoms to the diagnosis criteria of conventional medicine (see Table 6.1).

In this study, the pulsed electromagnetic field test was shown as an effective diagnostic tool for the detection of acupuncture meridian disorders. The stagnation of the meridian was related to the magnetic field changes. Chinese Kiwi-Fruit and Seaweed Extract (KFE) can regulate the function of the acupuncture meridians which have been proven through the pulsed electromagnetic tests. These approaches can easily be applied as early preventative and diagnostic strategies that are able to integrate approaches from both Chinese Medicine and Conventional Medicine.

The results indicated that Liver Qi Stagnation in Liver Meridians was a common phenomenon in this group of middle age women. A further development of polo light linked with computer graphic techniques may better illustrate a change with the image test for acupuncture meridians. The research has demonstrated that KFE regulated the acupuncture Liver Meridian and improved the Liver Qi Stagnation and Liver Kidney Yin Deficiency. Furthermore, KFE improved the general wellbeing of this group of middle age women. This research identified benefits of using KFE to assist middle-aged women with hormonal fluctuations. The findings of the research also supported the positive impact of KFE on their mental and physical wellbeing which can be interpreted by Chinese Medicine diagnosis patterns such as Liver Qi Stagnation and Liver Kidney Yin Deficiency.

Table 6.1 Comparison between the Chinese Medicine Pattern and Conventional medicine symptoms in peri-menopausal women

Chinese Medicine Pattern symptoms and signs (Long et al, 1998)	Conventional medicine symptoms and signs (Bastian et al, 2003; Huntley & Ernst, 2004)
Liver Qi Stagnation	Periods are very heavy, or accompanied by blood clots
Depression and fullness in the hypochondria area, a feeling of obstruction in the throat, dreaminess, fear	Periods last several days longer than usual
	Spotting between periods
Distending pain in the breasts, irregular menstruation, a bloated feeling in the lower abdomen	Experience spotting after sex
	Periods occur closer together
	Breast tenderness
Mood swings and a taut pulse	Mood swings
Liver Kidney Yin Deficiency	
Symptoms of heat, such as red in the zygomatic area (cheek rash), hot sensation in the Five Centres (Five Center Heat).	Hot flashes
	Worsening of premenstrual syndrome
	Decreased libido (sex drive)
	Fatigue
Vertigo, tinnitus, poor memory, scant menses in female	Irregular periods
	Difficulty sleeping
Insomnia, dry throat, night sweating	Vaginal dryness; discomfort during sex
Vexation, pain in the back region, lumbago and aches in the knees; while the tongue was red with little coating and the pulse was faint and rapid	Urine leakage when coughing or sneezing
	Urinary urgency

This study has also provided further laboratory and clinical evidence on the safety and effectiveness of Chinese Medicine, and its use of therapeutic food in preventing and treating peri-menopausal symptoms associated with hormone

disorders. The research has demonstrated the possibility of an anatomo-physiological foundation in Chinese Medicine theory and diagnostic methods, with the results illustrating the correspondence linking the concept of Chinese Medicine diagnosis patterns and hormonal changes in middle age women.

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APPENDIX

Appendix 1

INFORMATION TO PARTICIPANTS

We would like to invite you to participate in a study which is investigating the effectiveness of Kiwi-Fruit Seaweed Extract on the metabolism of female reproductive hormones. This is a study being undertaken by PhD student researcher, Dr Lulu Fu (Chinese Medicine) at Victoria University. The study is supervised by Dr Hong Xu and Associate. Prof. Dr Jack Antonas from the School of Biomedical and Health Sciences.

Why do we need to set up this study?

Kiwi-Fruit Seaweed Extract (KFE) as a food therapy has been used to safely and effectively regulate reproductive endocrine disorders in Chinese Medicine (CM) clinical practice for more than ten years. It has been used to improve the quality of life of women suffering from endocrine disorders.

This study will investigate the possible effects of KFE on Chinese Medicine Meridians (such as the Liver Meridian), which may indicate the relationship between a disorder in Meridian function and the change in female reproductive hormones. The disorder of Meridian function will be measured by using non-invasive breast thermography, non-invasive Magnetic tests and the change of female reproductive hormones will be tested by the ratio of bio-markers in the urine. The bio-markers may indicate preclinical breast changes, because these signs may indicate the early development of breast cancer.

This study also will investigate the impact of KFE on the physical and psychological well-being of women, using a self-estimate questionnaire form. All the research methods are safe and have been used in Australia and the USA for more than 10 years.

What is KFE?

KFE is a Chinese food formula, Kiwi-fruit – Seaweed Extract (KFE), which is a safe food therapy that has been on the market for more than ten years in China.

KFE contains isoflavones which are categorised as phytoestrogen, which reduce the risk of breast cancer. Phytoestrogen in KFE can benefit female hormone metabolism in peri menopausal women.

Who can participate?

- Female, Melbourne resident
- Aged from 40 to 55

Criteria for applicant exclusion

- Pregnant or breast feeding
- Positive mammogram or ultrasound diagnosis of breast cancer
- Undertaking hormone therapy or taking any form of contraceptive pills
- Taking tamoxifen or undergoing chemotherapy after surgical treatment of breast cancer
- Suffering from liver and/or kidney disease
- Being post-menopausal or experiencing amenorrhoea
- Food allergy or history of intolerance to Kiwi, Seaweed, Lactose or Tea
- Suffering from a major chronic disorder and taking medications

What will be required of the participant?

- To drink KFE or a placebo twice a day
- To take a morning urine collection on consultation day; a container (an urination collection kit) is provided for bring the sample to the consultation.
- To have Chinese Medicine diagnostic assessments five times during the research and three times of Magnetic test on Chinese Medicine Meridian

- To attend two non-invasive image tests of breast thermography which are at the beginning and the end of research
- To complete a self-assessment questionnaire on your general health at the beginning and at the end of the research

Procedure for urine collection kit

- Open a prepared bag which includes a collecting sample container and a large container, glove, and cleaning material for participant
- Clean the genital area with a swab provided before collecting the urine
- Put the glove on, collect urine sample in the sample container provided
- After collection of urine all the cleaning material and gloves will be discarded in a plastic bag
- Add ice in the large container provided by the researcher and stirred the ice for 2 seconds
- Sample container (provided) will be placed at the centre of the large container
- Samples will be stored in the –10 C^0 large container and delivered to a laboratory of Victoria University by the researcher. The biomedical and Health Science lab has a storage facility at -18 C^0

Procedure of Magnetic test

- The participants will prepare for the examination (take the top clothes off) in privacy then lying down in a supine position with the arm at 90 degree abduction.
- A polarized light will be used for detecting changes in the magnetic flux and the results will be recorded on a map which is an anatomical drawing of the human trunk. The polarized light instrument produces a low intensity light, which will be held to the skin about 1 cm away from the skin. The record will include depiction of any defective area found - in two dimensions, length and width.

- The area on the body, to be tested, will be restricted to between the mid-clavicular line and mid-axillary line, from the iliac crease to clavicula and the axillary line at the trunk area, which is the Chinese Medicine Liver Meridian area. The measurement will be performed at the beginning of study and repeated every 10 days.

Thermographic test

- The participants will need to expose their breast to the thermographics camera.
- The participants will prepare for the examination (take the top clothes off) in privacy then sitting at the front of the thermogram camera
- If the thermography result is positive, visiting GP is recommend, even though the tests are only used for research and not for clinical diagnosis.
- If the result of the last thermography is still positive, a further follow-up consultation by a GP will be recommended

Will you have any risk?

Although rare, you may be allergic to kiwi-fruit or seaweed products and you may have an allergic reaction. If you have allergic history of kiwi or seaweed intake, you should not attend the research. If you have any allergic reaction (e.g. itchy skin) after taking the material, you should report to the researcher and stop taking the material immediately. You may need to see local GP for anti allergic therapy.

How much time is involved?

Participation in this study will involve approximately one hour per 2 weeks for the consultation which will be five times totally during the research. The consultation will be free of charge and the time frame for the research will be two months.

Who do I contact?

- If you agree to participate in the research please call Dr Lulu Fu (Chinese Medicine) on 03 9919 2769 or 03 9331 3921

Important notes

All information such as personal details and test results will be kept confidential at Victoria University.

Any queries or concerns can be addressed to:

Principal Researcher: Dr Hong Xu 03 9919 2765
Associate Professor: Dr Jack Antonas 03 9919 2219

If you have any queries or complaints about the way you have been treated, you may contact the Secretary, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 phone (03) 9919 4148

Appendix 2

CONSENT FORM
FOR PARTICIPANTS
INVOLVED IN THE RESEARCH

INFORMATION TO PARTICIPANTS

We would like to invite you to be a part of a study into "A study of the effectiveness of kiwi-fruit-seaweed extract on the metabolism of female reproductive hormones"

The aim of this research is to investigate the effect of a Chinese food therapy formula-Kiwi-Fruit Seaweed Extract (KFE) on the metabolism of female reproductive hormones in middle aged women. The research also investigates the relationship between Chinese Medicine diagnostic patterns and hormonal disorders. This study will help us refine the Chinese Medicine diagnosis using hormonal changes and breast image tests, which include the breast's magnetic field and thermography changes. The benefits of this research aim to improve the general wellbeing of middle age women and to prevent the diseases which are related to female reproductive hormones, such as breast cancer.

CERTIFICATION BY SUBJECT

I,

_____(name in print)

of _____(street or road)_____(suburb)_____

(Post code)_____

Certify that I am at least 18 years old* and that I am voluntarily giving my consent to participate in the study:

"A study of the effectiveness of Kiwi-Fruit Seaweed Extract on the metabolism of female reproductive hormones"

being conducted at Victoria University by:

Dr Hong Xu, Ass. Prof. Jack Antonas,

I certify that the objectives of the study, together with any risks and safeguards associated with the procedures listed hereunder to be carried out in the research, have been fully explained to me by:

Dr Lulu Fu (Chinese Medicine)

I freely consent to participate, involving the use of these procedures on me.

- To drink a KFE or a placebo twice a day
- Required to take in a morning urine collection to the consultations
- To have a Chinese Medicine diagnosis assessment and a Magnetic test of the Chinese Medicine Meridian
- To attend one breast thermography image test.
- To complete a self-assessment questionnaire on your general health at the beginning and at the end of the research

I certify that I have had the opportunity to have any questions answered and that I understand that I can withdraw from this study at any time and that this withdrawal will not jeopardise me in any way.

I have been informed that the information I provide will be kept confidential.

Participant Name (in print): _____

Signed by participant: _____

Witness other than the researcher: (signature)

_____

Date: _____2007

Any queries about your participation in this project may be directed to;
Principal researcher: Dr Hong Xu 03 9919 2765, Associate Professor: Dr Jack Antonas 03 9919 2219

If you have any queries or complaints about the way you have been treated, you may contact the Secretary, Victoria University Human Research Ethics Committee, Victoria University, PO Box 14428, Melbourne, VIC, 8001 phone (03) 9919 4148

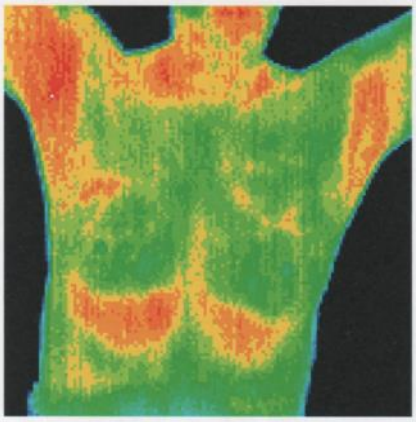
Appendix 3

Wellbeing

All normal protocols were observed.

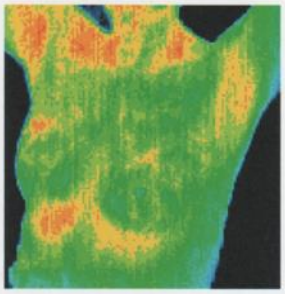
REPORT : THERE ARE NO SIGNIFICANT THERMAL ASYMMETRIES SEEN IN THE BREASTS.
THERE IS NO EVIDENCE TO SUGGEST NEOVASCULARITY.
THIS STUDY IS SUITABLE TO BE ARCHIVED FOR FUTURE ANNUAL COMPARISON.

PATTERNS OF HYPERTHERMIA ARE NOTED ON THE ANTERIOR RIGHT SHOULDER, THE MOUTH AND NASAL SINUSES. SUGGEST FOLLOW-UP.



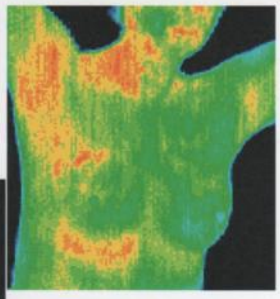
Left Breast
°C

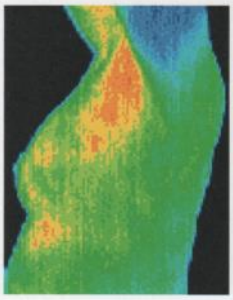
Maximum	32.4
Average	31.4
Minimum	30.1
Std Dev.	0.35

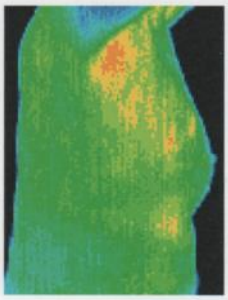


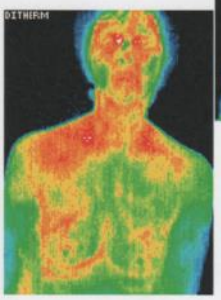
Right Breast
°C

Maximum	32.7
Average	31.5
Minimum	30.4
Std Dev.	0.44









Wellbeing Diagnostic Clinic
159 New South Head Rd Edgecliff, NSW 2027
Phone : 1300 366 779
Email : wellness@bigpond.com

Wellbeing
DIGITAL INFRARED THERMAL IMAGING

Patient: Karen FOGARTY Scan Date: 8-12-2008
Date of Birth: 7-10-66 Scan Ref: fogartykaren4
Referring Doctor: Dr Lulu FU Thermographer: Claude Penkemoun

Clinical Notes from patient: Routine check

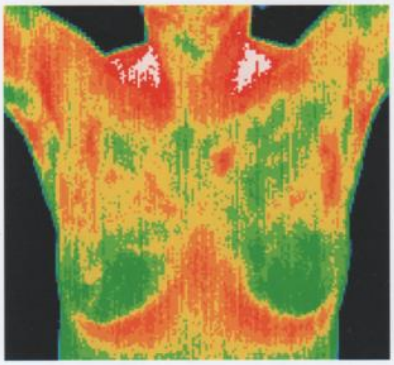
All normal protocols were observed.

REPORT : .THERE ARE NO SIGNIFICANT THERMAL ASYMMETRIES SEEN IN THE BREASTS

THERE IS NO EVIDENCE TO SUGGEST NEOVASCULARITY

THIS STUDY IS SUITABLE TO BE ARCHIVED FOR FUTURE ANNUAL COMPARISON.

FRONTAL SINUSITIS AND GINGIVITIS CONSPICUOUS. SUGGEST FOLLOW-UP.

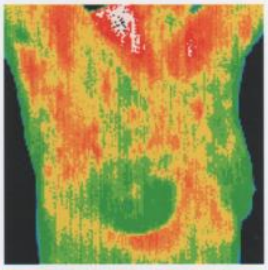


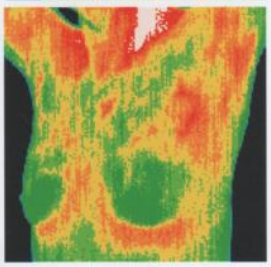
Left Breast
°C

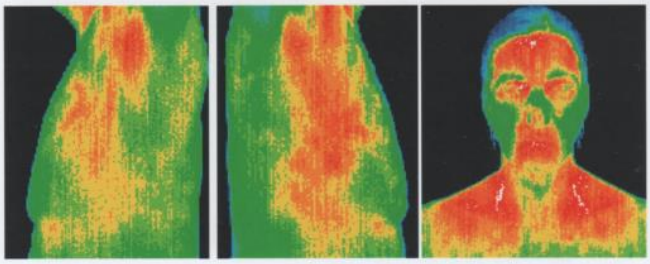
Maximum	32.6
Average	31.7
Minimum	30.5
Std Dev.	0.30

Right Breast
°C

Maximum	32.7
Average	31.9
Minimum	31.1
Std Dev.	0.34







Wellbeing Diagnostic Clinic
159 New South Head Rd Edgecliff, NSW 2027
Phone : 1300 366 779
Email : wellness@bigpond.com

Appendix 4: TABLES

Table 1: Results of the pre-trial and post-trial results between the magnetic test and the biomarker test

Group	Magnetic Test Pre-trial Liver meridian		Post-trial Liver meridian		Urinary test for 2OHE/16a-OHE			
	Left	Right	Left	Right	1st sample	2nd sample	3rd sample	4th sample
Control								
1	0	10	0	10	1.34	0.93	0.61	1.00
2	10	10	6	2	1.17	0.98	1.31	0.83
3	0	10	0	0	1.43	1.12	1.87	1.44
4	0	0	0	0	1.31	0.97	1.95	0.79
5	0	10	0	0	1.49	0.96	1.09	0.99
6	0	0	0	0	1.01	1.96	1.49	1.01
7	10	10	10	10	0.83	1.08	0.60	1.92
8	10	10	10	10	1.16	1.08	1.13	1.16
9	0	10	10	10	1.79	1.63	0.90	0.58
10	0	0	0	0	1.21	1.48	0.89	1.22
11	0	10	0	0	1.25	0.95	1.05	1.05
12	10	10	0	0	1.12	1.30	1.21	0.99
13	0	10	0	10	0.85	1.00	0.91	0.71
14	10	10	10	10	1.00	1.89	2.20	0.79
15	0	10	0	10	0.57	0.94	0.67	0.88
16	10	10	0	10	0.57	0.69	0.63	0.61
17	10	10	10	10	0.69	1.28	0.94	0.94
18	0	10	10	10	0.56	1.29	0.85	0.54
Intervention								
1	8	7	0	0	0.79	1.14	1.24	1.24
2	10	10	0	8	1.14	1.81	1.19	1.38
3	10	10	0	6	1.11	1.32	1.20	1.50
4	10	10	1	1	1.32	1.17	1.19	1.31
5	10	10	0	10	0.83	1.10	1.21	1.25
6	10	10	6	6	0.60	0.74	0.85	1.51
7	10	10	1	1	0.89	1.35	1.34	1.14
8	10	10	0	0	0.74	0.99	0.79	1.54
9	0	10	0	0	0.56	0.68	0.67	1.10
10	10	10	0	0	0.81	1.41	1.96	2.12
11	0	10	0	0	0.98	1.04	1.15	1.56
12	0	0	0	0	0.91	0.98	1.10	0.91
13	10	10	0	6	0.95	0.96	1.07	1.41
14	0	0	0	0	0.95	1.01	1.29	1.58
15	0	10	0	0	0.94	1.00	1.17	1.54
16	0	10	0	0	1.02	1.75	1.63	2.05
17	10	10	0	0	1.17	1.61	1.96	1.68
18	10	10	0	2	0.72	0.91	0.77	0.80

Note:* 1st urinary sample as the baseline, results obtained from triplicate tests for each sample

** 2nd urinary sample after the intervention of KFE and placebo

*** 3rd urinary sample

****4th urinary sample as the final urinary sample

Table 2: Comparison between the results of CM diagnosis and PEMF magnetic test

	Magnetic Test				**CM Pattern**		**Magnetic Test**				**CM Pattern**	
	Pre-trial		Post-trial				Pre-trial		Post-trial		Liver Kidney	
Goup	Liver meridian		Liver meridian		Liver Qi Stagnation		Kidney meridian		Kidney meridian		Yin Deficiency	
KFE	Left	Right	Leftr	Right	Pre-trial	Post-trial	Left	Right	Left	Right	Pre-trial	Post-trial
1	8	7	0	0	7.8	1.6	10	0	0	0	3.1	0.5
2	10	10	0	8	6.3	3.4	10	0	0	0	4.6	2.6
3	10	10	0	6	7.6	3.5	0	0	0	10	3.9	2.4
4	10	10	1	1	6.3	4.1	0	10	0	10	4.5	2.3
5	10	10	0	10	6.4	3.3	0	10	0	10	5.2	1.7
6	10	10	6	6	7	2.9	10	10	0	0	5.6	0.5
7	10	10	1	1	6.7	5.4	0	10	4	10	2.2	4.2
8	10	10	0	0	8.3	2.7	10	10	10	10	4.9	2.3
9	0	10	0	0	4.9	2.3	0	10	0	10	5.4	1.7
10	10	10	0	0	6.5	3.3	10	10	0	10	5.3	1.5
11	0	10	0	0	5.6	1.7	0	10	0	10	3.6	0.8
12	0	0	0	0	5.8	1.9	10	0	10		2.6	0.6
13	10	10	0	6	6.5	4.2	0	10	0	10	6.2	5.9
14	0	0	0	0	6.2	3.7	0	10	8	10	5.5	1.8
15	0	10	0	0	6.2	3.5	0	10		10	4.8	1
16	0	10	0	0	6.3	3.9	0	10	0	10	3.8	1.6
17	10	10	0	0	4.8	2.3	10	10	0	10	3.6	1.3
18	10	10	0	2	7.9	3.8	2	0	0	3	0.6	0.6
Control												
1	0	10	0	10	6.3	5.6	0	10	0	10	4.3	4.8
2	10	10	6	2	5.8	5.8	6	0	0	10	5.4	5.4
3	0	10	0	0	6.2	6.2	0	10	0	10	5	4.9
4	0	0	0	0	6.2	6	0	6	0	10	3.1	3.1
5	0	10	0	0	6.2	5.3	10	10	0	10	5	4.9
6	0	0	0	0	5.9	5.9	0	10	0	10	5.7	5.7
7	10	10	10	10	4.6	4.9	0	0	0	0	1.8	2
8	10	10	10	10	4.7	4.7	10	10	10	10	4.8	4.8
9	0	10	10	10	5.8	6.1	0	10	0	10	4.7	4.5
10	0	0	0	0	7.2	7.1	4	10	0	10	4.4	4.4
11	0	10	0	0	6.2	5.5	0	0	0	6	3.7	4.2
12	10	10	0	0	4.3	3.5	10	0	10	0	3	2.9
13	0	10	0	10	6.2	6	0	10	0	10	1.3	0.9
14	10	10	10	10	6.4	6.5	0	10	0	10	2.1	2.1
15	0	10	0	10	6.3	6.4	0	10	0	10	3.4	3.4
16	10	10	0	10	6.1	6.1	0	10	0	10	4.7	4.7
17	10	10	10	10	6.8	6.5	0	10	0	10	6.2	6.2
18	0	10	10	10	5.3	5.3	0	10	0	10	1.7	1.5

Table 3: Pre-trial and post-trial results between SF-36, Liver Qi Stagnation and Liver Kidney Yin Deficiency

SF-36 after improved value	SF-36 total before mean	SF-36 total after mean	Liver Qi ST before - After value	Liver Qi Stag before	after	L K Yin Deficiency before - After value	Liver Kidney Yin Deficiency before	after
0.5	7.9	8.4	8.0	7.8	1.6	2.0	3.1	0.5
1.1	6.9	8.0	5.0	6.3	3.4	3.0	4.6	2.6
0.7	7.6	8.3	6.0	7.6	3.5	4.0	3.9	2.4
0.9	7.3	8.2	4.0	6.3	4.1	4.0	4.5	2.3
0.0	8.8	8.8	3.0	6.4	3.3	2.0	5.2	1.7
0.2	7.9	8.1	6.0	7	2.9	7.0	5.6	0.5
-1.3	7.1	5.8	3.0	6.7	5.4	1.0	2.2	4.2
0.0	7.2	7.2	7.0	8.3	2.7	2.0	4.9	2.3
-0.1	5.6	5.5	2.0	4.9	2.3	5.0	5.4	1.7
0.9	8.7	9.6	5.0	6.5	3.3	2.0	5.3	1.5
0.3	6.7	7.0	5.0	5.6	1.7	1.0	3.6	0.8
2.2	3.8	6.1	5.0	5.8	1.9	3.0	2.6	0.6
1.1	5.3	6.4	6.0	6.5	4.2	3.0	6.2	5.9
1.1	7.5	8.6	6.0	6.2	3.7	3.0	5.5	1.8
0.9	7.3	8.1	5.0	6.2	3.5	3.0	4.8	1
0.3	7.7	8.0	0.0	6.3	3.9	4.0	3.8	1.6
-0.1	8.6	8.5	3.0	4.8	2.3	2.0	3.6	1.3
0.0	8.0	8.1	3.0	7.9	3.8	0.0	0.6	0.6
8.6			82.0			51.0		
0.7	8.1	8.9	0.0	6.3	5.6	0.0	4.3	4.8
0.1	8.4	8.5	-1.0	5.8	5.8	0.0	5.4	5.4
0.4	7.7	8.0	-1.0	6.2	6.2	0.0	5	4.9
-1.3	8.3	7.0	0.0	6.2	6	1.0	3.1	3.1
-0.3	8.5	8.1	0.0	6.2	5.3	0.0	5	4.9
-0.7	8.2	7.5	0.0	5.9	5.9	0.0	5.7	5.7
0.0	8.0	8.0	0.0	4.6	4.9	0.0	1.8	2
-0.1	7.5	7.4	0.0	4.7	4.7	0.0	4.8	4.8
2.0	5.5	7.5	0.0	5.8	6.1	-1.0	4.7	4.5
0.1	4.1	4.2	0.0	7.2	7.1	-1.0	4.4	4.4
-0.3	8.0	7.7	0.0	6.2	5.5	0.0	3.7	4.2
-1.4	8.9	7.6	0.0	4.3	3.5	0.0	3	2.9
0.0	6.1	6.1	0.0	6.2	6	0.0	1.3	0.9
0.9	4.5	5.3	0.0	6.4	6.5	0.0	2.1	2.1
-0.8	7.7	6.9	0.0	6.3	6.4	0.0	3.4	3.4
0.5	6.9	7.4	0.0	6.1	6.1	0.0	4.7	4.7
-0.2	6.9	6.7	0.0	6.8	6.5	0.0	6.2	6.2
0.2	7.7	7.9	0.0	5.3	5.3	0.0	1.7	1.5
-0.2			-2.0			-1.0		

Table 4: Pre-trial and post-trial PEMF results comparison between the KFE ngroup and the control group of peri-menopausal women (Refer table 4.5)

	Liver Meridian				**Kidney Meridian**			
	Before		**After**		**Before**		**After**	
Group								
	Left	Right	Left	Right	Left	Right	Left	Right
Control								
1	0	10	0	10	0	10	0	10
2	10	10	6	2	6	0	0	10
3	0	10	0	0	0	10	0	10
4	0	0	0	0	0	6	0	10
5	0	10	0	0	10	10	0	10
6	0	0	0	0	0	10	0	10
7	10	10	10	10	0	0	0	0
8	10	10	10	10	10	10	10	10
9	0	10	10	10	0	10	0	10
10	0	0	0	0	4	10	0	10
11	0	10	0	0	0	0	0	6
12	10	10	0	0	10	0	10	0
13	0	10	0	10	0	10	0	10
14	10	10	10	10	0	10	0	10
15	0	10	0	10	0	10	0	10
16	10	10	0	10	0	10	0	10
17	10	10	10	10	0	10	0	10
18	0	10	10	10	0	10	0	10
Intervention								
1	8	7	0	0	10	0	0	0
2	10	10	0	8	10	0	0	0
3	10	10	0	6	0	0	0	10
4	10	10	1	1	0	10	0	10
5	10	10	0	10	0	10	0	10
6	10	10	6	6	10	10	0	0
7	10	10	1	1	0	10	4	10
8	10	10	0	0	10	10	10	10
9	0	10	0	0	0	10	0	10
10	10	10	0	0	10	10	0	10
11	0	10	0	0	0	10	0	10
12	0	0	0	0	10	0	10	0
13	10	10	0	6	0	10	0	10
14	0	0	0	0	0	10	8	10
15	0	10	0	0	0	10	0	10
16	0	10	0	0	0	10	0	10
17	10	10	0	0	10	10	0	10
18	10	10	0	2	2	0	0	3

Notes: KFE group pre-trial PEMF test (Mean ± SD): Liver Meridian 7.64 ± 4.18 Kidney Meridian 5.61 ± 4.99

KFE group post-trial PEMF test (Mean ± SD): Liver Meridian 1.33 ± 2.68 Kidney Meridian 4.58 ± 4.88

Control group pre-trial PEMF test (Mean ± SD): Liver Meridian 6.11 ± 4.94 Kidney Meridian 4.89 ± 4.86

Control group post-trial PEMF test (Mean ± SD): Liver Meridian 4.67 ± 4.95 Kidney Meridian 4.89 ± 5.00