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DIETARY PHYTOESTROGENS AND HORMONE-RELATED HEALTH CONDITIONS IN MEN AND WOMEN

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Declaration of Authenticity

This thesis contains no material has been accepted for the award of any other degree or diploma in any university or institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by any other person, except where due reference is made.

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Andreanyta Meliala

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List of Publications and Presentations

List of publications and presentations arising during the period of this thesis:

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Dalais FS., **Meliala A.**, Wahlqvist ML. Maternal and Cord Blood Phytoestrogen Levels in Indonesian women. Third International Symposium on the Role of Soy in Preventing and Treating Chronic Disease. 1999 Washington DC, USA.

Meliala A. The Profile of Menopausal Women in Yogyakarta Indonesia. South East Asian Women Annual Workshop. 2000 Monash University, VIC Australia.

List of Abbreviations

°C	degree Celsius
>	greater than
<	less than
%	percent
BMI	body mass index
cm	centimetre
DHT	5α-dihydrotestosterone
EGF	epidermal growth factor
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
DMSO	dimethylsulfoxide
DMBA	dimethylbenz(a)anthracene
g	gram
GC	gas chromatography
h	hour
HPLC	high performance liquid chromatography
HRT	hormone replacement therapy
IGF	insuline-like growth factor
ISP	isolated soy protein
mg	milligram
mg/mL	milligram per millilitre
min	minute

mL	millilitre
n	number
ng	nanogram
nm	nanometre
PSA	prostate specific antigen
SD	standard deviation
SHBG	sex hormone binding globulin
UV	ultraviolet
µg	microgram
µL	microlitre

Summary

Phytoestrogens (plant oestrogens) have evolved in the area of hormone-related health conditions, due to their structural similarity to mammalian oestrogens. The potential beneficial effects of isoflavones and lignans, the most researched class of phytoestrogens, have triggered extensive research into investigating the role of phytoestrogens in a number of hormone-related health conditions, such as women's health (menopause and osteoporosis) cardiovascular disease and cancers.

The eating habits of different ethnicities play an important role in health outcomes. A range of dietary soy foods, which are rich in isoflavones, is part of the food culture in many Asian populations, but not in Western societies. The soy-culture difference has been reported to be associated with a different disease pattern. Published data show a low incidence of prostate cancer in Asian (Japanese, Chinese) men where the consumption of soy foods is relatively higher than that of Western men. Another class of isoflavones, the lignans, which are abundant in linseeds, has been reported to generate similar effects to those associated with isoflavones.

Two research projects included in this thesis add more novel data to this area. The first project was a clinical trial involving Australian men diagnosed with prostate cancer. Three different dietary manipulations, soy bread (high in isoflavones), soy and linseed bread (high in isoflavones and lignans) and

wheat bread (placebo/control), were performed to assess the effects of dietary phytoestrogens on prostate cancer biomarkers. Changes in PSA and free:total PSA ratio (prostate cancer biomarkers) of the soy bread group were significantly different from those of the wheat bread. A combination of soy and linseed (isoflavones and lignans) did not show a similar effect to the consumption of isoflavones alone.

Epidemiological evidence has shown different profiles of menopausal symptoms in women from different cultures and societies. Published studies have postulated that populations with high soy consumption experience less menopausal-related problems. The structural similarity of phytoestrogens to endogenous oestrogens has led to extensive research into phytoestrogens being used as an alternative to hormone replacement therapy (for the treatment of menopausal symptoms). The second research project in this thesis was carried out in Yogyakarta, Indonesia. Yogyakarta has its own unique culture in terms of tempe, a traditional fermented form of soy, originating from this precinct. Tempe has been part of its culture since the 1700s. Therefore it was assumed this population would be high consumers of soy foods. A cross-sectional study of among menopausal women in Yogyakarta was conducted to assess the intake of soy food intake, the urinary excretion of isoflavones and the menopausal profile. The average daily intake of soy-based foods was found to be higher than that assessed in other Asian populations. This high intake of soy was also reflected in the urinary excretion concentrations of isoflavones. In agreement with earlier published studies,

perimenopausal women were more symptomatic than the pre- and post menopausal women. Looking at different levels of intake, medium soy consumers were the least symptomatic compared to the low and high soy consumers. Psychological menopausal symptoms were predominant, while vasomotor symptoms were very mild.

In conclusion, firstly, among Australian men diagnosed with prostate cancer, dietary isoflavones (in the form of soy bread) were associated with improvements in the prostate biomarkers measured, but this association was not found when dietary isoflavones were combined with linseed. Secondly, the menopausal women in Yogyakarta who participated in this study were high consumers of soy foods and, these women displayed a different profile of menopausal symptoms from their Western counterparts. In particular, this study highlights that perimenopausal women in Yogyakarta appear to benefit from soy and its constituents at a certain dose range.

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CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

The role of phytochemicals, particularly phytoestrogens in human health has been an extensively investigated area in nutrition research. Phytoestrogens exhibit oestrogenic and anti-oestrogenic effects, and have been postulated to be of benefit particularly in several hormone dependent health conditions. Epidemiological studies have highlighted a protective link between the high consumption of two classes of phytoestrogens (the isoflavones and lignans), and certain cancers, menopausal symptoms and heart disease. This protective effect has been supported by *in vitro* and *in vivo* studies including biochemical and clinical intervention studies involving women's health and cardiovascular disease. The projects studied in this thesis further our knowledge about the role of dietary phytoestrogens, particularly isoflavones and lignans, in prostate cancer and menopausal health.

1.1 DIETARY PHYTOESTROGENS

Dietary factors are considered to be important environmental risk determinants for some Western diseases (Adlercreutz, 1998; Mazur et al, 1998). A diet that is low in fat and high in complex carbohydrates is associated with a low risk of chronic disease. In recent times, research has focused on phytochemicals, the non-nutrient components in food. Dietary phytoestrogens are one of the most researched phytochemicals. Soybeans, a

major source of isoflavones, also contain a number of other constituents that may have potential health benefits. These include the phytoesterols, protease inhibitors, inositol hexaphosphate and saponins. Soybeans and their derived products are common foods in many Asian countries where the incidence rates of Western disease are considered very low, such as Japan, China, Indonesia, and Korea (Adlercreutz, 1998).

Another major source of dietary phytoestrogens is linseed. Linseed is the richest plant source of lignans, another class of phytoestrogens considered important to human health.

1.2 HORMONE DEPENDENT HEALTH CONDITIONS :

With increasing life expectancy due to improved living conditions, the proportion of individuals aged 65 and over is also increasing. This demographic change may be accompanied by longer periods of morbidity unless the health problems in aging populations receive greater attention. Physical changes that occur with aging may be related to a decrease in hormonal activity. During normal aging there is a decrease in three hormonal systems; (1) oestrogen in women and testosterone in men, (2) dehydroepiandrosterone, and (3) growth hormone/insulin-like growth factor (Lamberts et al., 1997). Some individuals may age without symptoms, but for many, this decrease in hormone causes a number of health problems.

1.2.1 PROSTATE CANCER

Prostatic disease and its associated difficulties is a feature of the aging process (Griffiths et al., 1998). Cancer of the prostate is the most common cancer affecting elderly males in Western society, with the risk increasing with older age. The causes of this disease remain unclear, although it is known that in its early stages, prostate cancer is hormone dependent (Peeling & Griffiths, 1986). Interestingly, it is known that prostate cancer does not develop in men castrated before puberty, but paradoxically, it is clinically recognised at a period of life when plasma levels of androgen are generally declining (Griffiths et al., 1998).

Prostate cancer is a community problem for some developed countries namely the United States and Australia. In 1999, there was an estimated 179,000 new cases of prostate cancer in the US (www.prostate.com) In Australia, there are approximately 12,000 new cases anticipated yearly (www.prostatecancer.com.au).

Prostate cancer tends to affect men over 50, but genetics, ie a positive family-history, belonging to the Black-American race or eating a diet high in fat increases the risk (Pienta & Peggy, 1993). Epidemiological studies show a large variation in the incidence and mortality of prostate cancer among different geographic and ethnic groups, particularly between Eastern and Western societies. Although latent prostate cancer exists in quite similar proportions between the two, the clinical manifestation is strikingly different

(Arnot, 2000; Morton 1999; Peeling & Griffiths,1986). The association of dietary factors such as fat intake, vitamin D, phytoestrogens, carotenoids, fat soluble antioxidant vitamins, energy intake, and body composition on the risk of prostate cancer has been studied over the past decades. For example, phytoestrogens, have been shown to inhibit the growth of prostate cancer in both *in-vivo* and *in-vitro* models. However, despite the community burden caused by this type of cancer, prostate cancer studies, particularly in human subjects, are scarce.

1.2.2 MENOPAUSE

Menopause is defined as 12 consecutive months of natural amenorrhoea. The average age of menopause is 51 years in Western countries, with an age range of 40-60 years (Eden et al., 1996). There are a number of symptoms, both short and long-term symptoms associated with menopause. The incidence of hot flushes and vaginal dryness, represent short-term problems for most Western women, while long-term problems arise due to an oestrogen deficiency, such as increased risk of osteoporosis and cardiovascular disease. Epidemiological studies indicate that Asian women have less severe symptoms and fewer complaints at the time of menopause (Boulet et al., 1994). In Asian countries, soy consumption is usually greater than that consumed in Western countries (Adlercreutz et al., 1992). This has led to the hypothesis that phytoestrogens in soy, may behave as oestrogen agonists in an oestrogen deficient environment such as menopause.

1.3 HYPOTHESES

It was hypothesised that:

- A phytoestrogen-rich diet will favourably affect biomarkers of prostate cancer.
- There is an association between a phytoestrogen-rich diet and menopausal symptoms.

1.4 AIMS

- To determine the effects of dietary soy isoflavones on prostate cancer bio-markers in men diagnosed with prostate cancer.
- To study the combined action of dietary isoflavones and lignans on prostate cancer bio-markers in men diagnosed with prostate cancer.
- To assess the dietary phytoestrogen intake (focusing on soy-based foods) among women in Yogyakarta, Indonesia.
- To assess the urinary isoflavone excretion of postmenopausal women in Yogyakarta, Indonesia.
- To assess the profile of menopausal symptoms among menopausal women in Yogyakarta, Indonesia.
- To study the association between dietary phytoestrogen intake and the profile of menopausal symptoms.

CHAPTER TWO

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 FOOD AND HEALTH

In recent times, there has been an increasing interest in examining the relationship between certain food components and health. It is commonly known that an adequate consumption of macronutrients and a variety of vitamins and minerals (micronutrients) in the diet, coupled with regular exercise is required to achieve good health. Additionally, it is now considered other compounds found in food may be needed for optimum health. These compounds may protect against cell damage (resulting from exposure to environmental stresses such as free radicals) that may induce degenerative diseases such as cancers and cardiovascular diseases. These compounds are called phytochemicals. Phytochemicals, including phytoestrogens, function as antioxidants and phytoalexins in plants (Anderson & Garner, 1998) and express their protective mechanisms when under exogenous threat or attack from bacteria or physical damage (Dalais, 1998). These biologically active compounds in foods have been identified as having a number of beneficial health properties.

Despite phytochemicals being available from a variety of plant foods, there is a growing tendency to obtain them from a more isolated form such as a pill or

tablet. One possible reason for this trend is the practicality of commercialising a pill since much of the research is driven by industry. Moreover, certain health foods containing high concentrations of phytochemicals may not fit the taste profile of certain communities, hence making it rather difficult to introduce it as part of the regular diet. Phytochemicals contained in foods may be potentially protective for some populations since phytochemical-rich foods have been part of some food cultures for centuries. Furthermore, phytochemicals may only work as part of the food matrix and not in isolation. Therefore, the potentially desirable effects should not be identified with one simple component of a complex whole (Crotty, 1997).

Phytochemical-rich foods such as soy have been consumed for many generations in Japan, China, Korea and Indonesia (Adlercreutz, 1998). Epidemiological studies have shown that soy foods are associated with the lower incidence of certain cancers and other chronic diseases such as cardiovascular disease found in these communities (Adlercreutz et al., 1995).

2.1.1 FUNCTIONAL FOODS

Foods are a complex mixture of nutrients and other bioactive components. The potential of a particular food or food component to prevent some diseases has generated the development of functional foods. A food's primary function (according to the concept of a functional food) is its nutritive value and the secondary function is its sensory appeal or organoleptic properties (Astuti,

1999; Ichikawa, 1994). Its tertiary function refers to the physiological effect of the food, including its ability to neutralise harmful substrates, regulate body functions and physical conditions, prevent diseases and promote recovery and general good health (Ichikawa, 1994).

Functional foods include whole foods, fortified, enriched or enhanced with biologically active components that have been scientifically proven to prevent or treat certain diseases (especially in the early stages) when consumed as part of varied diet on a regular basis at effective levels (American Dietetic Association, 1999; Astuti, 1999). The development of functional foods so called pharmafoods or nutraceuticals, are still at an embryonic stage, although they have been popularly marketed in communities such as Japan (Goldberg, 1994). Soy-based foods for instance, are considered as functional foods in Japan (Astuti, 1999).

2.1.2 PHYTOESTROGENS

One of the most extensively researched phytochemicals is the phytoestrogen. Phytoestrogens are plant-derived compounds with an oestrogen-like action. In 1954, there were only 53 plants listed as possessing oestrogenic activity (Bradbury & White, 1954), but now there are more than 300 plants documented to contain these types of compounds (Mazur, 1998).

2.1.2.1 CLASSIFICATION AND STRUCTURE

2.1.2.1.1 CLASSIFICATION OF PHYTOESTROGEN

Phytoestrogens are classified into 3 categories, they are:

1. Isoflavones
2. Coumestans
3. Lignans

There is another class of partial phytoestrogens called the resorcylic acid lactones. Unlike isoflavones, coumestans (also known as isoflavonoids) and lignans, which are intrinsic oestrogenic components (since they are made in the plants), resorcylic acid lactones are not intrinsic components of food plants. Resorcylic acid lactones are secondary mold metabolites of certain fungal species (mainly *Fusarium*) (Price & Fenwick, 1985). They are therefore only partial phytoestrogens or mycoestrogens (Kurzer & Xu, 1997). The oestrogenic potency of phytoestrogens varies between classes, with coumestans being the most potent, followed by the isoflavones and the lignans as the least oestrogenic (Markiewicz, 1993; Welshons et al., 1987).

2.1.2.1.2 STRUCTURE OF PHYTOESTROGENS

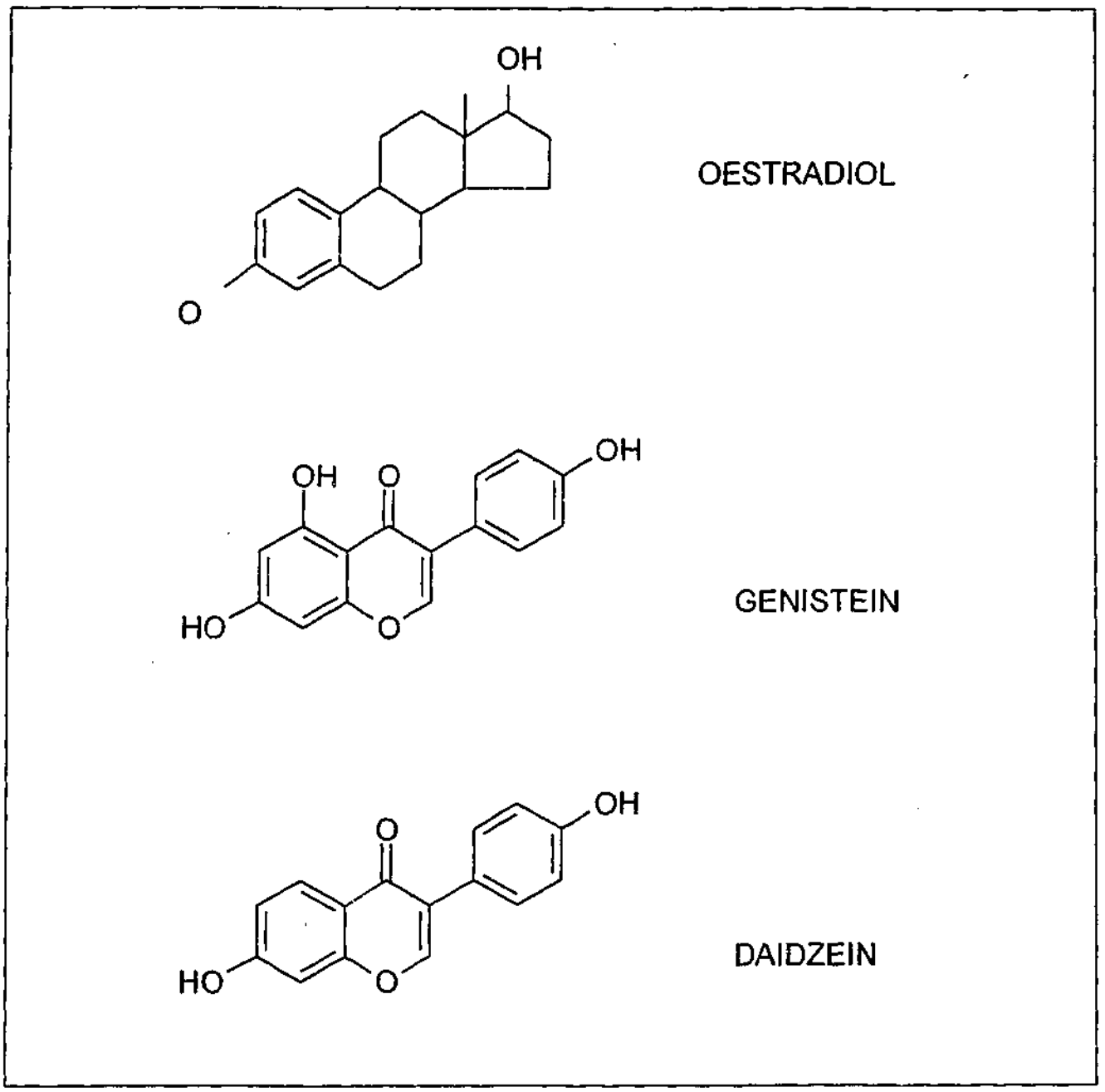
Phytoestrogens are characterised by a diphenolic ring structure, which allows them to bind to the oestrogen receptor, therefore enabling them to exert both an oestrogenic and anti oestrogenic hormonal effect (Tham et al., 1998; Adlercreutz et al., 1995; Setchell & Cassidy, 1999; Wahlqvist & Dalais, 1997).

However, the expected hormonal actions of phytoestrogens are influenced by the target tissue or organ, as well as the concentration dependency, receptor status and endogenous oestrogen concentration (Setchell & Cassidy 1999; Tham et al., 1998). This will be fu . .r described in Section 2.1.2.4.

2.1.2.1.2.1 ISOFLAVONE

The chemical structures of isoflavones (Figure 2.1) are strikingly similar to mammalian oestrogens (Setchell & Cassidy, 1999). The isoflavone aglucones, genistein and daidzein and their respective glucosides, genistin and daidzin are the most common naturally occurring phytoestrogens. Moreover, these isoflavones may be found as acetylglucosides and malonylglucosides, but occur predominantly as glucosides in plants (Price & Fenwick, 1985). Additionally, there are also methyl ether derivatives of isoflavones, such as formononetin (a daidzein methyl ether derivative) and biochanin A (a genistein methyl ether derivative).

Figure 2.1: Chemical structure of oestradiol, genistein and daidzein.



2.1.2.1.2.2 COUMESTANS

The oestrogenic coumestans include coumestrol and 4`methoxycoumestrol (Figure 2.2). Although a large number of coumestans have been isolated from plants, only the two mentioned above have been shown to exert oestrogenic activity (Kurzer & Xu, 1997).

Figure 2.2: Chemical structure 4`methoxycoumestrol and coumestrol (adapted from Kurzer and Xu, 1997)

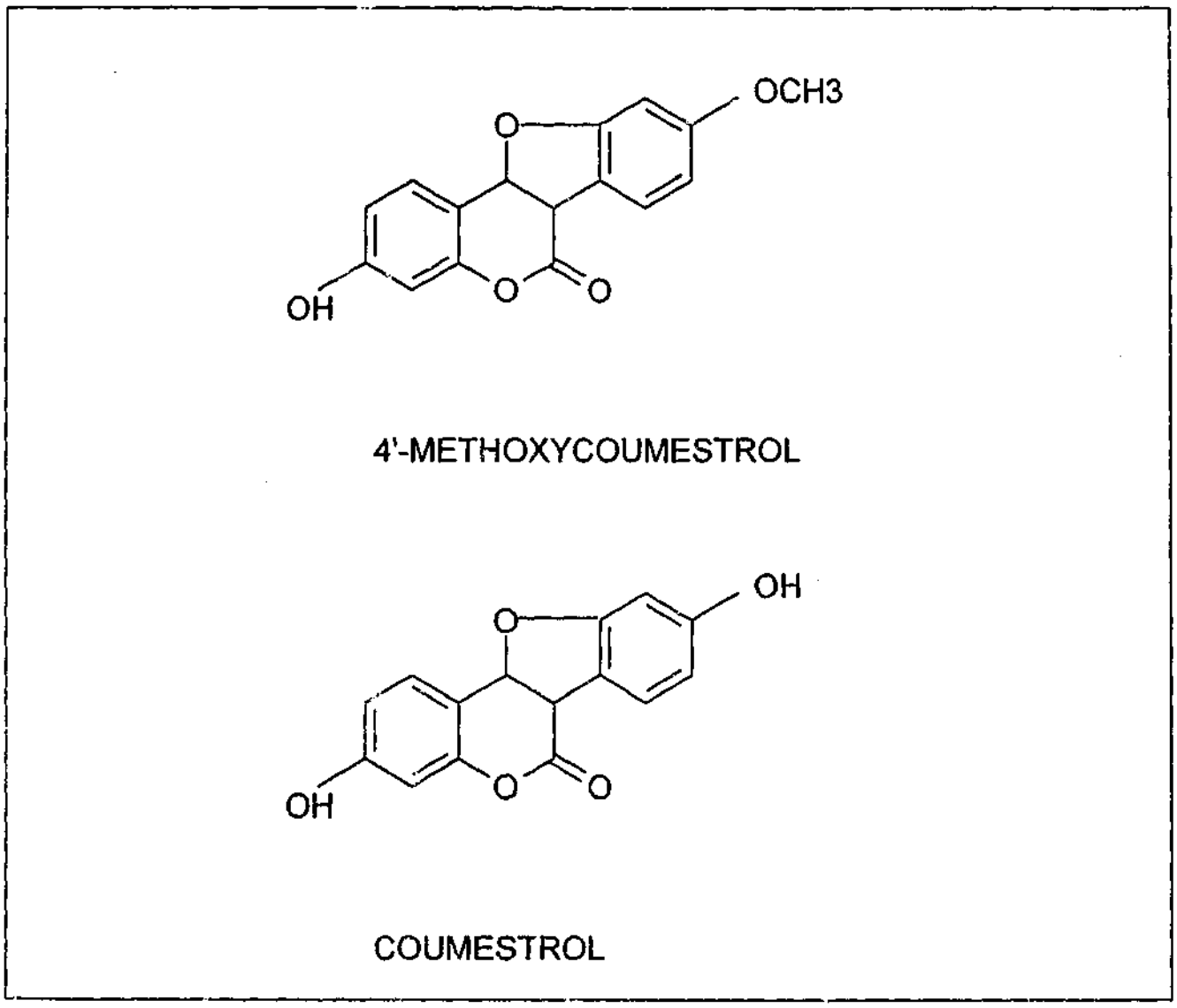
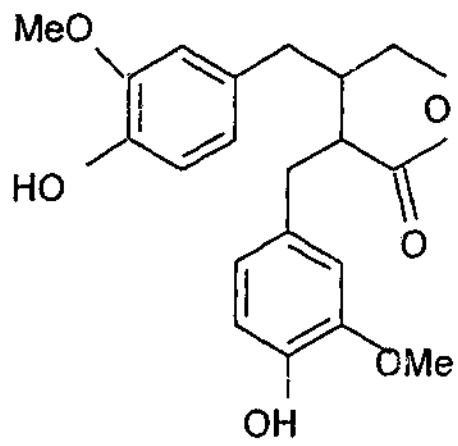
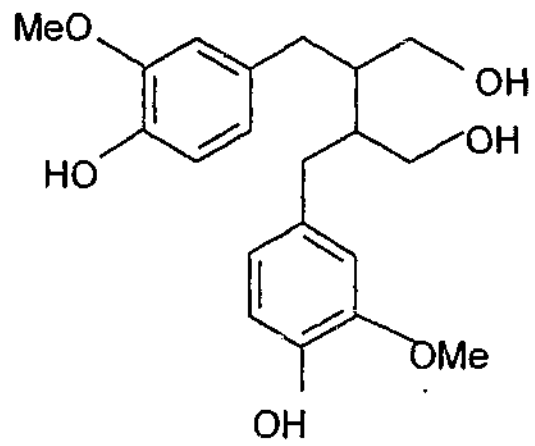


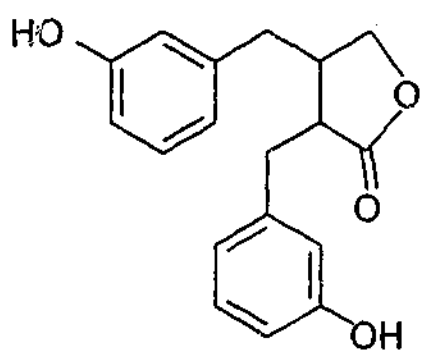
Figure 2.3. The chemical structures of lignan precursors (matairesinol and secoisolariciresinol) and mammalian lignans (enterolactone and enterodiol)



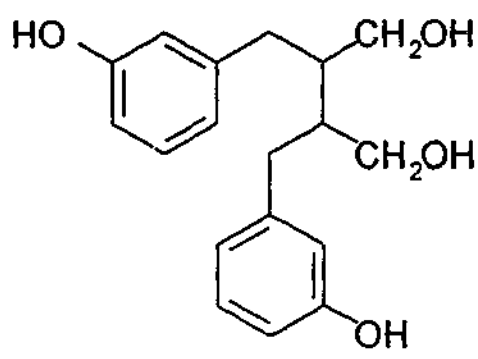
MATAIRESINOL



SECOISOLARICIRESINOL



Enterolactone



Enterodiol

2.1.2.1.2.3 LIGNANS

Lignans are present in plant foods (as plant lignans) as well as in human biological fluids (as mammalian lignans) (Kurzer & Xu, 1997). The two primary mammalian lignans include enterolactone and its reduction product, enterodiol (Setchell et al. 1981; Setchell & Bonney, 1981). The latter are formed in the gastrointestinal tract by bacterial action on its precursors, the plant lignans, matairesinol and secoisolariciresinol.

2.1.2.2 SOURCES OF PHYTOESTROGENS

Phytoestrogens are found in grains, legumes and nuts, fruits and vegetables, and some are also found in beverages such as tea, coffee, wine, beer and bourbon (Pillow et al., 1999).

2.1.2.2.1 SOURCE OF ISOFLAVONES

Isoflavones are found almost exclusively in legumes such as soybeans (*Glycine Max*), peas, and beans. They are also found in clover seeds and clover sprouts (Kurzer & Xu, 1997). As seen in Table 2.1, the highest dietary concentrations of isoflavones are found in kudzu roots, soybeans and chickpeas, respectively (Mazur, 1998). However, soybeans and its derived products are the most significant dietary source of isoflavones (Kurzer & Xu, 1997; Tham et al., 1998).

Table 2.1: Source of phytoestrogens isoflavones & lignans (µg/100 g dry weight)

Plant foods	Daidzein	Genistein	Secoisolariciresinol	Matairesinol	Reference
Grains and Cereal					
Rye	0	0	47.1	65	Adlercreutz and Mazur (1997)
Wheat	tr	tr	8.1	0	Adlercreutz and Mazur (1997)
Barley	14	7.7	58	0	Adlercreutz and Mazur (1997)
Oats	0	0	13.4	tr	Adlercreutz and Mazur (1997)
Maize	0	0	8	0	Unpublished observations
Rice	0	0	16	tr	Unpublished observations
Oilseeds and Nuts					
Flaxseed	***0***	***0***	***369900***	***1087***	***Mazur et al (1996)***
Sunflower seed	8	13.9	610	0	Mazur et al (1996)
Clover seed	178	323	13.2	0	Mazur et al (1996)
Caraway seed	tr	8	221	5.7	Adlercreutz and Mazur (1997)
Cashew	0	0	257	4	Mazur and Adlercreutz (1998)
Peanut	58	64	298	tr	Mazur and Adlercreutz (1998)
Walnut	5	tr	163	5	Mazur and Adlercreutz (1998)
Hazelnut	tr	tr	119	4	Mazur and Adlercreutz (1998)
Berries and Currants					
Bramble	tr	tr	3718	22.5	Unpublished observations
Strawberry	tr	tr	1500	78.1	Unpublished observations
Lingonberry	0	0	1510	0	Unpublished observations
Cranberry	0	0	1054	0	Adlercreutz and Mazur (1997)
Red raspberry	0	tr	139	0	Unpublished observations
Blackcurrant	0	0	388	9.5	Unpublished observations
Redcurrant	0	0	165.3	0	Unpublished observations
Fruits					
Apple	12.4	tr	tr	0	Mazur and Adlercreutz (1998)
Plum	0	0	5	0	Mazur and Adlercreutz (1998)
Banana	0	0	10	0	Mazur and Adlercreutz (1998)
Otaheite gooseberry	0	0	3040	5.8	Unpublished observations
Avocado	0	0	76.7	16	Unpublished observations
Tomato	0	0	51.6	6.5	Unpublished observations
Lychee	0	0	53.6	tr	Unpublished observations
Papaya	0	0	8.2	0	Unpublished observations
Guava	0	0	699.7	tr	Unpublished observations
Cantaloupe	0	0	183.9	0	Unpublished observations
Lemon	0	0	61.3	0	Unpublished observations
Orange	0	0	76.8	0	Unpublished observations

Plant foods	Daidzein	Genistein	Secoisolariciresinol	Lignans	Reference
Food legumes					
Soybeans	*10 500 - 85 000*	*26 800 - 102 500*	*13 - 273*	*tr*	*Mazur et al (1998)*
Kidney bean	7.0 - 40	18 - 518	56 - 153	tr	Mazur et al (1998)
Chickpea	11 - 192	69 - 214	7.0 - 8.0	0	Mazur et al (1998)
Pea	0 - 52.9	0 - 49.7	3.0 - 13	0	Mazur et al (1998)
Lentil	3.0 -10	7.0 -19	0 - 7	tr	Mazur et al (1998)
Kudzu leaf	375	2520	476	tr	Mazur et al (1998)
Kudzu root	185 000	12 600	31	tr	Mazur et al (1998)
Black gram sprouts	745	1900	468	0	Adlercreutz and Mazur (1997)
Alfalfa sprouts	62	5	33	0	Adlercreutz and Mazur (1997)
Cruciferous vegetables					
Cabbage	tr	tr	33	tr	Mazur and Adlercreutz (1998)
Broccoli	6	8	414	23	Adlercreutz and Mazur (1997)
Cauliflower	5	9	97	tr	Mazur and Adlercreutz (1998)
Allium vegetables					
Onion	0	0	83	8	Mazur and Adlercreutz (1998)
Garlic	tr	tr	379	3.6	Adlercreutz and Mazur (1997)
Chives	0	0	1254	tr	Unpublished observations
Other vegetables					
Potato	0	0	10	6	Mazur and Adlercreutz (1998)
Carrot	tr	tr	192	3	Adlercreutz and Mazur (1997)
Pepper	0	0	117	7	Mazur and Adlercreutz (1998)
Celery	0	0	111.4	3.5	Unpublished observations
Cucumber	0	0	25.1	tr	Unpublished observations
Eggplant	0	0	99.7	3	Unpublished observations
Radish	0	0	33.3	3	Unpublished observations
Beverages non alcoholic					
Prince of Wales black tea (brewed)	0	0	2420	305	Mazur et al (1997)
China Black tea (brewed)	0	0	1050	90	Mazur et al (1997)
China green tea (brewed)	tr	tr	2890	195	Mazur et al (1997)
Japanese Sencha green tea (brewed)	tr	tr	1890	277	Mazur et al (1997)
Maxwell coffee	0	0	500	nd	Mazur et al (1997)
Arabica coffee (Nescafe)	nd	nd	716	nd	Mazur et al (1997)
Beverages, wines					
Chardonnay, (France) White	nd	nd	174	22	Unpublished observation
Chardonnay (Italy) White	nd	nd	135.5	17.2	Unpublished observation
Cabernet Sauvignon (France) red	nd	nd	686	74.1	Unpublished observation
Chianti (Italy) red	nd	nd	1280	98	Unpublished observation

2.1.2.2.1.1 ISOFLAVONES IN SOY PRODUCTS

Soy and its derived products are the main contributors of dietary isoflavones in the human diet (Lampe et al., 1999). For more than 1000 years, Asian populations have been consuming soybeans in a variety of traditional soy products that are either non-fermented or fermented (Golbitz, 1995). The traditional non-fermented soy products include fresh green soybeans, whole dry soybean, soy nuts, soy sprouts, soy flour, soymilk, soymilk products, tofu, okara and yuba. The fermented products include tempe, miso, soy sauces, natto, dau chi, kinema, fermented tofu and soymilk products (Astuti et al., 2000; Golbitz, 1995). Some fermented soy products have a higher concentration of total isoflavones than non-fermented products such as tofu and soymilk (Wang & Murphy, 1994). It has been reported that fermented soy products contain a higher concentration of aglucones than glucosides. The reverse is seen for non-fermented soy products (Wang & Murphy, 1994; Wuryani, 1995).

2.1.2.2.1.1.1 ISOFLAVONES IN TEMPE (A TRADITIONAL FERMENTED SOY PRODUCT)

Soybeans were first cultivated in China approximately 2000 to 3000 years ago. Ancient Sanskrit suggested that soybeans were first traded in Indonesia about 2000 years ago (Shurtleff & Aoyagi, 1979). Indonesians consume soybeans as boiled beans, fresh or boiled sprouts, soymilk, tofu (tahu) or as tempe (pronounced tem-peh). The traditional fermented-soybean originated

from Java Island, in Indonesia. Tempe can be made not only from soybean, but it can also be manufactured from other kinds of legumes. In Indonesia, the soybean is the most common form of legume used to make tempe. The word "tempe" usually refers to soybean tempe, while other kinds of tempe are usually given the name of the raw material used. Tempe may be served in many different ways including snacks and side dishes. It can be fried, boiled, cooked as satay, as sauce, or as curry, or prepared as crackers (Astuti et al., 2000). Tempe is a food that holds deep cultural significance in Indonesia, especially in East and Central Java. Documents (Serat Centini) dating back to 1815 contain written historical evidence to suggest that the technology of tempe manufacturing and its role as part of food culture of the Central Javanese people appeared around the 1700's (Astuti, 1992).

2.1.2.2.1.1.1.1 TEMPE MANUFACTURING

Tempe is manufactured by placing an inoculum (*Rhizopus spp.*) on the cooked beans. The soybeans are then left to ferment in a warm environment for 1-2 days. The traditional way to make of tempe is to cover the beans with hibiscus leaves from a teak tree. These leaves have many fine hairs that actually contain the *Rhizopus* fungus. The hairs brush the beans with the inoculum. The more modern method is to place the inoculated beans in banana leaves or perforated plastic bags. During the fermentation process, the beans swell and develop a white mould covering of mycelia before binding together, to form a white cake-like appearance. Principally, the steps in tempe manufacturing are boiling, soaking and fermentation. However, there is no

standard method of preparing tempe in Indonesia, therefore variations exist in these basic steps (Astuti et al., 2000).

- Soybeans are washed, cleaned, and then soaked overnight.
- The bean-soaked water is discarded.
- The beans are boiled and de-hulled to separate the seed coat and the skin.
- The de-hulled beans are then soaked in water for approximately 24 hours before boiling until cooked.
- The beans are then drained and spread in a thin layer and cooled.
- The cooled beans are inoculated with a starter (containing *Rhizopus spp*) and wrapped in a banana leaf or a perforated plastic bag.
- The beans are incubated at room temperature (30-37°C) for 36-60 hours.

Fermentation is common to cultures with warm climates, since it is a way of preserving and improving the palatability and nutritional value of foods (Mollison, 1993). Fermented soybean foods found in other cultures, include kinema in Nepal, dau chi in China and miso in Japan. The fermentation process that occurs during the manufacture of tempe has been found to produce a variety of biochemical changes (Astuti et al., 2000). Tempe contains isoflavones (György et al., 1964; Murakami et al., 1984) that become more bioavailable with the fermentation process (Hutchins et al., 1995). The isoflavone concentrations in tempe are relatively higher compared to other

soybeans products such as tofu and soy beverages (Dwyer et al., 1994; Pillow et al., 1999; Wang & Murphy, 1994; Wuryani, 1995).

Table 2.2: Isoflavone concentrations in tempe, tofu and soy beverages

Soy product(s)	*Daidzein (μg/100g)*	*Genistein (μg/100g)*
Tempe	129*	138*
	137**	193**
	248***	345***
	167***	261***
	102***	140***
Tofu	46**	52**
Soy beverages	15-30**	32-50**

Sources: * Wuryani (1995)

** Wang and Murphy (1994)

*** Dalais, FS (personal comm., 1999)
(Various tempe samples were originally from local market in Yogyakarta, Indonesia)

2.1.2.2.2 SOURCE OF COUMESTANS

The most significant sources of coumestans are clover sprouts, ladino clover, and alfalfa sprouts. Others sources include split peas, kala chana seeds, pinto bean seeds andlima bean seeds. Small amounts of coumestans are also found in soybean sprouts and other fodder crops (Kurzer & Xu, 1997; Stob, 1983).

2.1.2.2.3 SOURCE OF LIGNANS

Unlike isoflavones that are derived mainly from the *Leguminosae Family,* plant lignans are ubiquitously distributed in nature. The most significant source of lignans is linseeds (flaxseeds) (Thompson et al., 1991). As can be seen in Table 2.2, dietary sources of lignan precursors include nuts, cereals, berries, various fruits and vegetables as well as various kinds of beverages, both alcoholic and non-alcoholic (Mazur, 1998).

2.1.2.3 METABOLISM OF PHYTOESTROGENS

2.1.2.3.1 ISOFLAVONES

The principal isoflavones found in food are genistin, daidzin, glycitin (bound to a glucoside group) and the methyl ether derivatives, biochanin A and formononetin. Biochanin A and formononetin are metabolised to genistein and daidzein, respectively. Genistein is further broken down to p-ethyl phenol and, daidzein to O-desmethylangolensin (O-DMA) and equol (Joannou et al., 1995; Setchell & Adlercreutz, 1988).

The presence of daidzein in urine indicates that if it is not converted to equol by bacterial action, it is absorbed from the gastrointestinal tract where it undergoes hepatic conjugation to glucuronic acid before being excreted in urine (Setchell & Adlercreutz, 1988). When ingested by mammals, isoflavones are modified by ruminal or intestinal microflora. The aglucones are released, absorbed from the gastrointestinal tract, transported to liver where they enter the enterohepatic circulation before being finally excreted through the kidneys (Setchell & Adlercreutz, 1988).

The identification of daidzein and equol in cow's milk, suggests that equol itself may be derived directly from the diet. Dietary equol maybe absorbed from the human intestinal tract and circulate without any action of intestinal bacteria. However, this is possibly only a minor route for its origin (Axelson et al., 1984; Setchell & Adlercreutz, 1988).

2.1.2.3.2 LIGNANS

The lignan precursors or plant lignans, secoisolariciresinol and matairesinol, are converted by human gut microflora to the mammalian lignans, enterodiol and enterolactone. Secoisolariciresinol diglucoside is transformed to enterodiol via a reaction involving hydrolysis of the sugar moiety, dehydroxylation, and demethylation. Enterodiol can be further oxidised into enterolactone (Kurzer & Xu, 1997).

Similar to isoflavones, lignans undergo enterohepatic circulation and are excreted as glucuronides and sulphate conjugates in urine and faeces (Axelson & Setchell, 1981).

2.1.2.3.3 PHYTOESTROGEN CONCENTRATIONS IN HUMAN BIOLOGICAL FLUIDS

Phytoestrogens have been identified in many physiological fluids found in humans (Kurzer & Xu, 1997). Those detected in urine include enterolactone, enterodiol, equol, daidzein, O-DMA, matairesinol, genistein and glycitein (Setchell et al., 1980). A number of studies reveal that soy consumption increases the urinary excretion isoflavones in humans in a dose-dependent manner (Hutchins et al., 1995; Karr et al., 1997). Urinary isoflavonoid excretion is associated with soy consumption, while urinary lignan excretion is

associated with the consumption of whole grains (Lampe et al., 1999; Maskarinec et al., 1998).

Daidzein, genistein, O-DMA, equol, enterolactone, enterodiol and matairesinol have been identified in human plasma, urine and faeces (Adlercreutz et al., 1994). Isoflavones have also been identified in other human fluids such as saliva, breast aspirate, breast milk and prostatic fluid (Bannwart et al., 1988 59; Dalais, 1998; Finlay et al., 1991; Morton et al., 1994), whilst lignans have been identified in human urine, serum, semen and bile (Axelson & Setchell, 1981; Dalais, 1998; Setchell et al., 1980).

Considering the importance of gut microflora in phytoestrogen synthesis, the use of antibiotics may significantly inhibit the conversion of isoflavone and lignan precursors into their oestrogenic forms (Adlercreutz et al., 1986; Borriello et al., 1985; Setchell & Adlercreutz, 1988).

For lignans, decreasing the particle size by chopping or cooking has been observed to make the precursor compounds in foods more accessible to the colonic bacteria that metabolise these substances into biologically active phytoestrogens. On the other hand, other forms of processing may significantly affect the concentration and metabolism of isoflavones. For example, although isoflavones and lignans are stable compounds, a combination of high temperatures and acid can destroy them (Hahn et al., 1998). This, in turn, may alter the biological action of isoflavones.

The onset and duration of isoflavone supplementation may also influence the expected biological outcome. The administration of genistein to neonatal rats led to a significant reduction in tumour formation when tumours were chemically induced at an adult phase (Hirayama, 1986; Lamartiniere et al., 1995).

2.1.2.4 PHYSIOLOGY OF PHYTOESTROGENS

Phytoestrogens may exert oestrogenic or in some cases, anti oestrogenic effects (through oestrogen receptor dependent actions). They are also capable of acting through an oestrogen receptor in an independent manner. The oestrogenic activity of isoflavones was first recognised in relation to a syndrome known as clover disease in sheep (Bennetts et al., 1946). A severe reproductive disorder that led to permanent infertility was reported in Australian ewes grazing on pastures of subterranean and red clover. Daidzein, genistein, biochanin A and formononetin were later found to be constituents of the red clover, but only formononetin was positively correlated with the oestrogenicity of the clover (Millington et al., 1964). The first published study to show the physiological oestrogenic effects of phytoestrogens in humans was by Wilcox and colleagues. This study revealed an increase in oestrogenicity in postmenopausal women who consumed phytoestrogens (Wilcox et al., 1990).

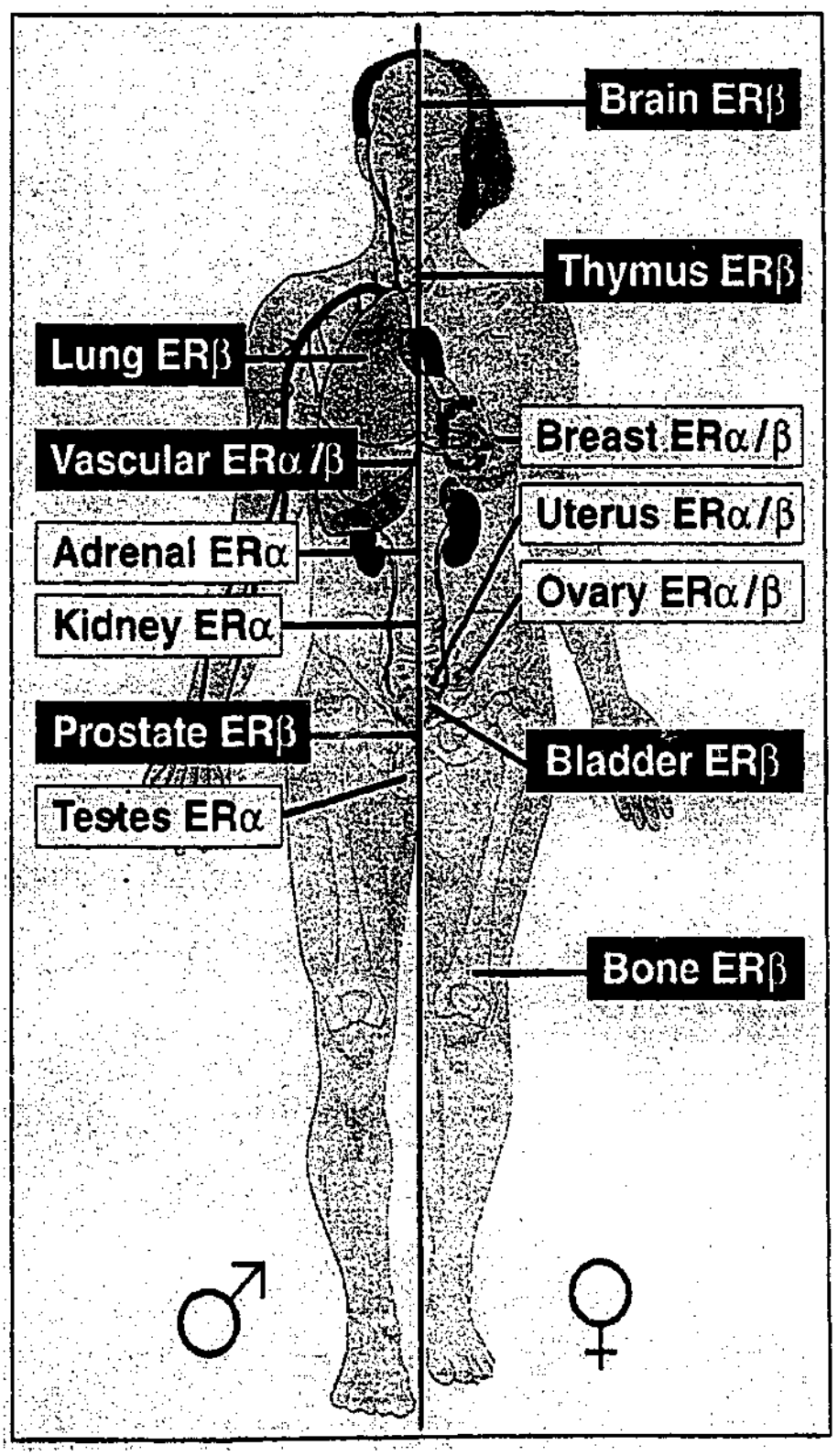
The physiological effects of phytoestrogens are mainly influenced by the route of administration, the chemical form (of the phytoestrogens), oestrogen receptor status, target tissue, intrinsic oestrogen state, metabolism and bioavailability, onset, duration and the level of exposure (Setchell, 1998; Setchell & Cassidy, 1999).

2.1.2.4.1 HORMONAL EFFECTS

Due to its structural similarity to steroidal oestrogens, isoflavones have been shown to bind to the oestrogen receptor α (ERα) (Price & Fenwick, 1985; Setchell et al., 1987), but weakly in comparison to oestradiol (Kurzer & Xu, 1997). At certain concentrations they can act as oestrogen agonists or antagonists, which may depend on many factors including receptor numbers, occupancy and the competing oestrogen concentration (Leclerq & Heuson, 1979). This paradoxical phenomenon is similarly demonstrated by selective oestrogen receptor modulators (SERMs), such as tamoxifen and raloxifene (Hahn et al., 1998).

Figure 2.4: Anatomical distribution of ERα and ERβ in the male and female

Source: (Setchell & Cassidy, 1999)



The discovery of ERβ and its presence in a number of tissues such as the brain, lung, bone, prostate and bladder (Figure 2.4) (Paech et al., 1997; Tetsuka et al., 1997) illustrates the notion that isoflavones may exert their action through pathways that are distinct and separate from that of classical steroidal oestrogens (Setchell, 1998). This was confirmed by Kuiper and colleagues, who demonstrated that genistein, and, to a lesser extent daidzein, have a higher affinity for ERβ than for ERα (Kuiper et al., 1998). Moreover, the lower affinity of several phytoestrogens for serum proteins would be expected to enhance the number of molecules available for receptor occupancy (Nagel et al., 1998).

2.1.2.4.2 NON HORMONAL EFFECTS

Apart from their oestrogen receptor dependent actions, isoflavones may also express non-hormonal effects. Isoflavonoids have been shown to act as antioxidants (György et al., 1964; Hodgson et al., 1996; Jha et al., 1985). The relative antioxidant activity of the isoflavones is as follows; genistein > daidzein = genistin ≈ biochanin A = daidzin > formononetin ≈ ononin (Ruiz-Larrea et al., 1997).

Studies have also demonstrated the antiproliferative activity of isoflavones and lignans in breast cancer cell lines (Hirano et al., 1989; Yanagihara et al., 1993) as well as anti angiogenic activities (Fotsis et al., 1993). In addition, isoflavones and lignans can inhibit the action of cytokines and growth factors

(Kim et al., 1998; Yamamoto et al., 1996). The antioxidant, antiproliferative, antiangiogenic and inhibitory nature of phytoestrogens contributes to their potential effectiveness as potential anticancer agents. These mechanisms may further explain how phytoestrogens influence cells that lack oestrogen receptors (Setchell, 1998).

2.2 PHYTOESTROGENS AND HUMAN HEALTH

2.2.1 PHYTOESTROGENS AND CANCER

Epidemiological studies have illustrated that certain dietary factors have been implicated as protective agents against a number of cancers. Colorectal, prostate, breast and other hormone-related cancers are often associated with a Western diet, which is typically high in fat and low in fibre (Morton et al., 1997; World Cancer Research Fund/American Institute of Cancer Research, 1997; Adlercreutz et al., 1995). On the other hand, the traditional Asian diet, which is lower in fat and higher in fibre, is associated with a low incidence and mortality of the above cancers (Morton et al., 1997). As stated before, the low fat and high fibre dietary profile attributed to the traditional Asian diet, is also similar to some Mediterranean and vegetarian diets. Apart from these dietary differences to a traditional Western type diet, Asian diets are also rich sources of phytochemicals, notably phytoestrogens (Adlercreutz, 1998; Morton et al., 1997).

The major contributors of dietary isoflavones in Asian and Western diets are soy products (Lampe et al., 1999). It was estimated that the average intake of traditional soy products among some Asian populations is around 1-2 servings per day (Hahn NI, 1998). In the United States, the intake of soy is reported to be 0.08 and 0.01 servings per day in "high" and "low" fruit and vegetables consumers, respectively (Lampe et al., 1999).

PROSTATE CANCER (Described in Section 2.3)

2.2.1.1 BREAST CANCER

The inherited genetic risk for breast cancer accounts for only 10-15% of all breast cancer cases, while the remaining 85% of cases diagnosed are in women who do not have a family history of breast cancer (Colditz & Frazier, 1995). It has been suggested that promotion of increased physical activity, diet, as well as other lifestyle in early life could reduce the risk of breast cancer. Phytoestrogens, notably isoflavones, have been observed to inhibit breast cancer growth via hormonal and non-hormonal related mechanisms of action.

2.2.1.1.1 HORMONE RELATED MECHANISM

Breast cancers as well as prostate cancers are initially hormone-dependent, and, therefore, the absence of related hormones may cause cancer growth regression (Morton et al., 1997). A large proportion of plasma oestradiol as

well as testosterone are bound to transport proteins such as sex hormone binding globulin (SHBG). However, a small proportion is left unbound and is diffused into the target cells as biologically active fractions (Vermeulen et al., 1972). The weakly oestrogenic isoflavones stimulate the synthesis of SHBG in the liver, therefore reducing the free fraction of the growth promoting steroid hormone (Adlercreutz et al., 1987).

The aromatase enzyme is known to be responsible for the irreversible conversion of C19 androgens into C18 oestrogens (Morton et al., 1997). The lignan, enterolactone, has been shown to be a moderate inhibitor of aromatase activity in placental microsomes (Adlercreutz et al., 1993). Moreover, it has been observed that isoflavonoids, biochanin A, coumestrol, genistein and equol are moderate aromatase inhibitors in genital skin fibroblasts (Evans et al., 1995). The metabolism of testosterone to androstenedione and the metabolism of oestrone to oestradiol is controlled by 17β-hydroxysteroid dehydrogenases (Morton et al., 1997). It has been observed that coumestrol and genistein inhibit the 17β-hydroxysteroid dehydrogenase type I enzyme which inhibits the conversion of oestrone to oestradiol (Makela et al., 1995).

2.2.1.1.2 NON-HORMONAL RELATED MECHANISM

Dietary phytoestrogens, mainly isoflavonoids and lignans, exhibit non-hormonal actions, which may affect carcinogenesis. The function of several

growth factors is partly dependent on tyrosine kinases. Genistein has been shown to be a specific inhibitor of tyrosine kinase activity (Akiyama et al., 1987). Angiogenesis, is normally restricted for wound healing, however, this mechanism is also involved in the new vascularisation and progression of carcinogenesis by invoking the proliferation and migration of endothelial cells (Morton et al., 1997). Genistein has been demonstrated to inhibit angiogenesis and endothelial cell proliferation (Fotsis et al., 1993). Isoflavonoids, flavonoids and lignans have been demonstrated to function as antioxidants (György et al., 1964; Hodgson et al., 1996; Jha et al., 1985) an essential anticancer agent characteristic.

Additionally, genistein has also been revealed to induce apoptosis in human breast tumour cells (Kiguchi et al., 1994), as well as inhibit DNA topoisomerases, the enzymes that alter the conformation of DNA and are crucial to cell division (Cummings & Smyth, 1993).

2.2.1.2 OTHER CANCERS

As stated before, phytoestrogens exert non-hormonal anticancer actions, therefore possibly exerting similar effects on other types of cancers. Table 2.3 presents some studies investigating the possible anticancer effects of isoflavones and lignans.

Table 2.3: Studies investigating the possible anticancer properties of phytoestrogens (isoflavones and lignans)

Author(s)-Year	*Type of cancer*	*Cell type*	*Compound(s)*	*Outcome(s)*
Hirano-1991	Mitogen-induced proliferation	Human lymphocytes	Plant lignans	Inhibition of proliferation
Traganos-1992	Leukemia	MOLT-4,HL-60 human cells	Genistein	Inhibition of: cell cycle, progression and growth
Adlercreutz-1992	Liver cancer	HepG2 cells	Enterolactone	Stimulation of SHBG synthesis
Fotsis- 1993	Endothelial cells	Several different endothelial cells	Genistein	Inhibition of angiogenesis
Piontek-1993	Gastric cancer	AGS human gastric Ca cells	Genistein	Inhibition of: growth and EGF-receptor tyrosine kinase
Mousavi & Adlercreutz-1991	Liver cancer	HepG2 cells	Genistein	Inhibition of proliferation
Matsukawa-1993	Gastric cancer	HGC-27 cells	Genistein	Growth inhibition; arrest of cell cycle at G(2)M
Rocchi-1995	Neuroblastoma	TS12 and SJNKP cells	Genistein	Growth inhibition; induction of differentiation
Serraino&Thompson-1992	Colon cancer	Rat colon cancer model	Flaxseed	Protective
Watanabe & Koessel-1993	Colon cancer	Japanese E& Γ	Soy intake	Reduced risk
Yanagihara-1993	Colon cancer	Colon cancer cells; stomach cancer cells	Genistein & biochanin A	Inhibition of proliferation
Kuo&Summers-1995	Colon cancer	Human colon cancer cells Caco 2 and HT-29	Genistein	Growth inhibition; apoptosis

2.2.2 PHYTOESTROGENS AND WOMEN'S HEALTH

PHYTOESTROGENS AND MENOPAUSE (described in Section 2.4)

2.2.2.1 PHYTOESTROGENS AND BONE HEALTH

Hip fracture and osteoporosis rates in Asian populations are approximately 50% lower than that of Western Caucasians (Cooper et al., 1992; Ho et al., 1993; Ross et al, 1991; WHO Study Group Report, 1994). Population studies found that the average intake of soy among Japanese is approximately 20-50g/day, which is higher than the average intake of Westerners, which is estimated 3g/day (Anderson & Garner, 1998). The bone mass and bone density of Asian populations are generally lower compared to Western populations and this difference may be due, in part, to soy. Soy isoflavones may take a different pathway to oestrogen in protecting against bone loss (Anderson & Garner, 1998). However, cell studies suggest that isoflavones may also have similar effects to oestrogen in bone cells. Genistein and oestrogen were shown to increase alkaline phosphatase at lower concentrations, but at higher concentrations they had a toxic effect in osteoblast-like cells. The similar effect of both compounds suggests a similar mechanism of action. In osteoclast-like cells, the mechanism of action seems to be different to that of osteoblast-like cells (Anderson & Garner, 1997). The recent discovery of oestrogen receptors in osteoblast cells increases our

understanding of the role of oestrogens in bone tissue (Eriksen et al., 1988). Isoflavones may exert similar actions to oestrogen, since ERβ was discovered to be the type of oestrogen receptor in bone tissue.

Soy protein has been shown to retard bone loss in ovariectomised rats (Arjmandi et al., 1998; Omi et al., 1994). Arjmandi and colleagues highlighted that soy protein containing high concentrations of isoflavones retarded bone loss at the femur and spine, but soy protein containing low concentrations of isoflavones did not show a similar effect.

Results from another study indicated that genistein exerts oestrogenic action in bone and bone marrow, regulates B-lymphopoiesis and prevents bone loss (Ishimi et al., 1999). Other studies have also highlighted that genistein may reduce trabecular and compact bone loss after ovariectomy by the stimulation of bone formation, rather than on the suppression of bone resorption (Fanti et al., 1998; Ishimi et al., 1999).

To date, there are not enough human studies to conclude that isoflavones may produce similar effects to oestrogen (as seen *in vitro)*, but the evidence to date tends to support a beneficial effect (Murkies et al., 1995; Scheiber et al., 1999; Wang & Murphy, 1994; Washburn et al., 1999; Wong, 1999).

2.2.3 PHYTOESTROGENS AND CARDIOVASCULAR HEALTH

Isoflavones and soy protein have been extensively investigated for their cardioprotective effects (Anderson et al., 1995; Anthony et al., 1996; Crouse et al., 1998; Hodgson et al., 1996; Teede et al., 2001). Experimental studies in this area provide evidence that isoflavone-rich food lowers low-density lipoprotein (LDL) cholesterol, total cholesterol, while increasing the high density lipoprotein (HDL) cholesterol concentration in plasma (Anthony et al., 1996). Dose-response studies using soy protein in humans have demonstrated a relationship between increasing isoflavone concentrations and decreasing total cholesterol (Crouse et al., 1998), while other studies using purified phytoestrogens pills have not shown the same effect (Nestel et al., 1997). Anthony and colleagues (1998) demonstrated that isoflavones inhibit the progression of atherosclerosis in the coronary, iliac, common and internal carotid arteries. Oxidised LDL particles are considered to be an essential component in enhancing atherogenesis. Isoflavones as good antioxidants (György et al., 1964; Jha et al., 1985; Ruiz-Larrea et al., 1997) have been shown to prevent the oxidation of the lipoprotein particles (Hodgson et al., 1996).

Isoflavones also favourably influence coronary artery reactivity, as indicated in a study where soy protein treatment provided an equivalent effect to

conjugated equine oestrogens in modulating coronary artery dilatation (Honoré et al., 1997).

2.3 PHYTOESTROGENS AND PROSTATE CANCER

2.3.1 PROSTATE CANCER

Prostate cancer is the most commonly diagnosed male malignancy and the second most common cause of cancer-related mortality in Western society, with the highest rates found in Europe, North America and Australia (World Cancer Research Fund/American Institute of Cancer Research, 1997). Another common disease of the prostate gland, benign prostatic hypertrophy (BPH) has a similar pattern of incidence and symptomatology as prostate cancer. The etiology of prostate cancer is still poorly understood, however, some of strong risk factors that have been established are presented below.

2.3.1.1 RISK FACTORS

Clinical symptoms of prostate cancer generally present in men beyond 50 years of age (Franks, 1954). Age beyond 50 years is therefore associated with an increased risk (Pienta & Peggy, 1993). The highest incidence rates worldwide are reported among African-American men, while the lowest are among Chinese men (World Cancer Research Fund/American Institute of Cancer Research, 1997). High intake of fat may also be associated with prostate cancer (Adlercreutz et al., 2000; McMichael, 1994; Skrabanek, 1994).

The prostate gland is androgen dependent and does not develop in castrated-male (Griffiths et al., 1998; Peeling & Griffiths, 1986).

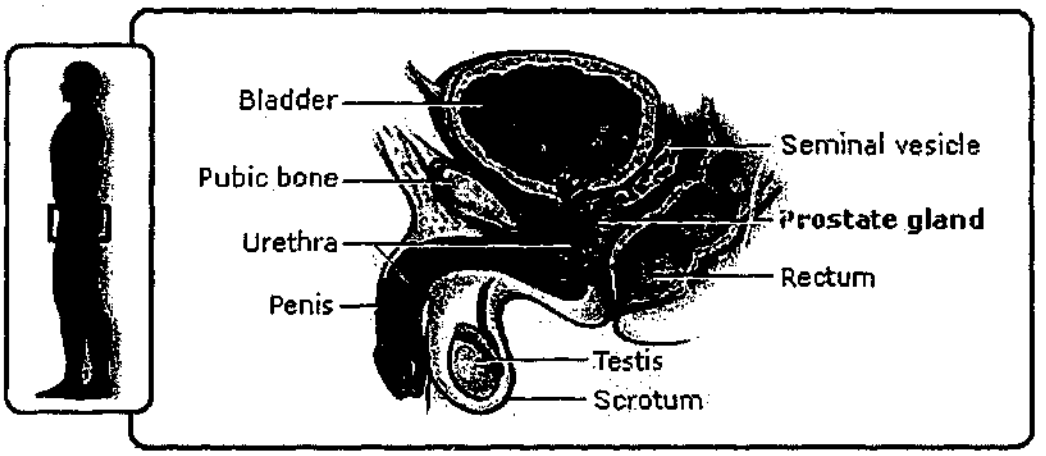


Figure 2.5: Anatomy of the prostate gland

2.3.1.2 SYMPTOMS

Prostate cancer and BPH share many similar symptoms (Griffiths et al., 1998; McMichael, 1994; Skrabanek, 1994). Both diseases occur among men aged over 50 years old. Most symptoms are related to micturition disturbances due to bladder outlet obstruction, resulting in frequent urination (especially at night), weak urinary stream, inability to urinate, urinary stream interruption (stopping and starting), pain or burning on urination and blood in the urine.

2.3.1.3 SCREENINGS

Prostate cancer patients are often diagnosed due to simple micturition complaints in the first place. The screening methods for prostate cancer are as follows:

2.3.1.3.1 DIGITAL RECTAL EXAMINATION (DRE)

DRE is a primary screening tool for physicians. This procedure allows the physician to determine whether the prostate texture is normal or abnormal. Detection rate of this method ranges from 0.8% to 1.5% (Long et al., 1997). As a sole screening method this is inadequate, although many studies report a benefit in the long-term outcome of prostate health from annual DRE screening.

2.3.1.3.2 PROSTATE-SPECIFIC ANTIGEN (PSA)

Prostate specific antigen (PSA) is a glycoprotein produced mainly by the epithelial component of the prostate gland (Michael, 2001). The accepted normal upper limit for serum total PSA is 4.0 ng/dL. Elevated PSA can indicate prostate cancer, but it may also increase with age, occur with a biopsy procedure of the prostate, a trans urethral prostatectomy, acute urinary retention, and acute prostatitis, BPH, or prostatitis (Arnot, 2000; Long et al., 1997). Men with prostatic disease may have a high serum PSA concentration because of an enhanced production of PSA as well as architectural distortions in the gland. These distortions allow PSA greater access to the circulation

(Long et al., 1997). Conversely, a low PSA concentration does not always mean that prostate cancer is not present, as an early stage of prostate cancer may be present but has not yet resulted in an increase in PSA in the bloodstream. PSA however is a useful tumour marker, with the most dramatic elevation occurring in prostate cancer cases. PSA is effective in following post-treatment prostate cancer patients to monitor any recurrence or persistence of the disease. Detection rate usually ranges from 1.6% to 4.6% (Brawer et al., 1993; Catalona et al., 1994), which is better than DRE alone.

Table 2.4: Age-specific PSA ranges (adapted from Oesterling et al., 1993)

Age (years)	*PSA level (ng/dL)*
40-49	0-2.5
50-59	0-3.5
60-69	0-4.5
>70	0-6.5

Measurement of PSA (considered to be the most sensitive and less invasive test for prostate cancer) and also performing a digital rectal examination (DRE) usually detects the presence of cancer that might otherwise be missed (Coley et al., 1997).

Serum PSA exists in several forms as PSA circulates both free and in complexes with macromolecules (i.e. PSA bound to α-1-antichymotrypsin, PSA bound to α-1-protease inhibitor) (Catalona & Southwick, 1998; Lilja et al., 1991). The free total PSA ratio has been suggested to be a better means of providing information about the aggressiveness of the cancer than PSA in the complex form alone. Free PSA is not bound to any protein and appears to be enzymatically inactive, but it is detectable by immunoassay. Most standard PSA assays detect the complex and free PSA types, yielding a PSA level referred to as total PSA (Ellis et al., 2001).

Catalona and Sothwick (1998), suggested from the results of their study that the probability of prostate cancer at biopsy among men with a PSA value of 4.0 to 10.0 ng/mL and normal findings on digital rectal examination ranged from 56% (for men with a ratio of free to total PSA of up to 10%), down to 8% (for men with a free:total PSA ratio of more than 25%). It has been further suggested that the likelihood of diagnosing prostate cancer by a prostate needle biopsy increases as the percentage of free PSA (free:total PSA ratio) decreases (Catalona & Southwick, 1998; Recker et al., 2001; Woodrum et al., 1998).

The measurement of the free:total PSA ratio may increase the specificity of cancer screening and has minimal loss of sensitivity as it detects 95% of cancers (Catalona & Southwick, 1998). It was also further suggested that the measurement of free PSA or PSA complexes might stratify the risk of prostate cancer for men with total PSA values ranging from 4.0 to 10 ng/mL. Although the reason is unclear, prostate cancer is associated with a lower percentage of circulating free PSA than is benign prostatic hypertrophy (Michael, 2001).

2.3.1.3.3 TRANS RECTAL ULTRASOUND (TRUS)

TRUS detection rate for prostate cancer is 2.6%, which is more reliable than a digital rectal examination (DRE), although the specificity and sensitivity are poor, 68% and 52% respectively (Long et al., 1997).

2.3.1.3.4 BIOPSY

A tissue biopsy is necessary when the previously described measurements provide suspicious results. A small amount of tissue is taken by performing a needle biopsy and then it is examined for cancerous cells. If prostate cancer is found, this procedure will provide the stage or grade of the cancer (Harrison, 2001).

Table 2.5: Cancer probabilities according to the range of percentages of free-PSA (Chan, 1999)

% free:total PSA range	*Prostate cancer probability*	*(95% confidence interval)*
≤ 9%	71%	(60% - 80%)
> 9 – 11 %	60%	(50% - 68%)
>11 – 15%	47%	(40 % - 54%)
>15 – 20%	34%	(29% - 40%)
>20 – 24%	24%	(19% - 30%)
>24 – 26%	16%	(11% - 22%)
> 26%	10%	(6% - 16%)

2.3.1.4 STAGING AND GRADING

Prostate cancer is often graded histologically by the Gleason grading system, the A to D staging system, TNM (tumour, nodes, metastases) staging and the conventional Whitmore-Jewett system (Harrison, 2001; D'Amico et al., 1997). A Gleason grade ranges from 1 to 5. For a Gleason score of 1 the cancer cell clusters must resemble the small, regular, evenly spaced glands of normal prostate tissue. When the cancer cells spread massively throughout the prostate, a Gleason grade of 5 is given. Since the stage or grade of cancer can differ throughout the prostate, a grade is assigned to the two areas most affected by the cancers. The two grades are then added together to yield a Gleason score between 2 and 10. Scores of 2 to 4 are grouped together and are considered as low, 5 and 6 are considered intermediate, and scores of 7 to 10 are considered high (Renshaw & Corless, 1997).

A – D staging system has the following criteria:

Stage A - if the tumour is located within the prostate gland and cannot be detected by digital rectal examination (DRE).

Stage B - if the tumour is located within the prostate, and may be detected by DRE.

Stage C - if the prostate cancer is more advanced and has spread outside the prostate to surrounding areas, but not to other organs.

Stage D - if the cancer has spread to other organs such as bones or lymph nodes.

Table 2.6: The similarity of commonly used staging systems for prostate cancer: TNM and Whitmore-Jewett methods

TNM (Tumour, Nodes, Metastases)		*Whitmore – Jewett*	
T0	No primary tumour evident	A	Tumour not clinically evident
T1a	≤3 microscopic foci of cancer	A1	<5% of resected specimen; Gleason ≤7
T1b	>3 foci of cancer	A2	>5% of specimen; Gleason >7
T1c	PSA elevation only (no nodule)	B	Clinically evident tumour
T2	Clinically evident tumour confined to gland	B0	PSA elevation only (no nodule)
T2a	Unilateral; <1.5cm	B1	Unilateral nodule; <2.0cm
T2b	Bilateral; >1.5cm	B2	Bilateral nodule; >2.0cm
T3	Extracapsular extension, seminal vesicle involvement	C	Extracapsular extension
T4	Invades adjacent structures (fixed)	C1	Minimal, not involving seminal vesicles
N1	Metastases to single lymph node <2cm	C2	Extensive or involving seminal vesicle
N2	Metastases to nodes 2-5cm	D	Metastatic
N3	Metastases to nodes >5cm	D1	Pelvic lymph nodes, organs or bone
M1	Distant metastases	D2	Distant lymph nodes, organs or bone

2.3.1.5 MANAGEMENT

The management of prostate cancer may include watchful waiting, curative treatment and palliation. Management of prostate cancer depends on the stage it is in and the characteristics of the patients, such as age and general health conditions (Catalona, 1989; Catalona, 1994). Treatment of the disease in stage A depends on the patient. If the life expectancy is greater than 10 years, the recommended treatment is radical prostatectomy (Long et al., 1997; Catalona, 1994). Otherwise, patients can be managed by 'watchful waiting', including serial exams and PSA tests. Brachy therapy or radiotherapy may be administered among older and less healthy patients with Gleason score ≤ 6, PSA ≤ 10, a clinical stage of T1C or T2A and have not had previous TURP (Catalona; 1994; Long et al., 1997).

2.3.2 EPIDEMIOLOGY OF PROSTATE CANCER

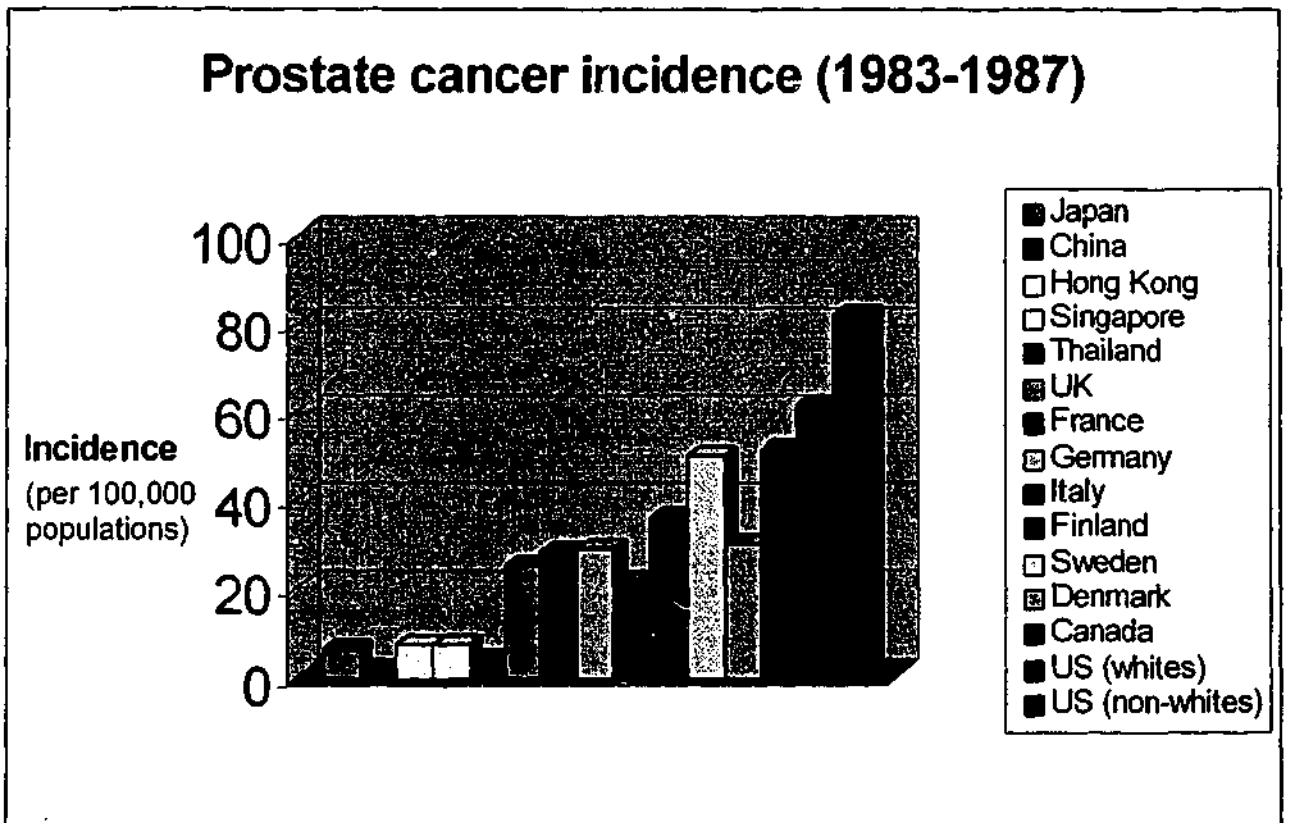
in most industrialised Western countries, prostate cancer is an extremely common malignancy where the incidence ranks second behind lung cancer. The incidence of prostate cancer in Australia is 50.4 per 100,000 population (Australian Institute Health and Welfare, 1998), while it represents 22.5% of all new cancer cases (Australian Institute Health and Welfare, 1997).

In 1990, it was estimated that about 106,000 new cases of prostate cancer would be diagnosed in the United States, of which about 30,000 would die of the disease. The facts however show that there were over 122,000 new cases

of prostate cancer in North America, with some 32,000 deaths from the disease (Boring et al., 1991). The prostate cancer incidence around the world is estimated to increase annually by 2-3% (Evans et al., 1995).

On the other hand, studies illustrate there are lower incidence and mortality rates of prostate cancer among Asian men. In China andJapan the incidence is between6.8 to 9.1 per 100,000 population, in Singapore the incidence is 9.8 per 100,000 populations and in countries like Thailand the incidence is low compared to Western populations (Australian Institute Health and Welfare, 1998; Rose et al., 1986). Prostate cancer is estimated to be 120 times less frequent in China than in Western countries (Zaridze et al., 1984; Miller, 1988). This indicates that there may be a protective mechanism to prevent Asian men from certain cancers. Further studies demonstrate that the potential protective mechanism is lost with a change in culture, as reflected in Japanese men who migrate to the United States. Within a generation, they were observed to have a similar prostate cancer incidence to that of Americans (Shimizu et al., 1991; Shimizu et al., 1987).

Figure 2.6: Prostate cancer incidence worldwide (adapted from World Cancer Research Fund/American Institute of Cancer Research, 1997)



2.3.3 POSSIBLE ROLE OF PHYTOESTROGENS IN PROSTATE CANCER

2.3.3.1 CELL AND ANIMAL STUDIES

Several cell culture studies looking at the possible action of phytoestrogens in prostate cancer have illustrated encouraging results. A study on human prostate cancer and BPH histo-cultured tissue demonstrated the potential of the isoflavone, genistein, as the therapeutic agent. Both prostate cancer and benign prostatic hypertrophy (BPH) tissue growth decreased in a dose-dependent manner starting from 1.25μg/ml and levelling off at 10μg/ml of genistein (Geller et al., 1998). The indicator used in this study was the decrease of ^{3}H-thymidine incorporation/μg protein for each genistein dose added. This study does not clearly address the mechanism of action for genistein. However, it is consistent with previous work conducted by Peterson and Barnes that showed genistein and its 4'-methyl ether derivative (biochanin-A) were able to inhibit EGF-stimulated growth, in hormone dependent (LNCaP) and hormone independent (DU-145) prostate cancer cell lines (Peterson & Barnes, 1993).

Using VeCaP cell lines (as a model to demonstrate the PSA expression in an androgen independent manner) and LNCaP cell lines, (as a model to demonstrate the PSA expression in an androgen dependent manner), Davis and colleagues (2000) were able to demonstrate the role of genistein as a

therapeutic agent for prostate cancer. Genistein was observed to inhibit cell growth in both cell lines, but had different effects on PSA expression. Using concentrations of genistein that have been detected in serum from humans consuming a soy rich diet, this study revealed that genistein decreases PSA and mRNA protein expression and secretion. This study found that genistein was able to inhibit cells proliferation, independent of PSA signalling pathways, providing evidence to support the role of genistein as a chemopreventive agent for prostate cancer irrespective of androgen responsiveness (Davis et al., 2000).

Studies looking at the association of rye bran, soy protein and human LNCaP prostate adenocarcinoma, demonstrate that rye bran and soy diets delay tumour growth and increase apoptosis. A smaller tumour size and a lower PSA concentration were detected post intervention. These effects were more apparent for rye than for soy (Bylund et al., 2000). A similar study carried out by Landström and colleagues (1998) found that both soy and rye diets delayed the development of transplanted Dunning R3327 prostatic adenocarcinoma in rats.

Zhou and colleagues (1999) conducted a study looking at the effect of soy protein isolate (SPI) and soy protein concentrate (SPC) on human prostate tumours grown in mice (LNCaP), and suggested that dietary SPC inhibited tumorous cell growth while SPI reduced tumour growth. The inhibitory effect was associated with a decrease in the cell proliferation index indicating a significant increase in tumour cell apoptosis and a reduction in vessel density.

The molecular mechanism of phytoestrogens against prostate cancer growth was examined by Davis and colleagues (1998). Davis and colleagues showed a novel effect of genistein on cell gene regulation resulting in the inhibition of cell growth and the ultimate demise of tumour cells. Genistein inhibited cultured prostate cancer cell growth in a dose dependent manner, which was accompanied by a G(2)/M cell cycle arrest. The inhibition was observed with the concomitant down regulation of cyclin B, the up-regulation of the p21(WAF1) growth inhibitory protein and the induction of apoptosis. Another study by Kyle et al, (1997) revealed that genistein has been found to block growth stimulation.

Pollard and Wolter (200) undertook serial studies using a soy protein isolate/isoflavone (SPII) diet in Lobund-Wistar rats with spontaneous cancer or induced prostate cancer. Rats were fed with SPII from 2–24 months, and the other group of rats were fed with a natural ingredient diet (L-485) containing soy meal. At age 24 months, 3% rats on SPII diet and 30% rats on the other diet developed spontaneous cancers of the prostate-seminal vesicle complex. Rats on SPII diet manifested a significant reduction of circulating testosterone, approaching physiological levels. On the other hand, the failure of rats fed with L485 to prevent the development of cancers in this study suggests that soy meal contained factors that blocked the antiandrogenic action of the phytoestrogens. This further suggests that the significant prevention of cancers of the prostate-seminal vesicle complex by SPII diet

from age 2 to 24 months, is possibly due to the agonist effect of the soy-derived phytoestrogens. The same authors further demonstrated a similar preventive effect of SPII against development of induced prostate-related cancer in Lobund-Wistar rats. It was revealed that SPII feeding significantly reduced circulating concentrations of testosterone and the weight of the testes. Elevated circulating testosterone found in a group of rats fed with L-485, suggests the soy meal in L-485 blocked the oestrogenic effects of the phytoestrogens (Pollard et al., 2000). Another dietary intervention study demonstrated that a combination diet of low fat, soy protein and isoflavone extract, significantly inhibited tumour growth rate and the weight of the human prostate LNCaP tumours in severe-combined immuno-deficient (SCID) mice, compared to the other 3 diets. These diets were: 1) high fat + casein, 2) high fat + soy protein + isoflavone extract and 3) low fat + casein (Aronson et al., 1999).

The chemopreventive effects of phytoestrogens have been illustrated in male F344 rats injected 10 times biweekly with the cancer causing agent 3,2`-dimethyl-4-aminobiphenyl (DMAB). They were given either genistin or daidzin in the diet at a concentration of 0.1% for 40 weeks. Both daidzin and genistin reduced the number and incidence of ventral prostate carcinor... ($P<0.05$). This experimental study illustrates that the anticancer effects of genistin and daidzin may be protective only at relatively early stage of prostate cancer development (Kato et al., 2000).

2.3.3.2 HUMAN STUDIES

The following studies illustrate the possible association between phytoestrogen intake and prostate cancer risk based on epidemiological studies. Japanese men consuming a low fat diet with a high content of soy products have a lower incidence of prostate cancer. A good biomarker of isoflavone consumption in humans is the urinary or plasma concentrations of isoflavones. The plasma concentration of isoflavones, daidzein, genistein, O-DMA, and equol, among 14 Japanese men was 7-110 times greater than those in Finnish men (Adlercreutz et al., 1993a & 1993b). A later study by Morton et al., (1997) measured the concentration of isoflavones and lignans in the prostatic fluid of Asian and European men. This study observed that men of Asian origin (Hong Kong and China) had a higher isoflavone concentration compared to Europeans (Portugal and UK).

A case study (Stephens, 1997) showed that the administration of a high dose of isoflavones for one week prior to radical prostatectomy resulted in a histological profile of tumour regression. Apoptotic changes seen in the prostate cancer tissue was typical of those seen among patients treated with oestrogens. A case-control study conducted by Strom et al., (1999) that compared the relationship between phytoestrogen intake and prostate cancer risk in prostate cancer patients (n=83) and controls (n=107), found that the control group consumed higher amounts of foods containing genistein, daidzein and coumestrol. After adjustment for age, family history of prostate cancer, alcohol consumption and total calorie intake, there was an inverse

association between prostate cancer risk and coumestrol (p=0.03) and daidzein (p=0.07) (Strom et al., 1999).

Table 2.7: Effects of soy or soybean isoflavones on spontaneous and chemically induced prostate cancers

Author(s), Year	*Media*	*Product(s) tested*	*Cancer causing agent(s)*	*Finding(s)*
Makela, 1991	Mice	Soy product	DES-induced prostate cancer (PC)	Protective
Sharma, 1992	SD Rats	Soy product	Spontaneus PC cells	Protective
Peterson, 1993	N/A	Isolated genistein and biochanin A	LNCaP – DU-145	Protective
Geller, 1998	N/A	Isolated genistein	Histocultured human BPH and cancer cell	Protective
Davis, 1998	N/A	Isolated genistein	Cultured PC	Protective
Aronson, 1999	SCID mice	Soy protein+isoflavone extract	LNCaP	Protective
Bylund, 2000	Nude mice	Soy protein	LNCaP	Protective
Kato, 2000	F344 rats	Isolated genistin and daidzin	LS10-rat prostate carcinoma	Protective
Davis, 2000	N/A	Isolated genistein	LNCaP VeCaP	Protective
Pollard, 2000	Lobund Wistar rats	WSB	MNU induced PC	Protective
Pollard, 2000	Lobund Wistar rats	WSB	Spontaneus PC cells	Protective

2.3.4 MECHANISMS OF ACTION

2.3.4.1 HORMONAL RELATED MECHANISMS

Polyphenolic phytoestrogens possess weak oestrogenic activity and therefore have the potential to influence hormone dependent cancers such as breast and prostate cancers. An analysis of expressed prostatic fluid found the constituents, enterolactone and equol, suggesting that oestrogens of dietary origin may accumulate in the prostate (Griffiths et al., 1998a; Griffiths et al., 1998b). As weak oestrogens, phytoestrogens compete with endogenous oestrogens for binding with the nuclear oestrogen receptors. However, one study found that genistein binds to the newly identified receptor, ERβ, with 36% of the relative binding affinity (RBA) of oestradiol. While the RBA of genistein for ERα is only 5% of that of oestradiol, some of the effects of genistein may still be mediated via ERβ (Kuiper et al., 1997; Griffiths et al., 1998a). Unlike the classic oestrogen receptor, the ERα that is located predominantly in the stromal compartment of the human prostate, is the major oestrogen receptor in the secretory epithelial cells of the gland where prostate cancer occurs (Habenicht & El Etreby, 1989; Krieg et al., 1983; Kuiper et al., 1996).

The prostate gland is androgen dependent and, plasma testosterone is necessary for the growth, development and function of this gland (Griffiths et al., 1993; Morton et al., 1999). It is specifically dependent on the free, non-protein bound form of testosterone (Cunha et al., 1983; Griffiths et al., 1993;

Morton et al., 1999). Approximately 98% of plasma testosterone is bound to SHBG and other transport proteins, while the remaining 2% is in a free form and this is passively diffused into the target cells as the biologically active fraction (Morton et al., 1999; Vermeulen et al., 1989). The ingestion of the weakly oestrogenic isoflavones stimulates the synthesis of SHBG in the liver, increases the concentration of SHBG, therefore reduces the free fraction of testosterone, a growth promoting steroid hormone (Adlercreutz et al., 1987; Morton et al., 1999).

A metabolite of testosterone, 5α-dihydrotestosterone (DHT) is another essential hormone for prostate growth. Testosterone is metabolised to DHT by the enzyme 5α-reductase (Griffiths et al., 1993; Imperato-McGinley et al., 1974). Lowering the conversion of testosterone to DHT would reduce the intracellular androgen effect on the prostate thus inhibit the growth of prostate cancer cells. Isoflavonoids and lignans have been shown to inhibit 5α-reductase (Evans et al., 1995; Griffiths et al., 1993). Population studies have illustrated lower levels of 5α-reductase activity among Japanese men compared to white and black Americans (Ross et al., 1992).

The aromatase enzyme system in adipose and muscle tissue is known to be responsible for the peripheral conversion of male plasma oestrogens from adrenal C19-steroids, dehydroepiandrosterone (DHEA) and androstenedione (Griffiths et al., 1998a; Morton et al., 1999). Declining testicular activity and increasing aromatisation in aging men sustain the level of plasma free-

estradiol-17β concentration. The changed oestrogen-androgen balance has been suspected to be the predominant factor in the induction of prostatic stromal hyperplasia (Vermeulen et al., 1989). The lignan, enterolactone, was shown to be a moderate inhibitor of the aromatase enzyme and was found to bind to the active site of aromatase, thus competing with the androgen substrate of the enzyme (Adlercreutz et al., 1993; Morton et al., 1999). Moreover, it has been observed that isoflavonoids, biochanin A, coumestrol, genistein and equol are moderate aromatase inhibitors in genital skin fibroblasts (Evans et al., 1995).

The metabolism of testosterone to androstenedione and the metabolism of oestrone to oestradiol is controlled by 17β-hydroxysteroid dehydrogenases (Morton et al. 1997). It has been observed that coumestrol and genistein inhibit the 17β-hydroxysteroid dehydrogenase type I enzyme which therefore inhibits the conversion of oestrone to oestradiol (Evans et al., 1995; Makela et al., 1995).

2.3.4.2 NON HORMONAL RELATED MECHANISMS

Genistein has been demonstrated to be a specific inhibitor of tyrosine kinase activity (Akiyama et al., 1987). The function of several growth factor receptors, including the epidermal growth factor (EGF), platelet-derived growth factor, insulin and insulin like growth factor, is partly dependent on tyrosine kinases (Hunter & Cooper, 1985). Cell proliferation and cell transformations are

influenced by tyrosine phosphorylation and therefore the inhibition of tyrosine kinase activity may be an anticancer mechanism (Kenyon & Garcia, 1987).

Angiogenesis is a physiological response to wound healing (Morton et al., 1997; Morton et al., 1999). However when cancer persists, new capillary blood vessels are necessary for cancer growth (Folkman et al., 1989; Folkman, 1985). The generation of new vascularisation will progress carcinogenesis, as this mechanism invokes the proliferation and migration of endothelial cells (Morton et al., 1997). Genistein has been demonstrated to inhibit angiogenesis and endothelial proliferation (Fotsis et al., 1993; Hodgson et al., 1996; Jha et al., 1985). Antioxidants are an essential anticancer characteristic and, isoflavonoids, flavonoids and lignans have been demonstrated to possess antioxidative effects as well as act as free radical scavengers (György P et al., 1964; Hodgson et al., 1996; Jha et al., 1985).

DNA topoisomerases are enzymes that alter the conformation of DNA and are crucial to cell division, as they are able to untangle supercoiled DNA (Cummings & Smyth, 1993; Morton et al., 1997). Phytoestrogens may generate its protective action through this mechanism, since genistein has been found to inhibit the work of DNA topoisomerases I and II (Constantinou et al., 1995).

All the possible mechanism of phytoestrogens against prostate cancer presented appear to warrant their use in a number of clinical trials as potential protective, preventive and treatment agents for prostatic diseases.

2.4 PHYTOESTROGENS AND MENOPAUSE

2.4.1 MENOPAUSE

Menopause is defined as 12 consecutive months of natural amenorrhoea (absence of menstruation) (Greendale, 1999; Hill, 1996; North American Menopause Society, 2000). Premenopausal women are considered to have regular menstruation or a similar menstrual pattern to preceding years, while perimenopausal women are described as having irregular menstruation during the previous 12 months. Postmenopausal women are considered as having no menstrual period during the past 12 months (Jaszmann, 1973).

Published population studies indicate that the average age of menopause ranges from 41 to 52 years (Boldsen & Jeune, 1990; Greendale et al., 1999; Martin et al., 1993; North American Menopause Society, 2000). Using the average age of menopause as 50 years, the estimated number of menopausal and postmenopausal women worldwide in 1990 was 467 million, and it is projected to be 1.2 billion in the Year 2030 (Hill, 1996).

Some scientists describe menopause as an ovarian failure or oestrogen deficiency disease (Wilson & Wilson, 1963). On the other hand, others define the menopausal phase not as a disease but as a normal part of the life cycle, a normal developmental transition (Greer, 1991). The rapid change in hormonal balance within this phase is accompanied by symptoms that occur before, during and after menopause, so called the climacteric syndrome or menopausal symptoms (Hill, 1996).

2.4.1.1 SYMPTOMS OF MENOPAUSE

In order to identify and scale the severity of menopausal symptoms, several measurements have been developed. One of the scaling system that has been commonly used is the Greene climacteric scale (Table 2.8) that was developed by Greene (Greene, 1998). The symptoms are assessed by clustering them into:

- Somatic
- Vasomotor
- Psychological (this is further divided into depression and anxiety)
- Sexual

Among many of the symptoms of menopause, some of the primary symptoms include hot flushes, night sweats, mood swings, decreased or loss of libido and vaginal dryness (McKinlay & Jefferys, 1974).

Table 2.8: The Greene Climacteric Scale

Please indicate the extent to which you are bothered at the moment by any of these symptoms by placing a tick in the appropriate box.

SYMPTOMS	Not at all	A little	Quite a bit	Extremely	Score 0-3
1.Heart beating quickly or strongly					
2. Feeling tense or nervous					
3. Difficulty in sleeping					
4. Excitable					
5. Attacks of panic					
6. Difficulty in concentrating					
7. Feeling tired or lacking energy					
8. Loss of interest in most things					
9. Feeling unhappy or depressed					
10.Crying spells					
11.Irritability					
12.Feeling dizzy or faint					
13.Pressure or tightness in head or body					
14.Parts of body feel numb or tingling					
15.Headaches					
16.Muscle or joint pains					
17.Loss of feeling in hands or feet					
18.Breathing difficulties					
19.Hot flushes					
20.Sweating at night					
21.Loss of interest in sex					

2.4.1.2 HORMONE REPLACEMENT THERAPY

In many Western countries, hormone replacement therapy (HRT) is often used as a treatment to alleviate menopausal symptoms (Carusi, 2000; Kotsopoulos et al., 2000; Pearce et al., 1997). Data from randomised clinical trials have suggested oestrogen replacement therapy is a highly effective approach in controlling vasomotor and genitourinary symptoms (Manson & Martin, 2001). Apart from relieving the ongoing menopausal symptoms, HRT has also been shown to decrease long-term health problems associated with menopause, which may lead to morbidity in older age (Brzezinski & Debi, 1999). The sites of adverse effects of prolonged oestrogen depletion can be structured into (Anthony, 2000):

- brain: decreased cognitive function, increased risk of dementia
- cardiovascular: increased risk of coronary atherosclerosis and constriction
- genito-urinary dysfunction
- bone: osteopenia, osteoporosis, hip and vertebrae fractures

Research has highlighted HRT to be useful for postmenopausal women:

- to control vasomotor symptoms
- to maintain normal cognitive function
- to reduce the risk of Alzheimer's disease
- to prevent coronary heart disease
- to prevent osteoporotic fractures
- to maintain normal genitourinary function

However, as presented in Figure 2.7, only a small percentage of postmenopausal women ever use HRT (Albertazzi et al., 1998; Anthony, 2000; Fitzpatrick, 1999; Glazier et al., 2001). Many women prefer to terminate its use and/or choose other alternatives (Eisenberg et al., 1998) to avoid the possible risks and side effects associated with HRT, including venous thrombo-embolism, endometrial and breast cancer risk (Albertazzi et al., 1998; Brzezinski & Debi, 1999; Carusi, 2000; Fitzpatrick, 1999).

The development of a newer class of drugs called selective oestrogen receptor modulators (SERMs) provides both oestrogen agonist and antagonist properties, depending on the target tissue (Brzezinski & Debi, 1999; Carusi, 2000; Fitzpatrick, 1999). Included in this group are tamoxifen, droloxifene and raloxifene. These types of drugs have been developed to preserve the benefits of traditional hormone replacement therapy while avoiding unwanted side effects. As described earlier, phytoestrogens may have similar characteristics to SERMs, (Brzezinski & Debi, 1999) given their agonist/antagonist properties in oestrogen sensitive tissues (Hahn et al., 1998). The structural similarity of raloxifene, oestradiol and genistein is shown in Figure 2.8. The similarity between phytoestrogens and SERMs is illustrated by the former having antiproliferative effects on the breast, and a positive effect on lipoprotein profiles and bone, as well as potentially alleviating some of the climacteric symptoms (Anthony et al., 1996; Brzezinski & Debi, 1999; Fotsis et al., 1993).

Figure 2.7: The usage of HRT in some countries.

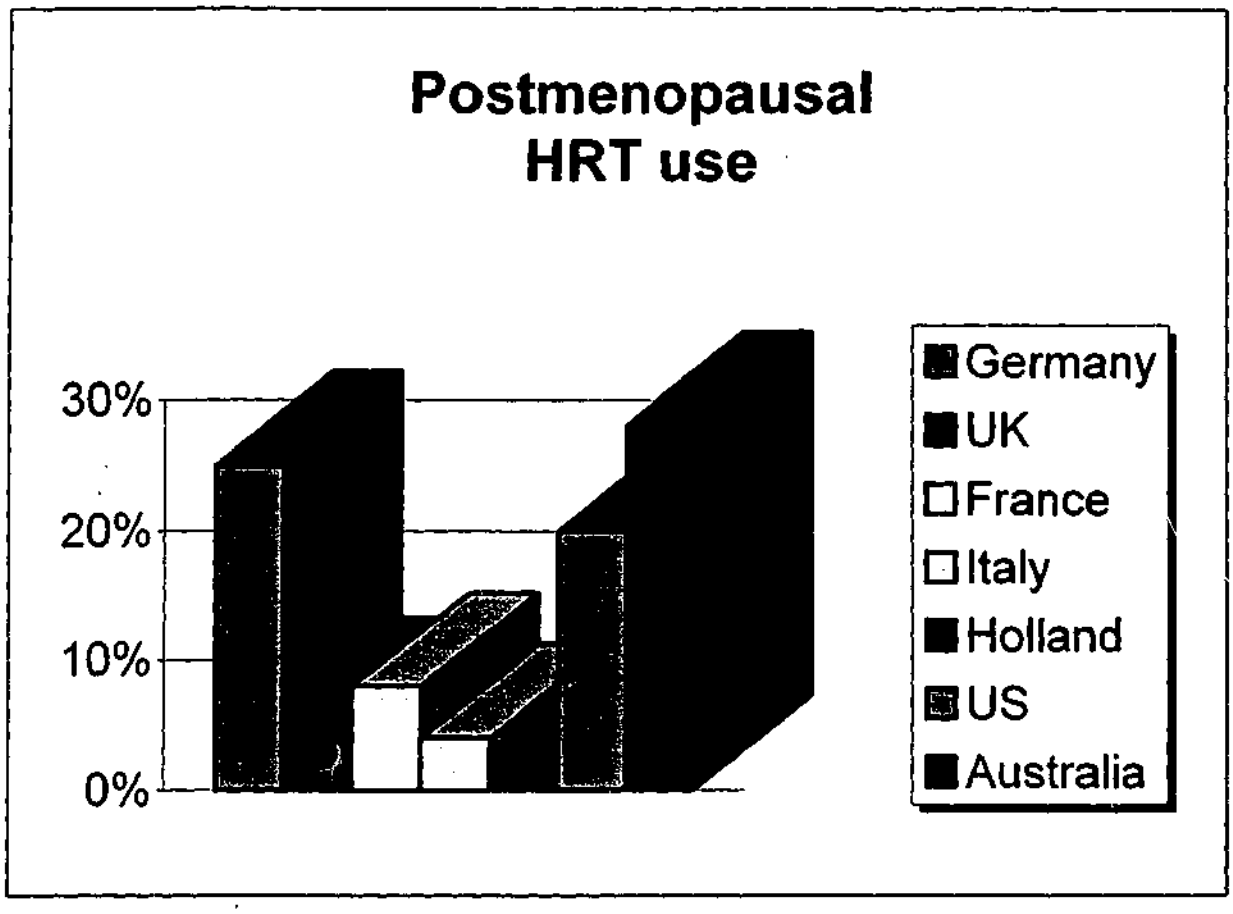
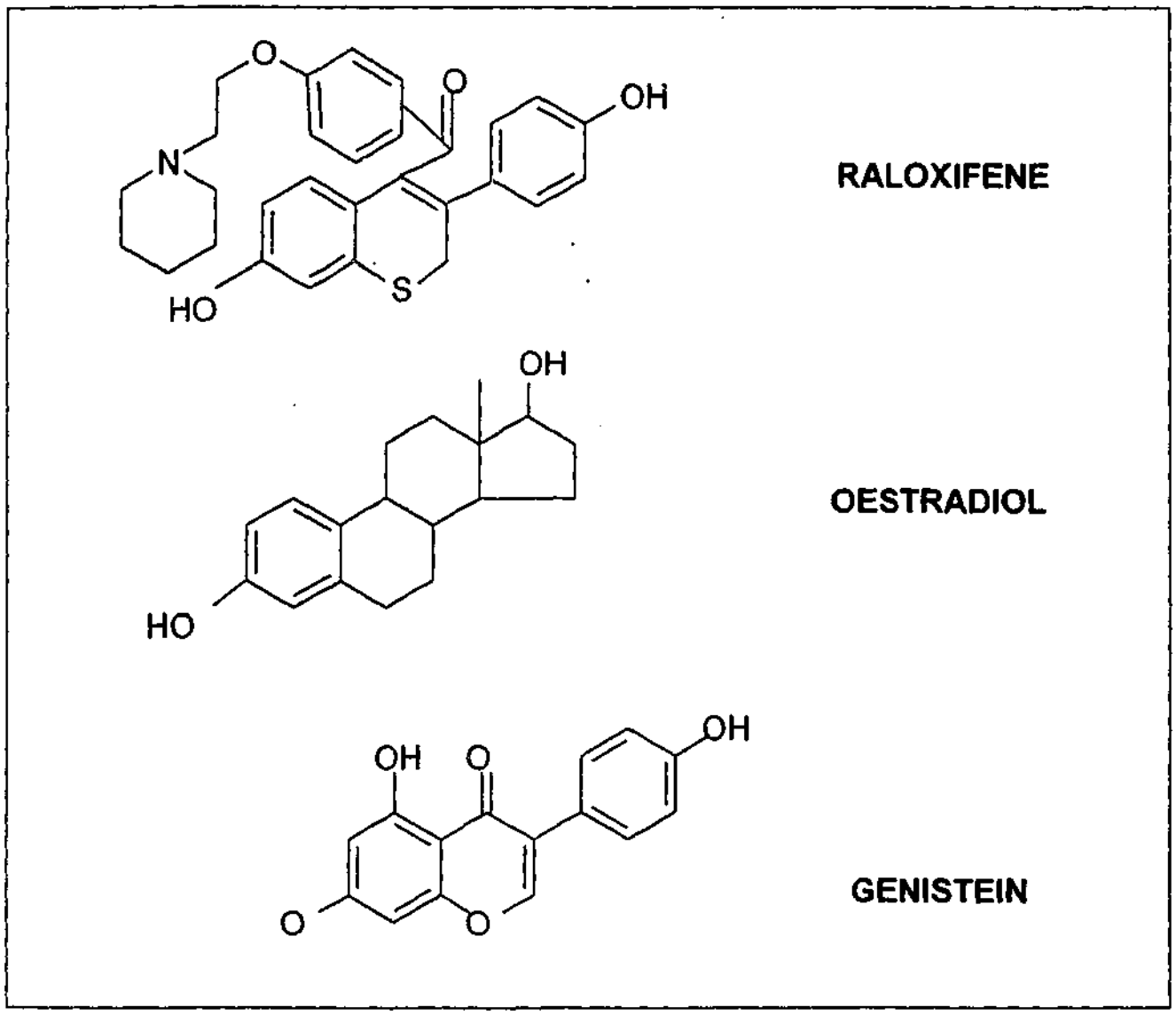


Figure 2.8: The similarity of raloxifene, oestradiol and genistein chemical structure (Brzezinski & Debi, 1999).



2.4.2 MENOPAUSE IN DIFFERENT CULTURES

Despite menopause being a universal hormonal event, the menopausal transition is interpreted and experienced differently by different cultures. Epidemiological studies indicate that Asian women experience fewer and less severe complaints, compared to their Western counterparts (Boulet et al., 1994; Hilditch et al., 1999; Oddens, 1994).

The proportion of women experiencing hot flushes may be as great as 85% in western countries, but as low as 10% in some east Asian countries (Greendale et al., 1999; North American Menopause Society, 2000; Oddens, 1994). Surprisingly, hot flushes were reported to be absent among Mayan Indian women (Martin et al., 1993). These remarkable differences may be related to several factors, such as:

- Socio-demographic and cultural variables (Brzezinski et al., 1997; Dennerstein, 1996; Kotsopoulos et al., 2000).
- Health conditions (McKinlay et al., 1987; Van Keep & Kellerhals, 1976)
- Diet and lifestyle (Boulet et al., 1994; Brzezinski et al., 1997; Brzezinski & Debi, 1999; Dalais et al., 1998; Kotsopoulos et al., 2000; Scambia et al., 2000).

The majority of the studies suggest that the lower occurrence of menopausal complaints is associated with a different perspective of menopause, with Asian women having a more positive expectation of the transition (Beyene, 1986; Boulet et al., 1994; Flint, 1975; Hilditch et al., 1999; Kaufert, 1982).

Positive expectations could be based partially on social and cultural backgrounds. From an Asian society's point of view, menopause means freedom from menstruation, contraception and pregnancy, since women in developing countries typically have high gravidity during their reproductive years. Furthermore, menopause is a symbol of higher social status due to older age. Therefore, there is a socio-cultural context of menopause that it is perceived as a reward (Flint, 1975; Kaufert, 1982). However, there is a suggestion that the stability of vaso-somatic menopausal symptoms are independent of the ethnic variable and thought to be closely related to reduced ovarian activity (Maoz et al., 1977; Mercer, 1999).

2.4.2.1 POPULATIONS WITH LOW MENOPAUSAL SYMPTOMS

Boulet and colleagues (1994) examined the occurrence of menopausal symptoms in seven South-East Asian countries namely Hong Kong, Indonesia, Korea, Malaysia, Philippines, Singapore, and Taiwan. The aim of this study was to determine the existence and the profiles of menopausal symptoms in the above countries. The frequency and severity of symptoms was less than that experienced in Western society. Psychological complaints appear to be the major symptoms found, while some minor vasomotor symptoms such as hot flushes and night sweats occur mostly during the perimenopausal period (Boulet et al., 1994). Perimenopause as the most symptomatic period is in accordance with data assessed from western women (Greendale et al., 1999). Table 2.9 displays the percentages of Asian women affected by hot flushes.

The median age at menopause assessed from the above countries was 51 years, with the lower and higher ranges being 47 and 52 years. This finding is in agreement with other population studies that have found the average age of menopause is 49, 48 and 50 years in Taiwan, Indonesia and Thailand women, respectively (Chow et al., 1997; Agoestina. & Van Keep, 1984; Punyahotra et al., 1997).

Migrating Filipino women to the United States show a similar average menopausal age of 49 years as Filipino women living in the Philippines (Berg, 1999). A study of Mayan Indian women demonstrates a relatively early age of the onset of menopause (aged 41 to 45 years), although menopause-related illnesses or symptoms are not recognised in this community. The social and cultural beliefs of the Mayan Indian community are that menopause is a phase with minimal connotations and is one that is welcomed. There are however, similarities in the changes to the endocrine profile that are shown in menopausal women in the United States. The endocrine profile in these Mayan Indian women showed an oestrogen-deprived status with bone mineral density measurements indicating the occurrence of bone demineralisation (Martin et al., 1993).

Table 2.9: Hot flush rates in Asian women

Countries	*Hot flush rate*
Japan	12-25%
China	15-17%
Hong Kong	10%
Taiwan	21-49%
Indonesia	10-11%
Thailand	26%
Korea	38%
Malaysia	30%
Filipino	30%
Singapore	14%

Figure 2.9: Hot flush rates of Asian and Western women

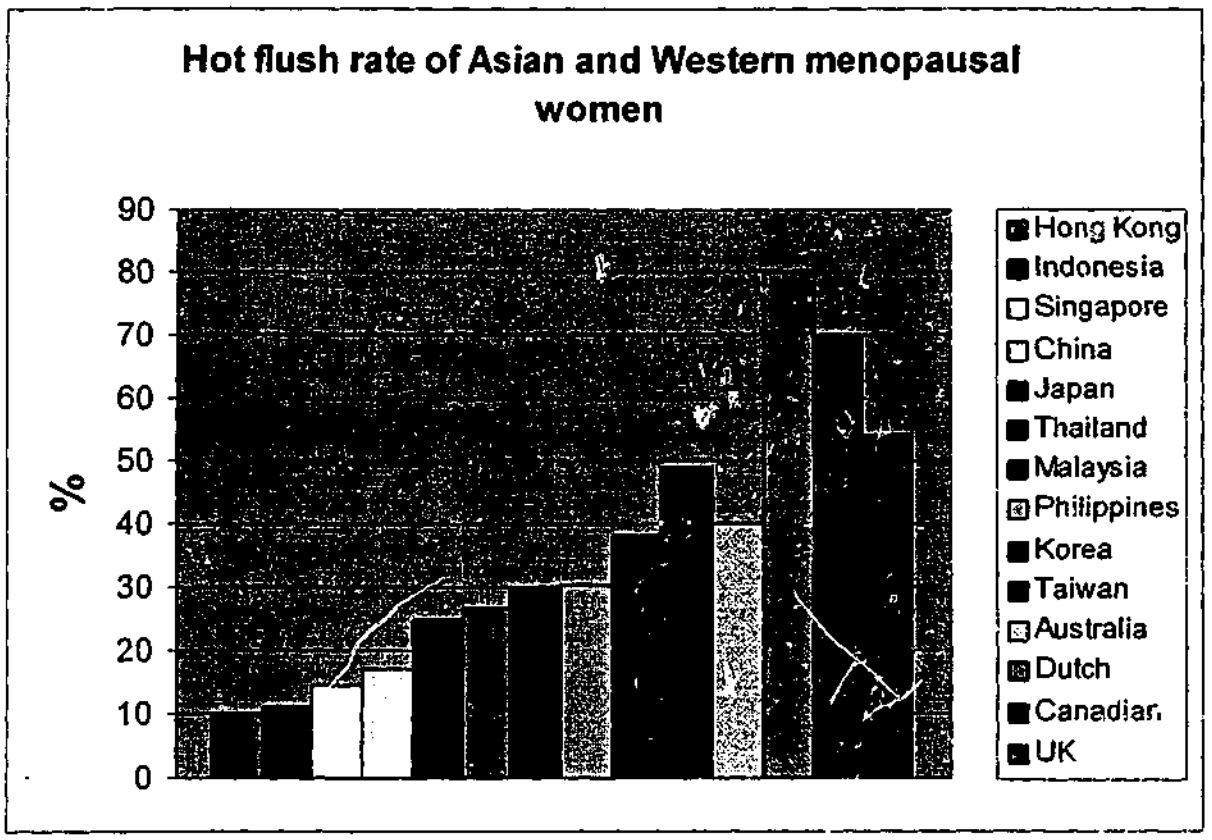
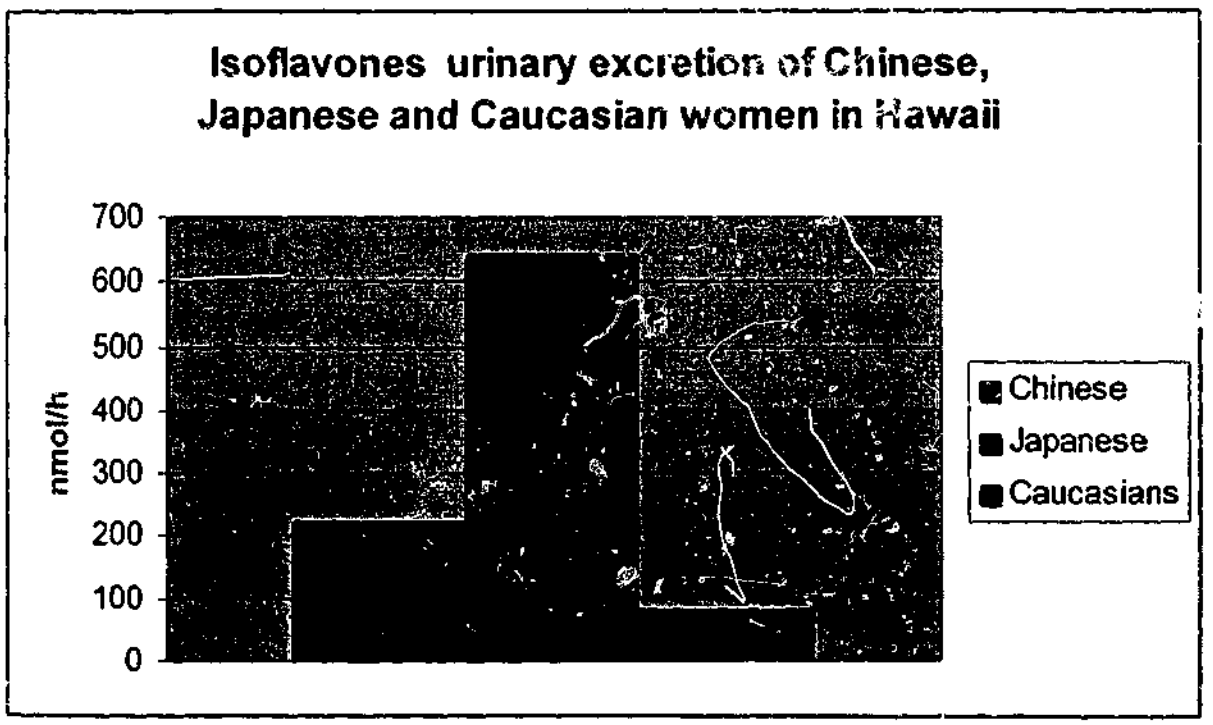


Figure 2.10: The mean urinary excretion of isoflavones of women in Hawaii



2.4.2.2 MENOPAUSAL SYMPTOMS AMONG INDUSTRIALISED-WESTERN SOCIETY

In contrast, menopausal symptoms in an industrialised Western society include hot flushes, night sweats and vaginal dryness. Such vasomotor symptoms are estimated to affect 75% - 85% of menopausal women living in North America and northern Europe (Jaszmann, 1973; North American Menopause Society, 2000; Oldenhave & Jaszmann, 1991). Population studies among Australian women also illustrate patterns of vasomotor symptoms, affecting approximately 40% of menopausal women (Ballinger, 1985; Dennerstein et al., 1993; Dennerstein, 1996). Recent studies among menopausal women in United States reported the median age of natural menopause to be 51 years (Gold et al., 2001; Greendale et al., 1999).

2.4.3 POSSIBLE ROLE OF PHYTOESTROGENS IN MENOPAUSE

During menopause, oestradiol concentrations are low. This condition may allow phytoestrogens to exert a more agonistic effect due to the low circulating concentrations of oestradiol (Carusi, 2000). Furthermore, as described earlier, phytoestrogens may act similarly to SERMs.

Epidemiological studies provide supportive evidence of the possible protective role of phytoestrogens in Asian menopausal women. The urinary excretion of

isoflavonoid phytoestrogens in Japanese women consuming a traditional diet was found to be 10-100 fold higher than that of their American and Finnish counterparts (Adlercreutz et al., 1982 & 1992). The authors of these studies were also the first to hypothesise that the low frequency of hot flushes among Japanese women was associated with the high urinary excretion of phytoestrogens. Since then, a number of trials have been conducted to elucidate whether phytoestrogens (primarily isoflavones and lignans) could act similarly to HRT in alleviating the symptoms of menopause.

Wilcox and colleagues (1990) conducted a study of 25 postmenopausal women who consumed soy, red clover sprout and linseed. With a randomised Latin square design and blinded endpoints, this study was the first to discover a significant improvement in vaginal cytology and a fall in FSH levels with a high intake of phytoestrogens.

The effect of soy supplementation in reducing hot flushes was investigated by Murkies and colleagues (1995) in a study involving 58 postmenopausal women who had at least 14 hot flushes per week. Subjects were randomised into 2 groups, one consumed soy flour, while the control group consumed wheat flour for 12 weeks. This study demonstrated the effect of soy flour supplementation in reducing hot flushes by 40% compared with a 25% reduction in the wheat flour group. However, there were no significant differences between the two groups for vaginal maturation index, lipoprotein profile, serum FSH levels, or urinary calcium.

A study carried out by Baird and colleagues (1995) compared urinary isoflavones and blood levels of LH, FSH, SHBG and oestradiol as well as vaginal cytology in a soy diet group (n=66) versus control group (n=25). After 4 weeks supplementation, increased levels of urinary isoflavones in soy diet group were identified. However, there was no impact on either the hormonal parameters or vaginal cytology measurements conducted in the subjects on the soy diet.

The administration of a 12-week phytoestrogen-rich diet (consisting of tofu, soy drinks, miso and flaxseed) or a control diet in 145 women with climacteric complaints were examined in one study conducted by Brzezinski et al., (1997). The serum concentration of phytoestrogens significantly increased in the group consuming the phytoestrogen-rich diet while remaining unchanged in the control group. This study is the first to show a significant increase in serum levels of SHBG on a phytoestrogen-rich diet. There was no such effect in the control group. Hot flushes and vaginal dryness were significantly reduced on the phytoestrogen-rich diet, but no change was observed when using the menopausal scoring system.

Dalais et al., (1998) conducted a study looking at the effects of soy, linseed and wheat in a double blind, randomised, cross-over study design. Consumption of linseed and wheat showed a decrease of 41% in the rate of hot flushes. Consumption of soy showed an increase of 103% in vaginal

cytology and a significant increase of 5.2% in bone mineral content (Dalais et al., 1998).

A 12 week randomised double-blind placebo-controlled study conducted by Albertazzi et al., (1998) showed that women consuming 60g of isolated soy protein (about 76mg isoflavones) showed a 45% reduction in hot flushes compared to 30% in the placebo group. Baber et al, (1999) in their study on red clover extract (Promensil^{TM}) in 50 postmenopausal women demonstrated a negative correlation between the increasing level of urinary daidzein and menopausal symptoms. However there were no significant differences in all parameters measured (blood levels of FSH, oestradiol, SHBG, endometrial thickness and vaginal cytology) between the two groups.

In another study using a red clover extract (Promensil^{TM}), 36 postmenopausal women were randomised into 3 groups to take either a placebo, 40mg, or 160mg of isoflavones for 12 weeks. There were no significant differences among the three groups. However, this study found a strong correlation between the urinary excretion of isoflavones (particularly daidzein) and hot flush response. Women with a 24-hour urinary excretion > 7mg/day had a greater response than the placebo group (Knight et al., 1999).

Scambia et al., (200) performed a 12-week double-blind randomised study to evaluate the activity of a standardised soy extract (50 mg of isoflavones) or soy extract in combination with conjugated equine oestrogens (CEE --

Premarin™) compared to a placebo. Compared to pre-treatment data, there was a significant reduction of hot flushes in soy extract group.

The effect of a 12-week period of soy protein administration in postmenopausal women was studied by conducting a double blind, placebo-controlled trial. Improvements in libido, facial hair and dry skin were observed in both the soy and the placebo groups, while only vaginal dryness was significantly improved in the soy group (Kotsopoulos et al., 2000).

CHAPTER THREE

METHODS

3. METHODS

3.1 DIETARY PHYTOESTROGEN SUPPLEMENTATION IN MEN WITH PROSTATE CANCER (THE PEPCA STUDY)

3.1.1 INTRODUCTION

The two major classes of phytoestrogens found in the typical human diet, the isoflavones and the lignans, have been shown to have beneficial effects against prostate cancer in epidemiological, cell and animal studies. These data demonstrate that dietary phytoestrogens are strong candidates for playing a role as protective compounds with regard to prostate disease. However, more human studies are required to further elucidate the role of dietary phytoestrogens on prostate cancer protection, prevention and treatment.

3.1.1.1 AIMS

The aim of this project was to determine the effects of isoflavones from soy and, a combination of isoflavones and lignans from linseed (in men diagnosed with prostate cancer) on prostate cancer biomarkers such as total prostate specific antigen (PSA), free PSA and the free:total PSA ratio.

3.1.1.2 HYPOTHESIS

The hypothesis being that isoflavones from soy, with or without lignans from linseed, will favourably affect total PSA, free PSA and free:total PSA ratio in Australian men diagnosed with prostate cancer.

3.1.1.3 ETHICAL CLEARANCE

Ethical permission was obtained from The Standing Committee on Ethics for Research involving Humans (SCERH) at Monash University and from Cabrini Hospital, The Alfred Hospital, Monash Medical Centre, the Border Urology Clinic in Albury-Wodonga and two private urology clinics that were used as recruitment-fields for this study.

3.1.2 STUDY DESIGN AND STUDY LOGISTICS

3.1.2.1 RECRUITMENT

In this randomised, double-blind, placebo-controlled study, subjects were recruited through a number of hospitals and medical centres listed below:

- Cabrini hospital
- The Alfred hospital
- Monash Medical Centre (Southern healthcare network)
- Border Urology Clinic (Albury-Wodonga)
- Bendigo hospital
- Geelong hospital

In each centre, patients diagnosed with prostate cancer and scheduled for radical prostatectomy were asked to participate in this study. A pamphlet containing information (patient information statement) about the study was given to the potential participant by a nurse at each centre or was sent by mail (Appendix 3). Potential patients were called to confirm whether they were interested in participating.

3.1.2.2 INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria for the study participants were as follows:

- Men of any age with histologically diagnosed prostate cancer and who had not undergone radiotherapy.
- Men of any age about to undergo a radical prostatectomy after being histologically diagnosed with prostate cancer and who had not undergone radiotherapy.

The exclusion criteria for the study participants were as follows:

- Subjects on a high phytoestrogen diet or taking pills containing phytoestrogens.
- Subjects on antibiotic treatment in the last 3 months prior to being included in the study.

3.1.2.3 RANDOMISATION

Subjects were randomised to one of three dietary treatment groups while awaiting surgery (radical prostatectomy). High phytoestrogen intake was achieved by using special bread manufactured by George Weston Foods (Enfield, NSW) (Appendix 1).

The randomised groups were assigned to the following bread-supplementation diets:

A. Standard wholegrain wheat bread 4 slices per day (placebo group).
B. Wholegrain wheat bread with soy grits (40g in 4 slices per day).
C. Wholegrain wheat bread with soy grits (40g in 4 slices per day) and whole linseeds (20g in 4 slices per day).

3.1.2.4 SAMPLE COLLECTIONS

Biological samples collected in this study included:

- 20mL of urine and
- 9mL of blood (temporarily stored in a plain tube).

Urine samples were stored at -20°C, while blood specimens were centrifuged and stored at –70°C.

Urine samples were used for measuring the concentration of isoflavones and lignans. Baseline urine was compared to urine collected post intervention (bread supplementation) to determine study participant dietary compliance. Sera samples were used to measure the PSA and free PSA concentration of the subjects. The baseline concentration values were then compared to the post intervention concentration values to determine the level of change during the study intervention.

Once informed consent had been obtained, a baseline visit was organised and the following measurements and endpoints collected:

- Demographic data (Appendix 2).
- Physical measurements: height, weight, abdominal circumference, hip circumference, blood pressure and pulse rate.
- Biological sample collection:
    - Blood samples (9mL) and approximately 20-30mL of midstream urine were collected at baseline
    - The second collection of biological samples (9mL of blood and 20-30mL of midstream urine) was collected 1 or 2 days prior to surgery.

Height was measured in centimeters and weight was measured in kilograms and used to calculate body mass index (BMI).

$$BMI = \text{weight (kg)} / \text{height (m)}^2$$

BMI measurement is a simple method of assessing whether a patient is at risk of under or overweight. The cut offs for BMI classifications for adult Caucasians aged 18 and over, recommended by the World Health Organisation (Western Pacific Regional Office of the World Health Organization, 2000) as follows:

- BMI < 18.5 is classified as underweight
- BMI =18.5 – 24.9 is classified as a healthy weight
- BMI =25 – 29.9 is classified overweight
- BMI =30 - 39.9 is classified as obesity
- BMI ≥ 40 is classified as morbid obesity

Abdominal and hip circumference were measured in centimeters and used as predictors of health risk together with BMI. Abdominal circumference was measured midway between the lower rib margin and the iliac crest/hip bone, while hip circumference was measured at the greatest gluteal protuberance (Willet, 1998). Abdominal and hip circumferences were used to calculate the abdominal hip ratio. For Caucasian men, a ratio of > 0.9 indicates increased

health risk and central (abdominal) obesity. Abdominal circumference alone has been shown to be a better predictor of health risk than BMI and waist:hip ratio (WHR) in Caucasian populations (Western Pacific Regional Office of the World Health Organization, 2000). In men, abdominal circumference > 94 cm is indicating increased risk.

Study participants were then instructed to consume 4 slices of bread every day prior to the surgery date.

Of the 38 patients referred, 32 were eligible and 3 patients refused to be randomised as they wanted to be on the 'active' bread, leaving 29 participants eligible. Of the 29 patients, one patient did not provide biological sample at the end of the study and 28 completed the study.

3.1.2.5 DIETARY QUESTIONNAIRE

The Food Frequency Questionnaire developed by the Anti-Cancer Council of Victoria (ACCVFFQ) (Appendix 4) was used in this study to determine whether there were any dietary differences between the intervention groups. The completed questionnaires were optically scanned by the AACV at the end of recruitment stage. Information about the average nutrient intake of the participant over the past 12 months was obtained from this questionnaire (Hodge et al., 2000).

3.1.3 ANALYTICAL METHODS

3.1.3.1 ANALYSIS OF PHYTOESTROGENS EXCRETION

Analysis of the urinary isoflavone and lignan concentrations was done using ultraviolet, high-performance liquid chromatography (HPLC).

3.1.3.1.1 URINE SAMPLE PREPARATION

One ml of urine was incubated at 37°C for 20 hours with 1ml of 2mg/ml glucuronidase (glucuronidase activity 400,000-600,000/g solid and sulfatase activity 15,000-40,000g/solid (Sigma Chemical Company, St. Louis, MO, USA) in 0,1M acetate buffer (pH5). Two µl of tritiated estrone sulphate (Amersham, Melbourne, VIC, Australia) was added to estimate extraction recovery and 100µl of butyrophenone (Sigma Chemical Company, St Louis, MO, USA) was used as an internal standard. The mixture was extracted using 10ml of diethyl ether (BDH Chemicals, Melbourne, Victoria, Australia). The remaining diethyl ether was dried down under nitrogen and resuspended in 1ml of 80% aqueous methanol (Aldrich Chemical Company, Milwaukee, WI, USA) and anaylsed using HPLC.

3.1.3.1.2 HPLC ANALYTICAL METHOD

Isoflavones were analysed by reverse-phase HPLC (Shimadzu system LC 10A) using a 25cm x 4.6mm x 0.5 µm Supelcosil LC-18 column (Activon,

Pleasant Hills, NSW, Australia). Isoflavones (genistein and daidzein) and lignans (enterolactone) were detected by UV absorbance at 230 and 254nm, respectively. A 100μL volume was loaded onto the column. Genistein, daidzein and enterolactone were quantified by comparing the area under the curve with known reference standards, genistein (Sigma Chemical Company, St Louis, MO, USA), daidzein (ICN Biomedicals, Aurora, OH, USA) and enterolactone (Plantech, Reading, UK).

The percentage recovery for the urinary extraction procedure varied between 70-75%. The intra-assay co-efficients of variation for the HPLC analysis of the samples was 1.3% with the sensitivity of 1.0ng for both isoflavones (genistein and daidzein) and lignans (enterolactone). The elution time for standards were 19.35 ± 0.05 min for daidzein, 23.32 ± 0.01 min for genistein and 20.42 ± 0.02 min for enterolactone. All the urine samples were processed in one assay.

3.1.3.1.3 ANALYTICAL METHOD FOR CREATININE

Creatinine measurements were carried out at Southern Cross Pathology, Clayton, Victoria, Australia. The creatinine analysis involves an automated colorimetric method carried out on a Dade Behring Dimension RxL Clinical Chemistry system with reagents and calibrators supplied by Dade Behring Diagnostics (Sydney, Australia) (Dade Dimension CREA kit Cat No. DF33A kit, insert dated October 1999). The method is based on a modified kinetic Jaffe reaction.

3.1.3.2 ANALYTICAL METHOD FOR PSA

The analysis of both total PSA and free PSA were carried out at Southern Cross Pathology. All samples were processed in one assay. AxSym PSA (total PSA) recognises both free and complex PSA (non-equimolar assay) and is based on Microparticle Enzyme Immunoassay (MEIA) technology carried out on an Abbott Diagnostics AxSym with reagents and calibrators supplied by Abbott Diagnostics. Reagents used for PSA analysis were the AxSym Reagent pack (Cat No. 3C19-20, Calibrators Cat No. 3C19-30, Standardised to Stanford 90:10 PSA Reference).

3.1.3.3 ANALYTICAL METHOD FOR FREE-PSA

Measurement of free PSA is also based on MEIA technology carried out on an Abbott Diagnostics AxSym with reagents and calibrators supplied by Abbott Diagnostics. Reagents used for this analysis were AxSym Reagent pack (Cat No. 3C20-20 Calibrators Cat No. 3C20-30 Standardised to Stanford 90:10 PSA Reference).

3.1.3.4 DATA ANALYSIS

3.1.3.4.1 DATA ENTRY

All data was entered into Microsoft Excel 1997 (Microsoft Corp.) spreadsheets and converted to SPSS 10.0 for Windows and/or SAS System data sets for further analysis. To facilitate the statistical analysis, data was log transformed as it was not normally distributed. Following log transformation, two outliers remained and were excluded from analyses leaving 26 pairs of data.

3.1.3.4.2 STATISTICAL ANALYSES

A Statistical Analysis System (SAS) software (SAS Institute, Cary, NC, USA) and SPSS statistical package version 10 for Windows (SPSS Inc., Chicago, IL, USA) were used to convert database files into data set files to perform all the statistical analyses for the studies. The significance level was set at 5% unless otherwise specified.

3.1.3.4.2.1 DESCRIPTIVE STATISTICS

Descriptive statistics are used to show the distribution of variables of interest. An SPSS procedure (Descriptive Analysis) was used to obtain values of mean and standard deviations.

3.1.3.4.2.2 MULTIPLE COMPARISON

The analysis of variance (ANOVA) using SPSS and SAS procedure (Proc GLM with Class variable) was performed to compare differences in the group means. The variables of interest were age, BMI, WHR, abdominal circumference, duration of dietary intervention, nutrient intake and baseline value of PSA, free:total PSA ratio, urinary excretion of phytoestrogens and the mean changes in urinary excretion of phytoestrogens, PSA and the ratio of free : total PSA from baseline to post intervention.

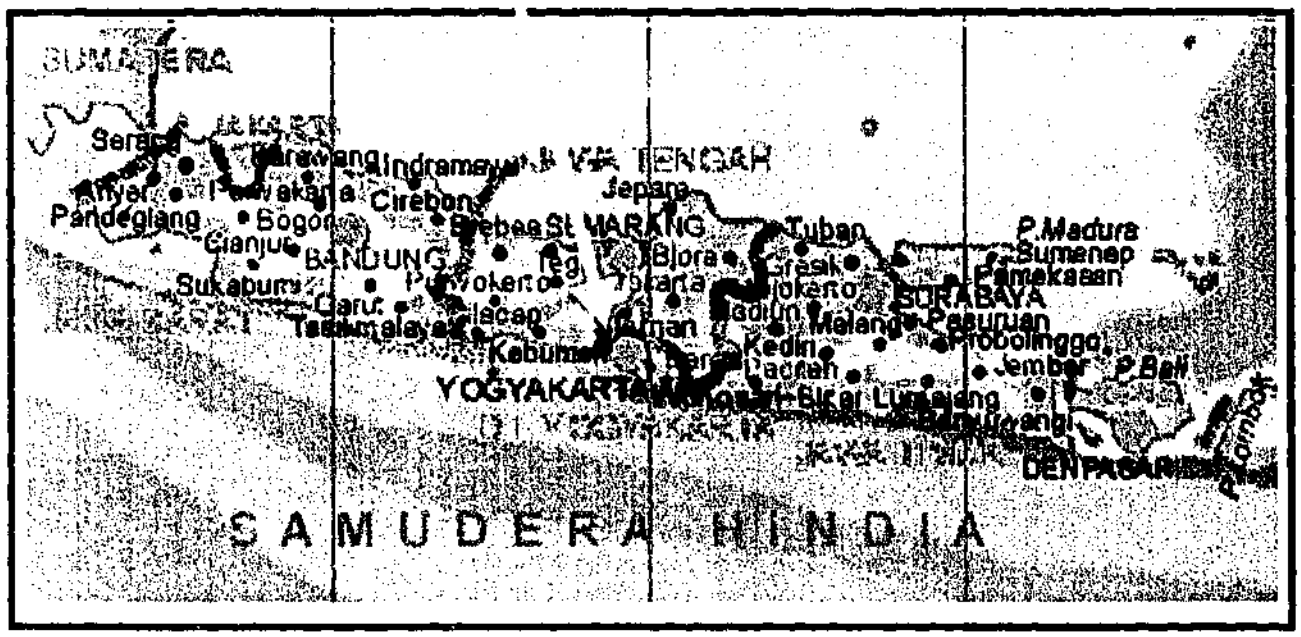
3.2 DIETARY PHYTOESTROGEN CONSUMPTION AND MENOPAUSE IN INDONESIA (THE MENOINDO STUDY)

3.2.1 INTRODUCTION

Yogyakarta is located in the central part of Java Island in Indonesia (Figure 3.1). The population of Yogyakarta regularly consume soy products, particularly in the form of tempe and tofu. Yogyakarta is the largest tempe producer in Indonesia. There are approximately 3000 tempe producers in Yogyakarta (Astuti, 1992) and most of these producers are house-hold industries that may produce between 10kgs to 4 tons of tempe per day. This production rate may reflect the tempe consumption rate of this population. Urban Yogyakarta is estimated to be the highest tempe consumer in Indonesia and in the world (Hermana & Karyadi, 1985).

The aim of the study was to describe the relationship between the dietary intake of soy products and menopausal symptoms in a population known to regularly consume soy foods as part of their daily diet. The high intake of soybean-based food products, particularly in the form of tempe and tofu (locally known as *tahu*) in Yogyakarta, makes Yogyakarta an ideal population to assess the potential effects of a phytoestrogen-rich diet on menopausal symptoms.

Figure 3.1: Map of Yogyakarta (source: www.theodora.com/maps)



3.2.1.1 STUDY OBJECTIVES

To date, there are no data available on phytoestrogen intake and menopausal symptoms in women living in Yogyakarta. The objectives of this project were to assess (1) dietary phytoestrogen intake (particularly from soy-based foods) amongst women in Yogyakarta, (2) the urinary excretion of isoflavones in women in Yogyakarta and (3) the menopausal symptom profile of women in Yogyakarta. These data will be used to determine whether there is an association between high dietary phytoestrogen (soy food) consumption and menopausal symptoms.

3.2.1.2 HYPOTHESIS

The hypothesis of this study was that there is an association between a long-life exposure to a phytoestrogen-rich diet and menopausal symptoms.

3.2.1.3 ETHICAL CLEARANCE

Ethical approval was obtained from the Standing Committee on Ethics and Research involving Humans (SCERH), Monash University and the Ethics Committee of the Faculty of Medicine at Gadjah Mada University, Yogyakarta, Indonesia. Other administrative permits to conduct the study were sought from each local council and each community health centre one week prior to the commencement of the study.

3.2.2 STUDY DESIGN

This cross sectional study was a collaborative project between the International Health and Development Unit, Monash University, Australia and the Centre of Women's Studies, Gadjah Mada University, Yogyakarta, Indonesia.

3.2.2.1 RECRUITMENT

Recruitment and collection of samples were conducted in Yogyakarta Indonesia from May to July 1999. Recruitment was carried out through community health centres and based on the following criteria:

- Subjects were recruited from community health centres that had a large turn over of people.
- Subjects were recruited from community health centres that had administrative procedures to record all visitors.
- Subjects were recruited from those people who had not recently been seen by a health professional.
- Subjects were recruited from those people with low to moderate health problems that utilised the community health centre facility.

3.2.2.2 INCLUSION AND EXCLUSION CRITERIA

Women aged 40 and above who were attending the health centres for any reason were asked to participate in the study. Pregnant and lactating women

and nuns were excluded, as well as women who were hysterectomised or had both ovaries removed.

3.2.2.3 INTERVIEWERS SELECTION AND TRAINING

3.2.1.1.1 INTERVIEWERS

Information from the participants was assessed by interviewers since there was a relatively high level of illiteracy and the nature of many questions required clarification. Interviewers were recruited from the Centre of Women's Studies at Gadjah Mada University and trained to conduct interviews based on one section of the questionnaire. Each interviewer was assigned a particular section of the questionnaire. Alternative ways of addressing particular questions were discussed and standardised sentences used to accommodate both the local (Javanese) and national (Bahasa Indonesia) languages. In order to minimise interviewer bias, it was agreed that all questions be asked in the same way and so standardised sentences for each question were established.

3.2.2.4 SURVEY QUESTIONNAIRE

The questionnaire was designed in English and translated into Bahasa Indonesian and then re-translated back into English by a qualified English-Indonesian translator from Gadjah Mada University to confirm its translation

accuracy. The questionnaire was divided into 4 sections and colour-coded (Appendix 4). These sections included questions relating to:

1. Background (socio-demographic).
2. Women's health status including menopausal status.

Height and weight measurements were included in this section and used to calculate BMI. In this study, a cut off value for adult Asians (Western Pacific Regional Office of the World Health Organization, 2000) was used as follows:

- BMI < 18.5 is classified as underweight
- BMI 18.5 – 22.9 is classified as normal or average weight
- BMI ≥ 23 is classified as overweight
- BMI 23-24.9 classified at risk,
- BMI 25-29.9 classified as obese I, and ≥ 30 as obese II.

Waist and hip circumference were also measured to assess the waist circumference and waist to hip ratio (WHR), an indicator of abdominal obesity. The suggested normal cut off range for waist circumference in adult Asians is <80 cm for women. There is no validated specific cut off value for WHR in adult Asians. In Caucasian women a WHR>0.85 identifies those with abdominal fat accumulation (James, 1996). However, waist circumference is suggested as the preferred measure of abdominal obesity compared to the WHR (WHO, 1998).

3. Menopausal symptoms score
4. Food intake

3.2.2.4.1 CONSTRUCTION OF A CLIMACTERIC SCALE

Menopausal symptoms were assessed using a modified Greene Climacteric Scale (Appendix 5). This scaling system measured a total of 21 symptoms similar to the Greene climacteric scale (Figure 2.7) but with one additional question about dyspareunia. It was decided that the question about loss of sexual interest could be due to two factors; not being in the mood or dyspareunia. Therefore, If participants indicated they had lost interest in having sex (question number 21), the interviewer would ask an additional question about dyspareunia.

The study participants were asked to rate the menopausal symptoms according to their severity, using a four-point rating scale. Each question was phrased according to whether or not the symptoms bothered them:

- 0 = not at all
- 1 = a little
- 2 = quite a bit
- 3 = extremely

Questions were phrased in Bahasa Indonesia, but some words or phrases were structured in Javanese, to assist the study participants who did not understand the Bahasa Indonesian words or phrases..

3.2.2.4.1.1 SCORING SYSTEM

The mean score for each symptom was calculated by summing the individual scores and dividing by the number of subjects. Symptoms were also clustered into six categories (see below). The mean score for each cluster is the sum of mean scores of the symptoms within that cluster. Each question of the climacteric scale was clustered in the following way:

Cluster(s)	Score – total of questions
Psychological	1-11
Anxiety	*1-6*
Depression	*7-11*
Somatic	12-18
Vasomotor	19-20
Sexual	21-22
Total score	**1-22**

3.2.2.4.2 DIETARY QUESTIONNAIRE

Dieticians from the Nutrition Unit at Prof. Sardjito Hospital (Gadjah Mada University teaching hospital), Yogyakarta, assisted in the administration of the dietary questionnaire in order to estimate the amount of soy foods consumed.

3.2.2.4.2.1 PHYTOESTROGENS FOOD FREQUENCY TABLE AND 24 HOUR DIETARY RECALL

A phytoestrogen food frequency table (of relevant foods for Yogyakarta) was devised to assess the amount and frequency of specific soy- products consumed. Subjects were asked to record their intake of soy foods using a 24-hour food recall for three non-consecutive days. It was decided to use non-consecutive days, as the habit to keep left overs from dinner for breakfast the next morning is common. Soy based foods recorded by participants over the 24 hour dietary recall period were entered in a database using Microsoft Excel 1997 (Microsoft Corp. 1997).

3.2.3 ANALYTICAL METHODS

3.2.3.1 ANALYSIS OF ISOFLAVONES EXCRETION

3.2.3.1.1 URINE SAMPLE PREPARATION

The method of preparing urine samples was similar to that of used in the PEPCA study (Chapter 3.1.3.1.1).

3.2.3.1.2 HPLC ANALYTICAL METHOD

The analytical method using a 50μL injection volume, was similar to that of used in the PEPCA study (Section 3.1.1.2). The elution times for the standard

were, 19.35 ± 0.05 min for daidzein and 23.32 ± 0.01 min for genistein. All the urine samples were processed in one assay. The urinary isoflavone excretion analysis was only done in 154 of the 191 postmenopausal women. Some urine volumes were insufficient (n=4) to be used for HPLC analysis. Five of the samples appeared to have unusually low creatinine concentrations and were omitted from analyses, leaving 145 samples for analysis.

3.2.3.1.3 CREATININE EXCRETION

The urine creatinine analysis was carried out to assess the individual's excretion rate and was conducted at Southern Cross Pathology using a similar method used in the PEPCA study (Section 3.2.1).

3.2.3.2 DATA ANALYSIS

3.2.3.2.1 DATA ENTRY

The data obtained from the studies were entered into database files using Microsoft Excel 1997.

3.2.3.2.2 STATISTICAL ANALYSIS

Data was converted from Microsoft Excel spreadsheets to dataset files. Statistical analyses for the studies were performed using SPSS statistical package (SPSS Inc., Chicago, IL, USA) version 10.0 for Windows and

Statistical Analysis System (SAS) package software (SAS Institute, Cary, NC, USA).

3.2.3.2.2.2.1 DESCRIPTIVE STATISTICS

Descriptive statistics were used to examine the distribution of variables of interest. A SAS procedure, *Proc Univariate*, was used to obtain means, standard deviations (SD) and percentiles.

3.2.3.2.2.2.2 CORRELATION ANALYSIS

The degree and direction of a linear relationship between two variables were estimated using a Pearson correlation coefficient.

3.2.3.2.2.2.3 PAIRED COMPARISON

The paired *t*-test or Wilcoxon rank-sum test was used to evaluate changes in the variables of interest.

3.2.3.2.2.2.4 MULTIPLE COMPARISON

General linear models and one way ANOVA analysis, Wilcoxon scores (Rank Sums) were used to measure the difference of in symptom-variables according to menopausal status and level of soy-intake. Pearson correlation coefficients analysis was used to measure the correlation of age to other variables.

In order to examine dependent variables between different menopausal states, study participants were broken down into premenopausal, perimenopausal and postmenopausal groups according to their menopausal status. Furthermore, to facilitate analyses of the symptom profile according to the level of soy food intake, subjects were broken down into 3 categories. They were classified as low, medium and high soy consumers according to the tertile values of the average daily intake of soy foods.

Low soy consumers were those with an average daily intake of soy foods less than 68.38 g/day (low < 68.38) (n = 109). Medium soy consumers were those with an average daily intake of soy foods between 68.38 and 114.6 g/day (n = 109), while high soy consumers were those with an average daily intake ≥ 114.6 g/day (n = 110). Those with a soy intake > 300 g/day were not included in these analyses.

CHAPTER FOUR

DIETARY PHYTOESTROGEN SUPPLEMENTATION IN MEN WITH PROSTATE CANCER (The PEPCA Study)

4. HIGH DIETARY PHYTOESTROGEN SUPPLEMENTATION IN MEN WITH PROSTATE CANCER (The PEPCA STUDY)

4.1 SUBJECT PROFILE

The mean ± SD age of the study participants was 60 ± 5 years (minimum = 46 and maximum = 66). The mean ± SD BMI was 27 ± 3 kg/m^2 (minimum = 21 and maximum = 34). The mean ± SD abdominal hip ratio was 0.95 ± 0.03, while the mean ± SD abdominal circumference was 98.7 ± 8.4. The mean ± SD duration of intervention was 24.5 ± 9 days (minimum = 8 and maximum = 45). There were no significant differences between the three groups for age, BMI, abdominal hip ratio, abdominal circumference and duration of intervention (Table 4.1).

4.2 NUTRIENT INTAKE PROFILE

The mean nutrient intake profile of each intervention group is presented in Table 4.2. There were no significant differences between the groups for any dietary parameters assessed.

Table 4.1: Characteristics of the study participants

VARIABLE	*Soy*	*Soy & linseed*	*Placebo*	*P value Anova*
Sample size	8	10	8	*
Age (years)	61.7	58.4	60.5	0.39
BMI (kg/m2)	27.1	27.9	25.98	0.43
Waist/hip ratio (%)	0.94	0.95	0.94	0.86
Abdominal circumference (cm)	99.5	99.9	96.62	0.69
Pulse rate (beats/minute)	73.5	72	73.5	0.92
Systolic blood pressure (mmHg)	149	141	146	0.72
Diastolic blood pressure (mmHg)	88	81	84	0.37
Duration of dietary intervention (days)	23.2	27.4	22.2	0.44

Table 4.2: Nutrient intake profile

		SOY GROUP (n=8)		SOY & LINSEED GROUP (n=10)		CONTROL GROUP (n=8)	
Variable	*Unit*	*Mean*	*Std Dev*	*Mean*	*Std Dev*	*Mean*	*Std Dev*
Energy	kJ/day	10110	± 6200	9991	± 2315	8868	± 2723
Total fat	g/day	93	± 86	91	± 30	85	± 29
Saturated fat	g/day	34	± 34	33	± 14	32	± 11
Monounsaturated	g/day	33	± 33	32	± 11	30	± 12
Polyunsaturated fat	g/day	17	± 11	17	± 4	16	± 6
Protein	g/day	119	± 113	108	± 24	90	± 22
Carbohydrate	g/day	228	± 99	262	± 61	219	± 68
Sugar	g/day	85	± 37	105	± 42	83	± 22
Starch	g/day	143	± 71	155	± 33	135	± 51
Dietary fibre	g/day	23	± 11	29	± 5	24	± 8
Alcohol	g/day	29	± 25	14	± 18	18	± 27
Betacarotene	ug/day	2565	± 1017	3271	± 892	2658	± 1306
Calcium	mg/day	870	± 400	850	± 129	887	± 288
Cholesterol	mg/day	279	± 349	244	± 102	211	± 48
Folate	ug/day	319	± 112	391	± 128	300	± 134
Iron	mg/day	18	± 13	18	± 5	14	± 5
Magnesium	mg/day	366	± 183	286	± 69	323	± 89
Niacin	mg/day	28	± 20	32	± 12	25	± 13
Niacin equivalent	mg/day	51	± 43	52	± 15	43	± 16
Phosporus	mg/day	1881	± 1346	1775	± 260	1555	± 440
Potassium	mg/day	3304	± 1547	3645	± 589	3050	± 799
Retinol	ug/day	348	± 185	366	± 124	458	± 231
Retinol equivalent	ug/day	776	± 271	912	± 217	901	± 318
Riboflavin	mg/day	2.4	± 1.3	3.1	± 1.1	3.1	± 1.5
Sodium	mg/day	3203	± 2535	3188	± 925	2798	± 930
Thiamin	mg/day	1.7	± 0.8	2.3	± 0.8	2.2	± 1.2
Vitamin C	mg/day	131	± 54	196	± 154	114	± 47
Vitamin E	mg/day	7.3	± 3.3	8.5	± 1.8	7.2	± 2.6
Zinc	mg/day	17	± 19	14	± 3	12	± 3

4.3 URINARY EXCRETION OF ISOFLAVONES AND LIGNANS

Urinary excretion of isoflavones and lignans were used as biomarkers of isoflavones and lignans consumption and to measure subject compliance. Table 4.3 displays urinary phytoestrogen excretion at baseline measurement. While there were significant increases in urinary excretion of isoflavones in the soy group (8 ± 7 to 288 ± 184 ng/μmol creatinine) and soy and linseed groups (8 ± 14 to 208 ± 99 ng/μmol creatinine), no significant change was found in the wheat group (7 ± 10 to 23 ± 35 ng/μmol creatinine).

Table 4.3: Urinary excretion of phytoestrogens (mean ± SD, ng/μmol creatinine)

Variable	*Soy*		*Soy & linseed*		*Control*		*P value (Anova)*
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***	
Daidzein	8±7	288±184	8±14	208±99	7±10	23±35	0.001
Genistein	4±5	221±215	4±9	124±67	7±12	3±4	0.007
Enterolactone	7±11	46±41	11±8	163±88	11±15	26±33	0.000

Pre = baseline

Post = post dietary intervention

Table 4.4: Comparison of urinary excretion of phytoestrogen between baseline and post intervention

Phytoestrogens	*P value Soy vs Soy & linseed*	*P value Soy vs Placebo*	*P value Placebo vs Soy & linseed*
Daidzein	0.001	0.000	0.003
Genistein	0.007	0.002	0.04
Enterolactone	0.000	0.5 (ns)	0.000

ns = not significant

Figure 4.1: Urinary excretion of daidzein at baseline and post intervention

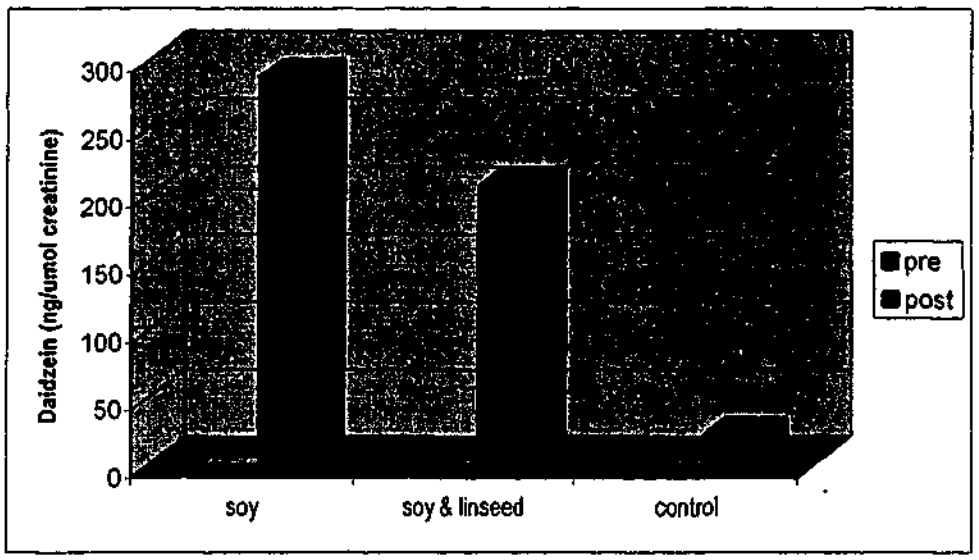


Figure 4.2: Urinary excretion of genistein at baseline and post intervention

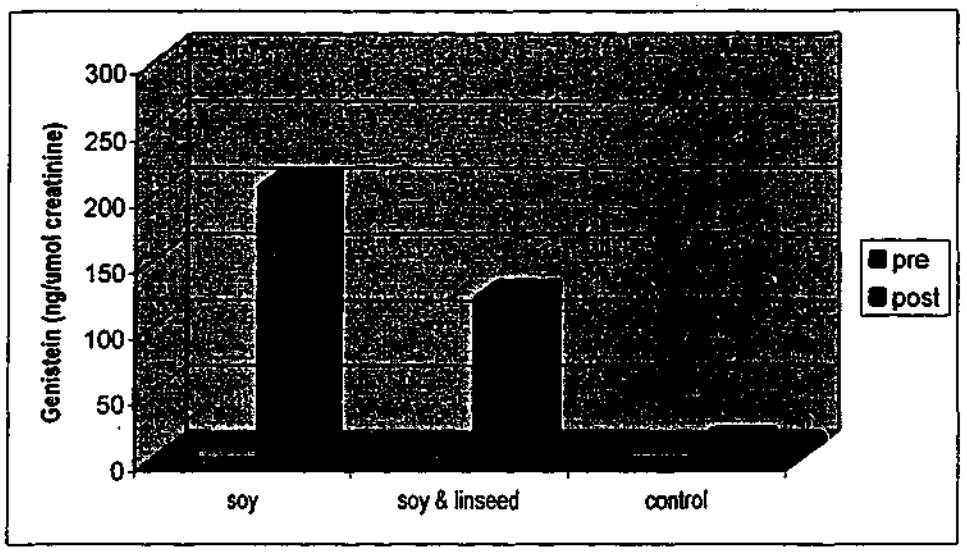
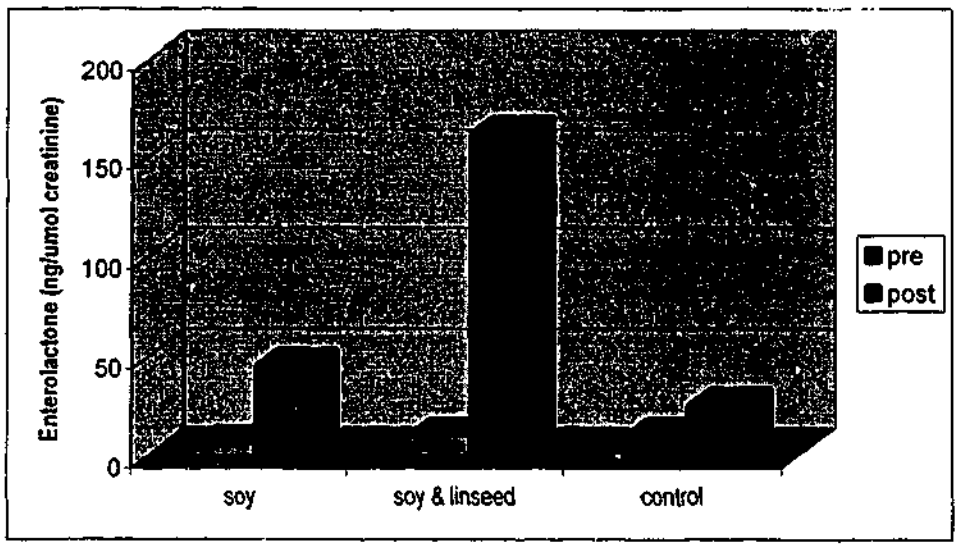


Figure 4.3: Urinary excretion of lignans at baseline and post intervention



4.4 PROSTATE CANCER BIOMARKERS

To assess the effects of the three dietary interventions on prostate cancer, changes in total and free PSA between pre and post dietary intervention were measured.

4.4.1 CHANGES IN TOTAL PSA

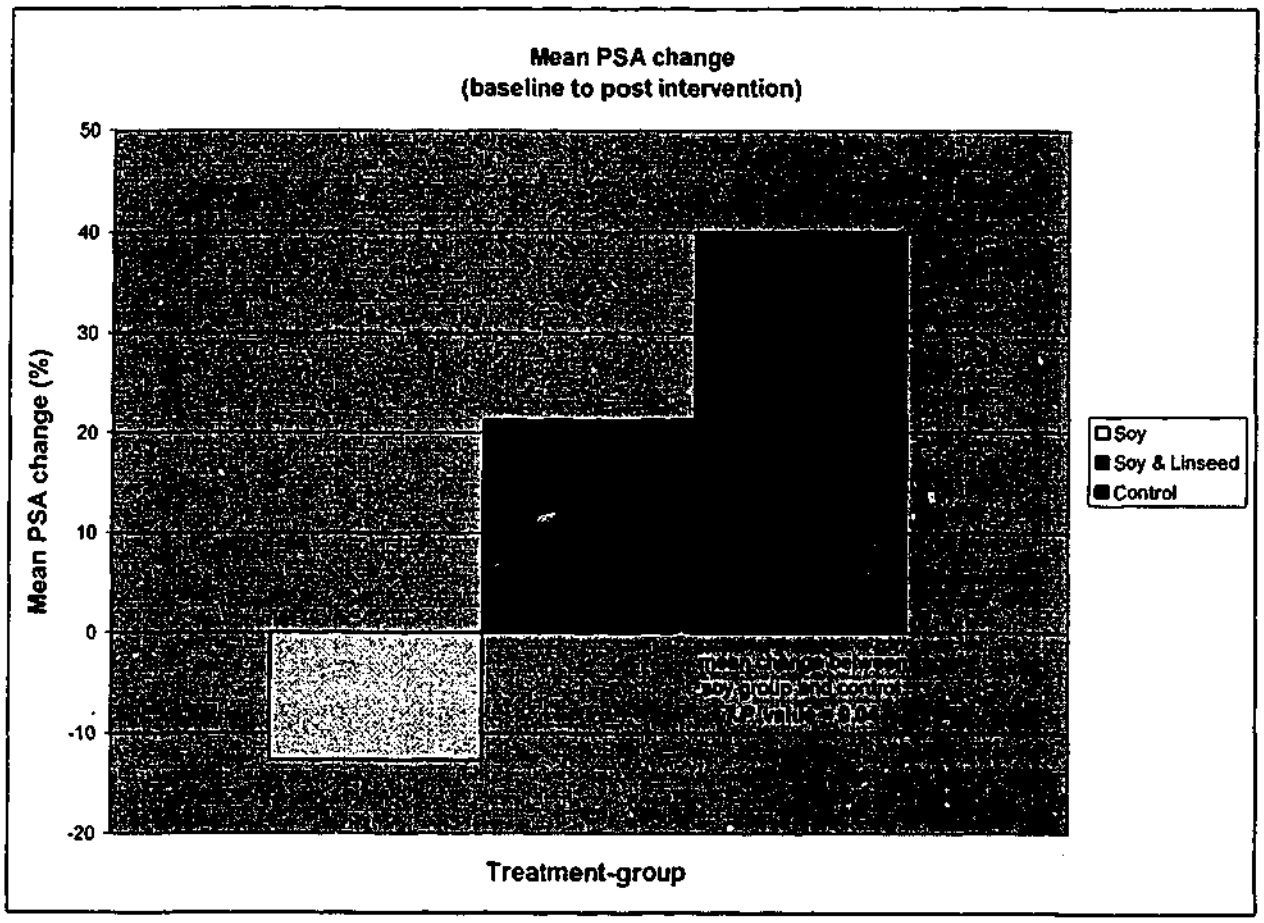
The mean changes of total PSA from baseline to post intervention in all groups are presented in Table 4.5. A significant difference was found between soy group (12.6% decrease) and control group (40% increase) (*p value* = 0.04), while there was no significant difference between soy group (12.6% decrease) to soy & linseed group (21.3% increase) and between soy & linseed group (21.3% increase) and control group (40% increase).

Table 4.5: Total PSA concentration at baseline and post intervention (μg/mL)

Variable	*Soy*		*Soy & linseed*		*Control*		*P value*
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*(Anova)*
PSA	7.1±3.2	6.3±3.0	6.3±4.0	6.9±3.2	5.8±3.7	7.1±4.2	0.04

Pre = baseline; Post = post dietary intervention

Figure 4.4: Percent PSA-change from baseline to post intervention (*Significant difference, *p value* = 0.04)



4.4.2 CHANGES IN FREE PSA

There were no significant differences between the groups for changes in free PSA. The change for the soy, soy & linseed and control groups were: 8.6%, 11.9% and 4.7%.

Table 4.6: Free PSA concentrations at baseline and post intervention (μg/mL)

Variable	*Soy*		*Soy & linseed*		*Control*		*P value (Anova)*
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	
Free-PSA	0.69±0.70	0.73±0.30	0.62±0.60	0.65±0.40	0.64±0.50	0.63±0.40	0.95

Pre = baseline; Post = post dietary intervention

4.4.3 CHANGES IN THE FREE:TOTAL PSA RATIO

As seen in Table 4.7, there were significant differences in the change of free:total PSA ratio between soy group (22.1% increase) and control group (17% decrease) (*p value* =0.01) and between soy group (22.1% increase) and soy & linseed group (11% decrease) (*p value* = 0.03). No significant difference was found between soy & linseed group (11% decrease) and control group

(17% decrease). The free:total PSA ratio changes between baseline and post intervention are presented in Figure 4.5.

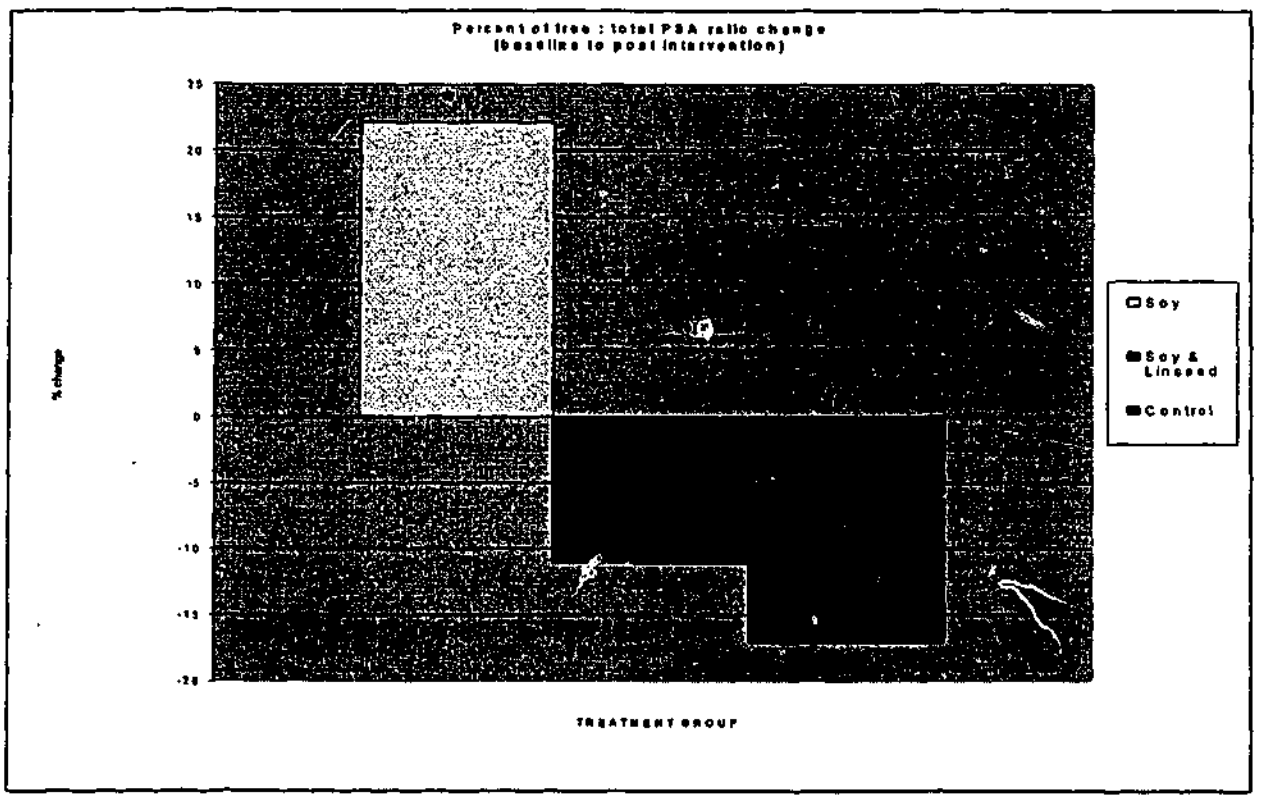
Table 4.7: Free:total PSA ratio at baseline and post intervention (*P value* between groups = 0.03)

Variable	*Soy*		*Soy & linseed*		*Control*		*P value*
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*(Anova)*
Free : total PSA ratio	0.10±0.03	0.12±0.04	0.11±0.04	0.10±0.04	0.11±0.04	0.08±0.02	0.03

Pre = baseline; Post = post dietary intervention

Figure 4.5: % Change of free:total PSA ratio between baseline and post intervention

*Significant difference (*P value* between groups = 0.03)



CHAPTER FIVE

HIGH PHYTOESTROGEN CONSUMPTION AND MENOPAUSE IN INDONESIA

(The MENDOINDO Study)

5. DIETARY PHYTOESTROGEN CONSUMPTION AND MENOPAUSE IN INDONESIA (THE MENOINDO STUDY)

5.1 SUBJECT PROFILE

5.1.1 AGE

There were 337 women who gave consent to take part in this study. Of these, 7 were excluded because of incomplete information, leaving 330 women participated in this study. Twenty percent (n = 66) were premenopausal women, 22.2% (n = 73) were perimenopausal women, and 57.8% (n = 191) were postmenopausal women. The age range of total study participants was between 41 and 75 years with a mean ± SD value of 53 ± 8 years.

5.1.2 MARITAL STATUS

Of the 330 women in the study, 1.8% (n = 6) were single and never married, 2.7% (n = 9) were divorced or separated, 23.3% (n = 77) widowed and 72.2% (n = 238) married.

5.1.3 EDUCATION

Thirty two percent (n = 105) of study participants had not completed primary school, 24.9% (n = 82) had completed primary school, 20.3% (n = 67) had some secondary education, and 17.4% (n = 58) had completed secondary school. Only 5.4% (n = 18) of study participants had a diploma or bachelor degree.

5.1.4 OCCUPATION

Seventy two percent (n = 238) of the study participants was housewives, 14% (n = 46) had a small businesses (small shop), 8% (n = 26) were working at governmental institutions (teachers, civil administration staff, health care staff) and 6% (n = 20) were working at private institutions.

5.1.4 MEDICATION

Eighty three percent (n = 274) of all study participants used traditional medicines (traditionally called = *jamu*). There were no significant relationships found between "jamu usage" and the menopausal symptoms score.

5.1.5 LIFESTYLE

5.1.5.1 PHYSICAL ACTIVITY

Of all the study participants, 40.1% exercised regularly, with the majority of the time spent doing house works (4.5 hours per week). There were no significant differences between the groups.

5.1.5.2 SMOKING

Only 1.8% (n = 6) of the study participants claimed to be currently smoking, 5.5% (n = 18) had smoked in the past. 60.6% (n = 144) of the married-participants had a husband who smoked.

5.1.5.3 ALCOHOL DRINKING

Only 0.3% or 1 participant claimed to currently drink alcohol regularly. Three percent (n = 9) claimed to have been regular drinkers in the past.

5.1.6 ANTHROPOLOGICAL MEASUREMENTS

The mean ± SD BMI was 23 ± 4 and the mean ± SD waist to hip ratio (WHR) was 1.2 ± 0.1. The mean ± SD BMI of pre, peri and postmenopausal women were 23.0 ± 3.6, 23.4 ± 4.3 and 22.9 ± 4.0, respectively. While the mean±SD WHR were 1.2 ± 0.9, 1.1 ± 0.1 and 1.2 ± 0.1, respectively. There were no

significant differences in BMI between pre, peri and postmenopausal women (Table 5.1).

5.2 MENSTRUAL RELATED INFORMATION

5.2.1 MENSTRUAL STATUS

The mean age at menarche was 14.6 years, while only 37% of the study participants were able to recall the length of their menstrual cycle. The mean menstrual cycle length was 28.6 days.

5.2.2 MENOPAUSAL STATUS

The mean age for pre, peri and postmenopausal women was 45.1, 48.1, 48.1 years, with medians of 45, 47 and 49 years respectively (Table 5.1). The mean number of years post menopause was 10.6 years.

5.1 SOY FOODS INTAKE

Soy foods were a common part of the study participant's diet with 92% (n = 304), indicating that they had tempe and tofu in their diet more than 5 days every week. 24 hours food recall diary (for three non-consecutive days) found that the mean ± SD daily intake of soy-foods (tempe and tofu-based products only) was 104.0 ± 72.2g. The mean ± SD daily soy foods intake in pre, peri

and postmenopausal women were 85.4 ± 76.4, 95.8 ± 52.5 and 107.2 ± 77.0 g/day, respectively, while there were no significant differences found between different menopausal groups (Table 5.1).

Table 5.1: Characteristics of the study participants

Variables	*Pre menopausal*	*Peri menopausal*	*Post menopausal*	*Total*	*P value (ANOVA)*
Sample size	66	73	191	330	< 0.05
Mean age (years)	45.1	47.4	58.02	53.08	< 0.05
Mean BMI (kg/m2)	23	23.3	22.9	23	0.39
Mean WHR	1.2	1.1	1.2	1.2	0.88
Mean daily intake of soy foods (g/day)	105.3	95.8	107.5	104.4	0.79
Mean score of Traditional medication (*jamu*) usage	1.19	1.13	1.18	1.17	0.48

5.2 MENOPAUSAL SYMPTOM PROFILE

5.2.1 SYMPTOM SCORE ACCORDING TO THE MENOPAUSAL STATUS

Out of all the symptoms assessed only excitable, loss of feeling in hands or feet and vaginal dryness (dyspareunia) showed significant differences according to the menopausal status (Table 5.2). Mean excitable score was higher in perimenopausal women compared to pre and postmenopausal women. Mean loss of feeling in hands or feet score was higher in premenopausal women compared to peri and postmenopausal women. The mean score of dyspareunia were as follows, postmenopausal > perimenopausal > premenopausal.

Table 5.2: Mean score of each symptom according to menopausal status

* *P* value = 0.01 ** *P* value = 0.02 *** *P* value = 0.04

Symptoms	*Premenopausal*	*Perimenopausal*	*Postmenopausal*
Heart beating strongly/quickly	0.34	0.47	0.46
Feeling tense or nervous	0.39	0.56	0.49
Difficulty in sleeping	0.92	1.15	0.95
Excitable	0.67*	1.09*	0.65*
Attacks of panic	0.42	0.67	0.4
Difficulty in concentrating	0.58	0.67	0.7
Feeling tired or lacking	1.08	1.26	1.1
in energy			
Loss interest in most things	0.73	0.86	0.73
Feeling unhappy or	0.58	0.64	0.53
distressed			
Crying spells	0.98	1.07	0.81
Irritability	0.54	0.84	0.67
Feeling dizzy or faint	0.48	0.45	0.51
Pressure or tightness in	0.34	0.38	0.35
head or body			
Parts of body feel numb or	0.25	0.25	0.2
tingling			
Headaches	0.93	1.01	0.72
Muscle or joint pains	1.02	1.05	1.1
Loss of feeling in hands	0.07**	0.01**	0.01**
or feet			
Breathing difficulties	0.05	0.09	0.06
Hot flushes	0.15	0.08	0.15
Sweating at night	0.15	0.35	0.3
Loss of interest in sex	1.4	1.47	1.37
Vaginal dryness/dyspareunia	0.31***	0.53***	0.6***

5.2.2 MEAN SCORE OF MENOPAUSAL SYMPTOM CLUSTERS ACCORDING TO THE MENOPAUSAL STATUS

As per the Greene Climacteric Scale scoring system, symptoms were divided into clusters. The mean of psychological symptom score was significantly higher in perimenopausal women compared to pre and postmenopausal women. A similar result can be observed in the anxiety symptom subcluster, but not for the depression symptoms sub cluster (Table 5.3).

Table 5.3: Mean score of every symptom cluster according to menopausal status

	PRE	*PERI*	*POST*	*Pre vs Peri*	*Pre vs Post*	*Post vs Peri*
Psychological cluster	7.25	9.31	7.56	0.04	ns	0.03
Anxiety subcluster	3.32	4.63	3.67	0.02	ns	0.02
Depression subcluster	3.92	4.68	3.89	ns	ns	ns
Somatic cluster	3.16	3.26	2.96	ns	ns	ns
Vasomotor cluster	0.37	0.56	0.54	ns	ns	ns
Sexual interest	1.71	2.01	1.97	ns	ns	ns
Total score	12.5	15.15	13.05	ns	ns	ns

ns = not significant

5.2.3 MEAN SYMPTOM SCORES IN LOW, MEDIUM AND HIGH SOY-CONSUMERS

Table 5.4: Menopausal symptom scores in low, medium and high soy consumers

Variable	*Level of Soy intake*			*P value*	*Low vs*	*Low vs*	*High vs*
	Low	*Medium*	*High*	*Anova*	*Medium*	*High*	*Medium*
PSYCHOLOGICAL	7.78	7.1	8.73	0.07	ns	ns	p=0.04
ANXIETY	3.74	3.3	4.38	0.05	ns	ns	p=0.01
DEPRESSION	4.05	3.77	4.35	0.2	ns	ns	ns
SOMATIC	3.05	2.7	3.45	0.4	ns	ns	ns
VASOMOTOR	0.5	0.42	0.61	0.4	ns	ns	ns
SEXUAL	1.95	2.14	1.7	0.4	ns	ns	ns
TOTAL SYMPTOMS	13.26	12.37	14.57	0.2	ns	ns	ns

ns = not significant

5.2.4 MEAN SYMPTOM SCORES ACCORDING TO THE LEVEL OF SOY INTAKE AND MENOPAUSAL STATUS

5.2.4.1 LOW SOY CONSUMERS

Table 5.5: Mean symptom scores according to menopausal status in **'low soy consumers'** (Pre = premenopausal women; Peri = perimenopausal group; Post = postmenopausal group)

Variable	*Menopausal Status*			*P value*	*P value*	*P value*	*P value*
	PRE	*PERI*	*POST*	*(ANOVA)*	*Pre vs Peri*	*Pre vs Post*	*Post vs Peri*
PSYCHOLOGICAL	6.31	11.12	6.93	**0.002**	**0.003**	ns	**0.001**
ANXIETY	2.47	6.32	3.12	**0.0004**	**0.0001**	ns	**0.0001**
DEPRESSION	3.84	4.8	3.83	0.3	ns	ns	ns
SOMATIC	2.52	4.36	2.71	**0.06**	**0.04**	ns	**0.02**
VASOMOTOR	0.57	0.68	0.4	0.6	ns	ns	ns
SEXUAL	1.47	1.76	2.1	0.4	ns	ns	ns
TOTAL	10.89	17.92	12.16	**0.004**	**0.004**	ns	**0.002**

ns = not significant

5.4.4.2 MEDIUM SOY CONSUMERS

Table 5.6: Mean symptom scores according to menopausal status in **'medium soy consumers'**

Variable	*Menopausal Status*			*P value*	*P value*	*P value*	*P value*
	PRE	*PERI*	*POST*	*(ANOVA)*	*Pre vs Peri*	*Pre vs Post*	*Post vs Peri*
PSYCHOLOGICAL	6.18	8.2	7.05	0.42	ns	ns	ns
ANXIETY	2.81	3.52	3.43	0.55	ns	ns	ns
DEPRESSION	3.37	4.68	3.56	0.38	ns	ns	ns
SOMATIC	2.59	3.2	3.56	0.68	ns	ns	ns
VASOMOTOR	2.59	3.2	2.55	0.14	ns	ns	ns
SEXUAL	1.7	2.24	2.31	0.53	ns	ns	ns
TOTAL	10.66	13.92	12.5	0.47	ns	ns	ns

ns = not significant

5.4.4.3 HIGH SOY CONSUMERS

Table 5.7: Mean symptom scores according to menopausal status in **'high soy consumers'**

Variable	*Menopausal Status*			*P value (ANOVA)*	*P value Pre vs Peri*	*P value Pre vs Post*	*P value Post vs Peri*
	PRE	*PERI*	*POST*				
PSYCHOLOGICAL	9.47	8.56	8.56	0.9	ns	ns	ns
ANXIETY	4.76	4	4.4	0.83	ns	ns	ns
DEPRESSION	4.71	4.56	4.16	0.83	ns	ns	ns
SOMATIC	4.47	2.13	3.58	0.1	0.02	ns	ns
VASOMOTOR	0.42	0.73	0.62	0.59	ns	ns	ns
SEXUAL	1.95	2.04	1.51	0.18	ns	ns	ns
TOTAL	16.33	13.47	14.39	0.71	ns	ns	ns

ns = not significant

5.4.4.4 PREMENOPAUSAL WOMEN

Table 5.8: Mean symptom scores according to the level of soy intake in premenopausal women (Low = low soy consumers; Med = medium soy consumers; High = high soy consumers)

VARIABLE	*SOY INTAKE*			*P value*	*low vs medium*	*low vs high*	*high vs medium*
	LOW	*MED*	*HIGH*	*ANOVA*			
PSYCHOLOGICAL	6.31	6.18	9.47	0.1	ns	ns	**0.03**
ANXIETY	2.47	2.81	4.76	0.1	ns	**0.01**	**0.02**
DEPRESSION	3.84	3.37	4.71	0.3	ns	**0.04**	**0.03**
SOMATIC	2.52	2.59	4.47	0.1	ns	ns	ns
VASOMOTOR	0.57	0.18	0.42	0.2	ns	ns	ns
SEXUAL	1.47	1.7	1.95	0.4	ns	ns	ns
TOTAL SYMPTOMS	10.89	10.66	16.33	0.1	ns	**0.03**	**0.01**

ns = not significant

5.4.4.5 PERIMENOPAUSAL WOMEN

Table 5.9: Mean symptom scores according to the level of soy intake in perimenopausal women

VARIABLE	SOY INTAKE			P value	low vs medium	low vs high	high vs medium
	LOW	MED	HIGH	ANOVA			
PSYCHOLOGICAL	11.12	8.2	8.56	0.1	ns	ns	ns
ANXIETY	6.32	3.52	4	0.02	**0.004**	**0.001**	ns
DEPRESSION	4.8	4.68	4.56	0.9	ns	ns	ns
SOMATIC	4.36	3.2	2.13	0.07	ns	**0.01**	ns
VASOMOTOR	0.68	0.28	0.73	0.3	ns	ns	ns
SEXUAL	1.76	2.24	2.04	0.6	ns	ns	ns
TOTAL SYMPTOMS	17.92	13.92	13.47	0.1	ns	ns	ns

ns = not significant

5.4.4.6 POSTMENOPAUSAL WOMEN

Table 5.10: Mean symptom scores according to the level of soy intake in postmenopausal women.

VARIABLE	SOY INTAKE			P value	low vs medium	low vs high	high vs medium
	LOW	MED	HIGH	ANOVA			
PSYCHOLOGICAL	6.93	7.05	8.56	0.1	ns	ns	ns
ANXIETY	3.12	3.43	4.4	0.06	ns	**0.03**	ns
DEPRESSION	3.83	3.56	4.16	0.3	ns	ns	ns
SOMATIC	2.71	2.55	3.58	0.3	ns	ns	ns
VASOMOTOR	0.4	0.5	0.62	0.5	ns	ns	ns
SEXUAL	2.16	2.31	1.51	0.1	ns	ns	**0.04**
TOTAL SYMPTOMS	12.16	12.5	14.39	0.3	ns	ns	ns

ns = not significant

5.2.5 SIGNIFICANT FINDINGS IN SYMPTOM CLUSTERS

5.2.5.1 PSYCHOLOGICAL SYMPTOMS

The 'psychological symptoms score' was found to be significantly less in the medium soy consumer compared to the high soy consumers (*p value* = 0.04), but not significant when compared to the low soy consumers. When the data were broken down by menopausal status, a similar pattern appeared in premenopausal women, where the psychological score was significantly lower in the medium soy consumers, compared to the high soy consumers (*p value* = 0.03) but not significant when compared to the low soy-consumer.

Examining the data by soy intake level revealed that in low soy consumers, perimenopausal women were more symptomatic than premenopausal (*p value* = 0.001) and postmenopausal (*p value* = 0.003).

Table 5.11: Significant finding for **'psychological symptom cluster'**

GROUP ANALYSED	*SIGNIFICANT FINDINGS FOR PSYCHOLOGICAL SYMPTOMS*
All subjects according to the level of soy foods intake	Medium soy consumer < high soy consumer (P value = 0.04)
Premenopausal women only	Medium soy consumer < high soy consumer (P value = 0.03)
Low soy consumer group only	Perimenopausal > premenopausal (P value = 0.001) Perimenopausal > postmenopausal (P value = 0.003)

5.2.5.2 ANXIETY SYMPTOMS (AS A SUBCLUSTER OF PSYCHOLOGICAL SYMPTOMS)

The medium soy consumers were found to experience less anxiety symptoms than the high soy consumer groups (*p value* = 0.01). Statistical analyses within different menopausal status group showed that in the premenopausal group, the high soy consumers had a higher score compared to the medium (*p value* = 0.01) and low soy consumers (*p value* = 0.02). Inversely, within the perimenopausal group, the low soy consumers had a significant higher score than the medium (*p value* = 0.004) and high soy consumers (*p value* = 0.001). In the postmenopausal group, the high soy consumers had a higher score than the low and medium soy consumers (*p value* = 0.03).

Similar to the results from the psychological cluster, the perimenopausal women were significantly more symptomatic than the premenopausal (*p value* = 0.0001) and postmenopausal women (*p value* = 0.001).

Table 5.12: Significant findings for the **'anxiety symptom cluster'**

GROUP ANALYSED	*SIGNIFICANT FINDINGS FOR THE ANXIETY SYMPTOMS*
All subjects according to the level of soy-foods intake	Medium soy consumer < high soy-consumer (P value = 0.01)
Premenopausal women only	High soy-consumer > medium soy-consumer (P value = 0.01) High soy-consumer > low soy-consumer (P value = 0.02)
Perimenopausal women only	Low soy-consumer > medium soy-consumer (P value = 0.004) Low soy-consumer > high soy-consumer (P value = 0.001)
Postmenopausal women only	High soy-consumer > low soy-consumer (P value = 0.03)
Low soy-consumer group only	Perimenopause > premenopause (P value = 0.0001) Perimenopause > postmenopause (P value = 0.0001)

5.4.5.3 DEPRESSION SYMPTOMS

Within the premenopausal group, the high soy consumers were significantly more symptomatic than the low and medium soy consumers groups (Table 5.13).

Table 5.13: Significant finding for the **'depression symptom cluster'**

GROUP ANALYSED	*SIGNIFICANT FINDINGS FOR DEPRESSION SYMPTOMS*
Premenopausal women only	High soy consumer > medium soy consumer (P=0.03) High soy consumer > low soy consumer (P=0.04)

5.2.5.4 SOMATIC SYMPTOMS

The somatic symptoms in the low soy consumers were significantly more in the perimenopausal group compared to the premenopause group (*p value* =0.004) and postmenopause group (*p value* = 0.002). Within the high soy consumers, perimenopausal women had less symptomatic compared to premenopausal women (*p value* = 0.02). Examining the perimenopausal

women only, the high soy consumers were significantly less symptomatic compared to the low soy consumers (*p value* = 0.01) (Table 5.14).

Table 5.14: Significant findings for the **'somatic symptom cluster'**

GROUP ANALYSED	*SIGNIFICANT FINDINGS FOR THE SOMATIC SYMPTOMS*
Low soy consumers group only	Perimenopause > premenopause (P value =0.004) Perimenopause > postmenopause (P value = 0.002)
High soy consumers only	Perimenopause < premenopause (P value = 0.02)
Perimenopausal women only	High soy-consumer < low soy consumers (P value = 0.01)

5.2.5.5 VASOMOTOR SYMPTOMS

As displayed in Table 4.6, the score of vasomotor symptom cluster were very low. Unlike other clusters, there were no significant differences in vasomotor symptoms score.

5.2.5.6 SEXUAL SYMPTOMS

For postmenopausal women, the medium soy consumers were more symptomatic than the high soy consumers (*p value* = 0.04) (Table 5.15).

Table 5.15: Significant finding for the **'sexual symptom cluster'**

GROUP ANALYSED	*SIGNIFICANT FINDING FOR SEXUAL SYMPTOMS*
Postmenopausal women only	Medium soy consumer > high soy consumer (P=0.04)

5.2.5.7 SIGNIFICANT FINDINGS FOR TOTAL SYMPTOM SCORES

Within low soy consumers, the perimenopausal women's total symptoms score was significantly higher compared to premenopausal (*p value* =0.004) and postmenopausal women *(p value* = 0.002). While within premenopausal women only, the total symptoms score of the high soy consumers was significantly higher than the low (*p value* = 0.03) and medium soy consumers (*p value* = 0.01) (Table 5.16).

Table 5.16: Significant findings for the **'total symptom scores'**

GROUP ANALYSED	*SIGNIFICANT FINDINGS FOR TOTAL SYMPTOM SCORE*
Low soy consumers only	Perimenopausal women > premenopausal women (p value = 0.004) Perimenopausal women > postmenopausal women (p value = 0.002)
Premenopausal women only	High soy consumers > low soy consumers (p value = 0.03) High soy consumers > medium soy consumers (p value = 0.01)

5.3 URINARY EXCRETION OF ISOFLAVONES

Table 5.17: Results of the urinary isoflavones excretions in postmenopausal women only (n=145).

	Daidzein	*Genistein*
Mean±SD (ng/μmol creatinine)	627.9±58.0	522.9±153.0
CV (%)	1.78	1.19

CHAPTER SIX

GENERAL DISCUSSION

6. GENERAL DISCUSSION

6.1 PHYTOESTROGEN SUPPLEMENTATION IN MEN WITH PROSTATE CANCER (THE PEPCA STUDY)

The Pepca Study was a double blind, randomised, placebo-controlled study designed to evaluate the effects of dietary phytoestrogen supplementation in Australian men diagnosed with prostate cancer. The study demonstrated a significant increase in urinary phyoestrogen excretion with soy and with soy & linseed ingestion; favourable differences in % changes in total PSA between soy and control groups, and in free:total PSA ratio with the soy diet compared to the soy & linseed and control groups.

The study participant demographics highlighted the fact that these were men at greater risk of prostate cancer due to their age and body morphometry measurements. These were men who were overweight and abdominally obese as reflected by their BMI (27 ± 3 kg/m^2), their abdominal to hip ratio (0.95 ± 0.3) and abdominal circumference (98.7 ± 8.4 cm).

For ethical reasons we could not impose a specific duration for the dietary intervention, and as a result obtained a wide variation in the mean duration of intervention which was 24.5 ± 9 days. This wide variation was principally due

to the fact that study participants were recruited from public and private hospitals, with different waiting periods prior to surgery. Despite being a limitation of the study, the three intervention groups were relatively well-matched.

Study participants were also well-matched for their dietary behaviour. The food intake patterns fell within the dietary recommendations. Nutrient intake ranges were mostly close or within recommended values and there were no significant differences in macronutrient or micronutrient profiles. Additionally, the average mono-unsaturated, poly-unsaturated and saturated fat intakes, implicated in prostate cancer, were within the acceptable ranges for percentage of energy intake.

The dietary phytoestrogen interventions (n = 8 in soy group and n = 10 in soy & linseed group) resulted in increased urinary excretions of isoflavones and lignans from baseline to post dietary intervention, indicating study participants' compliance. Isoflavone excretion in urine is reflective of isoflavone intake in the diet (Morton et al., 1994; Kirkman et al., 1995; Xu et al., 1995).

These data are comparable to previously published data by Dalais et al (1998) who performed a 12-week dietary soy supplementation in Australian postmenopausal women. Increases in urinary excretions of isoflavones from less than 50ng/μmol of creatinine at baseline to almost 800ng/μmol of creatinine at post dietary manipulation were observed (Dalais et al., 1998).

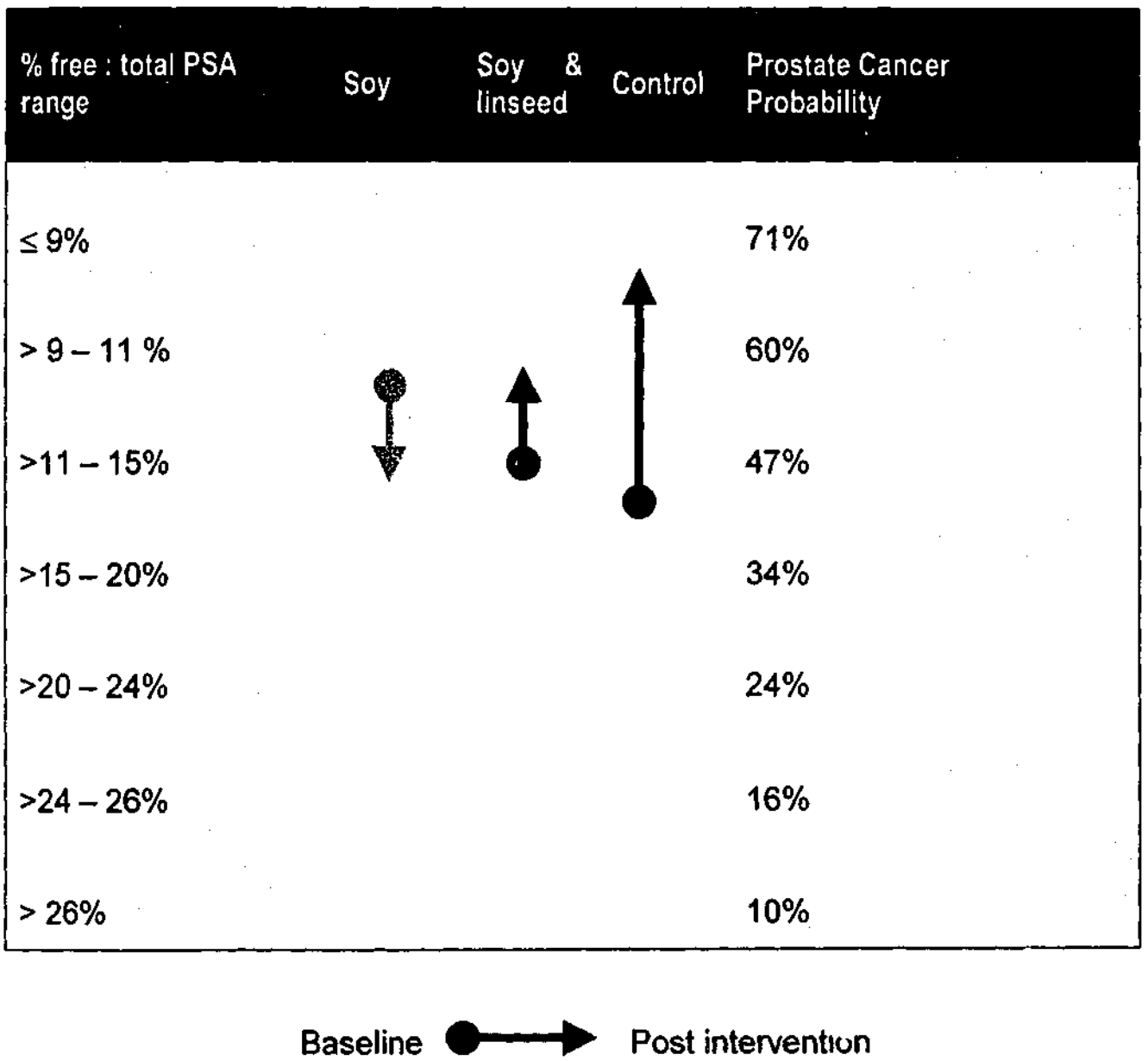
Study participants were known not to add any foods containing high concentrations of phytoestrogens such as soy foods to their diet, so that any increase could be attributed to the study intervention.

This is the first study to demonstrate a beneficial effect of dietary supplementation with phytoestrogens on the biomarkers of prostate cancer. The significant difference in the percent change in total PSA between the soy group and the placebo group, supports previous published cell studies investigating the effect of soy isoflavones where PSA expression is inhibited (Davis et al., 2000).

Significant differences were also observed in the percent change in free:total PSA ratio. Soy, soy & linseed and placebo groups showed a 22.1% increase, 11% decrease, and 17% decrease, respectively. Recently, free:total PSA ratio has been shown to be a better predictor of prostate cancer probability compared with total PSA alone (Chan et al., 1999). However, despite being supported by a number of studies, this end-point remains to be fully validated for patient management strategies. Using the probability analysis of Chan et al (1999), the present study shows a shift in percent free:total PSA ratio from baseline to post intervention in all groups (Table 6.1). There is a change in the soy group from 10% to 11%, which would mean no change in prostate cancer probability. A change in the opposite direction is observed in the soy & linseed group from 11% to 10%, with no change in prostate cancer probability. while the change in the placebo group was from 11% to 8%, resulting in a shift in

prostate cancer probability from 47% to 71%. While the use of this marker is still embryonic, it could be that the soy diet reduced prostate cancer probability. More data will be required on the use of this marker before any firm conclusions can be made.

Figure 6.1: Prediction of the change in prostate cancer probability based on the change in the ratio of free:total PSA from baseline to post intervention



These results do not support the hypothesis that the combination of soy and linseed, on the basis of cell culture studies using isoflavones and lignans, would inhibit 5α-reductase and 17β-hydroxysteroid dehydrogenase *in vivo* (Evans et al., 1995; Griffiths et al.,1998).

Published data on the combination of soy and linseed or isoflavones and lignans on prostate cancer are scarce. However, data from Evans and colleagues have suggested that the isoflavones genistein and daidzein in isolation have stronger inhibitory effects on 5α-reductase when combined with the lignans enterolactone (Evans et al., 1995).

There were no significant differences within or between all three groups for the change in free PSA between baseline and post dietary intervention. This could be due to the lack of sensitivity of free PSA as a marker to show a change, the short duration and the variation in the dietary intervention, a small number of participants, or possibly was differences in phytoestrogens dose response with genetic polymorphisms.

Ultimately, these data encourage a further investigation of prostate cancer patients who are in "watchful waiting", to plan further interventions and to evaluate more extensively of prostate cancer biomarkers.

6.2 High Phytoestrogens Consumption and Menopause in Indonesia (The MenoIndo Study)

In a soy-based food culture, the MenoIndo study investigated the intake of dietary phytoestrogens and the urinary excretion of isoflavones in relation to menopausal symptoms. This study was conducted during May and July 1999, was limited because of the first 'democratic' general election in Indonesia held on July 1999.

The mean age at menarche of the study participants was 14 years, which is older than that of contemporary Western women (12 years) (Strom et al., 1998). However, this was within the average range of age at menarche observed in other Asian populations. Age at menarche was found to be a relevant predictor of age at menopause in two studies conducted in Asian women (Boulet et al., 1994; Nagata et al., 1998).

The mean age at menopause was 48 years, which is lower than that of Caucasian women and Asian women participating in seven South East Asian countries study (51 years). However, it is consistent with findings of Agoestina and van Keep who surveyed 1025 West Javanese women and showed that the mean age at menopause was 48 years in 1984 (Greendale et al., 1999; Boulet et al., 1994). Further, the present findings support the notion that a

"later age of menarche" is a predictor of an "earlier onset of menopause" (Boulet et al., 1994).

The average BMI of the study participants was 23kg/m^2. Other health outcome data in relation to metabolic and cardiovascular disease indicate that a preferred BMI range for adult Asian would be up to 23, and of an overweight at BMI ≥ 23. The mean waist-to-hip ratio (WHR) in the present study was 1.2, which exceeded the preferred WHR for women (WHR < 0.8) (WHO, 1998). These data need interpretation in the light of study participants wearing traditional outfits with several layers. The study was carried out in an open plan area of health centres, and for cultural reasons, clothing could not be removed or adjusted, so that accuracy will have been compromised.

The average intake of soy foods was 104g per day. These data are based on the two principal soy foods consumed in Yogyakarta, tempe and tofu. In comparison with other Asian populations, it has been shown that Japanese consume between 20-50g of soy products, which is equivalent to a daily intake of 20-80mg of isoflavones (Adlercreutz et al, 1991). The daily intake of isoflavones in Western food-culture is estimated to be less than 1mg/day (de Kleijn et al., 2000). There has been a report by Nagata et al of an association between early onset of menopause and a high intake of soy products (Nagata et al., 1998). This study, conducted in Japanese women, revealed that women with an earlier age of menopause reported a higher intake of soy products compared to women with a later age of menopause. Phytoestrogens are

capable of acting both as antioestrogenic and oestrogenic compounds. The antioestrogenic effects produced by a high intake of soy foods in the present study could potentially suppress normal reproductive function through disruption of ovarian function, and in turn, result in an early onset of menopause (Nagata et al., 1998). Additionally, a similar mechanism may lower the risk of breast cancer, as Asian women have a lower incidence of breast cancer and early menopause is also associated with a lower risk of breast cancer (Kelsey et al., 1993).

Urinary excretions of isoflavones from the spot urine specimens were 627 ng/μmol of creatinine and 522 ng/μmol of creatinine for daidzein and genistein, respectively. Soy foods are the primary contributors to isoflavone intake and excretion in the human diet of both Asian and non-Asian populations (Horn-Ross et al., 2000; Lampe et al., 1999). Such high urinary excretion of isoflavones seen in the present study confirms the high dietary intake of soy foods.

Of all the menopausal symptoms assessed, psychological symptoms were dominant in this population, with anxiety rather than depression being the prime contributor to this symptomatology. Menopausal vasomotor symptoms that are the most common problems for most Western women were, on the other hand, minimal in women in Yogyakarta. This supports previously published data, which suggest that, even though vasomotor symptoms are experienced by Asian women, they are very uncommon (Boulet et al., 1994).

The very low or zero vasomotor cluster score in most Yogyakartan women could possibly explain why our study failed to demonstrate any association between soy food intake and vasomotor symptoms, as a very low score (= zero in most women) would not be sensitive enough to allow such an association.

As expected, and in agreement with previously published data which have assessed both Asian and Western populations, the most symptomatic group in this study was the perimenopausal (Dennerstein et al., 1993; Barentsen et al., 2001). It may be that perimenopausal women benefit more from eating soy as the additional isoflavones may balance endogenous oestrogen effects, whereas pre and postmenopausal women may not demonstrate enough menopausal symptoms to observe an effect of a soy-based diet.

By evaluating the menopausal symptom profile at different levels of soy intake, this study demonstrated that medium soy consumers were less symptomatic compared to low and high consumers. The 'somatic cluster score' in the low soy consumer group was shown to be significantly higher in perimenopausal women compared to pre and postmenopausal women. Those Yogyakartan women, with a low soy intake, could reasonably be expected to have a menopausal symptom profile like populations of Western women with a low phytoestrogen intake, where, again, perimenopausal women demonstrate relatively more symptoms. Inversely, in the high soy consumers, perimenopausal women had significantly lower somatic symptom scores than

did premenopausal women. This illustrates that high soy intake during the perimenopausal period may balance endogenous oestrogens or act in an oestrogenic manner, resulting in less symptoms.

The 'somatic cluster score' was significantly higher in perimenopausal women who were low soy consumers compared to those who were high soy consumers. However, intake of soy was not enough to eliminate symptoms. These associations may be causally linked.

The 'sexual cluster score' was also significantly higher in postmenopausal women who were medium soy consumers compared to those who were high soy consumers. If causal, this may provide an opportunity for higher intake of soy foods to alleviate sexual symptoms associated with the menopause.

For the total symptom score, perimenopausal women who were low soy consumers had significantly higher score than pre and postmenopausal women. This highlights the fact that perimenopausal women who are more symptomatic (even in Yogyakarta) behave in a similar manner to Western women on a low phytoestrogen diet. However, in premenopausal women, high soy consumers demonstrate a significantly higher total symptom score compared to medium and low soy consumers. It may be that there is a certain endogenous oestrogen status at which isoflavones act favourably. It also seems that, in regard to menopausal symptoms, premenopausal women in Yogyakarta do not gain any significant benefit from high soy consumption,

given their higher symptomatology compared to premenopausal women who are medium and low soy consumers.

As previously hypothesised, the isoflavones in soy foods may only have an effect in oestrogen deficient states or where there is an exogenous and endogenous oestrogen imbalance, as in peri and postmenopausal women although not during the premenopausal period.

All-in-all, the observations among Yogyakartan women, menopausal (pre, peri and post) symptoms, usually attributable to endogenous oestrogen deficiency in European women, indicate that there may be an optimal intake of soy and its oestrogenic constituents at various points in the life cycle (at least at the end of the reproductive lifespan).

CHAPTER SEVEN

CONCLUSIONS

7. CONCLUSIONS

7.1 HIGH PHYTOESTROGEN SUPPLEMENTATION IN MEN WITH PROSTATE CANCER (THE PEPCA STUDY)

The effects of a dietary phytoestrogen intervention on biomarkers of prostate cancer were assessed. Dietary phytoestrogen supplementation increased urinary excretion of isoflavones and lignans, which confirmed study participants' compliance. Increases in isoflavone excretion were seen in the two active intervention groups, the soy and the soy & linseed, with a significant increase of lignan excretion in the soy & linseed group. No changes were seen in the placebo group. A high soy diet caused a significant relative decrease in PSA compared to the placebo group. Furthermore, the soy diet was favourably changed the free:total PSA ratio.

This is the first study to show how a food-based phytoestrogen intervention improved the biomarkers of prostate cancer. However, these benefits were not detected when soy was combined with linseed. These data are consistent with findings from cell biology and animal experimentation. However, the mechanism by which soy alters PSA status need further study. The findings encourage prospect for the food-based prevention or complementary management of prostate cancer.

7.2 High Phytoestrogen Consumption and Menopause in Indonesia (The MenoIndo Study)

The menopausal women who took part in this study had a high intake of soy products as evidenced by the data from dietary questionnaires and urinary excretion of isoflavones. Published studies in other Asian populations demonstrate that the average intake of soy foods is between 20-80g/day. Therefore, the soy food intake of this population is among the highest in the world.

Whilst the present study has assessed menopausal symptoms, higher intakes of isoflavones may also be a protective factor against breast cancer if they contribute to the late age of menarche and early age of menopause compared to Western women. Similar to Western women, the perimenopausal women in this study were the most symptomatic in all symptom cluster groups. The profile of menopausal symptoms in Yogyakartan women demonstrated that psychological symptoms were dominant, and vasomotor symptoms were mild. As expected, there were no beneficial effects in low level of soy consumption, but benefit in consumption of between 68 – 114g/day.

This study is the first to provide an estimate of optimal intake of soy and its constituents in the diet to alleviate menopausal symptoms (in Yogyakartan

women), rather than the present relentless quest for higher intakes evident in some commercial and community sector.

7.3 Integrating Information About Phytoestrogenic Foods and Health by Gender and Food Culture

The present thesis has added new data on the background phytoestrogen intakers and status of men and women in different cultural settings. For European Australian men, the status s probably at the lower end and the Yogyakarta Indonesian women at the upper end of human intakes. Some communities, with health patterns possibly amenable to such an increase with relative safety, is provided by scrutiny of the growing number of such studies (Table 7.1). However, ongoing monitoring and surveillance of this area of dietary change, especially where there is an inadequate regulatory framework for food and pharmaceutical phytoestrogen sources, is clearly important.

It is unlikely that all health consequences of isoflavones and lignans in different cultural settings and different genetic background, have been identified as yet.

Table 7.1a: Studies investigated the association of phytoestrogenic foods and some hormone-related health conditions

*unpublished data ** published data	Studied-subjects	Type of diet	Urinary excretion of isoflavones (ng/µmol Cr) (mean±SD)		Urinary excretion of lignans (ng/µmol Cr) (mean±SD)	
			Daidzein	Genistein	Enterodiol	Enterolactone
***Meliala et al, 2001** (Melbourne, Australia)	8 European Australian ° diagnosed with prostate cancer	*(Their) regular diet*	7.9±6.8	3.8±5.3	-	-
		+ 40g soy grits/day	288.0±184.0	221.2±214.6	-	-
	10 European Australian ° diagnosed with prostate cancer	*(Their) regular diet*	8.2±14.4	3.8±8.7	-	10.8±8.4
		+ 40g soy and 20g linseed/ day	208.1.8±98.8	123.6±66.7	-	162.6±88.0
****Teede et al, 2001** (Melbourne, Australia)	55 healthy Australian ° & 50 healthy Australian postmenopausal †	*(Their) regular diet*	44.0±8	15.0±3.0	-	-
		+ 40g soy protein (≈ 118mg isoflavones/day)	316.0±37.0	186.0±23.0	-	-
		(Their) regular diet	≈50	≈10	-	-
****Dalais et al, 1998** (Melbourne, Australia)	44 Australian postmenopausal †	**+ 45g soy grits/day**	≈800	≈300	-	-
		(Their) regular diet	-	-	- nd (not detected)	≈200
		+ 45g linseed/day	-	-	≈900	≈2500

Table 7.1b: Studies investigated the level of phytoestrogenic food intake in different culture settings

* unpublished data ** published data	Studied-subjects	Type of diet	Urinary excretion of isoflavones (nmol/24 hr) (mean±SD)		Urinary excretion of lignans (nmol/24hr) (mean±SD)	
**Murkies, 1995 (Melbourne, Australia)	28 healthy postmenopausal Australian †	(Their) regular diet	2126±539	-	-	6128±1150
		+ 45g soy flour	44,902±6122	-	-	11,191±3285
**Dalais, 1998 (Melbourne, Australia)	95 postmenopausal Chinese † in Melbourne	(Their) regular diet	≈600	≈650	-	
	80 postmenopausal Anglo-Celt Australian †	(Their) regular diet	≈100	≈150	-	
**De Kleijn et al, 2001 (The Framingham Study)	964 Caucasian postmenopausal †	(Their) regular diet	Estimated daily isoflavone intake (mean)		Estimated total lignans intake (mean)	
			39µg	70µg	578µg	
			Urinary excretion of isoflavones (ng/µmol Cr) (mean±SD)			
*Meliala et al, 2001 (Yogyakarta, Indonesia)	145 healthy Yogyakartan postmenopausal †	Regular Yogyakartan diet (85±76 g soy foods/day)	627.9±58.0	522.9±153.0	-	-

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APPENDICES

Appendix 1 Bread formulations (The Pepca Study)

Appendix 2 Patient consent form (The Pepca Study)

Case report forms (The Pepca Study)

Appendix 3 Patient information statement (The Pepca Study)

Appendix 4 Dietary questionnaire (ACCV, 1996) (The Pepca Study)

Appendix 5 Participant explanatory statement

(The MenoIndo Study)

Informed consent form (The MenoIndo Study)

Appendix 6 Questionnaires used for The MenoIndo Study;

Questionnaire 1: Sociodemographic and general health conditions

Questionnaire 2: Women's health

Questionnaire 3: Menopausal symptom assessment (modified Greene's climacteric scale)

Questionnaire 4: 24hour food recall table

APPENDIX 1

Bread formulation developed by George Weston Foods (Enfield, NSW) to make 3 loaves of bread.

Bread	Flour	Salt	Improver	Yeast	Gluten	Kibbled soy	Toasted soy	Linseed	Oil	Water	Final water	water (soak)	Vinegar
Soy	1140	36	20	48	160	430	430	0	20	1380			0
Soy & linseed	860	36	20	48	220	430	430	320	20	1460			0
Control	1140	36	20	36	160	0	0	0	20		600	860	9.6

APPENDIX 2

PEPCA Study
Patient Consent Form

Subject No.				Subject Initials			
Date							

NAME _____
DATE OF BIRTH ____/ ____/ ____
ADDRESS_____
_____

I agree to take part in the above Cabrini Hospital research project. I have had the project explained to me, and I have read the Explanatory Statement, which I keep for my records. I understand that agreeing to take part means that I am willing to:

- be interviewed by the researchers
- provide samples of blood, urine, and prostate tissue twice during the study
- complete questionnaires relating to my food intake
- make myself available for a further interview should that be required
- consume a specially designed bread daily for the duration of the study
- allow the researchers to have access to my medical records

I understand that whilst I am participating in this trial I must ask my doctor before I take any other medication, including non-prescription items such as vitamins, minerals or herbal medicines

I understand that any information I provide is confidential, and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any other party.

I also understand that my participation is voluntary, that I can choose not to participate in part or all of the project, and that I can withdraw at any stage of the project without being penalized or disadvantaged in any way.

Please tick the appropriate box

- ☐ The information I provide can be used by other researchers as long as my name and contact information is removed before it is given to them
- ☐ The information I provide cannot be used by other researchers without asking me first
- ☐ The information I provide cannot be used except for this project

Signature: _____ Date: ____/____/____

PEPCA Study
Case Report Form

Subject No.				Subject Initials			
Date							

Demographics

Family Name:_____

First Name:_____

Address: _____

Suburb: Postcode:

Telephone Number (h):_____

(w):_____

(mob):_____

	Day	Month	Year
Date of Birth			

Age | | | Yrs

	Day	Month	Year
Date of Surgery			

Randomisation Gp		Checked by person I		by person II	

PEPCA Study
Case Report Form

Subject No. | | | | Subject Initials | | | |

Date | | | |

Physical Examination

1. Height | | | | Cm

2. Weight | | | | Kg

3. BMI | | | kg/m^2

4. Max abdo circ | | | | Cm

5. Hip circ | | | | Cm

6. Blood pressure | | | | / | | | | mm/Hg

I. | | | | | | | |

II. | | | | | | | |

Mean | | | | | | | |

7. Pulse Rate | | | | Beats/minute

PEPCA Study
Case Report Form

Subject No.				Subject Initials				
Date								

Exclusion Criteria

Are you currently:

1. On any drug for the treatment of prostate cancer? Yes ☐ No ☐
If yes, which drug? _____

2. Undergoing radiotherapy? Yes ☐ No ☐

3. Consuming a high phytoestrogens diet? Yes ☐ No ☐
Eg Soy products
If yes, which foods? _____

4. Consuming any other alternative treatment? Yes ☐ No ☐
Eg Trinovin, Proseren
If yes, which treatment? _____

5. Are questions 1 - 4 all ticked "NO"? Yes ☐ No ☐

Additional Questions (Not part of Exclusion Criteria)

6. Have you used antibiotics in the previous 3 months? Yes ☐ No ☐

7. How many bread slices do you consume per day? _____

8. What type of bread do you normally consume? _____

PEPCA Study
Case Report Form

Subject No.				Subject Initials			
Date							

Check Form

Consent form signed	
All three (3) pages of CRF completed	
Food Frequency Questionnaire given and explained	
Blood sample collected	
Urine sample collected	
Bread given	
Date of starting the 1st Bread	
Pathology Requisition form given	
Organised (2nd)bread delivery location	

Location & time _____

APPENDIX 3

How long will the study last?

The duration of the study will be variable, you will only be asked to consume the specially designed bread from the period when you agree to be involved in the study until your surgery date, usually between 3 to 6 weeks. This time period will have no implications on the waiting list for surgery.

At the end of the trial, the results from the tests of people who were on the high phytoestrogen diet will be compared with those of the placebo bread (without phytoestrogens). This way we will be able to tell whether phytoestrogens have a positive effect on prostate cancer and PSA level.

Details and results will be kept confidential

All information provided by you and the results of tests will be treated in the strictest confidence and will be available only to the researchers directly involved in the study. The results of your medical tests will be labelled only with a code number and will be stored separately from identifying information. When the results are analyzed we will be looking for difference between groups of people, not at the results of individuals. No information that could identify any person taking part in the study will be revealed when the results are reported.

Withdraw from the study

Participation in the study is voluntary and you will be free to withdraw from the study at any time if you so choose. Should you choose not to participate in the study, this will have no implications on your treatment whatsoever. If you have any questions or problems please feel free to contact us at the following phone number:

International Health and Development Unit, Monash University

Dr Andreanyta Meliala
Or
Ms Joanne Ferguson on
0408 343 633

Should you have a concern regarding the conduct of the trial, you should discuss it with the doctor/nurse in charge of the trial or contact the

The Secretary
The Standing Committee on Ethics in Research on Human
Monash University
Wellington Road, Clayton Vic 3800
Telephone (03) 9905 2052 Fax (03) 9905 1420

The PhytoEstrogen and Prostate CAncer Study
The PEPCA Study

The PhytoEstrogen and Prostate Cancer Study

(The PEPCA Study)

Patient Information Statement

MONASH UNIVERSITY

APPENDIX 4

WHAT IS THE STUDY ABOUT?

It is becoming more apparent that diet plays an important role in disease prevention. There is increasing evidence that prostate cancer is one of those diseases that is influenced by a number of dietary factors. One of these dietary factors that may reduce the risk of prostate cancer is phytoestrogens.

Phytoestrogens are plant compounds that have a similar structure to our own estrogen hormone, but are much weaker in potency. Populations that consume diets high in phytoestrogens such as the Japanese and the Chinese have among the lowest rates of prostate cancer in the world. Soybeans and linseed are foods that contain high levels of phytoestrogens and to date these compounds have been shown to act as protective agents in prostate cancer development. This study will examine the effects of a diet high in phytoestrogens on prostate cancer. It will determine whether increasing the intake of phytoestrogens influences the development of prostate cancer. Prostate Specific Antigen (PSA) and free PSA, current markers for prostate cancer screening, will also be measured.

WHO IS DOING THE STUDY?

The study is directed by Prof Mark Wahlqvist (International Health and Development Unit, Monash University). The other senior investigators involved in this trial are Mr Greg Neerhut (159 Myers St Geelong), Dr Don Mass (Ballarat Urology Clinic), Dr Stephen Lindsay (Bendigo), Dr Chee Wee Cham (Frankston & Mentone), Dr Fabien Dalais (International Health and Development Unit, Monash University) and Dr Andreanyta Meliala (International Health and Development Unit, Monash University).

WHO CAN TAKE PART?

We are seeking the help of men of any age with diagnosed prostate cancer who will be undergoing a radical prostatectomy who have not undergone hormonal or radiotherapy and are not on a high phytoestrogen diet or tablet eg soy and linseed bread or Trinovin.

STUDY PROCEDURE

If you are eligible for the trial, and are willing to take part, please call us on the number at the back of this pamphlet. If convenient, Dr Andreanyta Meliala (PhD student) and Ms Joanne Ferguson (Research Nurse) will organise a visit to your home. The visit will take approximately 30 minutes. We will perform a simple physical examination, measuring your height, weight, pulse rate and blood pressure, and then ask you some questions regarding your general health and medications. If you are willing to proceed with the study, you will be supplied with one of three specially designed grain breads. Two of the breads will have high levels of phytoestrogens and the other low levels (this bread will still contain the normal levels of all nutrients compared to the other two breads in order to compare the effect of phytoestrogens on prostate cancer. The levels of phytoestrogens that you will be consuming will be similar to that of a typical Asian diet. You will be asked to consume four slices of the study bread per day. This bread will be supplied until the time of your prostatectomy. During your own time, you will also be required to complete a food intake questionnaire, which will take approximately 60 minutes. This will help us determine your usual food consumption.

MEASUREMENTS

A small volume of blood (10ml) and urine (30ml) will be collected at the beginning of the study and at the time of surgery. The following will be measured: changes in prostate cancer cells (measured from the pathology report), body weight, height, blood pressure, PSA (Prostate Cancer Antigen - a prostate cancer marker), free PSA and the level of urinary phytoestrogens.

Dietary Questionnaire

Questions about what you usually eat and drink

Please fill in the date you completed this questionnaire:

DAY	MTH	YEAR
	JAN	1996
	FEB	1997
	MAR	1998
	APR	1999
	MAY	2000
	JUN	2001
	JUL	2002
	AUG	2003
	SEP	2004
	OCT	2005
	NOV	2006
	DEC	2007

INSTRUCTIONS:

This questionnaire is about your **usual** eating habits **over the past 12 months**. Where possible give only **one answer per question** for the type of food you eat **most often**. If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS: ●

NOT LIKE THIS: ✓ x —○

1. **How many pieces of fresh fruit do you usually eat per day?** *(Count 1/2 cup of diced fruit, berries or grapes as one piece.)*
   - I don't eat fruit
   - less than 1 piece of fruit per day
   - 1 piece of fruit per day
   - 2 pieces of fruit per day
   - 3 pieces of fruit per day
   - 4 or more pieces of fruit per day

2. **How many different vegetables do you usually eat per day?** *(Count all types, fresh, frozen or tinned.)*
   - less than 1 vegetable per day
   - 1 vegetable per day
   - 2 vegetables per day
   - 3 vegetables per day
   - 4 vegetables per day
   - 5 vegetables per day
   - 6 or more vegetables per day

3. **What type of milk do you usually use?**
   - none
   - full cream milk
   - reduced fat milk
   - skim milk
   - soya milk

4. **How much milk do you usually use per day?** *(Include flavoured milk and milk added to tea, coffee, cereal etc.)*
   - none
   - less than 250 ml *(1 large cup or mug)*
   - between 250 and 500 ml *(1-2 cups)*
   - between 500 and 750 ml *(2-3 cups)*
   - 750 ml *(3 cups)* or more

5. **What type of bread do you usually eat?**
   - I don't eat bread
   - high fibre white bread
   - white bread
   - wholemeal bread
   - rye bread
   - multi-grain bread

6. **How many slices of bread do you usually eat per day?** *(Include all types, fresh or toasted and count one bread roll as 2 slices.)*
   - less than 1 slice per day
   - 1 slice per day
   - 2 slices per day
   - 3 slices per day
   - 4 slices per day
   - 5-7 slices per day
   - 8 or more slices per day

7. **Which spread do you usually put on bread?**
   - I don't usually use any fat spread
   - margarine of any kind
   - polyunsaturated margarine
   - monounsaturated margarine
   - butter and margarine blends
   - butter

8. **On average, how many teaspoons of sugar do you usually use per day?** *(Include sugar taken with tea and coffee and on breakfast cereal etc.)*
   - none
   - 1 to 4 teaspoons per day
   - 5 to 8 teaspoons per day
   - 9 to 12 teaspoons per day
   - more than 12 teaspoons per day

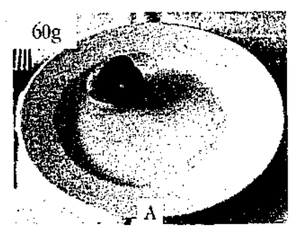
9. **On average, how many eggs do you usually eat per week?**
   - I don't eat eggs
   - less than 1 egg per week
   - 1 to 2 eggs per week
   - 3 to 5 eggs per week
   - 6 or more eggs per week

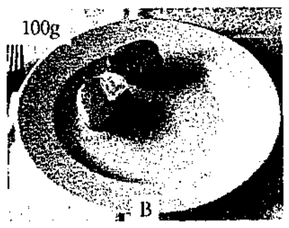
10. **What types of cheese do you usually eat?**
    - I don't eat cheese
    - hard cheeses, e.g. parmesan, romano
    - firm cheeses, e.g. cheddar, edam
    - soft cheeses, e.g. camembert, brie
    - ricotta or cottage cheese
    - cream cheese
    - low fat cheese

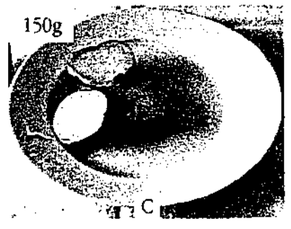
*For each food shown on this page, indicate **how much on average you would usually have eaten at main meals during the past 12 months.** When answering each question, think of the **amount** of that food you usually ate, even though you may rarely have eaten the food on its own.*

*If you usually ate more than one helping, fill in the oval for the serving size closest to the **total amount** you ate.*

11. When you ate potato, did you usually eat: ○ I never ate potato

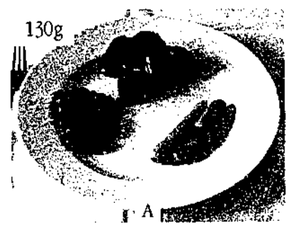


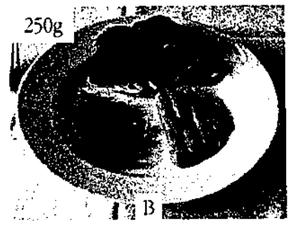


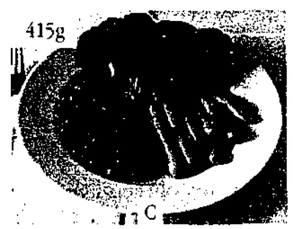


○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables

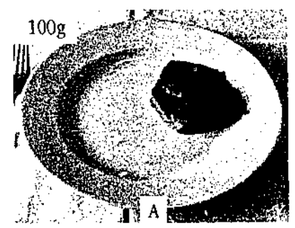


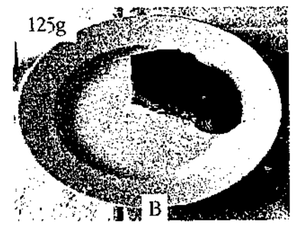


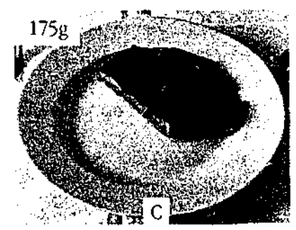


○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

13. When you ate steak, did you usually eat: ○ I never ate steak

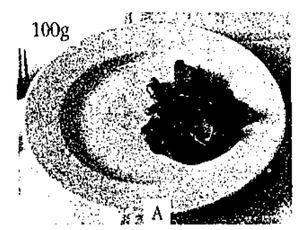


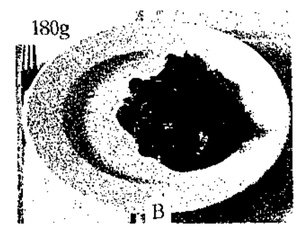


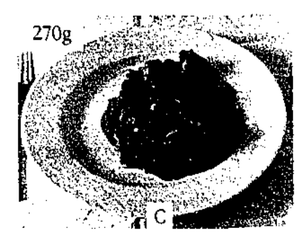


○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

14. When you ate meat *or* vegetable casserole, did you usually eat: ○ I never ate casserole







○ Less than A ○ A ○ Between A & B ○ B ○ Between B & C ○ C ○ More than C

15. Over the last 12 months, on average, *how often* did you eat the following foods? *Please completely fill one oval in every line.* Please MARK LIKE THIS: ○■○○ NOT LIKE THIS: ✓ ✗ =○

Times You Have Eaten	NEVER	less than once per month	1 to 3 times per month	1 time per week	2 times per week	3 to 4 times per week	5 to 6 times per week	1 time per day	2 times per day	3 or more times per day
CEREAL FOODS, SWEETS & SNACKS										
All Bran™	○	○	○	○	○	○	○	○	○	○
Sultana Bran™, FibrePlus™, Branflakes™	○	○	○	○	○	○	○	○	○	○
Weet Bix™, Vita Brits™, Weeties™	○	○	○	○	○	○	○	○	○	○
Cornflakes, Nutrigrain™, Special K™	○	○	○	○	○	○	○	○	○	○
Porridge	○	○	○	○	○	○	○	○	○	○
Muesli	○	○	○	○	○	○	○	○	○	○
Rice	○	○	○	○	○	○	○	○	○	○
Pasta or noodles (include lasagne)	○	○	○	○	○	○	○	○	○	○
Crackers, crispbreads, dry biscuits	○	○	○	○	○	○	○	○	○	○
Sweet biscuits	○	○	○	○	○	○	○	○	○	○
Cakes, sweet pies, tarts and other sweet pastries	○	○	○	○	○	○	○	○	○	○
Meat pies, pasties, quiche and other savoury pastries	○	○	○	○	○	○	○	○	○	○
Pizza	○	○	○	○	○	○	○	○	○	○
Hamburger with a bun	○	○	○	○	○	○	○	○	○	○
Chocolate	○	○	○	○	○	○	○	○	○	○
Flavoured milk drink (cocoa, Milo™ etc.)	○	○	○	○	○	○	○	○	○	○
Nuts	○	○	○	○	○	○	○	○	○	○
Peanut butter or peanut paste	○	○	○	○	○	○	○	○	○	○
Corn chips, potato crisps, Twisties™ etc.	○	○	○	○	○	○	○	○	○	○
Jam, marmalade, honey or syrups	○	○	○	○	○	○	○	○	○	○
Vegemite™, Marmite™ or Promite™	○	○	○	○	○	○	○	○	○	○
DAIRY PRODUCTS, MEAT & FISH										
Cheese	○	○	○	○	○	○	○	○	○	○
Ice-cream	○	○	○	○	○	○	○	○	○	○
Yoghurt	○	○	○	○	○	○	○	○	○	○
Beef	○	○	○	○	○	○	○	○	○	○
Veal	○	○	○	○	○	○	○	○	○	○
Chicken	○	○	○	○	○	○	○	○	○	○
Lamb	○	○	○	○	○	○	○	○	○	○
Pork	○	○	○	○	○	○	○	○	○	○
Bacon	○	○	○	○	○	○	○	○	○	○
Ham	○	○	○	○	○	○	○	○	○	○
Corned beef, luncheon meats or salami	○	○	○	○	○	○	○	○	○	○
Sausages or frankfurters	○	○	○	○	○	○	○	○	○	○
Fish, steamed, grilled or baked	○	○	○	○	○	○	○	○	○	○
Fish, fried (include take-away)	○	○	○	○	○	○	○	○	○	○
Fish, tinned (salmon, tuna, sardines etc.)	○	○	○	○	○	○	○	○	○	○
FRUIT										
Tinned or frozen fruit (any kind)	○	○	○	○	○	○	○	○	○	○
Fruit juice	○	○	○	○	○	○	○	○	○	○
Oranges or other citrus fruit	○	○	○	○	○	○	○	○	○	○
Apples	○	○	○	○	○	○	○	○	○	○
Pears	○	○	○	○	○	○	○	○	○	○
Bananas	○	○	○	○	○	○	○	○	○	○
Watermelon, rockmelon (cantaloupe), honeydew etc.	○	○	○	○	○	○	○	○	○	○
Pineapple	○	○	○	○	○	○	○	○	○	○
Strawberries	○	○	○	○	○	○	○	○	○	○
Apricots	○	○	○	○	○	○	○	○	○	○
Peaches or nectarines	○	○	○	○	○	○	○	○	○	○
Mango or paw paw	○	○	○	○	○	○	○	○	○	○
Avocado	○	○	○	○	○	○	○	○	○	○

Times You Have Eaten CONTINUED	NEVER	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
		per month		per week				per day		
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)										
Potatoes roasted or fried (include hot chips)	○	○	○	○	○	○	○	○	○	
Potatoes cooked without fat	○	○	○	○	○	○	○	○	○	
Tomato sauce, tomato paste or dried tomatoes	○	○	○	○	○	○	○	○	○	
Fresh or tinned tomatoes	○	○	○	○	○	○	○	○	○	
Peppers (capsicum)	○	○	○	○	○	○	○	○	○	
Lettuce, endive, or other salad greens	○	○	○	○	○	○	○	○	○	
Cucumber	○	○	○	○	○	○	○	○	○	
Celery	○	○	○	○	○	○	○	○	○	
Beetroot	○	○	○	○	○	○	○	○	○	
Carrots	○	○	○	○	○	○	○	○	○	
Cabbage or Brussels sprouts	○	○	○	○	○	○	○	○	○	
Cauliflower	○	○	○	○	○	○	○	○	○	
Broccoli	○	○	○	○	○	○	○	○	○	
Silverbeet or spinach	○	○	○	○	○	○	○	○	○	
Peas	○	○	○	○	○	○	○	○	○	
Green beans	○	○	○	○	○	○	○	○	○	
Bean sprouts or alfalfa sprouts	○	○	○	○	○	○	○	○	○	
Baked beans	○	○	○	○	○	○	○	○	○	
Soy beans, soy bean curd or tofu	○	○	○	○	○	○	○	○	○	
Other beans (include chick peas, lentils etc.)	○	○	○	○	○	○	○	○	○	
Pumpkin	○	○	○	○	○	○	○	○	○	
Onion or leeks	○	○	○	○	○	○	○	○	○	
Garlic (not garlic tablets)	○	○	○	○	○	○	○	○	○	
Mushrooms	○	○	○	○	○	○	○	○	○	
Zucchini	○	○	○	○	○	○	○	○	○	

16. Over the last 12 months, how often did you drink beer, wine and/or spirits?

Times That You Drank	NEVER	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)	○	○	○	○	○	○	○	○	○	
Beer (full strength)	○	○	○	○	○	○	○	○	○	
Red wine	○	○	○	○	○	○	○	○	○	
White wine (include sparkling wines)	○	○	○	○	○	○	○	○	○	
Fortified wines, port, sherry, etc.	○	○	○	○	○	○	○	○	○	
Spirits, liqueurs, etc.	○	○	○	○	○	○	○	○	○	

When answering the next two questions, please convert the amounts you drink into glasses using the examples given below
For spirits, liqueurs, and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 can or stubby of beer = 2 glasses
1 large bottle beer (750 ml) = 4 glasses
1 bottle wine (750 ml) = 6 glasses
1 bottle of port or sherry (750 ml) = 12 glasses

17. Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more
	○	○	○	○	○	○	○	○	○	

18. Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more
	○	○	○	○	○	○	○	○	○	

Thank You for completing this questionnaire

APPENDIX 5

Participant Explanatory Statement

Project title:

High phytoestrogen consumption and menopause in Indonesia (The MenoIndo Study)

What is this study about:
The aim of this research is to determine if there is an association of the phytoestrogen (in soy foods) consumption and menopause. Soy foods are high in chemical called isoflavones and protect some women from low oestrogen levels. Low oestrogen levels in women occur at the time of menopause and are generally associated with many of the symptoms associated with the menopause. This study will be asking questions about the intake of soy foods and symptoms of the menopause.

Who is doing the study?
Dr Andreanyta Meliala is conducting this study towards a doctorate degree at faculty of medicine, Monash University. The study is under supervision of Professor Mark Wahlqvist and Dr Fabien Dalais from the International Health and Development Unit at Monash University and Professor Mary Astuti from Women's Study Centre at Gadjah Mada University.

Who can take part?
We are looking for menopausal women between the ages of 40-75.

What do you need to do?
You will be asked questions about any symptoms of the menopause that you might have experienced and questions about your diet. You will be required to give a urine sample. Measurements of height, weight, hip and waist circumference will be assessed.

What is the urine sample for?
The urine sample is to measure the levels of isoflavones that indicate soy foods consumption.

How long will it take and is there any harm involved?
The questionnaire will take approximately 30 minutes to complete, while all other measurements will take approximately 5-10 minutes. This is a routine procedure and should cause a minimal harm.

All details are confidential and you will not be able to be identified in any way.
No findings that could identify any individual participant will be published. Your name is not required as your responses and samples will be replaced by numbered-code. Only my supervisors and I will have access to this data that will be stored for five years as prescribed by the university regulations.

You may withdraw from the study at anytime.
If you agree to participate you may withdraw your consent anytime. If you are uncomfortable with giving urine sample, or any other measurements, you do not have to participate.

Your results may be used for other studies only if you give consent.
The consent form contain tic box, in which you may allow your samples and results to be stored and used for other studies. Your results will not be used unless you specifically for this to be so.
If you have any queries or would like to be informed of the aggregate research finding, please contact telephone:

In Indonesia: +62-274-583 546
In Australia: +61-3-9903 0555

Thank you.

Dr Andreanyta Meliala

Informed Consent Form

For project title:

(1) Association of tempe consumption with oestrogen beta receptor gene polymorphisms and variation in menopausal symptoms

(2) High phytoestrogen consumption and menopause in Indonesia

I agree to take part in the above Monash University research projects. I have had the projects explained to me, and I have read the Explanatory Statement, which I keep for my records. I understand that agreeing to take part means that I am willing to:

- Provide one sample of blood
- Provide one sample of urine
- Complete a questionnaire asking me about my diet, background, general health and any symptoms, which might be related with the menopause.

I understand that any information I provide is confidential and that no information that could lead to the identification of any individual will be disclosed in any reports on the projects, or to any other party.

I also understand that my participation is voluntary, that I can choose not to participate in part or all of the projects, and that I can withdraw at any stage of the projects without being penalised or disadvantaged in any way.

If you wish to allow your data to be used for future projects please tick the box.

☐ The information I provide can be used in further research projects, which have ethics approval.

Name:.....

Signature:.....Date:

Independent witness to participant's voluntary and informed consent:

I believe that:..... understand the above projects and gives her consent voluntarily.

Name:.....

Signature:..... .....Date:

Address:.....

APPENDIX 6

Interviewer ID [ ][ ] – Participant ID [ ][ ][ ]

DATE [ ][ ] [ ][ ] *1999*

PART I: SOCIO DEMOGRAPHIC & GENERAL HEALTH CONDITIONS QUESTIONNAIRE

1. DATE OF BIRTH:

2. PLACE OF BIRTH:

3. MARITAL STATUS:

   1 SINGLE
   2 MARRIED
   3 DIVORCED
   4 WIDOWED

4. How many biological children:

5. EDUCATION LEVEL:

   1 NOT EDUCATED AT ALL
   2 PRIMARY SCHOOL
   3 JUNIOR HIGH SCHOOL
   4 SENIOR HIGH SCHOOL
   5 DIPLOMA
   6 BACHELOR DEGREE
   7 OTHERS PLEASE SPECIFY:

6. What is your daily activity or professional task ?(ie. housewife, Government officer, private officer, merchant, own small business, etc.)

7. HEALTH CONDITION

   Please specify whether you are suffering from the type of disease listed below:

   1 High blood pressure
   2 High cholesterol
   3 Heart disease
   4 Depression
   5 Overweight (obese)
   6 Diabetic
   7 Osteoporosis
   8 Cancer of the breast, ovary

9 Other cancers
10 Others, please specify:

8. Which from the list below would describe your current health condition:

1 I don't have any health problems
2 my activity is not disturbed by my health problem
3 my activity is a little disturbed by my health problem
4 My health problem is a burden

9. Are you currently on regular medication?

1 Yes
2 No

If yes, please specify the drugs and what disease is it for?

10. Are you consuming traditional medicine (jamu)?

1 Yes
2 No

If yes, please specify the name of traditional medicine (jamu) and why do you consume it?

11. For the past month, do you regularly exercise?

12. Approximately how long do you spend time for physical activity, such as walking, doing domestic duties, and exercising?

(Interviewer was asked to help subject to explore what activities she normally had per day and sum the approximate hours spent.)

13. Do you currently smoke? If yes, how many cigarette per day in average and how long has it been going for?

14. Did you have smoking habit in the past? If yes, how long ago was it and for how long had it been going for?

15. Does anyone living at the same house currently smoke?

16. Do you currently drink alcohol? If yes, how frequent is it? (Please specify the amount drank per day or week and how long it the habit has going for)

17. Did you have alcohol-drinking habit in the past? If yes, how long ago was it and for how long had it been going for?

Interviewer ID ☐☐ **Participant ID** ☐☐☐

DATE ☐☐ ☐☐ *1999*

PART II: WOMEN'S HEALTH QUESTIONNAIRE

18. How old were you at menarche?

19. Do you know what's your cycle day?

    1 Yes, please state:
    2 No

20. For the past 12 months, have you been menstruating?

    1 Yes
    2 No → When was the last menstruation?

21. If you have been menstruating in the past 12 months, has it been in the same pattern as before?

    1 Yes → pre menopause
    2 No → peri menopause

22. Have you been using contraceptive device listed below:

    1 pills
    2 injection
    3 implant
    4 IUD
    5 Permanent (tube resection)

23. Do you know about hormone replacement therapy? Have you ever been on hormone replacement therapy?

24. Have you ever consumed any particular compound (in the forms of food, drinks, fruit, etc.) due to menopause-related conditions?

    1 Yes, please specify
    2 No

25. Have you ever avoided consuming any particular compound (in the forms of food, drinks, fruit, etc.) due to menopause-related conditions?

    1 Yes, please specify
    2 No

Interviewer ID ☐☐ Participant ID ☐☐☐

DATE ☐☐ ☐☐ 1999

PART III. MENOPAUSAL SYMPTOMS ASSESSMENT – GREENE'S CLIMACTERIC SCALE

Please indicate the extent to which you are bothered at the moment by any of these symptoms by placing a tick in the appropriate box.

SYMPTOMS	Not at all	A little	Quite a bit	Extremely	Score 0-3
1.Heart beating quickly or strongly					
2. Feeling tense or nervous					
3. Difficulty in sleeping					
4. Excitable					
5. Attacks of panic					
6. Difficulty in concentrating					
7. Feeling tired or lacking energy					
8. Loss of interest in most things					
9. Feeling unhappy or depressed					
10.Crying spells					
11.Irritability					
12.Feeling dizzy or faint					
13.Pressure or tightness in head or body					
14.Parts of body feel numb or tingling					
15.Headaches					
16.Muscle or joint pains					
17.Loss of feeling in hands or feet					
18.Breathing difficulties					
19.Hot flushes					
20.Sweating at night					
21.Loss of interest in sex					
22.Dyspareunia					

Interviewer ID [ ][ ] Participant ID [ ][ ][ ]

24 HOURS FOOD RECALL TABLE

DATE [ ][ ] [ ][ ] *1999*

MEAL TIME	FOOD NAME	PORTION	ADDITIONAL INFORMATION
BREAKFAST TODAY			
ANYTHING EATEN BETWEEN: AFTER DINNER (LAST NIGHT) AND BEFORE BREAKFAST (TODAY)			
DINNER (LAST NIGHT)			
ANYTHING EATEN BETWEEN: AFTER LUNCH TIME AND BEFORE DINNER (YESTERDAY)			
LUNCH (YESTERDAY)			
ANYTHING EATEN YESTERDAY BETWEEN: AFTER BREAKFAST AND BEFORE LUNCH			