Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 17 (2006) 211–215

Journal of Nutritional Biochemistry

Short-term phytoestrogen supplementation alters insulin-like growth factor profile but not lipid or antioxidant status

Jayne V. Woodside[a,*], Maeli J. Campbell[b], Erica E. Denholm[b], Lynne Newton[b], John W. Honour[c], Michael S. Morton[d], Ian S. Young[a], Anthony J.C. Leathem[b]

[a]*Department of Medicine, Centre for Cardiovascular and Genetics Research, The Queen's University of Belfast, Belfast BT12 6BJ, UK*
[b]*Department of Surgery, Royal Free and University College London Medical School, London W1W 7EJ, UK*
[c]*Department of Chemical Pathology, University College London Hospitals, London, W1P 6DB, UK*
[d]*Department of Medical Biochemistry, University of Wales College of Medicine, Heath Hospital, Tenovus Building, Cardiff CF4 4XN, UK*

Abstract

Phytoestrogens are plant compounds that have been proposed to have a variety of health benefits. The aim of this study was to assess the effects of these compounds on a number of physiological endpoints. Subjects were given a single intake of a phytoestrogen-rich (80 mg total phytoestrogens) supplement containing soy, rye and linseed (Phase 1), followed by a week-long intervention using the same supplement (Phase 2) (80 mg total phytoestrogens daily). A number of biochemical endpoints were assessed including urinary phytoestrogen metabolites, lipids, antioxidant status, DNA damage and insulin-like growth factor-1 (IGF-1) and IGF binding protein-1 (IGFBP-1) and -3 (IGFBP-3). Ten healthy female subjects took part in the study. Excretion of the isoflavones genistein, daidzein and equol in urine increased in both phases of the study. No other endpoint was altered in Phase 1. However, in Phase 2, concentrations of IGF-1 and IGFBP-3 were increased by phytoestrogen supplementation [IGF-1, median (IQ range), baseline 155 (123, 258), postweek 265 (228, 360) ng/ml, $P<.05$; IGFBP-3, baseline 3725 (3631, 4196), postweek 4420 (4192, 4935) ng/ml, $P<.05$]. There was no effect of supplementation on lipids or markers of antioxidant status. Short-term phytoestrogen supplementation increases urinary phytoestrogen excretion and increases IGF-1 and IGFBP-3. These results require elucidation in further controlled studies.

Keywords: Phytoestrogens; Nutrition; Lipids; Antioxidants; DNA damage; Insulin-like growth factor

1. Introduction

Phytoestrogens may protect against many diseases including hormone-dependent cancers and coronary heart disease. Found in many plant products, but particularly soy, legumes and vegetables, they have a wide variety of proposed biochemical effects including estrogenic and antioxidant effects [1].

Many studies have examined the effect of phytoestrogen supplementation in vitro, but fewer have looked at their physiological effects in vivo. We have examined the effects of a single intake of phytoestrogens and a week-long intervention. We have assessed a variety of biochemical endpoints, including phytoestrogen metabolites in urine, antioxidant status and lipid status. These all are potentially altered by phytoestrogen consumption [1]. We have also examined the effects of phytoestrogen supplementation on insulin-like growth factor (IGF) profile and on DNA damage, as assessed by the comet assay. Levels of IGF-1 and its related binding proteins are altered by tamoxifen [2], and phytoestrogens have a similar structure to tamoxifen [1]. Phytoestrogens have antioxidant properties [1] and may therefore protect against oxidative DNA damage.

2. Methods and materials

Subjects were healthy female volunteers, employed within University College London. The study was fully explained to the subjects, and written informed consent obtained. This study received ethical approval from the Royal Free and University College London Medical School Research Ethics Committee.

* Corresponding author. Department of Medicine, Mulhouse Building, Grosvenor Road, Belfast, BT12 6BJ, UK. Tel.: +44 028 90632585; fax: +44 028 90235900.
E-mail address: j.woodside@qub.ac.uk (J.V. Woodside).

0955-2863/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.08.001

Table 1
Estimated nutritional content of phytoestrogen supplement

	Per 100 g	Per daily dose supplement
Energy (kJ)	1656	1988
Energy (kcal)	395	476
Protein (g)	15.1	18
Carbohydrate (g)	56.7	68.0
of which sugars (g)	29.2	35.2
Fat (g)	12.0	14.4
of which saturates (g)	1.9	2.4
Fibre (g)	4.2	5.2
Sodium (g)	0.1	0.03

The supplement used was a soy and linseed bar, each bar containing a total 20 mg of isoflavones and lignans. The nutritional information is shown in Table 1.

2.1. Phase 1

Subjects were asked to provide a 24-h urine collection prior to the study. After an overnight fast, subjects gave a baseline blood sample. They were then given a breakfast containing 80 mg of phytoestrogens (4× supplement bars). Blood samples were taken at 8 and 24 h (fasting) after the meal with spot urine samples collected during the hours 1–2, 2–4, 4–6, 6–8 and 24 h after the meal. Meals or snacks, excluding soy products, were permitted throughout the 8-h postmeal period, but only liquids were consumed until 2 h after the meal.

2.2. Phase 2

Six days later, subjects collected a further 24-h urine sample and had a further fasting baseline blood sample taken. They were then asked to take an 80-mg phytoestrogen supplement daily for a period of 7 days after which they collected a final 24-h urine sample and had a final fasting blood sample taken.

2.3. Collection and preparation of biological samples

Urine samples (24 h) were stabilised with 1–2 g/L ascorbic acid and sodium azide (0.1 g/L) and stored at −80°C until analysis. Spot urine samples were not stabilised but immediately frozen after collection. Blood samples either kept at 4°C for a maximum of 30 min (plasma) or were allowed to clot for 30 min in the dark (serum) and then centrifuged at 3000 rpm for 10 min. Serum or plasma was aliquoted and stored at −80°C until analysis. Lymphocytes were collected into a Vacutainer Cell Preparation Tube (Becton Dickinson, New Jersey), extracted and washed according to manufacturer's instructions and stored at −80°C.

Table 3
Effect of 1 week of phytoestrogen supplementation on urinary phytoestrogen excretion—Phase 2

	Baseline [median (IQ range)]	Postweek [median (IQ range)]
Genistein (ng/ml)	206 (88, 306)	2775 (1818, 3101)*
Daidzein (ng/ml)	385 (195, 599)	4571 (3934, 5803)*
Equol (ng/ml)	96 (53, 131)	899 (501, 1540)*
Enterolactone (ng/ml)	224 (95, 403)	392 (181, 565)*
Enterodiol (ng/ml)	46 (19, 81)	83 (58, 114)*

* $P<.05$ when compared to baseline 2, Wilcoxon signed rank test.

2.4. Endpoint measurement

Urine samples were analysed for the isoflavonoids genistein and daidzein, the isoflavan equol and the lignans enterolactone and enterodiol levels, using gas chromatography–mass spectrometry following enzyme hydrolysis, extraction into ether and silylation according to Morton et al. [3].

Ascorbic acid concentrations were determined in EDTA plasma as described by Vuillemier and Keck [4]. Concentrations of lipid-soluble antioxidants were measured by HPLC in serum according to the method of Thurnham et al. [5]. Levels of aqueous phase lipid hydroperoxides were assessed in plasma by the FOX 1 (ferrous oxidation in xylenol orange) assay according to Wolff [6]. Susceptibility of LDL to oxidation was assessed in Phase 2 according to McDowell et al. [7]. Endogenous DNA damage was analysed in Phase 2 by the comet assay in lymphocytes according to Spanswick et al. [8].

Serum total cholesterol was estimated using an enzymatic CHOD-PAP kit, while serum triglycerides were measured using the Peridochrom GPO-PAP kit (both from Boehringer Mannheim). Precipitation for HDL-cholesterol estimation employs phosphotungstic Mg^{2+} reagents.

Serum samples were analysed for IGF-1 and IGFBP-1 and IGFBP-3 using ELISA kits from Diagnostic System Laboratories (London, UK). For all laboratory methods, daily quality controls were utilised to ensure both within- and between-assay reproducibility.

Table 2
Effect of one phytoestrogen meal on urinary phytoestrogen excretion—Phase 1

	Baseline [median (IQ range)]	1–2 h [median (IQ range)]	2–4 h [median (IQ range)]	4–6 h [median (IQ range)]	6–8 h [median (IQ range)]	24 h [median (IQ range)]
Genistein (ng/ml)	196 (146, 308)	217 (169, 377)	313 (201, 416)*	392 (344, 558)*	1257 (1013, 2711)*	289 (139, 417)
Daidzein (ng/ml)	428 (236, 593)	484 (388, 714)*	642 (375, 854)*	952 (681, 1837)*	3283 (2463, 4365)*	408 (252, 634)
Equol (ng/ml)	91 (48, 192)	104 (31, 268)	191 (25, 328)	356 (36, 523)*	757 (207, 915)*	136 (42, 264)
Enterolactone (ng/ml)	284 (105, 334)	280 (149, 307)	226 (153, 308)	242 (146, 305)	301 (206, 335)	284 (169, 461)
Enterodiol (ng/ml)	69 (22, 101)	66 (42, 88)	79 (47, 88)	77 (40, 95)	75 (48, 95)	77 (46, 145)

* $P<.05$ when compared to baseline, Wilcoxon signed rank test.

Table 4
Effect of phytoestrogen supplementation on lipids and antioxidant status

	Phase 1 [median (IQ range)]			Phase 2 [median (IQ range)]	
	Baseline	8 h	24 h	Baseline	postweek
Total cholesterol (mmol/L)	4.59 (4.07, 5.31)	4.47 (4.05, 4.82)	4.19 (3.89, 4.79)	4.63 (4.16, 5.54)	5.00 (4.32, 5.27)
HDL cholesterol (mmol/L)	1.49 (0.91, 1.68)	1.43 (0.96, 1.62)	1.43 (0.93, 1.87)	1.48 (1.16, 1.58)	1.57 (1.10, 1.73)
Triacylglycerol (mmol/L)	1.00 (0.83, 1.58)	1.44 (1.31, 2.39)	0.92 (0.66, 1.20)	1.19 (0.84, 1.78)	1.19 (0.86, 1.45)
LDL cholesterol (mmol/L)	2.59 (2.24, 3.39)	2.27 (2.13, 2.86)	2.36 (2.16, 2.51)	2.86 (2.29, 3.41)	2.96 (2.70, 3.31)
FOX1 (μmol/L)	1.65 (1.15, 2.57)	1.42 (1.37, 1.64)	1.20 (1.10, 1.37)	1.35 (1.19, 1.45)	1.34 (1.29, 1.46)
Retinol (μmol/L)	1.00 (0.74, 1.49)	0.79 (0.48, 1.47)	1.01 (0.69, 1.30)	0.99 (0.61, 1.10)	0.91 (0.52, 1.19)
α-Tocopherol (μmol/L)	20.9 (17.6, 23.9)	19.1 (14.8, 22.8)	21.4 (16.3, 24.8)	18.9 (15.5, 24.2)	21.0 (12.6, 23.3)
Lycopene (μmol/L)	0.09 (0.04, 0.16)	0.09 (0.06, 0.11)	0.09 (0.06, 0.11)	0.06 (0.04, 0.09)	0.07 (0.03, 0.16)
α-Carotene (μmol/L)	0.14 (0.06, 0.29)	0.12 (0.06, 0.21)	0.15 (0.09, 0.27)	0.08 (0.04, 0.11)	0.07 (0.05, 0.41)
α-Carotene (μmol/L)	0.14 (0.09, 0.20)	0.13 (0.09, 0.18)	0.16 (0.08, 0.19)	0.12 (0.09, 0.16)	0.10 (0.07, 0.17)
DNA damage (olive tail moment)	–	–	–	4.21 (1.50, 5.07)	2.11 (1.31, 2.74)
Lag time (min)	–	–	–	44.2 (37.4, 53.6)	44.5 (38.8, 48.4)

2.5. Statistical methods

Comparisons between subjects pre- and postintervention were carried out using a nonparametric Wilcoxon signed rank test due to the small numbers of subjects. Associations between continuous variables were tested using Spearman correlation coefficients. All analyses were carried out using SPSS.

3. Results

Subjects had a mean age of 33.1 years ranging from 23 to 50 years. Eight were premenopausal, of whom three were on the oral contraceptive pill. One of the subjects was postmenopausal and was also on hormone replacement therapy. One subject was perimenopausal. All subjects were nonsmokers.

Concentrations of genistein, daidzein, equol and enterodiol were elevated, both after a single 80-mg dose and after 1 week of supplementation as shown in Tables 2 and 3.

The effect of phytoestrogen supplementation on lipid- and antioxidant-related endpoints is shown in Table 4. Neither acute nor chronic phytoestrogen supplementation had any effect on any of the lipid or antioxidant-related endpoints assessed.

All IGF-1 and IGFBP-3 levels fell within the range of expected values given for healthy females in this age group. As fasting samples are required for these assays, measures of IGF-1 and IGFBP-3 status were limited to baseline and 24-h postsupplement in the first phase of the study.

Levels of IGF-1 were unchanged following the single 80-mg load. However, in Phase 2, IGF-1 and IGFBP-3 concentrations were significantly elevated after a week of supplementation. Levels of IGFBP-1 were not altered by phytoestrogens in either phase of the study (Table 5).

The change in IGF-1 in Phase 2 was significantly associated with the change in IGFBP-3 ($r=.721$, $P<.05$; Spearman correlation coefficient). However, if the ratio of IGF-1/IGFBP-3 was compared pre- and postsupplementation, the ratio was also significantly increased postsupplementation, suggesting that IGF-1 has increased independently of the increase seen in IGFBP-3 (Table 5).

4. Discussion

This study has assessed the effect of phytoestrogen supplementation, in the form of a soy, rye and linseed bar, on a variety of biochemical endpoints. Concentrations of phytoestrogen metabolites in urine reached their highest levels 6–8 h after supplementation for isoflavones, and 24 h for lignans in Phase 1. This is generally in agreement with other studies examining the effect of supplementation on urinary excretion of phytoestrogens [9]. This study, however, was not designed to be a rigorous study of phytoestrogen pharmacokinetics.

Phytoestrogens can act as antioxidants [1] and may inhibit the oxidation of LDL [10], although the data from this study have not confirmed this in terms of lag time to oxidation. A protective/antioxidant effect has been shown by phytoestrogen supplementation on the susceptibility of

Table 5
Effect of phytoestrogen supplementation on insulin-like growth factor and binding protein concentrations

	Phase 1 [median (IQ range)]		Phase 2 [median (IQ range)]	
	Baseline	24 h	Baseline 2	Postweek
IGF-1 (ng/ml)	218 (151, 293)	250 (179, 310)	155 (123, 258)	265 (228, 360)*
IGFBP-1 (ng/ml)	22.6 (19.0, 36.8)	17.4 (15.8, 34.6)	24.8 (14.6, 57.7)	14.7 (10.0, 36.0)
IGFBP-3 (ng/ml)	4564 (3954, 5306)	4110 (3887, 4533)	3735 (3631, 4196)	4420 (4192, 4935)*
IGF-1/IGFBP-3 ratio	0.05 (0.04, 0.06)	0.06 (0.04, 0.07)	0.04 (0.03, 0.06)	0.06 (0.05, 0.07)*

* $P<.05$ when compared to baseline, Wilcoxon signed rank test.

LDL to oxidation in another study [11]. Another study, however, in common with this one, has failed to show an inhibition of lipid peroxidation after 8 weeks of isoflavonoid supplementation in subjects with high-normal blood pressure [12], measuring urinary F2-isoprostanes, a reliable indicator of oxidative stress. The study measuring F2-isoprostanes was randomised, double-blind and placebo-controlled, in contrast to the present study, and was carried out in relatively large numbers ($n=59$). Our subjects were healthy and therefore had normal antioxidant status. Therefore the lag times of these subjects may not have been easily affected by this supplement.

We have also observed no significant change in DNA damage in lymphocytes after 1 week of phytoestrogen supplementation. We have previously shown that genistein and equol within physiological range can reduce oxidative DNA damage in vitro in lymphocytes [13]. Mitchell and Collins [14] carried out a 4-week feeding study in healthy men to assess the effects of a phytoestrogen supplement in the form of soy milk on levels of DNA damage. There was no significant effect on H_2O_2-induced DNA damage in lymphocytes in those receiving the soy milk supplement as assessed by the comet assay. However, the levels of oxidised pyrimidine base damage over the 4-week period progressively decreased. A recent study found no effect of rye crispbread (rich in lignans secoisolariciresinol and matairesinol) over 2 weeks on oxidative DNA damage [15]. We have only evaluated endogenous DNA damage in this study, where the damage may or may not have been oxidative in origin, and our intervention period was shorter than that which previously showed an effect. The antioxidant potential of these foods and compounds in vivo clearly requires further investigation.

This study found no effect of either a single dose or week's supplementation with 80 mg of phytoestrogens daily on serum lipids. This is in contrast to a meta-analysis of studies published in 1995 [16]. Of the 38 studies reviewed, 89% reported a net decrease in plasma cholesterol that averaged around 9% for an intake of around 47 g soy protein daily. However, it is notable that 77% of the studies included in the meta-analysis had 95% confidence intervals that included zero.

Since the meta-analysis, several well-controlled studies have been carried out, and the results of these have been variable [17]. There are several potential explanations for this variability. Merz-Demlow et al. [18] suggest that the effect of isoflavones on lipids may be related to menstrual cycle phase. The food matrix may also be important, as Lichtenstein [17] suggests that the effect of isoflavones appears to be somewhat dependent on whether the isoflavones are ingested in isolation or as an enriched preparation of those endogenously present in soy.

A recent study observed a reduction in LDL cholesterol in 156 men and women by an average of 6%. This was due entirely, however, to a mean reduction of 9% among those with a raised baseline LDL cholesterol (>4.29 mmol/L) and was only observed in men and postmenopausal women [19]. It may be that we observed no difference in our largely premenopausal sample set because they had low-normal lipid status initially. The original meta-analysis also found a greater lowering effect in those with initially raised total cholesterol levels [16].

This study has shown that 1 week of phytoestrogen supplementation can alter IGF status, producing a significant increase in both IGF-1 and IGFBP-3 levels. The vast majority of IGF-1 is carried through the circulation bound to a complex containing IGFBP-3 and, until released by proteolysis, is relatively unreactive. Thus the strong correlation between increasing levels of IGF-1 and IGFBP-3 was as expected. However, when the ratio of IGF-1 to IGFBP-3 was considered, this ratio was significantly increased postsupplement, and this finding requires further elucidation. Tamoxifen, a selective estrogen receptor modulator, has been shown to lower IGF-1 and increase IGFBP-3 concentrations [2]; therefore the phytoestrogen supplement appears to be behaving differently to tamoxifen. Oral estrogen treatment for menopausal women has also been shown to reduce IGF-1 [20] through inhibition of hepatic IGF-1 synthesis. The mechanism by which this supplement has increased IGF-1 requires further study.

One possible reason for the increase in this ratio is that IGF-1 is closely linked to nutritional status [21]. Our observation of an increase in both IGF-1 and IGFBP-3 could well be attributed to the increase in kilocalories and protein consumed in the phytoestrogen bar (476 kcal daily; 18 g protein daily; Table 1), as IGF-1 appears to be closely related to protein and energy metabolism [21]. No dietary intake data were collected in the present study so it is unclear whether total protein and energy intake increased or whether subjects unconsciously decreased their intake to compensate for the extra calories over the week. Intervention studies using a phytoestrogen extract or, as a control, a food supplement from which phytoestrogens have been removed, will be required to exclude the possibility that an increase in protein and/or energy intake produced the observed alteration in IGF-1 and IGFBP-3.

It may also be important to take into account the effects of the menstrual cycle as it has been shown that IGF-1 can vary throughout the menstrual cycle [22]. Oestrogenic, and therefore confounding, effects of oral contraceptives and hormone replacement therapy should also be considered as numbers were not large enough in this study to examine their effect.

The supplement bar we used contained two classes of phytoestrogen—isoflavones (found predominantly in soy) and lignans (found predominantly in linseed) [1]. The biological effects of these two classes may be different, and research has, to date, concentrated on the isoflavones. From this study, we cannot distinguish the effect of the two different classes or, indeed, conclusively attribute the biological effects we have observed to the phytoestrogens independent of any other supplement constituent.

Nevertheless, these findings may have future implications for health and disease. Phytoestrogens have been proposed to protect against breast cancer, as Japanese women, who consume their traditional diet with a high intake of phytoestrogens, have a very low incidence of breast cancer [23]. IGF-1 has been shown to be a risk factor for breast cancer in premenopausal women [24], and, if it can be confirmed that phytoestrogens increase circulating IGF-1 concentrations, then this would actually indicate an increased risk of breast cancer in those consuming high-phytoestrogen diets. Obviously, further work is required before such conclusions can be drawn, but the data presented here do highlight the need for further, well-designed trials.

In conclusion, this small study suggests that 1 week of supplementation with a phytoestrogen-rich bar alters concentrations of IGF-1 and IGFBP-3. The supplement had no effect on antioxidant parameters or lipid status in healthy female subjects.

Acknowledgment

This study was funded by Action Against Breast Cancer, registered charity number 1020967.

References

[1] Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A. Phytoestrogens: where are we now? Br J Nutr 1998;79:393–406.
[2] Campbell MJ, Woodside JV, Secker-Walker J, Titcomb A, Leathem AJC. Insulin-like growth factor (IGF) status is altered by tamoxifen in breast cancer patients. Mol Pathol 2001;54:307–10.
[3] Morton MS, Wilcox G, Wahlqvist ML, Griffiths K. Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. J Endocrinol 1994;142:252–9.
[4] Vuillemier JP, Keck E. Fluorometric assay of vitamin C in biological materials using a centrifugal analyser with fluorescence attachment. J Micronutr Anal 1989;5:25–34.
[5] Thurnham DI, Smith E, Flora PS. Concurrent liquid-chromatographic assay of retinol, α-tocopherol, β-carotene, α-carotene, lycopene, and β-cryptoxanthin in plasma, with tocopherol acetate as internal standard. Clin Chem 1988;34:377–8.
[6] Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. Methods Enzymol 1994;233:182–9.
[7] McDowell IFW, McEneny J, Trimble ER. A rapid method for measurement of the susceptibility to oxidation of low-density lipoprotein. Ann Clin Biochem 1995;32:167–74.
[8] Spanswick VJ, Hartley JM, Ward TH, Hartley JA. Measurement of drug-induced DNA interstrand crosslinking using the single cell gel electrophoresis (comet) assay. In: Brown R, Brown U, editors. Methods in molecular medicine: cytotoxic drug resistance mechanisms. USA: Humana Press; 1999. p. 143–54.
[9] King RA, Bursill DB. Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. Am J Clin Nutr 1998;67:867–72.
[10] Lissin LW, Cooke JP. Phytoestrogens and cardiovascular health. J Am Coll Cardiol 2000;35:1403–10.
[11] 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 U S A 1998;95:3106–10.
[12] Hodgson JM, Puddey IB, Croft KD, et al. Isoflavonoids do not inhibit in vivo lipid peroxidation in subjects with high-normal blood pressure. Atherosclerosis 1999;145:167–72.
[13] Sierens J, Hartley JA, Campbell MJ, Leathem AJC, Woodside JV. Effect of phytoestrogen and antioxidant supplementation on oxidative DNA damage assessed using the comet assay. Mutat Res 2001; 485:169–76.
[14] Mitchell JH, Collins AR. Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men — a pilot study. Eur J Nutr 1999;38:143–8.
[15] Pool-Zobel BL, Adlercreutz H, Glei M, et al. Isoflavonoids and lignans have different potentials to modulate oxidative genetic damage in human colon cells. Carcinogenesis 2000;21:1247–52.
[16] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;332:276–82.
[17] Lichtenstein AH. Got soy? Am J Clin Nutr 2001;73:667–8.
[18] Merz-Demlow BE, Duncan AM, Wangen KE, et al. Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. Am J Clin Nutr 2000;71:1462–9.
[19] Crouse JR, Morgan T, Terry TG, et al. A randomised trial comparing the effect of casein with that of soy protein containing various amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch Intern Med 1999;159:2070–6.
[20] Kelly JJ, Rajkovic IA, O'Sullivan AJ, Sernia C, Ho KKY. Effects of different oral estrogen formulations on insulin-like growth factor-1, growth hormone and growth hormone binding protein in postmenopausal women. Clin Endocrinol 1993;39:561–7.
[21] Clemmons DR, Underwood LE. Nutritional regulation of IGF-1 and IGF binding proteins. Annu Rev Nutr 1991;11:393–412.
[22] Helle SI, Anker GB, Meadows KA, Holly JMP, Lonning PE. Alterations in insulin-like growth factor system during the menstrual cycle in normal women. Maturitas 1998;28:259–65.
[23] Woodside JV, Campbell MJ. Isoflavones and breast cancer. J Br Menopause Soc 2001;7:S17–S21.
[24] Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor 1 and risk of breast cancer. Lancet 1998;351:1393–6.