DIETARY MANAGEMENT OF MENOPAUSAL SYNDROME: ROLE OF PHYTOESTROGENS

DIGITIZED

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MPhil Thesis

GIFT

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December 2004

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DECLARATION

The thesis titled 'Dietary management of menopausal syndrome: role of phytoestrogens' is submitted in partial fulfillment of the requirement for MPhil degree under the Institute of Nutrition and Food Sciences, University of Dhaka. This work had been carried out in the Institute of Nutrition and Food Sciences, University of Dhaka and Biomedical Research Group, BIRDEM, Dhaka during the period of June 2003 to November 2004. To the best of my knowledge no part of the work has been submitted for another degree or qualification in any other Institutes.

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Dedicated

To

Maj M Saleh Raheem (Rtd)

Mrs Rokeya Nargis

My loving parents

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ABBREVIATIONS

IHD	Ischaemic Heart Disease
AD	Alzheimer Disease
HDL	High Density Lipoprotein- cholesterol
FSH	Follicle-stimulating hormone
LH	Luteinizing Hormone
E2	Estradiol
E1	Estrone
A	Androstenedione
HRT	Hormone Replacement Therapy
NHANES	National Health and Nutrition Examination Survey
LDL-cholesterol	Low Density Lipoprotein-cholesterol
CVD	Cardiovascular Disease
BIRDEM	Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders
INFS	Institute of Nutrition and Food Sciences
NHN	National HealthCare Network
FFQ	Food Frequency Questionnaire
EDTA	Ethylene Diamine Tetra Acetic Acid
rpm	Rotation per minute
^{0}C	Degree Celsius
nm	Nanometere
µl	Microlitere

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OD	Optical Density
GK	Glucoseoxidase
POD	Per oxides
mmol/l	Milimole per liter
U/ml	Unit per milliliter
mg/dl	Milligram per deciliter
ml/dl	Milliliter per deciliter
mIU/ml	Micro International Unit per milliliter
BMI	Body Mass Index
Kg/m^2	Kilogram per miter square
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
mmHg	millimeter mercury
Kcal	Kilocalorie
g	Gram
µg	Microgram
mg	Milligram

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ABSTRACT

Menopause is the transitional event of female life creating a considerable degree of clinical, psychological as well as social problem. Hormone Replacement Therapy (HRT) was thought to be a cornerstone in the management of menopause, but evidences accumulated in the recent past have raised serious questions regarding its safety and usability. In this context, phytoestrogens are getting increasingly more attention for therapeutic (as an alternate of HRT) and dietary interventions. Menopause is a special problem for women in developing countries and intake of phytoestrogens can be highly useful also from the economic point of views. The present study aims to explore the quantitative and qualitative intake of phytoestrogens in Bangladeshi postmenopausal women and its relation to clinical outcome. A total of 112 postmenopausal subjects [Age, 46.2±4.8 (yrs, M±SD)] were studied. The dietary intake of phytoestrogens by postmenopausal women was calculated by a specific food frequency questionnaire (FFQ). Serum glucose was estimated by glucose-oxidase method. Serum total cholesterol, triglycerides and HDL-C were analyzed by enzymatic-colorimetric methods. LDL-C was estimated by the Friedewald's formula. Plasma fibrinogen was measured by clotting method and serum FSH was measured by chemiluminescence-based ELISA technique. Mainly two types of phytoestrogen were taken by the study subjects and those were isoflavones and lignans. The intake of total phytoestrogens, isoflavones and lignans (mean ± SD, mg/day) were 9.16 ± 3.35, 8.91 ± 3.35 and 0.24 ± 0.08 respectively. The intake of daidzein, genistein, formononetin, Biochanin A (mean ± SD, mg/day) were 4.34 ± 1.66, 4.56 ± 1.69, 0.0009 ± 0.001, and 0.009 ± 0.01 respectively. The intake of matairesinol, secoisolaiciresinol (SILR) (mean ± SD, mg/day) were 0.005 ± 0.002, 0.24 ± 0.079 respectively. Significant differences were found between optimum intake and low intake of total lignans group in hot flushes (p=0.05), night sweat (p=0.02), sleep disturbance (p= 0.02), burning sensation (p=0.05) and headache (p=0.04). A significant positive association of SILR

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was found with night sweat (p=0.026) and burning sensation (p=0.033). The conclusions of the study are as follows: a) the dietary intake of total phytoestrogens, lignans and isoflavones are 9.16, 0.24 and 8.91 mg/day respectively in postmenopausal women in Bangladesh. b) total isoflavones intake is about 10 times higher compared to western population and 19 to 50 times lower to oriental population. On the other hand, total lignans intake is about 5 times lower compared to western population. c) the main sources of isoflavones are soyabean oil, beans and peas and those of lignans are ruti (wheat), bhat (cooked rice), fruits, onion and garlic. d) phytoestrogen, more particularly lignans, reduces the menopausal symptoms such as hot flushses, night sweat, sleep disturbance, burning sensation and headache. SILR has a consistent effect on night sweat and burning sensation. e) phytoestrogens do not operate postmenopausal symptoms through FSH.

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INTRODUCTION

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1. Introduction

1.1. Menopause

Menopause is the transitional event of female life from reproductive to non-reproductive stages. The menopause is the counterpart of the menarche and refers only to cessation of menstruation; it is merely one manifestation of the climacteric and precedes complete cessation of ovarian function by several months or years. The terms 'menopause' and 'climacteric' are often used synonymously but they refer to essentially different conditions. The age of the menopause does not depend on the type of menstrual cycle, and the number of pregnancies, which a woman has had, marriage, climate or environment[1]. In the absence of general or pelvic diseases, and with the possible exception of economic and social status, the only known factors governing it are familial and racial. The age at which menopause occurs varies from country to country and among individuals in the same country. In developed countries the usual age for the onset of menopause is between 50 and 51 years. The average age, which used to be 47 years in Britain and the USA, is now said to be 51 years. Reliable data are not available for developing countries but, generally, it is considered to occur at an early age in this world. In India, it is still estimated at 48 years [1,2]. Menstrual function may cease suddenly without warning, but the menopause is most often heralded by a gradual decrease in the amount and frequency of blood loss during several months or years. Every women from her late reproductive period passes through different stages of menopause, known as premenopause, perimenopause and postmenopause; but, a lot of controversies are present regarding the perimenopausal status which is also known as 'menopausal transition'. In this menopausal transition some sort of menstrual irregularities and menopausal symptoms may be seen in women[3]. Although it is an obligatory physiological phenomenon, it creates a number of biological, psychological and social problems imposing additional risk and challenge in the life.

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1.2. Burden of Menopause

The prevalence and incidence of menopausal problems vary according to ethnic groups. For instance, Asian women seem to have fewer problems after menopause than their western counterparts. Nevertheless, the problem do exist and seem to be increasing due to longer life expectancy and lifestyle changes of people in the south-east Asian region[4]. The sufferings from menopause in respect to frequency and severity of symptoms and the number of women seeking medical help varies widely depending upon social, personal, economical and cultural background.

The period of menopause and post-menopause of a women's life is a universal event, but the sufferings from menopause in respect to frequency and severity of symptoms vary widely. Probably these symptoms are not specific to menopause and are multifactorial in origin and the deficiency of estrogen play an important contributory role. Many symptoms have been attributed to the endocrine changes of the postmenopausal state. Symptoms possibly related to specific autonomic nervous system instability, but equally attributable to anxiety or other emotional disturbances, are paresthesias (pricking, itching, formication), dizziness, tinnitus, fainting, scotomas and dyspnea. Symptoms clearly not of endocrine origin are weakness, fatigue, nausea, vomiting anorexia, diarrhea[2].

a) Hot flushes and night sweats - Hot flushes and night sweats are the most common characteristics of the menopause. Hot flushes arise as sudden feeling of heat in the face, neck and chest. Most women indicate that hot flushes begin with a sensation of pressure in the head, much like a headache. This increases in intensity until the physiologic flush occurs. Palpitations may also be experienced. The actual flush is characterized as a feeling of heat or burning in the face, neck and chest, followed immediately by an outbreak of sweating that affects the entire body but is particularly prominent over the head, neck, upper chest, and back. The duration of the whole episode varies from momentary to as long as 10 minutes; the average length is 4 minutes.

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The frequency varies from 1-2 per hour to 1-2 per week. Investigators have now characterized the physiologic changes associated with hot flushes and have shown that the symptoms result from true alterations in cutaneous vasodilation, perspiration, reductions of core temperature, and elevation of pulse rate. Changes in heart rhythm and blood pressure have not been observed. The patient's awareness of symptoms does not correspond exactly with physiologic changes. Women become conscious of symptoms approximately 1 minute after the onset of measurable cutaneous vasodilation, and discomfort persists for an average of 4 minutes, whereas physical changes persists for several minutes longer. The exact mechanism responsible for hot flushes is not known. Hot flushes occur after the spontaneous cessation of ovarian function or following oophorectomy, it has been presumed that the underlying mechanism is endocrinologic, related either to reduction of ovarian estrogen secretion or to enhancement of pituitary gonadotropin secretion[2].

Different authors have presented evidences that prevalence of hot flushes associated with menopause varies in different culture. For example, the prevalence has been reported to be 0% in Mayan women, 10-20% in Hong Kong women, around 70% in Japanese women, 23% in Thai women, 45% in North –American women and up to 80% in Dutch women[5, 6].

Night sweats are the night time manifestation of hot flushes. It has been observed in about 75% of women who go through the physiologic menopause. Of those having flushes, 82% experience the disturbance for more than 1 year and 25-50% complain of the symptom for more than 5 years[2].

b) Sleep disturbance - Insomnia is often cited as a menopausal complain, but it usually a secondary effect of sleep disruption caused by the night sweats[2].

c) Changes in reproductive tract - Alteration of menstrual function is a clinical symptom of the climacteric symptoms which causes decreased in

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cervix size, along with a reduction of secretion of cervical mucus. This may contribute to excessive vaginal dryness which may cause dyspareunia[2]. A study was conducted on Singaporean population and showed that social and life style are associated with menopausal symptoms, as well as the average age at menopause[7].

d) Changes in urinary tract - Estrogen plays an important role in maintaining the epithelium of the bladder and urethra. Due to menopause, atrophic cystitis occurs which is characterized by urinary urgency, incontinence, and frequency without pyuria or dysuria[2].

e) Changes in mammary glands - Regression of breast size during and after menopause is psychologically distressing to some women. To those who have been bothered by cyclic symptoms of breast pain and cystic formation, the disappearance of these symptoms postmenopausally is a great relief[2].

f) Osteoporosis - Osteoporosis is a systemic skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in fragility of bone and susceptibility to risk of fracture. Bone loss occurs from around menopause because estrogen deficiency results in increased bone remodeling within the skeleton. Postmenopausal bone loss disproportionately affects cancellous bone which is found in vertebral bodies and at the end of long bones[2].

g) Cardiovascular system changes ischemic heart disease (IHD) - Estrogen deficiency is associated with a reduction in the plasma level of the protective cholesterol, HDL. Postmenopausal women are two and four times more likely to suffer IHD than their premenopausal counterparts. Early surgical menopause increases the risk of IHD[8].

h) Alzheimer's disease (AD) - Dementia refers to the loss of cognitive or mental abilities. AD is subdivided into early-onset (below 65 years) and late-onset (after age 65 years) diseases. For women one such factor which influences AD risk may be postmenopausal estrogen deficiency[8]. A variety of

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symptoms, occurring either singly or together, are frequently reported as being part of a menopausal syndrome. Some frequently mentioned symptoms are depression, nervous tension, palpitation, headache, insomnia, fluid retention, backache, difficulty in concentrating and dizzy spells. In most studies, occurrence of these symptoms is not highly correlated with menopausal status.

1.3. Hormonal Change in Menopause

In the menopause, there are major changes in androgen, estrogen, progesterone, and gonadotropin secretion.

1. Estrogens - The naturally occurring estrogens are 17β-estradiol, estrone, and estriol. They are secreted by the granulosa and the thecal cells of the ovarian follicles, the corpus luteum, and the placenta. The biosynthetic pathway involves their formation from androgens and by aromatization of androstenedione in the circulation. Aromatase also catalyzes the conversion of testosterone to estradiol. Estrogens facilitate the growth of the ovarian follicles, increase uterine blood flow and have important effects on the smooth muscle of the uterus. Estrogens increase the amount of uterine muscle and its content of contractile proteins. It decreases FSH secretion. Estrogen exerts both negative and positive feedback in LH secretion. It lowers plasma cholesterol level. and prevents initiation of atherosclerosis. These actions may account for the low incidence of myocardial infraction and other complications of atherosclerotic-vascular disease in premenopausal women. There is considerable evidence that small doses of estrogen may reduce the incidence of cardiovascular disease after menopause[9]. After a woman has passed the menopause, there is good clinical evidence of reduced endogenous estrogen production in most subjects. The greatest decrease is in estradiol. Its concentration is distinctly lower than that found in young women during any phase of their menstrual cycle and is similar to the level seen in premenopausal women following oophorectomy.

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2. Progesterone - Progesterone is secreted in large amounts by the corpus luteum and the placenta. It is an important intermediate in steroid biosynthesis in all tissues that secrete steroid hormones, and small amounts enter the circulation from the testes and adrenal cortex. In women, the plasma progesterone level is approximately 0.9 ng/ml during the follicular phase of the menstrual cycle. Progesterone is responsible for the progestational changes in the endometrium and the cyclic changes in the cervix and vagina. It decreases the number of estrogen receptors in the endometrium and increases the rate of conversion of 17β-estradiol to less active estrogens. Large doses of progesterone inhibit LH secretion and potentiate the inhibitory effects of estrogens, preventing ovulation. During the follicular phase of the cycle, progesterone levels are low. With ovulation the levels rise greatly, reflecting the secretory activity of the corpus luteum. In postmenopausal women, the levels of progesterone are only 30% of the concentrations seen in young women during the follicular phase. Since postmenopausal ovaries do not contain functional follicles, ovulation does not occur and progesterone levels remain low [2].

3. Gonadotropins - The gonadotropins FSH and LH act in concert to regulate the cyclic secretion of the ovarian hormones. FSH from the pituitary is responsible for early maturation of the ovarian follicles, and FSH and LH together are responsible for final follicle maturation. A burst of LH secretion triggers ovulation and the initial formation of the corpus luteum. There is also a smaller midcycle burst of FSH secretion, the significance of which is uncertain. LH stimulates the secretion of estrogen and progesterone from the corpus luteum[9]. With the menopause, both LH and FSH levels rise substantially, with FSH usually higher than LH. This is thought to reflect the slower clearance of FSH from the circulation. Concentration of FSH are strikingly elevated during the early follicular phase and fall as estradiol increase during follicular maturation. FSH levels at the midcycle peak and late in the luteal phase are also consistently higher than those found in younger

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women and decrease during the midluteal phase. LH concentration are indistinguishable from those observed in younger women. The mechanism responsible for this early rise of FSH is probably related to inhibin. Inhibin is a polypeptide hormone that is synthesized and secreted by granulosa cells. It causes negative feedback on FSH release by the pituitary. As the oocyte number decreases, inhibin levels fall, resulting in a rise in FSH levels[2]. Low estrogen levels alone do not appear to trigger hot flushes. Hot flushes appear to be related to gonadotropins. A close temporal association between the occurrence of flushes and the pulsatile release of LH has been demonstrated. The observation that flushes occur after hypophysectomy suggests that they are not due directly to LH release.

4. Androgens - During reproductive life, the primary ovarian androgen is androstenedione, the major secretory product of developing follicles. In postmenopausal women, there is a reduction of circulating androstenedione to approximately 50% of the concentration found in young women, reflecting the absences of follicular activity. For testosterone, the level found in postmenopausal women is minimally lower than that found in premenopausal women before oophorectomy and is distinctly higher than the level observed in ovariectomized young women.

In a study, the normal ranges of the concentration of FSH, estradiol (E2), estrone (E1), and androstenedione (A) were established in healthy women. The hormone levels of normal post-menopausal women were compared with post-menopausal women with severe obesity. There was no significant difference between the E2 levels of the normal and obese women. In the obese women, A and E1 levels were significantly lower than in the normal women. A weight reduction in the obese women had no influence on the concentrations of A and E2, whereas E1 levels tended to increase. FSH levels increased significantly during weight reduction[10].

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1.4. Management of Menopause

Management of menopause can be divided into three types such as: psychosocial, therapeutic and nutritional management.

1.4.1. Psychosocial

Anxiety, depression, postmenopausal symptoms, fatigue, sexual issues, relationship and family become important problem areas for the subjects. It is encouraging that the social awareness about menopause is gradually increasing. Menopause is now receiving more attention from researchers, physicians and media than ever before. Women today are much more open about menopause and more willing to take measures to relieve symptoms and prevent long-term problems. The postmenopausal women of our population is not getting enough support due to poor socio-economic condition, illiteracy, ignorance and inadequate health care system. During this period women are not well accepted in the society and family and they consider themselves as a burden. So, good pre-care is just as important as good aftercare to reduce problems and increase well being. Addressing information and support in a timely fashion is important in providing success to psychological aftercare.

1.4.2. Therapeutic Management

1.4.2.1. Drug

Small doses of sedatives can be help to control hot flushes. Hypotensive agents such as clonidine hydrochloride are sometimes advised. Tranquillizers may be helpful when nervous symptoms predominate. Osteoporosis can be corrected by bisphosphonates. Other agents are etidronate, paradronate and risedronate. Salmon calcitonin has the advantage of relieving the acute pain caused by subclinical vertebral fractures[1].

1.4.2.2. Hormone Replacement Therapy

Since menopausal symptoms are directly related to the result of deprivation of estrogen, the administration of this hormone should relieve these symptoms

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completely. About 1/3rd of a women's life is spent in the postmenopausal era and as a consequence the role of HRT is of relevance. For the symptomatic women, HRT with estrogen is clearly beneficial in improving quality of life in the menopausal years. The benefits are mainly the prevention or control of vasomotor symptoms like insomnia and genital atrophy. In a study it was found that in women with coronary disease, hormone therapy reduced the incidence of diabetes by 35%. This observation provided important insights into the metabolic effects of postmenopausal hormone but it was insufficient to recommend the use of hormones for secondary prevention of heart disease[11]. It was also found that diabetic women taking HRT had better glycemic control than never users of HRT[12]. In another study on postmenopausal women with type 2 diabetes it was found that hormone replacement therapy with either oral or transdermal estrogen plus micronized progesterone has no harmful influence on glucose metabolism and there was no increase in HDL and also triglyceride levels[13]. The NHANES III study suggested that diabetic and non-diabetic postmenopausal women taking HRT had better lipoprotein profile than never users of HRT[12].

On the contrary, despite some positive effects of these synthetic hormones on postmenopausal women, they also have some negative effects. Usually, estrogen acts by inhibiting or reducing the output of gonadorophins by the hypothalamic-pituitary system. It is being prescribed for the relief of vasomotor symptoms. Its dose is gradually increased to achieve this effect. When the appropriate level is found, the same dose is maintained for one month and is then very slowly reduced. A disadvantage of this form of treatment is that many women fail to obey instructions to reduce the dose and, since it relieves symptoms, they continue to take them haphazardly over the course of many years. In such circumstance estrogen is likely to cause uterine haemorrhage, endometrial hyperplasia and possible carcinoma[1]. The use of unopposed estrogen by itself without progesterone in women with uterus, causes increased risk of endomatrial hyperpalasia and cancer[8]. It is

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now generally accepted that progesterone should always be given with estrogen therapy. Patients receiving estrogen and progesterone therapy must have regular examination of the breasts and genital tract, blood pressure monitoring, blood sugar, liver function tests and lipid profile[1]. The main reasons for discontinuing HRT are vaginal bleeding, breast tenderness, nausea, vomiting, weight gain, fear of breast cancer and general belief of harmfulness of hormones. The risk of breast cancer is still debatable. It is accept that short term HRT did not increase breast cancer risk significantly. In the Million Women Study it was found that the effects of specific types of HRT was associated with an increased risk of incidence and fetal breast cancer and the effect was substantially greater for estrogen-progesterone combinations than for other types of HRT[14]. However, in another study, it was found that estrogen use in American Indian postmenopausal women was related to deterioration of glucose tolerance. Longer duration of estrogen users compared to current users were related to an increased risk of type 2 diabetes[15]. Some relief of symptoms may be obtained in these women by lifestyle and dietary modifications and natural estrogen.

1.4.3. Nutritional Management

The popularity of nutritional management among menopausal women is increasing day by day. This includes the use of vitamin and mineral supplements according to specific nutritional recommendations in addition to dietary modifications. The diet should balance with adequate calcium. Milk, beans, broccoli, fish are good dietary sources of calcium but a supplement may be required. Vitamin E is an antioxidant, which has been shown to decrease the risk of coronary artery disease by inhibiting the oxidation of LDL-cholesterol and inhibiting platelet aggregation. Vitamin E supplementation has been shown to decrease fatigue, nervousness, dizziness, headaches, palpitations, joint pains and backache. Supplementation of vitamins B_6, B_{12}, and folic acid protect against cardiovascular disease[1].

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1.4.3.1. Phytoestrogen

Phytoestrogens are compounds derived from plants. These are structurally and functionally comparable to 17-β estradiol. Phytoestrogens can be classified in three groups, i.e., isoflavones, coumestans and lignans. The major isoflavones are genistein, daidzein, formononetin and biochanin A. Coumestrol is the most important coumestan. The major lignans are enterolactone and enterodiol, which are produced by colonic bacteria from their dietary precursors matairesinol and secoisolariciresinol[16].

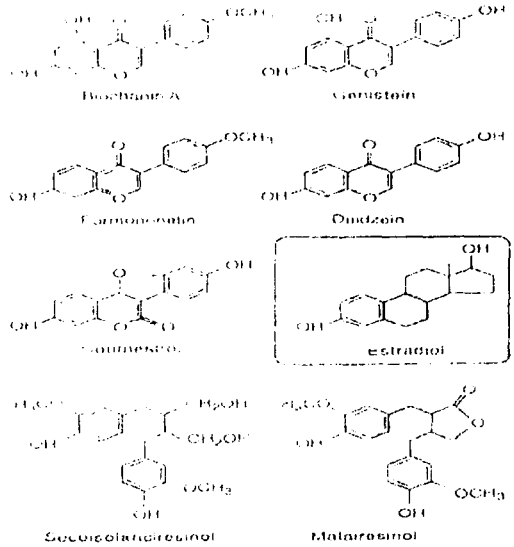

FIGURE 1 Chemical structures of phytoestrogens and estradiol.

There are many classes of phytoestrogens but the most important are the isoflavones which are found in legumes, especially soyabeans and chickpeas, and the lignans which are found in many fiber-rich foods, whole –grain cereals and nuts. However, small concentrations of phytoestrogens have been measured in several fruits and vegetables[17-20]. Phytoestrogens have a weak affinity for the estrogen receptors. They have estrogenic and anti-estrogenic activity. In women, 40g of soya protein per day for a period of 6 months has been reported to increase the bone density by up to 2.2%, but the beneficial effect of phytoestrogens on bone density is still to be established.

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Phytoestrogens have been found to be useful in reducing hot flushes, and may induce favorable changes in lipid metabolism and improve carbohydrate metabolism. High intake of phytoestrogens in the diet (>40 mg soya/day) has been correlated epidemiologically with a lower incidence of endometrial, breast and prostate cancers[1]. Lignans protect against certain cancers, particularly hormone – sensitive cancers such as those of the breast, endometrium and prostate, by interfering with sex hormone metabolism. Lignans have been shown to stimulate hepatic synthesis of sex hormone binding globulin (SHBG), thus enhancing the clearance of circulating estrogen[21], and to bind to estrogen receptors on SHBG in a dose – dependent manner, thereby inhibiting estrogen and testosterone binding[22]. As SHBG is found in breast cancer cells, the binding of mammalian lignans to SHBG may interfere with estrgen-mediated tumorigenic processes.

In the Framingham study, the association between dietary phytoestrogen intake and metabolic cardiovascular risk factors in postmenopausal women was studied. It was found that high intake of phytoestrogens in postmenopausal women appears to be associated with a favorable metabolic cardiovascular risk profile[23]. A serum lipid change by a phytoestrogen dietary supplement compared with oral estrogen-progesterone replacement in hypercholesterolemic menopausal women was assessed. It was found that flaxseed was as effective as oral estrogen-progesterone to improve mild menopausal symptoms and to lower glucose and insulin levels[24]. In another study, it was found that phytoestrogens had a protective effect on the risk of atherosclerosis[25]. On the other hand, few epidemiological studies were suggested that diets rich in phytoestrogens might be associated with low risk of breast and prostate cancer [26]. In a study, it was found that cream with phytoestrogens had an effect in climacteric symptoms in postmenopausal women[27]. In another study, it was found that Pueraria mirifica, containing phytoestrogens, relatively alleviated the climacteric symptoms in perimenopausal women[28].

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In a study, it was found that dietary supplementation with soy phytoestrogens favorably alters insulin resistance; glycemic control and serum lipoproteins in postmenopausal women with type 2 diabetes, thereby improving their cardiovascular risk profile[29]. In prospective study, the findings were suggested that consumption of soy products had a protective effect against hot flushes[30]. In another study, it was found that daily diet supplemented with soy flour could reduce flushes compared with wheat flour over 12 weeks. Hot flushes significantly decreased in the soy and wheat flour groups 40% and 25% respectively[31]. Effects of genistein on the endometrium: ultrasonographic evaluation was studied. It was found that genistein administration reduces climacteric symptoms in postmenopausal women and does not increase endometrial thickness[32]. In another study, it was found that consumption of daidzein was more efficient than genistein in preventing ovariectomy-induced bone loss in rats[33]. Dietary intervention studies indicated that in women soya and linseed had beneficial effects on the risk of breast cancer and alleviated postmenopausal symptoms[34].

In a study it was found that the soy beverage containing phytoestrogens did not alleviate hot flushes in women with breast cancer[35]. In another study, it was found that phytoestrogens supplementation with150mg/d over a 6 month period did not significantly alter serum lipoproteins in postmenopausal women and might not effectively reduce the risk of CAD in this population[36].

In above context, some studies were observed maximum positive effect for phytoestrogens with negligible negative effect. Therefore, more research is required on this field. In Bangladesh, the diet is rich in rice, wheat, legumes, beans, fruits and vegetables. No study, however, has so far been conducted to investigate the effect of phytoestrogens-rich diet on menopausal symptoms of our women. The present study aims to explore the quantitative and qualitative intake of phytoestrogens in Bangladeshi menopausal women and its relation to clinical outcome.

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OBJECTIVES

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2. Objectives

2.1. General Objective

- To find an alternative for hormone replacement therapy in menopausal women.

2.2. Specific Objectives

- To explore the quantity and nature of phytoestrogens in common Bangladeshi diet.
- To investigate the nature and severity of postmenopausal symptoms in relation to the quantity and nature of phytoestrogens consumed.
- To study the status of female reproductive hormone (FSH) and phytoestrogen intake.

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SUBJECTS AND METHODS

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3. SUBJECTS AND METHODS

3.1. Place of the study

The study was conducted in the Institute of Nutrition and Food Sciences (INFS), University of Dhaka and in the Biomedical Research Group, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) during the period of August 2003 to July 2004.

3.2. Subjects

One hundred and twelve subjects were enrolled from 4 different hospitals named BIRDEM, National Health Care Network (NHN)-Mirpur, National Health Care Network (NHN)-Dhanmondi and National Health Care Network (NHN)-Rampura on the basis of availability.

3.2.1. Inclusion criteria

1. All types of postmenopausal women (menopause within last 5 years).
2. Age between 40 to 60 years.

3.2.2. Exclusion criteria

1. Patients having other medical complications such as nephropathy, retinopathy, cardiovascular diseases.
2. Patients taking hormone replacement therapy.

3.3. Preparation of the subjects

After selection the purpose of the study was explained in details to each subject and written consent was taken from each of them. Subjects were requested to fast overnight (10-12 hours).

4. METHODS

4.1. Study Design

It was a descriptive cross-sectional study. The subjects were selected purposively.

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4.2. Determination of Sample Size

Sample size for detecting a correlation between phytoestrogen levels and clinical hormonal outcomes was calculated by the formula as given by Bland (2002)[37].

It is convenient to treat the relationship between two continuous variables as an estimation of or test of a correlation coefficient. The correlation coefficient has an awkward distribution, which tends only very slowly to the Normal, even when both variables themselves follow a Normal distribution. We can use Fisher's z transformation

$$z = 1/2 \log_e (1+r/1-r)$$

which follows a Normal distribution with mean

$$z_p = 1/2 \log_e (1+p/1-p) + p/ 2 (n-1)$$

and variance $1/(n - 3)$ approximately, where p is the population correlation coefficient and n is the sample size (§11.10). For sample size calculations we can approximate z_p by

$$z_p = 1/2 \log_e (1+p/1-p)$$

The 95% confidence interval for z will be $z_p \pm 1.96 [1/(n - 3)]^{1/2}$ approximately. Given a rough idea of p we can estimate n required for any accuracy. For example, suppose we want to estimate a correlation coefficient, which we guess to about 0.5, and we want it to within 0.1 either way, *i.e.* we want a confidence interval like 0.4 to 0.6. The z transformations of these values of r are $z_{0.4} = 0.42365$, $z_{0.5} = 0.54931$, $z_{0.6} = 0.69315$, the differences are $z_{0.5} - z_{0.4} = 0.12566$ and $z_{0.6} - z_{0.5} = 0.14384$ and so to get the sample size we want we need to set 1.96 standard errors to the smaller of these differences. We get $1.96 [1/(n - 3)]^{1/2} = 0.12566$ giving $n = 246$.

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We more often want to see whether there is any evidence of a relationship. When r = 0, z_r = 0, so to test the null hypothesis that p = 0 we can test the null hypothesis that z_p = 0. The difference we wish to test is $\mu_d = z_p$, which has SE(d) = $[1/(n-3)]^{1/2}$. Putting this into the formula of §18.3 we get

$$z^2_p = f(\alpha,P)\ 1/(n-3)$$

Thus we have

$$(1/2\ \log_e\ (1+p/1-p))^2 = f(\alpha,P)\ 1/n-3$$

and we can estimate n, p or P given the other two. Table 1 shows the sample size required to detect a correlation coefficient with a power of P = 0.9 and a significance level α = 0.05.

Table 1: Approximate sample size required to detect a correlation at the 5% significance level with power 90%

p	*n*	*p*	*n*	*p*	*n*
0.01	100000	0.1	1000	0.6	25
0.02	26000	0.2	260	0.7	17
0.03	12000	0.3	110	0.8	12
0.04	6600	0.4	62	0.9	8
0.05	4200	0.5	38		

4.3. History and Clinical examination

The histories of the subjects were collected by an interviewer-administered questionnaire and clinical examination done by a qualified physician.

4.4. Dietary Assessment

The dietary intake of phytoestrogens and other nutrients was calculated by a specific food frequency questionnaire (FFQ) for 7 days[38]. Phytoestrogen contents of the already known foods were found by literature search[39-43]. The content of phytoestrogens in Bangladeshi foods was calculated from the values from literature. All values in the literature were converted to mg per

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100 g food. Each phytoestrogen content of a food item was scored in seven categories (Table 1) in the literature. Our study food items were also categorized according to the literature. Then score in milligrams of each of our food item was multiplied by the serving size of the food. This final phytoestrogen amount of each food item was mulitiplied by the frequency of the consumption of that food and then summed across foods to obtain the total individual intake of each phytoestrogen. The subjects taking phytoestrogens below the median value of total phytoestrogens, isoflavones and total lignans were considered to be low intake group and those taking phytoestrogens higher the median value of total phytoestrogens, isoflavones and total lignans were considered to be optimum intake group. The median value of total phytoestrogens, isoflavones and total lignans was determined by frequency test.

Table 2: Scoring of phytoestrogen concentrations of food items

Phytoestrogen, mg/100g wet weight	Score, mg/100 g
Nondetectable, 0	0
$0 < * < 0.001$	0.0005
$0.001 \leq * < 0.01$	0.005
$0.01 \leq * < 0.1$	0.05
$0.1 \leq * < 1$	0.5
$1 \leq * < 10$	5
≥ 10	50

4.5. Collection of blood sample

Fasting samples for the measurement of serum glucose, lipid profile, fibrinogen and FSH was taken between 8.00-9.00 a.m. Venous blood (10 ml) was collected by venepuncture with the subject lying supine in a quiet room. Blood was taken into a test tube containing anticoagulant (EDTA). After 10-15

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minutes sample blood was centrifuged for 5 minutes at 3000 rpm to obtain plasma. All plasma were frozen at – 70 ^{0} C until analysis.

5. Laboratory Procedures

5.1. ESTIMATION OF FASTING PLASMA GLUCOSE

Serum glucose was estimated by Glucose-Oxidase (GOD-PAP) method by an Auto analyzer (Auto lab, Analyzer Medical System, Rome, Italy) using reagents of Randox Laboratories, UK[44].

5.1.1. Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4- aminophenazone to form a red - violet quinoneimine dye as indicator.

5.1.2. Reaction Principle

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

5.1.3. Reagents composition

1. **Buffer:** Phosphate Buffer (0.1 mol/l, pH 7.0) and phenol (11 mol/l)
2. **GOD-PAP Reagent:** 4-aminophenazone (0.77 mmol/l), Glucose oxidase (≥1.5 kU/l) and Peroxidase (≥1.5 kU/l).
3. **Standard:** Glucose (5.55 mmol/l)

5.1.4. Procedure

The Auto lab Unit was calibrated before the assay. Serum was taken in the sample cup and GOD-PAP reagent was taken in the reagent container. Then the sample cups and reagent containers were placed in the sample and reagent holder. The Auto lab was programmed for the estimation of glucose and allowed to run with the following procedure:

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5 μl sample and 500 μl reagent were taken to the reaction cell and mixed. The mixture was then incubated for 10 minutes at 37°C within the Auto lab. Reading was taken 500 nm.

Calculation of result for unknown sample is as follows:

Result of unknown sample = [(Standard Concentration / OD for Standard) × OD of unknown sample]

5.2. ESTIMATION OF FASTING PLASMA TRIGLYCERIDES

Serum triglyceride was measured by enzymatic colorimetric (GPO-PAP) method in Auto analyzer (Analyzer Medical System, Rome, Italy) using reagents of Randox Laboratories, UK[45].

5.2.1. Principle

The triglyceride is determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

5.2.2. Reaction Principle

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxy acetone phosphate} + H_2O_2$$

$$2H_2O_2 + 4\ \text{aminophenazone} + 4\ \text{chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + HCl + 4H_2O$$

5.2.3. Reagents:

1. **Buffer**: Pipes Buffer (40mmol/l, pH 7.6), 4-choloro-phenol (5.5 mmol/l), Magnesium-ions (17.5 mmol/l).
2. **Enzyme Reagent**: 4-aminophenazone (0.5 mmol/l), Glycerol-3-phosphate oxidase (1.5 U/ml), Lipases (>150 U/ml), ATP (1.0 mmol/l), Peroxidase (0.5 U/ml).

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3. **Standard**: 2.29 mmol/l (200 mg/dl).

5.2.4. Procedure

Serum and reagents were taken in specific cup or cell. They were arranged serially. Then ID number for test was entered in the AUTOLAB. Five (5) μl sample and 500 μl reagent were mixed and incubated at 37^0C for 5 minutes within the AUTOLAB. Reading was taken at 500 nm wavelength.

Calculation of result for unknown sample is as follows:

Concentration of unknown sample = [(Standard Concentration / OD for Standard) × OD of unknown sample]

5.3. ESTIMATION OF PLASMA TOTAL CHOLESTEROL

Total cholesterol was measured by enzymatic endpoint method (cholesterol Oxidase/ Peroxidase) method in Auto analyzer (Analyzer Medical System, Rome, Italy) using reagents of Randox Laboratories, UK[46].

5.3.1. Principle

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantiphyrine in the presence of phenol and peroxidase.

5.3.2. Reaction Principle:

$$\text{Cholesterol-ester} + H_2 \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestene- 3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{phenol} + \text{4–Aminoantipyrine} \xrightarrow{\text{Peroxidasee}} \text{quinoneimine} + H_2O$$

5.3.3. Reagent composition

1. Enzyme Reagent: Cholesterol oxides (≥ 0.1 U/ml), Cholesterol esterase (> 0.15 U/ml), Peroxidase **(>** 0.5 U/ml**),** 4-Aminoantipyrine (0.30 mmol/l), Phenol (6 mmol/l) and Pipes Buffer (80 mmol/l; pH 6.8).

2. Standard: 5.17 mmol/l (200mg/dl)

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5.3.4. Procedure

The equipment was calibrated before assay. Serum was taken in the sample cup and enzyme reagent was taken in the reagent container. Then the sample cups and reagent containers were placed in the Auto lab analyzer (Analyzer medical system, Rome, Italy). The Auto lab was programmed for the estimation serum cholesterol and allowed to run with the following steps: 5 μl sample and 500 μl reagent were taken to the reaction cell and mixed .The mixture was then incubated for 10 minutes at 37°C within the unit. Reading was taken at 500 nm wavelength.

Calculation of result for unknown sample is as follows:

Concentration of unknown sample = [(Standard Concentration / OD for Standard) × OD of unknown sample]

5.4. ESTIMATION OF PLASMA HIGH DENSITY LIPOPROTEION (HDL) CHOLESTEROL

Serum High density Lipoprotein (HDL) was measured by enzymatic colorimetric (cholesterol CHOD-PAP) method in Autoanalyzer (Analyzer Medical System, Rome, Italy) using reagents of Randox Laboratories, UK[47].

5.4.1. Principle

HDL (High Density Lipoproteins) was separated from chylomicrons, VLDL (very low density lipoproteins) and LDL (Low density lipoproteins) by the addition of a precipitating reagent phosphotungstic acid in the presence of magnesium ions to serum or plasma. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, was determined by the enzymatic colorimetric method using CHOD- PAP.

5.4.2. Procedure

Samples (200 ml) and precipitating reagent (500 μl) were taken in a microcentrifuge tube. Then it was mixed and allowed to sit for 10 minutes at room temperature. Then it was centrifuged at 4000 rpm for 10 minutes.

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The supernatant was used as sample for determination of cholesterol content by the CHOD-PAP method. The sample and reagents were taken in specific cup or cell. They were arranged serially then ID number for test was entered in the AUTOLAB. Then 5 μl sample and 500 μl reagent were mixed and incubated at 37^0C for 5 minutes within the AUTOLAB. The reaction occurred in reaction cell. Reading was taken at 500 nm.

Calculation of result for unknown sample is as follows:

Concentration of unknown sample = [(Standard Concentration / OD for Standard) × OD of unknown sample]

5.5. ESTIMATION OF FIBRINOGEN

5.5.1. Principle

Fibriquik is based on a method described by Clauss[48]. When thrombin is added to a sample of plasma, fibrinogen is converted enzymatically to fibrin. Fibrin, in turn, undergoes polymerization to form a fibrin network. Factor XIII, activated by thrombin, catalyzes the formation of stabilizing cross links to produce a visible clot. The elapsed time, from addition of thrombin to the formation of a clot, is inversely proportional to fibrinogen level.

5.5.2. Reagents

Thrombin Reagent (6x1ml): Contains lyophilized bovine thrombin (approximately 100 10x3ml NIH units per ml) with stabilizers and buffer.

Fibrinogen Calibration Reference (1x1 ml): Contains lyophilized human plasma.

Owren's Veronal Buffer (2x25ml): Contains 0.028M Sodium Barbital.

5.5.3. Caution:

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The reagent should be handled with care, as it contains potentially infectious material. This product includes materials that have been prepared from human plasma or serum which have been tested using FDA-licensed methods and found to be nonreactive for HIV-1, HIV-2, and HCV antibodies and for hepatitis B surface Antigen (HBsAg). However, as no test method can offer

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complete assurance that infectious agent are absent, all specimens of human origin should be considered potentially infectious and handled with care.

5.5.4. Additional materials required

- Micropipet with disposable tips
- Collection tubes containing sodium citrate at 3.2% (or 3.8%) concentration.
- Hemostasis instrumentation
- Purified water, USP or equivalent

5.5.5. Storage instructions

All reagent should be stored at 2-8^{0}C until the expiration date.

5.5.6. Specimen collection and preparation

A ratio of nine parts blood to one part anticoagulant (3.2% sodium citrate) was used[48]. After collection, specimens was stored capped at room temperature (18-24^0 C) and centrifuged within 10-20 minutes. Then the plasma was frozen rapidly (-20^0 C) to prevent denaturation of fibrinogen.

5.5.7. Quality Control

The use of both normal and abnormal control materials is used. Control materials was tested by the same procedure as that used for patient samples. Verify^R 1 and Verify ^{R} Low Fibrinogen Control were used for monitoring the Fibriquik quantitative determination of fibrinogen .

5.5.8. Fibriquik Test Procedure

5.5.8.1. Preliminary comments and precautions

1. No materials was pipetted by mouth.
2. Disposable gloves was used to handle all blood specimens.
3. Spillage (if any) of specimens was immediately cleaned up with a 1:10 dilution of 5% sodium hypochlorite.

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a) Blood products was autoclaved for 60 minutes at 121^{0}C and Incinerated disposable materials.

b) Liquid waste was mixed with 5% sodium hypochlorite solution so that the final concentration was approximately 1% sodium hypochlorite. It was allowed to stand 30 minutes before disposal.

5.5.8.2. Reagent preparation

Thrombin Reagent with 1.0 (3.0) ml of purified water was reconstitute and mixed gently. It was stored in the original (tightly-capped) vial at 2-8^{0}C for not more than 3days.

Fibrinogen Calibration Reagent with 1.0 ml of purified water was reconstituted, allowed to stand 30 minutes and mixed gently. It was stored in the original (tightly-capped) vial at 2-8^{0}C for not more than 24 hours.

5.5.8.3. Test Procedure

1. A test tube for each sample to be tested was labeled.
2. Fibriquik Thrombin Reagent was allowed to warm to room temperature (20-25^{0}C) before use.
3. A 1:10 dilution of each patient was prepared and control sample was tested.
4. 0.2ml of a sample dilution was added to an appropriate tube or sample tray and warmed to 37^{0}C for at least 2 minutes before testing.
5. 0.1 ml of Fibriquik Thrombin Reagent was dispense into the 0.2 ml of plasma dilution in each tube.
6. the time required for clot detection was record to the nearest 0.1 second.
7. fibrinogen concentration (ml/dl) was interpolate from calibration curve.

5.6. ESTIMATION OF SERUM FSH

Serum FSH was measured by Immulite chemiluminescent immunoassay system (DPC, USA) [50].

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5.6.1. Principle

Luminescence is the emission of light or radiant energy when an electron returns from an excited or higher energy level to a lower energy level. There are several types of luminescence phenomena, including fluorescence, phosphorescence and chemiluminescence. Chemi-luminescence involves the oxidation of an organic compound by an oxidant. Light is emitted from the excited product formed in the oxidation reaction. These reactions occur in the present of catalysts such as enzymes (eg. alkaline phosphatase), metal ions or metal complexes and hemin.

5.6.2. Reagent composition for Follicle stimulating hormone (FSH)

5.6.2.1. Contents

FSH test units (LFS1): Each bar code labeled unit contains one bead coated with monoclonal murine anti FSH.

FSH Reagent Wedge (LFS2): With barcode 6.5 ml alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat ant-FSH in buffer.

FSH Adjustors (LFSL, LFSH): Two vials (low and high), 3.0 ml each, of FSH in a nonhuman serum matrix.

5.6.2.2. Kit Components

- FSH sample diluent
- Chemiluminescence substrate
- Probe wash module
- Probe cleaning kit
- Sample cup holder
- Sample cups
- Sample cup caps

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5.6.3. Procedure

Each kit was adjusted in the IMMULITE by using respective adjustors. FSH controls and control Con 6 were used for calibrating the equipment before the analysis was started. More than 350μl sample was given in the sample cup and FSH reagents were taken in the reagent container at a time. Against each sample cup, test unit for FSH was given. This is placed in reaction cell of immulite. The instrument was programmed for the estimation of plasma FSH and the immulite system automates the entire assay process in the following way:

Sample and reagent were automatically pipetted into the test tube unit which was then incubated at 37^0C with intermittent agitator. After incubating the sample with alkaline phosphatase reagent, the liquid reaction mixture in the test unit was rapidly separated from the bead. It was washed and the test unit was spun at high speed on it's vertical axis. The entire fluid contents transferred to a coxacial waste chamber in the test unit. A series of washes efficiently remove unbound material from the bead unit inner tube. The bound label was then came in contact with chemiluminescent substrate which is added to the test unit. Light emission was detected with a high sensitivity photon counter or photomultiplier tube and printed report, for each sample was generated by the system's computer. Concentration of FSH is expressed as mIU/ml.

5.7. Ethical issues

Ethical guidelines of Declaration of Helsinki IV (2001) were followed throughout the study. Informed written consent was taken from every subject. The questionnaire was designed considering the privacy of the subjects. The subjects' personal information was kept confidential. The subjects were not deprived of any treatment or facility that deserved. The study did not involve collection of any biological specimen from the subjects.

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5.8. Statistical analysis

The data were managed and analyzed using a computer program Statistical Package for Social Science (SPSS) (Windows version 10.0). P value less than or equal to 0.05 was considered significant.

X^2 test was done to compare means between postmenopausal symptoms and total phytoestrogens, isoflavones and total lignans.

Correlation was performed to find the association of total phytoestrogens with clinical and biochemical parameters.

Regression was performed to find the association between individual phytoestrogens and menopausal symptoms.

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RESULTS

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6. Results

6.1. Phytoestrogen concentration of food items in the food frequency questionnaire (Table 3)

Table 2 lists the phytoestrogen level per 100 g of the food item in the Bangladeshi foods. The richest sources of daidzein (mg/100g dry weight) are soyabean, pea, chickpea, moshur dal, blackgram, cabbage, cauliflower and carrot. Genistein, another isoflavone, is found in soyabean, pea, chickpea, moshur dal, blackgram, cabbage, cauliflower and carrot. The isflavone formononetin is present mainly in cabbage. The isoflavone biochanin A is also found in moshur dal. Lignans concentrate in cereals and some vegetables; matairesinol is found mainly tomato, onion, garlic, potato, pepper, carrot, and the richest sources of secoisolaiciresinol (SILR) are ruti, bhat, apple, banana, tomato, lychee, papaya, guava, lemon, plum, pea, chickpea, moshur dal, black gram, cabbage, cauliflower, onion, garlic, potato, pepper and carrot.

6.2. Clinical characteristics of the study subjects (Table 4)

A total of 112 postmenopausal subjects were studied. Age (years, mean ± SD) of those subjects was 51.4 ± 5.3, age (years, mean ± SD) at menopause was 46.2 ± 4.8 and their BMI (Kg/m^2, mean ± SD) was 27.1 ± 4.1. Systolic blood pressure (mmHg, mean ± SD), diastolic blood pressure (mmHg, mean ± SD) and pulse (occasions/minute, mean ± SD) of the study subjects were 127.8 ± 17.1, 79.8 ± 8.1 and 78 ± 11 respectively. Fasting plasma glucose [mmol/L, median (range)], serum triglycerides [mg/dl, median (range)], total cholesterol [mg/dl, median (range)], HDL cholesterol [mg/dl, median (range)], fibrinogen [mg/dl, median (range)], FSH [mIU/mL, median (range)] of the study subjects were 7.3 (4.4 – 33.2), 159 (73 - 655), 197 (113 - 301), 36 (17 - 52), 282 (101 - 847) and 56.8 (0.57 - 154) respectively. Among the study subjects 95 (85%) were diabetic and 75 (67%) were hypertensive. The family history of diabetes mellitus in the study subjects was 73 (65%) and that of hypertension was 78 (70%).

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6.3. Nutrient intake by the study subjects (Table 5)

The average daily intake of various nutrients by the subjects is shown in the table. The intake of energy, iron and vitamin C (mean ± SD) were 1705 ± 228 kcal /day, 20 ± 9 mg/day, 103 ± 31 mg/day respectively.

6.4. Phytoestrogens intake by postmenopausal subjects (Table 6)

The intake of total phytoestrogens, isoflavones and lignans (Mean ± SD) were 9.16 ± 3.35, 8.91 ± 3.35 and 0.24 ± 0.08 mg/day respectively. The intake of daidzein, genistein, formononetin and Biochanin A (mean ± SD, mg/day) were 4.34 ± 1.66, 4.56 ± 1.69, 0.0009 ± 0.001, and 0.009 ± 0.01 mg/day respectively. The intake of matairesinol, secoisolaiciresinol (SILR) (mean ± SD, mg/day) were 0.005 ± 0.002, 0.24 ± 0.079 mg/day respectively.

6.5. Sources of intake of phytoestrogens in the daily diet of the study subjects (Table 7)

The main sources of dietary isoflavones in the study subjects were soyabean, beans and peas. The main sources of dietary lignans in the study subjects were ruti (wheat), bhat (cooked rice), fruits, onion, garlic, beans and peas.

6.6. Difference of optimum intake vs low intake groups (Total phytoestrogens) regarding postmenopausal symptoms (Table 8)

6.6.1. Hot flush

It was found that the subjects taking less amount of phytoestrogens had higher hot flush. Among the subjects who took phytoestrogens >9 mg/day (median value), 36 (61%) had hot flush and 23 (39%) had no hot flush. But among the subjects who took ≤9 mg/day (median value), 35 (66%) had hot flush and 18 (34%) had no hot flush. There was no significant difference between total phytoestrogens consumption and hot flush (X^2 = 0.303, p=0.362).

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6.6.2. Night sweat

The study showed that the subjects taking less amount of phytoestrogens had higher night sweat. Among the subjects who took phytoestrogens >9 mg/day (median value), 26 (49%) had night sweat and 27 (51%) had no night sweat. But among the subjects who took ≤9 mg/day (median value), 30 (51%) had night sweat and 29 (49%) had no night sweat. There was no significant difference between total phytoestrogens consumption and night sweat (X^2= 0.03, p=0.50).

6.6.3. Sleep disturbance

It was found that the subjects taking less amount of phytoestrogens had higher sleep disturbance. Among the subjects who took phytoestrogens >9 mg/day (median value), 22 (42%) had sleep disturbance and 31 (58%) had no sleep disturbance. But among the subjects who took ≤9 mg/day (median value), 27 (46%) had sleep disturbance and 32 (54%) had no sleep disturbance. There was no significant difference between total phytoestrogens consumption and sleep disturbance (X^2= 0.205, p=0.397).

6.6.4. Mood change

Among the subjects who took phytoestrogens >9 mg/day (median value), 43 (73%) had mood change and 16 (27%) had no mood change. But among the subjects who took ≤9 mg/day (median value), 43 (81%) had mood change and 10 (19%) had no mood change. There was no significant difference between total phytoestrogens consumption and mood change (X^2=1.06, p=0.210).

6.6.5. Burning sensation

It was found that the subjects taking less amount of phytoestrogens had higher burning sensation. Among the subjects who took phytoestrogens >9 mg/day (median value), 32 (54%) had burning sensation and 27 (46%) had no burning sensation. But among the subjects who took ≤9 mg/day (median value), 30 (57%) had burning sensation and 23 (43%) had no burning

sensation. There was no significant difference between total phytoestrogens consumption and burning sensation (X^2=0.06, p=0.47).

6.6.6. Headache

It was found that the subjects taking less amount of phytoestrogens had higher headache. Among the subjects who took phytoestrogens >9 mg/day (median value), 25 (42%) had headache and 34 (58%) had no headache. But among the subjects who took ≤9 mg/day (median value), 28 (53%) had headache and 25 (47%) had no headache. There was no significant difference between total phytoestrogens consumption and headache (X^2= 1.22, p= 0.18).

6.7. Difference of optimum intake vs low intake groups (Total isoflavones) regarding postmenopausal symptoms (Table 9)

6.7.1. Hot flush

It was found that the subjects taking less amount of isoflavones had higher hot flush. Among the subjects who took isoflavones >9 mg/day (median value), 33 (61%) had hot flush and 21 (39%) had no hot flush. But among the subjects who took ≤9 mg/day (median value), 38 (66%) had hot flush and 20 (34%) had no hot flush. There was no significant difference between total isoflavones consumption and hot flush (X^2 = 0.234, p=0.387).

6.7.2. Night sweat

Among the subjects who took isoflavones >9 mg/day (median value), 27 (50%) had night sweat and same number had no night sweat. And same was the case when their daily intake of total isoflavones was ≤9 mg/day (median value). There was no significant difference between total isoflavones consumption and night sweat (X^2=0.000, p=0.575).

6.7.3. Sleep disturbance

It was found that the subjects taking less amount of isoflavones had higher sleep disturbance. Among the subjects who took isoflavones >9 mg/day (median value), 23 (40%) had sleep disturbance and 35 (60%) had no sleep

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disturbance. But among the subjects who took ≤9 mg/day (median value), 26 (48%) had sleep disturbance and 28 (52%) had no sleep disturbance. There was no significant difference between total isoflavones consumption and sleep disturbance (X^2= 0.82, p=0.237).

6.7.4. Mood change

Among the subjects who took isoflavones >9 mg/day (median value), 38 (70%) had mood change and 16 (30%) had no mood change. But among the subjects who took ≤9 mg/day (median value), 48 (83%) had mood change and 10 (17%) had no mood change. There was no significant difference between total isoflavones consumption and mood change (X^2=2.408, p=0.092).

6.7.5. Burning sensation

It was found that the subjects taking less amount of isoflavones had higher burning sensation. Among the subjects who took isoflavones >9 mg/day (median value), 28 (52%) had burning sensation and 26 (48%) had no burning sensation. But among the subjects who took ≤9 mg/day (median value), 34 (59%) had burning sensation and 24 (41%) had no burning sensation. There was no significant difference between total isoflavones consumption and burning sensation (X^2=0.518, p=0.298).

6.7.6. Headache

It was found that the subjects taking less amount of isoflavones had higher headache. Among the subjects who took isoflavones >9 mg/day (median value), 25 (46%) had headache and 29 (54%) had no headache. But among the subjects who took ≤9 mg/day (median value), 28 (48%) had headache and 30 (52%) had no headache. There was no significant difference between total isoflavones consumption and headache (X^2= 0.044, p= 0.492).

6.8. Difference of optimum intake vs low intake groups (Total Lignans) regarding postmenopausal symptoms (Table 10)

6.8.1. Hot flush

It was found that the subjects taking less amount of lignans had higher hot flush. Among the subjects who took lignans >0.2 mg/day (median value), 59 (60%) had hot flush and 39 (40%) had no hot flush. But among the subjects who took ≤0.2 mg/day (median value), 12 (86%) had hot flush and 2 (14%) had no hot flush. There was a significant difference between total lignans consumption and hot flush (X^2 = 3.435, p=0.05).

6.8.2. Night sweat

The study showed that the subjects taking less amount of lignans had higher night sweat. Among the subjects who took lignans >0.2 mg/day (median value), 45 (46%) had night sweat and 53 (54%) had no night sweat. But among the subjects who took ≤0.2 mg/day (median value), 11 (79%) had night sweat and 3 (21%) had no night sweat. There was a significant difference between total lignans consumption and night sweat (X^2= 5.22, p=0.02).

6.8.3. Sleep disturbance

It was found that the subjects taking less amount of lignans had higher sleep disturbance. Among the subjects who took lignans >0.2 mg/day (median value), 39 (40%) had sleep disturbance and 59 (60%) had no sleep disturbance. But among the subjects who took ≤0.2 mg/day (median value), 10 (71%) had sleep disturbance and 4 (29%) had no sleep disturbance. There was a significant difference between total lignans consumption and sleep disturbance (X^2= 4.98, p=0.02).

6.8.4. Mood change

Among the subjects who took lignans >0.2 mg/day (median value), 10 (71%) had mood change and 4 (29%) had no mood change. But among the subjects who took ≤0.2 mg/day (median value), 76 (77%) had mood change and 22

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(23%) had no mood change. There was no significant difference between total lignans consumption and mood change (X^2=0.25, p=0.415).

6.8.5. Burning sensation

It was found that the subjects taking less amount of lignans had higher burning sensation. Among the subjects who took lignans >0.2 mg/day (median value), 51 (52%) had burning sensation and 47 (48%) had no burning sensation. But among the subjects who took ≤90.2 mg/day (median value), 11 (79%) had burning sensation and 3 (21%) had no burning sensation. There was a significant difference between total lignans consumption and burning sensation (X^2=3.48, p=0.05).

6.8.6. Headache

It was found that the subjects taking less amount of lignans had higher headache. Among the subjects who took lignans >0.2 mg/day (median value), 43 (44%) had headache and 55 (56%) had no headache. But among the subjects who took ≤0.2 mg/day (median value), 10 (71%) had headache and 4 (29%) had no headache. There was a significant difference between total lignans consumption and headache (X^2= 3.73, p= 0.04).

6.9. Association between total phytoestrogens and variables of interest in the study subjects (Table 11)

Correlation was tested with log total phytoestrogens, biochemical parameters and blood pressure. But total phytoestrogens showed no significant association with systolic blood pressure, diastolic blood pressure, fasting plasma glucose, triglyceride, total cholesterol, HDL-cholesterol and fibrinogen respectively.

6.10. Logistic regression analysis of factors affecting hot flush and burning sensation in the study subjects (Table 12)

Logistic regression analysis of hot flush and burning sensation was performed against the confounding independent variables of 6 types of phytoestrogens. There was no significant association of type of phytoestrogens with hot flush.

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But there was a significant association between SILR and burning sensation (p=0.033).

6.11. Logistic regression analysis of factors affecting sleep disturbance and headache in the study subjects (Table 13)

Logistic regression analysis of sleep disturbance and headache was performed against the confounding independent variables of 6 types of phytoestrogens. There was no significant association of type of phytoestrogens with sleep disturbance and headache.

6.12. Logistic regression analysis of factors affecting night sweat in the study subjects (Table 14)

Daidzein, genistein, matairesinol, biochanin A and formononetin showed no significant association with night sweat. But SILR showed a significant association with night sweat (p=0.026).

6.13. Association between FSH and phytoestrogens intake in the study subjects

There was no relationship between FSH and total phytoestrogens (Figure 2).

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Table 3: Phytoestrogen concentration of food items in the food frequency questionnaire

Food name as in FF Questionnaire	Food name as in literature	Daidzein	Genistein	Formononetin	Biochanin A	Coumestrol	Matairesinol	SILR
		mg/100g dry weight						
Ruti	Wheat whole grain[39]	0	0	0	0	0	0.0026	0.0329
Bhat	Rice[40]	0	0	0	0	0	0	0.016
Apple	Apples[40]	0.013	0	0	0	0	0	trace
Banana	Bananas[41]	0	0	0	0	0	0	0.005
Tomato	Tomatoes[40]	0	0	0	0	0	0.0065	0.0516
Lychee	Lychee[40]	0	0	0	0	0	0	0.0536
Papaya	Papaya[40]	0	0	0	0	0	0	0.0082
Guava	Guava[40]	0	0	0	0	0	0	0.6997
Lemon	Lemon[40]	0	0	0	0	0	0	0.0613
Orange	Oranges[40]	0	0	0	0	0	0	0.0768
Plum	Plum[41]	0	0	0	0	0	0	0.005
Pea	Peas[40]	0.053	0.0497	0	0	0	0	0.013
Chickpea	Chickpea[40]	0.011	0.069	0	0	0	0	0.007
Lentil	Navy beans[42]	0.014	0.408	0	0.04	0	0	0.0588
Black gram	Black gram[40]	0.745	1.9	0	0	0	0	0.468
Soybean	Tofu[43]	25.34	42.15	0	0	0	0	0
Cabbage	Red cabbage[41]	0.005	0.014	0.011	0	0	0	0.141
Cauliflower	Cauliflower[41]	0.005	0.009	0	0	0	0	0.097
Broccoli	Broccoli[41]	0.006	0.008	0	0	0.008	0.023	0.414
Onion	Onion[40]	0	0	0	0	0	0.008	0.083
Garlic	Garlic[40]	0	0	0	0	0	0.004	0.379
Potato	Potato peeled[41]	0	0	0	0	0	0.006	0.010
Pepper	Pepper[41]	0	0	0	0	0	0.007	0.117
Carrot	Carrots[39, 41]	0.002	0.0017	0	0	0	0.0029	0.192

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Table 4:Clinical and Biochemical Characteristics of the study subjects

Characteristics	
Age, years	51.4 ± 5.3
Age of menopause, years	46.2 ± 4.8
Body Mass Index (BMI), Kg/m2	27.1 ± 4.1
Systolic Blood Pressure (SBP), mmHg	127.8 ± 17.1
Diastolic Blood Pressure (DBP), mmHg	79.8 ± 8.1
Pulse, occasions/min	78 ± 11
Fasting plasma glucose (mmol/L)	7.3 (4.4 – 33.2)
S Triglycerides (mg/dl)	159 (73 - 655)
Total Cholesterol (mg/dl)	197 (113 - 301)
HDL Cholesterol (mg/dl)	36 (17 - 52)
Fibrinogen (mg/dl)	282 (101 - 847)
FSH (mIU/mL)	56.8 (0.57-154)
Diabetes Mellitus of the study subjects, n(%)	95 (85)
Hypertension of the study subjects, n(%)	75 (67)
Family history of diabetes mellitus, n(%)	73 (65)
Family history of hypertension, n(%)	78 (70)

Result are expressed as mean ± SD, median (range) or n (%)

Table 5: Nutrient intake of the study subjects

Name of the nutrients	Mean ± SD	Median (range)
Energy intake, Kcal/day	1705.3 ± 228.3	1652.6 (1510.5-3651.9)
Carbohydrate intake, g/day	160.3 ± 61.1	155.1 (100.3-687.0)
Protein intake, g/day	50.1 ± 22.0	41.4 (30.0-182.2)
Fat intake, g/day	16.7 ± 5.0	15.9 (10.0-37.5)
Iron intake, mg/day	20.1 ± 9.4	17.3 (10.0-67.3)
Vitamin B_1 intake, mg/day	1.3 ± 0.8	1.1 (0.6-5.8)
Vitamin B_2 intake, mg/day	0.5 ± 0.3	0.4 (0.1-1.6)
Calcium intake, mg/day	417.4 ± 156.4	471.3 (102.6-600.0)
β-carotene intake, μg/day	5093.3 ± 3508.7	4238.4 (1062.9-26931.8)
Retinol intake, IU/day	1733.5 ± 187.4	1702.9 (1022.0-2763.0)
Vitamin C intake, mg/day	103.2 ± 30.5	104.6 (42.0-150.0)

Cooking loss is not included

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Table 6: Phytoestrogens intake from Bangladeshi diet by postmenopausal subjects participating in the study

	Mean ± SD	Median (interquartile range) (mg/day)
Daidzein	4.34 ± 1.66	4.26 (3.56 – 5.37)
Genistein	4.56 ± 1.69	4.61 (3.66 –5.51)
Formononetin	0.0009 ± 0.001	0.0005 (0.0003-0.001)
Biochanin A	0.009 ± 0.01	0.007 (0.003 - 0.011)
Matairesinol	0.005 ± 0.002	0.004 (0.004 - 0.006)
Secoisolariciresinol (SILR)	0.24 ± 0.079	0.20 (0.20 - 0.30)
Total isoflavones	8.91 ± 3.35	8.91 (7.31 – 10.96)
Total lignans	0.24 ± 0.08	0.207 (0.204 - 0.305)
Total Phytoestrogens	9.16 ± 3.35	9.25 (7.57 - 11.13)

Table 7: Sources of intake of phytoestrogens (isoflavones and lignans) in the daily diet of the study subjects

Sl No	Food Groups	Isoflavons	Lignans
		% Daily intake	
1	Ruti and Bhat	0.0	37.48
2	Fruits	0.02	22.38
3	Beans and peas	11.11	13.00
4	Soyabean oil	88.84	0.0
5	Cabbage, cauliflower, carrot	0.03	7.26
6	Onion, garlic, pepper	0.0	16.67
7	Potato	0.0	1.79
8	Tomato	0.0	1.40

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Table 8: Difference of optimum intake vs low intake groups regarding postmenopausal symptoms

Phytoestrogens Consumed (mg)	Hot flash		Night sweat		Sleep disturbance		Mood change		Burning sensation		Headache		Total (%)
	Yes (n)	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	
>9	36 (61%)	23 (39%)	26 (49%)	27 (51%)	22 (42%)	31 (58%)	43 (73%)	16 (27%)	32 (54%)	27 (46%)	25 (42%)	34 (58%)	59 (100)
<8.99	35 (66%)	18 (34%)	30 (51%)	29 (49%)	27 (46%)	32 (54%)	43 (81%)	10 (19%)	30 (57%)	23 (43%)	28 (53%)	25 (47%)	53 (100)
X^2/ P value	*303/0.362*		*0.03/0.50*		*0.205/0.397*		*1.06/0.210*		*0.06/0.47*		*1.22/0.18*		

X^2 – test was performed as the test of significance

Table 9: Difference of optimum intake vs low intake groups regarding postmenopausal symptoms

Total Isoflavones Consumed (mg)	Hot flash		Night sweat		Sleep disturbance		Mood change		Burning sensation		Headache		Total (%)
	Yes (n)	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	
>9	33 (61%)	21 (39%)	27 (50%)	27 (50%)	23 (40%)	35 (60%)	38 (70%)	16 (30%)	28 (52%)	26 (48%)	25 (46%)	29 (54%)	54 (100)
<8.99	38 (66%)	20 (34%)	29 (50%)	29 (50%)	26 (48%)	28 (52%)	48 (83%)	10 (17%)	34 (59%)	24 (41%)	28 (48%)	30 (52%)	58 (100)
X^2/ P value	*0.234/0.387*		*0.000/0.575*		*0.82/0.237*		*2.408/0.092*		*0.518/0.298*		*0.044/0.492*		

X^2 – test was performed as the test of significance

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Table 10: Difference of optimum intake vs low intake groups regarding postmenopausal symptoms

Total Lignans Consumed (mg)	Hot flash		Night sweat		Sleep disturbance		Mood change		Burning sensation		Headache		Total (%)
	Yes (n)	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	*Yes (n)*	*No (n)*	
>0.2	59 (60%)	39 (40%)	45 (46%)	53 (54%)	39 (40%)	59 (60%)	10 (71%)	4 (29%)	51 (52%)	47 (48%)	43 (44%)	55 (56%)	98 (100)
<0.199	12 (86%)	2 (14%)	11 (79%)	3 (21%)	10 (71%)	4 (29%)	76 (77%)	22 (23%)	11 (79%)	3 (21%)	10 (71%)	4 (29%)	14 (100)
X^2/ P value	*3.435/0.055*		*5.22/0.02*		*4.98/0.026*		*0.25/0.415*		*3.48/0.05*		*3.73/0.04*		

X^2 – test was performed as the test of significance

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Table 11: Relationship between total phytoestrogens and variables of interest in the study subjects

Variables	r	*p*
Fasting plasma glucose	0.003	*0.976*
S Triglyceride	-0.098	*0.304*
Total Cholesterol	-0.063	*0.513*
HDL Cholesterol	0.075	*0.432*
Fibrinogen	0.021	*0.827*
FSH	-0.006	*0.953*
Systolic blood pressure	0.071	*0.460*
Diastolic blood pressure	-0.032	*0.735*

Table 12: Logistic regression analysis of factors affecting hotflash and burning sensation in the study subjects

Independent variables	Hot flush		Burning sensation	
	β values	*p values*	β values	*p values*
Daidzein	0.362	*0.726*	0.701	*0.484*
Genistein	-0.408	*0.689*	-0.717	*0.468*
Secoisolariciresinol (SILR)	11.420	*0.164*	17.206	*0.033*
Matairesinol	-168.544	*0.083*	-141.01	*0.099*
Biochanin A	3.108	*0.955*	-13.95	*0.793*
Formononetin	238.509	*0.219*	-188.30	*0.32*
R^2	**0.064**		**0.051**	

β for standardized regression coefficients). R^2 for Cox & Snell R square (Multiple coefficient of determination).

Table 13: Logistic regression analysis of factors affecting sleep disturbance and headache in the study subjects

Independent variables	Sleep disturbance		Headache	
	β values	*p values*	β values	*p values*
Daidzein	-0.554	*0.611*	-0.895	*0.395*
Genistein	0.482	*0.654*	0.892	*0.391*
Secoisolariciresinol	7.98	*0.344*	3.864	*0.62*
Matairesinol	134.77	*0.164*	-107.10	*0.20*
Biochanin A	-70.55	*0.202*	-29.73	*0.577*
Formononetin	448.16	*0.091*	156.48	*0.419*
R^2	**0.117**		**0.039**	

β for standardized regression coefficients. R^2 for Cox & Snell R square (Multiple coefficient of determination).

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Table 14: Logistic regression analysis of factors affecting night sweat in the study subjects

Independent variables	Night sweat	
	β values	*p values*
Daidzein	1.140	*0.260*
Genistein	-1.145	*0.253*
Secoisolariciresinol	18.18	*0.026*
Matairesinol	-90.07	*0.267*
Biochanin A	36.44	*0.502*
Formononetin	-42.76	*0.820*
R^2	0.058	

β for standardized regression coefficients. R^2 for Cox & Snell R square (Multiple coefficient of determination).

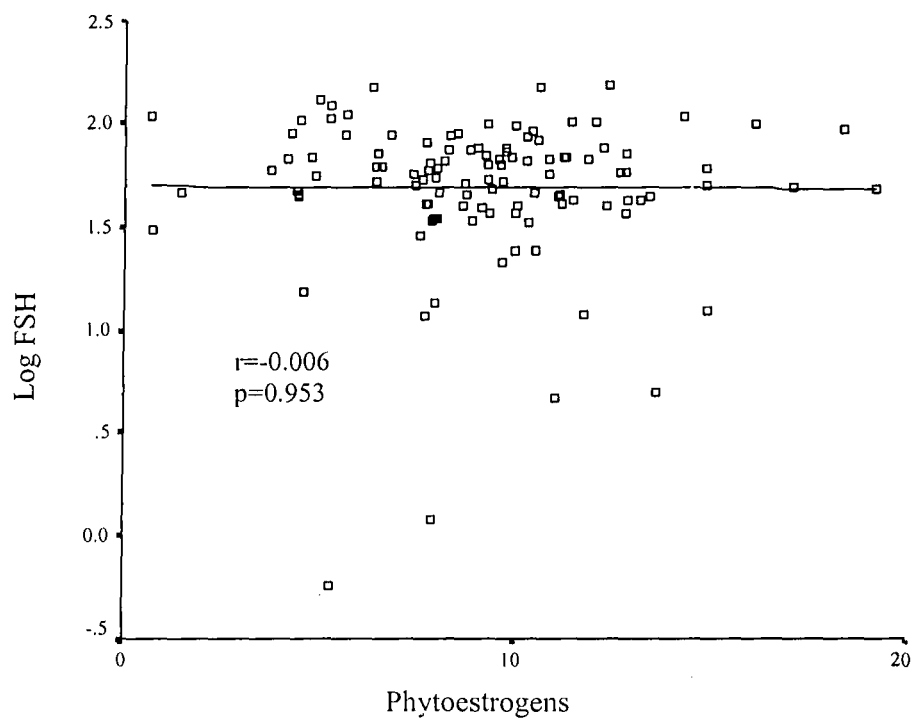

Figure 2: Relationship between FSH and phytoestrogens intake

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DISCUSSION

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7. Discussion

Menopause is an important but obligatory physiological phenomenon which creates a number of biological, psychological and social problems imposing additional risk and challenge in the life of a woman. Now a days hormone replacement therapy (HRT) is used to improve the quality of life of menopausal women, but due to its expensiveness and probable side effects it is now seriously thought that an alternative therapy is necessary. Recent studies suggest that phytoestrogens, which are contained in our daily diet, could be an alternative of HRT to reduce the menopausal symptoms. As we know so far, no study has been conducted to find out the effect of phytoestrogens-rich diet on menopausal symptoms in our population and even in women in this subcontinent. The present study aimed to explore the quantitative and qualitative intake of phytoestrogens in Bangladeshi menopausal women and its relation to clinical outcome.

With the growing interest in the potential health benefits of phytoestrogen, more and more data are being published on the phytoestrogen content of foods. It was the first study to determine the quality and nature of phytoestrogens in common Bangladeshi diet. In this study, the phytoestrogens contents of food were based on only literature. Therefore, our results probably express only the approximate intake of phytoestrogens.

Phytoestrogens are classified in three groups, i.e., isoflavones, coumestans and lignans. The major isoflavones are genistein, daidzein, formononetin and biochanin A. Coumestrol is the most important coumestan. The major lignans are enterolactone and enterodiol, which are produced by colonic bacteria from their dietary precursors matairesinol and secoisolariciresinol[16].In this study, the dietary intake of total phytoestrogens, lignans and isoflavones were found to be 9.16, 0.24 and 8.91 mg/day respectively in postmenopausal women. In USA, the corresponding value were <1, 0.64 and 0.76 mg/day respectively[16]. Total dietary lignans and isoflavones intake in Dutch white women was found to be 1.11 and 0.88 mg/day[51]. On the other hand, daily intake of isoflavones

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in Chinese women in Shanghai was 40mg[52] and in Korean subjects it was 14.9mg/ day[54]. Thus, it is evident that the daily intake of total phytoestrogens and isoflavones in our subjects is about 9 and 10 fold higher compared to that of western population, but the daily intake of total lignans is about 5 times lower[16,51]. In contrast, the daily intake of total isoflavones in our population is about 19 to 50 fold lower compared to that of oriental population[16,51].

The present data shows that two types of phytoestrogen are mainly consumed by our population and those are isoflavones and lignans. Phytoestrogens mainly consumed are isoflavones. On the other hand, most phytoestrogens in western population are in the forms of lignans[51].

Not only are intakes of isoflavones among our subjects is substantially higher compared to western subjects, the types and sources of phytoestrogens consumed differ as well. The phytoestrogens, largely in the form of isoflavones, are mainly derived from soyabean, beans and peas. These foods contain high amounts of phytoestrogens per gram of food and are consumed frequently. However, among Japanese, Chinese and Korean subjects, traditional soy foods, such as tofu invariant forms, constitute the main, and sometimes only, sources of isoflavones[51-55]. The main sources of lignans intake in this study are ruti (wheat), bhat (cooked rice), fruits, onion and garlic. Ruti and bhat are the common foods of Bangladeshi population. Among western subjects lignans are mainly derived from grain products, coffee and tea[52-56].

We have no recommended value for phytoestrogens intake; thus, we cannot ascertain whether the current intake is optimum. No significant difference was found between the groups having optimum and low intake of total phytoestrogens or isoflavones regarding hot flushes, night sweat, sleep disturbance, mood change, burning sensation and headache. In a study, it was found that daily diet supplemented with soy flour could reduce flushes compared with wheat flour over 12 weeks. Hot flushes were significantly decreased in the soy and wheat flour groups 40% and 25% respectively[31]. In

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another study, it was found that genistein administration reduced climacteric symptoms in postmenopausal women [32].

Significant differences were found between the groups having optimum and low intake of lignans regarding hot flushes (p=0.05), night sweat (p=0.02), sleep disturbance (p= 0.02), burning sensation (p=0.05) and headache (p=0.04). To investigate the issue in more details, regression analysis was done. A significant positive association of SILR, one specific type of lignans, was found with night sweat (p=0.026) and burning sensation (p=0.033), whereas matairesinol, another specific type of lignans, showed no significant association with any of those symptoms. So it may be suggested that SILR and matairesinol have synergistic effect on postmenopausal symptoms. It may also be concluded that total lignans is biologically more effective than isoflavones because total isoflavones as previously described have no significant effect on symptoms. It has been reported that both soya and linseed help to alleviate postmenopausal symptoms[34].

In the present study a correlation was made between total phytoestrogens and serum lipid levels but we could not find any significant relation. In the Framingham study, the association between dietary phytoestrogen intake and metabolic cardiovascular risk factors in postmenopausal women was studied. It was found that high intake of phytoestrogens in postmenopausal women appeared to be associated with a favorable metabolic cardiovascular risk profile[57]. In another study, it was found that soya had beneficial effects on blood lipids which might help to reduce the risk of cardiovascular disease and atherosclerosis[34].

Numerous unanswered questions remain concerning the effects of phytoestrogen intake for women. Further studies should be performed to clarify the most effective types and forms of phytoestrogens; optimal intake; the significance of individual variation in phytoestrogen metabolism and long term effects, particularly on symptoms.

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CONCLUSIONS

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8. Conclusions

- The dietary intake of total phytoestrogens, lignans and isoflavones are 9.16, 0.24 and 8.91 mg/day respectively in postmenopausal women in Bangladesh.
- Total isoflavones intake is about 10 times higher compared to western population and 19 to 50 times lower to oriental population. On the other hand, total lignans intake is about 5 times lower compared to western population.
- The main sources of isoflavones are soyabean oil, beans and peas and those of lignans are ruti (wheat), bhat (cooked rice), fruits, onion and garlic.
- Phytoestrogen, more particularly lignans, reduces the menopausal symptoms such as hot flushses, night sweat, sleep disturbance, burning sensation and headache. SILR has a consistent effect on night sweat and burning sensation.
- Phytoestrogens do not operate postmenopausal symptoms through FSH.

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RECOMMENDATIONS

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9. Recommendations

- Phytoestrogens containing food intake should be encouraged for the management of menopausal symptoms.
- The content of phytoestrogens in Bangladeshi foods should be chemically measured using appropriate tools.
- Mode of action of phytoestrogens should be explored further.
- Bioavailability of phytoestrogens should be measured to see the corresponding estradiol level.
- Further, this study should be conducted on general population especially on those who are non-diabetic.

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STUDY LIMITATIONS

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10. Study Limitations

- It was, in fact, very difficult to collect the subjects with postmenopausal symptoms for conducting the study because they won't like to expose themselves to the society which is, in general, still conservative. So, they were collected purposively when they came to BIRDEM or other branches of DAB in their critical stages. Actually, their presence was found very rare in other hospitals and clinics. So, most of the subjects in the study were diabetic.
- Large number of subjects should have been included in the study.
- Due to limited resources, financial and technical support, it has not been possible to do the chemical analysis of phytoestrogens in our local food.
- In case of interview regarding past information, recall bias may be present in the dietary history.
- Since the interviews were administered by four interviewers, there may have been subjective variation in recording data.

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APPEDICES

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APPENDIX I

CASE RECORD FORM

Sl. No: Date:

1. Name: Age:
2. Address:
   a) Present:

   b) Permanent:
3. Patient source:
4. Habitat:
5. Religion: Muslim-1/ Hindu-2/ Christian-3/ Other-4
6. Education: Illiterate-Primary-1/ SSC-HSC-2/ Graduate & Above-3
7. Current marital status: Married- 1/ Unmarried -2/ Widowed –3/ Separated –4/ Others (specify)
8. No of Children:
9. Socioeconomic status:
   a) No of Family member:
   b) Yearly income:
10. Occupation:
11. Family history of hypertension_____ present/absent
12. Family history of D.M_____ present/absent
13. Present history:
    a) Hypertension: present/absent
    b) D.M: present/absent
    c) Other
14. Past history
    a) Any surgical history (e.g. hysterectomy)
    b) Thyroid
    c) Other

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15. Past Drug History (including Vitamins & Minerals)

Trade Name	Generic Name	mg	dose

16. Menopause_____(Age)____

17. How old were you when you had your hysterectomy/uterus/womb (protection area for female organ) removed?

18. Have you had one or both of yours ovaries removed?

19. Do you feel hot flash?
    1Yes / 2No

20. How many times / day_____ Duration_____

21. How many days (In months) ?

22. How severely do you feel hot flash?
    1 Severe / 2 Moderate / 3 Mild/ Occasional

23. Do you ever experience night sweat?
    1Yes / 2No
    If yes,
    Frequency-
    Duration-
    For how many days (In months) ?

24. How severely do you feel night sweat?
    1 Severe / 2 Moderate / 3 Mild/ Occasional

25. Have you any sleeping disturbance?
    1Yes / 2No

26. How long do you experience sleep disturbance (In months) ?

27. How severely do you feel this?
    1 Severe / 2 Moderate / 3 Mild/ Occasional

28. Is there any mood change than before?
    1Yes / 2No

29. What type of mood change do you feel?
    Irritable 1 / Emotional upset 2/ Depression 3

30. How long do you feel the complain of mood change (In months) ?

31. How severely do you feel this?
    1 Severe / 2 Moderate / 3 Mild/ Occasional

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32. Any urinary complain
    micturition
    dysuria
    frequency
    urgency
    other

33. Do you feel headache?
    1Yes / 2No

34. Do you feel burning sensation?
    1Yes / 2No

35. How many times / day_____ Duration_____

36. How many days (In months)?

37. How severely do you feel this?
    1 Severe / 2 Moderate / 3 Mild/ Occasional

38. Do you feel dysparunia?
    1Yes / 2No

39. **General examination:**
    a) Ht (m)_____ b) Wt (Kg)_____ Desire wt (kg)_____
    c) BMI (Kg/m^2)_____
    d) Anemia_____
    e) Pulse_____ f) BP_____

40. Systematic examination:
    a) Thyroid_____
    b) Other gynecological problem _____
    c) Heart_____
    d) Lung_____
    e) Other_____-

41. Investigations:
    a) S Glucose (Fasting)
    Lipid Profile:
    a) TG
    b) TC
    c) LDL-C
    d) HDL-C
    e) VLDL-C
    f) Fibrinogen
    g) Serum FSH

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42. Present Drug History (including Vitamins & Minerals)

Trade Name	Generic Name	mg	dose

Interviewer Signature

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CASE RECORD FORM (Dietary)

Registration No: Date:

1. Name:
2. Address:
   a) Present:

   b) Permanent:

Food Frequency Questionnaire

Phytoestrogens rich Foods

Food	Portion size (g)/day	Never	Limited	Unlimited	Time/wk
Cereals					
Ruti					
Bhat					
Fruits					
Apple					
Banana					
Tomato					
Lychee					
Papaya					
Guava					
Lemon					
Orange					
Plum					
Pulses					
Chickpea					
Pea					
Dal/Lentil					
Blackgram					
Soybean					
other					

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Food	Portion size (g) /day	Never	Limited	Unlimited	Time/wk
Vegetables					
Cabbage					
Cauliflower					
Broccoli					
Onion					
Garlic					
Potato					
Pepper					
Carrot					
Other					

Food Frequency Questionnaire

Other Foods

Food	Portion size (g) /day	Never	Limited	Unlimited	Time/wk
Cereals					
Chira					
Moori					
Suzi					
Shemai					
Others					
Fruits					
Atafol					
Amra					
Pakaam					
Tal					
Nashpatee					
Bel					
Tarmuz					
Others					

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Food	Portion size (g) /day	Never	Limited	Unlimited	Time/wk
Vegetables					
Shak					
Mula					
Shim					
Begun					
Potol					
Korola					
Lau					
Mishtikumra					
Kakrol					
Others					
Fish					
Meat					
Beef					
Mutton					
Chicken					
Others					
Egg					
Milk					
Sugar					
Others					

Interviewer Signature _____

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APPENDIX II

সম্মতি পত্র

ক্রমিক নং ঃ-

আমি ----------*মেনোপোজ মহিলাদের খাদ্য ব্যবস্থাপনা* সংক্রান্ত গবেষণায় অংশগ্রহনকারী হিসাবে তথ্য প্রদানে আমার সম্মতি প্রদান করিতেছি।

আমার দেওয়া সকল তথ্য গবেষণার কাজে ব্যবহৃত হবে এবং সেবার ধরণ উন্নত করার ক্ষেত্রে অবদান রাখবে।

স্বাক্ষরঃ-

তারিখঃ-

ঠিকানাঃ-