EFFECT OF ISOFLAVONES ON SERUM HOMOCYSTEINE AND C-REACTIVE PROTEIN LEVELS IN POSTMENOPAUSAL WOMEN

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Dedicated

To

Late Abul Khair Bhuiyan
Anwara Begum

My Beloved Parents

CERTIFICATE

The thesis titled 'EFFECT OF ISOFLAVONES ON SERUM HOMOCYSTEINE AND C-REACTIVE PROTEIN LEVELS IN POSTMENOPAUSAL WOMEN' is submitted in partial fulfillment of the requirement for MPhil degree under the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. This work had been carried out in the Department of Biochemistry & Cell Biology, Bangladesh University of Health Sciences (BUHS) and Biomedical Research Group, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic disorder (BIRDEM) during the period of June 2012 to July 2013. 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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DECLARATION

I hereby declare that this thesis entitled as 'EFFECT OF ISOFLAVONES ON SERUM HOMOCYSTEINE AND C-REACTIVE PROTEIN LEVELS IN POSTMENOPAUSAL WOMEN' is based on work carried out by me and no part of it has been presented previously to any academic institute or university for any higher degree.

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TABLE OF CONTENTS

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The Author
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LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

HDL	High Density Lipoprotein- cholesterol
TG	Triglycerides
HRT	Hormone Replacement Therapy
LDL-cholesterol	Low Density Lipoprotein-cholesterol
CVD	Cardiovascular Disease
INFS	Institute of Nutrition and Food Sciences
FFQ	Food Frequency Questionnaire
rpm	Rotation per minute
^{0}C	Degree Celsius
µl	Microlitere
GK	Glucoseoxidase
POD	Per oxides
mmol/l	Milimole per liter
U/ml	Unit per milliliter
mg/dl	Milligram per deciliter
BMI	Body Mass Index
Kg/m^2	Kilogram per miter square
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
mmHg	millimeter mercury
g	Gram
µg	Microgram
mg	Milligram

WHO	World Health Organization
MS	Metabolic Syndrome
BUHS	Bangladesh University of Health Sciences
FFA	Free fatty acid
CRP	C-reactive protein
Hcy	Homocysteine
WC	Waist circumference
WHR	Waist hip ratio
BDT	Bangladeshi taka
IPAQ	Physical Activity Questionnaire
m	meter
GOD-PAP	Glucose-Oxidase
SD	Standard deviation
CI	Confidence intervals
OR	Odds ratios
IR	Insulin resistance
apoB	Apolipoprotein B
NEFA	Nonesterified fatty acids
IRAS	Insulin Resistance Atherosclerosis Study
NHANES III	Third National Health and Nutrition Examination Survey
ISI	Insulin sensitivity index
E2	Estradiol
E1	Estrone

Abstract

Cardiovascular diseases (CVDs) are the leading cause of mortality and morbidity among the postmenopausal women in developed as well as developing countries. The incidence of CVD is lower in premenopausal women than in men; however, CVD risk in postmenopausal women is 3.4 times than that in premenopausal women. These differences in risk are partially related to increases in homocysteine (Hcy) and C-reactive protein (CRP). Elevated Hcy is an independent, modifiable risk factor for atherosclerotic CVD. Elevated CRP, a marker of acute inflammation, is a reliable predictor of CVD. Soy milk is rich in phytoestrogen, it has been claimed that phytoestrogens, particularly soy isoflavones, have beneficial effects on cardiovascular diseases (CVDs) and diabetes; however, the underlying mechanism for these effects still need to be established. In the present study the effect of soy milk on homocysteine (Hcy, an important risk for CVDs), C-reactive protein (CRP, one of the marker of inflammatory status) and insulin resistance (a major defect in diabetes & CVDs) were explored in response to short-term intake of soy milk. Soy beans, cultivated in Bangladesh, were collected directly from the producers, and milk was prepared by following a standardized manual procedure.

It was an un-blinded open ended trial where 36 women, under a randomized cross-over design, consumed 350ml of the milk 2 times/day (calculated with a target 30 mg isoflavone daily) for 21 days with a wash out period of 10 days. The subjects were advised to follow the usual lifestyle during the period. The Control Group did not receive any other placebo product. Fasting (10-12 h) serum samples were analyzed for glucose & lipids (enzymatic-colorimetric method), insulin and CRP (ELISA method) and Hcy (Fluorescence Polarization Immunoassay). Pooled data for the two groups were used to calculate the baseline and final values which were then compared by paired t-test. Insulin sensitivity (HOMA%S) was calculated by homeostatic model assessment. Among the lipidemic profile serum HDL (mg/dl) was significantly higher ($p=0.005$) at the time point of 21st days compared to 0 day in Soy milk group. Serum triglycerides (mg/dl) and NEFA (mmol/l) were significantly lower ($p=0.088$ and $p=0.010$ respectively) in Soy milk group at 21st days compared to 0 day. However the insulinemic profile (fasting insulin, HOMA%B, HOMA%S and HOMA-IR) and inflammatory status (CRP and Hcy) did not show any significant difference between the two time points. When considering the time point of 31st and 52nd days (after 10 days wash out period) serum glucose (mmol/l) was significantly lower ($p=0.005$) in Soy milk group at 52nd

days compared to 31st days. Serum triglyceride was significantly lower (p=0.049) in both groups at 52nd days compared to 31st days. Among inflammatory status and insulinemic profile there were no significant difference between the two time points (p=ns). On the other hand, at the time point of baseline and endpoint serum blood sugar, triglycerides and NEFA were significantly lower (p=0.009; p=0.008 and p=0.013) in endpoint compared to the baseline values. On Pearson's correlation analysis CRP showed significant positive correlation with BMI (r=0.416, p=0.012), WHR (r=0.330, p=0.049) and fasting insulin (r=0.286/p=0.015) at baseline, while at the endpoint CRP showed significant positive correlation with BMI (r=0.538, p=0.001) and WHR (r=0.343, p=0.041). On the other hand, Hcy showed significant positive correlation with age (r=0.451, p=0.006) and NEFA (r=0.407/p=<0.001) at baseline while at endpoint it showed correlation only with age (r=0.508, p=0.002). Logistic regression analysis of CRP was done with age, BMI, WHR, lipid profile and HOMA%B confounding independent variables. A negative significant association was found with HOMA%B (β=-0.352 [95% CI: -0.021-<0.002], p=0.043) at baseline. At end point CRP showed significant negative association with HOMA%B (β=-0.360 [95% CI: -0.017-<0.001], p=0.041) after adjusting the effects of major confounders (age, BMI, W/P ratio and lipid profile). End point group Hcys showed significant negative association with HOMA%S (β=-0.521 [95% CI: -0.157-(-0.019)], p=0.041) and HOMA IR (β=-0.382 [95% CI: -1.111-(-0.001)], p=0.050) after adjusting the effects of major confounders (age, BMI, W/P ratio), these associations disappeared after adjusting the same confounders at baseline.

From the above results it may concluded that Soy Isoflavones seem to have beneficial effects on hyperglycemia and dyslipidemia among postmenopausal women and the effects seems to be associated through lowering of insulin resistance and chronic subclinical inflammation.

INTRODUCTION

1.1. Menopause

Menopause (as defined by the World Health Organization) is the permanent cessation of menstruation due to loss of ovarian follicular activity [1]. This definition uses both, a symptom that can be identified by a woman (the end of menstruation) and a sign that can be measured (loss of follicular activities resulting in changes in levels of hormones). There remain, however, shortcomings in this definition. First, follicular activity can continue even in the absence of menstruation, for example, in case of hysterectomy, ovaries may remain functional. Second, follicular activity can end, but menstruation can continue through the use of cyclic hormonal therapy. Third drawback of this definition is how women experience menopause, which may vary within and between the social groups. Most women perceive menopause to be a marker for the end of childbearing. The end of menstruation can, therefore, be an emotional event. Some women may react to the cessation of menstruation with relief (no more birth control); other may describe deep sadness because they can no longer bear children [2]. The term menopause (or climacteric) is use widely in clinical practice and in the medical literature to describe a period of time during which spontaneous menstruation normally ceases. This period is characterized endocrinologically by evidence of decreasing ovarian activity, biologically by decreasing fertility and clinical by alterations in menstrual cycle intervals and by a variety of symptoms [1].

Problems related to menopause were given scanty attention till the 1980's. The concept of menopause got its importance only in 1981 when a report of the World Health Organization (WHO), Scientific Group, based on its meeting on Research on the Menopause held in Geneva during December 1980 says there are virtually no data on the age distribution of the menopause and no information on its socio-cultural significance in the developing countries. Further the Scientific Group made some specific recommendations as WHO sponsored research should be undertaken to determine the impact on health service needs of the rapidly increasing numbers of postmenopausal women in developing countries; uniform terminology should be adopted by health care workers with regard to the menopause; uniform endocrine standards should be developed which can be applied to the description of peri and postmenopausal conditions and diseases; and descriptive epidemiological studies of the age at menopause should be performed

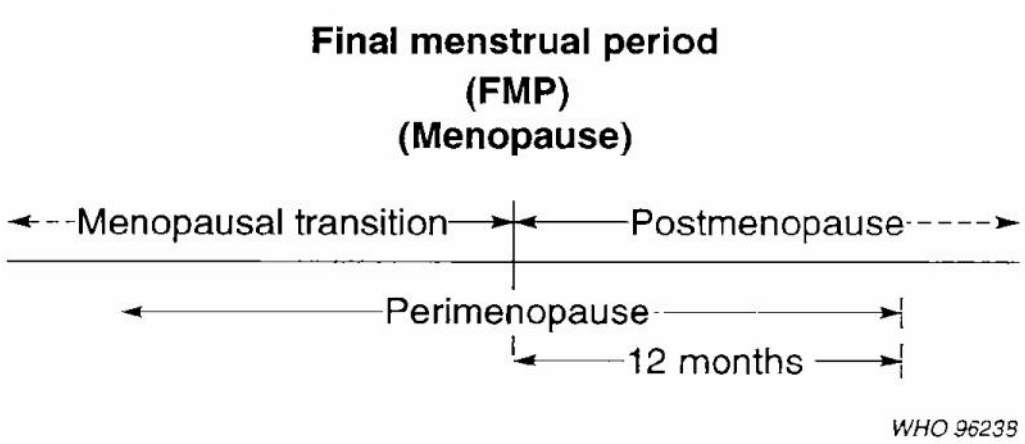
in a variety of settings [3]. The age at which menopause occurs varies from country to country and among individuals in the same country. In the Western world, the most typical age range for menopause (last period from natural causes) is between the ages of 40 and 61 [4] and the average age for last period is 51 years [5]. The average age of natural menopause in Australia is 51.7 years [6], although this varies considerably from one woman to another. In India and the Philippines, the median age of natural menopause is considerably earlier, at 44 years [7]. According to one study, women who smoke cigarettes experience menopause significantly earlier than non-smokers [8]. Women who have undergone hysterectomy with ovary conservation go through menopause on average 3.7 years earlier than the expected age [9]. Although, there were reported that geography, secular trends, marital status, occupation, parity, use of oral contraceptives, weather, altitude, socioeconomic factors influencing the age at menopause [1]. 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 [10].

The following definitions have been recommended by a WHO Scientific Committee in 1980 [1]:

- **menopause:** the permanent cessation of menstruation resulting from loss of ovarian follicular activity;
- **perimenopause (or climacteric):** the period immediately prior to the menopause (when the endocrinological, biological and clinical features of approaching menopause commence) and at least the first year after the menopause;
- **postmenopause:** dating from the menopause, although it cannot be determined until after a period of 12 months of spontaneous amenorrhea has been observed;
- **premenopause:** the whole of the reproductive period prior to the menopause.

Figure 1

Relationship between different time periods surrounding the menopause



World Health Organization. Research on Menopause in the 1990s: report of WHO Scientific group. Geneva: WHO Technical series 866;1996; page 13.

1.2.Indications and signs

During the menopause transition years, as the body responds to the rapidly fluctuating and dropping levels of natural hormones, a number of effects may appear [9]. With the general increase in life expectancy, many women are likely to live for more than 20 years after menopause, spending about one quarter of their lives or more in a state of estrogen deficiency [11]. Owing to lack of estrogen woman may experience decrease physical and mental well being that's why postmenopausal woman can be considered a risk population [12,13]. The overall health and well being of mid-aged women has become a major public health concern around the world. More than 80% of women experience physical or psychological symptoms in the year approaching menopause with various distress and distribution in their lives, leading to decrease in quality of life [14]. World Health Organization (WHO) defines Quality of life (QoL) as an individual's perception of their position in life in the context of culture and values system in which they live and in relation to their goal expectations, standards and concerns [15]. The study of QoL in the post menopause has become an essential component in clinical practices. Most studies on QoL of postmenopausal women were conducted in developed countries with different sociocultural realities, which may influence not only the perception of QoL but also

the experience of menopausal symptoms. Very little information exists about QoL of postmenopausal women in developing countries [16].

The average woman also has increasingly erratic menstrual periods, due to skipped ovulations. Typically, the timing of the flow becomes unpredictable. In addition the duration of the flow may be considerably shorter or longer than normal, and the flow itself may be significantly heavier or lighter than was previously the case, including sometimes long episodes of spotting. Early in the process it is not uncommon to have some 2-week cycles. Further into the process it is common to skip periods for months at a time, and these skipped periods may be followed by a heavier period. The number of skipped periods in a row often increases as the time of last period approaches. At the point when a woman of menopausal age has had no periods or spotting for 12 months, she is considered to be one year into post-menopause [9].

One way of assessing the impact on women of some of these menopause effects are the Greene Climacteric Scale questionnaire[17], the Cervantes Scale [18], and the Menopause Rating Scale[19].

- Vascular instability
- Hot flashes or hot flushes, including night sweats and, rarely, cold flashes
- Possible but contentious increased risk of atherosclerosis [20].
- Migraine
- Rapid heartbeat

Urogenital atrophy, also known as vaginal atrophy

- Thinning of the membranes of the vulva, the vagina, the cervix, and also the outer urinary tract, along with considerable shrinking and loss in elasticity of all of the outer and inner genital areas.
- Itching
- Dryness
- Young women who are approaching menopause may experience dysfunctional bleeding due to the hormonal changes that accompany the menopausal transition. Genital bleeding is an alarming symptom for postmenopausal women that requires an appropriate study to

discard malignant diseases. Spotting or bleeding may be related to a benign sore (polyp or lesion) or functional endometrial response. The European Menopause and Andropause Society has released Guidelines for assessment of the endometrium which is main origin of the spotting or bleeding [21].

- Watery discharge
- Urinary frequency
- Urinary incontinence may worsen the menopause-related quality of life, although urinary incontinence is more related to obstetric events than to menopause [22].
- Urinary urgency
- Increased susceptibility to inflammation and infection, for example vaginal candidiasis, and urinary tract infections

Skeletal

- Back pain
- Joint pain, Muscle pain
- Osteopenia and the risk of osteoporosis gradually developing over time

Skin, soft tissue

- Breast atrophy
- Breast tenderness, swelling
- Decreased elasticity of the skin
- Formication (itching, tingling, burning, pins, and needles, or sensation of ants crawling)
- Skin thinning and becoming drier

Psychological

- Depression and/or anxiety [23].
- Fatigue
- Irritability
- Memory loss,and problems with concentration
- Mood disturbance
- Sleep disturbances, poor or light sleep, insomnia, and sleepiness [19],[24],[25].

Sexual

- Dyspareunia or painful intercourse
- Decreased libido [26],[27],[28].
- Problems reaching orgasm
- Vaginal dryness and vaginal atrophy

Cohort studies have reached mixed conclusions about medical conditions associated with the menopause. For example, a 2007 study found that menopause was associated with hot flashes; joint pain and muscle pain; and depressed mood [29]. In the same study, it appeared that menopause was not associated with poor sleep, decreased libido, and vaginal dryness [29]. However, in contrast to this, a 2008 study did find associations with poor sleep quality [30].

1.3 Hormonal changes during Menopause

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

a. 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 infarction 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 [31]. 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.

b. 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 [32].

c. 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[31]. 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 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[32]. 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.

d. 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 [33].

1.4 Health problems after menopause

1.4.1 Risk of Cardiovascular disease (CVD) after menopause

Cardiovascular diseases (CVDs) are the leading cause of mortality and morbidity among the postmenopausal women in developed as well as developing countries. The incidence of CVD is lower in premenopausal women than in men; however, CVD risk in postmenopausal women is 3.4 times of that in premenopausal women1[34]. Estrogen deficiency has been linked to the rapid increase in CVD in women who have undergone natural or surgical menopause [35]. More than 450,000 women succumb to heart disease annually, and 250,000 die of coronary artery disease [36]. CVD risk increases after the menopause, which may be related to metabolic and hormonal changes [37]. Premenopausal women appear to be protected from CVD compared with men of similar age. Although women below the age of 50 yr rarely develop CVD, by age 70 yr the incidence of CVD is equal in men and women, suggesting that estrogen deficiency causes a rapid acceleration in CVD risk. Controversy exists about whether menopause increases the risk of

CVD independent of normal aging. Some studies have demonstrated increased risk of CVD after menopause, and others have not [38]. For example, Framingham investigators found a 4-fold increase in CVD in the 10 yr following natural menopause. Premature, surgically induced menopause has been shown to increase the risk for CVD [39]. Yet the question of whether natural menopause is an independent risk factor for CVD has not been answered, as it is very difficult to design studies that can separate the effects of the normal aging process from menopause. Statistical adjustment for age or body weight in longitudinal studies may erase the influence of other closely related factors and underestimate the effect of estrogen deficiency on CVD risk. The metabolic and hormonal changes of menopause occur.

1.4.2 Metabolic syndrome

The metabolic syndrome may not be a single disease entity, but, rather, a constellation of closely related risk factors that together convey substantially increased cardiovascular risk after accounting for traditional CVD risk factors[40]. The features of the metabolic syndrome include the accumulation of visceral (abdominal) adiposity, insulin resistance, hypertension, and dyslipidemia (hypertriglyceridemia, reduced high density lipoprotein (HDL), and small dense LDL particles [41]. The metabolic syndrome is estimated to affect approximately 20–30% of the middle-aged population, and prevalence appears to be increasing in the U.S. population with increasing obesity and sedentary lifestyle [42]. Postmenopausal status is associated with a 60% increased risk of the metabolic syndrome, even after adjusting for confounding variables, such as age, body mass index (BMI), household income, and physical inactivity. The risk of CVD attributed to the metabolic syndrome appears to be especially high in women, and it is estimated that half of all cardiovascular events in women are related to the metabolic syndrome [43].

1.4.3 Effects of menopause on body composition

Epidemiological studies have found a progressive increase in the prevalence of cardiovascular risk factors (dyslipidaemia, elevated blood pressure, disturbances in glycaemic control) with increasing body fatness [44-46]. Estrogen promotes the accumulation of gluteo-femoral fat [47], and the loss of estrogen with menopause is associated with an increase in central fat [48]. The sexual dimorphism in adipose tissue distribution may partially explain the greater CVD risk in men compared with premenopausal women [49]. In recent decades, many prospective and cross-

sectional studies using anthropometric measures have been undertaken in order to understand the relationship between obesity and cardiovascular risk factors. Various obesity measurements such as body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR) were investigated. BMI is the most commonly used and simple measure of body size, especially for estimating the frequency of obesity in large epidemiological studies [50]. This index cannot however be used for the evaluation of body fat distribution and abdominal fat mass. It has been shown that intra-abdominal fat has a stronger relationship with risk of obesity-related morbidity than with overall adiposity [51]. High fasting TG and increased WC levels have been recognized as high-risk metabolic abnormality [52]. Elevated TG elevated WC that mean WC more than 90 cm in males or 85 cm in females together with fasting TG levels higher than 177 mg/dL is defined as hyper triglyceridemic waist phenotype has been revealed as strong predictor for CVD [53,54]. Therefore WHR and WC measurements can be used as valid alternatives to BMI for the evaluation of intraabdominal mass and total fat [55]. A study by Huang and co-workers showed that WHR and WC measurements were strongly associated with incidence of coronary heart disease, independent of BMI [56]. However the validity of WHR measurements has been questioned as an indicator of abdominal adipose tissue distribution [57].

1.4.4 Effects of menopause on Lipid Profile

Many longitudinal studies have shown that TG levels increase with the transition through the menopause [58], and the increase in TG also appears early in the postmenopausal period [59]. The prospective transition to postmenopause was associated with a 16% increase in TG. Although men generally have higher TG levels than women, TG increases in middle-age (between 40–69 yr) in women, but not in men [49,60], and TG appears to be a better predictor of CVD risk in women than in men. Lindquist [31] reported a prospective increase in TG levels in women who became postmenopausal during a 6-yr period, whereas there was no change in TG in the similarly aged women who remained either premenopausal or postmenopausal. Increasing TG with menopause may be related to the fact that TG levels are highly correlated with increasing abdominal fat content and insulin resistance.

Postmenopausal women have higher total cholesterol, LDL cholesterol, triglycerides (TG), and lipoprotein (a) [Lp(a)] levels and lower HDL cholesterol levels than premenopausal women [58].

Although elevated LDL is not a component of the metabolic syndrome, LDL levels increase by 10–20% [49,61], with menopause, and the greatest change.

Most studies show that total HDL levels fall slightly with menopause [49,62], whereas others reveal no changes [63]. Menopausal changes in HDLmetabolism are more complex than the measurement of total HDL reveals, because the more antiatherogenic HDL2 levels decrease (by 25%), whereas HDL3 levels increase [64], HDL2 particles are the large, buoyant, and more cardioprotective subspecies of total HDL. The strong inverse relationship between HDL cholesterol and abdominal adiposity appears to be largely dependent on variations in HDL2 levels [65].

1.4. 5 Insulin Resistance

Two of the most important pathophysiological components of the metabolic syndrome are increased visceral fat accumulation and insulin resistance. Abdominal obesity is closely associated with increased insulin resistance, compensatory hyperinsulinemia, and increased risk of type 2 diabetes, independent of an individual's total body fat content [66]. The pathophysiology underlying the insulin-resistant state is complex. Insulin resistance, with inadequate compensatory hyperinsulinemia, diminishes the normal suppression of FFA arising from adipose tissue by insulin. The increased levels of FFA may impair peripheral glucose uptake, increase hepatic gluconeogenesis, and reduce hepatic clearance of insulin. The literature to date is not clear as to whether menopause is associated with increased insulin resistance. What little data there are remain contradictory. Several groups have shown increased fasting insulin and increased fasting glucose levels [67] in postmenopausal compared with premenopausal women, which would imply worsened insulin resistance with the menopause. However, insulin sensitivity is known to worsen with advancing age and increasing central obesity, making it difficult to tease out the effect of menopause from these processes. Studies using accurate measures of insulin resistance, such as the euglycemichyperinsulinemic clamp or the frequently sampled glucose tolerance test, are scarce [68,69].

1.4.6 Inflammation

The CVD risk in postmenopausal women is 3.4 times of that in premenopausal women. These differences in risk are partially related to increases in total homocysteine (tHcy) and C-reactive

protein (CRP). Elevated tHcy is an independent, modifiable risk factor for atherosclerotic CVD. Elevated CRP, a marker of acute inflammation, is a reliable predictor of CVD. CVD patients from the Women's Health Study [70] who were in the highest quartile for tHcy concentrations (>13.26mol/L) were twice as likely to experience a future cardiovascular event as were those in the lowest quartile (<9.54 mol/L). Whereas premenopausal women typically have lower tHcy concentrations than do men of all ages, the women's values increase by 7–20% after menopause [71,72] and become comparable to the values in similarly aged men. Although tHcy may affect atherosclerotic CVD risk by several mechanisms, strong evidence exists that hyperhomocysteinemia suppresses the production of nitric oxide [73], an important vasodilator and antioxidant, and that suppression indirectly causes damage to the vasculature [74-76]. The mechanism by which tHcy is involved in the initiation and progression of atherosclerotic CVD should be studied further. Elevated CRP, a marker of acute inflammation, is a reliable predictor of CVD and concentrations >75th percentile (ie, >2.11 mg/dL) are associated with a 1.5-fold risk of myocardial infarction [77]. Studies have shown that hormone therapy results in a short-term rise in CRP [78,79]. CRP may stimulate the incorporation of monocytes into atherosclerotic lesions [80,81] and thus contribute to endothelial dysfunction [82,83].

1.5 Management

Management of menopause can be divided into five types such as: psychosocial, lifestyle modification, therapeutic management, nutritional management and education.

1.5.1 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 are 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.

1.5.2 Lifestyle Modification

Weight loss and physical exercise are both mainstays of therapy, as they address the underlying etiology of the metabolic syndrome (visceral obesity and insulin resistance). Even modest weight loss has been shown to improve visceral adiposity and insulin resistance. There is a preferential loss of abdominal fat with aerobic exercise, as visceral adipocytes appear to respond more quickly to exercise-induced weight loss than subcutaneous adipocytes [84]. Regular endurance exercise may improve insulin sensitivity independent of total weight loss. Therefore, the aim of lifestyle modification therapy is to promote regular prolonged low intensity exercise (i.e. walking) to maintain weight and reduce visceral adipose tissue, rather than to set unobtainable weight loss goals.

1.5.3 Therapeutic Management

1.5.3.1 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 bisphosphorates. Other agents are etidronate, paradronate and risedronate. Salmon calcitonin has the advantage of relieving the acute pain caused by subclinical vertebral fractures [85].

1.5.3.2 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 completely. About $1/3^{rd}$ 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 addition to relief from hot flashes, hormone therapy can alleviate vaginal dryness; improve sleep quality and joint pain. It is also extremely effective for preventing bone loss and osteoporotic fracture[86]. 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.[87]

Beginning in 1987 the National Institutes of Health sponsored PEPI, the Postmenopausal Estrogen/Progestin Interventions Trial, to try to find out the effect of estrogen on risk factors for heart disease. The investigators gave 875 postmenopausal women either estrogen alone, estrogen with synthetic progestin cyclically, estrogen with natural progesterone cyclically, estrogen with synthetic progestin continuously, or a placebo. They found that there was a beneficial effect on HDL and LDL levels, but an unfavorable effect on TG for all the forms of oral hormone replacement studied. The hormone therapy did not increase blood pressure or cause weight gain. The therapy also seemed to decrease levels of a substance known as fibrinogen which has a role in blood clotting. Increased fibrinogen levels can contribute to heart disease and stroke. The study also reinforced the importance of using progesterone in women with a uterus to reduce the risk of endometrial cancer. Lastly, it showed that HRT slowed menopausal bone loss and even increased bone mass.[88]

It was also found that diabetes women taking HRT has better glycemic control than never users of HRT [89]. 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 TG levels [90]. The NHANES III study suggested that diabetic and non-diabetic postmenopausal women taking HRT had better lipoprotein profile than never users of HRT [89].

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 does is gradually increased to achieve this effect. When the appropriate level is found, the same does 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 hemorrhage, endometrial hyperplasia and possible carcinoma [91].

Several recent studied have examined whether the addition of progestin to hormone therapy changes the risk of developing breast cancer. In the year 2000 scientists at the NIH'S National Cancer Institute (CNI) reported on a review of medical histories of more than 46,000 postmenopausal women between 1980 and 1995; 2,082 of whom had breast cancer. The NCI report showed that both estrogen and estrogen/progestin therapy led to a small increase in the risk of breast cancer, but the increase was greater with estrogen and progestin than with estrogen alone. Their analysis suggested that estrogen/progestin therapy over a 4-year period increased a woman's risk of developing breast cancer by about 30 percent-a result similar to that reported by the Collaborative Group for hormone use for 5 years or longer. Lean women also appeared to be a greater risk than heavier women. That same year a study at the University of Southern California reported similar results in a group of 3,534 women, 1,897 of whom had breast cancer. The long-term nationwide WHI study may provide further information about the possible role of postmenopausal estrogen and estrogen/progestin therapy in breast cancer.[88] The 2002 and 2003 announcements of the Women's Health Initiative of the American National Institute of Health and The Million Women Study of the UK Cancer Research and National Health Service collaboration respectively, that HRT treatment coincides with an increased incidence of breast cancer, heart attacks and strokes, lead to a sharp decline in HRT prescription throughout the world [92],[93],[94], which was followed by a decrease in breast cancer incidence[95,96]. However, in another study, it was found that estrogen use in American India postmenopausal women was related to deterioration of glucose tolerance. Longer duration of estrogen users compared to current users was related to an increased risk of type 2 diabetes [97]. Some relief of symptoms may be obtained in these women by lifestyle and dietary modifications and natural estrogen.

The pros and cons for hormone replacement therapy [88]

PRO	CON
HRT and ERT reduce the risk of osteoporosis. HRT and ERT relieve hot flashes and night sweats. HRT and ERT relieve vaginal dryness. HRT and ERT improve cholesterol levels. HRT and ERT may reduce the risk of heart disease. HRT and ERT may improve mood and psychological well-being. HRT and ERT may prevent the decline of mental abilities with age.	ERT, without the use of a progestin, modestly increases the low risk of cancer of the uterus (endometrial cancer). HRT and ERT can have unpleasant side effects, such as bloating or breast tenderness. HRT and ERT may increase risk of breast cancer while they are being used; long-term use may pose the greatest risk. When hormones are discontinued, risk gradually returns to almost normal. HRT may increase cardiovascular events such as heart attack and stroke at first, but this increased risk appears to grow smaller over time. HRT and ERT increase the risk for blood clots. HRT and ERT in pill form may raise TG levels and might contribute to gallbladder disease.

1.5.4 Nutritional Management

Vasomotor menopausal symptoms (VMSs), commonly referred to as night sweats and hot flushes, are extreme thermoregulatory responses resulting from an inability to keep body temperature within a specific optimal range—the so-called thermoneutral zone [98]. VMSs are viewed largely as symptoms affecting quality of life and are the leading reason women seek menopause-related health care. Currently, hormone therapy is the only effective treatment for VMSs, but whether it is safe with regard to breast cancer risk, for example, is in serious doubt [99]. Therefore, the identification of modifiable lifestyle factors that might prevent or alleviate VMS has become increasingly important. Until now, many modifiable lifestyle risk factors for VMSs have been identified, such as BMI [100], physical activity [101], smoking [100], and alcohol consumption [102]. Another lifestyle factor that has been suggested as a potential risk factor is diet [103]. Support for this comes from the several trials of soy supplementation that have reported statistically significant reductions in hot flushes [104]. Furthermore, evidence

indicates that high fiber [105], and low fat [106], intakes may be associated with a reduced risk of VMSs. A recent study found that women with VMSs who followed a diet low in fat and high in whole grains, fruit, and vegetables were more likely to reduce or eliminate VMSs after 1 year [107]. Until now, however, very little was known about the associations between overall dietary patterns and risk of VMSs. The dietary pattern approach is complementary to analyses using individual foods or nutrients because it can capture the potential for interactions or synergistic effects of combinations of foods [108]. Evidence for the effects of dietary patterns may also translate more effectively into targeted public health advice and dietary interventions because they relate to how people tend to perceive their diet rather than the consumption of individual food items.

1.5.4.1 Soy and Menopause:

A great deal of attention has been focused on soy for menopause relief. Women in Asian cultures have a very low incidence of hot flashes and other menopausal symptoms, such as night sweats. Dietary differences may be one possible reason for the low incidence of menopausal symptoms in these cultures. Asian women typically consume more vegetables, grains, and beans and less animal protein compared to women in Western cultures. Since soy is a major component of Asian diets, soy and menopause has become an active area of investigation.

Over 25 human studies have been conducted on soy and menopause to determine the effectiveness of soy for menopause relief. A recent analysis of 19 studies on soy and menopause revealed a significant relationship between the initial frequency of hot flashes and soy or soy isoflavone effectiveness [109]. The results of this analysis suggest that women with frequent hot flashes may receive the greatest benefit. Soy and menopause have recently reported that soy isoflavones, while not a replacement for HRT, are a viable option for women who do not wish to take hormone therapy.

1.5.4.2 Isoflavones

Isoflavones represent one of the classes of the so-called "phytoestrogens." Phytoestrogens are weakly estrogenic, diphenolic compounds found in plant foods. These heterocyclic phenols are structurally similar to mammalian estrogens, and they have weak estrogenic activity attributable to their affinity for estrogen receptors[110]. Phytoestrogens can be classified in three group, i.e.,

isoflavones, coumestans and lignans. The major isoflavones are genistein, daidzein, formononetin and biochanin A. Compared with 17ß-estradiol (arbitrarily assigned an affinity value of 100), however, their relative affinities are comparatively weak: 0.084 for genistein and 0.013 for daidzein [111].

After ingestion, soybean isoflavones are hydrolyzed by intestinal glucosidases, which releases the aglycones, daidzein, genistein and glycitein. These may be absorbed or further metabolized to many specific metabolites, including equol and p-ethylphenol [112,113]. The extent of this metabolism appears to be highly variable among individuals and is influenced by other components of the diet. A high carbohydrate milieu, which causes increased intestinal fermentation, results in more extensive biotransformation of phytoestrogens, with greatly increased formation of equol, a mammalian isoflavone metabolite. Like endogenous estrogens, isoflavones undergo an enterohepatic circulation; they are secreted in bile. This has been shown in rats [114,115,116], and pharmacokinetic studies in humans (unpublished) indicate that absorption takes place along the entire length of the intestine, presumably by nonionic passive diffusion [117].

It has been claimed that phytoestrogens, particularly soy isoflavones, are effective in the alleviation of estrogen deficiency -Related health problems and may be effective in enhancing memory and cognitive functions [118]. Soy isoflavones were reported to increase the percent of women who no longer suffered hot flashes and reduced the number of hot flashes in other women [119]. In another study on soy and menopause, it was reported that soy isoflavones reduced a number of menopausal symptoms including insomnia and mood [120].

Cardiovascular disease (CVD) remains the leading cause of mortality and morbidity in postmenopausal women [121]. Changes in lipids and lipoproteins at the time of menopause may contribute significantly to the increased risk for the development of CVD over the lifetime of women. Research has documented that serum lipids and lipoprotein are altered as a consequence of menopause, resulting in a more atherogenic lipid profile [122-128]. Evidence also suggests that this may include shifts in atherogenic lipoprotein subclasses [124,125]. Although the results have been mixed, some clinical trial have demonstrated a beneficial effect of dietary soy protein on plasma lipids and lipoproteins. A 1995 meta-analysis of 38 published controlled clinical trials showed a significant reduction in low-density lipoprotein (LDL) cholesterol of 12.9%,

significant lowering of TG by 10.5% and a nonsignificant increase in high-density lipoprotein (HDL) cholesterol of 2.4% [129]. In a more recent meta-analysis to quantify the effects of soy protein-containing isoflavones on the lipid profile, 23 randomized, controlled trials published from 1995 to 2002 were reviewed [130]. Soy protein with isoflavones was associated with significant decreases in serum total cholesterol (3.77%), LDL cholesterol (5.25%) and TG (7.27%), and significant increases in serum HDL cholesterol (3.03%) [131]. Another study reported data from an intervention study indicating that isoflavones may reduce baseline measures of several CVD risk factors, including systolic and diastolic blood pressures, total cholesterol, and non-HDL cholesterol [132].

One possible reason for this increased CAD risk after menopause may be a decrease in glucose tolerance and insulin sensitivity and an increase in plasma insulin levels, all of which may be the result of increased total body and abdominal adiposity that begins to occur at menopause [133–136]. Besides lipids, phytoestrogens could also affect insulin sensitivity, which, when reduced, is an independent risk factor for CVDs [137]. Insulin sensitivity is largely genetically determined [138], but, additionally, it is regulated by a number of steroid band peptide hormones [139]. A fall in insulin sensitivity is accompanied by estrogen deficiency after natural menopause [140].

Soy is a staple in the diet of the Japanese population, and consumption of soy has been shown to have an inverse relationship with mortality from CVD. These legumes contain complex carbohydrates, vegetable protein, soluble fibers, oligosaccharides, minerals, and phytoestrogens, particularly the isoflavones genistein and daidzein, that may be beneficial in the management of diabetes [141]. In vitro studies have shown isoflavones to have antidiabetic properties such as inhibiting intestinal brush border uptake of glucose, having α-glucosidase inhibitor actions, and also demonstrating tyrosine kinase inhibitory properties [142–144]. Diets containing soy protein rich in isoflavones have been shown to improve insulin resistance in ovariectomized cynomolgus monkeys [145] and to reduce insulin levels in healthy postmenopausal women [146,147]. Previous studies on the effects of using a soy-based diet in individuals with diabetes [148–151] have been performed on a heterogenous population using differing soy preparations, and there are few data focusing specifically on the effect of soy phytoestrogens on postmenopausal women with type 2 diabetes.

One of the most widely distributed phytoestrogens currently sold is a red clover botanical supplement [152]. Much of the positive data regarding the effects of phytoestrogens on menopausal symptoms, cardiac risk factors and cancer prevention are derived from observational studies. Prospective studies to date suggest that red clover based phytoestrogens may have similar effects to oestrogen therapy to improve metabolic risk factors such as vascular endothelial function in postmenopausal women with type 2 diabetes, arterial compliance in healthy postmenopausal women and possibly the lipid profile. Previous prospective studies on the effects of red clover based phytoestrogens on insulin resistance, however, have not had consistent results and are more difficult to interpret because insulin resistance was not the primary outcome. These limited studies on insulin resistance were also performed in different study populations, postmenopausal women with type 2 diabetes [153] or premenopausal women [154], therefore difficult to compare.

Elevated circulating homocysteine level is an independent risk factor for cardiovascular disease [155,156]. Total homocysteine is significantly higher in postmenopausal women than in premenopausal women [157]. It is already well documented that HT has positive effects on plasma tHcy concentrations in menopausal years [158]. Contrary to the HT results, some studies report no effect of isoflavone supplementation on plasma tHcy levels in menopausal women [159, 160] while one study reports a modest reduction [161] and another one reports a significant reduction of plasma tHcy in postmenopausal women [162].

The levels of C-reactive protein (CRP), as detected by highly sensitive assays, can be elevated in individuals at increased risk of myocardial infarction [163–165]. This has led to the theory that atherosclerotic diseases in coronary and other arteries can be a reflection of inflammation, which, in turn, through the release of cytokines, leads to increased synthesis of CRP in the liver [166]. However, it is possible that CRP itself can act as a promoter of atherosclerotic diseases [167–171]. No data exist on the effect of phytoestrogens on CRP, except for one study in which a 1-month course of phytoestrogen caused no change in CRP concentrations [172].

1.5.5 Education

The postmenopausal population in the United States will be increasing dramatically in future decades, with the US census estimating 50 million postmenopausal women by year 2020. The average age of menopause is 51 years, and life expectancy for women in the United States is 85 years; thus, women will live one third of their lives after menopause. Medical care of postmenopausal women will be critical to enable the 'baby boomer' generation to remain active physically, emotionally, and professionally while maintaining a high quality of life [173,174].

Many women arrive at their menopause transition years without knowing anything about what they might expect, or when or how the process might happen, and how long it might take. As a result, a woman who happens to undergo a strong perimenopause with a large number of different effects may become confused and anxious, fearing that something abnormal is happening to her. There is a strong need for more information and more education on this subject [175].

1.6 RATIONALE OF THE PRESENT STUDY:

Cardiovascular diseases (CVDs) are the leading cause of mortality and morbidity among the postmenopausal women in developed as well as developing countries. The incidence of CVD is lower in premenopausal women than in men; however, CVD risk in postmenopausal women is 3.4 times than that in premenopausal women. These differences in risk are partially related to increases in homocysteine (Hcy) and C-reactive protein (CRP). Soy milk is rich in phytoestrogen, it has been claimed that phytoestrogens, particularly soy isoflavones, have beneficial effects on cardiovascular diseases (CVDs) and diabetes; however, the underlying mechanism for these effects still need to be established. In the present study the effect of soy milk on homocysteine (Hcy, an important risk for CVDs), C-reactive protein (CRP, one of the marker of inflammatory status) and insulin resistance (a major defect in diabetes & CVDs) were explored in response to short-term intake of soy milk. Soy bean products are now marketed in Bangladesh and those can contribute to the health and well being of our population. The popularization of the products are, however, slow and a major reason for the lower rate of market growth is the lack/shortage of evidence on the health consequences of these products. Postmenopausal women create substantial family and social burden. In Bangladesh, so far no study has addressed the reduction of postmenopausal problems through diet based strategies. Under this context the present study has been under taken to investigate the effect of isoflavones on the underlying markers of inflammation in postmenopausal women.

1.7 HYPOTHESIS:

Isoflavones in Soy milk reduce serum homocysteine and C-reactive protein among postmenopausal women.

1.8 OBJECTIVES:

1.8.1 General Objective

The general objective of the study was to determine the effect of Soy milk Isoflavones on CVDs risk factors in postmenopausal women.

1.8.2 Specific Objectives

The specific objectives of the study were:

- To explore the effect of Soy milk Isoflavones on CVDs related clinical parameters of postmenopausal women; and
- To determine the effect of Soy milk Isoflavones on two important biochemical markers (Hcy and CRP) of subclinical inflammation and insulin resistance which play a vital role in the pathophysiology of CVDs.

SUBJECTS AND METHODS:

2.1 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 (BMRG), Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh.

2.2 Subjects

Thirty Six postmenopausal women

2.3 Inclusion criteria

- Aged ≥ 40 yrs
- Menopause within the last 1 year
- Women who are non-users of hormone therapy and have an intact uterus

2.4 Exclusion criteria

- Subjects with chronic illness advised for hospitalization.
- Subjects unable to answer (mental illness, severe neuro complications, disability etc)

2.5 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).

2.6 Study design

It was an un-blinded open ended trial where 36 women, under a randomized cross-over design, consumed 350 ml of the soy milk 2 times/day (calculated with a target 30 mg isoflavones daily) for 3 weeks. Soybeans, cultivated in Bangladesh, were collected directly from the producers, and milk was prepared by following a standardized manual procedure. The subjects were advised to follow the usual lifestyle during the period. The control group did not receive any other placebo product. At 10-12 hrs overnight fasting the blood samples were collected from the women at the day of '0'. They were then divided into two groups randomly. Group-A consumed the milk for 3 weeks and Group–B were on their usual diet. Their fasting blood samples were collected on 21st

days. After 10 days of wash out period the fasting blood samples were again collected on the 31st days. The groups were then crossed over and the subjects who consumed *Soy milk* in the first 21 days started their usual diet and the other subjects started to consume the *Soy milk* for the next 21 days. The final blood sampling was done on 52nd days. All blood samples were preserved at -20ºC for future analysis.

Figure 2A: Soy Bean

Figure 2B: Soy Milk

2.7 Study period: This study was carried during May-2012 to May-2013.

2.8 Determination of sample size: Sample size was determined by the following formula (Bland 2002):

$(\mu 1 - \mu 2)^2 = f(\alpha, \rho)\sigma^2 (1/n1 + 1/n2)$

Where,

n1 is the sample size of the Control group;

n2 is the sample size of the Soy milk group;

$(\mu 1 - \mu 2)$ is the difference of the mean values between Control and Soy milk group assumed to be clinically meaningful for the purpose of the study; σ is the standard deviation of the parameter;

$f(\alpha,\rho) = \{u\alpha + u2(1-p)\}^2$

With α as the significance level, p is the power of the study and u is the value of the Standard Normal Distribution.

Taking serum glucose as the main outcome of interest and putting

$\mu 1 - \mu 2 = 1$, $n1 = n2 = n$ (sample size equal for both groups), $\sigma = 1.0$ (from experience in the same population), and $f(\alpha, \rho)$ as 10.5 (table 18.1 from Bland with α value 0.05, $\rho = 0.90$)

$$12 = 10.5 \times 1^2 \times (1/n + 1/n)$$

$$n = (10.5 \times 1 \times 2)/1$$

$$= 21$$

2.9 History of the subjects

The history of the subjects was collected by an interviewer-administered questionnaire.

2.9.1 Questionnaire:

Basic core, expanded and optional variables with regards to socio-economic and demographic data (age, location, education, occupation and monthly income), family history of diseases, physical activity was incorporated into the questionnaire (Appendix-).

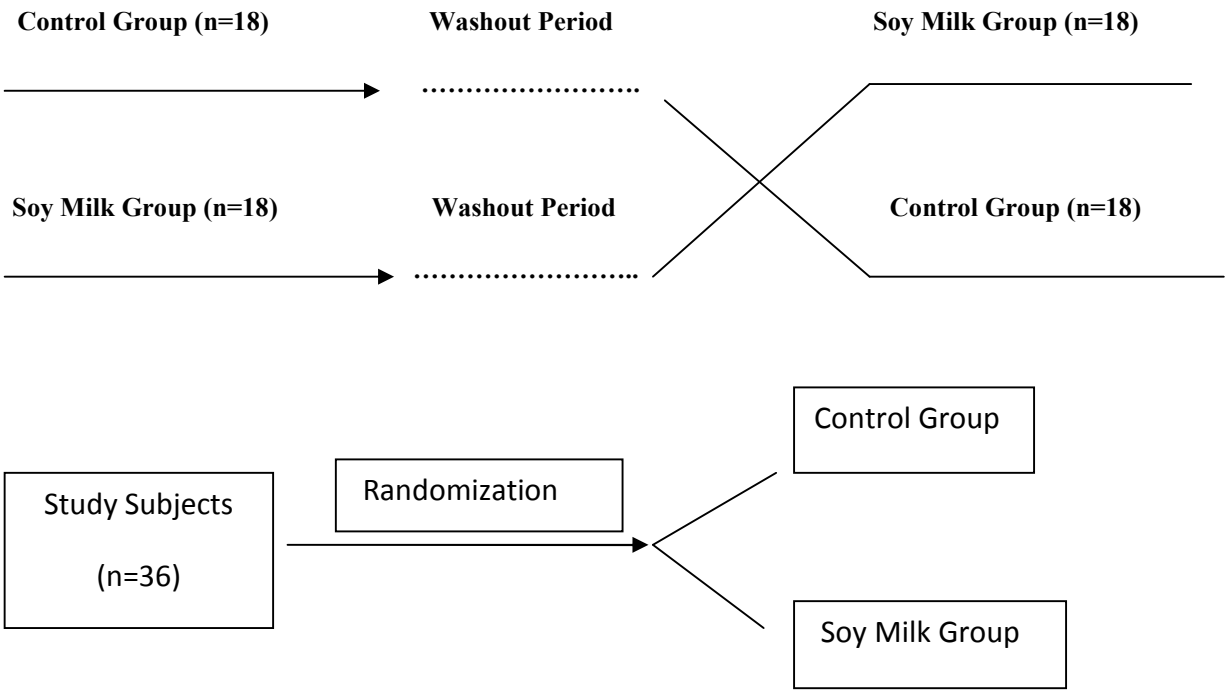


Figure 3: Flow chart of cross-over design

2.9.2 Anthropometric measurements

2.9.2.1 *Height (m)*

Standing height was measured using appropriate scales (Detect-Medic, Detect scales INC, USA) without shoes. The subject was positioned fully erect, with the head in the Frankfurt plane (with the line connecting the outer canthus of the eyes and the external auditory meatus perpendicular to the long axis of the trunk); the back of the head, thoracic spine, buttocks, and heels touched the vertical axis of the anthrop meter and the heels were together. Height was recorded to the nearest 5 mm.

2.9.2.2 *Weight (kg)*

The balance was placed on a hard flat surface and checked for zero balance before measurement. The subjects were in the center of the platform wearing light cloths without shoes. Weight was recorded to the nearest 0.5 Kg.

2.9.2.3 *Calculation of BMI (Kg/m^2)*

Body mass index (BMI) of the subjects was calculated using following formula:

BMI = Weight (kg)/ Height (m^2)

2.9.2.4 *Waist circumference (cm)*

Waist circumference was measured to the nearest 0.5 cm with a soft non-elastic measuring tape. The tape was snug, but not so tight as to cause skin indentation or pinching. The waist circumference was taken to the nearest standing horizontal circumference between the lower border of the 12th rib and the highest point of the iliac crest on the mid-axillary line at the end of normal expiration.

2.9.2.5 *Hip circumference (cm)*

Hip circumference was measured on the maximum circumference over the buttocks using soft non-elastic measuring tape and reading was taken to the nearest 0.5 cm. Participants were asked to breath normally, the reading were taken after gentle exhaling. The measuring tape was held firmly, ensuring its horizontal position. The tape was loose enough to allow the observer to place one figure between the tape and subject's body.

2.9.2.6 *Calculation of WHR*

Waist to hip ratio (WHR) of the study subjects was calculated as the ratio of waist circumference divided by hip circumference.

2.9.3 Measurement of blood pressure

Blood pressure was measured using Barometric Sphygmomanometer. Standard protocol was followed to record blood pressure data. Blood pressure was measured in sitting position, with calf at the level of the heart. After 10 minutes of rest a second reading was taken and average was recorded. Recorded Korotkoff sound I (the first sound) and V (the disappearance of sound) denoted the systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively (according to WHO-IHS).

2.10 Laboratory Methods

2.10.1 Sample collection and storage

After overnight fasting (10-12 hours) blood was collected between 8.00-9.00 am. Venous blood (~6 ml) was obtained by venipuncture following standard procedure. Subjects were then allowed to drink glucose (75 g in 300 ml of water). They were requested not to take any food and be rested for two hours. After 2 hours of glucose intake the second blood sample (3.00 ml venous blood) was taken. Fasting and postprandial blood samples were taken into plain tube (~6 cc), allowed to clot for 30 minutes and serum was separated by centrifugation for 10 min at 3000 rpm and then the serum was collected at least 600 µl in each four aliquot. Blood samples were maintained at 4^0c until separation and serum was frozen at -30^0c within an hour of sample collection. One aliquot was used for measuring OGTT, lipid profile, liver enzymes, creatinine, second aliquot for Insulin and the third aliquot for inflammatory marker (hs-CRP & tHcy) measurement respectively. The remaining aliquot was frozen at $–30^0$ C for further measurement. Serum was not allowed to be thawed until the assay is performed.

2.10.2 Biochemical Parameter:

- Serum glucose fasting was analyzed by glucose oxidase-method (Randox, UK).
- Serum lipid profile (Total cholesterol, TG, and HDL) was measured by enzymatic-colorimetric methods (Randox, UK).
- SGPT was measured by enzymatic-colorimetric method (Randox, UK).
- Serum insulin was determined by an ELISA method (DRG-International, Germany).
- Serum CRP was measured by an ELISA method (DRG-International, Germany).
- Insulin secretory function (HOMA%B) and insulin sensitivity (HOMA%S) was calculated by Homeostasis Model Assessment (HOMA) using HOMA Sigma software.
- tHcy was measure by Fluorescence Polarization Immunoassay.

2.11 Data analysis:

All analysis was done by appropriate statistical methods using SPSS 11.5 (SPSS, Inc. Chicago. IL. USA) Windows package. Pooled data for the two groups were used to calculate the baseline and final values which were then compared by paired t-test. Univariate or multivariate analysis was done where as appropriate.

2.12 Ethical Considerations:

Ethical clearance was taken from appropriate authority prior to the commencement of data collection. Informed written consent was obtained from the patients. Confidentially of the information collected from subjects were maintained with utmost care.

RESULTS:

3.1 Sociodemographic characteristics of the study subjects (Table-1)

The sociodemographic characteristics of the study subjects are given in Table 1. A total number of thirty Six (36) postmenopausal subjects were included in the study. Among them age [yrs, (Mean ± SD)] was 50±5.16. Majority were urban residents (94.4%). Among the subjects 72.2% were service holder and 27.8% were housewife. On the basis of education, the study subjects were divided into three groups- illiterate- primary (66.7%), secondary (13.9%) and graduate (19.4%).

Table-1: Sociodemographic characteristics of the study subjects (n=36)

Variable	Frequency	percent
Age in yr (M±SD**)**	50±5.16	
Education		
Illiterate-Primary	24	66.7
Secondary	5	13.9
Graduate & Above	7	19.4
Occupation		
Housewife	10	27.8
Service	26	72.2
Family Expenditure		
5,000-10,000	15	41.7
10,000-20,000	11	30.6
<20,000	10	27.8
Residence		
Urban	34	94.4
Rural	2	5.6
Religion		
Muslim	34	94.4
Hindu	2	5.6

Values are expressed as M±SD or numbers and percentages as appropriate.

3.2 Frequency distribution of the study subjects by BMI (n=36)

Figure 1 shows the distribution of the study according to different BMI categories (adapted from WHO guideline - 2004). Among the subjects 6% under wt (n=2), 39% normal (n=14), 44% over wt (n=16) & 11% obese (n=4).

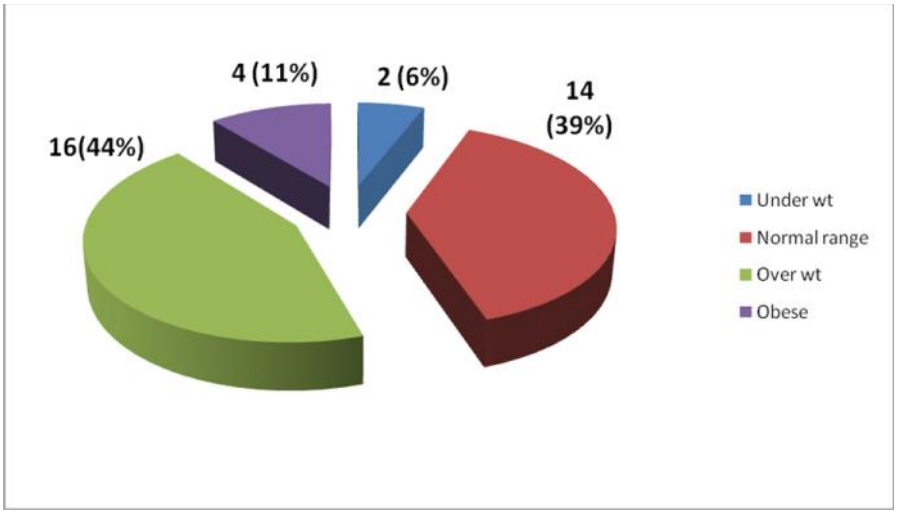


Figure 4: Pie Chart shows the percentage of BMI of the study subjects

3.3 Frequency distribution of the study subjects by W/H ratio (n=36)

Figure 2 shows the distribution of the study subjects according to different W/H ratio range (n=36). Most of the study subjects were obtain w/h ratio to 0.8-0.9 (50%; n=18), <0.8 (11%; n=4) and >0.9 (39%; n=14).

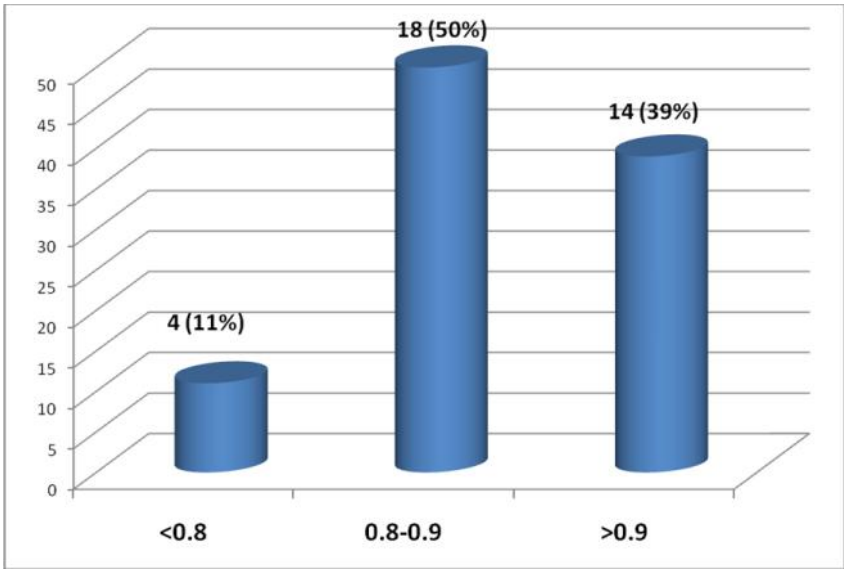


Figure 5: Distribution of the study subjects by W/H ratio

3.4 Frequency distribution of the study subjects by physical activity (n=36)

Figure 3 shows the frequency distribution of the study subjects according to different physical activity level per minutes. Among the subjects 44% (n=16) were low, 17% (n=6) moderate and 39% (n=14) were attain high physical activity.

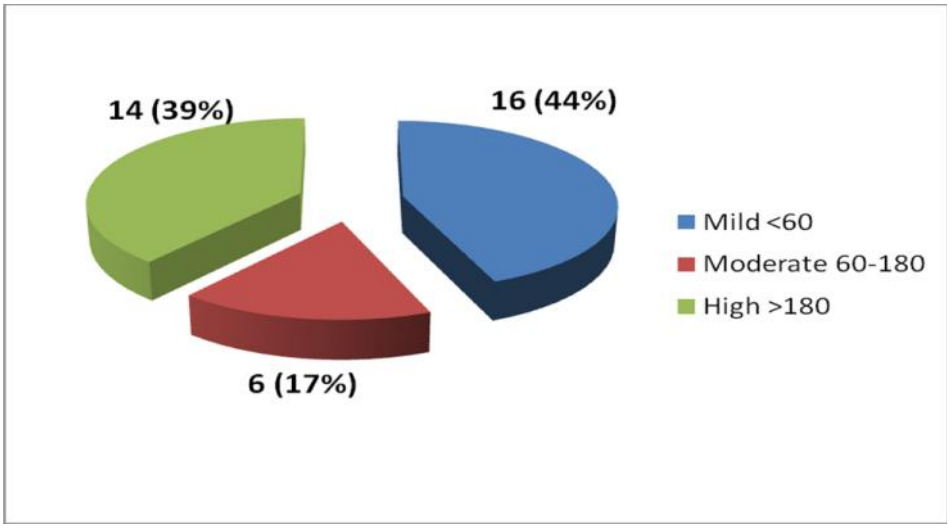


Figure 6: Frequency distribution of the study subjects by physical activity (n=36)

3.5 Clinical characteristics of the study subjects (n=36)

The clinical characteristics of the study subjects are given in Table 2. Among the postmenopausal women systolic blood pressure (Mean ± SD) was 114±11.61 and diastolic blood pressure (Mean ± SD) was 75±10.03.

Table-2: Clinical characteristics of the study subjects (n=36)

Variable	Mean±SD
SBP	114±11.61
DBP	75±10.03

Values are expressed as M±SD.

3.6 Biochemical characteristics of the study subjects (Table 3)

Biochemical characteristics of the study subjects (n=36) are given in Table 3. Variables were expressed as Mean±SD, of which fasting blood glucose (FBG) was 5.37±0.68; post prandial glucose (PPG) was 6.42±1.02; serum triglyceride (S TG) was 159.92±63.83; serum cholesterol (S Chol) was 215.03±44.55; serum high density of lipoprotein (S HDL) was 41.97±8.08; serum low density of lipoprotein (S LDL) was 140.56±36.97; serum non esterified fatty acid (S NEFA) was [median (range)] 0.43(0.32); c-reactive protein (CRP) was 3.30±2.43; homocysteine Hcy was 10.40±4.60; serum fasting insulin (S Insulin) was 17.08±9.41; homeostasis model assessment ß-cell function (HOMA%B) was 160±80.22; homeostasis model assessment insulin sensitivity (HOMA%S) was 48±20.95; serum IR (S IR) was 4.06±2.19; serum glutamate pyruvate transaminase (SGPT) was 21.83±9.20; serum creatinine (S Creat) was 0.94±0.16; serum urea (S Urea) was 22.89±7.86; serum uric acid (S Uric Acid) was 5.80±0.96 and serum total protein was 7.60±1.09.

Table-3: Biochemical characteristics (Baseline) of the study subjects (n=36)

Variable	Mean±SD
FBG (mmol/l)	5.37±0.68
AG (mmol/l)	6.42±1.02
S TG (mg/dl)	159.92±63.83
S Chol (mg/dl)	215.03±44.55
S HDL (mg/dl)	41.97±8.08
S LDL (mg/dl)	140.56±36.97
S NEFA (mmol/l)	0.43(0.32)
CRP (mg/l)	3.30±2.43
Hcy (µmol/L)	10.40±4.60
S Insulin (µIU/ml)	17.08±9.41
HOMA IR	4.06±2.19
HOMA%B	160±80.22
HOMA%S	48±20.95
SGPT (U/L)	21.83±9.20
S Creatinine (mg/dl)	0.94±0.16
S Urea (mg/dl)	22.89±7.86
S Uric Acid (mg/dl)	5.80±0.96
Total Protein (gm/l)	7.60±1.09

Results are expressed as mean ± SD, median (range)

3.7 Effect of soy milk on glycemic and lipidemic status of subjects during 0-21 days (Table 4)

Table 4 shows effects of soy milk on glycemic and lipidemic status of postmenopausal women subjects during 0 to 21 days time period. Fasting blood glucose levels (mmol/l, Mean ± SD) of the Control group was 5.28±0.65 (on `0´ day) and 5.39±0.69 (on `21´ day); soy milk group was 5.46±0.72 (on `0´ day) and 5.33±0.57 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, On `0´ day: Control group was 5.28±0.65 and soy milk group was 5.46±0.72; On `21´ day: Control group was 5.39±0.69 and soy milk group was 5.33±0.57. No significant difference was found there.

Serum triglyceride levels (mg/dl, Mean ± SD) of the Control group was 160.11±65.89 (on `0´ day) and 152.28±28.35 (on `21´ day); soy milk group was 159.72±63.62 (on `0´ day) and 133.72±23.36 (on `21´ day), during 0 to 21 days. Low significant difference was found in soy milk group between '0' to '21' days where p value was 0.088. But no significant difference was found between Control and soy milk group, On `0´ day: Control group was 160.11±65.89 and soy milk group was 159.72±63.62; On `21´ day: Control group was 152.28±28.35 and soy milk group was 133.72±23.36.

Serum cholesterol levels (mg/dl, Mean ± SD) of the Control group was 212.44±42.15 (on `0´ day) and 120.67±41.25 (on `21´ day); soy milk group was 217.61±47.91 (on `0´ day) and 120.17±50.89 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, On `0´ day: Control group was 212.44±42.15 and soy milk group was 217.61±47.91; On `21´ day: Control group was 210.67±41.25 and soy milk group was 220.17±50.89. No significant difference was found there.

Serum HDL levels (mg/dl, Mean ± SD) of the Control group was 44.94±7.90 (on `0´ day) and 46.00±6.24 (on `21´ day); soy milk group was 39.00±7.30 (on `0´ day) and 45.22±6.20 (on `21´ day), during 0 to 21 days. Significant difference was found in soy milk group. P vable of the soy milk group was 0.005. But between Control and soy milk group, On `0´ day: Control group was 44.94±7.90 and soy milk group was 39.00±7.30; On `21´ day: Control group was 46.00±6.24 and soy milk group was 45.22±6.20. No significant difference was found there.

Serum LDL levels (mg/dl, Mean ± SD) of the Control group was 135.56±33.76 (on `0´ day) and 131.28±32.76 (on `21´ day); soy milk group was 145.56±40.26 (on `0´ day) and 151.11±44.46 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, On `0´ day: Control group was 135.56±33.76 and soy milk group was 145.56±40.26; On `21´ day: Control group was 131.28±32.76 and soy milk group was 151.11±44.46. No significant difference was found there.

Serum NEFA levels (mmol/l, Mean ± SD) of the Control group was 0.48±0.64 (on `0´ day) and 0.32±0.18 (on `21´ day); soy milk group was 0.37±0.22 (on `0´ day) and 0.19±0.17 (on `21´ day), during 0 to 21 days. Significant difference was found in soy milk group. P value of the soy milk group was 0.010. But no significant difference was found between Control and soy milk group on `0´ day and `21´ day. On `0´ day, Control group was 0.48±0.64 and soy milk group was 0.37±0.22. On `21´ day, Control group was 0.32±0.18 and soy milk group was 0.19±0.17.

Table-4: Effect of Soy milk on glycemic and lipidemic status of subjects during 0-21 days (n= 18 in each group)

Parameters	0 day	21 days	t/P value
FBG (mmol/l)			
Control Group	5.28±0.65	5.39±0.69	-0.743/0.467
Soy Milk Group	5.46±0.72	5.33±0.57	0.924/0.369
t/p value	-0.78/0.442	0.29/0.774	
S TG (mg/dl)			
Control Group	160.11±65.89	152.28±28.35	0.53/0.602
Soy Milk Group	159.72±63.62	133.72±23.36	1.81/0.088
t/p value	-0.02/0.986	2.143/0.039	
S Chol (mg/dl)			
Control Group	212.44±42.15	210.67±41.25	0.29/0.777
Soy Milk Group	217.61±47.91	220.17±50.89	-0.25/0.803
t/p value	0.344/0.733	-0.62/0.542	
S HDL (mg/dl)			
Control Group	44.94±7.90	46.00±6.24	-0.75/0.461
Soy Milk Group	39.00±7.30	45.22±6.20	-3.25/0.005
t/p value	-2.35/0.025	0.48/0.633	
S LDL (mg/dl)			
Control Group	135.56±33.76	131.28±32.76	0.60/0.554
Soy Milk Group	145.56±40.26	151.11±44.46	-0.83/0.419
t/p value	0.81/0.425	-1.52/0.137	
S NEFA (mmol/l)			
Control Group	0.48±0.64	0.32±0.18	1.04/0.312
Soy Milk Group	0.37±0.22	0.19±0.17	2.89/0.010
t/p value	-0.60/0.514	2.187/0.036	

Comparison between 0 day 21 day values of the same individual were compared by Paired- t test (n=36).On the other hand comparison between Control and soy milk Groups on 0 day and 21 day were done by unpaired t-test (n=18). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant, n= number of subjects.

3.8 Effect of soy milk on glycemic and lipidemic status of postmenopausal women subjects during 31-52 days (Table 5)

Table 5 shows effects of soy milk on glycemic and lipidemic status of the subjects during 31 to 52 days time period. Fasting blood glucose levels (mmol/l, Mean ± SD) of the Control group was 5.39±0.51 (on `31´ day) and 5.23±0.49 (on `52´ day); soy milk group was 5.56±0.43 (on `31´ day) and 5.18±0.49 (on `52´ day), during 31 to 52 days. Serum FBG of soy milk group decreased significantly (p=0.005) during 31 to 52 day. But between Control and soy milk group, On `31´ day: Control group was 5.39±0.51 and soy milk group was 5.56±0.43; On `52´ day: Control group was 5.23±0.49 and soy milk group was 5.18±0.49. No significant difference was found there.

Serum triglyceride levels (mg/dl, Mean ± SD) of the Control group was 149.67±45.23 (on `31´ day) and 122.22±39.40 (on `52´ day); soy milk group was 180.61±98.93 (on `31´ day) and 138.33±53.03 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, On `31´ day: Control group was 149.67±45.23 and soy milk group was 180.61±98.93; On `52´ day: Control group was 122.22±39.40 and soy milk group was 138.33±53.03. No significant difference was found there.

Serum cholesterol levels (mg/dl, Mean ± SD) of the Control group was 207.56±40.62 (on `31´ day) and 215.17±39.01 (on `52´ day); soy milk group was 212.22±34.15 (on `31´ day) and 208.11±43.91 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, On `31´ day: Control group was 207.56±40.62 and soy milk group was 212.22±34.15; On `52´ day: Control group was 215.17±39.01 and soy milk group was 208.11±43.91. No significant difference was found there.

Serum HDL levels (mg/dl, Mean ± SD) of the Control group was 42.78±5.81 (on `31´ day) and 41.06±6.51 (on `52´ day); soy milk group was 39.50±3.18 (on `31´ day) and 43.29±12.54 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, On `31´ day: Control group was 42.78±5.81 and soy milk group was 39.50±3.18; On `51´ day: Control group was 41.06±6.51 and soy milk group was 43.29±12.54. No significant difference was found there.

Serum LDL levels (mg/dl, Mean ± SD) of the Control group was 134.72±39.57 (on `31´ day) and 143.17±33.21 (on `52´ day); soy milk group was 131.22±35.31 (on `31´ day) and 134.44±33.45 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, On `31´ day: Control group was 134.72±39.57 and soy milk group was 131.22±35.31; On `52´ day: Control group was 143.17±33.21 and soy milk group was 134.44±33.45. No significant difference was found there.

Serum NEFA levels (mmol/l, Mean ± SD) of the Control group was 0.39±0.23 (on `31´ day) and 0.29±0.19 (on `52´ day); soy milk group was 0.30±0.19 (on `31´ day) and 0.26±0.17 (on `52´ day), during 31 to 52 days. Serum NEFA of control group decreased significantly (p= 0.060) during 31 to 52 day. On `31´ day: Control group was 0.39±0.23 and soy milk group was 0.30±0.19. On 52´ day: Control group was 0.29±0.19 and soy milk group was 0.26±0.17. No significant difference was found there.

Table-5: Effect of Soy milk on glycemic and lipidemic status of subjects during 31-52 days (n= 18 in each group)

Parameters	31 days	52 days	t/P value
FBG (mmol/l)			
Control Group	5.39±0.51	5.23±0.49	0.99/0.338
Soy milk Group	5.56±0.43	5.18±0.49	3.21/0.005
t/p value	-1.06/0.297	0.31/0.761	
S TG (mg/dl)			
Control Group	149.67±45.23	122.22±39.40	2.87/0.011
Soy milk Group	180.61±98.93	138.33±53.03	2.12/0.049
t/p value	-1.21/0.236	-1.03/0.308	
S Chol (mg/dl)			
Control Group	207.56±40.62	215.17±39.01	-1.28/0.219
Soy milk Group	212.22±34.15	208.11±43.91	0.68/0.507
t/p value	-0.37/0.711	0.51/0.614	
S HDL (mg/dl)			
Control Group	42.78±5.81	41.06±6.51	0.99/0.333
Soy milk Group	39.50±3.18	43.29±12.54	-1.35/0.195
t/p value	2.10/0.043	-0.67/0.506	
S LDL (mg/dl)			
Control Group	134.72±39.57	143.17±33.21	-1.73/0.102
Soy milk Group	131.22±35.31	134.44±33.45	-0.43/0.672
t/p value	0.28/0.781	0.79/0.438	
S NEFA (mmol/l)			
Control Group	0.39±0.23	0.29±0.19	2.02/0.060
Soy Milk Group	0.30±0.19	0.26±0.17	0.78/0.448
t/p value	1.28/0.208	0.57/0.570	

Comparison between 31 day 52 day values of the same individual were compared by Paired- t test (n=36).On the other hand comparison between Control and soy milk Groups on 0 day and 21 day were done by unpaired t-test (n=18). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant, n= number of subjects.

3.9 Effect of Soy milk on insulinemic status of subjects during 0-21 days (Table 6)

Table 6 shows effect of soy milk on insulinemic status of postmenopausal women subjects during 0 to 21 days time period. Serum Insulin (Mean ± SD) of the Control group was 16.26±6.60 (on `0´ day) and 16.36±7.41 (on `21´ day); soy milk group was 17.89±11.72 (on `0´ day) and 22.06±21.05 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, on `0´ day: Control group was 16.26±6.60 and soy milk group was 17.89±11.72; On `21´ day: Control group was 16.36±7.41 and soy milk group was 22.06±21.05. No significant difference was found there.

HOMA IR (Mean ± SD) of the Control group was 3.79±1.55 (on `0´ day) and 3.85±1.63 (on `21´ day); soy milk group was 4.33±2.70 (on `0´ day) and 5.22±4.93 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, on `0´ day: Control group was 3.79±1.55 and soy milk group was 4.33±2.70; On `21´ day: Control group was 3.85±1.63 and soy milk group was 5.22±4.93. No significant difference was found there.

HOMA%B levels (percent, Mean ± SD) of the Control group was 161±71.44 (on `0´ day) and 145±58.87 (on `21´ day); soy milk group was 159±90.24 (on `0´ day) and 176±123.47 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, on `0´ day: Control group was 161±71.44 and soy milk group was 159±90.24; On `21´ day: Control group was 145±58.87 and soy milk group was 176±123.47. No significant difference was found there.

HOMA%S levels (percent, Mean ± SD) of the Control group was 48±20.31 (on `0´ day) and 51±35.93 (on `21´ day); soy milk group was 48±22.16 (on `0´ day) and 50±28.12 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, On `0´ day: Control group was 48±20.31 soy milk group was 48±22.16; On `21´ day: Control group was 51±35.93 and soy milk group was 50±28.12. No significant difference was found.

Table-6: Effect of Soy milk on insulinemic status of subjects during 0-21 days (n= 18 in each group)

Parameters	0 day	21 days	t/P value
S Insulin (µIU/ml)			
Control Group	16.26±6.60	16.36±7.41	-0.046/0.964
Soy Milk Group	17.89±11.72	22.06±21.05	-1.27/0.222
t/p value	-0.51/0.611	-1.084/0.286	
HOMA IR			
Control Group	3.79±1.55	3.85±1.63	-0.159/0.876
Soy Milk Group	4.33±2.70	5.22±4.93	-1.130/0.274
t/p value	-0.737/0.466	-1.120/0.271	
HOMA%B			
Control Group	161±71.44	145±58.87	0.88/0.392
Soy Milk Group	159±90.24	176±123.47	-1.02/0.324
t/p value	0.08/0.933	-0.97/0.34	
HOMA%S			
Control Group	48±20.31	51±35.93	-0.46/0.650
Soy Milk Group	48±22.16	50±28.12	-0.42/0.679
t/p value	-0.001/0.999	0.13/0.90	

Comparison between 0 day 21 day values of the same individual were compared by Paired- t test (n=36).On the other hand comparison between Control and soy milk Groups on 0 day and 21 day were done by unpaired t-test (n=18). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant, n= number of subjects.

3.10 Effect of Soy milk on insulinemic status of subjects during 31-52 days (Table-7)

Table 7 shows effect of soy milk on insulinemic status of postmenopausal women subjects during 31 to 52 days time period. Serum Insulin (Mean ± SD) of the Control group was 24.16±17.94 (on `31´ day) and 19.77±18.98 (on `52´ day); soy milk group was 17.27±5.96 (on `31´ day) and 18.49±10.22 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, on `31´ day: Control group was 24.16±17.94 and soy milk group was 17.27±5.96; On `52´ day: Control group was 19.77±18.98 and soy milk group was 18.49±10.22. No significant difference was found there.

HOMA IR (Mean ± SD) of the Control group was 5.88±4.42 (on `31´ day) and 4.78±5.28 (on `52´ day); soy milk group was 4.25±1.47 (on `31´ day) and 4.34±2.62 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, on `31´ day: Control group was 5.88±4.42 and

soy milk group was 4.25±1.47; On `52´ day: Control group was 4.78±5.28 and soy milk group was 4.34±2.62. No significant difference was found there.

HOMA%B levels (percent, Mean ± SD) of the Control group was 171±98.22 (on `31´ day) and 155±80.04 (on `52´ day); soy milk group was 146±50.84 (on `31´ day) and 161±60.17 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, On `31´ day: Control group was 171±98.22 and soy milk group was 146±50.84; On `52´ day: Control group was 155±80.04 and soy milk group was 161±60.17. No significant difference was found.

HOMA%S levels (percent, Mean ± SD) of the Control group was 41±22.70 (on `31´ day) and 47±19.99 (on `52´ day); soy milk group was 42±15.40 (on `31´ day) and 45±20.04 (on `52´ day), during 31 to 52 days. Between Control and soy milk group, On `31´ day: Control group was 41±22.70 and soy milk group was 42±15.40; On `52´day, Control group was 47±19.99 and soy milk group was 45±20.04. No significant difference was found.

Table-7: Effect of Soy milk on insulinemic status of subjects during 31-52 days (n= 18 in each group)

Parameters	31 days	52 days	t/P value
S Insulin (µIU/ml)			
Control Group	24.16±17.94	19.77±18.98	1.37/0.188
Soy Milk Group	17.27±5.96	18.49±10.22	-0.58/0.571
t/p value	1.55/0.131	0.253/0.802	
HOMA IR			
Control Group	5.88±4.42	4.78±5.28	1.02/0.324
Soy Milk Group	4.25±1.47	4.34±2.62	-0.13/0.899
t/p value	1.49/0.147	0.32/0.752	
HOMA%B			
Control Group	171±98.22	155±80.04	0.82/0.422
Soy Milk Group	146±50.84	161±60.17	-0.94/0.361
t/p value	0.93/0.360	-0.28/0.783	
HOMA%S			
Control Group	41±22.70	47±19.99	-1.09/0.290
Soy Milk Group	42±15.40	45±20.04	-0.56/0.581
t/p value	-0.21/0.838	0.36/0.721	

Comparison between 31 day 52 day values of the same individual were compared by Paired- t test (n=36).On the other hand comparison between Control and soy milk Groups on 0 day and 21 day were done by unpaired t-test (n=18). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant, n= number of subjects.

3.11 Effect of Soy milk on C-reactive protein, homocysteine of subjects during 0-21 days (Table-8)

Table 8 shows effects of soy milk on h C-reactive protein and homocysteine of postmenopausal women subjects during 0 to 21 days time period. Serum CRP levels (mg/L, Mean ± SD) of Control group was 3.28±2.03 (on `0´ day) and 3.12±2.43 (on `21´ day); soy milk group was 3.32±2.84 (on `0´ day) and 2.94±0.20 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, On `0´ day: Control group was 3.28±2.03 and soy milk group was 3.32±2.84; On `21´ day: Control group was 3.12±2.43 and soy milk group was 2.94±0.20. No significant difference was found between Control and soy milk group.

Serum tHcy (mg/dl, Mean ± SD) of Control group was 11.81±5.93 (on `0´ day) and 11.67±7.19 (on `21´ day); soy milk group was 8.98±2.05 (on `0´ day) and 8.93±1.86 (on `21´ day), during 0 to 21 days. Between Control and soy milk group, On `0´ day: Control group was 11.81±5.93 and soy milk group was 8.98±2.05; On `21´ day: Control group was 11.67±7.19 and soy milk group was 8.93±1.86. No significant difference was found between Control and soy milk group.

Table-8: Effect of Soy milk on C-reactive protein, homocysteine of subjects during 0-21 days; (n= 18 in each group)

Parameters	0 day	21 days	t/P value
CRP (mg/l)			
Control Group	3.28±2.03	3.12±2.43	0.43/0.672
Soy Milk Group	3.32±2.84	2.94±0.20	1.15/0.264
t/p value	0.05/0.959	0.23/0.817	
Hcy (mg/dl)			
Control Group	11.81±5.93	11.67±7.19	0.27/0.791
Soy Milk Group	8.98±2.05	8.93±1.86	0.14/0.890
t/p value	1.91/0.065	1.57/0.127	

Comparison between 0 day 21 day values of the same individual were compared by Paired- t test (n=36).On the other hand comparison between Control and soy milk Groups on 0 day and 21 day were done by unpaired t-test (n=18). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant, n= number of subjects.

3.12 Effect of Soy milk on C-reactive protein, homocysteine of subjects during 31-52 days (Table-9)

Table 9 shows effects of soy milk on C-reactive protein and homocysteine of the postmenopausal women subjects, during 31 to 52 days time period. Serum CRP levels (mg/L, Mean ± SD) of Control group was 3.23±2.35 (on `31´ day) and 2.69±2.31 (on `52´ day); soy milk group was 3.05±2.43 (on `31´ day) and 3.58±2.25 (on `52´ day), during 31 to 52 day. Between Control and soy milk group, On `31´ day: Control group was 3.23±2.35 and soy milk group was 3.05±2.43; On `52´ day: Control group was 2.69±2.31and soy milk group was 3.58±2.25. No significant difference was found.

Serum Hcy (mg/dl, Mean ± SD) of Control group was 8.32±1.89 (on `31´ day) and 8.37±1.92 (on `52´ day); soy milk group was 10.96±5.33 (on `31´ day) and 10.94±5.68 (on `52´ day), during 31 to 52 day. Between Control and soy milk group, On `31´ day: Control group was 8.32±1.89 and soy milk group was 10.96±5.33; On `52´ day: Control group was 8.37±1.92 and soy milk group was 10.94±5.68. No significant difference was found.

Table-9: Effect of Soy milk on C-reactive protein, homocysteine of subjects during 31-52 days; (n= 18 in each group)

Parameters	31 days	52 days	t/P value
CRP (mg/l)			
Control Group	3.23±2.35	2.69±2.31	1.11/0.283
Soy Milk Group	3.05±2.43	3.58±2.25	-1.19/0.250
t/p value	0.23/0.820	-1.18/0.247	
Hcy (mg/dl)			
Control Group	8.32±1.89	8.37±1.92	-0.15/0.882
Soy Milk Group	10.96±5.33	10.94±5.68	0.05/0.963
t/p value	-1.98/0.056	-1.82/0.078	

Comparison between 31 day 52 day values of the same individual were compared by Paired- t test (n=36).On the other hand comparison between Control and soy milk Groups on 0 day and 21 day were done by unpaired t-test (n=18). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant, n= number of subjects.

3.13 Effect of soy milk on glycemic and lipidemic status of study subjects (Base line Vs End point) (Table 10)

Table 10 shows effect of soy milk on glycemic and lipidemic status of the subjects (Base point Vs End point). Fasting blood glucose levels (mmol/l, Mean ± SD) of Control group: at base line was 5.34±0.58 and at end point was 5.31±0.59; soy milk group: At base line was 5.51±0.59 and at end point was 5.26±0.53. Serum FBG of soy milk group decreased significantly (p=0.009) during base line to end point. But no significant difference was found between Control and soy milk group, At base line: Control group was 5.34±0.58 and soy milk group was 5.51±0.59; At end point: Control group was 5.31±0.59 and soy milk group was 5.26±0.53.

Serum triglyceride levels (mg/dl, Mean ± SD) of Control group, at base line was 154.89±55.95 and at end point was 137.25±37.11; soy milk group: At base line was 170.17±82.66 and at end point was 136.03±40.45. Serum triglyceride of soy milk group decreased significantly (p=0.008) during base line to end point. But no significant difference was found Between Control and soy milk group, At base line: Control group was 154.89±55.95 and soy milk group was 170.17±82.66; At end point: Control group was 137.25±37.11 and soy milk group was 136.03±40.45.

Serum cholesterol levels (mg/dl, Mean ± SD) of Control group, at base line was 210.00±40.87 and at end point was 212.92±39.63; soy milk group: At base line was 214.92±41.10 and at end point was 214.14±47.24. Between Control and soy milk group, At base line: Control group was 210.00±40.87 and soy milk group was 214.92±41.10; At end point: Control group was 212.92±39.63 and soy milk group was 214.14±47.24. No significant difference was found.

Serum HDL levels (mg/dl, Mean ± SD) of Control group, at base line was 43.86±6.92 and at end point was 43.53±6.77; soy milk group: At base line was 39.25±5.56 and at end point was 44.15±9.79. Serum HDL of soy milk group increased significantly (p=0.006) during base line to end point. Between control and soy milk group, At base line: Control group was 43.86±6.92 and soy milk group was 39.25±5.56; At end point: Control group was 43.53±6.77 and soy milk group was 44.15±9.79. No significant difference was found. Serum HDL of soy milk group decreased significantly at base line (p=0.003).

Serum LDL levels (mg/dl, Mean ± SD) of Control group, at base line was 135.14±36.26 and at end point was 137.22±33.06; soy milk group: At base line was 138.39±38.02 and at end point was 142.78±39.68. Between Control and soy milk group, At base line: Control group was 135.14±36.26 and soy milk group was 138.39±38.02; At end point: Control group was 137.22±33.06 and soy milk group was 142.78±39.68. No significant difference was found.

Serum NEFA levels (mmol/l, Mean ± SD) of Control group, at base line was 0.43±0.48 and at end point was 0.30±0.19; soy milk group: At base line was 0.33±0.21 and at end point was 0.22±0.17. Serum NEFA level of soy milk group decreased significantly where P value was 0.013 respectively from base line to end point. Between Control and soy milk group, At base line: Control group was 0.43±0.48 and soy milk group was 0.33±0.21; At end point: Control group was 0.30±0.19 and soy milk group was 0.22±0.17.

Table-10: Effect of Soy milk on glycemic and lipidemic status of subjects (Base line Vs End point) (n= 36 in each group)

Parameters	Base line	End point	t/P value
FBG (mmol/l)			
Control Group	5.34±0.58	5.31±0.59	0.22/0.824
Soy Milk Group	5.51±0.59	5.26±0.53	2.75/0.009
t/p value	-1.25/0.214	0.42/0.676	
S TG (mg/dl)			
Control Group	154.89±55.95	137.25±37.11	2.00/0.053
Soy Milk Group	170.17±82.66	136.03±40.45	2.80/0.008
t/p value	-0.92/0.362	0.13/0.894	
S Chol (mg/dl)			
Control Group	210.00±40.87	212.92±39.63	-0.68/0.503
Soy Milk Group	214.92±41.10	214.14±47.24	0.13/0.895
t/p value	-0.51/0.612	-0.12/0.906	
S HDL (mg/dl)			
Control Group	43.86±6.92	43.53±6.77	0.30/0.768
Soy Milk Group	39.25±5.56	44.15±9.79	-293/0.006
t/p value	3.12/0.003	-0.31/0.756	
S LDL (mg/dl)			
Control Group	135.14±36.26	137.22±33.06	-0.48/0.637
Soy Milk Group	138.39±38.02	142.78±39.68	-0.89/0.382
t/p value	-0.37/0.712	-0.65/0.521	
S NEFA (mmol/l)			
Control Group	0.43±0.48	0.30±0.19	1.61/0.116
Soy Milk Group	0.33±0.21	0.22±0.17	2.60/0.013
t/p value	1.15/0.253	1.94/0.056	

Comparison between 0 day 21 day values of the same individual were compared by Paired- t test (n=72).On the other hand comparison between Control and Soy Milk Groups on 0 day and 21 day were done by unpaired t-test (n=30). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant.

3.14 Effect of Soy milk on insulinemic status of subjects (Base line Vs End point) (Table-11)

Table 11 shows effect of soy milk on insulinemic status of the postmenopausal women (Base point Vs End point). Fasting serum Insulin levels (Mean ± SD) of Control group, at base line was 20.21±13.91 and at end point was 18.06±14.30; soy milk group: At base line 17.58±9.17 and at end point was 20.27±16.41. Between Control and soy milk group, At base line: Control group was 20.21±13.91 and soy milk group was 17.58±9.17; At end point: Control group was 18.06±14.30 and soy milk group was 20.27±16.41. No significant difference was found.

HOMA IR levels (Mean ± SD) of Control group, at base line was 4.83±3.43 and at end point was 4.32±3.88; soy milk group: at base line 4.29±2.15 and at end point was 4.78±3.91. Between Control and soy milk group, At base line: Control group was 4.83±3.43 and soy milk group was 4.29±2.15; At end point: Control group was 4.32±3.88and soy milk group was 4.78±3.91. No significant difference was found.

HOMA%B levels (percent, Mean ± SD) of Control group, at base line was 166±84.77 and at end point was 150±69.42; soy milk group: At base line 153±72.47 and at end point was 169±96.01. Between Control and soy milk group, At base line: Control group was 166±84.77 and soy milk group was 153±72.47; At end point: Control group was 150±69.42 and soy milk group was 169±96.01. No significant difference was found.

HOMA%S levels (percent, Mean ± SD) of Control group, at base line was 44±21.50 and at end point was 49±28.73; soy milk group: At base line was 45±19.01 and at end point was 47±24.20. Between Control and soy milk group, At base line: Control group was 44±21.50 and soy milk group was 45±19.01; At end point: Control group was 49±28.73 and soy milk group was 47±24.20. No significant difference was found.

Table-11: Effect of Soy milk on insulinemic status of subjects (Base line Vs End point) (n= 36 in each group)

Parameters	Base line	End point	t/P value
S Insulin (µIU/ml)			
Control Group	20.21±13.91	18.06±14.30	0.909/0.369
Soy Milk Group	17.58±9.17	20.27±16.41	-1.29/0.206
t/p value	0.948/0.346	-0.609/0.544	
HOMA IR			
Control Group	4.83±3.43	4.32±3.88	0.89/0.380
Soy Milk Group	4.29±2.15	4.78±3.91	0.96/0.345
t/p value	0.806/0.423	-0.505/0.615	
HOMA%B			
Control Group	166±84.77	150±69.42	1.22/0.231
Soy Milk Group	153±72.47	169±96.01	-1.40/0.170
t/p value	0.71/0.478	-0.96/0.342	
HOMA%S			
Control Group	44±21.50	49±28.73	-1.02/0.312
Soy Milk Group	45±19.01	47±24.20	-0.69/0.492
t/p value	-0.14/0.889	0.30/0.762	

Comparison between 0 day 21 day values of the same individual were compared by Paired- t test (n=72).On the other hand comparison between Control and Soy Milk Groups on 0 day and 21 day were done by unpaired t-test (n=36). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant.

3.15 Effect of Soy milk on C-reactive protein, homocysteine of subjects (Base line Vs End point) (Table-12)

Table 12 shows Effect of soy milk on C-reactive protein and homocysteine of study subjects (Base line Vs End point). Serum CRP levels (mg/l, Mean ± SD) of Control group: at base line was 3.25±2.16 and at end point was 2.91±2.34; soy milk group: At base line was 3.18±2.61 and at end point was 3.26±2.22. Between Control and soy milk group, At base line: Control group was 3.25±2.16 and soy milk group was 3.18±2.61; At end point: Control group was 2.91±2.34 and soy milk group was 3.26±2.22. No significant difference was found.

Serum Hcy levels (mg/l, Mean ± SD) of Control group: at base line was 10.07±4.68 and at end point was 10.02±5.45; soy milk group: At base line was 9.97±4.10 and at end point was 9.93±4.29. Between Control and soy milk group, At base line: Control group was 10.07±4.68 and soy milk group was 9.97±4.10; At end point: Control group was 10.02±5.45 and soy milk group was 9.93±4.29. No significant difference was found.

Table-12: Effect of Soy milk on C-reactive protein, homocysteine of subjects (Base line Vs End point); (n= 36 in each group)

Parameters	Base line	End point	P value
CRP (mg/l)			
Control Group	3.25±2.16	2.91±2.34	1.15/0.256
Soy Milk Group	3.18±2.61	3.26±2.22	-0.28/0.784
t/p value	0.12/0.902	-0.67/0.508	
Hcy (mg/dl)			
Control Group	10.07±4.68	10.02±5.45	0.15/0.878
Soy Milk Group	9.97±4.10	9.93±4.29	0.13/0.898
t/p value	0.09/0.927	0.07/0.940	

Comparison between 0 day 21 day values of the same individual were compared by Paired- t test (n=72).On the other hand comparison between Control and Soy Milk Groups on 0 day and 21 day were done by unpaired t-test (n=36). Values were expressed as Mean± SD. P<0.05 was considered as statistically significant.

3.16 Pearson's correlation analysis of CRP & Hcy with some significant variables at two different time points (n= 36 in each group) (Table-14)

On Pearson's correlation analysis of CRP showed significant positive correlation with BMI (r=0.416, p=0.012), WHR (r=0.330, p=0.049) and fasting insulin (r=0.286/p=0.015) and at baseline while at the endpoint CRP showed significant positive correlation with BMI (r=0.538, p=0.001) and WHR (r=0.343, p=0.041). On the other hand, Hcy showed significant positive correlation with age (r=0.451, p=0.006) and NEFA (r=0.407/p=<0.001) at baseline while in endpoint it showed with age (r=0.508, p=0.002) only.

Table-13: Pearson's correlation analysis of CRP & Hcy with some significant variables at two different time points (n= 36 in each group)

Characteristics (r/p)	**Age**	**BMI**	**WHR**	**Insulin**	**HOMA-IR**	**NEFA**
Baseline						
CRP	-0.040/0.818	0.416/**0.012**	0.330/**0.049**	0.286/**0.015**	0.217/0.683	-0.137/0.252
Hcy	0.451/**0.006**	0.038/0.828	0.045/0.794	-0.011/0 .925	-0.026/0.827	0.407/**<0.001**
Endpoint						
CRP	-0.002/0.992	0.538/**0.001**	0.343/**0.041**	0.131/0.273	0.161/0.177	-0.119/0.320
Hcy	0.508/**0.002**	0.010/0.955	0.081/0.638	-0.081/0.497	-0.120/0.315	-0.204/ 0.086

3.17 Multiple linear regression analysis taking CRP as dependent variable after adjusting the effects of major confounders at base line (Table 14)

Table 13 shows Logistic regression analysis of CRP according to the confounding independent variables (age, BMI, WHR, lipid profile, HOMA%B). There was a negative significant association was found of HOMA%B (β=-0.352 [95% CI: -0.021-0.002], p=*0.043*) at base line.

Table 14: Multiple linear regression analysis taking CRP as dependent variable after adjusting the effects of major confounders at base line

Variables	β	t	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
(Constant)		-2.068	0.048	-26.253	-0.101
Age (yrs)	-0.181	-1.079	0.290	-0.248	0.077
BMI (kg/m^2)	0.499	3.052	0.005	0.112	0.572
WHR	0.369	2.360	0.026	1.935	27.740
TG (mg/dl)	0.560	1.234	0.228	-0.014	0.057
CHOL (mg/dl)	-0.875	-0.617	0.543	-0.207	0.111
HDL-c (mg/dl)	0.147	0.498	0.622	-0.138	0.226
LDL-c (mg/dl)	0.637	0.537	0.596	-0.118	0.202
HOMA%B	-0.352	-2.121	***0.043***	-0.021	<0.001

Regression coefficient R^2=0.432; p=0.032

3.18 Multiple linear regression analysis taking CRP as dependent variable after adjusting the effects of major confounders at end point (Table 15)

At end point group CRP showed significant negative association with HOMA%B (β=-0.360 [95% CI: -0.017-<0.001], p=0.041) after adjusting the effects of major confounders (age, BMI, W/P ratio and lipid profile).

Table 15: Multiple linear regression analysis taking CRP as dependent variable after adjusting the effects of major confounders at end point

Variables	β	t	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
(Constant)		-2.581	0.016	-27.843	-3.180
Age (yrs)	-0.096	-0.573	0.571	-0.196	0.110
BMI (kg/m^2)	0.554	3.730	0.001	0.161	0.554
WHR	0.349	2.128	0.043	0.470	25.921
TG (mg/dl)	0.149	0.937	0.357	-0.015	0.040
CHO (mg/dl)	-0.106	-0.323	0.749	-0.039	0.028
HDL-c (mg/dl)	-0.062	-0.360	0.722	-0.154	0.108
LDL-c (mg/dl)	0.237	0.779	0.443	-0.022	0.049
HOMA%B	-0.360	-2.016	***0.041***	-0.017	<0.001

Regression coefficient R^2=0.491; p=0.010

3.19 Multiple linear regression analysis taking Hcy as dependent variable after adjusting the effects of major confounders at end point (Table 16)

At end point group Hcy showed significant negative association with HOMA%S (β=-0.521 [95% CI: -0.157-(-0.019)], p=0.041) and HOMA IR (β=-0.382 [95% CI: -1.111-(-0.001)], p=0.050) after adjusting the effects of major confounders (age, BMI, W/P ratio).

Table 16: Multiple linear regression analysis taking tHcy as dependent variable after adjusting the effects of major confounders at end point

Variables	β	t	p-value	95% Confidence Interval	
				Lower Bound	Upper Bound
(Constant)		-0.218	0.829	-30.844	24.885
Age (yrs)	0.630	4.139	<0.001	0.332	0.978
BMI (kg/m^2)	0.025	0.166	0.869	-0.420	0.495
WHR	-0.171	-1.094	0.283	-43.392	13.125
HOMA%S	-0.521	-2.604	***0.014***	-0.157	-0.019
HOMA-IR	-0.382	-2.045	***0.050***	-1.111	-0.001

Regression coefficient R^2=0.408; p=0.006

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 day's hormone replacement therapy (HRT) is used to improve the quality of life among menopausal women. However estrogens, influencing coagulation parameters, may increase the risk of thrombosis [176], and enhancing markers of inflammation, such as C-reactive protein (CRP) [177], may increase the risk of myocardial diseases [176]. Furthermore, the modest but important increased prevalence of breast cancer in women taking HRT, is another major limitation of this therapy [178,179]. Phytoestrogens may be an alternative of conventional HRT. The particular plant molecules of interest are isoflavones, which are found in abundance in soybeans and their derivative foods. After menopause, women experience changes in body composition, especially increase in fat mass. Additionally, advancing age, decreased physical activity and increased inflammation may predispose them to develop type-2 diabetes. Isoflavones have been shown to improve metabolic parameters in postmenopausal women. However, the effect of isoflavones on adipo-cytokines remains unclear. Reports about isoflavones effects on plasma homocysteine and serum CRP in postmenopausal women are very few. Recently, plasma homocysteine reduction has been reported in postmenopausal women treated with soy foods [180]. Recent studies suggest that Soy milk Isoflavones, could be an alternative of HRT, to create effect on two important biochemical markers (Hcy and CRP) of subclinical inflammation and insulin resistance which play a vital role in the pathophysiology of CVDs.

Controlled clinical and experimental studies have been performed to clarify the role of isoflavones in the prevention of cardiovascular diseases and particularly their effect on cholesterolemia in humans. Most studies have been performed in postmenopausal women. The results are inconsistent, however; some studies show a decrease in total cholesterol and low-density lipoprotein concentrations, and an increase in high-density lipoprotein levels, and other investigations fail to show any beneficial effect of soy isoflavones on lipid profiles. Many mechanisms may explain the beneficial effects of soy protein on cholesterolemia. A decrease in the absorption of cholesterol and biliary acids [181] and an increase in the activity of low-density lipoprotein (LDL) receptors [182] may be implicated. However, the components of soy-based

preparations responsible for their hypocholesterolemic effect have not been identified with certainty. The protein and its constituent amino acids may have important roles to play [182]. One suggestion is that isoflavones naturally present in soy may contribute to a beneficial effect on cholesterolemia. However, the hypocholesterolemic effect of isoflavones and the mechanisms possibly implicated remain controversial. In this study, among the lipidemic profile serum HDL (mg/dl) was significantly higher (p=0.005) at the time point of 21st days compared to 0 day in Soy milk group. Serum triglycerides (mg/dl) and NEFA (mmol/l) were significantly lower (p=0.088) and (p=0.010) respectively in Soy milk group at 21st days compared to 0 day. After 10 days of wash out period serum triglycerides was significantly lower (p=0.049) in both groups at 52nd days compared to 31st days. On the other hand, at the time point of baseline and endpoint serum triglycerides, HDL and NEFA were significantly lower (p=0.008; p=0.006 and p=0.013) respectively in endpoint compared to the baseline values. In another epidemiologic study association between one-year dietary phytoestrogen intake and metabolic cardiovascular risk factors in 939 postmenopausal women participating in the Framingham Study [183]. Therefore, these results suggest that a high isoflavone intake may be associated with a slightly decreased risk of cardiovascular disease. Another study by Han et al. in which postmenopausal women received a placebo containing soy protein without isoflavones or a supplement of soy protein plus isoflavones showed a significant reduction in total cholesterol and LDL-C concentrations by the isoflavones supplementation in comparison with baseline values [184]. According to the authors, these beneficial effects may be attributed to the weak estrogenic action of isoflavones. Two recent studies in Asian postmenopausal women with normal baseline cholesterol levels showed no significant effect of isoflavones on total, LDL-C, and HDL-C concentrations at the end of the study in comparison with placebo37 or baseline38 values. In the study of Uesugi et al., a non significant decrease in total and LDL-C levels was observed [185]. In that study, the subjects had a high usual consumption of isoflavones [185].

In vitro and animal studies have suggested that soy protein and isoflavones have favorable effects on glucose and insulin regulation, but intervention studies in humans are limited. Among the various effects of isoflavones, the role of LDL reduction seems to be well documented; however, other metabolic effects, such as blood glucose control, are rarely investigated. In a study by showed soy isoflavones lower fasting blood glucose and insulin levels in

postmenopausal women [186]. Although most of studies claimed that soy isoflavones have hypoglycemic and insulinemic activity, so far the effects and mechanisms remain controversial. To our best knowledge this is the first report in Bangladesh investigating to clinical trial of soy isoflavones preparation used to reduce insulin resistance, improved glycemic control. In this study the effects on serum glucose did not showed any difference between 0-21 days, but after 10 days wash out period serum FBG of soy milk group decreased significantly (p=0.005) during 31 to 52 day. However, at the time point of baseline and endpoint serum FBG of soy milk group decreased significantly (p=0.009). In another study did not support the hypothesis that soy protein with isoflavones or soy isoflavone extracts could improve glycemic control or insulin sensitivity in postmenopausal Chinese women with prediabetes or initial untreated diabetes. An RCT noted a significant interaction between the isoflavone treatment and baseline FG concentration on the changes of FG. Several published trials that examined the effects of soy protein or isoflavones on FG or fasting insulin yielded inconsistent results, but glycemic control and insulin sensitivity were not the primary outcome measures in most of these trials. Only one trial that reported the results of a 2-h oral glucose tolerance test showed no significant changes in the FG and 2-h PG after 24 wk of supplementation with 30 g soy protein, 100 mg isoflavones, and 9 g fiber [187]. In addition, other studies showed the effect of soy on glycemic control in diabetes have shown inconsistent results that have been primarily attributed to the soluble fiber content of soybean preparation [149–151]. Our study results are inconsistent with this finding. In our study the insulinemic profile (fasting insulin, HOMA%B, HOMA%S and HOMA-IR) did not show any significant difference between the two time points. In a small study of 14 women and 6 men, 6 weeks of treatment with soy protein (50 g/day), isoflavones (165 mg/day), and cotyledon fiber (20 g/day) compared with placebo (casein 50 g/day and cellulose 20 g/day) showed an improvement in lipid parameters but no difference in glucose, insulin, or HbA1c [148].

In a subgroup of subjects, we also studied the effect of isoflavonoids on insulin resistance, which is often associated with abnormalities in the concentrations of lipids and lipoproteins [137]. To the best of our knowledge, there are no previous data on the concomitant effects of isoflavonoids on lipids and insulin in nondiabetic postmenopausal women in Bangladesh. Therefore, we are confident that an oral 2-h glucose tolerance test (75 g) would be efficient enough to detect

clinically significant changes in insulin resistance. We expected to see an improvement in insulin sensitivity during isoflavone intake in our postmenopausal nondiabetic women because previous data imply a hypoinsulinemic effect of soy protein rich in isoflavones [145, 188]. However, another study showed that isoflavonoids failed to affect the glucose-insulin balance in our normoinsulinemic subjects [189]. Our study results are in agreement with this study where we did not find any significant effect of isoflavones on lowering the insulin resistance as well as improving the insulin secretion and sensitivity among the post menopausal women in between the baseline and endpoint.

Since inflammation is believed to have a role in the pathogenesis of cardiovascular events, measurement of markers of inflammation has been proposed as a method to improve the prediction of the risk of these events. hs-CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events. Other study documented that CRP is primarily derived from the liver, and therefore it was of significance that phytoestrogen did not affect the levels of liver enzymes in menopausal patients [190] and the use of isoflavonoids by postmenopausal women failed to affect the concentrations of the vascular surrogate markers CRP. Previous research found no effect of soy protein or isoflavones on CRP [191]. The current study confirms these results by showing no effect of soy protein components on CRP concentrations in postmenopausal women. We may now conclude that phytoestrogens do not have any direct or indirect effect on the synthesis of CRP in the liver. Moreover, our data are in line with previous findings that a 1-month, high isoflavone regimen (73 mg/d) caused no changes in levels of CRP, in middle-aged women [191].

Consumption of soy foods has been associated with a reduced risk of coronary heart disease (CHD) in women [192]. However, the cardiovascular risk factors altered by soy consumption and mechanisms involved have yet to be conclusively established. Historically, the majority of research has focused on soy and plasma lipids, but as the paradigm of atherogenesis has shifted from a disease of simple lipid accumulation to a complex condition of chronic inflammation and vascular dysfunction, additional risk factors (that is, homocysteine (Hcy) and markers of vascular inflammation) have emerged as potential targets for soy. Although the present study did not find an effect of soy consumption on serum Hcy concentration which suggests that differences in

isoflavone bioavailability among subjects may influence the effect of soy on Hcy. Results of similar Hcy investigations have not been published stratified by plasma isoflavone concentration to refute or support this theory. However, studies reporting a change in Hcy [193, 194] provided 2- to 3-fold higher isoflavone doses than those without a significant effect [195-197]. While these investigations suggest a potential role for isoflavones in lowering Hcy, a recent study manipulating soy protein content of both isoflavones and phytate contradicts this theory [198]. In this study of postmenopausal women, soy protein with native phytate levels reduced Hcy concentrations regardless of isoflavone level, suggesting phytate instead of isoflavones as the responsible component [198].

However, soy protein and isoflavone intake are negatively related to serum tHcy in premenopausal women [199], but it is difficult to ascertain which component is responsible. Although the finding by is controversial, they reported that soy protein with isoflavones exerted a tHcy-lowering effect [194]. Other investigators suggested that, whereas soy protein reduces tHcy, this effect is independent of isoflavone content [200, 196] and thus may be due to another component of soy.

Another study, reported that measures of central adiposity (waist/hip ratio, waist girth, visceral fat) and overall adiposity (body mass index, subcutaneous fat, weight, percent body fat) were positively correlated with C-reactive protein levels among postmenopausal women [201]. This is inconsistent with our study where on Bivariate correlation analysis CRP showed significant positive correlation with BMI (r=0.416, p=0.012), WHR (r=0.330, p=0.049) and fasting insulin (r=0.286/p=0.015) at baseline while at the endpoint CRP showed significant positive correlation with BMI (r=0.538, p=0.001), WHR (r=0.343, p=0.041).

On the other hand, tHcys showed significant positive correlation with age (r=0.451, p=0.006) and NEFA (r=0.407/p=<0.001) at baseline while at endpoint it showed only with age (r=0.508, p=0.002) only. Other study showed that plasma homocysteine in middle-aged women is an independent risk factor for myocardial infarction and in particular mortality due to myocardial infarction [202]. On multiple linear regression analysis, CRP was found to be significant negative association with HOMA%B (β=-0.352, p=0.043) at base line after adjusting the confounding independent variables (age, BMI, WHR, lipid profile and HOMA%B). At end point

group CRP showed significant negative association with HOMA%B (β=-0.360, p=0.041) after adjusting the effects of major confounders (age, BMI, W/H ratio and lipid profile). While at end point group Hcy showed significant negative association with HOMA%S (β=-0.521, p=0.041) and HOMA IR (β=-0.382, p=0.050) after adjusting the effects of major confounders (age, BMI, W/P ratio). But this association disappeared after adjusting the same confounders at baseline. From the above mentioned data it may conclude that soya isoflavones seemed to have beneficial role on the reduction of cardiovascular risk markers among Bangladeshi postmenopausal women and further studies needs to be elucidated by considering a follow up study with large sample size.

CONCLUSIONS

Prevention through changes in lifestyle or early detection and treatment of elevated fasting blood glucose, hypertension, and hyperlipidemia are necessary for prevention of many chronic diseases in Bangladeshi women reaching after menopausal status. Health professionals should consider the post-menopausal women as a major target group for prevention of CVDs, which is an underlying condition of many noncommunicable diseases.

In conclusion, this study, have shown that the isoflavones, after 21 days of treatment, does not modify homocysteine and CRP plasma level from baseline, without any significant difference from endpoint. So it is reasonable to suggest that isoflavones does not increase the risk linked to elevated circulating levels of these independent factors of cardiovascular disease. However, soy Isoflavones seem to have beneficial effects on hyperglycemia and dyslipidemia among postmenopausal women and the effects seems to be associated through lowering of insulin resistance and chronic subclinical inflammation.

LIMITATIONS

- It is un-blinded trial
- Duration is relatively short-, particularly to find out the effects on the marker of inflammatory status.
- Patients were not under constant monitoring.
- Menopausal status was self-reported and could have recall bias.
- Study may have selection bias during cases recruitment due to selection of urban women from lower socioeconomic class and may not be generalized to the whole Bangladeshi women.

RECOMMENDATIONS

- Postmenopausal women can be advised to take regular soy milk.
- Further large scale and comprehensive clinical trials should be conducted with various constituents of soy milk.

BIOCHEMICAL ANALYSES

The following biochemical parameters were analyzed for the study.

7.1. ESTIMATION OF GLUCOSE

Glucose was estimated by enzymatic colorimetric (GOD-PAP) method in the Hitachi 704 Automatic Analyzer, Hitachi Ltd., Tokyo, Japan using reagents of RANDOX Laboratories Ltd., UK.

7.1.1. Principle

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts with phenol and 4-aminophenazone under catalysis of peroxidase to form a red violet quinoneimine dye as indicator (Trinder, 1969).

$$\text{Glucose} + H_2O \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

7.1.2. Reagents

Contents	Initial concentration of solution
Phosphate Buffer	0.1 mol/L, pH 7.0
Phenol	11 mol/L
4-aminophenazone	0.77 mmol/L
Glucose oxidase	≥1.5 kU/L
Peroxidase	≥1.5 kU/L
Glucose Standard	5.55 mmol/L (100 mg/dL)
Uranyl Acetate	0.16%

7.1.3. Materials

- Micro-centrifuge tube
- Micropipettes and pipettes
- Disposable tips
- Automatic Analyzer (Boehringer Mannheim, 704; HITACHI)

7.1.4. Procedure

The method determines glucose without deproteinization. The instrument was calibrated before estimation. Serum and reagent were taken in specific cup. They were arranged serially

into the Auto analyzer. The Auto analyzer was programmed for the estimation of glucose and allowed to run with following procedure:

5 µl sample and 500 µl reagent were mixed and incubated at 37° C for 10 minutes. The reaction occurred in reaction cell or cup. The absorbance of the sample and the standard against the reagent blank were measured at 500 nm within 60 minutes.

7.1.5. Calculation of the result

Optical densities or absorbances were fed into a computer and calculation was done using the software program. Values for the unknown samples were calculated by extrapolating the absorbance for the standard using following formula.

$$\text{Glucose concentration (mmol/L)} = \frac{A_{Sample}}{A_{Standard}} \text{ xx } 5.55$$

7.2. ESTIMATION OF TOTAL CHOLESTEROL

Total cholesterol was measured by enzymatic endpoint method (Cholesterol Oxidase/Peroxidase) method in the Hitachi 704 Automatic Analyzer, Hitachi Ltd., Tokyo, Japan using reagents of RANDOX Laboratories Ltd., UK.

7.2.1. Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantiphyrine in the presence of phenol and peroxidase (Richmond, 1973).

7.2.2. Reagents

Contents	Initial concentration of solution
Phosphate Buffer	0.1 mol/L, pH 7.0
Phenol	11 mol/L
4-aminophenazone	0.77 mmol/L
Glucose oxidase	≥1.5 kU/L
Peroxidase	≥1.5 kU/L
Glucose Standard	5.55 mmol/L (100 mg/dL)
Uranyl Acetate	0.16%

7.2.3. Materials

- Micro-centrifuge tube
- Micropipettes and pipettes
- Disposable tips
- Automatic Analyzer (Boehringer Mannheim, 704; HITACHI)

7.2.4. Procedure

Serum and reagents were taken in specific cup or cell. They were arranged serially. Then ID number for each test was entered in the Auto analyzer. 5 µl sample and 500 µl reagent were mixed and incubated at 37^0C for 5 minutes within the Auto lab. The reaction occurred in reaction cell or cup. The absorbance of the sample and the standard against the reagent blank were measured at 500 nm within 60 minutes.

7.2.5. Calculation of the result

Concentration of cholesterol in the sample was calculated by using software program with the following formula.

Cholesterol concentration (mg/dL) = $\frac{A_{Sample}}{A_{Standard}}$ x concentration of standard.

7.3. ESTIMATION OF TRIGLYCERIDE (TG)

Serum triglyceride was measured by enzymatic colorimetric (GPO-PAP) method in the Automatic Analyzer, Hitachi 704, Hitachi Ltd., Tokyo, Japan using reagents of RANDOX Laboratories Ltd., UK.

7.3.1. Principle

Sample triglycerides incubated with a lipoprotein lipase liberate glycerol and fatty acids. Glycerol is converted to glycerol-3-phosphate by glycerol kinase and ATP.Glycerol-3-phosphate oxidase (GPO) oxidizes glycerol-3-phosphate into dihydroxy acetone phosphate and H_2O_2. In the presence of peroxidase, hydrogen peroxide oxidizes the chromogen-4-aminoantipyrine and 4-chlorophenol to a violet colored complex. The quinone formed is proportional to the amount of triglycerides present in the sample. The principle is based on the following reaction system (Fossati and Prencipe, 1982).

$$\text{Triglyceride} \xrightarrow{Lipase} \text{glycerol + Fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow[Kinase]{Glycerol} \text{glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow[Oxidase]{Glycerol\ Phosphate} \text{dihydroxy acetone phosphate + } H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone + 4- chlorophenol} \xrightarrow{Peroxidase} \text{Quinoneimin+HCl+ } 4H_2O$$

7.3.2. Reagents

Content	Concentrations in the Test
Buffer	
Pipes Buffer	40 mmol/L, pH 7.6
4-choloro-phenol	5.5 mmol/L
Magnesium-ions	17.5 mmol/L
7.3.3. Enzyme Reagent	
4-aminophenazone	
ATP	1.0 mmol/L
Lipases	>150 U/ml
Glycerol-3-phosphate oxidase	1.5 U/ml
Standard	2.29 mmol/L (200 mg/dL)

7.3.4. Materials

- Micropipettes and pipettes
- Disposable tips
- Auto analyzer (Boehringer Mannheim, 704; HITACHI)

7.3.5. Procedure

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

7.3.6. Calculation of result

Triglyceride concentration was calculated by following formula:

$$\text{Triglyceride concentration (mg/dL)} = \frac{A_{Sample}}{A_{Standard}} \times \text{Concentration of standard.}$$

7.4. ESTIMATION OF HIGH DENSITY LIPOPROTEIN (HDL) CHOLESTEROL

High density lipoprotein cholesterol (HDL-C) was measured by Differential Precipitation, Enzymatic colorimetric test & Endpoint method using reagent of Linear Chemicals, Spain.

7.4.1. Principle

HDL (High Density Lipoprotein) is separated from chylomicrons, VLDL (very low density lipoprotein) and LDL (Low density lipoprotein) by precipitating reagent (phosphotungstic acid-magnesium chloride). After centrifugation, the cholesterol content of HDL fraction, which remains in the supernatant, was determined by the enzymatic colorimetric method using CHOD- PAP (Friedwald *et al*., 1972).

7.4.2. Materials and reagents

1. Precipitant Buffer
2. Lipid Controls
3. Randox aqueous Cholesterol Standard: 200 mg/dL
4. Reagent solution for cholesterol CHOP-PAP assay.
5. Pipettes (5 µl –50 µl, 100 µl-1000 µl) and Pipette Tips.
6. Multi-Channel Pipettes and Pipette Tips: 50-300 µl
7. Buffer and Reagent Reservoirs
8. Vortex Mixture
9. Deionized Water
10. Microtiter Plate Reader capable of reading absorbency at 450 nm 590 nm
11. Orbital Microtiter Plate Shaker
12. Absorbant Paper

7.4.3. Reagents composition

Phosphotungstic Acid: 0.55 mmol/L

Magnesium Chloride: 25 mmol/L

7.4.4. Standard Preparation

Dilute Randox aqueous cholesterol standard (200 mg/dL) with deionized water by volume of 0, 20, 40, 50, and 100 µl. The final volume was 200 µl.

7.4.5. Assay Procedure

1. 100 ml serum sample was taken in microcentrifuge tube.
2. 250 ml HDL-C precipitant was added.
3. Mixed well and allowed to sit for 10 minutes
4. The mix components were vortexed and centrifuged for 15 minutes at 4000 rpm.

5. 30 µl of each Standard was transferred in first six wells.
6. 30 µl of clear supernatant was transferred into the other wells.
7. 250 µl of cholesterol reagent was then added into all the 96 wells quickly using multi-channel pipettes.
8. Incubated for 5 minutes at 37^0C on orbital microtiter plate shaker.
9. Absorbance was read at 490 nm.

7.4.6. Calculation of the result

The HDL-C value of each sample was obtained as follows:

The net absorbance value for each level, obtained by subtracting the value for the HDLC concentration (mg/dL) from the value of individual. The smooth linear curve was drawn and the results of unknown samples were calculated using logistic function.

7.5. ESTIMATION OF LOW DENSITY LIPOPROTEIN (LDL) CHOLESTEROL

The LDL-Cholesterol level in serum was calculated by using by Friedewald formula (Friedwald *et al.,* 1972).

Formula

$$\text{LDL cholesterol} = \{\text{Total cholesterol} - (\text{HDL cholesterol} + \frac{1}{5} x \quad \text{Triglyceride})\}$$

7.6. ESTIMATION OF FASTING SERUM INSULIN

7.6.1. Principle

The DRG Insulin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the Insulin molecule. An aliquot of patient sample containing endogenous Insulin is incubated in the coated well with enzyme conjugate, which is an anti-Insulin antibody conjugated with Biotin. After incubation the unbound conjugate is washed off. During the second incubation step Streptavidin Peroxidase Enzyme Complex binds to the biotin-anti-Insulin antibody. The amount of bound HRP complex is proportional to the concentration of Insulin in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Insulin in the patient sample.

7.6.2. Reagents

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-Insulin antibody (monoclonal).
2. Zero Standard, 1 vial, 3 mL, ready to use 0 µIU/mL Contains non-mercury preservative.

3. Standard (Standard 1-5), 5 vials, 1 mL, ready to use; Concentrations: 6.25 - 12.5 – 25 - 50 and 100 µIU/mL, Conversion: µIU/mL x 0.0433 = ng/mL x 23.09 = µIU/mL The standards are calibrated against international WHO approved Reference material NIBSC 66/304.; Contain non-mercury preservative.
4. Enzyme Conjugate, 1 vial, 5 mL, ready to use, mouse monoclonal anti-Insulin conjugated to biotin; Contains non-mercury preservative.
5. Enzyme Complex, 1 vial, 7 mL, ready to use, Streptavidin-HRP Complex Contains non-mercury preservative.
6. Substrate Solution, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
7. Stop Solution, 1 vial, 14 mL, ready to use, contains 0.5 M H2SO4, Avoid contact with the stop solution. It may cause skin irritations and burns.
8. Wash Solution, 1 vial, 30 mL (40X concentrated).

7.6.3. Reagent Preparation

Wash Solution

Deionized water was added to the 40X concentrated Wash Solution. 30 mL of concentrated Wash Solution was diluted with 1170 mL deionized water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.

7.6.4. Procedure

1. Microtiter wells were secured in the frame holder.
2. 25 µL of each Standard, control and samples were dispensed with new disposable tips into appropriate wells.
3. 25 µL Enzyme Conjugate was dispensed into each well. Thoroughly mixed for 10 seconds.
4. Incubated for 30 minutes at room temperature.
5. The contents of the wells were shaked out briskly. The wells were rinsed 3 times with diluted Wash Solution (400 µL per well). The wells were striked sharply on absorbent paper to remove residual droplets.
6. 50 µL of Enzyme Complex was added to each well.
7. Incubated for 30 minutes at room temperature.
8. The contents of the wells were shaked out briskly. The wells were rinsed 3 times with diluted Wash Solution (400 µL per well). The wells were striked sharply on absorbent paper to remove residual droplets.
9. 50 µL of Substrate Solution was added to each well.

10. Incubated for 15 minutes at room temperature.
11. The enzymatic reaction was stopped by adding 50 µL of Stop Solution to each well.
12. The absorbance (OD) of each well was determined at 450 ± 10 nm with a microtiter plate reader.

7.6.5. Calculation

Optical densities obtained were used to calculate values of unknown samples using software, Kinetic Calculation. Results were calculated by expanding standard curve.

7.7. DETERMINATION OF INSULIN SECRETORY CAPACITY AND INSULIN SENSITIVITY

Homeostasis Model Assessment (HOMA) is a simple widely used method which derives separate indices of B cell secretion (HOMA B) and insulin sensitivity (HOMA S) from the Serum glucose and insulin concentrations under basal conditions by using mathematical formula or software. Using HOMA, insulin sensitivity (HOMA-IR) is calculated as [fasting insulin (mu/l) × fasting glucose (mmol/l)/22.5]. The HOMA model has been incorporated in a simple MS-DOS-based computer program (HOMA-CIGMA software) that allows rapid determination of % B (B cell secretion) and % S (insulin sensitivity) from measured values. Although the simple equation gives a qualitatively useful approximation of the model prediction, most authors prefer the computer model. In this study HOMA-CIGMA software was used.

7.8. DETERMINATION OF HIGH SENSITIVITY C-REACTIVE PROTEIN

7.8.1. Principle of the hs-CRP ELISA

Microtiter strips coated with anti-hs-CRPantibody are incubated with diluted standard sera and donor samples. During this incubation step hs-CRP is bound specifically to the wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated antibodies. After removal of the unbound conjugate, the strips are incubated with a chromogen solution containing tetra methyl benzidine and hydrogen peroxide: a blue colour develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 0.5M H2SO4 and the absorbance values at 450 nm are determined. A standard curve is obtained by plotting the absorbance values versus the corresponding standard values. The concentration of hs-CRP in donor samples is determined by interpolation from the standard curve.

7.8.2. Reagents

1. **Coated Microtiter strips**

12 x 8-well strips coated with monoclonal antibodies to human hs-CRP.

2. **Standard Sera** - 5 vials, each containing 1/10 prediluted hs-CRP standard solutions (0.2 ml): 0 - 0.4 - 1 - 5 - 10 µg/ml. Calibrated against the NIBSC 1st International Standard, 85/506.

Contain 0.09 % NaN_3 and antimicrobial agents as preservatives.

3. **Conjugate** - 1 vial, containing peroxidase conjugated monoclonal anti-human hs-CRP antibodies (12 ml). Contains antimicrobial agents and an inert red dye.

4. **Specimen Dilution Buffer** - 1 vial, containing 40 ml dilution buffer 5x concentrated.

Contains 0.09 % NaN_3 and antimicrobial agents and an inert green dye.

5. **Washing Solution -** 1 vial, containing 50 ml 20 x concentrated phosphate buffered washing solution.

6. **Chromogen Solution -** 1 vial, containing 15 ml of a solution containing H_2O_2 and tetramethylbenzidin.

7. **Stopping Solution** -1 vial, containing 12 ml of 0.5M H_2SO_4

7.8.3. Materials required

1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass tubes for the dilution of the samples.
4. A microtiterplate reader capable of measuring absorbencies at 450 nm

7.8.4. Reconstitution of the Reagents

Washing Solution: Dilute 50 ml of concentrated Washing Solution (5) to 1000 ml with distilled water. Reconstituted solution can be stored at least 1 month, store at 2 °C – 8 °C. At higher temperatures, the concentrated Washing solution (5) may appear cloudy, without affecting its performance. Upon dilution, the solution will be clear.

Sample Diluent: Dilute 40 ml of the concentrated Sample Diluent to 200 ml with distilled water. Reconstituted solution can be stored at least 3 months or as long as solution remains clear. Store at 2 °C – 8 °C.

7.8.5. Assay Procedure

1. The 10 x prediluted standard sera (2) are diluted 1:100 as follows: pipette 10 µl of each calibrator into separate glass dilution tubes. Add 990 µl of diluted Specimen Dilution Buffer (4) and mix carefully.

2. The donor samples are diluted 1:1000 in two consecutive steps: pipette 10 µl of each donor sample into separate glass dilution tubes and add 990 µl of diluted Specimen Dilution Buffer (4). Mix thoroughly. Add 450 µl of diluted Specimen Dilution Buffer to 50 µl of these 100 x prediluted samples. Mix thoroughly. Warning: do not store the diluted samples for more than 8 hours.

3. Pipette 100 µl of the diluted calibrators and samples into each of a pair of adjacent wells (1).

4. Incubate the covered microtiter strips for 30 ± 2 min at room temperature.

5. Wash the microtiter strips three times with Washing Solution. This can either be performed with a suitable microtiter plate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution is left in the strips for 2-3 min. Change washing solution for each cycle. Finally empty the microtiter strips and remove excess fluid by blotting the inverted strips on adsorbent paper.

6. Add 100 µl of Conjugate Solution (3) and incubate the covered microtiter strips for 30 ± 2 min at room temperature.

7. Repeat the washing procedure as described in step 5.

8. Add 100 µl of Chromogen (6) Solution to each well.

9. Incubate for 10 ± 2 min at room temperature. Avoid light exposure during this step.

10. Add 50 µl of Stopping Solution (7) to each well.

11. Determine the absorbance of each well at 450 nm within 30 min following the addition of acid.

7.8.6. Results

The average absorbance value of each calibrator is plotted against the corresponding CRP-value and the best calibration curve (e.g. log/linear) is constructed. Use the average absorbance of each donor sample obtained in the hsCRP ELISA to determine the corresponding value by simple interpolation from the curve. Depending on the experience and/or availability of computer capability, other methods of data reduction may be used.

Minimal detectable concentration

The minimal detectable concentration is approximately 0.02 µg/ml

7.9 MEASUREMENT OF HOMOCYSTEINE

The AxSYM homocysteine assay is a florescence polarization Immunoassay (FPIA) for the quantitative measurement of total L-homocysteine in human serum or plasma on the AxSYM system.

7.9.1 Principle:

Bounde homocysteine (oxidized form) is reduced to free homocysteine that is enzymatically converted to S-adenosyl-L-homocysteine (SAH) as follows:

7.9.2 Reduction: Homocysteine , mixed disulfide and prtine-bond froms of homocysteine in the sample are reduced to form free homocysteine by the use of dithiotreitol (DTT).

Homocysteine-SS- homocysteine

R1-SS- homocysteine(R1=residue) → homocysteine protine-SS-Hcy

7.9.3 Enzymatic conversion: Total free homocysteine is converted to SAH by the use of SAH Hydroase and excess adenosine.

Homocysteine + Adenosine SAH Hydrolase SAH

Under physiological condisions, SAH hydrolase converts SAH to homocysteine.Excess adenosine in the pretreatment solution drives the conversion of homocysteine to SAH the recombinant SAH hydrolase.

7.9.4 Reagents:

- S-adenosyl-L-cysteine Fluorescein Tracer in phosphate buffer with protein (bovine) stabilizer
- S-adenosyl-L-homocysteine homocysteine Hydrolase (recombinant) in phosphate buffer with protein (recombinant) stabilizer.
- Anti-S-adenosyl-homocysteine (mouse monoclonal in phosphate buffer with protein (Procine) stabilizer .
- Pretreatment solution containing dithiotheitol (DTT) and adenosine in citric acid.

Calibrators and controls.

Solution 4 (line diluent).

7.9.5 Procedure: 1(one) ml venous blood was collected in a heparin or EDTA containing tube.Serum or plasma specimens may be used in the AxSYM homocysteine assay. The samples were centrifuged at 1000× g for 10 minutes.50 µl of sample is the minimum volume require to perform the assay. Sample have been shown to be stable at -20°C for 8 month if measurement not done immediately. The refrigerated samples were mixed thoroughly thawing to ensure consistency of the results.

The AxSYM homocysteine reagents and sample were pipette in the following sequence:

7.9.6 Sampling Center

- Sample and all AxSYM homocysteine Regents required for one test were pipette by the sampling probe into various wells of a Reaction vessels (RV).
- Sample, Pretreatment Solution 4 (Line diluents) and SAH hydrolase Enzyme were pipetted into one well of the RV to make up the predilution mixture.

The RV was immediately transferred into the processing center. Further pipetting was done in the processing center by the processing probe.

7.9.7 Processing Center:

- An aliquot of the predilution mixture, Antibody, and solution 4 (Line diluent) were delivered to the cuvette of the RV.
- Tracer, Solution 4, and a second aliquot of the predilution mixture were transferred to the cuvette.
- SAH and labeled fluorescent light was measured by the FPIA optical assembly.

7.9.8 Expected Values: A majority of the scientific literature agress upon a range of normal values (adult male and female) between 5 and 15 µmol/L.

REFERENCE:

1. World Health Organization (WHO). (1981) Research on the Menopause. WHO Technical Report Series No.670. Geneva: World Health Organization.
2. Shailendra Kumar Mishra 2011**.** Menopausal transition and postmenopausal health problems: a review on its bio-cultural perspectives. J of Health.Vol.3, No.4, 233-237.
3. World Health Organization (WHO). Research on the Menopause in the 1990s: Report of a WHO Scientific Group. World Health Organ Tech Rep Ser 1996; 866: 1-107.
4. Minkin, Mary Jane; *et al.* (1997). *What Every Woman Needs to Know about Menopause*. Yale University Press. ISBN 0-300-07261-9.
5. Kato I, Toniolo P, Akhmedkhanov A, Koenig KL, Shore R, Zeleniuch-Jacquotte A (1998). "Prospective study of factors influencing the onset of natural menopause". *J Clin Epidemiol* **51** (12): 1271–1276. doi:10.1016/S0895-4356(98)00119-X. PMID 10086819.
6. Do KA, Treloar SA, Pandeya N *et al.* (1998). "Predictive factors of age at menopause in a large Australian twin study". *Hum Biol* **70** (6): 1073–91. PMID 9825597.
7. Ringa, V. (2000). "Menopause and treatments". *Quality of Life Research* **9** (6): 695–707. doi:10.1023/A:1008913605129. JSTOR 4036942.
8. Kaufman, D. L.; Slone, D.; Rosenberg, L.; Miettinen, O. S.; Shapiro, S. "Cigarette smoking and age of natural menopause". *American Journal of Public Health* **70** (4): 420–422. doi:10.2105/AJPH.70.4.420. PMC 1619390. PMID 7361965.
9. Menopause- Wikipedia.
10. S Fahmida: Menopausal status and reproductive behavior in rural women. MPH Thesis (Epidemiology). Dhaka: NIPSOM 2002-2003.
11. Bener A, Rizk DE, Shaheen H, Micallef R, Osman N, Dunn EV. Measurementspecific quality of life satisfaction during menopause in an Arabian Gulf country. Climacteric 2000; 3:43-9.
12. Fuh JL, Wang SJ, Lu SR, Juang KD. Quality of life and menopause transition for middle aged women on Kinmen Island. Quality Life Res 2003; 12:533-61.
13. Sulak PJ.The perimenopause: a critical time in a woman's life. International journal of fertility and menopausal studies 1996; 41: 85-9.

14. Whelan TJ, Goss PE, Ingle JN, Pater JL, Tu DS, Pritchard K, et al. Assessment of quality of life in MA.17: a randomized, placebo controlled trial of letrozole after 5 years of tamoxifen in postmenopausal women. J Clin oncol 2005; 23: 6931-40.
15. No author listed. Study protocol for the World Health Organization project to develop a quality of life assessment instrument (WHOQOL). Qual Life Res 1993; 2: 153-9.
16. Nusrat Nisar, Nisar Ahmed Sohoo. Frequency of menopausal symptoms and their impact on the quality of life of women: a hospital based survey 2009. **J Pak Med Assoc** Vol. 59 (11); 752-756.
17. Greene JG (1998). "Constructing a standard climacteric scale". *Maturitas* **29**: 25–31. PMID 9643514.
18. Monterrosa-Castro A, Romero-Pérez I, Marrugo-Flórez M, Fernández-Alonso AM, Chedraui P, Pérez-López FR (2012). "Quality of life in a large cohort of mid-aged Colombian women assessed using the Cervantes Scale.". *Menopause* **19** (8): 924–30. PMID 22549166.
19. Chedraui P, Pérez-López FR, Mendoza M, Leimberg ML, Martínez MA, Vallarino V, Hidalgo L. (2010). "Factors related to increased daytime sleepiness during the menopausal transition as evaluated by the Epworth sleepiness scale". *Maturitas* **65** (1). pp. 75–80. PMID 19945237.
20. Mitchell, Richard Sheppard; Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson (2007). *Robbins Basic Pathology: With Student Consult Online Access*. Philadelphia: Saunders. p. 344. ISBN 1-4160-2973-7. 8th edition.
21. Dreisler E, Poulsen LG, Antonsen SL, Ceausu I, Depypere H, Erel CT, Lambrinoudaki I, Pérez-López FR, Simoncini T, Tremollieres F, Rees M, Ulrich LG. (2013). "EMAS clinical guide: Assessment of the endometrium in peri and postmenopausal women". *Maturitas* **75** (2): 181–90. PMID 23619009.
22. Pérez-López FR, Cuadros JL, Fernández-Alonso AM, Chedraui P, Sánchez-Borrego R, Monterrosa-Castro A (2012). "Quality of life in a large cohort of mid-aged Colombian women assessed using the Cervantes Scale.". *Maturitas* **73** (4): 369–72. doi:10.1016/j.maturitas.2012.09.004. PMID 23041251.

23. Llaneza P, García-Portilla MP, Llaneza-Suárez D, Armott B, Pérez-López FR. (2012). "Depressive disorders and the menopause transition." *Maturitas* **71** (2): 120–30. PMID 22196311.
24. Arakane M, Castillo C, Rosero MF, Peñafiel R, Pérez-López FR, Chedraui P. (2011). "Factors relating to insomnia during the menopausal transition as evaluated by the Insomnia Severity Index.". *Maturitas* **69** (2). pp. 157–161. PMID 21444163.
25. Monterrosa-Castro A, Marrugo-Flórez M, Romero-Pérez I, Chedraui P, Fernández-Alonso AM, Pérez-López FR. (2013). "Prevalence of insomnia and related factors in a large mid-aged female Colombian sample.". *Maturitas* **74** (4): 346–51. PMID 23391501.
26. Llaneza P, Fernández-Iñarrea JM, Arnott B, García-Portilla MP, Chedraui P, Pérez-López FR. (2011). "Sexual function assessment in postmenopausal women with the 14-item changes in sexual functioning questionnaire.". *J Sex Med* **8** (8): 2144–51. PMID 21679299.
27. Pérez-López FR, Fernández-Alonso AM, Trabalón-Pastor M, Vara C, Chedraui P; MenopAuse RIsk Assessment (MARIA) Research Group. (2012). "Assessment of sexual function and related factors in mid-aged sexually active Spanish women with the six-item Female Sex Function Index.". *Menopause* **19** (11): 1224–30. PMID 22781787.
28. Ornat L, Martínez-Dearth R, Muñoz A, Franco P, Alonso B, Tajada M, Pérez-López FR. (2012). "Sexual function, satisfaction with life and menopausal symptoms in middle-aged women.". *Maturitas* **75** (3): 261–9. PMID 23684086.
29. Freeman EW, Sammel MD, Lin H, *et al.* (2007). "Symptoms associated with menopausal transition and reproductive hormones in midlife women". *Obstetrics and gynecology* **110** (2 Pt 1): 230–40. doi:10.1097/01.AOG.0000270153.59102.40. PMID 17666595.
30. Pien GW, Sammel MD, Freeman EW, Lin H, DeBlasis TL (July 2008). "Predictors of sleep quality in women in the menopausal transition". *Sleep* **31** (7): 991–9. PMC 2491505. PMID 18652094.
31. William F, Ganong MD. Physiology of reproduction in women. In: Alan HD, Lauren N, editors. Current obstetric and gynecologic diagnosis and treatment. 9th ed. New York: Mc Graw-Hill Publication: 1991. pp 130-153.

32. Norma LJ, Howard LJ. Menopause and postmenopause. In: Alan HD, Lauren N, editors. Current obstetric and gynecologic diagnosis and treatment. 9th ed. New York: Mc Graw-Hill Publication: 1991. pp1019-1040.
33. Klinga K, Holst T, Runnebaum B. Serum concentrations of FSH, oestradiol, oestrone and androstenedione in normal and obese women. Maturitus 1982; 4: 9-17.
34. Witteman JCM, Grobbee DE, Kok FJ, Hofman A, Valkenburg HA. Increased risk of atherosclerosis in women after menopause. BMJ 1989; 298:642– 4.
35. Harrison-Bernard LM, Raij L. Postmenopausal hypertension. Curr Hypertens Rep. 2000;2:202–7. [PubMed].
36. Giardina EG. Heart disease in women. Int J Fertil Womens Med. 2000;45:350–7. [PubMed].
37. Zárate A, Saucedo R, Basurto L, Martínez C. Cardiovascular disease as a current threat of older women.Relation to estrogens. Ginecol Obstet Mex. 2007;75:286–92. [PubMed]
38. Gohlke-Barwolf C 2000 Coronary artery disease: is menopause a risk factor? Basic Res Cardiol 95(Suppl 1):I77–I83.
39. Lerner DJ, Kannel WB. 1986 Patterns of coronary heart disease morbidity and mortality in the sexes: a 26 year follow-up of the Framingham population. Am Heart J. 111: 383-390.
40. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT 2002 The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288:2709–2716.
41. Despres JP 1993 Abdominal obesity as important component of insulinresistance syndrome. Nutrition 9:452–459.
42. Meigs JB 2002 Epidemiology of the metabolic syndrome, 2002. Am J Manag Care 8 (Suppl 11):S283–S296.
43. Wilson PW, Kannel WB, Silbershatz H, D'Agostino RB 1999 Clustering of metabolic factors and coronary heart disease. Arch Intern Med 159:1104–1109.
44. Denke MA, Sempose CT, Grundy SM. Excess body weight: An under-recognized contributor to high blood cholesterol levels in white American men. *Arch Intern Med* 1993; **153**: 1093–1103.

45. Kannel WB, D'Agostino RB, Cobb JL. Effects of weight on cardiovascular disease. *Am J Clin Nutr* 1996; **63**(Suppl): 419s–422s.
46. Eckel RH, Krauss RM. American Heart Association Call to Action: Obesity as a major risk factor for coronary heart disease. *Circulation* 1998; **97**: 2099–2100.
47. Krotkiewski M, Bjorntorp P, Sjostrom L, Smith U 1983 Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. J Clin Invest 72:1150–1162
48. Poehlman ET, Toth MJ, Gardner AW 1995 Changes in energy balance and body composition at menopause: a controlled longitudinal study. Ann Intern Med 123:673–675.
49. Poehlman ET, Toth MJ, Ades PA, Rosen CJ 1997 Menopause-associated changes in plasma lipids, insulin-like growth factor I and blood pressure: a longitudinal study. Eur J Clin Invest 27:322–326.
50. Colditz G, Willett W, Rotnitzky A, manson J. Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann Intern Med* 1995; **122**: 481–486.
51. Ho SC, Chen YM, Woo JL, Leung SS, Lam TH, Janus ED. Association between simple anthropometric indices and cardiovascular risk factors. *Int J Obes Relat Metab Disord* 2001; **25**: 1689–1697.
52. Egeland GM, Cao Z, Young TK. Hypertriglyceridemic-waistphenotype and glucose intolerance among Canadian Inuit: the International Polar Year Inuit Health Survey for Adults 2007–2008. *Canadian Medical Association Journal (CMAJ)*. 2011; **183(9):** 553 – 558.
53. Després JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E, et al. Abdominal obesityand the metabolic syndrome: contribution to global cardiometabolic risk. *Arterioscler Thromb Vasc Biol*. 2008; **28(6):** 1039 – 1049.
54. Arsenault BJ, Lemieux I, Després JP, Wareham NJ, Kastelein JJP, Khaw KT, et al. The hypertriglyceridemic-waist phenotype and the risk of coronary artery disease: results from the EPIC-Norfolk Prospective Population Study. *Canadian Medical Association Journal (CMAJ)*. 2010; **182(13):** 1427 – 1432.

55. Dalton M, Cameron AJ, Zimmet PZ, Shaw JE, Jolley D, Dunstan DW, et al. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *J Intern Med* 2003; **254**: 555–563.
56. Huang B, Rodreiguez BL, Burchfiel CM, Chyou PH, Curb JD, Sharp DS. Association of adiposity with prevalent coronary heart disease among elderly men: The Honolulu heart program. *Int J Obes* 1997; **21**: 340–348.
57. Lemieux S, Prudhomme D, Bouchard C, Tremblay A, Després JP. A single threshold value of waist girth identifies normal-weight and overweight subjects with excess visceral adipose tissue. *Am J Clin Nutr* 1996; **64**: 685–693.
58. Jensen J, Nilas L, Christiansen C 1990 Influence of menopause on serum lipids and lipoproteins. Maturitas 12:321–331.
59. Matthews KA, Kuller LH, Sutton-Tyrrell K, Chang YF 2001 Changes in cardiovascular risk factors during the perimenopause and postmenopause and carotid artery atherosclerosis in healthy women. Stroke 32:1104–1111.
60. Razay G, Heaton KW, Bolton CH 1992 Coronary heart disease risk factors in relation to the menopause. Q J Med 85:889–896.
61. Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR 1989 Menopause and risk factors for coronary heart disease. N Engl J Med 321:641–646.
62. LindquistO1982 Intraindividual changes of blood pressure, serum lipids, and body weight in relation to menstrual status: results from a prospective population study of women in Goteborg, Sweden. Prev Med 11:162–172.
63. Do KA, Green A, Guthrie JR, Dudley EC, Burger HG, Dennerstein L 2000 Longitudinal study of risk factors for coronary heart disease across the menopausal transition. Am J Epidemiol 151:584–593.
64. Kannel WB, Hjortland MC, McNamara PM, Gordon T 1976 Menopause and risk of cardiovascular disease: the Framingham study. Ann Intern Med 85: 447–452
65. Stevenson JC, Crook D, Godsland IF 1993 Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis 98:83–90.
66. Santamarina-Fojo S, Haudenschild C, AmarM1998 The role of hepatic lipase in lipoprotein metabolism and atherosclerosis. Curr Opin Lipidol 9:211–219.

67. Pouliot MC, Despres JP, Nadeau A, Moorjani S, Prud'Homme D, Lupien PJ, Tremblay A, Bouchard C 1992 Visceral obesity in men. Associations with glucose tolerance, plasma insulin, and lipoprotein levels. Diabetes 41:826–834
68. Dallongeville J, Marecaux N, Isorez D, Zylbergberg G, Fruchart JC, Amouyel P 1995 Multiple coronary heart disease risk factors are associated with menopause and influenced by substitutive hormonal therapy in a cohort of French women. Atherosclerosis 118:123–133
69. Lindheim SR, Buchanan TA, Duffy DM, Vijod MA, Kojima T, Stanczyk FZ, Lobo RA 1994 Comparison of estimates of insulin sensitivity in pre- and postmenopausal women using the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. J Soc Gynecol Invest 1:150–154.
70. Ridker PM, Manson JE, Bruing JE, Shih J, Matias M, Hennekens CH. Homocysteine and risk of cardiovascular disease among postmenopausal women. JAMA 1999;281:1817–21.
71. Hak AE, Polderman KH, Westendorp ICD, et al. Increased plasma homocysteine after menopause. Atherosclerosis 2000;149:163– 8.
72. Panagiotakos DB, Pitsavos C, Zeimbekis A, Chrysohoou C, Stefanadis C. The association between lifestyle-related factors and plasma homocysteine levels in healthy individuals from the "ATTICA" Study. Int J Cardiol 2005;98:471–7.
73. Fu WY, Dudman NPB, Perry MA, Wang XL. Homocysteine attenuates hemodynamic responses to nitric oxide in vivo. Atherosclerosis 2002; 161:169 –76.
74. Heydrick SJ, Weiss N, Thomas SR, et al. L-homocysteine and L-homocysteine stereospecifically induce endothelial nitric oxide synthase-dependent lipid peroxidation in endothelial cells. Free Radic Biol Med 2004;36:632– 40.
75. Nestel PJ, Chronopoulos A, Cehun M. Arterial stiffness is rapidly induced by raising the plasma homocysteine concentration with methionine. Atherosclerosis 2003;171:83– 6.
76. Zeng X, Dai J, Remick DG, Wang X. Homocysteine mediated expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human monocytes. Circ Res 2003;93:311–20.
77. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998;97:2007–11.

78. Sanada M, Tsuda M, Kodama I, Sakashita T, Nakagawa H, Ohama K. Substitution of transdermal estradiol during oral estrogen-progestin therapy in postmenopausal women: effects on hypertriglyceridemia. Menopause 2004;11:331– 6.
79. Yilmazer M, Fenkci V, Fenkci S, et al. Hormone replacement therapy, C-reactive protein, and fibrinogen in healthy postmenopausal women. Maturitas 2003;46:245–53.
80. Khreiss T, Jo´zsef L, Potempa LA, Filep JG. Conformational rearrangement in C-reactive protein is required for proinflammatory actions on human endothelial cells. Circulation 2004;109:2016 –22.
81. Devaraj S, Kumaresan PR, Jialal I. Effect of C-reactive protein on chemokine expression in human aortic endothelial cells. J Mol Cell Cardiol 2004;36:405–10.
82. Venugopal SK, Devaraj S, Jialal I. C-reactive protein decreases prostacyclin release from human aortic endothelial cells. Circulation 2003; 108:1676–8.
83. Verma S, Kuliszewski MA, Li SH, et al. C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function. Circulation 2004;109:2058–67.
84. Dallongeville J, Marecaux N, Isorez D, Zylbergberg G, Fruchart JC, Amouyel P 1995 Multiple coronary heart disease risk factors are associated with menopause and influenced by substitutive hormonal therapy in a cohort of French women. Atherosclerosis 118:123–133
85. Bhatla N. Jeffcoate's Principle of Gynaecology. 6th ed. London: Arnold Publication; 2001. Pp101-109.
86. Estrogen and progestogen use in postmenopausal women: 2010 position statement of The North American Menopause Society. Menopause. 2010 Mar;17(2):242–55
87. Kanaya AM, Herrington D, Vittinghoff E, Lin F, Grady D, Bittner V, et al. Glycemic effects of post menopausal hormone therapy: the heart and estrogen/progestin replacement study. A randomized, double blind, placebo-controlled trial. Ann Intern Med 2003; 138: 1-9.
88. Menopause; US Dept. of Health and Human Services, Public Health Services, National Institute of Health, NIH Publication No. 01-3886, February 2011.
89. Carlos J, Christopher T, Ellen S, Anastacia S, Ross EA. Hormone replacement therapy and its relationship to lipid and glucose metabolism in diabetic and nondiabetic postmenopausal women. Diabetes Care 2002; 25: 1675-1679.

90. Araujo DA, Farias ML, Andrade AT. Effects of trasdermal and oral estrogen replacement on lipid and glucose metabolism in postmenopausal women with type 2 diabetes mellitus. Climacteric 2002; 5: 286-292.
91. Bhatla N. Jeffcoate's Principle of Gynaecology. 6th ed. London: Arnold Publication; 2001. pp 101-109.
92. Menon U, Burnell M, Sharma A, *et al.* (2007). "Decline in use of hormone therapy among postmenopausal women in the United Kingdom". *Menopause* **14** (3 Pt 1): 462–7. doi:10.1097/01.gme.0000243569.70946.9d. PMID 17237735.
93. Du Y, Dören M, Melchert HU, Scheidt-Nave C, Knopf H (2007). "Differences in menopausal hormone therapy use among women in Germany between 1998 and 2003". *BMC Womens Health* **7**: 19. doi:10.1186/1472-6874-7-19. PMC 2233614. PMID 17945013.
94. Watson J, Wise L, Green J (September 2007). "Prescribing of hormone therapy for menopause, tibolone, and bisphosphonates in women in the UK between 1991 and 2005". *Eur. J. Clin. Pharmacol.* **63** (9): 843–9. doi:10.1007/s00228-007-0320-6. PMID 17598097.
95. Clarke CA, Glaser SL, Uratsu CS, Selby JV, Kushi LH, Herrinton LJ (November 2006). "Recent declines in hormone therapy utilization and breast cancer incidence: clinical and population-based evidence". *J. Clin. Oncol.* **24** (33): e49–50. doi:10.1200/JCO.2006.08.6504. PMID 17114650.
96. Ravdin PM, Cronin KA, Howlader N, *et al.* (April 2007). "The decrease in breast-cancer incidence in 2003 in the United States". *N. Engl. J. Med.* **356** (16): 1670–4. doi:10.1056/NEJMsr070105. PMID 17442911.
97. Zhang Y, Carl F. The effect of estrogen use on levels of glucose and insulin and the risk of type 2 diabetes in American Indian postmenopausal women. Diabetes Care 2002; 25:500-504.
98. Deecher DC, Dorries K. Understanding the pathophysiology of vasomotor symptoms (hot flushes and night sweats) that occur in perimenopause, menopause, and postmenopause life stages. Arch Womens Ment Health 2007;10:247–57.
99. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, et al. Risks and benefits of

estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321–33.

100. Gold EB, Colvin A, Avis N, Bromberger J, Greendale GA, Powell L, Sternfeld B, Matthews K. Longitudinal analysis of the association between vasomotor symptoms and race/ethnicity across the menopausal transition: study of women's health across the nation. Am J Public Health 2006;96:1226–35.
101. Whitcomb BW, Whiteman MK, Langenberg P, Flaws JA, Romani WA. Physical activity and risk of hot flashes among women in midlife. J Womens Health (Larchmt) 2007;16:124–33.
102. Sievert LL, Obermeyer CM, Price K. Determinants of hot flashes and night sweats. Ann Hum Biol 2006;33:4–16.
103. Gold EB, Block G, Crawford S, Lachance L, FitzGerald G, Miracle H, Sherman S. Lifestyle and demographic factors in relation to vasomotor symptoms: baseline results from the study of women's health across the nation. Am J Epidemiol 2004;159:1189–99.
104. Taku K, Melby MK, Kronenberg F, Kurzer MS, Messina M. Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials. Menopause 2012;19:776–90.
105. Gold EB, Flatt SW, Pierce JP, Bardwell WA, Hajek RA, Newman VA, Rock CL, Stefanick ML. Dietary factors and vasomotor symptoms in breast cancer survivors: the Whel Study. Menopause 2006;13:423–33.
106. Appling S, Paez K, Allen J. Ethnicity and vasomotor symptoms in postmenopausal women. J Womens Health (Larchmt) 2007;16:1130–8.
107. Kroenke CH, Caan BJ, Stefanick ML, Anderson G, Brzyski R, Johnson KC, Leblanc E, Lee C, La Croix AZ, Park HL, et al. Effects of a dietary intervention and weight change on vasomotor symptoms in the Women's Health Initiative. Menopause 2012;19:980–8.
108. Kant AK. Dietary patterns and health outcomes. J Am Diet Assoc2004;104:615–35.
109. Messina M, Hughes C. Efficacy of soy foods and soybean isoflavone supplements for alleviating menopausal symptoms is positively related to initial hot flash frequency. J Med Food 2003; 6:1-11
110. Setchell KDR, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. J Nutr 1999;129:758S-767S.

111. Markiewicz L, Garey J, Adlercreutz H, Gurpide E. In vitro bioassays of non-steroidal phytoestrogens. J Steroid Biochem Mol Biol 1993;45:399-405.

112. Axelson M, Sjovall J, Gustafsson B, Setchell KDR. Soya- A dietary source of the non-steroidal oestrogen equol in humans and animals. J Endocrin 1984;102:49-56.

113. Joannou GE, Kelly GE, Reeder AY, Waring MA, Nelson C. A urinary profile study of dietary phytoestrogens. The identification and mode of metabolism of new isoflavonoids. J Steroid Biochem Molec Biol 1995;54:167-184.

114. Axelson M, Setchell KDR. The excretion of lignans in rats - Evidence for an intestinal bacterial source for this new group of compounds. FEBS Lett 1981;123:337-342.

115. King RA, Broadbent JL, Head RJ. Absorption and excretion of the soy isoflavone genistein in rats. J Nutr 1996;126:176-182.

116. Sfakianos J, Coward L, Kirk M, Barnes S. Intestinal uptake and biliary secretion of the isoflavone genistein in the rat. J. Nutr 1997;127:1260-1268.

117. Kenneth D. R. Setchell, Ph.D. Absorption and Metabolism of Isoflavones .

118. Kim H, Li Xia H, Gewin L. Attenuation of neurodegeneration-relevant modification of brain proteins by dietary soy. Biofactors 2000;12: 243-250.

119. Nahas EP, Neto JN, De Luca L, Traiman P, Pontes A, Dalben I. Benefits of soy germ isoflavones in postmenopausal women with contraindication for conventional hormone replacement therapy. Maturitas 2004; 48:372-380.

120. Han KK, Soares Jr JM, Haidar MA, de Lima GR, Baracat EC. Benefits of soy isoflavone therapeutic regimen on menopausal symptoms. Obstet Gynecol 2002; 99:389-394.

121. American Heart Association. 1999 Heart and Stroke Statistical Update. Dallas, TX: American Heart Association, 1999.

122. Kannel WB. Metabolic risk factors for coronary heart disease in women: perspective from the Framingham Study. Am Heart J 1987;114:413-419.

123. Peters HW, Westendorp IC, Hak AE, et al. Menopausal status and risk factors for cardiovascular disease. J Intern Med 1999; 246:521-528.

124. Bonithon-Kopp C, Scarabin PY, Darne B, Malmejac A, Guize L. Menopause-related changes in lipoproteins and some other cardiovascular risk factors. Int J Epidemiol 1990;19:42<48.

125. Stevenson JC, Crook D, Godsland IF. Influence of age and menopause on serum lipids and lipoproteins in healthy women. Atherosclerosis 1993;98:83-90.

126. Jensen J, Nilas L, Christiansen C. Influence of menopause on serum lipids and lipoproteins. Maturitas 1990;12:321-331.

127. Akahoshi M, Soda M, Nakashima E, Shimaoka K, Seto S, Yano K. Effects of menopause on trends of serum cholesterol, blood pressure, and body mass index. Circulation 1996;94: 61-66.

128. Van Beresteijn E, Korevaar J, Huijbregts P, Schouten E, Burema J, Kok F. Perimenopausal increase in serum cholesterol: a 10 year longitudinal study. Am J Epidemiol 1993;137:383-392.

129. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333:276-282.

130. Zhan S, Ho S. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr 2005;81:397-408.

131. Jerilyn K. Allen, Diane M. Becker, Peter O. Kwiterovich, Kathleen A. Lindenstruth and Carol Curtis. Effect of soy protein-containing isoflavones on lipoproteins in postmenopausal women. Menopause 2007 Vol. 14, No. 1, pp. 106-114.

132. Sagara M, Kanda T, NJelekera M, et al. Effects of dietary intake of soy protein and isoflavones on cardiovascular disease risk factors in high risk, middle-aged men in Scotland. J Am Coll Nutr 2003; 23:85–91.

133. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173–194, 1991.

134. Reaven GM: Insulin resistance and compensatory hyperinsulinemia: role in hypertension, dyslipidemia, and coronary heart disease. *Am Heart J* 121:1283–1288, 1991.

135. Walton C, Godsland IF, Proudler AJ, Wynn V, Stevenson JC: The effects of the menopause on insulin sensitivity, secretion and elimination in non-obese, healthy women. *Eur J Clin Invest* 23:466–473, 1993.

136. Proudler AJ, Felton CV, Stevenson JC: Ageing and the response of plasma insulin, glucose, and C-peptide concentrations to intravenous glucose in postmenopausal women. *Clin Sci* 83:489–494, 1992.

137. **Reaven G, Lithell H, Landsberg L** 1996 Mechanisms of disease: hypertension and associated metabolic abnormalities—the role of insulin resistance and the sympathoadrenal system. N Engl J Med 334:374–381.

138. **McIntyre EA, Walker M** 2002 Genetics of type 2 diabetes and insulin resistance: knowledge from human studies. Clin Endocrinol (Oxf) 57:303–311

139. **Moller D, Flier J** 1991 Insulin resistance—mechanisms, syndromes, and implications. N Engl J Med 325:938–948.

140. **Gaspard UJ, Gottal J-M, den Brule FA** 1995 Postmenopausal changes of lipid and glucose metabolism: a review of their main aspects. Maturitas 21:171–178.

141. Anderson JW, Smith BM, Washnock CS: Cardiovascular and renal benefits of dry bean and soybean intake. *Am J Clin Nutr* 70:464S–474S, 1999.

142. Vedavanam K, Srijayanta S, O'Reilly J, Raman A, Wiseman H: Antioxidant action and potential antidiabetic properties of an isoflavonoid-containing soyabean phytochemical extract (SPE). *Phytother Res* 13: 601–608, 1999.

143. Lee DS, Lee SH: Genistein, a soy isoflavone, is a potent alpha-glucosidase inhibitor. *FEBS Lett* 501:84–86, 2001.

144. Sorenson RL, Brelje TC, Roth C: Effect of tyrosine kinase inhibitors on islets of Langerhans: evidence for tyrosine kinases in the regulation of insulin secretion. *Endocrinology* 134:1975–1978, 1994.

145. Wagner JD, Cefalu WT, Anthony MS, Litwak KN, Zhang L, Clarkson TB: Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesteryl ester content in ovariectomized cynomolgus monkeys. *Metabolism* 46:698–705, 1997.

146. Duncan AM, Underhill KE, Xu X, Lavalleur J, Phipps WR, Kurzer MS: Modest hormonal effects of soy isoflavones in postmenopausal women. *J Clin Endocrinol Metab* 84:3479–3484, 1999.

147. Goodman-Gruen D, Kritz-Silverstein D: Usual dietary isoflavone intake is associated with cardiovascular disease risk factors in postmenopausal women. *J Nutr* 131:1202–1206, 2001.

148. Hermansen K, Sondergaard M, Hoie L, Carstensen M, Brock B: Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. *Diabetes Care* 24:228–233, 2001.

149. Tsai AC, Vinik AI, Lasichak A, Lo GS: Effects of soy polysaccharide on postprandial plasma glucose, insulin, glucagon, pancreatic polypeptide, somatostatin, and triglyceride in obese diabetic patients. *Am J Clin Nutr* 45:596–601, 1987.

150. Mahalko JR, Sandstead HH, Johnson LK, Inman LF, Milne DB, Warner RC, Haunz EA: Effect of consuming fiber from corn bran, soy hulls, or apple powder on glucose tolerance and plasma lipids in type II diabetes. *Am J Clin Nutr* 39:25–34, 1984.

151. Thomas BL, Laine DC, Goetz FC: Glucose and insulin response in diabetic subjects: acute effect of carbohydrate level and the addition of soy polysaccharide in definedformula diets. *Am J Clin Nutr* 48:1048–1052, 1988.

152. Booth NL, Piersen CE, Banuvar S, Geller SE, Shulman LP, Farnsworth NR. Clinical studies of red clover (Trifolium pratense) dietary supplements in menopause: a literature review. Menopause 2006; **13**: 251–264.

153. Boonkasemsanti W, Reinprayoon D, Pruksananonda K et al. The effect of transdermal oestradiol on bleeding pattern, hormonal profiles and sex steroid receptor distribution in the endometrium of Norplant users. Hum Reprod 1996; **11**(Suppl. 2): 115–123.

154. Blakesmith SJ, Lyons-Wall PM, George C, Joannou GE, Petocz P, Samman S. Effects of supplementation with purified red clover (Trifolium pratense) isoflavones on plasma lipids and insulin resistance in healthy premenopausal women. Br J Nutr 2003; **89**: 467–474.

155. Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 1997; 277: 1775–81.

156. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997; 337: 230–6.

157. Hak AE, Polderman KH, Westendorp IC, Jacobs C, Hofman A, Witteman JC et al. Increased plasma homocysteine after menopause. Atherosclerosis 2000; 149: 163–8.
158. Gol M, Akan P, Dogan E, Karas C, Saygili U, Posaci C. Effects of estrogen, raloxifene, and hormone replacement therapy on serum C-reactive protein and homocysteine levels. Maturitas 2006; 53: 252-9.
159. Reimann M, Dierkes J, Carlsohn A, Talbot D, Ferrari M, Hallund J et al. Consumption of soy isoflavones does not affect plasma total homocysteine or asymmetric dimethylarginine concentrations in healthy postmenopausal women J Nutr 2006; 136: 100-5.
160. D'Anna R, Baviera G, Corrado F, Cancellieri F, Crisafulli A, Squadrito F. The effect of the phytoestrogen genistein and hormone replacement therapy on homocysteine and Creactive protein level in postmenopausal women. Acta Obstet Gynecol Scand 2005; 84: 474-7.
161. Jenkins DJ, Kendall CW, Jackson CJ, Connelly PW, Parker T, Faulkner D et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr 2002; 76: 365-72.
162. Hanson LN, Engelman HM, Alekel DL, Schalinske KL, Kohut ML, Reddy MB. Effects of soy isoflavones and phytate on homocysteine, C-reactive protein, and iron status in postmenopausal women. Am J Clin Nutr 2006; 84: 774-80.
163. Ridker PM, Buring JE, Shih J, Matias MBS, Hennekens CH 1998 Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 98:731–733.
164. Lagrand WK, Visser CA, Hermens WT, Niessen HWM, Verheugt FWA, Wolbink G-J, Hack CE 1999 C-reactive protein as a cardiovascular risk factor. Circulation 100:96–102.
165. Ridker PM, Hennekens CH, Buring JE, Rifai N 2000 C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836–843.
166. Ross R 1999 Atherosclerosis: an inflammatory disease. N Engl J Med 340:115–123
167. Lagrand WK, Niessen HWM, Wolbink G-J, Jaspars LH, Visser CA, Verheugt FWA, Meijer CJLM, Hack CE 1997 C-Reactive protein colocalizes with complement in human hearts during acute myocardial infarction. Circulation 95:97–103.

168. Pasceri V, Willerson JT, Yeh ETH 2000 Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation 102:2165–2168.

169. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM 2000 Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. Circulation 102:1000–1006.

170. Torzewski M, Rist C, Mortensen RF, Zwaka TP, Bienek M, Waltenberger J, Koenig W, Schmitz G, Hombach V, Torzewski J 2000 C-reactive protein in the arterial intima: role of C-reactive protein receptor-dependent monocyte recruitment in atherogenesis. Arterioscler Thromb Vasc Biol 20:2094–2099.

171. Libby P, Ridker PM, Maseri A 2002 Inflammation and atherosclerosis. Circulation 105:1135–1143.

172. Jenkins DJ, Kendall CW, Connelly PW, Jackson CJ, Parker T, Faulkner D, Vidgen E 2002 Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. Metabolism 51:919–924.

173. US Census Bureau. The older population in the United States: 2010 to 2050. May 2010. Available at: http://www.census.gov/prod/2010pubs/ p25-1138.pdf. Accessed May 15, 2012.

174. Soules MR, Sherman S, Parrott E, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW), Park City, Utah, July, 2001. Menopause 2001;8:402-407.

175. Twiss JJ, Wegner J, Hunter M, Kelsay M, Rathe-Hart M, Salado W (2007). "Perimenopausal symptoms, quality of life, and health behaviors in users and nonusers of hormone therapy". *J Am Acad Nurse Pract* 19 (11): 602–13. doi:10.1111/j.1745-7599.2007.00260.x. PMID 17970860.

176. Rossouw JE, Anderson JL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML et al. Risk and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. JAMA 2002; 288: 321–33.

177. Pradahan AD, Manson JE, Rossouw JE, Siscovick DS, Mouton CP, Rifai N et al. Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart

disease: prospective analysis from women's health initiative observational study. JAMA 2002; 288: 980–7.

178. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy; collaborative re-analysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 1997; 350: 1047–59.
179. Beral V, Million Women Study Collaborators. Breast cancer and hormone replacement therapy in the Million Women Study. Lancet 2003; 362: 419–27.
180. Jenkins DJ, Kendall CW, Jackson CJ, Connelly PV, Parker T, Faulkner D et al. Effects of high- and low-isoflavone soy foods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr 2002; 76: 365–72.
181. Beynen AC. Mode of cholesterolemic action of dietary proteins. Monogr Atheroscler. 1990;16:153–159.
182. Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr. 1995; 125:606S–611S.
183. De Kleijn MJJ, van der Schouw YT, Wilson PWF, Grobbee DE, Jacques PF. Dietary intake of phytoestrogens is associated with a favourable metabolic cardiovascular risk pro.le in postmenopausal U.S. women: the Framingham study. J Nutr. 2002; 132:276 – 282.
184. Uesugi T, Fukui Y, Yamori Y. Bene.cial effects of soybean iso avone supplementation on bone metabolism and serum lipids in postmenopausal Japanese women: a four-week study. J Am Coll Nutr. 2002;21:97–102.
185. Hsu C-S, Shen WW, Hsueh Y-M, Yeh S-L. Soy iso avone supplementation in postmenopausal women. J Reprod Med. 2001;46:221–226.
186. Shao-Yi Cheng, Ning-Sing Shaw, Ke-Sung Tsai, Ching-Yu Chen. The HHypoglycemic effects of Soy Isoflavones on Postmenopausal Women. J of Women's Health. 2004; 13(10):1080-86.
187. Zhao-min Liu, Yu-ming Chen, Suzanne C Ho, Yee Ping Ho, Jean Woo. Effects of soy protein and isoflavones on glycemic control and insulin sensitivity: a 6-mo double-bind, rabdomized, placebo-controlled trial in postmenopausal Chinese women with prediabetes or untreated early diabetes [1-4]. Am J Clin Nutr 2010; 91: 1394–401.

188. Goodman-Gruen D, Kritz-Silverstein D 2001 Usual dietary isoflavone intake is associated with cardiovascular disease risk factors in postmenopausal women. J Nutr 131:1202–1206.

189. Eini nikander, Aila tiitinen, Kalevi laitinen, Matti tikkanen, Olavi ylikorkala. Effects of Isolated Isoflavonoids on Lipids, Lipoproteins, Insulin Sensitivity, and Ghrelin in Postmenopausal Women. The Journal of Clinical Endocrinology & Metabolism 89(7):3567–3572.

190. Nikander E, Kilkkinen A, Metsa¨-Heikkila¨ M, Adlercreutz H, Pietinen P, Tiitinen A, Ylikorkala O 2003 A randomized placebo-controlled cross-over trial with phytoestrogens in treatment of menopause in breast cancer patients. Obstet Gynecol 101:1213–1220.

191. Jenkins DJA, Kendall CW, Connelly PW, et al. Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. Metabolism 2002;51:919 –24.

192. Zhang SX, Shu X, Gao YT, Yang G, Li Q, Li H et al. (2003). Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. J Nutr 133, 2874–2878.

193. Puska P, Korpelainen V, Hoie LH, Skovlund E, Lahti T, Smerud KT (2002). Soy in hypercholesterolaemia: a double-blind, placebocontrolled trial. Eur J Clin Nutr 56, 352–357.

194. Tonstad S, Smerud K, Hoie L (2002). A comparison of the effects of 2 doses of soy protein or casein on serum lipids, serum lipoproteins, and plasma total homocysteine in hypercholesterolemic subjects. Am J Clin Nutr 76, 78–84.

195. D'Anna R, Baviera G, Corrado F, Cancellieri F, Crisafulli A, Squadrito F (2005). The effect of the phytoestrogen genistein and hormone replacement therapy on homocysteine and C-reactive protein level in postmenopausal women. Acta Obstet Gynecol Scand 84, 474–477.

196. Reimann M, Dierkes J, Carlson A, Talbot D, Ferrari M, Hallund J et al. (2006). Consumption of soy isoflavones does not affect plasma total homocysteine or asymmetric dimethylarginine concentrations in healthy postmenopausal women. J Nutr 136, 100–105.

197. Roughead Z, Hunt J, Johnson J, Badger T, Lykken G (2005). Controlled substitution of soy protein for meat protein: effects on calcium retention, bone, and cardiovascular health indices in postmenopausal women. J Clin Endocrinol Metab 90, 181–189.

198. Hanson LN, Engelman HM, Alekel DL, Schalinske KL, Kohut ML, Reddy MB (2006). Effects of soy isoflavones and phytate on homocysteine, C-reactive protein, and iron status in postmenopausal women. Am J Clin Nutr 84, 774–780.

199. Nagata C, Shimizu H, Takami R, Hayashi M, Takeda N, Yasuda K. Soy product intake is inversely associated with serum homocysteine level in premenopausal Japanese women. J Nutr 2003;133:797– 800.

200. Jenkins JA, Kendall CWC, Jackson CJC, et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr 2002;76:365–72.

201. Emma Barinas-Mitchell, Mary Cushman, Elaine N. Meilahn, Russell P. Tracy, and Lewis H. Kuller. Serum Levels of C-reactive Protein Are Associated with Obesity,Weight Gain,and Hormone Replacement Therapy in Healthy Postmenopausal Women. *Am J Epidemiol* Vol. 153, No. 11, 2001.

202. Dimitri Edin Zylberstein, Calle Bengtsson, Cecilia Björkelund, Sverre Landaas, Valter Sundh, Dag Thelle, Lauren Lissner. Serum Homocysteine in Relation to Mortality and Morbidity From Coronary Heart Disease:A 24-Year Follow-Up of the Population Study of Women in Gothenburg. Circulation. 2004;109:601-606.

বাংলাদেশের Menopause (মেনোপজ বা ঋতুপরবর্তী) মহিলাদের রক্তে Homocysteine এবং C-reactive protein - এর পরিমাণের উপর Isoflavones- এর প্রভাব সম্পর্কিত গবেষণা প্রকল্প

সম্মতি পত্র

ক্রমিক নং ঃ-

আমি ---------- বাংলাদেশের ঋতুপরবর্তী মহিলাদের রক্তে Homocysteine এবং C-reactive protein - এর পরিমাণের উপর Isoflavones- এর প্রভাব সম্পর্কিত গবেষণায় অংশগ্রহনকারী হিসাবে তথ্য প্রদানে আমার সম্মতি প্রদান করিতেছি।

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

স্বাক্ষরঃ-

তারিখঃ-

ঠিকানাঃ-

Case Record Form

Sl No. - **Date:**

1. **Name:** _____

   Father's / Husband's Name: _____

   Mother's Name: _____

2. **Age:** _____years

3. **Gender:** Male/ Female

4. **Marital status:** Married _____yrs/ Unmarried

5. **No of Children**: ……Age (serially 1st -last)……

6. **LMP** (Last menstrual period):

7. **Area:** Rural/ Urban/ Semi urban

8. **Address**

Permanent:_____

_____

Phone: Off: _____ Cell: _____

Res: _____ Tnt: _____

9. Socioeconomic status

- Earning capacity (1. Earner 2. Dependent) _____
- No of earners in the family _____
- No of family members sharing the same kitchen_____
- Totally family monthly expenditure _____

10. Educational status: Illiterate-Primary-1/ SSC-HSC-2/ Graduate & Above-3

11. Occupational status: Housewife-1/ Service-2/ Others-3 (Specify)…….

12. Religion: Muslim/Hindu/Christian/ Buddhist

13. Any history of past illness or any chronic diseases

Patient history: Hypertension_____ Diabetes_____

Renal disease_____ Cardiac disease_____

Hepatic disease_____ Dislipidemia _____

GDM in previous pregnancy_____

No. of child: _____Before 28 weeks of gestation

After 28 weeks of gestation

Family history: Hypertension_____ Diabetes_____

Renal disease_____ Cardiac disease_____

Hepatic disease_____ Dislipidemia _____

14. Drug History

Trade Name	Generic Name	mg	dose	Duration

15. Physical Activity Level (minutes per day)

At Work: _____At Home: _____During Leisure Time: _____m/d

16. History of addiction

a. Smoking: Yes/ No (if yes; duration _____ yrs; Average ____ sticks/ day)

Past history: Yes/ No (if yes; duration _____yrs; Average ____ sticks/ day)

b. Tobacco leaf chewing/ using powder (Gull): Yes/ No (if yes; duration _____ yrs)

Past history: Yes/ No (if yes; duration _____ yrs)

c. Betel leaf chewing: Yes/ No (if yes; duration _____ yrs)

Past history: Yes/ No (if yes; duration _____ yrs)

d. Tobacco Powder sniffing: Yes/ No (if yes; duration _____ yrs)

Past history: Yes/ No (if yes; duration _____ yrs)

e. Drinking alcohol: Yes/ No (if yes; duration _____ yrs)

Past history: Yes/ No (if yes; duration _____ yrs)

17. Clinical examinations:

Blood pressure	**1st reading**	**2nd reading**	**3nd reading**	**Average reading**
Systolic				
Diastolic				
Pulse rate				

18. Anthropometrical examinations:

Ht (m)		Wt (Kg)	
Waist (cm)		Hip (cm)	

19. Biochemical Investigations:

Sl No.	**Biochemical parameters**	**Result**	**Result in SI unit**	**Reference Range**
1	S Glucose (F)	mg/dl	mmol/L	3.6- 5.6
2	S Glucose 2 hrs after 75 gm glucose load	mg/dl	mmol/L	<7.8
3	S Cholesterol	mg/dl	mmol/L	150- 200
4	S HDL-C	mg/dl	mmol/L	M:>55;F:>65
5	S LDL-C	mg/dl	mmol/L	<150
6	S TG	mg/dl	mmol/L	50- 150
7	S Creatinine	mg/dl	mmol/L	0.67- 1.2
8	S GPT	U/L		Upto 40