Soy isoflavones affect platelet thromboxane A_2 receptor density but not plasma lipids in menopausal women☆

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Abstract

Objectives: It has been suggested that isoflavones protect the cardiovascular system, in part by improving lipid profile. The purpose of the present research was to examine the effect of a 12-week soy isoflavone supplementation on lipoprotein status and platelet thromboxane A_2 receptor density.

Methods: Twenty-nine healthy postmenopausal women were invited to take part in a randomised study to receive either 100 mg/day isoflavone supplement ($n = 15$) or identical placebo capsules ($n = 14$). Blood samples obtained at baseline and after 12 weeks were analysed for isoflavones, total cholesterol, high density lipoprotein cholesterol, triglycerides, glucose, insulin, estradiol, testosterone, gonadotrophins, sex hormone-binding globulin (SHBG) and platelet thromboxane A_2 receptor density. Blood pressure measurements, body mass index, subcutaneous fat at entrance and at the end of treatment were also registered. Changes in variables between groups were compared by ANOVA for repeated measures.

Results: Blood pressure, body mass index, subcutaneous fat, insulin, serum lipoprotein, sex hormones and SHBG did not differ among groups. However, platelet thromboxane A_2 receptor density declined significantly (from 181.9 ± 30.9 to 115.2 ± 16.2 fmol/10^8 platelets) in the experimental group, remaining mostly unchanged in the placebo group (176.3 ± 27.3 to 170.4 ± 28.2 fmol/10^8 platelets). The dissociation constant (K_d) values were unchanged. The change in platelet thromboxane A_2 receptors correlated negatively with isoflavones serum concentration ($r = -0.59$, $p < 0.001$).

Conclusions: In this study we demonstrated that the beneficial effects of isoflavones in menopausal women could be more related to platelet function than to improving classical cardiovascular risk factors.

Keywords: Isoflavone; Cardiovascular risk; Platelet thromboxane A_2 receptor; Postmenopausal women

1. Introduction

Isoflavones, a group of biologically active compounds found in soybeans and other legumes, bind to both estrogen receptors ERα and ERβ with greater affinity demonstrated for ERβ [1–3]. Clinical studies have shown that low soy diets are associated with low

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cardiovascular disease risk [4], also that soy supplementation is associated with a reduction of lipoproteins in both hypercholesterolemic and normocholesterolemic subjects [5–7] and with an improvement in biomarkers of lipid peroxidation and vascular reactivity [8,9]. However, other studies have failed to demonstrate an effect on serum lipids using 80 mg isoflavone/day in postmenopausal patients [10]. Few previous clinical studies, however, have examined the effects of isoflavone supplement consumption on thromboembolic factors.

Platelet aggregation is an important component of the haemostatic mechanism that prevents undesired bleeding. Several aggregation pathways have been described, one of which is the thromboxane pathway. Thromboxane A_2 (TxA_2), main cyclooxygenase metabolite of arachidonic acid in platelets, acts through a membrane surface receptor to aggregate platelets and constrict vascular smooth muscle [11]. TxA_2 synthesis is increased in a variety of thrombotic cardiovascular conditions [12,13], and receptor density is increased during acute myocardial infarction and pregnancy-induced hypertension [14,15]. This study was designed to evaluate the effects of a 3-month soy isoflavone supplementation on sex hormones profile, lipid profile, and TxA_2 receptor density in postmenopausal women.

1.1. Subjects and methods

The study protocol was reviewed and approved by the Institute of Nutrition and Food Technology, University of Chile Review Board Human Subjects Committee.

1.1.1. Study participant

Twenty-nine women, aged 45–60 years, were recruited from Santiago Metropolitan Area, and gave written informed consent to participate in this study. To be eligible for this study, women had to be in menopause at least 6 months, have FSH levels over 20 IU/L, without any type of hormonal treatment during previous 6 months, and not currently using lipid-lowering drugs, soybean-derived products, or herbal supplements diets. Exclusion criteria included: no cigarette smoking within the last 5 years, diabetes, heavy alcohol consumption (more 30 g/day), hypertension, abnormal uterine bleeding, and coexistent major illnesses. Height and weight were measured by standard procedures and body mass index (BMI) was calculated as weight (kg) divided by height (m^2). The skinfold thickness at the triceps and subscapular region, for estimating subcutaneous fat, was measured twice on the right side by using a Lange skinfold caliper (Beta Technology Inc., Cambridge, MD, USA) and the average value was calculated, as was the subscapular-to triceps skinfold ratio (STR). A fasting blood sample was obtained to perform the following laboratory tests: routine clinical laboratory (serum glucose, insulin, hepatic and renal screening tests, and TSH, estradiol (E_2), testosterone, FSH, LH and SHBG, serum isoflavone levels (genistein and daidzein) and TxA_2 receptor density. Volunteers were randomly assigned to receive two daily capsules of a soybean isoflavones extract (50 mg isoflavones per capsule; SoyLife® Netherlands B.V.) or identical placebo during 12 consecutive weeks. The study coordinator and investigative team performing the blood collection, and assays were blinded to the group assignment.

1.2. Laboratory measurements

1.2.1. Routine laboratory and hormone serum levels

Commercially available RIA kits (Diagnostic Product, Los Angeles, CA, USA) were employed to measure concentrations of E_2, testosterone, insulin, FSH and LH. Circulating SHBG was determined by double antibody RIA, using [^{125}I]-labeled hormone (Diagnostic Product, Los Angeles, CA, USA). The inter-assay coefficients of variation were 7.7, 14.7, 6.3, 10.3 and 1.8% for E_2, testosterone, insulin, FSH–LH, and SHBG, respectively. Serum levels of total cholesterol and triglycerides were measured by enzymatic techniques, and HDL-cholesterol by precipitating the other lipoproteins with heparin and manganese chloride [16]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedwald equation. The results are based on duplicated assays.

1.2.2. Isoflavones determination

Total isoflavones in plasma and total isoflavones in soybean extract samples were analyzed by HPLC. In the present study, total daidzein and total genistein are each defined as the amount of nonconjugated analyte plus the amount of nonconjugated analyte that is released after treatment of the samples

with β-glucoronidase and aryl-sulphatase (Sigma St. Louis, MO, USA). The nonconjugated isoflavones were extracted as previously described [17], and isoflavones isolation was done by reverse-phase HPLC [18]. Detection was by dual-wavelength ultraviolet absorbance (250 nm for daidzein, 262 nm for genistein) and quantification by comparing the area under the curve with reference standards of genistein and daidzein (Sigma St. Louis, MO, USA). The efficiency of each extraction was calculated by the addition of 3000 dpm of [^{3}H]-estradiol as an internal standard.

1.2.3. Isolation of platelet for further TxA_2 receptor assays

Blood samples were collected in monoject tubes containing EDTA (5 mmol/L), immediately centrifuged at $200 \times g$ for 15 min at room temperature. The platelet-rich plasma was centrifuged at $400 \times g$ for 12 min to sediment the platelets. The platelets were resuspended in fresh medium containing 137 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10 mmol/L glucose, 10 mmol sodium-free HEPES, pH 7.4, and allowed to stand at room temperature for 30 min. The platelet count was adjusted to $\sim 2 \times 10^8$ ml^{-1} in a clear medium.

1.2.4. Equilibrium radioligand binding assay for TxA_2 receptor determination

It was performed according to the method of Kattelman et al. [19]. Aliquots of platelet suspensions (0.4 ml) were diluted to a final volume of 0.6 ml in the assay buffer (140 mmol/L NaCl, 5 mmol/L KCl, 5.6 mmol/L dextrose, 25 mmol/L Tris–HCl, pH 7.4) containing 1.0 mmol/L aspirin and 0.1% BSA in the presence of 8 nmol/L [^{3}H]SQ-29548 (38 Ci/mmol; New England Nuclear), and then incubated at 37 °C for 30 min. The reaction was terminated by the addition of 2 ml of ice-cold assay buffer followed by rapid filtration under reduced pressure through Whatman GFC glass fiber filters pre-washed with assay buffer. The tubes and filters were rapidly washed with assay buffer (four times with 2 ml) and the radioactivity was counted. Specific binding was defined as the amount of total bound radioactivity minus that observed in the presence of SQ-29548 (10 μmol/L). Saturation binding curves were constructed using increasing concentrations of [^{3}H]SQ-29548 (0.1–9 nmol/L). The dissociation constant (K_d) and maximum receptor density (B_{max}) were calculated from Scatchard-transformed binding data with iterative, mass action law-based curve-fatting program LIGAND.

1.3. Statistical analysis

Based on the studies of the effect of isoflavones on cardiovascular risk factors on monkey [20,21], we hypothesized that isoflavone supplement would be more effective than placebo in reducing plasma lipids concentration. Thus, this study was designed to have 90% power to detect at least 15% total cholesterol and LDL-cholesterol diminution in the isoflavone group. Results are expressed as means ± S.D. Significance was considered at $p < 0.05$. Comparison of variables between groups at baseline was performed using Student's *t*-test. ANOVA for repeated measurements was employed to study changes in parameters between and within treatment groups. Statistical analyses were done by Statistica for Windows® version 4.5.

1.4. Results

The two groups were well matched for baseline characteristics, including blood pressure, lipids, body weight, body mass index, subcutaneous fat (Table 1), sex hormones (Table 2) and age (53 ± 3 yr versus 54 ± 4 yr). All volunteers were healthy, except for mild hypertension detected in one woman. Hypercholesterolemia (total cholesterol over 6.2 mmol/L) was present in two cases and hypertriglyceridemia (serum fasting triglycerides over 2.8 mmol/L) in one subject. Women had been in amenorrhoea from 6 months to 16 years, and six reported occasional hot flashes. Variables exhibited a normal distribution, with exception of E_2 and gonadotrophins. Few adverse reactions to the soy supplement were reported, such as abdominal bloating (one), and nicturia (one). Hot flashes remained unchanged during the treatment.

Total isoflavones, measured in the soybean extract capsules, contained 23.4 ± 3.4 mg daidzein and 24.1 ± 4.6 mg genistein; thus, subjects in the experimental group ingested approximately 100 mg isoflavones daily. All women assigned to isoflavones group show a clear rise in plasma isoflavone levels; thus, from negligible levels at baseline, isoflavone serum levels rose 15-fold in the treat-

Table 1
Morphological characteristics, lipid profiles and blood pressure reading in postmenopausal women treated with placebo or isoflavone for 12 weeks

	Placebo group ($n = 14$)		Isoflavone group ($n = 15$)		p-Value
	Baseline	After treatment	Baseline	After treatment	
Weight (kg)	67.1 ± 6.8	67.6 ± 6.3	63.0 ± 5.0	63.7 ± 5.8	0.70
BMI (kg/m^2)	27.2 ± 2.5	27.4 ± 2.6	26.7 ± 2.2	26.8 ± 2.5	0.70
STR	0.92 ± 0.3	0.93 ± 0.3	0.93 ± 0.3	0.92 ± 0.4	0.70
Cholesterol (mmol/L)	4.8 ± 0.5	4.8 ± 0.6	5.5 ± 1.0	5.8 ± 0.7	0.31
HDL-cholesterol (mmol/L)	1.8 ± 0.6	1.7 ± 0.2	1.4 ± 0.3	1.8 ± 0.4	0.40
LDL-cholesterol (mmol/L)	2.9 ± 0.3	3.1 ± 0.4	3.4 ± 0.4	3.7 ± 0.3	0.70
Triglycerides (mmol/L)	1.4 ± 0.2	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	0.31
Apo A-1 (g/L)	1.3 ± 0.5	1.1 ± 0.4	1.2 ± 0.6	1.5 ± 0.5	0.32
Apo B (g/L)	1.78 ± 0.1	1.82 ± 0.2	1.86 ± 0.2	1.8 ± 0.2	0.41
SBP (mmHg)	124.0 ± 17	117.1 ± 18	119.0 ± 8	116.2 ± 14	0.14
DBP (mmHg)	78.3 ± 1.3	75.2 ± 0.9	76.2 ± 0.8	78.4 ± 1.1	0.40

Values are given as the mean ± S.D. BMI, body mass index; STR, subscapular-to-triceps skinfold ratio. SBP, systolic blood pressure; DBP, diastolic blood pressure.

ment group (69.2 ± 7.9–1000 ± 89.2 nmol/L), remaining unchanged in the placebo group.

No significant changes were observed in blood pressure and lipoprotein serum level (Table 1) nor insulin, glucose, sex hormones, gonadotrophins and SHBG (Table 2), within either the placebo or the isoflavone groups. Neither anthropometrics measurements (body mass index and skinfold thickness) revealed differences in fat distribution between the two groups.

We evaluated binding of [^{3}H]SQ-29548 to platelets, where the B_{max} and K_d were analysed (Fig. 1). As shown, [^{3}H]SQ-29548 bound to TxA_2 receptors in a saturable manner; isoflavone treatment significantly decreased the TxA_2 receptor density (B_{max}). In contrast, the Scatchard analysis of the binding reveals that the K_d before and after treatment was similar (1.7 nmol/L versus 1.5 nmol/L, respectively).

Platelet TxA_2 receptor density decreased significantly only in isoflavone treated subjects ($p < 0.02$), compared to the placebo group (Fig. 2). This decrease was not related to baseline levels, which varied amply, and was negatively correlated with the change in isoflavone serum levels (Pearson's correlation coefficient = −0.74, $p < 0.01$).

2. Discussion

The results of the present double-blind, randomised, placebo-controlled study indicate that supplementation with soy-derived isoflavones had no significant effect on body weight, body mass index or peripheral subcutaneous adipose tissue, and also was ineffective in reducing total serum cholesterol, triglycerides in healthy postmenopausal women. In addition, there

Table 2
Hormones profile and isoflavones level in postmenopausal women treated with placebo or isoflavone for 12 weeks

	Placebo group ($n = 14$)		Isoflavone group ($n = 15$)		p-Value
	Baseline	After treatment	Baseline	After treatment	
FSH (IU/L)	92.2 ± 45.7	77.6 ± 35.7	83.2 ± 37.7	90.9 ± 26.8	0.30
LH (IU/L)	35.5 ± 10.6	27.7 ± 8.5	43.9 ± 23.3	44.2 ± 11.4	0.41
SHBG (nmol/L)	55.3 ± 46.3	49.2 ± 22.6	50.0 ± 15.8	51.1 ± 19.1	0.15
E_2 (pmol/L)	37.5 ± 14.9	41.5 ± 15.1	41.6 ± 15.7	42.3 ± 16.3	0.53
Testosterone (nmol/L)	0.94 ± 0.24	0.86 ± 0.21	1.04 ± 0.22	1.14 ± 0.21	0.80
Fasting insulin (pmol/L)	71.4 ± 8.5	68.6 ± 6.8	64.8 ± 6.1	59.2 ± 5.7	0.18
Total isoflavones (nmol/L)	61 ± 5.1	52 ± 6.1	69.2 ± 7.9	1000 ± 89.2	<0.01

Values are given as the mean ± S.D.

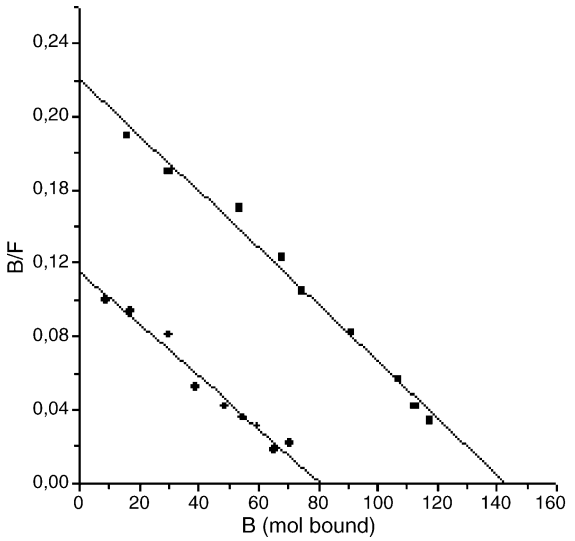

Fig. 1. Representative Scatchard analysis of equilibrium binding data of postmenopausal women who received isoflavone supplement. The two curves represent the pretreatment period (▲) and the 12-week treatment (●). The K_d values were 1.7 and 1.5 nmol/L, and the B_{max} value was 159 and 107 fmol/10^8 platelets for basal and isoflavone treatment, respectively. B, bound; F, free.

were no significant differences in the lipoprotein fraction (HDL or LDL cholesterol), and the isoflavones supplementation did not alter systolic or diastolic blood pressure. The hypothalamic–pituitary–gonadal axis and production of SHBG were also unaffected by isoflavones treatment. The only unexpected finding relative to thrombotic cardiovascular disease risk factors concerned platelet TxA_2 receptor density, which significantly decreased after 3 months of isoflavones supplementation.

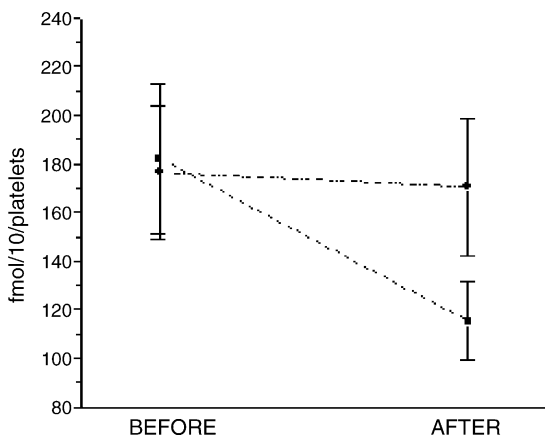

Fig. 2. Graph showing changes in platelet thromboxane A_2 (TxA_2) receptor density. Isoflavone treatment (■) was associated with significant decrease ($p < 0.02$) in B_{max} compared with placebo (●) and with the baseline values. Data are mean ± S.D. Isoflavone, $n = 15$; placebo, $n = 14$.

In the current study, baseline levels of serum isoflavones were similarly low in both groups. Twelve weeks of a soybean extract supplementation resulted in a significant 15-fold increase in genistein and daidzein serum concentrations, whereas, no change was noted in the placebo group. Despite some variability demonstrated with soybean and phytoestrogen equivalent intake, serum isoflavone concentration is still considered a reliable reflection of dietary phytoestrogen intake and their bioavailability.

Isoflavones have been promoted as good candidates for the beneficial lipoprotein changes attributed to soy protein [22]. However, our results along with previous investigations on the effect of isoflavones supplementation in postmenopausal women, does not support this general hypothesis [23,24]. It has been suggested that the cholesterol-lowering effects of soy are strongly associated with higher level of isoflavone of soy protein matrix [25]. In the present study, the subjects were mostly normocholesterolemic, and the dose of isoflavone used (100 mg/day) was 1.5–2 times higher that reasonably expected with a soy-based diet. In this aspect, the conditions of the present study should have been optimal for the detection of any hypocholesterolemic effect. These results strongly suggest that isoflavone treatment, as a supplement, probably plays a limited role in modifying serum cholesterol in middle-aged women.

In the present study, isoflavone supplementation had no effect on SHBG values, unlike a previous investigation in postmenopausal women, in which a positive relationship between serum isoflavone and SHBG concentration was found, following the consumption of soymilk for 10 weeks [17]. Several studies have provided evidence that isoflavones can modulate the SHBG concentration of postmenopausal women, but the data are not entirely consistent [26,27]. These contradictory results could be a consequence of differences in length of supplementation and type of isoflavone ingested [28]; however, the mechanism by which soy affect SHBG concentration remains unresolved.

Excessive platelet aggregation plays a role in the pathogenesis of several cardiovascular diseases events, including stroke and myocardial infarction. Evidence of platelet involvement in these syndromes includes increased TxA_2 synthesis and increased platelet TxA_2 receptor density [14,29]. The significant decrease in platelet TxA_2 receptor density associated with isoflavone supplementation observed in the present study was surprising. The incidence of myocardial infarction is significantly greater in men than in women who have not experienced menopause [30], but the difference disappears after menopause. Testosterone, the principal circulating androgen in men, has been implicated as one of the risk factors in this phenomenon. Interestingly, administration of testosterone to normal volunteers also increased platelet TxA_2 receptor density [31]. In experimental animals, the treatment of rats with testosterone increased platelet and aortic membrane TxA_2 receptors [32]. In those animals it has recently been reported that genistein prevented in vivo thrombogenesis and in vitro platelet aggregation induced by collagen [33].

In consideration to differential distribution of TxA_2 receptor in the human cardiovascular system, and the alteration of the receptor density in cardiovascular disease, it has been suggested that TxA_2 receptors represent a significant target for therapeutic intervention and highlights the importance for the development of novel selective products for prevention of cardiovascular conditions [34]. Our observations raise the possibility that the decrease in platelet TxA_2 receptor density, by isoflavone supplementation, contributes to reduce the risk of thrombogenesis. Although we did not study platelet function, the decrease in TxA_2 receptor density would imply an attenuation signal for other more potent agonists, such as thrombin and ADP.

To our knowledge, the present study is the first to study the relationship between the intake of an isoflavone supplement and platelet TxA_2 receptors in human. The molecular mechanism by which isoflavone decreases platelet TxA_2 receptor density cannot be elucidated by this study. However, it is unlikely to be due to binding to estradiol receptors, as other hormonal effects, since estradiol replacement therapy in postmenopausal women has no effect on platelet TxA_2 density receptor (unpublished observations). The decrease in receptor density is also unlikely to be a consequence of the action of isoflavones on androgen production. Testosterone and SHBG did not differ between placebo-group and isoflavone treatment women; in menopausal women, almost 100% of sex steroids are synthesized in peripheral tissues from precursors of adrenal origin, except for a small contribution from ovarian and/or adrenal testosterone and androstenedione [35]. Plasmatic dehydroepiandrosterone (DHEA) and its sulphate, DHEA-S, provide the level of substrates required for conversion into androgen and estrogen [36]. DHEA and DHEA-S were not measured in this study, but it should be explored in further investigations since it has been demonstrated that isoflavones are inhibitors of steroid-metabolising enzymes: steroid sulphatase and steroid sulphotransferase [37]. In summary, 100 mg/day of a soy isoflavone extract for 3 months had no effect on blood pressure, nor significant impact on lipid, lipoprotein and sex hormone levels in healthy middle-aged women. Additionally, the isoflavone supplement significantly reduced the platelet TxA_2 receptor density. These findings may be helpful in elucidating how dietary isoflavones may modulate platelet function and explain how phytoestrogen-rich foods are claimed to have beneficial effects on the cardiovascular system.

References

[1] Kurzer MS, Xu X. Dietary phytoestrogen. Annu Rev Nutr 1997;17:353–81.

[2] Kuiper GG, Carlson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 1997;138:863–70.

[3] Lorraine A, Fitzpatrick A. Soy isoflavone: hope or hype? Maturitas 2003;44(Suppl 1):S21–9.

[4] Clarkson TB, Anthony MS. Phytoestrogen and coronary heart disease. In: Adlercreutz H, editor. Phytoestrogen. London: BaillièreTindall/Saunder; 1998. p. 589–604.

[5] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333:276–82.

[6] Merz-Demlow BE, Duncan AM, Wanger KE, et al. Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. Am J Clin Nutr 2000;71:1462–9.

[7] Washburn S, Burke GL, Morgan T, Anthony M. Effect of soy protein supplementation on serum lipoprotein, blood pressure, and menopausal symptoms in perimenopausal women. Menopause 1999;6:7–13.

[8] Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low-density lipoprotein oxidation resistance. Proc Natl Acad Sci USA 1998;95:3106–10.

[9] Nestel PJ, Yamashita T, Sasahara T, et al. Soy isoflavone improve systemic arterial compliance but not plasma lipids in menopausal and perimenopausal women. Arterioscler Thromb Vasc Biol 1997;17:3392–8.
[10] Simons LA, von Konigsmark M, Simons J, Celermajer DS. Phytoestrogen do not influence lipoprotein levels or endothelial function in healthy, postmenopausal women. Am J Cardiol 2000;85:1297–301.
[11] Narumiya S, Sugimoto Y, Ushibuki F. Prostanoid receptors: structures, properties, and functions. Physiol Rev 1999;79: 1193–226.
[12] Halushka PV, Mais DE, Mayeux PR, Morinelli TA. Thromboxane, prostaglandin and leukotriene receptors. Annu Rev Pharmacol Toxicol 1989;29:213–39.
[13] Hirsh PD, Hillis LD, Campbell WB, Firth BG, Willerson JT. Release of prostaglandins and thromboxane into the coronary circulation in patients with ischemic heart disease. N Engl J Med 1991;304:685–91.
[14] Fitzgerald DJ, Roy L, Catella F, FitzGerald GA. Platelet activation in unstable coronary disease. N Engl J Med 1986;315:983–9.
[15] Liel N, Nathan I, Yermiyahu T, et al. Increased platelet thromboxane A2/prostaglandin H2 receptors in patients with pregnancy induced hypertension. Thromb Res 1993;70:205–10.
[16] DHEW. Manual of laboratory operations: Lipid Research Clinics Programs, vol. 1. Washington, DC: DHEW Publication (NIH); 1974. p. 75–628.
[17] Pino AM, Valladares LE, Palma MA, Mancilla AM, Yañez M, Albala C. Dietary isoflavones affect sex hormone-binding globulin levels in postmenopausal women. J Clin Endocrinol Metab 2000;85:2797–800.
[18] Busby MG, Jeffcoat AR, Bloedon LT, et al. Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration of healthy men. Am J Clin Nutr 2000;75:126–36.
[19] Kattelman EJ, Venton DL, Le Breton GC. Characterization of U46619 binding in unactivated intact human platelet and determination of binding site affinities of four TXA_2/PGH_2 receptor antagonist (13-APA, BM 13.177, ONO 3708 and SQ-29,548). Thromb Res 1986;41:471–81.
[20] Anthony MS, Clarkson TB, Hughes Jr CL, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular factors without affecting the reproductive system of peripubertal monkey. J Nutr 1996;126:43–50.
[21] Wagner JD, Cefalu WT, Anthony MS, Litwak KN, Zhang L, Clarkson TB. Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesteryl ester content in ovariectomized cynomologous monkey. Metabolism 1997;46:698–705.
[22] Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. J Nutr 1999;29:758S–67S.
[23] Potter S. Overview of proposed mechanism for the hypocholesterolemic effect of soy protein. J Nutr 1995;125:606S–11S.
[24] Dewell A, Hollenbeck CB, Bruce B. The effect of soy-derived phytoestrogen on serum lipids and lipoproteins in moderately hypercholesterolemic postmenopausal women. J Clin Endocrinol Metab 2000;87:118–21.
[25] Crouse III JR, Morgan T, Terry JG, Ellis J, Vitolins M, Burke GL. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentration of lipids and lipoprotein. Arch Intern Med 1999;159:2070–6.
[26] Duncan AM, Underhill KEW, Xu X, Lavalleur J, Phipps WR, Kurzer MS. Modest hormonal effects of soy isoflavones in postmenopausal women. J Clin Endocrinol Metab 1999;84:3479–84.
[27] Tedee AJ, Dalais FS, McGrath BP. Dietary soy containing phytoestrogens does not have detectable estrogenic effects on hepatic protein synthesis in postmenopausal women. Am J Clin Nutr 2004;79:396–401.
[28] Erdman Jr JW, Badger TM, Lampe JW, Setchell KDR, Messina M. Not all soy product are created equal: caution needed in interpretation of research results. J Nutr 2004;134:1229S–33S.
[29] Dorn II GW, Liel N, Trask JL, Mais DE, Assey ME, Halushka PV. Increased platelet thromboxane A_2/prostaglandin H_2 receptors in patients with acute myocardial infarction. Circulation 1990;81:212–8.
[30] Colditz GA, Willet WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. Menopause and the risk of coronary heart disease in women. N Engl J Med 1987;316:1105–10.
[31] Ajayi AAL, Mathur R, Halushka PV. Testosterone increases human platelet thromboxane A_2 receptor density and aggregation responses. Circulation 1995;91:2742–7.
[32] Matsuda K, Ruff A, Morinelli TA, Mathur R, Halushka PV. Testosterone increases thromboxane A_2 receptor density and responsiveness in rat aortas and platelet. Am J Physiol 1994;267:H887–93.
[33] Kondo K, Suzuki Y, Ikeda Y, Umemura K. Genistein an isoflavone included in soy, inhibits thrombotic vessel occlusion in the mouse femoral artery and in vitro platelet aggregation. Eur J Pharmacol 2002;455:53–7.
[34] Katugampola SD, Davemport AP. Thromboxane receptor density is increased in human cardiovascular disease with evidence for the inhibition at therapeutic concentration by the AT_1 receptor antagonist losartan. Br J Pharmacol 2001;134:1385–92.
[35] Labrie F, Belanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentration of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. J Clin Endocrinol Metab 1997;82:2396–402.
[36] Labrie F, Lu-The V, Labrie C, Simard J. DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. Front Neuroendocrinol 2001;22:185–212.
[37] Wong CK, Keung WM. Daidzein sulfo conjugates are potent inhibitors of sterol sulfatase (EC 3.1.6.2). Biochem Biophys Res Commun 1997;233:579–83.