# ผลของการให้เจนนิสทีนต่อระดับไขมันในพลาสมาและต่อการขยายตัว

# ของหลอดเลือดโคโรนารีในหนูแรทที่ถูกตัดรังไข่

นางสาว ขวัญหญิง มลศิริ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ปีการศึกษา 2545

# EFFECTS OF GENISTEIN ON PLASMA LIPID PROFILES AND CORONARY VASODILATION IN OVARIECTOMIZED RATS

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Physiology

Inter-departmental Program in Physiology

Graduate School

Chulalongkorn University

Academic year 2002

Thesis Title        Effects of genistein on plasma lipid profiles and
                    coronary vasodilation in ovariectomized rats
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ขวัญหญิง มลศิริ: ผลของการให้เจนนิสทีนต่อระดับไขมันในพลาสมา และต่อการขยายตัวของหลอดเลือดโคโรนารี ในหนูแรทที่ถูกตัดรังไข่ (EFFECTS OF GENISTEIN ON PLASMA LIPID PROFILES AND CORONARY VASODILATION IN OVARIECTOMIZED RATS) อ.ที่ปรึกษา:รศ.นพ.ประสงค์ ศิริวิริยะกุล, อ. ที่ปรึกษาร่วม: รศ.ดร.สุทธิลักษณ์ ปทุมราช; 104 หน้า ISBN 974-17-2984-7

วัตถุประสงค์ของการศึกษาครั้งนี้เพื่อศึกษาผลของเจนนิสทีนต่อระดับไขมันในพลาสมาและต่อการขยายตัวของหลอดเลือดโคโรนารีในหนูแรทที่ถูกตัดรังไข่ ซึ่งศึกษาการให้เจนนิสทีนที่ช่วงเวลา 4 และ 10 สัปดาห์ หัวใจที่ถูกทำให้หยุดเต้นแล้วตัดแยกออกมาเตรียมเพื่อศึกษาการตอบสนองของหลอดเลือดแดงรองโคโรนารีจากการให้สารอะซิทิลโคลิน $10^{-5}$โมลาร์และโซเดียม ไนโทรพรัสไซด์ $10^{-4}$ โมลาร์ การเปลี่ยนแปลงของหลอดเลือดหลังจากหยดสารเหล่านี้วิเคราะห์โดยใช้กล้องจุลทรรศน์ฟลูโอเร็สเซ็นซ์ ร่วมกับโปรแกรมการวิเคราะห์ภาพแบบดิจิตอล

ผลการทดลองของหลอดเลือดแดงรองโคโรนารีที่นำมาศึกษาจะแสดงในรูปร้อยละของค่าเฉลี่ยการเปลี่ยนแปลงของหลอดเลือด ซึ่งพบว่าความผิดปกติของการตอบสนองของหลอดเลือดแดงรองโคโรนารีต่อสารอะซิทิลโคลิน ในหนูกลุ่มที่ถูกตัดรังไข่แตกต่างจากกลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ สิ่งที่น่าสนใจคือเมื่อให้เจนนิสทีน 0.2 มก.ต่อนน.1 กก.ต่อวันโดยการฉีดทางใต้ผิวหนังทุกวันสามารถลดความผิดปกติเหล่านี้ได้ ผลการทดลองของโซเดียม ไนโทรพรัสไซด์ พบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญระหว่างกลุ่มควบคุม, กลุ่มตัดรังไข่ และกลุ่มตัดรังไข่ที่ได้รับเจนนิสทีน นอกจากนี้ยังพบว่าการตัดรังไข่ทั้งสองข้างและการให้เจนนิสทีนไม่ทำให้เกิดการเปลี่ยนแปลงระดับไขมันในพลาสมาและต่อการทำงานของหัวใจ ได้แก่ ความดันซิสโตลิค ความดันไดแอสโตลิค ความดันเลือดแดงเฉลี่ย อัตราการเต้นของหัวใจ

จากผลการทดลองสรุปได้ว่าการให้เจนนิสทีนน่าจะสามารถป้องกันความผิดปกติของการตอบสนองของหลอดเลือดแดงรองโคโรนารีในหนูแรทที่ถูกตัดรังไข่

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ปีการศึกษา................2545.....................................     ลายมือชื่ออาจารย์ที่ปรึกษา................................................................

                                                             ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.........................................................

##4289653920:MAJOR PHYSIOLOGY

KEYWORD: GENISTEIN/LIPID PROFILES/VASODILATION/OVARIECTOMIZED
RATS

KHUANYING MONSIRI: EFFECTS OF GENISTEIN ON PLASMA LIPID
PROFILES AND CORONARY VASODILATION IN OVARIECTOMIZED
RATS. THESIS ADIVISOR: ASSOC.PROF.PRASONG
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PATUMRAJ, Ph.D.104 pp. ISBN 974-17-2984-7

The purpose of this study was to examine the effects of genistein on plasma lipid profiles and coronary vasodilation in ovariectomized rats at 4 and 10 weeks. The isolated arrested hearts were prepared to investigate coronary arteriolar responses to acetylcholine (Ach;$10^{-5}$ M) and sodium nitroprusside (SNP; $10^{-4}$ M). Changes in diameter to topical applications of these agents were determined by using intravital fluorescence microscopy and digital image processing analysis.

The results assessed by means percent changes of studied arteriolar diameter indicated that the impairment of coronary arterioles to Ach were significantly obtained in ovariectomized rat as compared to their age-matched controls. Interestingly, these abnormalities of arteriolar responses were attenuated by daily injection of genistein (0.2 mg/kg bw/day). However, in the experiments using SNP found that there was no significant difference among those groups of sham ovariectomized rats, ovariectomized rats and ovariectomized injection with genistein. Moreover, bilateral ovariectomy and treatment with genistein did not alter the lipid profiles and the cardiovascular functions includings systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate.

It may conclude that genistein could prevent endothelial dysfunction in ovariectomized rats.

Field of study…….Physiology…….          Student's signature……………………………...

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Academic year……2002…………...          Co-adivisor's signature………………………

# ACKNOWLEDGEMENTS

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| AA | = | arachidonic acid |
| Ach | = | acetylcholine |
| CAD | = | coronary artery disease |
| DBP | = | diastolic blood pressure |
| EDRF | = | enothelium-derived relaxation factor |
| ER | = | estrogen receptors |
| ER$\beta$ | = | estrogen receptor $\beta$ |
| ER$\alpha$ | = | estogen receptor $\alpha$ |
| HDL-C | = | high density lipoprotein cholesterol |
| HR | = | heart rate |
| LDL-C | = | low density lipoprotein cholesterol |
| L-NAME | = | N$\omega$-Nitro-L-arginine methyl ester |
| M | = | molar |
| MAP | = | mean arterial blood pressure |
| mg/kg bw | = | milligram per kilogram body weight |
| ml/min | = | millimeter per minute |
| mmHg | = | millimeter of mercury |
| NE | = | norepinephrine |
| NO | = | nitric oxide |
| PGI$_2$ | = | prostacyclin |
| SBP | = | systolic blood pressure |

| | |
|---|---|
| sham-ovx rats | = sham ovariectomized rats |
| SNP | = sodium nitroprusside |
| ovx-genistein | = ovariectomized genistein rats |
| ovx-rats | = ovariectomized rats |

# CHAPTER I

# INTRODUCTION

During menopause, the hormonal changes can cause a variety of uncomfortable symptoms such as hot flashes, night sweats, insomnia, vaginal dryness, or headaches.  Especially, it can increase the risk of heart disease and osteoporosis (Lock, 1994; American Heart Association, 1997; National Osteoporosis Foundation, 1997).  There are two kinds of estrogen receptor, alpha (ER$\alpha$) and beta (ER$\beta$), and they are founded in almost every organs of the body (Kuiper et al., 1997).  Some organs have predominantly ER$\alpha$, such as kidney, uterus, pituitary, and epididymis.  Others have equal or greater amounts of ER$\beta$, including ovary, prostate, and brain (Kuiper et al., 1997).  Estrogen receptors are present in the cardiovascular system and bone, and that estrogen plays an important role in the health of these tissues.

The incidence of coronary artery disease (CAD) is lower in premenopause women than in age-matched men (McGill and Stern, 1979; Kalin and Zumoff, 1990).  After the menopause, the risk of coronary artery disease rises to levels equivalent to those of men (Kalin et al., 1990; Stampfer et al., 1991; Bush et al., 1987; Gruchow et al., 1998).  However, the risk of CAD in women rapidly increases and gradually approaches that in men at older ages.  The annual incidence of CAD in women aged 55 to 64 is seven times higher than that in woman aged 35 to 44 (Walsh et al., 1991).  Although CAD is more aged-dependent in women than in men, a number of well-established risk factors contribute to CAD in women as they do in men.

These include smoking, diabetes, hypertension, obesity, and physical inactivity. In addition, hyperlipidemia plays a significant role in the development of atherosclerotic heart disease in men and women. Changes in lipid levels after menopause are associated with increased cardiovascular risks. Lipid parameters gradually change to effect a 25% increase in low density lipoprotein cholesterol (LDL-C) and apolipoprotein B and a 15% increase in total cholesterol (Bonithon et al., 1990). Although lipid levels in men also gradually increase with age, the elevations in total cholesterol and LDL-C are not of the same magnitude as those in women. Additionally, modest elevations in triglyceride and lipoprotein (a) [Lp(a)] levels also occur in postmenopausal women (Schriewer et al., 1984). In contrast, levels of high density lipoprotein cholesterol (HDL-C) and its subclass $HDL_2$ remain unchanged or decline only slightly after menopause. Overall, postmenopausal lipoprotein changes result in a significant increase in cardiovascular risk for women.

Epidemiological and clinical evidence suggests that estrogen is cardioprotective, because hormone replacement therapy reduces the risk of coronary artery disease in women (Stampfer et al., 1991; Barrett et al., 1978; Lobo et al., 1994). However, the mechanisms by which estrogen is cardioprotective are incompletely understood. Although estrogen lower LDL-C and increases HDL-C, these changes account for only 25% of its cardioprotecteve effect in women (Bush et al., 1987; Gruchow et al., 1998). The remaining 75% is accounted by the alternative mechanisms. Estrogen may be cardioprotective by enhancing vasodilation of the coronary circulation. Functional estradiol receptors are present on both endothelial

(Colburn et al., 1978) and vascular smooth muscle cells (Karas et al., 1994; Losordo et al., 1994).

More and more evidences suggest that estrogen has a direct effect on the blood vessel wall. It indicates that vascular endothelium may play a key role in mediating these effects by producing several vasoactive factors and more specifically nitric oxide (NO)( Kleinert et al., 1988). In fact estrogen replacement therapy (HRT) in postmenopausal improved the impaired endothelial-dependent relexation ( Gerhard et al., 1998). However, estrogens also have adverse effects on the reproductive system of female (Grady et al., 1995) and male (Robinson et al., 1963) that limit their therapeutic potential. Those adverse effects of estrogen are the induction of breast and uterine neoplasms and its tendency to increase coagulability (Lissin and cooke, 2000).

Soy contains phytoestrogens in the form of isoflavones, genistein and daidzein. Phytoestrogens have similar structure to estradiol and have weak affinity for the estrogen receptors. Several studies have indicated that a total daily intake of 25 g of soy protein paired with a low-fat diet resulted in clinically important reductions of total cholesterol and LDL-C levels. Soybeans are a rich source of isoflavones, a class of phytoestrogens found predominantly in legumes and beans. Soy isoflavones are heterocyclic phenols with structural similarity to 17β-estradiol and selective estrogen receptor modulators. Actions at the cellular level depend on the target tissue, receptor status of the tissue, and the level of endogenous estrogen. Studies of soy-based diets evaluating the relationship between soy consumption and serum lipid concentrations revealed that soy consumption

significantly decreased total cholesterol, LDL-C and triglyceride levels. However, soy isoflavones do not increase HDL-C or triglyceride levels. The effects of soy protein on other target tissues reflect estrogen like agonist and antagonist effects (ANN and Lorraine, 2000).

A traditional Asiatic phytoestogen-rich diet is associated with a lower incidence of breast and uterine cancer, menopausal symptoms, and much evidence indicates that phytoestrogens prevent bone resorption, increase bone density and reduce cholesterol. The estrogenic effects of phytoestrogens can be useful in preventing postmenopausal osteoporosis and cardiovascular disease (Chiechi, 1999). In summary, phytoestrogens, in the form of dietary isoflavones, might be a new nutritional approach to cardiovascular protection.

According to the literature reviewed as indicated above, it has been demonstrated that phytoestrogens has its good role on cardioprotection like estrogen. However, mechanisms of both estrogen and phytroestogens have not yet been clarified. Whether it is based directly on lipid lowering property or on its direct effect on endothelial function. Therefore, our objectives are:

1. To study the effect of genistein on lipid profiles in bilateral ovariectomized rats.
2. To study the effect of genistein on coronary vasodilation in bilateral ovariectomized rats.

# CHAPTER II

# REVIEWS OF LITERATURE

## Phytoestrogens

Phytoestrogens are naturally occurring estrogens that may have beneficial effects on the cardiovascular system and may also alleviate common illnesses afflicting women, such as menopausal symptoms, osteoporosis and breast cancer.  Phytoestrogens may have advantages over conventional estrogens in that they may lower LDL-C without inducing hypertriglyceridemia (Anderson et al, 1995); they may relieve menopausal symptoms without increasing the risk of uterine or breast neoplasia (Giidman et al., 1996); they may enhance vascular function without accelerating pathological angiogenesis (Fotsis et al., 1993); and there are no reports of thrombotic events.  There is not yet enough evidence from large randomized trials to make an unqualified recommendation about the use of phytoestrogens, but accumulating data indicate that phytoestrogens may be an alternative therapy for postmenopausal women at risk for cardiovascular disease.

Isoflavones, lignans and coumestans are major sources of phytoestrogens. Common and significant sources of phytoestrogens are soybeans (isoflavones), cereals and oilseeds such as flaxseed (lignans) and alfalfa sprouts (coumestans).   The most common and best studied phytoestrogens is the class of isoflavones.  The most abundant active components of isoflavones are genistein and daidzein.  These agents appear

to have selective estrogenic actions, for exsample in some tissues they display proestrogenic responses, whereas in other, they inhibit estrogenic effects. The recent identification of a second subtype of estrogen receptor lends support to the theory of selective estrogenic action (Paech et al., 1997; Foegh et al., 1998). Estrogenic activity is dependent on the affinity of binding to the estrogen receptors, which is determined by the presence of the aromatic ring as well as hydroxyl groups at specific cites (Martucci and Fishman, 1993). Compared with estradiol, genistein and daidzein bind estrogen receptors (ER) with 100 and 1,000 times less affinity, respectively (Adlercruetz et al., 1995). Nevertheless, in the quantities that can be consumed in the diet, isoflavones can have biological effects. Antiestrogenic effects may be due to the competitive inhibition at the estrogen receptor, interference with gonadotrophins, the inhibition of estrogen synthesis or increased synthesis of estrogen-binding protein. Phytoestrogens do not have feminzing effects in male primates as reflected by unchanged weight of the reproductive organs in these animals, although prenatal exposure of rats resulted in diminished weights of ovaries and uteri in female (Awoniyi et al., 1998).

### Isoflavones

Soy contains phytoestrogens in the form of the isoflavones, genistein and daidzein. These are known to have weak estrogenic effects when consumed by animals and humans (Knight et al., 1996). Researchers are studying the physiological effects of the isoflavones to find out whether they can serve some of the same functions as the physiological estrogens, and

thereby decrease the health risks associated with menopause.  Soyfoods are commonly consumed in the Asian countries, providing an estimated 25 to 45 mg of isoflavones per day for the average person (Knight et al., 1996). Japan has the highest consumption of soy, and an estimated 200 mg per day intake of isoflavones.  In the US and Canada, average isoflavone consumption is less than 5 mg per day (Knight et al., 1996).  A cross-cultural study of menopause found that women in Japan rarely reported the symptoms of perimenopause which are common in the West (Lock, 1994). Post-menopausal Japanese women also have lower rates of osteoporosis and heart disease, and a longer life-expectancy (Lock, 1994).  These facts have fueled an interest in research designed  to clarify the relationship between soy consumption and health.
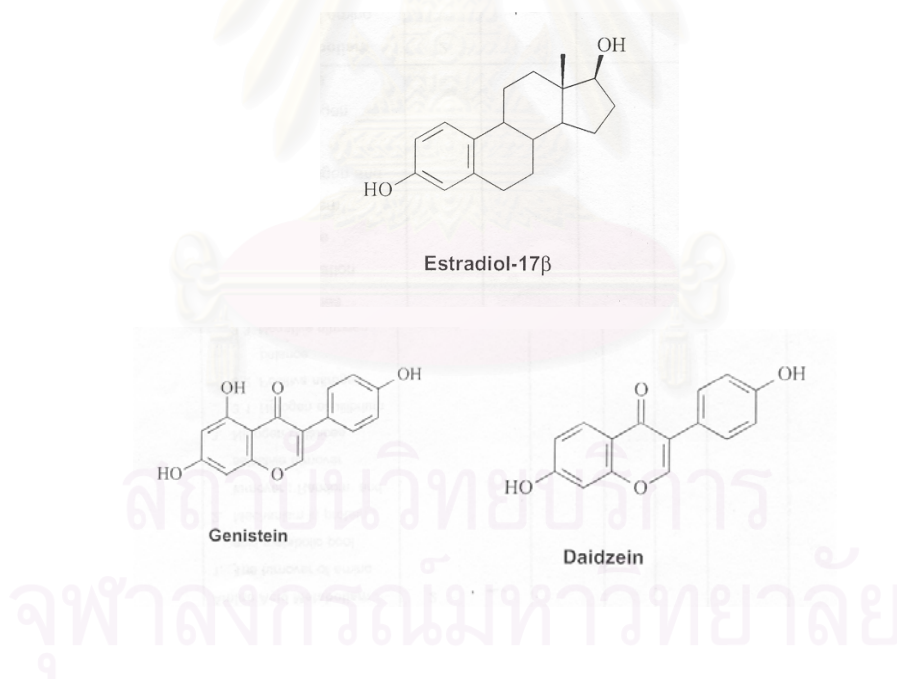


Figure 1. Structural formulars of 17β-estradiol genistein and daidzein.

*Isoflavones absorption and metabolism*

Isoflavones have a similar chemical structure to the mammalian estrogens. After ingestion, the conjugated form of isoflavones are hydrolyzed by the intestinal b-glucosidases, which release the principal bioactive aglycones, daidzein and genistein. These compounds may be absorbed or further metabolized in the distal intestine with the formation of specific metabolites, such as equol and *p*-ethylphenol (Setchell, 2000). Three native b-glucosidases have been identified in humans (Day et al., 1998). The first is glucocerebrosidase, being a lysosomal enzyme which hydrolyses glucoceramide from endogenous membrane glycolipids. Another is lactase phlorizin hydrolase, which is a membrane bound enzyme found in the brush-border of the small intestine, and is primarily responsible for the hydrolysis. The third b-glucosidase is a broad-specificity cytosolic enzyme found in abundance in the liver, kidney, and small intestine of mammals. Some intestinal bacteria produce b-glucuronidases, which can deconjugate these isoflavone metabolites when they pass through the intestine (Setchell, 2000). The aglycones along with any bacterial metabolites are absorbed from the intestinal tract and transported via the portal venous system to the liver, where the isoflavones and their metabolites are efficiently conjugated with glucuronic acid (95%), and to a lesser extent are found as sulfate conjugates (Lundh, 1995). They are then excreted in the urine or in the bile (Zhang et al., 1999). Some isoflavones undergo enterohepatic recycling. It has been proposed that the intestinal metabolism is essential for their subsequent absorption and bioavailability in the body. However, Andlauer et al. (Andlauer et al., 2000) reported that genistin was partly absorbed without previous cleavage. Piskula et al. (Piskula et al., 1999) also demonstrated

that both aglycones and their glucosides are absorbed very fast. These results contradict the above assumption. The results from Izumi et al. (Izumi et al., 2000) showed that the isoflavone aglycones were absorbed faster and in greater amounts than their glucosides in humans. The peak concentrations of isoflavones in the blood are seen generally 4-8 h after dietary intake (Setchell, 2000; Setchell et al., 2001). Most of the daidzein and genistein are excreted in the urine within the first 24 h after food intake (Xu et al., 1994; Lu and Anderson, 1998). The rate of urinary excretion of daidzein was greater than that of genistein throughout the postmeal period (King and Bursill, 1998). Differences are observed in the elimination half-life for different studies. Watanabe et al. (Watanabe et al., 1998) found that after ingestion of 60 g baked soybean powder, the half-lives of plasma genistein and daidzein were 8.36 and 5.79 h, respectively. While with the same foods, from King and Bursill's (King and Bursill, 1998) research, the elimination half-lives were 4.7 and 5.7 h for daidzein and genistein, respectively. More rapid elimination is observed for isoflavones in a liquid matrix than in a solid matrix (Setchell, 2000). In ruminant animals, the absorption of isoflavones takes place mainly in the rumen, where the gastrointestinal epithelium is the major site of metabolism. The liver contributes very little to the total degradation of isoflavones in ruminants (Lundh, 1995). Metabolism of isoflavones in pigs is not as well documented. The pig seems to differ markedly in comparison with ruminants regarding the conjugation of equol. Only 50-70% of equol was found in the conjugated form whereas the corresponding figure for the conjugated equol in plasma from cow and sheep is 95-99%.

The metabolism of isoflavones is also influenced by other components in the diet. For example, a diet rich in carbohydrates that causes increased intestinal fermentation results in more extensive biotransformation of isoflavones and higher levels of equol. The metabolism is blocked in the presence of antibiotics, which alters the intestinal flora (Setchell et al., 1984). However, the presence of different population of microflora in the human gut may also influence the bioavailability of soy isoflavones.

The ability of isoflavones to bind to the ER and produce estrogenic responses was demonstrated in a breast cancer cell line treated with genistein and daidzein. The ability of these compounds to effectively compete with estradiol-17$\beta$, although these compounds were much less effective on a molar basis than estradiol-17$\beta$ itself (Miksicek et al., 1995). Relative to estradiol-17$\beta$, soy isflavones have a 7 times greater affinity for estrogen receptor $\beta$ (ER$\beta$) than estogen receptor $\alpha$ (ER$\alpha$). The relative binding affinity of genistein compared with that of estradiol is about 36% for ER$\beta$ and 5% for ER$\alpha$ (Kuiper et al., 1997). Tissue-specific responses to isflavones, much like estrogen and the SERMs, may be explained, in part, by the relative expression of ER$\alpha$ and ER$\beta$ present and varying receptor-ligand interactions that have differing transcriptional activities (Paech et al., 1997; Fitzpatrick et al., 1999).

Phytoestrogens have demonstrated numerous biochemical actions. Actions at the cellular level depend on the target tissue, receptor status of the tissue, and the level of endogenous estrogen. For example, in premenopausal women in whom circulating levels of endogenous estrogen

are high, isoflavones may act as estrogen antagonists. In postmenopausal women with lower levels of circulating estrogen, isoflavones may have the opposite effect and have as estrogen agonists (Adlercreutz, 1997). These observations led to the hypothesis that isoflavones would be biologically active, conferring health benefits that could explain the relatively low incidence of hormone-dependent diseases in countries in which soy is a dietary staple.

### Hormonal effects of isoflavones

Phytoestogens act as estrogen receptor (ER) agonists or antagonists, depending on the hormonal status of the animal or man. Isoflavonoids at concentrations 100-1000 times higher than that of 17β-estradiol have been considered to compete with endogenous mammalian estrogens, to bind ER, and to prevent estrogen-stimulated growth in mammals (Adlercreutz et al., 1995). It is therefore possible that the consumption of a diet rich in phytoestogens could affect endogenic hormone production. The mid-cycle peaks of the luteinizing hormone and the follicle stimulating hormone are suppressed (Cassidy et al., 1995) or sometimes unaffected (Lu et al., 2000), and in premenopausal women the length of the follicular phase of the menstruals cycle is increased during an isoflavones-rich diet (Cassidy et al., 1995; Lu et al., 1996). The serum 17β- estradiol concentration is unaffected (Cassidy et al., 1995; Honore et al., 1997) or decreased (Lu et al., 1996; Lu et al., 2000), the progesterone level is

decreased (Lu et al., 1996; Lu et al., 2000), and the serum testosterone concentration is unaffected (Hornore′ et al., 1997), or reduced (Strauss et al. 1998) by dietary isoflavones supplementation.

Menopause is frequently accompanied by unpleasant symtoms such as hot flashes, emotional disturbances and compromised sexual activity. Epidemiological observations of Asian females, who typicallly consume a diet high in soy, have a much lower incidence of menopausal symptoms than Western women do. Asian women who lived in the Western worked and did not consume soy did not display this lowered incidence of menopausal symptoms. The mild estrogenic activity may ease menopause symptoms for some women, without creating estrogen related problems. Abundant evidence exists implicating that soy isoflavones may help relieve menopausal symptoms, and the recent scientific studies have confirmed this hypothesis. One study was composed by fifty-eight menopausal women, who supplemented their diets with soy everyday for three months. Prior to the study, the women experienced an average of fourteen hot flashes per week. The women taking the soy reduced their hot flashes by 40 percent (Cassidy, 1994).

Another important menopausal symptom is vaginitis, which is due to epithelial atrophy.When postmenopausal women consume a mixture of soy, linseed and clover, an increase in cell proliferation in the vaginal epithelium occurs (Wilcox et al., 1990), indicating estrogenic activity. However, opposite findings also exist. Soy isoflavones have no estrogenic

effect on vaginal cytology in postmenopausal macaques (Cline et al., 1996) or women (Murkies et al., 1995).

Osteoporosis is a condition in which bone density is decreased, although the composition remains unaltered. Bone becomes porous due to an imbalance between the bone cells responsible for the formation and resorption of the bone. This results in structural failure and an increased likelihood of fracture. Osteoporosis is particularly a problem in women postmenopause due to the acceleration of bone loss associated with the estrogen loss. Hormone replacement therapy (HRT) is effective in preventing the bone loss, up to the age of at least 75 years, provided it is taken for several years early on in the postmenopausal phase (Bingham et al,, 1998)

The incidence of osteoporosis differs within populations, and according to the World Health Organaization report (1994) the incidence is lower in Asian women than in western women. One of the reasons for this could be the dietary differences between the areas, which are partly related to the consumption of soy products. Soy isoflavones have been shown to attenuate bone loss in perimenopausal women (Alekel et al., 2000) and in ovariectomized rats (Arjmandi et al., 1998a). This may be due to enhanced bone formation rather than the slowed bone resorption (Arjmandi et al., 1998b). Although both genistein and daidzein are effective in preventing bone loss, daidzein is the more potent of these two compounds (Picherit et al., 2000).

The incidence of breast, endometrial and ovarian cancer is lower in Asian and eastern Europe than in western countries (Rose et al., 1986). All these cancers are hormone-dependent. Migrants from Asia who maintain their traditional diet have a decreased risk even when living in western countries (Kolonel, 1988), whereas the increased risk of these diseases follows a change towards a westernized diet (Lee et al., 1991) An increased soy intake, for example, is associated with reduced breast cancer risk in both pre- and postmenopausal women (Wu et al., 1996) and with lowered prostate cancer risk in men (Jacobsen et al., 1998; Strom et al., 1999).

*Cardiovascular effect of isoflavones*

Cardiovascular disease is the leading cause of death among women in developed nations. It is recognized that its incidence increase substantially after menopause, purportedly due to the loss of estrogen's protection. The increase incidence of cardiovascular events in younger, surgically postmenopausal women supported this hypothesis. The role of estrogen deficiency in cardiovascular disease was further indicated by preclinical studies demonstrating that estrogen could inhibit atherosclerosis. Population-based, observational studies rivealed that the risk of cardiovascular events in women taking hormones was cut in half (i.e., a relative risk of 0.5), providing support for a benefit of estrogen (Grodstein et al., 1996; Barrett and Bush, 1991; Stampfer and Colditz, 1991).

Postmenopausal estrogens and hormone replacement therapy (HRT) may also be protective against CAD by specifically decreasing the plasma LDL-C and/or increasing of HDL-C and causing vascular effects in both vasomotor tone and vessel wall compliance. Lp(a) is also acknowledged as a primary predictor of heart disease. Levels of Lp(a) are genetically determined and are decreased up to 35% by estrogens and other sex steroids (Knight and Eden, 1996).

The role of estrogens in the prevention of cardiovascular disease remains controversial (Fitzpatrick, 2000). Although the retrospective studies suggested a cardioprotective effect, the prospective trials have indicated a lack of secondary prevention in women with established heart disease. It is hoped that the use of a more natural estrogen like compound may provide protection against CAD, the No.1 killer of postmenopausal women.

Epidemiological studies have demonstrated a reduced rate of mortality due to CAD in Japanese populations consuming a traditional Japanese diet compared to a western diet (Kagan et al., 1974). Expatriate Japanese living in the United Kingdom have higher blood pressure and cholesterol levels and lower triglyceride levels than the Japanese still living in Japan (Robinson et al., 1995), which suggests that these differences are not of genetic origin but may be due to diet. The Japanese diet is rich in soy products, fish and fibre. The beneficial effects of soy are thought to be caused primarily by isoflavones and appear to be mediated by many mechanisms. Most of researchers consider that these effects resulted from a reduction of plasma LDL-C (Tovar et al., 1998; Crouse et al., 1999; Ashton

and Ball, 2000) and triglyceride concentrations (Ashton and Ball, 2000; Ho et al., 2000). Hamilton and Carroll (Hamilton and Carroll, 1976) were the first to report that soy protein lowered plasma cholesterol in hypercholesterolemic rabbits. Many reports confirmed these findings to some extent.

Animal studies demonstrated the important lipid-lowering effects of soy isoflavones. Monkeys were fed by soy isolates high in isoflavones and compared in a cross-over trial with a soy isolate in which the isoflavones had been removed via alcohol extraction.LDL-C, very low density lipoprotein cholesterol (VLDL-C), and total cholesterol:HDL-C ratios were significantly lowered, while HDL-C was significantly elevated in the group on the isoflavone-rich diet (Anthony et al., 1996). No lipid lowering effect occurred in the group on a casein diet.

Anthony et al (1997) demonstrated a robust effect on serum lipid levels in both male and female monkeys fed soy diets for 14 months. Eighty five male and 75 female cynomolgus monkeys were assigned to 1 of 3 treatment groups-casein, soy protein devoid of isoflavones, or soy protein with intact isoflavones. The LDL-C and VLDL-C levels were lowered by 30% to 40% , and HDL-C levels increased by 50% in the soy isoflavone group compared with the casein-fed group. The prevalence of coronary artery atherosclerosis in these 3 treatment groups was also evaluated, and the group provided with soy protein containing phytoestogens had the least atherosclerosis (Anthony et al., 1997).

The meta-analysis allowed researchers to combine the results of 38 smaller studies to strengthen and validate their findings. It was shown that consuming soy protein rather than animal protein significantly decreased blood levels of total cholesterol and LDL-C. Results showed that soy protein was most effective in people at the highest risk level. The higher the initial levels of total and LDL-C, the greater the amount of lowering. It is now widely accepted that soy protein consumption decreases high blood LDL-C levels. As little as 25 g of soy protein per day have been shown to lower cholesterol in individuals with high cholesterol levels. Genistein is the isoflavone most prevalent in soy. It's thought that it may play a role in the prevention of the arterial wall changes present when atherosclerosis begins. Genistein may also interfere with the formation of blood clots that can lead to arterial blockage. In addition, genistein and daidzein are antioxidants, and researchers are investigating the possible role of soy isoflavones in reducing the oxidation of LDL-C. Tikkanen et al.(1998) reported that the intake of soy protein containing 60 mg isoflavones per day may provide the protection against oxidative modification of LDL-C. The oxidative modification of LDL-C particles is considered to be a prerequisite for the uptake of LDL-C by macrophages in the artery wall, which is an initial step in the formation of atheroma. Thus, this may be one of the mechanisms of soy protein inhibition of the atherosclerosis.

Soy isoflavones inhibit the atherosclerotic plaque formation by intervening at several steps in the thrombus formation. Arterial thrombus formation is generally initiated by an injury to the endothelial cells lining the blood vessels. One of the first events after an injury is the thrombin formation. This leads to a cascade of events, including the platelet

activation, resulting in the thrombus formation. Genistein has been found to inhibit the thrombin formation and platelet activation (Wilcox and Blumenthal, 1995). The pathogenesis of the atherosclerotic plaque formation also involves, in addition to lipid accumulation, the infiltration of monocytes and T-lymphocytes into the arterial wall, contributing to the thickening of the wall and occlusion of the vessel. Monocytes and lymphocytes adhere to the endothelial cell surfaces via the expression of certain "adhesion molecules." The infiltration and proliferation appear to be controlled by the peptide growth factors. The increased levels of isoflavones, genistein particular, appear to alter the growth factor activity, and inhibit cell adhesion and proliferation, all activities necessary for the lesion formation in the intima of blood vessels.

Preclinical studies suggest that the vascular reactivity may be favorably influenced by isoflavones. In the atherosclerotic macaque, dietary isoflavones enhance the endothelium-dependent relaxation to acetycholine (Ach) in the coronary arteries (Homore at al., 1997), and the treatment with genistein augments the endothelium-dependent arterial relaxations in ovx-rats (Squadrito et al., 2000). In vitro studies of isolated vessels, the mechanisms of isoflavones-induced vasodilation have been examined (Nevala et al., 1998).

**<u>Endothelium</u>**

The vascular endothelium is a highly active endocrine organ covering the inner surface of the arteries and veins. Endothelial cells are multifunctional cells playing a key role in the control of the vascular tone (Furchgott and Vanhoutte, 1989).The endothelium is an important regulator of the arterial tone because it secretes various vasodilating and contracting substances.

***Endothelium-derived vasodilatory factors***

*Nitric oxide*

In 1980 Furchgott and Zawadzki showed that the endothelium must be intact for Ach to induce the arterial smooth muscle relaxation. A substance originating from a vessel with an intact endothelium caused the relaxation in an arterial ring with a denuded endothelium; it was named "the endothelium-derived relaxing factor" (EDRF). Later the EDRF was confirmed to be nitric oxide (NO) (Ignarro et al., 1987; Palmer et al., 1987). NO is a gaseous free radical which is synthesized from the amino acid L-arginine by a family of NO syntethases (NOSs). NO relaxes vascular smooth muscle cell (VSMCs) by increasing the production of cyclic guanosine 3´,5´ monophosphate (cGMP).

A normal endothelium constantly releases the small amounts of NO. The extra NO is released in response to the physiological stimuli such as an increased shear stress and reduced oxygen tension and to substances such as Ach , bradykinin, histamine, thrombin, ADP, ATP and the substance P. So far, NO is the most potent vasodilator known (Umans and Levi, 1995). NO also inhibits the platelet aggregation, neutrophil adesion to the endothelium, VSMC proliferation, and adhesion molecule expression. The synthesis of NO is impaired in many diseases, such as in hypertension, diabetes, hypercholesterolemia and atherosclerosis (Cannon, 1998). In human endothelial cells, NO production is enhanced by $17\beta$-estradiol but not by testosterone (Hishikawa et al., 1995), and the physiological levels of circulating $17\beta$-estradiol could elevate the basal NO release from the endothelial cells (Wellman et al., 1996). Pharmacologically, NO synthesis can be blocked by the L-arginine analogs such as N$\omega$-Nitro-L-arginine methyl ester (L-NAME).

*Prostacyclin*

Prostacyclin ($PGI_2$) is formed from arachidonic acid (AA) by the cyclo-oxygenase enzyme. The endothelial cells are the highest producers of $PGI_2$, but VMSCs and fibroblast are also able to synthetize $PGI_2$. $PGI_2$ is produced in response to shear stress and to substances that stimulate NO formation. The contribution of $PGI_2$ to vasodilation is less than that of NO. However, $PGI_2$ inhibits the platelet aggregation and

promotes the fibrinolysis. Estrogens stimulate $PGI_2$ in cultured human endothelial cells (Mikkola et al., 1996) and in the rat endothelium (Wakasugi et al., 1989), whereas testosterone reduces it (Wakasugi et al., 1989). The synthesis of $PGI_2$ is inhibited by common anti-inflammatory drugs such as acetylsalicylic acid, diclofenac, ibuprofen and tolfenamicacid.

*Endothelium-derived hyperpolarizing factor (EDHF)*

Endothelium-dependent relaxations and hyperolarizations can be partially or totally resistant to the inhibitors of cyclo-oxygenase and NO synthetase, suggesting the existence of an additional endothelial relaxing mechanism. These NO-and $PGI_2$-independent relaxations appear to be without an increase in the intracellular levels of cyclic nucleotides in smooth muscle cells, and the relaxations are antagonized by apamin and ChTX. The inhibitors of $Ca^{2+}$ sensitive $K^+$ channels is responsible for these relaxation, and the relaxing agent is called an endothelium-derived hyperpolarizing factor. The nature of EDHF was for a long time unclear, but quite recently, it had been discovered that EDHF may be an 11, 12-epoxyeicosatrienoic acid formed by cytochrome P450 2C from arachidonic acid , at least in the porcine coronary artery (Fisslthaler et al., 1999).
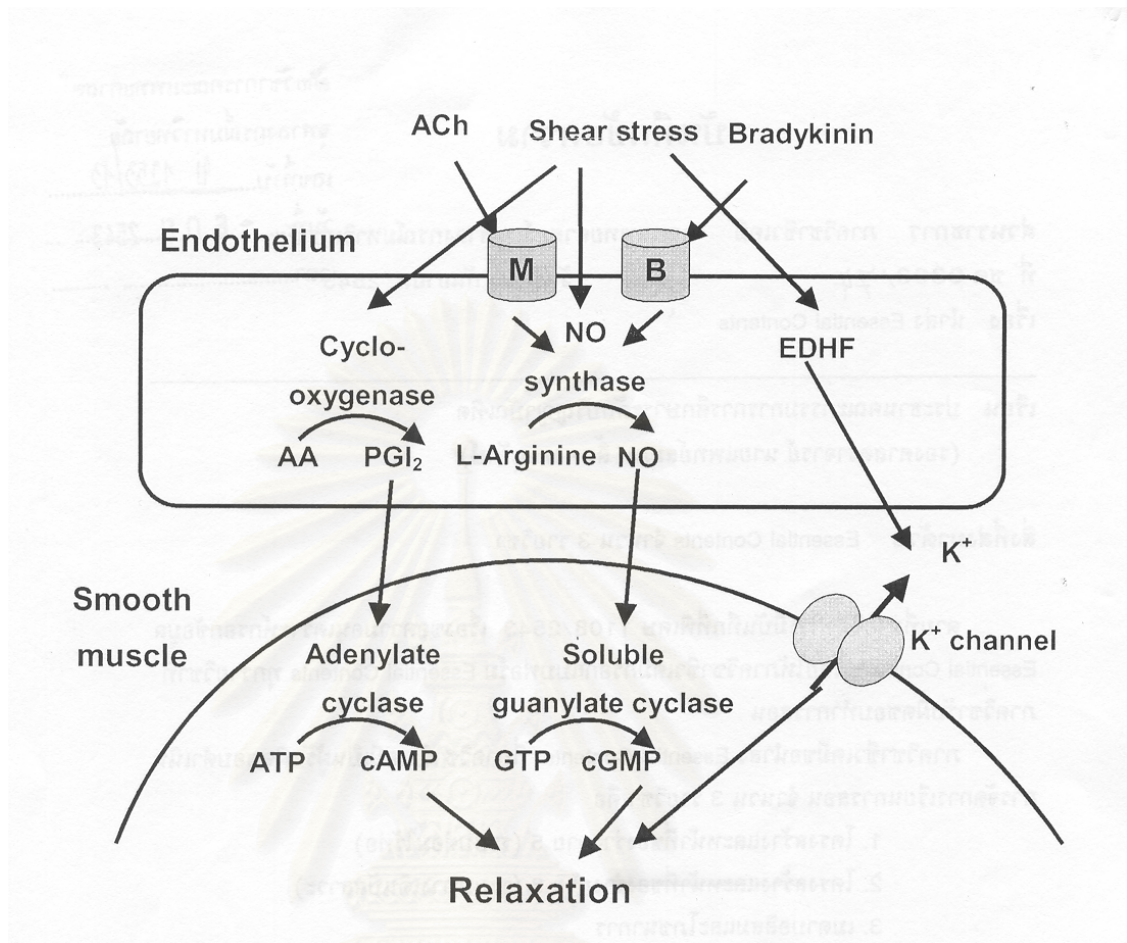
Figure 2. Simplified endothelium-dependent relaxing mechanisms of vascular smooth muscle. (Modified from Nevala et al, 1998).

# CHAPTER III

# MATERIALS AND METHODS

This experiment was designed to investigate the effects of Genistein on coronary arteriolar responses to vasoactive agents in ovariectomized rats (ovx-rats). Females Wistar rats were used in this study. These rats were separated into two groups: sham ovariectomized rats (sham-ovx) and ovx-rats. Each group was also separated for study at 4 and 10 weeks. In this study, the modified Langendroff method was used. The isolated arrested heart was prepared to investigate coronary arteriolar responses to acetylcholine ($10^{-5}$M) and sodium nitroprusside ($10^{-4}$M). Responses to topical application of these agents were recorded with real time intra-vital fluorescence microscopic system. After that the diameter of arterioles were determined using the computer program called "Global Lab Image Software".

**Chemical substances**

Genistein

Acetylcholine ( Ach )

Norepinephrine ( NE )

Sodium nitroprusside ( SNP )

Dimethyl sulfoxide

Bovine serum albumin

Fluorescein isothiocyanate-labeled dextran, molecular weight 150,000

( FITC-Dx-150 )

Pentobarbital sodium

Chemical for preparation of  Kreb-Hansenleit

Bicarbonate solution

Normal saline solution

Gas mixture ( 95 % $O_2$, 5% $CO_2$ )

**Perfusate composition**

| | |
|---|---|
| NaCl | 80.00 mM |
| KCl | 39.70 mM |
| $CaCl_2$ | 2.52 mM |
| $MgSO_4$ | 1.66 mM |
| $NaHCO_3$ | 24.88 mM |
| $KHPO_4$ | 1.18 mM |
| $C_6H_{12}O_6$ | 5.85 mM |
| Bovine serum albumin | 2.00 g/100 ml |
| pH = 7.4 | |
| $O_2 : CO_2$ = 95% :5% | |

Female Wistar Rats

(N=36)

Injection of genistein 4 weeks

Sham+gen.vehicle  ovx+gen.vehicle  ovx+genistein
n=6            n=6            n=6

Injection of genistein 10 weeks

Sham+gen.vehicle  ovx+gen.vehicle  ovx+genistein
n=6            n=6            n=6
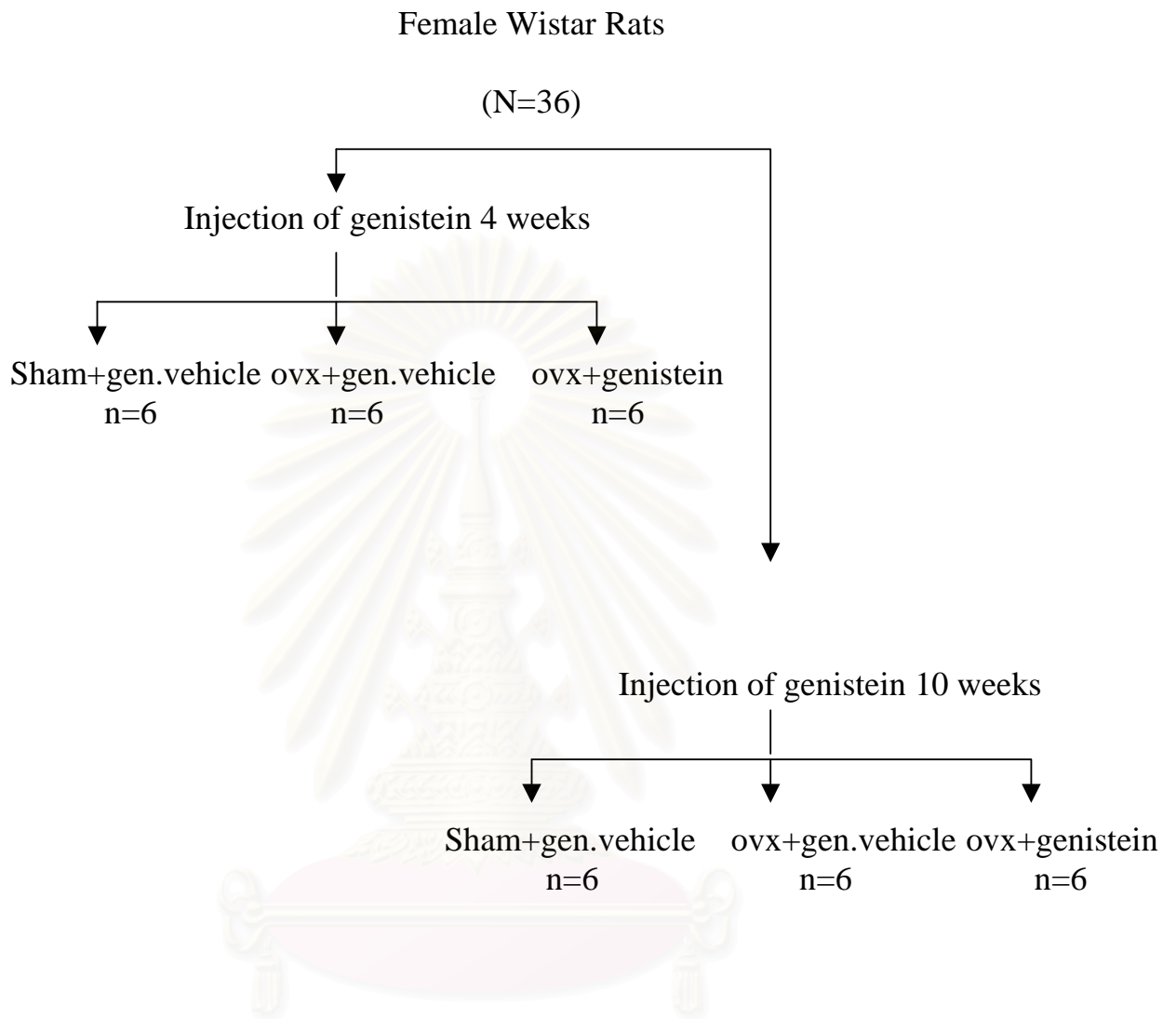
Figure 3. Diagram of experimental animal groups

**Animal preparations**

All rats were supplied by the National Laboratory Animal Center of Salaya Campus, Mahidol University. Females Wistar rats (n=36) aged of 10 weeks were subjected to a bilateral ovariectomy (ovx-rats). Sham operated animals (sham-ovx) were used as controls. The animals were maintained at temperature 28-32°C and light cycle 12 hour light: 12 hour dark cycle at the Experimental Animal Center, Faculty of Medicine, Chulalongkorn University. All rats were given free access to standard laboratory chow and drinking water.

Three weeks after surgery, ovx-rats and sham-ovx animals were separated into two groups of 4-week and 10 week treatment periods :

1. Studies of treatment with 4-week injection of genistein (n=18)

    1.1. Sham ovx (control) (n=6) received daily injection of genistein vehicle (100 µl of DMSO subcutaneous)

    1.2. Ovx rats (n=6) received dialy injection of genistein vehicle (100 µl of DMSO subcutaneous)

    1.3. Ovx rats (n=6) received dialy injection of genistein (0.2 mg/kg body weight in 100 µl of DMSO subcutaneous)

2. Studies of treatment with 10-week injection of genistein (n=18)

    2.1 Sham ovx (control) (n=6) received daily injection of genistein vehicle (100 µl of DMSO subcutaneous)

2.2 Ovx rats (n=6) received dialy injection of genistein vehicle (100 μl of DMSO subcutaneous)

2.3 Ovx rats (n=6) received dialy injection of genistein (0.2 mg/kg body weight in 100 μl of DMSO subcutaneous)

The animals were separated into group as shown in figure 3. After treatment in each group, the animals were used in the performing of isolated heart experiment.

## The Isolated Arrested Heart Model

The isolated heart preparation developed by Langendorff is a model which is free of central neural and hormonal effects. It has been employed to study basic questions in cardiac function, metabolism, ultrastructure and hemodynamics. Moreover, pharmacologic studies have also been performed using isolated heart preparation. In this preparation, coronary perfusion is started in situ to avoid ischemic damage to the heart. The studies of coronary circulation by using perfusate containing elevated potassium (39.7 mM) have found that the heart was arrested. Cardiac arrest greatly reduces myocardial metabolism and allows direct visualization of coronary microcirculation. This model is appropriate for both physiologic and pharmacologic studies. It is particularly well for determining the direct effects of an intervention on coronary tone and the coronary microcirculation. Since the heart is arrested, changes in heart rate and

contractility do not complicate measurements of coronary tone (McDonagh, 1983; McDonagh et al., 1984).

According to the beneficial effects of isolated heart model as mentioned above. Therefore, our study would like to use this model for investigation the effect of genistein on coronary response of ovariectomized rats.

**Experimental of Isolated Rat Heart**

On the day of experiment, the animal was not allowed for food for twelve hours. To prevent thrombosis of the coronary arteries in the remainder of the experiment, the animals were pre-injected with approximately 500 USP-units of heparin per 100 g body weight, intraperitoneally ( i.p. ), one hour before the surgery to inhibit blood coagulation. Experimental rats were anesthetized for the duration of the experiments by intraperitoneal administration of 60 mg/kg of sodium pentobarbital. After tracheostomy, the ventilation was assured by using positive pressure ventilator (ventilator 141). Then, a catheter was inserted into the carotid artery for recording blood pressure and heart rate by using pressure transducer (Nihon model TP-300T) that connected to the polygraph (Nihon RM 600).

The skin was incised by a longitudinal cut from the middle of the abdomen up to the throat. Then the abdomen was opened up to the

diaphragm. The diaphragm was cut off following the anterior part of the inferior thoracic aperture. The thorax was cut open on the left and right side following to bone-cartilage-border on a line parallel to the sternum starting at the diaphragm and proceeding as far cranial as to the first rib. The complete anterior thoracic wall was turned upwards over the animal's head and fixed in this position.

The pericardium was removed then free the ascending aorta of any connective tissue and separate from the pulmonary artery. In order to prevent large amounts of blood from pouring out of the opened aorta and impeding the view on the surgical area, the inferior vena cava was clamped with a vessel clamp. The insertion of the aortic cannula was facilitated and flooding of the surgical area with blood was reduced by sprinkling the heart with cold physiological saline ( 4 to 8°C ) so that the heart stop beating or slow down.

The pulmonary artery was incised so that the right ventricle would not be overstretched by continuing blood flow during the time when the aortic cannula has been inserted but the heart has not been removed from the thorax. The aortic cannula was filled with perfusate and was connected to the heat exchanger. The ascending aorta was incised and the cannula was inserted. At this moment a slight perfusion of the coronary arteries commenced, any blood in the coronary arteries would be washed out and the re-supply of oxygen to the heart begins.

The heart, which was now attached to the cannula,was slightly elevated and cut out. The right atrium was then quickly cut open. The retrograde perfusion is performed by the Langendorff technique, with perfusion pressure ( PP ) of 90 mmHg. The whole apparatus was enclosed in a thermostatic chamber at 37.5°C. The heart rapidly arrested upon perfusion because the perfusate contained 39.7 $mMK^+$ . After coronary perfusion was established the heart was carefully cut out of the thoracic cavity. It was placed on chamber for intravital fluorescence microscopy of the left ventricular epicardial microcirculation.Then, serum were collected from inferior vena cava. At the end of each experiment, the hearts, livers and uteruses were disconnected and weighted.

**Studies of coronary arteriolar responses to vasoactive agents**

In this experiment, the third-order of arterioles ranging in size for 40-55 μm were selected to study the responses of coronary arterioles to vasoactive agents. After isolated heart preparation and equilibration period 10 min which time the heart were washed with Krebs solution to reach a steady-state level of base-line tone of the arterioles. The responses of coronary arterioles to 1 ml of $10^{-4}$ M norepinephine ( NE, preconstriction ), 1 ml of $10^{-5}$ M acetylcholine ( Ach ) and 1 ml of $10^{-4}$ M sodium nitroprusside were determined on the selected arteriole. In this study, interval of action of NE, Ach and SNP were used about 3, 3 and 3 min, respectively. Between each application, for the selected arterioles were washed with Krebs solution and allowed 10 min washout period was used to allow the vessel to return to

its control diameter. The protocol used for studies of coronary arteriolar responses as showed in Figure 5. The video images of the arterioles were obtained by fluorescent microscopy using fluorescein isothiocyanate-labeled dextran of 150,000 molecular weight ( FICT-Dx-150 ). In this experiment, FITC-Dx-150 was mixed with the perfusate solution to make the concentration equal to 1 mg/100 ml for label the diameter of arterioles by using the infusion pump rate 0.25 ml/min that connected with Isolated Heart Apparatus. After the FITC-Dx-150 reached to the heart, images of vessels could observed by fluorescence microscopy (Nikon, Model Eclipse E 600). The isolated heart preparation for direct visualization of coronary microcirculation showed in Figure 4a and 4b. The images of selected arterioles in responses to all vasoactive agents and their base-line diameter were recorded on the videotape. The diameter of arterioles were determined by using the computer program "Global Lab Image" . The diameter of each selected arteriole was assessed by the software indicated by  number of pixels (n). Then the software convert number of pixels to micrometer. Figure 6 show the measurement of distance between two points (B to C) by using point A as a reference point and  the detial for analysing the percent changes of arteriolar diameter.
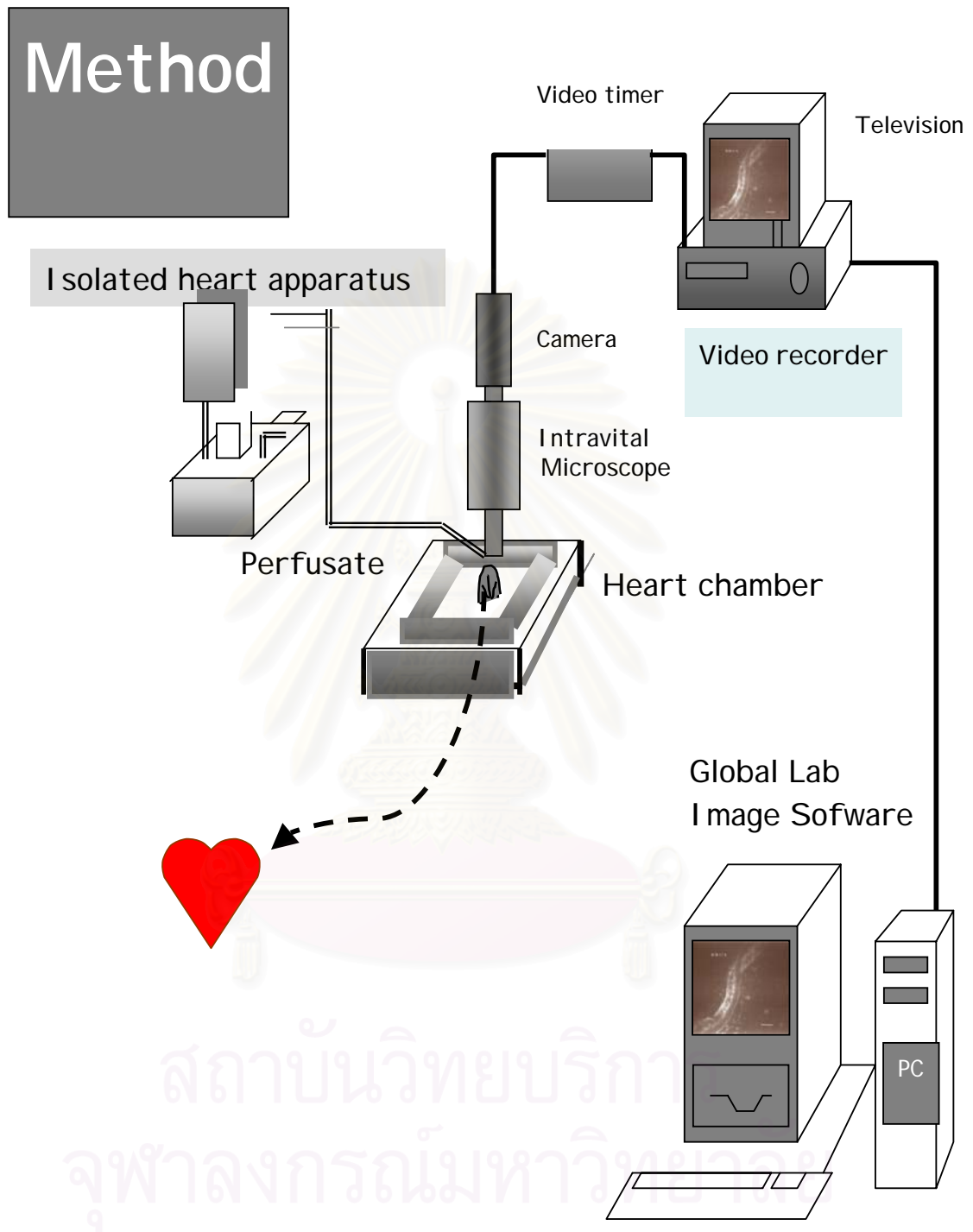
Figure 4a. Isolated heart preparation for direct visualization of coronary
microcirculation.
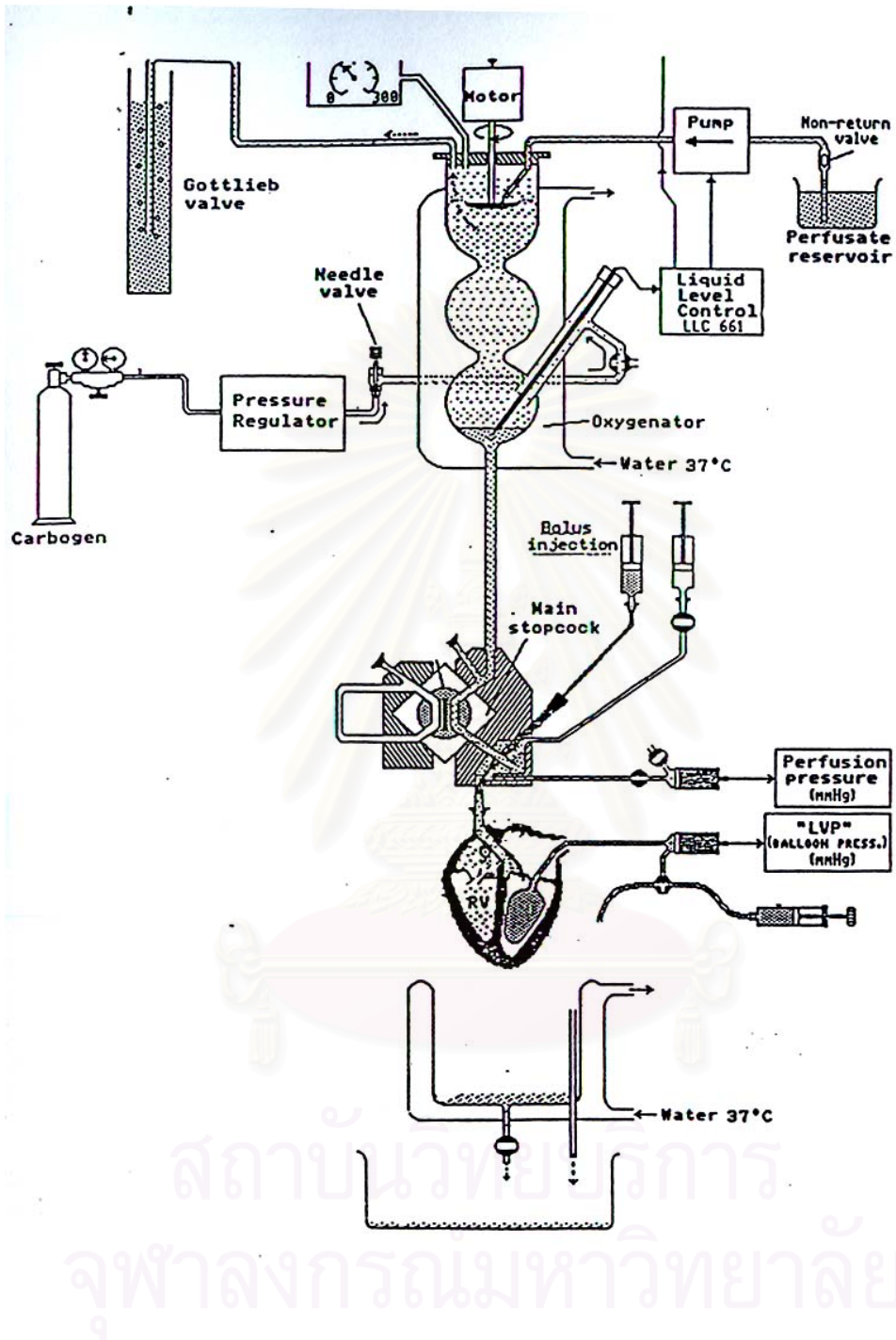
Figure 4b. Isolated heart apparatus.
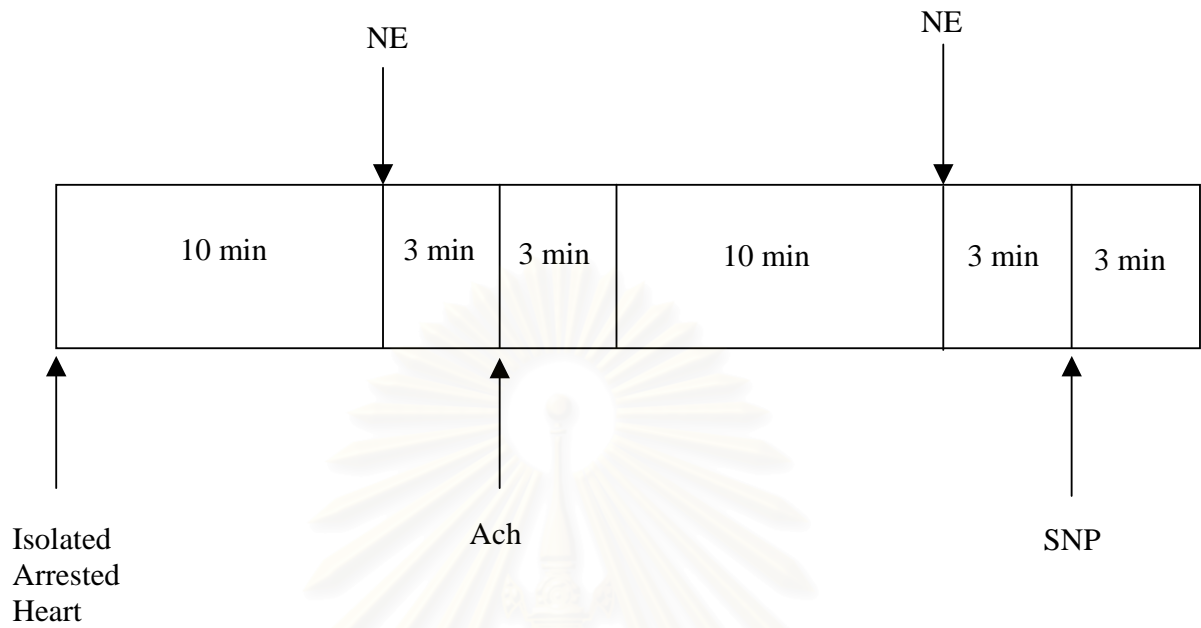
Figure 5. The protocol used for studies of coronary arteriolar responses to vasoactive agents.
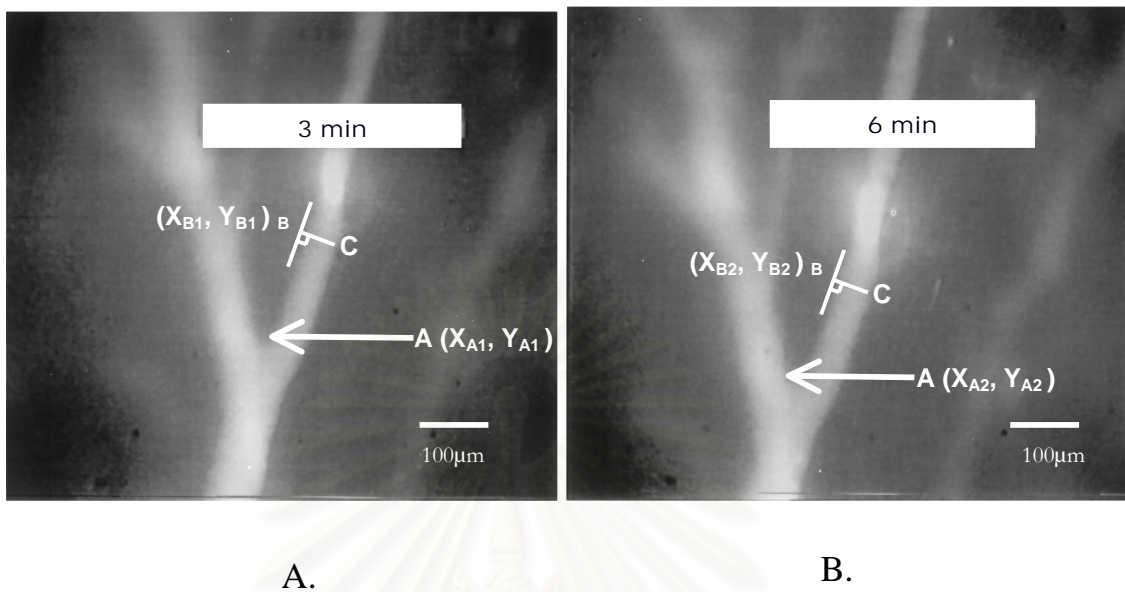
Figure 6. The reference point A and the defined point B and C. The diameter of arteriole was measured as the length of B-C.

$$\text{Mean Diameter} = \frac{B_1C_1 + B_2C_2 + B_3C_3}{3}$$

$$\%\text{Change of Diameter} = \frac{\overline{BC_v} - \overline{BC_b}}{\overline{BC_b}} \times 100\%$$

$\overline{BC_v}$ mean diameter of coronary arteriolar responses to topical application of Ach or SNP.

$\overline{BC_b}$ mean diameter of coronary arteriolar responses to topical application of NE.

**Plasma total cholesterol, triglyceride HDL-C and LDL-C levels**

After performing the isolated heart, plasma was collected from inferior vena cava. Plasma was separated from total blood by centrifugation with 3,500 rpm at 4º for 15 minutes, then it was stored at a temperature of 4ºC until being analyzed at the King Chulalongkorn Memorial Hospital Laboratory. The analysis of total cholesterol, triglyceride, HDL-C and LDL-C in the blood was determined by using an automatic analysis technique on a chemical analyzer from Roche Diagnostics (Hitachi analyzer 911).

**Plasma estradiol ($E_2$)**

Blood samples were collected during anestrous period at the end of the experiment. In order to evaluate plasma $E_2$ levels, blood samples (3 ml) were collected in polyprophylene tubes containing heparin (50,000 IU) and after centrifugation at 3500 rpm at 4ºC for 10 minutes, each sample was stored at -70ºC until analysis. Plasma $E_2$ levels were determine by electrochemiluminescence immunoassay (ECLIT) with a commercial available kit. Functional sensitivity assay is 44 pmol/1 (12 pg/ml) and reproducible measured with an inter-assay coeffecient of variation of $\leq 20\%$.

**Statistical analysis**

The results are expressed as means ± S.E.. Statistical analysis of differences between groups was performed using ANOVA followed by repeated measurement with post hoc Bonferroni's test. Probability values of less than 0.05 were considered to be statistically significant.

# CHAPTER IV

# RESULTS

## General observation

In this study, the ovariectomized rat was used as an experimental model of postmenopause.  In order to confirm the inclusion criteria, the plasma $E_2$ level were determined and demonstrated in Table 1.  The results indicated that plasma $E_2$ in ovx-rats and ovx-genistein rats at 3-week period were significantly decreased as compared to sham-ovx.

## Body weight

In this experiment, the changes in body weight (g) were determined. All ovariectomized groups (4 and 10 weeks) had significantly increased body weight compared to the age-matched sham-ovx groups.  The result was shown in Table 2 and Figure 8.

## Uterine weight

All ovariectomized groups (4 and 10 weeks) had significantly decreased uterus weight compared to the age-matched sham-ovx groups. The result was shown in Table 3 and Figure 9.

## Studies of cardiovascular functions

In this experiment, cardiovascular functions including Systolic blood Pressure(SP), Diastolic blood Pressure (DP), Mean Arterial blood Pressure (MAP) and Heart Rate (HR) were determined for all animal groups.

Bilateral ovariectomy did not modify SP, DP, MAP and HR in any ovx-rats. In addition, supplementation with genistein did not cause any significant change in all parameters throughout the study period.

All of the results were shown in Table 4-7 and also demonstrated graphically as showed in Figure 9-12.

## Studies of coronary arteriolar responses to vasoactive agents

*Endothelium dependent relaxation*

Endothelium dependent relaxation determined by measurement the coronary arteriolar responses to topical application of Ach ($10^{-5}$M). Intravital fluorescent microscopy was used to examine those arteriolar responses in sham-ovx rats, ovx-DMSO rats and ovx-genistein rats at 4 and 10 weeks of treatment.  In ovx-DMSO rats at 10 weeks of treatment, there was significantly decreased response of Ach induced relaxation compared with age-matched sham-ovx rats. Significant improvement of Ach induced relaxation was observed in coronary arterioles from the genistein supplement

in ovx-genistein at 10 weeks treatment compared with the genistein vehicle treated rats. The changes in arteriolar diameter in responses to Ach in ovx-DMSO 4 weeks was lower than sham-ovx 4 weeks but was not significant. However, supplementation of genistein 4 weeks could improve Ach induced relaxation. The results were summarized in Table 8 and Figure 14.

*Endothelium-independent relaxation*

The responses to topical application of SNP ($10^{-4}$M) were investigated on coronary arterioles of sham-ovx rats, ovx-DMSO rats and ovx-genistein rats at 4 and 10 weeks. The endothelium-independent relaxation were then determined. The result was shown in Table 9 and Figure 15. The topical SNP increased on coronary arteriolar diameters of sham-ovx, ovx-DMSO and ovx-genistein rats at 4 and 10 weeks. Moreover, no significant difference of SNP induced relaxation was observed all among animal groups.

**Lipid profiles**

Plasma total cholesterol, triglyceride, HDL-C and LDL-C in ovariectomized rats were not significant changed when compared to sham-ovx rats. Moreover the four and ten weeks genistein supplementation did not change the total cholesterol, triglyceride, HDL-C and LDL-C levels. All these assessed lipid profiles were also demonstrated graphically as shown in Figure 16-19 and Table 10-13.

Table 1. Plasma $E_2$ of sham-ovx, ovx-DMSO and ovx-genistein at after washout period 3 weeks.

| Group | Plasma $E_2$ (pg/ml) |
|---|---|
| sham-ovx | $37.11 \pm 1.85$ |
| Ovx-DMSO | $17.56 \pm 0.12^{a}$ |
| ovx-genistein | $15.88 \pm 3.16^{b}$ |

Values are means $\pm$ SE ; n = 6

[a] $p < 0.05$ comparing between ovx-DMSO and sham-ovx

[b] $p < 0.05$ comparing between ovx-genistein and sham-ovx

Table 2. Changes in Body weight (g) of sham-ovx, ovx-DMSO and ovx-
genistein at 4 and 10 weeks

| groups | Changes in Body weight (g) | |
|--------|----------------------------|----------------------------|
| | 4 weeks | 10 weeks |
| sham-ovx | $15.00 \pm 4.14$ | $37.33 \pm 3.14$ |
| ovx-DMSO | $76.83 \pm 6.34^{*}$ | $122.67 \pm 9.0^{*}$ |
| ovx-genistein | $75.33 \pm 4.65^{NS^{b}}$ | $103.17 \pm 9.8^{NS^{b}}$ |

Values are means $\pm$ SE ; n = 6

*  Significant difference as compared to sham-ovx ( P<0.05 )

NS[b] Nonsignificant difference as compared to ovx-DMSO ( P<0.05 )

Figure 7 . Body weight (g) of sham-ovx, ovx-DMSO and ovx-genistein at 4
and 10 weeks

Values are means ± SE ; n = 6

* Significant difference as compared to sham-ovx ( P<0.05 ).

NS[b] Nonsignificant difference as compared to ovx-DMSO
( P<0.05 ).

Table 3. Changes in Uterine weight (g) of sham-ovx, ovx-DMSO and ovx-
genistein at 4 and 10 weeks

| groups | Changes in Uterine weight (g) | |
|--------|---------------|---------------|
| | 4 weeks | 10 weeks |
| sham-ovx | $0.51 \pm 0.06$ | $0.72 \pm 0.10$ |
| ovx-DMSO | $0.10 \pm 0.01^{*}$ | $0.12 \pm 0.01^{*}$ |
| ovx-genistein | $0.11 \pm 0.01^{NS^b}$ | $0.12 \pm 0.03^{NS^b}$ |

Values are means $\pm$ SE ; n = 6

\* Significant difference as compared to sham-ovx ( P<0.05 )

$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( P<0.05 )
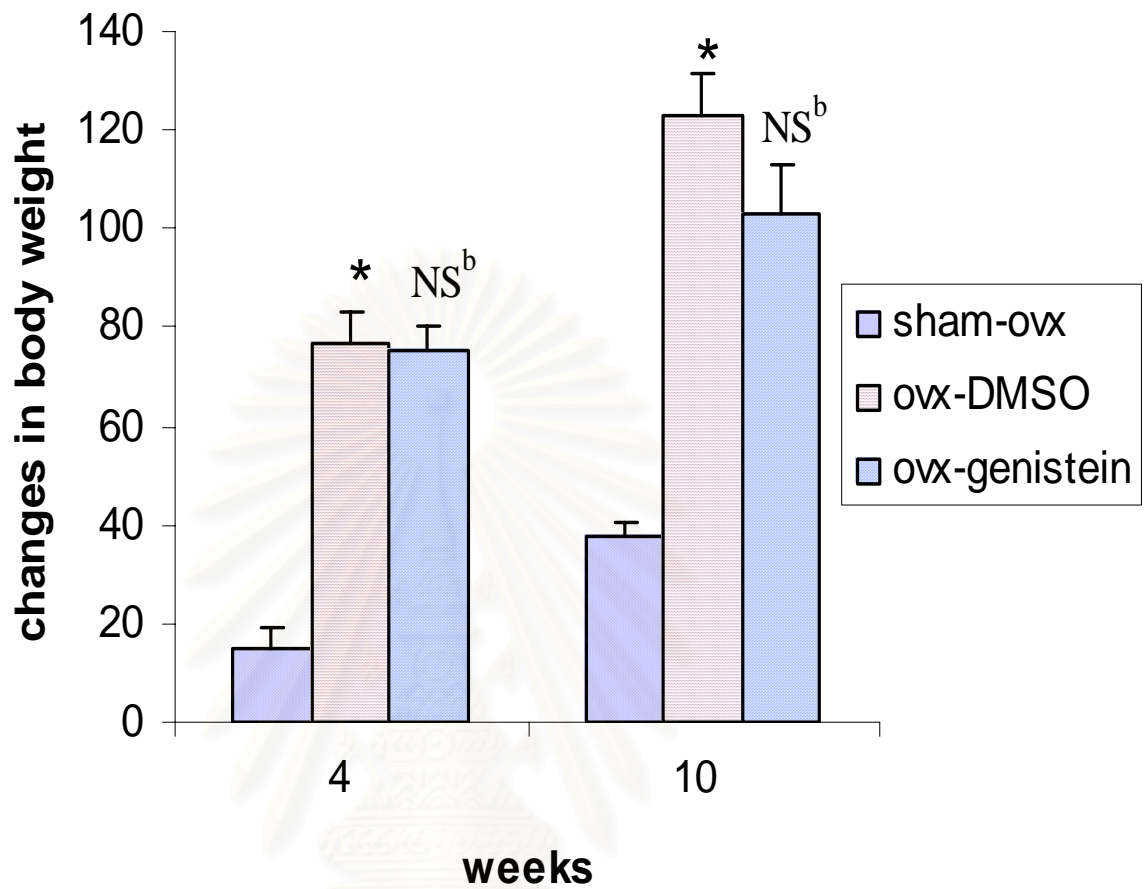
Figure  8.  Uterine weight (g) of sham-ovx, ovx-DMSO and ovx-genistein at
4 and 10 weeks

Values are means ± SE ; n = 6

* Significant difference as compared to sham-ovx ( P<0.05 )

NS[b]  Nonsignificant difference as compared to ovx-DMSO
( P<0.05 )

Table  4. Systolic blood pressure (mmHg) of sham-ovx, ovx-DMSO and  ovx-
genistein at 4 and 10 weeks

| groups | systolic blood pressure (mmHg) | |
| --- | --- | --- |
| | 4 weeks | 10 weeks |
| sham-ovx | $114.83 \pm 8.94$ | $125.00 \pm 4.28$ |
| ovx-DMSO | $124.17 \pm 3.00^{NS^a}$ | $126.67 \pm 10.06^{NS^a}$ |
| ovx-genistein | $118.33 \pm 2.47^{NS^b}$ | $121.67 \pm 7.82^{NS^b}$ |

Values are means $\pm$ SE ; n = 6

$NS^a$  Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

$NS^b$  Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).
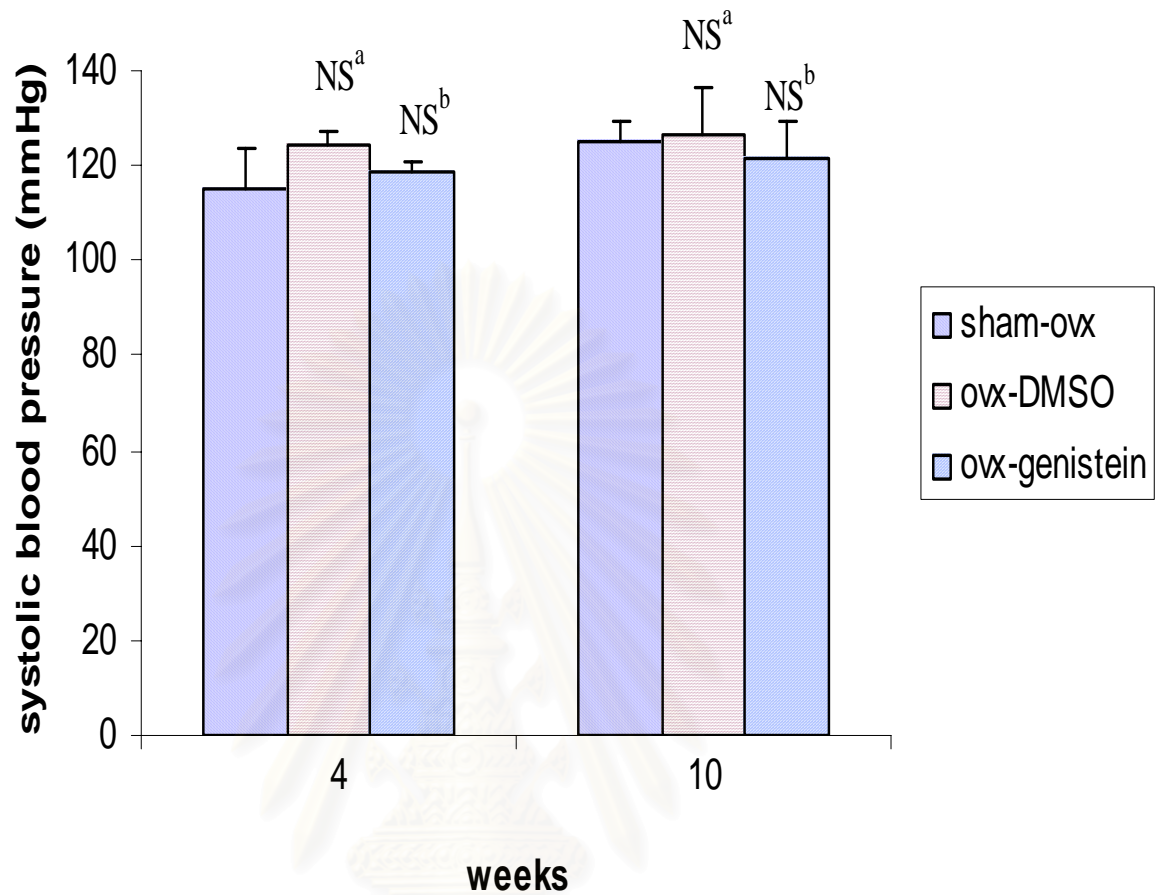
Figure 9. Systolic blood pressure (mmHg) of sham-ovx, ovx-DMSO and ovx-genistein at 4 and 10 weeks

Values are means ± SE ; n = 6

$NS^a$ Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Table  5. Diastolic blood pressure (mmHg) of sham-ovx, ovx-DMSO and
ovx-genistein at 4 and 10 weeks

| groups | diastolic blood pressure (mmHg) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $84.17 \pm 9.26$ | $87.5 \pm 4.96$ |
| ovx-DMSO | $93.33 \pm 3.07^{NS^a}$ | $97.5 \pm 7.83^{NS^a}$ |
| ovx-genistein | $90 \pm 1.83^{NS^b}$ | $90.33 \pm 5.83^{NS^b}$ |

Values are means $\pm$ SE ; n = 6

$NS^a$ Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Figure 10. Diastolic blood pressure (mmHg) of sham-ovx, ovx-DMSO and
　　　　　ovx-genistein at 4 and 10 weeks

　　　　　Values are means ± SE ; n = 6

　　　　　NS$^a$ Nonsignificant difference as compared to sham-ovx
　　　　　　　( P<0.05 ).

　　　　　NS$^b$ Nonsignificant difference as compared to ovx-DMSO
　　　　　　　( P<0.05 ).

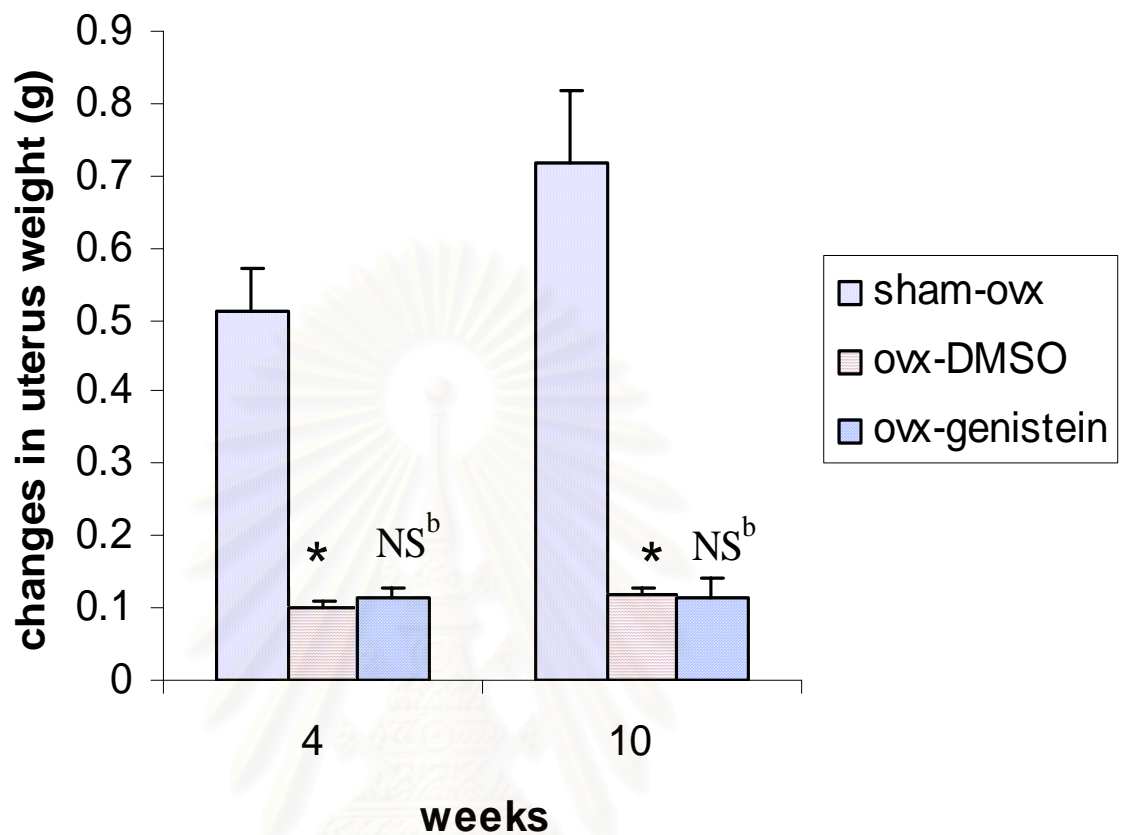Table  6.  Mean arterial blood pressure of sham-ovx, ovx-DMSO and

ovx-genistein at 4 and 10 weeks

| groups | Mean arterial blood pressure | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $94.39 \pm 9.02$ | $99.72 \pm 4.62$ |
| ovx-DMSO | $103.61 \pm 2.56$ NS[a] | $107.22 \pm 8.54$ NS[a] |
| ovx-genistein | $99.44 \pm 1.91$ NS[b] | $101.11 \pm 6.44$ NS[b] |

Values are means $\pm$ SE ; n = 6

NS[a] Nonsignificant difference as compared to sham-ovx ( $P<0.05$ ).

NS[b] Nonsignificant difference as compared to ovx-DMSO ( $P<0.05$ ).
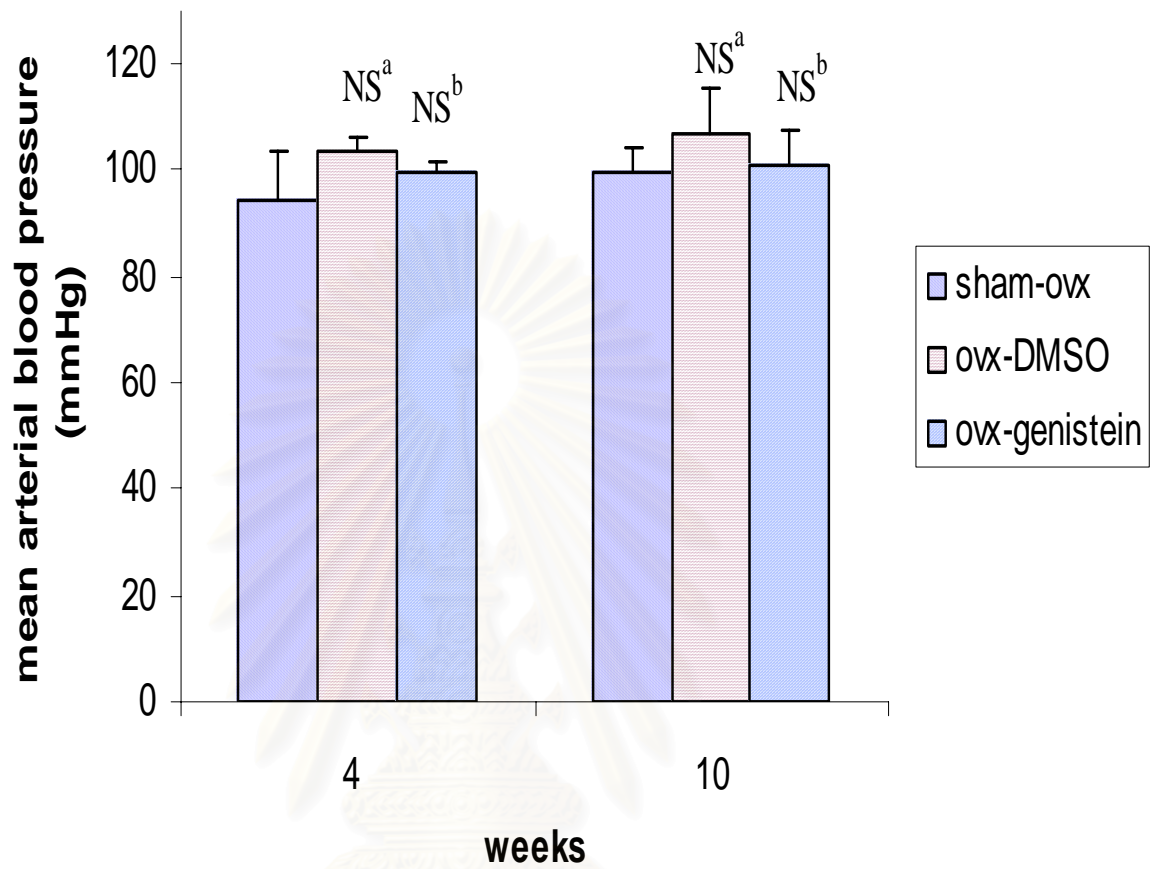
Figure 11. Mean arterial blood pressure of sham-ovx, ovx-DMSO and
ovx-genistein at 4 and 10 weeks

Values are means ± SE ; n = 6

NS[a]  Nonsignificant difference as compared to sham-ovx
( P<0.05 ).

NS[b]  Nonsignificant difference as compared to ovx-DMSO
( P<0.05 ).

Table 7. Heart rate (beats / minute) of sham-ovx, ovx-DMSO and
ovx-genistein at 4 and 10 weeks

| Groups | Heart rate (beats / minute) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $310 \pm 18.44$ | $325 \pm 12.04$ |
| ovx-DMSO | $NS^a$ $315 \pm 16.88$ | $NS^a$ $332.5 \pm 13.65$ |
| ovx-genistein | $NS^b$ $325 \pm 14.32$ | $NS^b$ $312.5 \pm 19.14$ |

Values are means $\pm$ SE ; n = 6

$NS^a$ Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Figure 12. Heart rate (beats / minute) of sham-ovx, ovx-DMSO and
ovx-genistein at 4 and 10 weeks
Values are means ± SE ; n = 6
$NS^a$  Nonsignificant difference as compared to sham-ovx
( P<0.05 ).
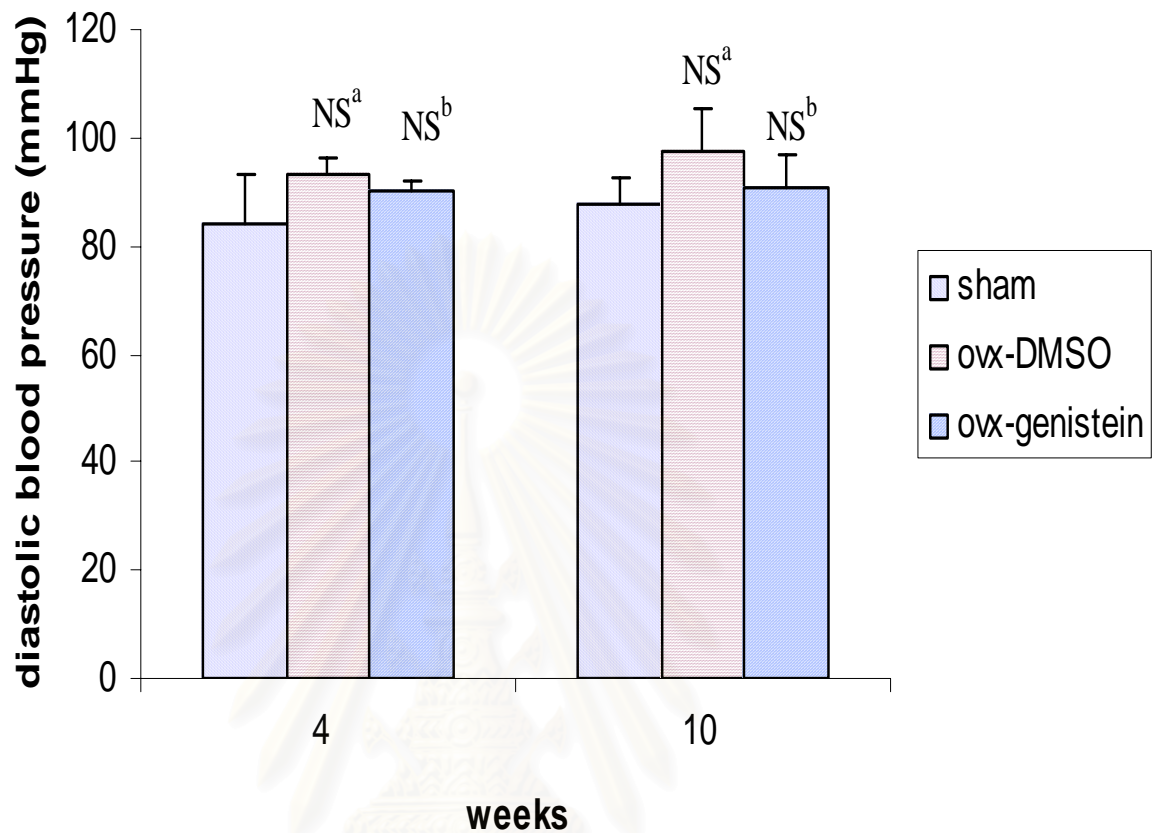$NS^b$ Nonsignificant difference as compared to ovx-DMSO
( P<0.05 ).
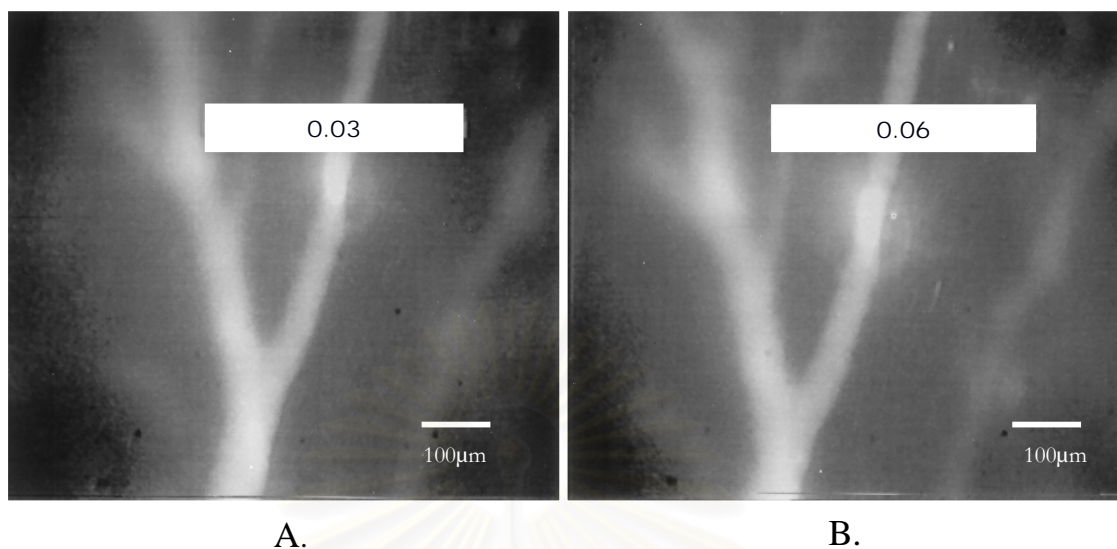
Figure 13. Coronary arteriolar responses to topical application of acetylcholine
$(10^{-5})$ in a isolated arrested heart of control rat.

A. Before topical application of acetylcholine.

B. Coronary arterioles dilate after topical application of acetylcholine.

Table 8. Changes in arteriolar diameter in responses to acetylcholine

$(10^{-5}M)$ of sham-ovx, ovx-DMSO and ovx-genistein at 4 and 10 weeks

| groups | Changes in arteriolar diameter (%) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $12.02 \pm 1.55$ | $10.96 \pm 1.23$ |
| ovx-DMSO | $NS^a$<br>$7.64 \pm 0.99$ | $3.2 \pm 0.77^*$ |
| ovx-genistein | $NS^b$<br>$12.69 \pm 1.95$ | $11.45 \pm 1.84^{\#}$ |

Values are means $\pm$ SE ; n = 5

  *   Significant difference as compared to sham-ovx ( P<0.05 ).

  #   Significant difference as compared to ovx-DMSO ( P<0.05 ).

$NS^a$ Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Figure 14. Changes in arteriolar diameter in responses to acetylcholine

(10^{-5}M) of sham-ovx, ovx-DMSO and ovx-genistein at 4 and 10

weeks

Values are means ± SE ; n = 5

* Significant difference as compared to sham-ovx ( P<0.05 ).

# Significant difference as compared to ovx-DMSO ( P<0.05 ).

NS^a Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

NS^b Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Table 9. Changes in arteriolar diameter in responses to sodium nitroprusside

(10-4M) of sham-ovx, ovx-DMSO and ovx-genistein at 4 and 10 weeks

| groups | Changes in arteriolar diameter (%) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | 12.68 ±2.03 | 16.05 ± 2.82 |
| ovx-DMSO | NS$^a$<br>12.43 ± 1.17 | NS$^a$<br>12.73 ±2.72 |
| ovx-genistein | NS$^b$<br>16.79 ± 3.16 | NS$^b$<br>16.4 ± 4.71 |

Values are means ± SE ; n = 5

NS$^a$  Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

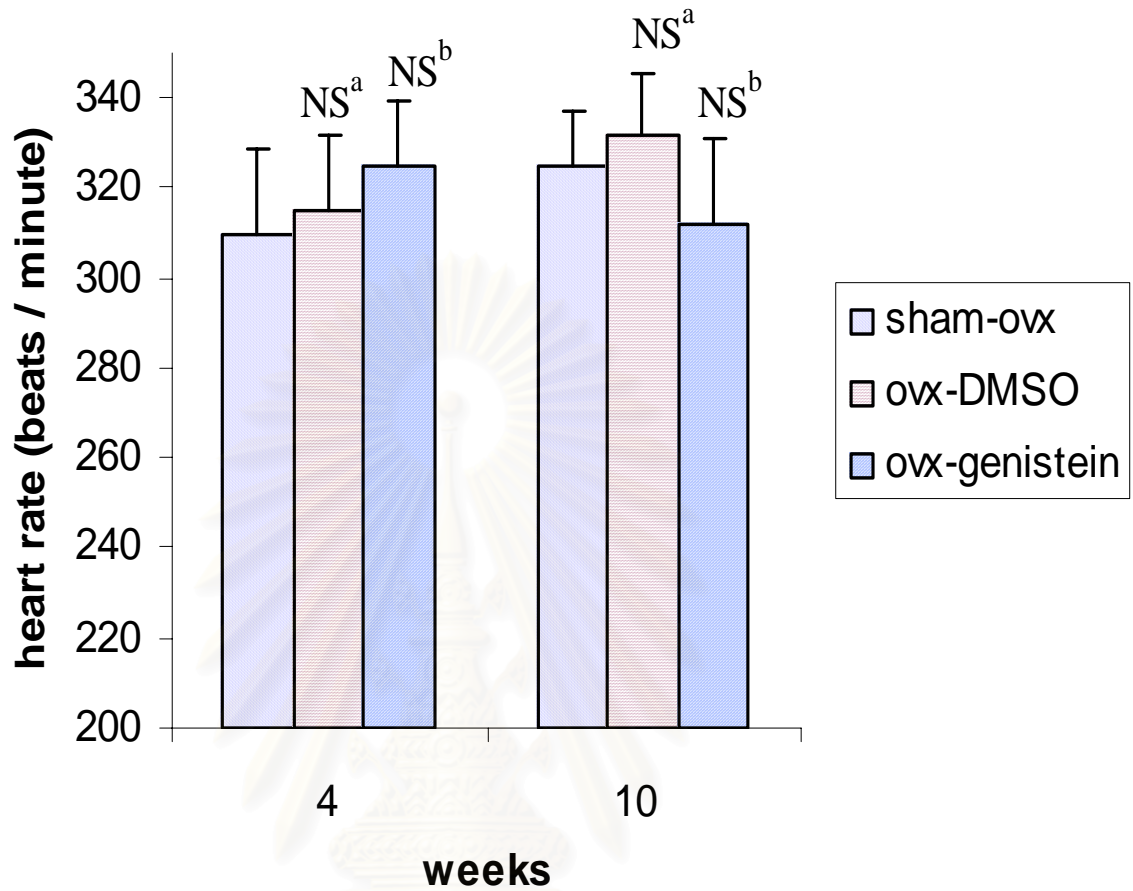NS$^b$  Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Figure 15. Changes in arteriolar diameter in responses to sodium

nitroprusside($10^{-4}$M) of sham-ovx, ovx-DMSO and ovx-genistein

at 4 and 10 weeks

Values are means ± SE ; n = 5

$NS^a$ Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Table  10. Total cholesterol (mg/dl) of of sham-ovx, ovx-DMSO and ovx-
genistein at 4 and 10 weeks

| groups | Total cholesterol (mg/dl) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $55.17 \pm 3.8$ | $61.33 \pm 4.65$ |
| ovx-DMSO | $70.17 \pm 4.81^{NS^a}$ | $67.67 \pm 2.67^{NS^a}$ |
| ovx-genistein | $70.83 \pm 4.08^{NS^b}$ | $72.00 \pm 3.71^{NS^b}$ |

Values are means $\pm$ SE ; n = 6

NS[a]  Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

NS[b]  Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Figure 16. Total cholesterol (mg/dl) of of sham-ovx, ovx-DMSO and ovx-
genistein at 4 and 10 weeks

Values are means ± SE ; n = 6

NS[a] Nonsignificant difference as compared to sham-ovx
( P<0.05 ).

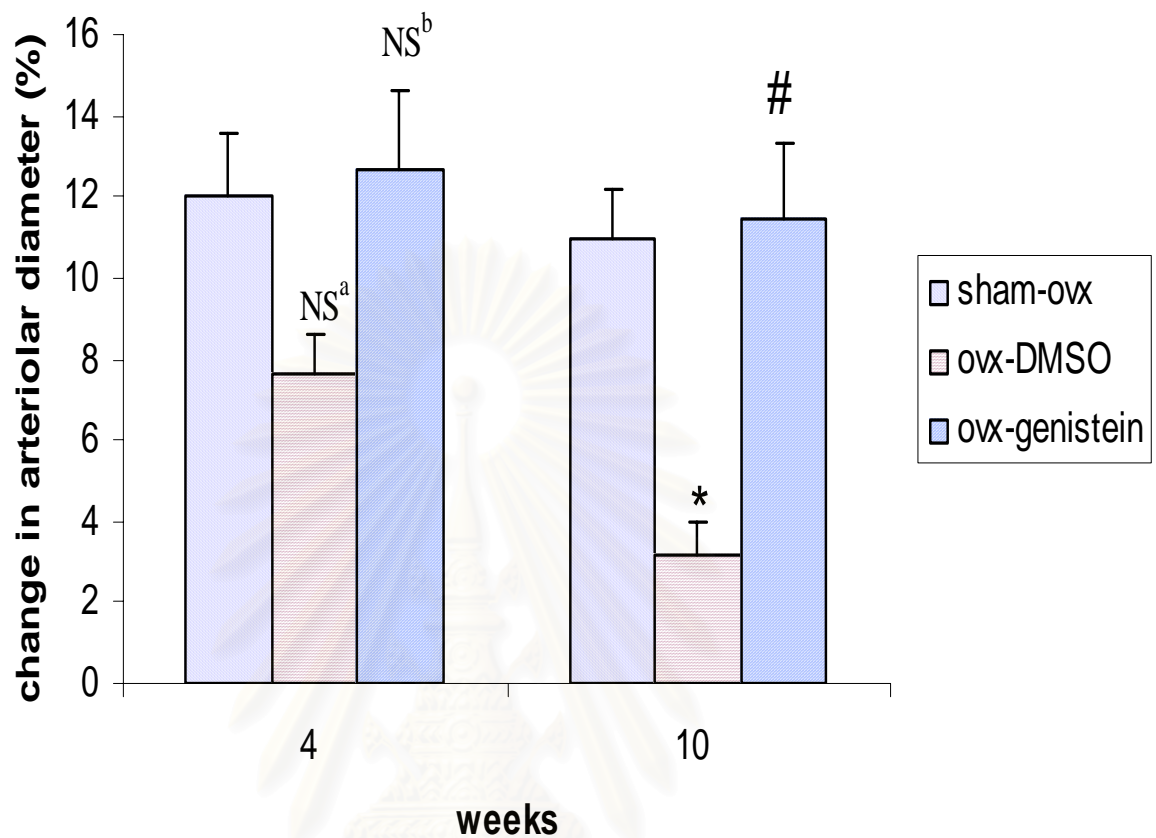NS[b] Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Table  11. Triglyceride (mg/dl) of of sham-ovx, ovx-DMSO and ovx-
genistein at 4 and 10 weeks

| groups | Triglyceride (mg/dl) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $35.17 \pm 5.73$ | $33.00 \pm 3.83$ |
| ovx-DMSO | $29.67 \pm 4.16$ NS[a] | $27.67 \pm 2.95$ NS[a] |
| ovx-genistein | $31.17 \pm 5.08$ NS[b] | $22.67 \pm 2.10$ NS[b] |

Values are means $\pm$ SE ; n = 6

NS[a]  Nonsignificant difference as compared to sham-ovx ( $P<0.05$ ).

NS[b]  Nonsignificant difference as compared to ovx-DMSO ( $P<0.05$ ).

Figure 17. Triglyceride (mg/dl) of of sham-ovx, ovx-DMSO and ovx-
genistein at 4 and 10 weeks

Values are means ± SE ; n = 6

NS[a] Nonsignificant difference as compared to sham-ovx
( P<0.05 ).

NS[b] Nonsignificant difference as compared to ovx-DMSO
( P<0.05 ).

Table  12. HDL-C (mg/dl) of of sham-ovx, ovx-DMSO and ovx- genistein at
4 and 10 weeks

| groups | HDL-C (mg/dl) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $54.67 \pm 2.74$ | $59.5 \pm 3.75$ |
| ovx-DMSO | $58.5 \pm 3.27^{NS^a}$ | $64.5 \pm 2.01^{NS^a}$ |
| ovx-genistein | $64.83 \pm 2.44^{NS^b}$ | $65.33 \pm 2.20^{NS^b}$ |

Values are means $\pm$ SE ; n = 6

$NS^a$ Nonsignificant difference as compared to sham-ovx ( P<0.05 ).

$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).
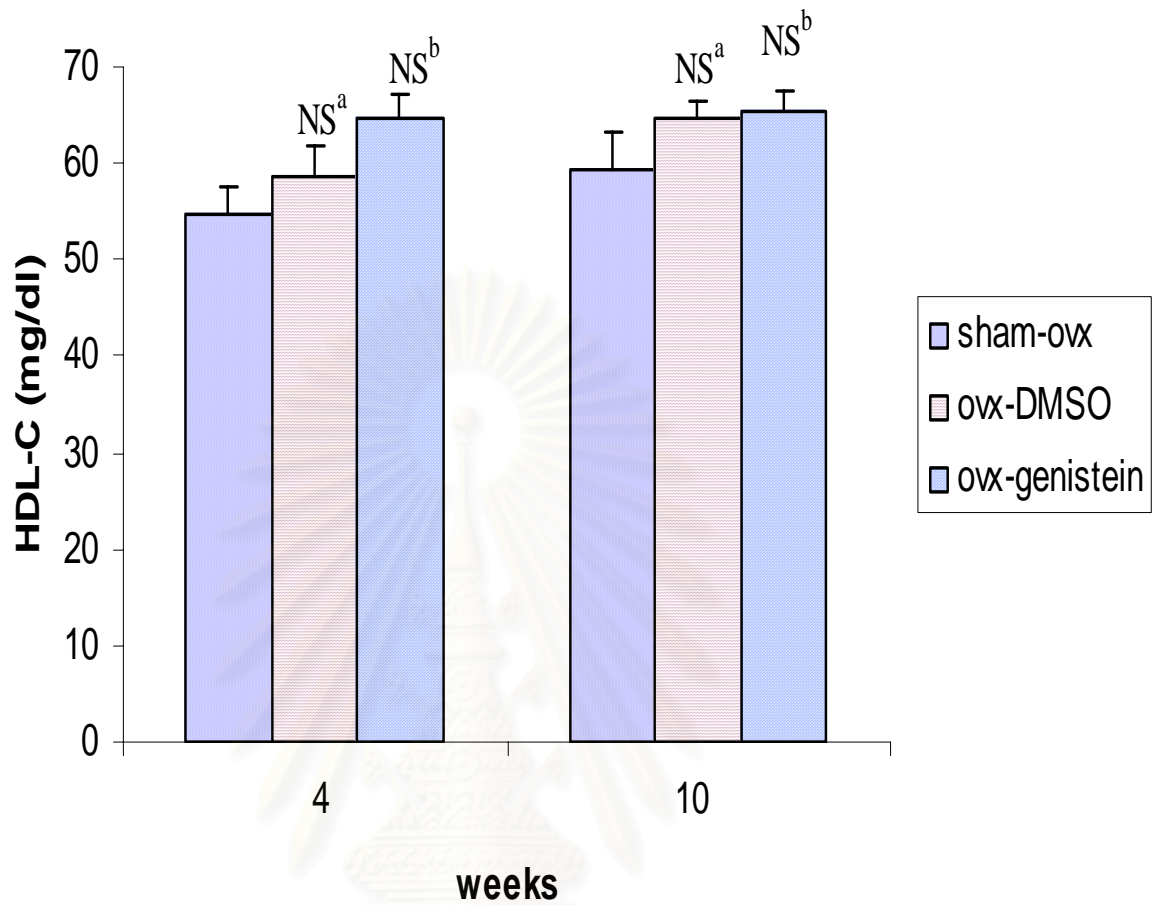
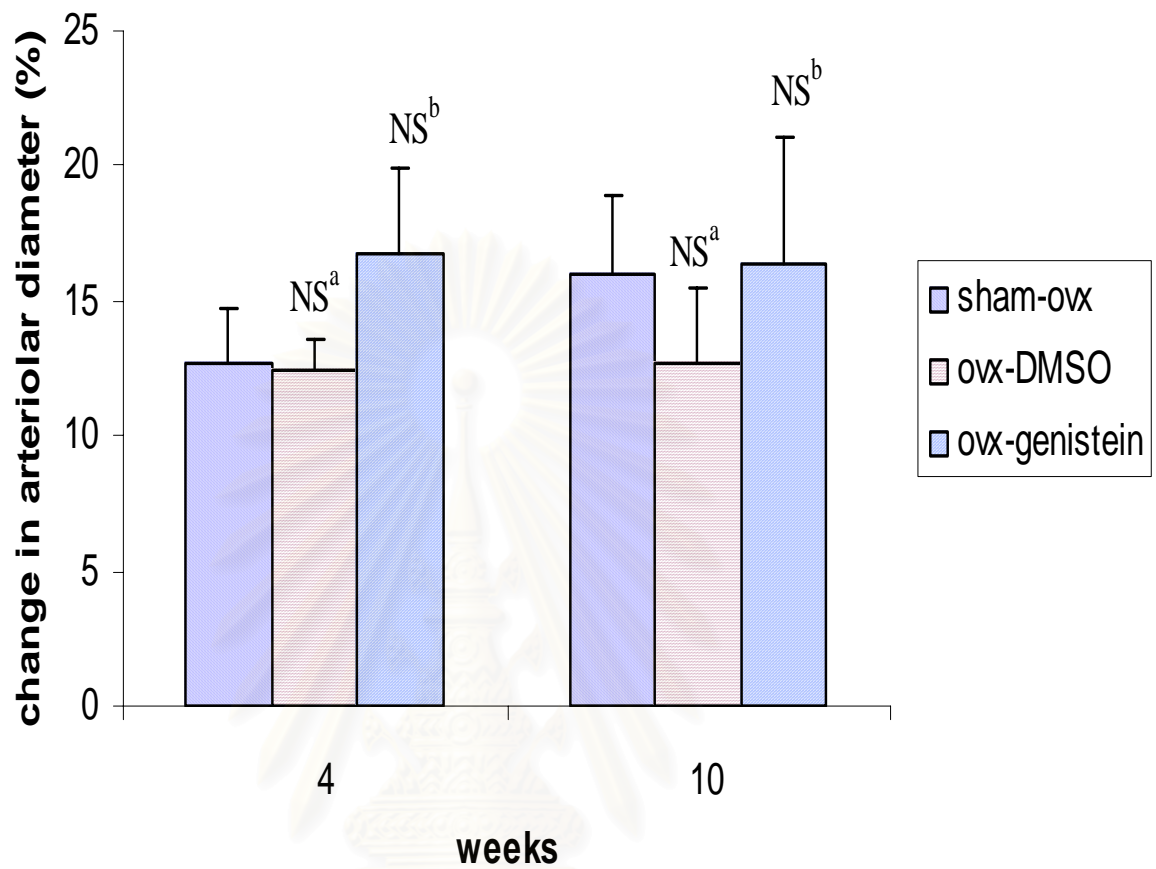Figure 18.  HDL-C (mg/dl) of of sham-ovx, ovx-DMSO and ovx- genistein

at 4 and 10 weeks

Values are means ± SE ; n = 6

NS[a]  Nonsignificant difference as compared to sham-ovx

( P<0.05 ).

NS[b]  Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

Table  13. LDL-C (mg/dl) of of sham-ovx, ovx-DMSO and ovx- genistein at
4 and 10 weeks

| groups | LDL-C (mg/dl) | |
|---|---|---|
| | 4 weeks | 10 weeks |
| sham-ovx | $3.50 \pm 0.43$ | $6.83 \pm 2.10$ |
| ovx-DMSO | $7.50 \pm 0.62^{NS^a}$ | $8.00 \pm 1.61^{NS^a}$ |
| ovx-genistein | $7.17 \pm 1.49^{NS^b}$ | $9.50 \pm 1.95^{NS^b}$ |

Values are means $\pm$ SE ; n = 6

$NS^a$ Nonsignificant difference as compared to sham-ovx ( $P<0.05$ ).

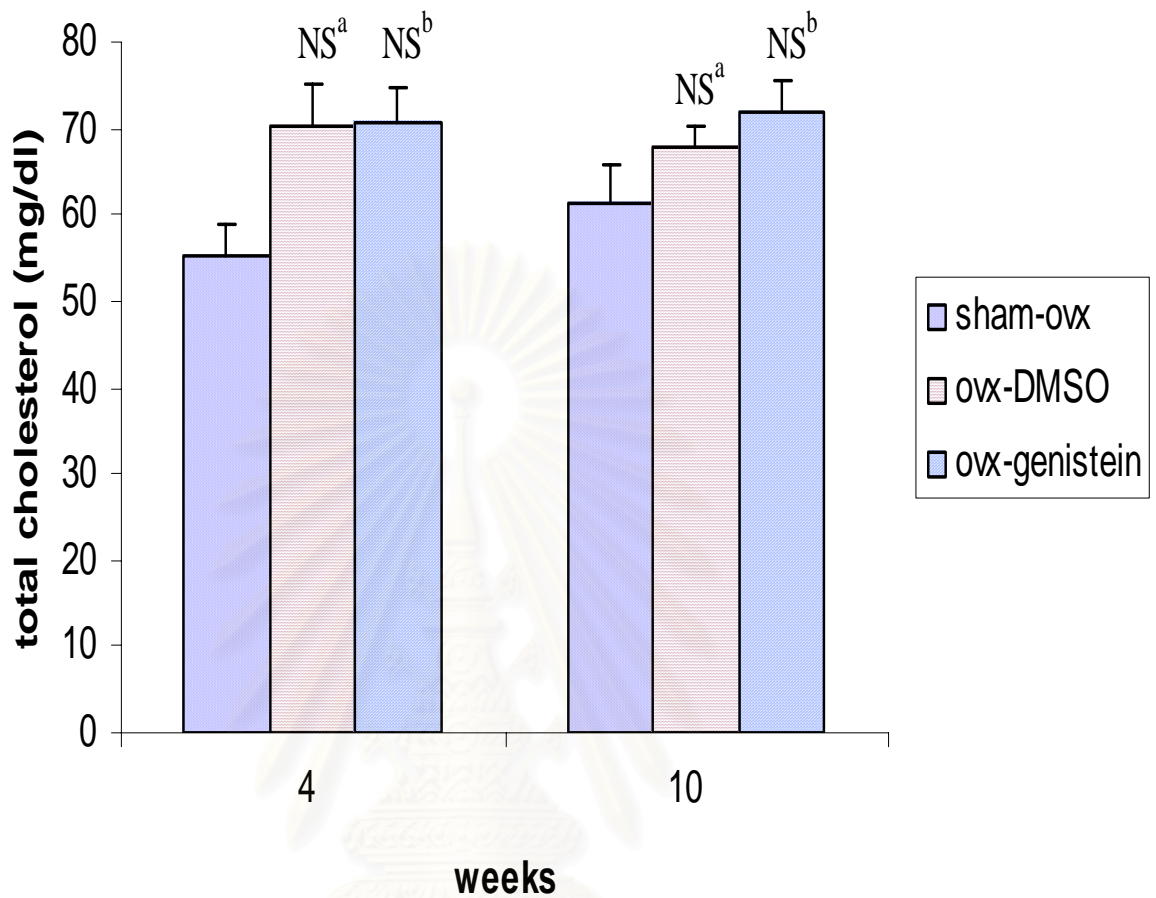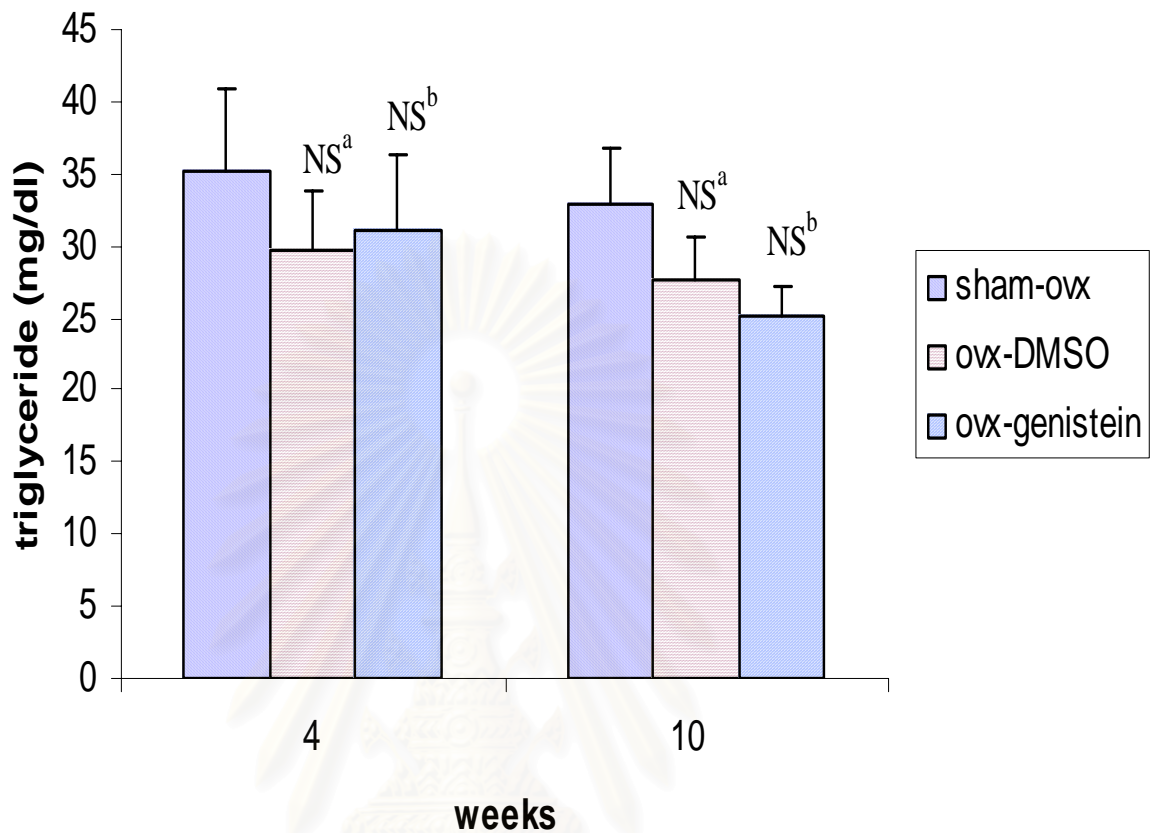$NS^b$ Nonsignificant difference as compared to ovx-DMSO ( $P<0.05$ ).
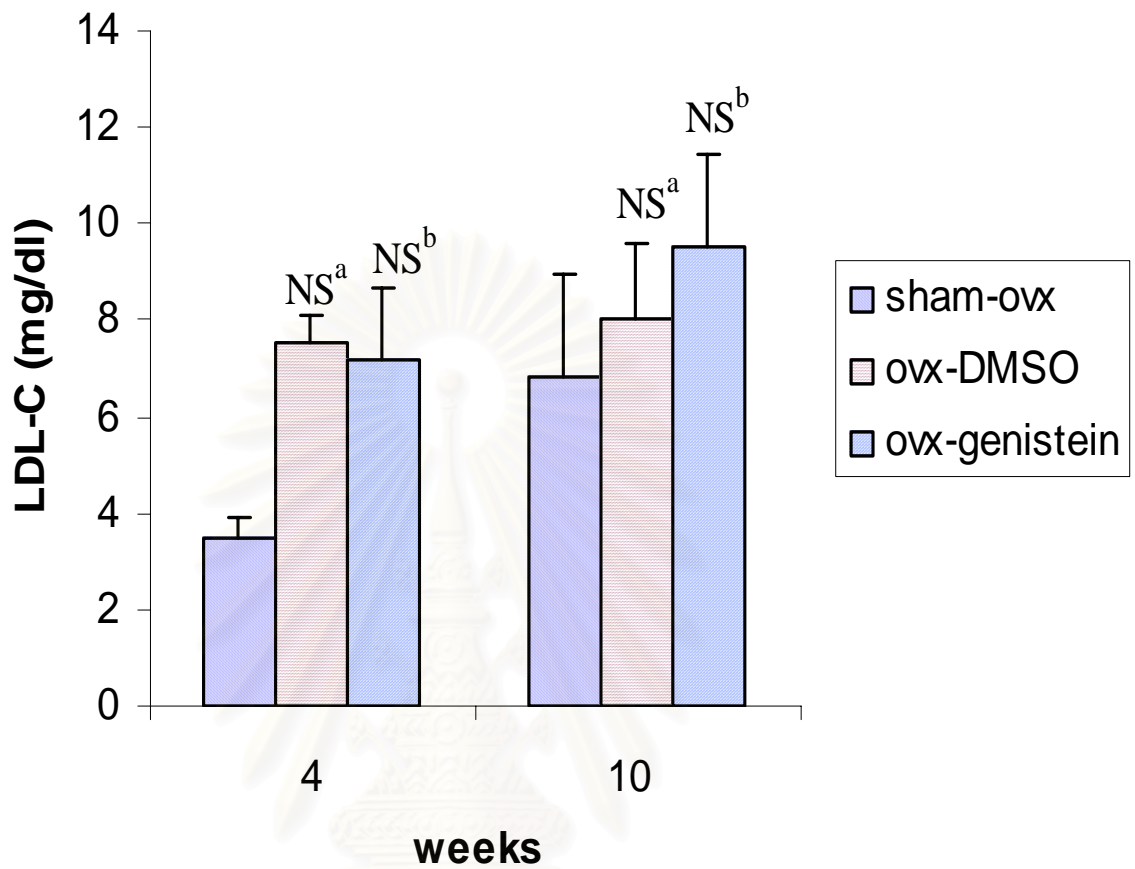
Figure 19. LDL-C (mg/dl) of of sham-ovx, ovx-DMSO and ovx- genistein

at 4 and 10 weeks

Values are means ± SE ; n = 6

NS[a] Nonsignificant difference as compared to sham-ovx

( P<0.05 ).

NS[b] Nonsignificant difference as compared to ovx-DMSO ( P<0.05 ).

# CHAPTER V

# DISCUSSION

In the present study, bilateral ovariectomy in female rats was used as a model represented the menopausal condition. The coronary endothelial functions of these ovariectomized rats were studied using Langindroff's isolated heart model. On 4 and 10 weeks after the ovarian surgery, the experiments were performed and the lipid profile was examined as well. The effects of the genistein on plasma lipid profiles and coronary endothelial functions in ovariectomized rats were also investigated during 4 and 10 seeks of experimental periods.

## Study of cardiovascular functions

In our study, the experimental results have demonstrated that the bilateral ovariectomy did not modify cardiovascular function which were characterized by unchanged systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and heart rate. It also showed that there was no changes on blood pressure in both groups of bilateral ovariectomize genistein supplementation. In 1999-2000, Squadrito et al and Tamaya et al had also showed that there were not any differences on systolic blood pressure and diastolic blood pressure between the sham ovariectomized and ovariectomized rats.

In 1947, Taylor showed no evidence of a rise in blood pressure in women at the time of the menopause. This was comfirmed in Framingham study (Hjortland et al., 1976). Other data are conflicting one prospective study a temporary decrease on the age-related rise in blood pressure at the time of the menopause (although only for systolic blood pressure) (Lindquist, 1982), while the 1960-62 health examination survey showed a rise in diastolic blood pressure with the onset of the menopause (weiss, 1972). Thus, there is no consistent evidence that the menopause, a state of estrogen deficiency, has any effect on blood pressure.

In our study, the experiment results have shown that bilateral ovariectomy could cause a changing of increase body weight in ovariectomized groups. It found that estrogen was important in regulating weight gain. Animals with their ovaries surgically removed gained weight, even if they were fed the same diet as the animals with intact ovaries. Moreover, supplementation with genistein did not decrease weight gain

Postmenopausal women are at increased risk of coronary heart disease (CHD), partly because of the decline in estrogen production and concurrent elevations in total and LDL-C level. Obesity, weight gain, and adverse changes in body fat distribution and composition are part of this phenomenon (Poehlman et al., 1995; Wing et al., 1991). Moreover, the rise in LDL-C levels and onset of other CHD risk factors (eg, high blood pressure, high total cholesterol and triglyceride levels, insulin resistance) is directly influenced by weight gain (Wing et al., 1991; Denke et al., 1994).

Weight gain in menopausal women appears to be more closely related to physiologic and behavioral changes associated with aging than to hormonal shifts. Changes in resting metabolic rate (RMR) have been observed, which may be due in part to a decline in fat-free mass. However, loss of ovarian function and the luteal phase of the menstrual cycle may also contribute to decreased RMR (Heymsfield et al., 1994). The age-related decline in RMR and decreased physical activity, with or without increased caloric intake, could easily result in weight gain.

The factor most consistently related to weight gain in this age-group is decreased physical activity (Wing et al., 1991). Poehlman and associates showed that postmenopausal women were less physically active during leisure time than age-matched premenopausal women (Poehlman et al., 1995). In the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial, women who were more physically active at baseline gained less weight (Espeland et al., 1997). In the Healthy Women's Study (Wing et al., 1991), women who decreased their physical activity by at least 300 kcal per week over 3 years gained 2.4 kg (5.28 lb), compared with a gain of only 1.58 kg (3.48 lb) in women who increased their activity by at least 300 kcal per week ($P<0.05$). Women whose physical activity remained unchanged over the 3 years gained, on average, 2.75 kg (6 lb) (Wing et al., 1991).

Decreased physical activity is not only strongly related to weight gain but is also related to the loss of fat-free mass and increased body fat observed in postmenopausal women (Heymsfield et al., 1994). Moreover, evidence suggests that regular exercise in postmenopausal older women can attenuate the accumulation of adipose tissue in the upper and central body regions (Kohrt et al., 1992).

The result of uterus weight in this experiment show that bilateral ovariectomy decreased uterus weight in ovariectomized groups and did not increase when supplementation of genistein. It showed that genistein did not have effect on reproductive system.

Genistein's effects appear to be tissue-specific, with estrogen agonist effects on plasma lipid concentrations, plasma lipoprotein distributions (Anthony et al., 1994; Balmir et al., 1995) that are similar in magnitude to mamalian estrogens, but without estrogenic effects such as endometrial proliferation on the uterus at these same doses.

In 1996, Anthony et al. reported that the phytoestrogens had no adverse effects on the reproductive systems of nither the males nor females, as evaluated by reproductive hormone concentrations and organ weights at necropsy. Thus, the isoflavones in soy protein improve cardiovascular disease risk factors without apparent deleterious effects on the reproductive system of peripubertal rhesus monkeys.

**Study of coronary arteriolar responses**

*Endothelium-dependent relaxation*

Our study demonstrated that the ovariectomized rats exhibited the endothelial dysfunction which were characterized by decreased vasodilation responses to acethycholine, but unchanged response to sodium nitroprusside.

This data suggested that ovariectomized rats show a marked impairment in the L-arginine/NO pathway characterized by blunting production of endothelial NO on 10 weeks after ovariectomy significantly.

Interestingly, the result of this experiment showed that supplementation of genistein 4 and 10 week in ovariectomize group enhanced the response of vasodilation of arteriolar to acethycholine and significant in 10 weeks supplementation of genistein.

In 1980, Furchgott and Zawadzki showed that the presence or absence of an intact endotheoium determined the response of the underlying smooth muscle to acetylcholine. In the presence of the endothelium, a precontracted blood vessel ring in an organ bath relaxed when exposed to acetylcholine. Conversely, when the endothelium was denuded by gently rubbing the vessel, there was a contractile response to acetylcholine. When rubbing the adventitial surface did not reproduce this phenomenon, they suggested that it was due to a substance they called endothelium-derived relaxation factor (EDRF) or nitric oxide (NO) (Furchgott and Zawadzki, 1980). From the studies by using light/dye treatment, resulting in selective impairment of the endothelium without affecting vascular smooth muscle reactivity, demonstrated that arteriolar dilation to acetycholine is endothelium-dependent and is mediated exclusively via endothelial factor (Koller et al., 1989a; 1989 b). The action of EDRF on blood vessels has been demonstrated to be mediated by the stimulation of guanylate cyclase, leading to elevated levels of cyclic GMP in vascular smooth muscle which initiates the process of relaxation (Rapoport and Murad, 1983).

Honor et al (1997) reported that genistein enhanced the dilator response to acethycholine of the atherosclerotic arteries in female monkeys. Nevala et al (1998) suggested that the isoflavones enhanced the dilator response to acethycholine in the atherosclerotic arteries. In 1999, Squadrito et al. had showed that an injection of genistein supplementation for 4 weeks could improve endothelium-dependent relaxation in the ovariectomized rats.

However, in vitro studies showed that neither the removal of endothelium nor the inhibition of nitric oxide synthesis had any effect on the genistein-induced relaxation responses (Nevala et al., 1998). This would suggested that perhaps there is a genomic and non-genomic effect the genistein similar to the genomic and non-genomic effect of estrogen.

Because of the traditional estrogen-signaling pathway involving nuclear interaction takes minutes to hours to increase the protein synthesis by the transcriptional activation. Estrogens have other effects that cannot be explained by a transcriptional mechanism because of their rapid onset. These effects are the result of direct estrogenic action on cell membranes and are mediated by the cell-surface forms of the estrogen receptor. Although these receptors remain largely uncharacterized, they are thought to resemble their intracellular counterparts (Watson et al., 1999). Example of the effect mediated by this alternative pathway are the short-term vasodilation of the coronary arteries (Kim et al., 1999).

There is a direct link between the estrogen cell-surface receptors and the mitogen-activated protein kinase signaling cascade (Kato et al., 1995).

Coupling of the bound membrane estrogen receptor to the mitogen activated protein kinase pathway has been demonstrated in the endothelial cells.

Estogens receptors have been detected in the smooth-muscle cells of the coronary arteries (Karas et al., 1994) and endothelial cells in various sites(Venkov et al., 1996). Estrogens cause short-term vasodilation by increasing the formation and release of nitric oxide and prostacyclin in the endothelial cells(67). They also reduce vascular smooth-muscle tone by opening the specific calcium channels through a mechanism that is dependent on cyclic guanosine monophosphate.

From our study, it showed that genistein supplementation could improve endothelial dysfunction observed in the ovariectomized rats by increasing the activity of endothelial NO synthase. The phytoestrogen was able to restore the endothelium response likely through and increased production of the basal endothelial NO release. From a mechanistic point of view it can be proposed that genistein increased the activity of the endothelial NO synthase: indeed lung homogenates prepared from phytoestrogen-supplemented ovariectomized rats showed an increased activity of this enzyme isoform (Squadrito et al., 1999).

Genistein behaves as a tyrosine kinase inhibitor at higher doses up to 1 mg/kg bw (Akyama et al., 1987) while at lower doses 0.2 mg/kg Bw (Squadrito et al., 1999) as the dose used in the present study, it exerts estrogenic activity (Akyama et al., 1987). The lower doses may be easily reached from a nutritionally-based treatment since the concentration of genistein in most soy food ranges from 1-2 mg/g protein.

Genistein has been shown to mimic many of the biological activities of 17β-estradiol.  The research findings of Barnes et al. (1990), Setchell et al. (1984) and other investigators (Makela et al., 1994) have suggested that genistein acts via estrogen receptor and exhibits the estrogenic properties in some tissue (Martin et al., 1978). The protective effects of estrogen supplementation on the cardiovascular apparatus may be mediated by preventing the endothelial cell to become dysfunction. Endothelial dysfunction has been well characterized by decreasing in NO production NO release is reduced in cardiovascular disease such as hypertension and atherosclerosis (Luscher and Dbey, 1994).  NO has been documented widely for its important actions such as vasodilatior (Furchgott and Zawadzki, 1980; Ignarro et al., 1987), the inhibition of platelet adhesion and aggregation (Mellion et al., 1981) and the inhibition of smooth muscle cell proliferation and migration (Dubey, 1994).

***Endothelium-independent relaxation***

In our study, the endothelium-independent response was investigated by analyzing the relaxant effect of sodium nitroprusside. Because of sodium nitroprusside which causes relaxation in a vessel via endothelium-independent pathway directly on smooth muscle cell by the activation of guanylate cyclase.This activation lead to increasing the rate of formation of cyclic GMP in the vascular smooth muscle cells which initiates the process of relaxation (Rapoport and Murad, 1983).  From the study by using light/dye treatment, resulting in the selective impairment of the endothelium

without affecting vascular smooth muscle reactivety, demonstrated that the arteriolar dilation to sodium nitroprusside was not altered by this treatment (Koller et al., 1989a).This result indicated that arteriolar dilation to sodium nitroprusside is endothelium-independent.

In our result, the coronary arteriolar dilation caused by sodium nitroprusside was not impaired in the ovariectomized rats. The result of this study suggested that the vascular smooth muscle cells in the ovariectomized groups were not impaired by bilateral ovariectomy and did not altered by the treatment.

**Lipid profiles**

During 1960 to 1993, several studies on the hypocholesterolemic effects of soy protein in both humans and animal model, had been performed. However, the American Heart Association (AHA) had concluded that soy protein could lower serum cholesterol only in animals but not humans (Chait et al., 1993). Formal recognition of the cholesterol-lowering properties of soy protein, however, has come one year earlier, in 1999, the U.S.Food and Drug Adiministration (FDA) approved a health claim for the cholesterol-lowering effects of soy protein. They have set 25 g/day as the target goal for cholesterol reduction (Food and Drug Administration, 1999). However, some of the studies and limited clinical research suggests fewer than 25 g soy protein was needed for cholesterol reduction (Nagata et al., 1998; Ho et al., 2000; Tonstad et al., 2002; Teixeira

et al., 2000).  It is clear that the effects of soy protein are most pronounced in those with elevated serum cholesterol (Anderson et al., 1995).

In rats, no difference was reported in total cholesterol levels between 2 and 12 months of age but a significant increase in total cholesterol was observed at the age of 24 months (Lacko and Davis, 1979).  Which is in accordance with Aguila et al (2002).  It is important to note that there is a general similarity between the cardiovascular system of rats and that of other mammals, man inclusive (Campbell et al., 1986).  Despite the difficulty in producing hyperlipidemia and atherosclerosis in rats, special diets may induce an increase in the serum levels of cholesterol, and also induce arterial hypertension (Gill et al., 1989).

Phytoestrogens have been reported for their good effects on lipid profiles.  Soy protein inhibits atherosclerosis in animals.  This effect seems to be mediated in large part by effects on plasma lipoprotein concentrations, that is, by reducing LDL-C by about 13%, lowering plasma triglycerides by about 10%, and possibly increasing HDL-C by 2% (Anderson et al., 1995). These beneficial effects of soy protein on plasma  lipoprotein concentrations culminated recently in the U.S.Food and Drug Administration's approval of a health claim that 25 g of soy protein a day, as part of a diet low in saturated fat and choesterol, may reduce the risk of heart disease.

From the results of our study, the plasma levels of total cholesterol, triglyceride, HDL-C and LDL-C were unchanged in ovariectomized rats. Thus it suggested that the present model, 4 and 10 weeks of estrogen dificiency, caused a selective  impairment in the L-arginine /NO pathway

(endothelial dysfunction) without any correlation with the alteration of lipid profile that previous study by Squatridro (1999). It suggested that the time period of endothelial dysfunction did not correlate with changing of lipid profiles. And our study showed that endothelial dysfunction occurred before lipid profiles changing in case that did not fed with hypercholesterolemic food.

Therefore, from our findings, it might be further implied that the cardioprotective effect of estrogen, as well as phytoestrogens, is actually direct on endothelial function not by its indirect effect on lipid profiles. Therefore in the following, the effects on endothelial function would be continued discussion.

# CHAPTER VI

# CONCLUSION

This study investigated the effects of genistein on plasma lipid profiles and coronary vasodilation in ovariectomized rats. The overall results of this investigation indicated that:

1.The injection of genistein could attenuate the impairment of endothelium-dependent relaxation and preventing the impairment in endothelium dependent relaxation of ovariectomized rats.

2.The lipid profiles in this study did not change in both groups of 4 and 10 weeks. At this moment, therefore, it implied that the endothelial dysfunction did not primarily cause by the lipid profiles changing in the ovariectomized rats..

3.Bilateral ovariectomy and the treatment with genistein did not alter the cardiac functions including HR and MAP in both 4 and 10 weeks groups.

4.The impairment of endothelial functions in the ovariectomized rats could be prevented by daily injection of 0.2 mg/kg body weight of genistein.

These effect of genistein on menopause model in this study indicated that genistein might be the great benefit to postmenopasaul women in

prevent of endothelial dysfunction. However, the suitable dose and its mechamism of action need to be confirmed in the furture.

# REFERENCES

Adlercreutz H, Fotsis T, Lampe J. 1993. Quantitative determination of lignans and isoflavones in plasma of omnivorous and vegeterian women by isotope-dilution das chromatography-mass spectrometry. Scand Clin Lab Invest. 53: 5-18.

Adlercreutz H, Goldin B, Gorbach S, Höck erstedt K, Watanabe S, Hämäläinen E, Markkanen M, Mäkelä T, Wähälä K, Hase T, Fotsis T. 1995. Soybean phytoestrogen intake and cancer risk. J Nutr. 125: 757-770.

Adlercruetz H, Goldin BR, Gorbach SL, et al. 1995. Soybean phytoestrogen intake and cancer risk. J Nutr. 125: 757S-70S.

Adlercreutz H, Markkanen H, Watanabe S. 1993. Plasma concentration of phyto-oestrogens in Japanese men. Lancet. 342: 1209-1210.

Aguila MB, Loureiro CC, Pinheiro ADR. 2002. Lipid metabolism in rats fed diets containing different typed of lipids. Arq Bras Cardiol. 78: 32-8.

Alekel DL, Germain AS, Peterson CT, Hanson KB, Stewart JW, Toda T. 2000. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr. 72: 844-852.

Akyama T, Ishida J, Nakahawa S et al. 1987. Genistein,a specific inhibitor of tyrosne-specific protein dinases. <u>J Biol Chem</u>. 262: 5592-5595.

American Heart Association. 1997. Heart and Stroke Statistical Update. Dallas, TX.

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

Andlauer W, Kolb J, Furst P. 2000. Absorption and metabolism of genistin in the isolated rat small intestine. <u>FEBS Lett</u>. 475: 127-130.

Anthony MS, Clarkson TB, Bullock BC, Wagner JD. 1997. Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. <u>Arterioscler Thromb Vasc Biol</u>. 17: 2524-2531.

Anthony MS, Clarkson TB, Hughes CL, Morgan TM, Burke GL. 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. <u>J Nutr</u>. 126: 43-50.

Anthony MS, Clarkson TB, Hughes CL. 1994. Plant and mammalian estrogen effects on plasma lipids of female monkeys. <u>Circulation</u>. 90: I-235 (abs).

Arjmandi BH, Birnbaum R, Goyal NV, Getlinger MJ, Juma S, Alekel L, Hasler CM, Drum ML, Hollis BW, Kukreja SC. 1998a. Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. Am J Clin Nutr. 68: 1364-1368.

Arjmandi BH, Getlinger MJ, Goyal NV, Alekel L, Hasler CM, Juma S, Drum ML, Hollis BW, Kukreja SC. 1998b. Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by overian hormone deficiency inrats. Am J Clin Nutr. 68: 1358-1363.

Ashton E, Ball M. 2000. Effects of soy as tofu vs meat on lipoprotein concentrations. Eur J Clin Nutr. 54: 14-19.

Awoniyi CA, Roberts D, Veeramachaneni DNR, et al. 1998. Reproductive sequelae in female rats after in utero and neonatal exposure to the phytoestrogen genistein. Fertil Steril. 70: 440-7.

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

Balmir F, Staack RF, Jeffery EH, Potter SM. 1995. Soy bean isoflavones influence serum lipid levels in rats. J Nutr. 125:805s (abs).

Barnes S, Grubbs C, Setchell KDR, Carlson J. 1990. Soybeans inhibit nannary tumors in moddels of vreast cancer. Prog Clin Biol Res. 347: 239-253.

Barrett-Connor E, Bush TL. 1991. Estrogen and coronary heart disease in women. JAMA. 265: 1861-7.

Barrett-Connor E, Bush TL. 1978. Estrogen replacement and coronary heart Disease. Cardiovasc Clin. 19: 259-161.

Bingham S, Atkinson C, Liggins J et al. 1999. Phyto-oestrogens: where are we now? British J. Nutr. 79: 393-406.

Bonithon-Kopp C, Scarabin PY, Darne B, et al. 1990. Menopause-related changes inlipoproteins and some other cardiovascular risk factors. Int Jepidemiol. 19(1): 42-8

Bush TL, Barrett-Connor E, Cowan LD, et al. 1987. Cardiovascular mortali:y and non-contaceptive estrogen use in women: results from the Lipid Research Clinic's Program Follow – Up Study. Circulation. 75: 1002-1012.

Cannon R. 1998. Role of nitric oxide in cardiovascular diseases: Focus on the endothelium. Clin Chem. 44: 1809-1819.

Cassidy A, Bingham S, Setchell K. 1995. Biological effects of isoflavones in young women: importance of the chemical composition of soybean products. Br J Nutr. 74: 587-601.

Chang HC, Churchwell MI, Delclos KB, Newbold RR, Doerge DR. 2000. Mass spectrometric determination of Genistein tissue distribution in diet-exposed Sprague-Dawley rats. J Nutr. 130: 1963-1970.

Chait A, Brunzell JD, Denke MA, et al. 1994. Rationale of the diet-heart statement of the American Heart Association . Report of the Nutrition Committee. Circulation. 90: 2564-5.

Chiechi LM. 1999. Dietary phytoestrogens in the prevention of long term postmenopausal disease. Int J Gynaecol Obstet. 67(1):39-40.

7Cline J, Obasanjo I, Paschold J, Adams M, Anthony M. 1996. Effects of hormonal therapies and dietary soy phytoestrogens on vaginal cytology in surgically postmenopausal macaques. Fertil Steril. 65: 1031-1035.

Colburn P, Buonassisi V. 1978. Estrogen-binding sites in endothelial cell cultures. Science. 201: 817-819.

Crouse JR, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. 1999. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of iso flavones on plasma concentration of lipids and lipoproteins. Arch Int Med. 159: 2070-2076.

Day AJ, DuPont MS, Ridley S, Rhodes M, Rhodes MJ, Morgan MR, Williamson G. 1998. Deglycosylation of flavonid and isoflavonoid

glycosides by human small intestine and liver beta-glucosidase activity. <u>FEBS Lett</u>. 436: 71-75.

Denke MA, Sempos CT, Grundy SM. 1994. Excess body weight. An under-recognized contributor to dyslipidemia in white American women. <u>Arch Intern Med</u>. 154(4): 401-10.

Dong Z, Qi X, Xie K, Fidler IJ. 1993. Protein tyrosine kinase inhibitors decrease induction of nitric oxide synthase activity in lipopolysaccharide-reponsive and lipopolysaccharide-nonresponsive murine macrophages. <u>J immunol</u>. 151: 2717-24.

Dubey RK. 1994. Vasidilator-derived nitric oxide inhibits angiotensin II and fetal calf serum induced growth of arteriolar smooth muscle cells. **J Pharmacol Exp Ther**. 269: 402-408.

Erdman JW, Jr. 2000. Soy protein and cardiovascular disease: A statement for healthcare professionals from the nutrition committee of the AHA. <u>Circulation</u>. 102: 2555-9.

Espeland MA, Stefanick ML, Kritz-Silverstein D, et al. 1997. Effect of postmenopausal hormone therapy on body weight and waist and hip girths. Postmenopausal Estrogen-Progestin Interventions Study Investigators. <u>J Clin Endocrinol Metab</u>. 82(5): 1549-56.

Fisslthaler B, Popp R, Kiss L, Potente M, Harder D, Fleming I, Busse R. 1999. Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature. 401: 493-497.

Fitzpatrick LA, Litin SC, Bell MR. 2000. The Women's Health Initiative: a heart-to-HRT converstion (editorial). Mayo Clin Proc. 75: 559-561.

Fitzpatrick LA. 1999. Selective estrogen receptor modulators and phytoestrogens: new therapies for the postmenopausal woman. Mayo Clin Proc. 74: 601-607.

Foegh ML, Ramwell PW. 1998. Cardiovascular effects of estrogen: implications of the discovery of the estrogen receptor subtype beta. Curr Op Neph HTN. 7: 83-9.

Food and drug Administration. 1999. Food labeling, health claims, soy protein, and coronary heart disease. Fed Reg. 57: 699-733.

Fotsis T, Pepper M, Adlercreutz H, et al. 1993. Genistein, a dietary derived inhibitor of in-vitro agniogenesis. PNAS. 90: 2690-4.

Furchgott RF, Vangoutte PM. 1989. Endothelium-derived relaxing and contracting factors. FASEB. 3: 2007-18.

Furchgott RF, Zawakzki JV. 1980. The obigatory role of endothelial cells in the relaxatiion of arterial smooth muscle by acethylcholine. Nature. 288: 373-376.

Gerhard M, Walsh BW, Tawakol A et al. 1998. Estradiol therapy combined with progesterone and endothelium-dependent vasodilation in postmenopausal women. Circulation. 12: 1158-1163.

Goodman MT, Wilken LR, Hankin JN, Kolonel LN. The Association of Dietary Phytoestrogens with the Risk for endometrial Cancer. Proceedings of Second International Symposium on the Role of Soy in Preventing and Treating Chronic Diseases. St. Louis (MO): Protein Technology International, 1996;36.

Grady D, Gebretsadik T, Kerlikowske K, Ernster V, Pettiti D. 1995. Hormone replacement therapy and endometrial cancer risk: a metaanlysis. Obstet Gynecol. 85: 304-313.

Grodstein F, Stampfer MS, Manson JE, et al. 1996. Postmenopatusal estrogen and progestin use and the risk of cardiovascular disease. N Engl J Med. 335: 453-61.

Gruchow HW, Anderson AJ, Barboriak JJ, et al. 1988. Postmenopausal use of estrogen and occlusion of coronary arteries. Am Heart J. 115: 954-959.

Hamilton RM, Carroll KK. 1976. Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets: effects of dietary proteins, carbohydrates and fibre from different sources. Ateroscloerosis. 24: 47-62.

Heymsfield SB, Gallagher D, Poehlman ET, et al. 1994. Menopausal changes in body composition and energy expenditure. Exp Gerontol. 29(3-4): 377-89.

Hishikawa K, Nakaki T, Marumo T, Suzuki H, Kato R, Sarula S. 1995. Up-regulation of nitric oxide synthesis by estradiol in human aortic endothelial cells. FEBS lett. 360: 291-293.

Hjortland MC, McNamara PM, Kannel WB. 1976. Some atherogenic concomitants of menopause: the Framing ham Study. American Journal of Epidemiology. 103(3): 304-11.

Hodges RE, Krehl WA, Stone DB, Lopez A. 1967. Dietary carbohydrates and low cholesterol diets: effects on serum lipids on man. Am J Clin Nutr. 20: 198-208.

Honoré EK, Williams JK, Anthony MS, Clarkson TB. 1997. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril. 67: 148-154.

Ho SC, Woo JL, Leung SS, Sham Al, Lam TH, Janus ED. 2000. Intake of soy products is associated with better plasma lipid profiles in the Hong Kong Chinese population. J Nutr. 130: 2590-2593.

Ignarro L, Buda g, Wood K, Byrns R, Yamori Y. 1987. Enddothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. <u>Proc Natl Acad Sci USA</u>. 84: 9265-9269.

Ignarro LJ, Byrns RE, Buga GM, Wood KS. 1987. Endothelium-derived relaxing factor fron pulmonary and vein possesses pharmacologic and chemmical properties identical to those of nitric oxide radical. <u>Circ Res</u>. 61: 866-879.

Izumi T, Piskula MK, Osawa S, Obata A, Tobe K, Saito M, Kataoka S, Kubota Y, Kikuchi M. 2000. Soy isoflavone aglycones are absorbed faster and in higher amounts than threir glucosides in humans. <u>J Nutr</u>. 130: 1695-1699.

Jacobsen BK, Knutsen SF, Fraser GE. 1998. Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States) <u>Cancer Causes Control</u>. 9: 553-557.

Janning P, Schuhmacher US, Upmeier A, Diel P, Michna H, Degen GH, Bolt HM. 2000. Toxicokinetics of the Phytoestrogen daidzein in female DA/Han rats. <u>Arch Toxicol</u>. 74: 421-430.

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

Kalin MF, Zumoff B. 1990. Sex hormones and coronary disease: a review of the clinical studies. Steroids. 55: 330-352.

Kagan A, Harris B, Winkelstein W, Johnson K, Kato H, Syme S, Rhoads G, Gay M, Nichaman M, Hamilton H, Tillotson J. 1974. Epidemiologic studies of coronary heart disease and strike in Japanese men living in Japan, Hawaii and California: demographic, physical, dietary and biochemical characteristics. J Cronic Dis. 27: 345-364.

Kalin, MF, Zumoff B. 1990. Sex Hormones and coronary disease: a review of the clinical studies. Steroids. 55: 330-352.

Karas RH, Patterson BL, Mendelsohn ME. 1994. Human vascular smooth muscle cells contain functional estrogen receptor. Circulation. 89: 1943-1950.

Kato S, Endoh H, Masuhiro Y, et al. 1995. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science. 270: 1491-4.

Kim HP, Lee JY, Jeong JK, Bae SW, Lee HK, Jo I. 1999. Nongenomic stimulation of nitric oxide release by estrogen is mediated by estrogen receptor α localized in caveolae. Biochem Biophys Res Commun. 263: 257-62.

King RA, Bursill DB. 1998. Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. <u>Am J Clin Nutr</u>. 67: 867-872.

Kleinert H, Wallerath T, Euchenhofer C, Lhrig-Biedert I, Li H, Forstermann U. 1988. Estrogens increase transciption of the human endothelial NO synthase gene: analysis of the transcription factors involved. <u>Hypertension</u>. 31: 582-584.

Knight, DC, Eden, JA. 1996. A review of the clinical effects of phytoestrogens. <u>Obstet Gynecol</u>. 87: 897-904.

Kohrt WM, Malley MT, Dalsky GP, et al. 1992. Body composition of healthy sedentary and trained, young and older men and women. <u>Med Sci Sports Exerc</u>. 24(7): 832-7.

Koller A, Messina EJ, Wolin MS and Kaley G. 1989a. Effects of endothelial impairment on arteriolar dilator responses in vivo. <u>Am J Physiol</u>. 257 (Heart Circ Physiol 26): H1485-H1489.

Koller A, Messina EJ, Wolin MS and Kaley G. 1989b. Endothelial impairment inhibits prostaglandin and EDRF-mediated arteriolar dilation in vivo. <u>Am J Physiol</u>. 257 (Heart Circ Physiol 26): H1966-H1970.

Kolonel L. 1988. Variability in diet and its ralation to risk in ethnic and migrant groups. <u>Basic Life Sci</u>. 43: 129-135.

Krauss RM, Eckel RH, Howard B, et al. 2000. AHA dietary guidelines: revision 2000: A statement for healthcare professionals from the nutrition committee of the American Heart Association. Circulation. 102: 2284-99.

Kuiper GG, Carlsson B, Grandien K, et al. 1997. Comparison of the ligand binding specificity and transcipt tissue distribution of estrogen receptors α and β. Endocrinology. 138: 863-870.

Lamartiniere CA, Moore JB, Brown NM, jThompson R, Hardin MJ, Barnes S. 1995. Genistein suppresses mammary cancer in rats. Carcinogenesis. 16: 2833-40.

Lee H, Gourley L, Duffy S. 1991. Dietary effects on breast cancer risk in Singapore. Lancet. 337: 1197-1200.

Lindquist O. 1982. Influence of the menopause on ischaemic heart disease and its risk factors on bone mineral content. Acta Obstetrica et Gynecologica Scandinavica. S110: 1-32.

Lissin LW, Cooke JP. 2000. Phytoestrogens and cardiovascular health. J Am Coll Cardiol. 35(6): 1403-1410.

Lobo RA, Speroff L. 1994. International consensus conference on postmenopausal therapy and the cardiovascular system. Fertil Steril. 61:592-595.

Lock, M. 1994. Menopause in cultural context. Exp Gerontol. 29(3-4): 307-317.

Losordo DW, Kearney M, Kim EA, et al. 1994. Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. Circulation. 89: 1501-1510.

Lu L, Anderson K, Grady J, Nagamani M. 1996. Effects of soy consumption for one month on steroid hormones in premenopausal women: implications of breast cancer risk reduction. Cancer Epidemiol Biomark Prev. 5: 63-70.

Lu L, Anderson K, Grady J, Kohen F, Nagamani M. 2000. Decreased ovarian hormones during a soya diet:implications for breast cancer prevention. Cancer Res. 60: 4112-4121.

Lu L, Anderson K . 1988. Sex and long-term soy diets affect the metabolism and excretion of soy isoflavones in humans. Am J Clin Nutr. 68: 1500S-1504S.

Lundh T. 1995. Metabolism of estrogenic isoflavones in domestic animals. Pro Soc Exp Biol Med. 208: 33-39.

Luscher TF, Dbey RK. 1994. Endothelium and platelet-derived vasoactive substances: role in the regylation of vascular tone and growth. In:Laragh JH, Brenner BM, editors 2 nd ed, Hypertension:

pathophysiology, diagnosis and management, New York: Raven Press. pp. 609-630.

Matthews KA, Meilahn E, Kuller LH, et al. 1989. Menopause and risk factors for coronary heart disease. N Engl J Med. 321(10): 641-6.

McDonagh, PF. 1983. Both protein and blood cells reduce coronary vascular permeability to macromolecular. Am J Physiol. 14: H698-H706.

McDonagh, PF, Niven AT, and Roberts D. 1984. Direct visualization of the coronary microcirculation for pharmacologic and physoologic studies. Microvasc Res. 28: 180-196.

Makela S, Davis VL, Tally WC et al. 1994. Dietary esttriges act through estrogen receptor-mediated processses and show and show no antiestrogenicity in cultrred breast cancer cells. Environ Health Perspecy. 102: 572-578.

Masaki T. 1995. Possible role of endothelin in endothelial regulation of vascular tone. Annu Rev Pharmcol Toxicol . 35: 235-255.

Martin PM, Horwitz KB, Ryan DS, Mcguire WL. 1978. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology. 103: 1860-1867.

Martucci CP, Fishman J. 1993. P450 enzymes of estrogen metabolism. Pharmacol Ther. 57: 237-57.

McGill HC, Stern MP. 1979. Sex and atherosclerosis. <u>Atheroscler Rev</u>. 4: 157-242.

Mellion BT, Ignarro LJ, Ohlstein EH, Pontecorvo EG, Hyman AL, Kasowitz PJ. 1981. Evidence for the inhibitory role of guanosine 3´5´ monophoshate in ADP-induced human plateley aggregation in the precence of nitric oxide and related vasodilators. <u>Blood</u>. 57: 946-955.

Messina M. 1994. Diet-heart statement [letter; comment]. <u>Circulation</u>. 90: 2564-5.

Mikkola T, Ranta V, Orpana A, Viinikka L, Yli-Korkala O. 1996. Effect of physiological concentration of estradiol on $PGl_2$ and NO in endothelial cells. <u>Maturitas</u>. 25: 141-147.

Miksicek RJ. 1995. Estrogenic flavonoids: structural requirements for biological activity. <u>Proc Soc Exp Biol Med</u>. 208: 44-50.

Murkies A, Lombard C, Strauss B, Wilcox G, Burger H, Morton M. 1995. Dietary flour supplementation decreases hot flushes: Effect of soy and wheat. <u>Maturitas</u>. 21: 189-195.

Nagata C, Takatsuka N, Kurisu Y, Shimizu H. 1998. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. <u>J Nutr</u>. 128:209-13.

National Osteoporosis Foundation. 1997. Fast Facts on Osteoporosis. Washington, DC.

Nevala R, Korpela R, Vapaatalo H. 1998. Plant derived estrohens relax rat mesenteric artery in vitro. <u>Life Sci</u>. 63: 95-100.

Paech K, Webb P, Kuiper GG, et al. 1997. Differential ligand activition of estrogen receptors ERα and ERβ at API sites. <u>Science</u>. 277: 1508-1510.

Palmer RM, Ferrige AG, Moncada S. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. <u>Nature</u>. 327: 524-526.

Picherit C, Cxam V, Bennetau-Pelissero C, Kati-Coulibaly S, Davicco MJ, Lebecque P, Barlet JP. 2000. Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. <u>J Nutr</u>. 130: 1675-1681.

Piskula MK, Yamakoshi J, Iwai Y. 1999. Daidzein and genistein but not their glucosides are absorbed from the rat stomach. <u>FEBS Lett</u>. 447:287-291.

Poehlman ET, Toth MJ, Gardner AW. 1995. Changes in energy balance and body composition at menopause: a controlled longitudinal study. <u>Ann Intern Med</u>. 123(9): 673-5

Pueyo M, Michel J. 1997. Angiotensin II receptors in endothelial cells. <u>Gen Pharmacol</u>. 29: 691-696.

Rapoport RM, Murad F. 1983. Agonist-induced endothelium-dependent relaxation in rat thracic aorta may be mediated throught cGMP. <u>Circ Res</u>. 52: 352-357.

Robinson RW, Higano N, Cohen WD. 1963. Long-term effects of high dosage estrogen therapy in men with coronary heart disease. <u>J Chron Dis</u>. 16: 155-161.

Robinson D, Kawamura T, Hinohara S, Sakamoto Y, Takahashi T. 1995. Levels of cardiovascular risk factors in Japanese people living in the UK. <u>J Cardiovasc Risk</u>. 2: 449-458.

Rose DP, Boyer AP, Wynder El. 1986. International comparison of mortality rates for cancer of the breast, ovary, prostate, and colon, per capita fat consumption. <u>Cancer</u>. 58: 2363-2371.

Rowland IR, Wiseman H, Sanders TA, Adlercreutz H, Bowy EA. 2000. Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. <u>Nutr Cancer</u>. 36: 27-32.

Schriewer H, Assmann G, Sandkamp M, et al. 1984. The relationship of lipoprotein (a) (Lp(a)) to risk factors of coronary heart disease: initial results of the prospective epidemiological study on company employees in Westfalia. <u>J Clin Chem Clin Biochem</u>. 22(9):591-6.

Setchell, KD. 2000. Absorption and metabolism of soy isoflavones-from food to dietary supplements and adults to infants. <u>J Nutr</u>. 130: 654S-655S.

Setchell KD, Brown NM, Desai P, Zimmer-Nechemias L, Wolfe BE, Brashear WT, Kirschner AS, Cassidy A, Heubi JE. 2001. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. <u>J Nutr</u>. 131: 1362S-1375S.

Setchell KD, Cassidy A. 1999. Dietary isoflavones: biological effects and relevance to human health. <u>J Nutr</u>. 129: 758S-767S

Setchell KDR, Borrielo SP, Hulme P, Kirk DN, Axelson M. 1984. Nonsteroidal estrogins of dietary origin: Possible roles in hormone dependent disease. <u>Am J Clin Nutr</u>. 40: 569-578.

Squdrito F, Altavilla D, Squadrito G, Saitta A, Cucinotta D, Minutoli L, Deodato B, Ferlito M, Campo G, Bova A, Caputi A. 2000. Genistein supplementation and estrogen replacement therapy improve endothelial dysfunction induced by ovariectomy in rats. <u>Cardiovasc Res</u>. 45: 454-462.

Stampfer MJ, Colditz GA. 1991. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. <u>Prev Med</u>. 20: 47-63.

Stampfer MJ, Colditz GA, Willet WC, et al. 1991. Postmenopausal estrogen therapy and cardiovascular disease: ten-year follow-up from the nurses' health study. N Engl J Med. 325: 756-762.

Strauss L, Mäkela S, Joshi S, Huhtaniemi I, Santti R. 1998. Genistein exerts estrogen-like effects in male mouse reproductive tract. Mol Cell Endocrinol. 144: 83-93.

Strom SS, Yamamura Y, Duphorne CM, Spitz MR, Babaian RJ, Pillow PC, Hursting SD. 1999. Phytoestrogen intake and prostate cancer: a case-control study using a new database. Nutr Cancer. 33: 20-25.

Stroth U, Unger T. 1999. The renin-angiotensin system and its receptors. J Cardiovasc Pharmacol. 33: 21-28.

Tamaya N, Uemura K, Ignchi A. 2002. Gender differences in the dietary lard-induced increase in Blood pressure in rats. Hypertension. 39: 1015-1020.

Taylor RD, Corcoran AC, Page IH. 1947. Menopausal hypertension: a critical study. American Journal of the Medical Sciences. 213: 475-6.

Teixeira SR, Potter SM, Weigel R, Hannum S, Erdman JW, Jr., Hasler CM. 2000. Effects of feeding 4 levels of soy protein for 3 and 6 wk on blood lipids and apolipoproteins in moderately hypercholesterolemic men. Am J Clin Nutre. 71: 1077-84.

Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. 1998. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci U S A. 95: 3106-3110.

Tonstad S, Smerud K, Hoie L. 2000. 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.

Tovar-Palacio C, Potter SM, Hafermann JC, Shay NF. 1998. Intake of soy protein and soy protein extracts influences lipid metabolism and hepatic gene expression in gerbils. J Nutr. 128: 839-842.

Umans J, Levi R. 1995. Nitric oxide in the regulation of blood flow and arterial pressure. Annu Rev Physiol. 57: 771-790.

Venkov CD, Rankin AB, Vaughan DE. 1996. Identification of authentic estrogen receptor in cultured endothelial cells: a potential mechanism for steroid hormone regulation of endothelial function. Circulation. 94: 727-33.

Wakasugi M, Noguchi T, Kazama Y-I, Kanemaru Y, Onya T. 1989. The effects of sex hormones on the synthesis of prostacyclin ($PGI_2$) by vascular tissues. Prostaglandins. 37: 401-410.

Walsh BW, Schiff I, Rosner B, et al. 1991. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med. 325(17): 1196-204

Wang HJ, Murphy PA. 1994. Isoflavone content in commercial soyberan foods. J Agric Food Chem. 42: 1666-1673.

Watanabe S, Yamaguchi M, Sobue T, Takahashi T, Miura T, Arai Y, Mazur W, Wahala K, Adlercreutz H. 1998. Pharmacokinetics of soybean isoflavones in plasma, urine and feces of men after ingestion of 60 g baked soybean powder (kinako). J Nutr. 128: 1710-1715.

Watson CS, Norfleet AM, Pappas TC, Gametchu B. 1999. Rapid actions of estrogens in $GH_3/B_6$ pituitary tumor cells via a plasma membrane version of estrogen receptor-α. Steroids. 64: 5-13.

Weiss NS. 1972. Relationship of menopause to serum cholesterol and arterial blood pressure: the United States health examination survey of adults. American Jounal of Epidemiology. 96: 37-47.

Wellman G, Bonev A, Nelson M, Brayden J. 1996. Gender differences in coronary diameter involve estrogen, nitric oxide, and $Ca^{2+}$ -dependent $K^+$ channels. Circ Res. 79: 1024-1030.

Wijetunge S, Aalkjaer C, Schachter M, Hughes AD. 1922. Tyrosine kinase inhibitors block calcium channel currents in vascular smooth muscle cells. Biochem Biophys Res Comm. 189: 1620-3.

Wilcox G, Wahlqvist M, Burger H, Medley G. 1990. Oestrogenic effects of plant foods in postmenopausal women. Br Med J. 301: 905-906.

Wilcox JN, Blumenthal BF. 1995. Thrombotic mechanisms in atherosclerosis: potential impact of soy proteins. J Nutr. 125: 631S-638S.

Williams JK, Honoré EK, Washburn SA, Clarkson TB. 1994. Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. J Am Coll Cardiol. 24: 1757-61.

Wing RR, Matthews KA, Kuller LH, et al. 1991. Weight gain at the time of menopause. Arch Intern Med. 151(1): 97-102.

Wiseman H. 1999. The bioavailability of non-nutrient plant factors: dietary flavonoids and phytooestrogens. Proc Nutr Soc. 58: 139-146.

Wu AH, Ziegler RG, Horn-Ross PL, Nomura AM, West DW, Kolonel LN. Rosenthal JF, Hoover RN, Pike MC. 1996. Tofu and risk of breast cancer in Asian-Americans. Cancer Epidemiol Biomark Prev. 5: 901-906.

Xu X, Harris KS, Wang H-J, Murphey PA, Hendrich S. 1995. Bioavailability of soybean isoflavones depends upon gut microflora in women. J Nutr. 125: 2307-2315.

Xu X, Wang HJ, Murphy PA, Cook L, Hendrich S. 1994. Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. <u>J Nutr</u>. 124: 825-832.

Yueh TL, Chu HY. 1997. The metabolic fate of daidzein. <u>Sci Sin</u>. 20: 513-521.

Zhang Y, Wang G-J, Song TT, Murphey PA, Hendrich S. 1999. Urinary disposition of the soybean isoflavones daidzein, genistein and glycitein differs among humanes with moderate fecal isoflavones degradation activity. <u>J Nutr</u>. 129: 957-962.

# BIOGRAPHY

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