# Isolated Isoflavones Do Not Affect the Circulating Insulin-Like Growth Factor System in Men at Increased Colorectal Cancer Risk[1]

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## Abstract

Epidemiological studies show that increased insulin-like growth factor (IGF)-I concentrations are related to increased colorectal cancer risk. A reduced colorectal cancer risk has been associated with isoflavones, which might affect the IGF-system because of their weak estrogenic activity. We conducted a randomized, placebo-controlled, double-blind crossover study to investigate the effect of an 8-wk isolated isoflavone supplementation (84 mg/d) on serum concentrations of total IGF-I, free IGF-I, total IGF-II, IGF binding protein (BP)-1, IGFBP-2, and IGFBP-3. Additionally, we investigated whether IGF-system component differences were related to concentrations of the more potent estrogenic isoflavone metabolite, equol. Our study population consisted of 37 men with a family history of colorectal cancer or a personal history of colorectal adenomas. Isoflavone supplementation did not significantly affect serum total IGF-I concentrations (relative difference between serum total IGF-I concentrations after isoflavone supplementation and after placebo: −1.3%, 95% CI −8.6 to 6.0%). Neither free IGF-I, nor total IGF-II, IGFBP-1, IGFBP-2, or IGFBP-3 concentrations were significantly altered. Interestingly, the change in serum IGF-I concentrations after isoflavone supplementation was negatively associated with serum equol concentrations ($r = -0.49$, $P = 0.002$). In conclusion, isolated isoflavones did not affect the circulating IGF-system in a male high-risk population for colorectal cancer. However, to our knowledge, this is the first study that suggests isoflavones might have an IGF-I lowering effect in equol producers only. This underlines the importance of taking into account equol status in future isoflavone intervention studies. J. Nutr. 137: 379–383, 2007.

## Introduction

Insulin-like growth factors (IGF)[11] are involved in cell proliferation and apoptosis and are important in both normal and tumor growth (1). Prospective epidemiological studies indicate that higher circulating concentrations of IGF-I are associated with increased colorectal cancer risk, whereas, for IGF binding protein (IGFBP)-3, inconsistent associations have been found (2). In addition, higher concentrations of IGF-II and reduced concentrations of IGFBP-1 and IGFBP-2 may be associated with increased colorectal cancer risk (3). Circulating concentrations of IGF-I and IGFBP are known to be modifiable by exogenous factors (e.g., dietary habits or other lifestyle factors) (4).

Estrogenic substances, such as oral estrogen replacement therapy (OERT), are associated with decreased colon cancer risk (5). Both OERT (6,7) and selective estrogen receptor modulators (SERM) (8–10) reduce serum IGF-I concentrations. Isoflavones are a class of phytoestrogens mainly present in soy products and extensively studied for their potential anticarcinogenic effects (11). Isoflavones structurally resemble estrogens and possess weak estrogenic activity (12). Therefore, these compounds may induce an estrogen-like reduction of IGF-I in the circulation.

In vitro and in vivo animal studies indicate that (soy) isoflavones may decrease serum IGF-I concentrations, whereas cross-sectional studies in humans did not find clear associations between soy isoflavone intake and serum IGF-I concentrations

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[11] Abbreviations used: IGF, insulin-like growth factor(s); IGFBP, IGF binding protein(s); IP, isoflavones-placebo; PI, placebo-isoflavones; SERM, selective estrogen receptor modulator(s).

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(4). Several randomized controlled supplementation studies of soy protein isolates containing isoflavones in humans found either no effect (13–15) or an increase (16–20) in serum IGF-I concentrations by soy isoflavones. The inconsistency in these results may be explained by potential IGF-increasing effects of soy protein itself, which can mask a potentially IGF-lowering effect of isoflavones alone (4).

Isolated isoflavones can be derived from red clover, which has a different isoflavone composition, but may result in a similar isoflavone blood profile compared with soy products (21). To our knowledge, red clover isoflavones have not been previously studied for their IGF effects in men or in relation to the risk of colorectal cancer. Therefore, we investigated the effect of a 2-mo isolated red clover isoflavone supplementation (84 mg/d) on serum concentrations of IGF and various IGFBP in a male population at increased risk of colorectal cancer that could potentially benefit most from this intervention. Because the biological effectiveness of isoflavones may depend on the individual ability to biotransform isoflavone metabolites to the more potent estrogenic metabolite equol (22), we also evaluated whether effects of isoflavones on circulating IGF were related to the ability to produce equol.

## Materials and Methods

*Study population.* We selected men aged 40–75 y with a personal history of colorectal adenomas or with at least one 1st-degree family member with a history of colorectal cancer. Asymptomatic men, scheduled to undergo a colonoscopy for screening purposes, were selected from the medical registries and pathology databases and were sent an invitation letter to participate in our study. Exclusion criteria were a history of cancer, familial adenomatous polyposis syndrome, familial Li Fraumeni syndrome, chronic inflammatory bowel disease, diabetes mellitus, acromegaly, significant liver or renal disease, (partial) bowel resection, nonremissive celiac disease, diverticulitis, laxative abuse, other severe comorbidity, and the use of food supplements containing isoflavones. Participants were recruited between July 2003 and January 2005. In total, 154 men were invited to participate in this trial. Of 128 eligible men, 40 were included in this trial and 15 were assigned to another trial (43% response). We obtained written informed consent from all participants.

The study was conducted in 4 hospitals in the Netherlands: the Antoni van Leeuwenhoek Hospital in Amsterdam, the Gelderse Vallei Hospital in Ede, the Slotervaart Hospital in Amsterdam, and the Sint Antonius Hospital in Nieuwegein. The study protocol was approved by the Medical-Ethical Committees of all participating centers.

*Design.* We conducted a randomized, placebo-controlled, double-blind crossover study. The total duration of the study was ~6 mo, consisting of two 8-wk intervention periods separated by an 8-wk washout period. Surveillance colonoscopies in study participants were always planned at the end of the 1st intervention period. Subjects were allocated to receive isoflavone tablets in the 1st intervention period and placebo tablets in the 2nd [isoflavones-placebo (IP group)] or vice versa [placebo-isoflavones (PI group)], according to a randomization scheme with permuted blocks. The isoflavone tablets (Promensil, Novogen) contained an isoflavone extract derived from red clover containing 42 mg of total isoflavones (25 mg biochanin, 8 mg formononetin, 4 mg genistein, and 5 mg daidzein). Subjects were asked to take 2 tablets/d, one with breakfast and one with dinner (total dose, 84 mg/d). Subjects were asked to maintain their habitual diet and lifestyle.

*Data collection.* Subjects visited the hospital at the beginning and end of both intervention periods. At each visit, blood samples were drawn after an overnight fast, and weight, waist, and hip circumference were measured. To assess habitual diet of the study population, a 24-h recall was conducted at each study visit. The method of interviewing and coding of foods and portion sizes was standardized and was performed by trained nutritionists and graduate students in nutrition. Energy and nutrient intakes were calculated using the VBS food calculating system (BAS Nutrition Software) based on the Dutch food composition table (23). Habitual physical activity over the 2 mo preceding each visit was assessed using the validated self-administered Short Questionnaire to Assess Health-enhancing physical activity (SQUASH) (24). During both intervention periods, subjects kept a daily notebook in which they recorded information about their health, medicine use, smoking, and consumption frequency of products rich in isoflavones (i.e., soy products, legumes, and nuts). Compliance was measured by counting returned tablets, self-reported supplement intakes from daily notebooks, and serum genistein concentrations at the beginning and end of both intervention periods.

*Laboratory analyses.* Fasting serum and EDTA-plasma samples were frozen and stored at –30°C until further analysis. All IGF-system components were measured at the end of both intervention periods. Serum total IGF-I was measured using an immunometric technique on the Advantage Chemiluminescense System (Nichols Institute Diagnostics). The sensitivity was 6.0 $\mu$g/L, intra-assay CV were 8.0% and 6.0% ($n = 25$) at 30 and 450 $\mu$g/L mean serum IGF-I, and interassay CV were 8.7%, 5.8% and 6.5% ($n = 115$) at 33, 174, and 445 $\mu$g/L mean serum IGF-I, respectively. Plasma free /IGFBP dissociable IGF-I was measured using a highly sensitive 2-site immunoradiometric free IGF-I kit (Diagnostic Systems Laboratories). The sensitivity was 0.2 $\mu$g/L, and the interassay CV was 12% at a mean plasma free IGF-I concentration of 1.6 $\mu$g/L. Serum IGF-II concentrations were determined in Sep-Pak C18 extracts of serum by RIA, as described previously (25,26). The sensitivity was 0.09 $\mu$g/L and intra- and interassay CV were 6.7 and 8.8% ($n = 12$) at 505 $\mu$g/L mean serum IGF-II, respectively. Serum IGFBP-1, IGFBP-2, and IGFBP-3 were determined by specific RIA. Relevant technical details were described previously (25,27,28). Total IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 assays were performed in the same laboratory.

Serum genistein was measured at all 4 time points using reversed phase HPLC with UV detection according to methods adapted from Supko et al. (29) and Busby et al. (30). The sensitivity was 37 nmol/L, and the accuracy and precision were within 100 ± 15% and 15%, respectively.

Serum equol concentrations were measured in samples collected after isoflavone intervention. TR-FIA kits (Labmaster) were used as described previously (31) and fluorescence was measured with a Victor 2 model 1420 spectrofluorometer (Wallac). The sensitivity was 3.3 nmol/L and intra-assay CV was 12.3% at a mean equol concentration of 134 nmol/L.

*Statistical analyses.* The parameter of interest in our statistical analysis was the relative crossover difference (see formulas below), expressed as a percentage relative to the concentration after placebo. The mean crossover difference for each IGF system component was calculated for both intervention groups (IP and PI) and then pooled over the 2 intervention groups, to adjust for period effects. We tested whether the pooled crossover difference significantly deviated from null with a $t$ test (2-sided $\alpha = 0.05$, df = 37) using the pooled SEM crossover differences (32).

$$\text{Crossover difference}\,(\Delta) = \text{concentration after intervention}\,(C_I) - \text{concentration after placebo}\,(C_P);$$

$$\text{Pooled crossover difference} = \frac{1}{2}(\text{mean}_{\Delta IP} + \text{mean}_{\Delta PI});$$

$$\text{Standard error of the pooled crossover difference} = \frac{1}{2}\sqrt{[(s^2/n_{IP}) + (s^2/n_{PI})]};$$

$$\text{where } s^2 = [(n_{IP} - 1)\,sd^2_{\Delta IP} + (n_{PI} - 1)\,sd^2_{\Delta PI}]/(n_{IP} + n_{PI} - 2).$$

Descriptive characteristics were computed for both randomized groups separately. We calculated whether relevant changes occurred in dietary and lifestyle factors that are known to influence the IGF-system (i.e., dietary intake of macronutrients, weight, waist, and hip circumference, total physical activity score, and dietary intake of products relatively rich in isoflavones) during the study period for both intervention



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groups separately. To evaluate whether the relative crossover differences correlated with serum genistein and equol concentrations after isoflavone supplementation, Spearman correlation coefficients were calculated. Logarithmic curve estimation was performed to obtain **Figure 1**.

## Results

Forty men were included in this trial. After randomization, one individual was ineligible because of a history of prostate carcinoma. One participant was diagnosed with esophageal cancer in the 1st intervention period, and one participant dropped out in the 2nd intervention period because of bowel complaints (drop-out rate 5%). This resulted in 17 men in the IP group and 20 men in the PI group who finished the complete study protocol.

Men in the IP group were slightly heavier, had a greater BMI and waist circumference, and were more often nonsmokers than those in the PI group (**Table 1**). The number of participants with a family history of colorectal cancer and/or a personal history of colorectal adenomas was equally distributed over the 2 groups.

Isoflavone supplementation did not significantly affect serum total IGF-I concentrations (mean relative difference between concentration after isoflavones and after placebo: −1.3%, 95% CI −8.6 to 6.0%) (**Table 2**). Concentrations of free IGF-I, total IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 also did not differ after isoflavone supplementation, with mean differences ranging from –1.2 to 4.0% (Table 2). The change in serum IGF-I concentration did not correlate with serum genistein concentrations (Spearman $r = -0.12$, $P = 0.49$). However, the change in serum IGF-I concentrations and serum equol concentrations after isoflavone intervention were negatively correlated ($r = -0.49$, $P = 0.002$) (Fig. 1). Using a cutoff value of 83 nmol/L (22), 9 of 37 men (24%) were classified as equol producers and, for 8 of these 9 men, serum IGF-I decreased after isoflavone intervention compared with placebo (median –15.1%; range –27.2 to –4.1%). For free IGF-I, a similar but weaker association with equol was found ($r = -0.37$, $P = 0.03$). Correlations of equol with changes in the other IGF parameters were in the same direction, but were not significant ($P = 0.05$–$0.78$; data not shown).

Based on returned tablet counts and recordings in the daily record books, 95% of participants were compliant (≥80% of tablets taken). For 29 of 37 men (78%), mean serum genistein concentrations after isoflavone intervention (252 ± 252 nmol/L; range 46 to 1274 nmol/L) had increased compared with all other time points (generally below detection limit of 37 nmol/L).

Body weight, waist, and hip circumference, total physical activity score, dietary macronutrient intake, and the number of days that products rich in isoflavones were consumed did not materially differ for the isoflavone vs. the placebo intervention period (data not shown).

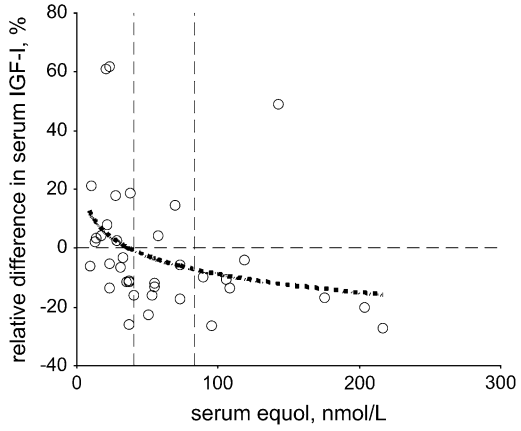


**Figure 1** Serum equol concentrations vs. the relative difference (%) in serum IGF-I concentrations after isoflavone and after placebo supplementation in 37 men. Individuals with equol concentrations ≤40 nmol/L were previously defined as equol nonproducers (22), and individuals with equol concentrations ≥83 nmol/L as equol producers (dashed vertical lines).

**TABLE 1** General characteristics of the IP and PI groups of men[1]

| | IP[2] | PI[2] |
|---|---|---|
| *n* | 17 | 20 |
| Age, *y* | 59.9 ± 9.7 | 59.8 ± 7.2 |
| Weight, *kg* | 90.0 ± 14.3 | 83.3 ± 12.7 |
| Height,[3] *cm* | 179.2 ± 9.9 | 178.3 ± 6.5 |
| BMI, *kg/m²* | 27.9 ± 3.1 | 26.2 ± 3.0 |
| Waist circumference,[4] *cm* | 105.3 ± 9.0 | 99.4 ± 9.9 |
| Hip circumference, *cm* | 106.7 ± 7.9 | 104.9 ± 7.1 |
| Waist-to-hip ratio | 0.99 ± 0.06 | 0.95 ± 0.06 |
| Smoking, *n (%)* | | |
| Yes | 2 (12) | 5 (25) |
| Never | 4 (24) | 2 (10) |
| Past | 11 (65) | 13 (65) |
| Family history of colorectal cancer and/or adenomas, *n (%)* | | |
| Family history and adenomas | 3 (18) | 5 (25) |
| Family history only | 4 (24) | 5 (25) |
| Adenomas only | 10 (59) | 10 (50) |
| Food supplement use | 3 (18) | 3 (15) |

[1] Values are means ± SD or n (%).
[2] IP and PI indicate treatment order; IP started with isoflavones, and PI with placebo.
[3] Height of 2 persons in the PI group was self-reported.
[4] Waist circumference of 1 person in the IP group was missing.

## Discussion

In our randomized, placebo-controlled, double-blind crossover study, isolated isoflavone supplementation of 84 mg/d for 2 mo did not influence circulating IGF concentrations (total IGF-I, free IGF-I, and total IGF-II) and IGFBP (IGFBP-1, -2, and -3) in men at high risk of colorectal cancer. However, a relative

**TABLE 2** Circulating IGF system component concentrations after placebo and isoflavone treatment and within-person crossover difference between isoflavone and placebo treatment

| | Concentration after placebo[1] | Concentration after isoflavones[1] | Within-person difference | |
|---|---|---|---|---|
| | | | Absolute[2] | Relative[3] |
| Total IGF-I, *μg/L* | 141.6 ± 57.5 | 138.1 ± 56.2 | −3.5 | −1.3 (−8.6 to 6.0) |
| Free IGF-I,[4] *μg/L* | 0.63 ± 0.34 | 0.59 ± 0.26 | −0.04 | −1.2 (−9.0 to 6.7) |
| Total IGF-II, *μg/L* | 562.1 ± 177.6 | 572.4 ± 163.9 | 10.3 | 4.0 (−2.2 to 10.2) |
| IGFBP-1,[4] *μg/L* | 40.5 ± 23.0 | 37.9 ± 24.2 | −2.6 | 1.6 (−15.8 to 19.1) |
| IGFBP-2, *μg/L* | 306.0 ± 142.0 | 310.5 ± 153.5 | 4.5 | 3.4 (−4.1 to 10.8) |
| IGFBP-3, *mg/L* | 2.31 ± 0.54 | 2.32 ± 0.53 | 0.01 | 1.0 (−2.9 to 4.9) |

[1] Values are pooled means ± SD, $n = 37$.
[2] Values are means, $n = 37$.
[3] Values are means (95% CI), $n = 37$.
[4] Two (free IGF-I) and one (IGFBP-1) subjects in the IP group were excluded because concentrations were below the detection limit.



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decrease in serum total IGF-I concentrations after isoflavone intervention was observed with increasing individual serum equol concentrations, suggesting an IGF-I lowering effect of isoflavones in equol producers only.

To our knowledge, this is the first randomized controlled trial that correlates the effects of isolated isoflavones with both serum IGF-I and equol status in men. We used a crossover design, which has the important advantage of obtaining results unaffected by the high interindividual variation in circulating IGF-concentrations relative to the much lower intraindividual variation. Our study population consisted of men at high-risk for colorectal cancer who could potentially benefit most from this isoflavone intervention. The number of recruited individuals for our study was more than sufficient, and the dropout rate was low (5%) and unrelated to supplement intake, which resulted in an adequately powered study. Compliance was high (≥95%) and genistein concentrations were increased in 78% of the participants. Blinding was confirmed by the fact that only 22% of the participants correctly guessed the period in which they received the isoflavone supplements. Furthermore, factors thought to potentially influence circulating concentrations of IGF and IGFBP did not materially change during the study; therefore, changes in various parameters of the IGF-system could mainly be attributed to the isoflavone intervention.

To our knowledge, only 1 pilot crossover study investigated the effect of an isolated red clover isoflavone supplementation on the IGF-system in humans. This study did not observe any effects on serum IGF-I and IGFBP-3 after 1 mo in both pre- and postmenopausal women (33), which is in line with our results in men. The 2-mo duration of isoflavone supplementation in our study was likely sufficient to affect the circulating IGF-system, insofar as human intervention studies on oral estrogens and SERM also observed a decrease in serum IGF-I after 2 mo (6,8). The differential effects on IGF for isoflavones compared with that of estrogens and SERM may be explained by their much weaker estrogenic activity (34). However, serum genistein concentrations in subjects with high soy consumption can far exceed those of endogenous estrogens, reaching levels sufficient to produce relevant biological effects (12).

The biological effects of isoflavones may also depend on the source and metabolism of isoflavones. In previous randomized controlled studies, the separate effects of isoflavones, soy protein, and calcium on the circulating IGF-system were difficult to disentangle (4). Therefore, we investigated a source of isolated isoflavones using a red clover supplement. Red clover isoflavones mainly consist of biochanin A and formononetin, which are metabolized to genistein and daidzein, respectively. Soy mainly contains the glucoside isoflavones genistin and daidzin, which are also metabolized to genistein and daidzein in the intestines (34). Differences in composition between soy and red clover have been shown not to affect bioavailability within individuals (35). Plasma genistein and daidzein concentrations after red clover isoflavone supplementation are within the range of plasma concentrations in subjects who traditionally consume a high-isoflavone soy-based diet (50–800 $\mu$g/L) (21). The bioavailability of isoflavones, however, may vary up to 10-fold between individuals (35). We also encountered large interindividual differences in serum genistein concentrations after isoflavone intervention. However, the relative difference in serum IGF-I concentrations after isoflavones and placebo did not correlate with serum genistein concentrations.

Recently, it has been hypothesized that only equol producers may benefit from isoflavone consumption (22). Approximately 30–50% of individuals are able to convert daidzein, one of the main isoflavone metabolites, to the more potent estrogenic isoflavone equol (36). Mice and rats metabolize the majority of daidzein to equol (22), and their serum IGF-I concentrations decreased with physiological dietary intakes of soy or genistein (37,38). Accordingly, the majority of equol producers in our study had decreased IGF-I concentrations after consuming isoflavones compared with placebo. IGF-I concentrations after placebo did not differ between equol producers and nonproducers, suggesting that equol production, rather than host factors associated with equol production, may be important with respect to IGF modulation (36).

In conclusion, isolated isoflavones did not influence serum IGF-I concentrations in our adequately powered, randomized, placebo-controlled, double-blind crossover trial in a male population at increased risk of colorectal cancer. These results suggest that the increased serum IGF-I concentrations observed in previous studies investigating soy food or soy protein supplementation are most likely due to soy protein, and not to isoflavones. Although based on small numbers of equol producers, our data suggest that isoflavones might have an IGF-lowering effect in equol producers only. Therefore, it is important that our results are confirmed in larger human-intervention studies among men and women using isolated isoflavones and giving consideration to equol status.

## Literature Cited


1. Khandwala HM, McCutcheon IE, Flyvbjerg A, Friend KE. The effects of Insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev. 2000;21:215–44.
2. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet. 2004; 363:1346–53.
3. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr. 2001;131:3109S–20S.
4. Voskuil DW, Vrieling A, van't Veer LJ, Kampman E, Rookus MA. The insulin-like growth factor system in cancer prevention: potential of dietary intervention strategies. Cancer Epidemiol Biomarkers Prev. 2005; 14:195–203.
5. Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. JAMA. 2002;288:872–81.
6. Weissberger AJ, Ho KK, Lazarus L. Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-hour growth hormone (GH) secretion, insulin-like growth factor I, and GH-binding protein in postmenopausal women. J Clin Endocrinol Metab. 1991; 72:374–81.
7. Decensi A, Bonanni B, Baglietto L, Guerrieri-Gonzaga A, Ramazzott F, Johansson H, Robertson C, Marinucci I, Mariette F, et al. A two-by-two factorial trial comparing oral with transdermal estrogen therapy and fenretinide with placebo on breast cancer biomarkers. Clin Cancer Res. 2004;10:4389–97.
8. Decensi A, Bonanni B, Guerrieri-Gonzaga A, Gandini S, Robertson C, Johansson H, Travaglini R, Sandri MT, Tessadrelli A, et al. Biologic activity of tamoxifen at low doses in healthy women. J Natl Cancer Inst. 1998;90:1461–7.
9. Shewmon DA, Stock JL, Rosen CJ, Heiniluoma KM, Hogue MM, Morrison A, Doyle EM, Ukena T, Weale V, Bakker S. Tamoxifen and estrogen lower circulating lipoprotein(a) concentrations in healthy postmenopausal women. Arterioscler Thromb. 1994;14:1586–93.
10. Duschek EJ, Gooren LJ, Netelenbos C. Comparison of effects of the rise in serum testosterone by raloxifene and oral testosterone on serum insulin-like growth factor-1 and insulin-like growth factor binding protein-3. Maturitas. 2005;51:286–93.
11. Lechner D, Kallay E, Cross HS. Phytoestrogens and colorectal cancer prevention. Vitam Horm. 2005;70:169–98.
12. Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. J Nutr. 1999;129:758S–67S.




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13. Adams KF, Newton KM, Chen C, Emerson SS, Potter JD, White E, Lampe JW. Soy isoflavones do not modulate circulating insulin-like growth factor concentrations in an older population in an intervention trial. J Nutr. 2003;133:1316–9.
14. Gann PH, Kazer R, Chatterton R, Gapstur S, Thedford K, Helenowski I, Giovanazzi S, Van Horn L. Sequential, randomized trial of a low-fat, high-fiber diet and soy supplementation: effects on circulating IGF-I and its binding proteins in premenopausal women. Int J Cancer. 2005;116: 297–303.
15. Maskarinec G, Takata Y, Murphy SP, Franke AA, Kaaks R. Insulin-like growth factor-1 and binding protein-3 in a 2-year soya intervention among premenopausal women. Br J Nutr. 2005;94:362–7.
16. Wangen KE, Duncan AM, Merz-Demlow BE, Xu X, Marcus R, Phipps WR, Kurzer MS. Effects of soy isoflavones on markers of bone turnover in premenopausal and postmenopausal women. J Clin Endocrinol Metab. 2000;85:3043–8.
17. Khalil DA, Lucas EA, Juma S, Smith BJ, Payton ME, Arjmandi BH. Soy protein supplementation increases serum insulin-like growth factor- I in young and old men but does not affect markers of bone metabolism. J Nutr. 2002;132:2605–8.
18. Arjmandi BH, Khalil DA, Smith BJ, Lucas EA, Juma S, Payton ME, Wild RA. Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary calcium excretion. J Clin Endocrinol Metab. 2003;88:1048–54.
19. Arjmandi BH, Lucas EA, Khalil DA, Devareddy L, Smith BJ, McDonald J, Arquitt AB, Payton ME, Mason C. One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. Nutr J. 2005;4:8.
20. Woodside JV, Campbell MJ, Denholm EE, Newton L, Honour JW, Morton MS, Young IS, Leathem AJ. Short-term phytoestrogen supplementation alters insulin-like growth factor profile but not lipid or antioxidant status. J Nutr Biochem. 2006;17:211–5.
21. Howes J, Waring M, Huang L, Howes LG. Long-term pharmacokinetics of an extract of isoflavones from red clover (Trifolium pratense). J Altern Complement Med. 2002;8:135–42.
22. Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr. 2002;132:3577–84.
23. Foundation NEVO. Dutch food composition table 2001. The Hague, The Netherlands: 2001.
24. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. J Clin Epidemiol. 2003;56:1163–9.
25. de Boer L, Hoogerbrugge CM, van Doorn J, Buul-Offers SC, Karperien M, Wit JM. Plasma insulin-like growth factors (IGFs), IGF-Binding proteins (IGFBPs), acid-labile subunit (ALS) and IGFBP-3 proteolysis in individuals with clinical characteristics of Sotos syndrome. J Pediatr Endocrinol Metab. 2004;17:615–27.
26. Rafferty B, Rigsby P, Gaines-Das RE. Multicentre collaborative study to calibrate IGF-II by bioassay and immunoassay: establishment of the First WHO Reference Reagent. Growth Horm IGF Res. 2001;11: 18–23.
27. Buul-Offers SC, van Kleffens M, Koster JG, Lindenbergh-Kortleve DJ, Gresnigt MG, Drop SL, Hoogerbrugge CM, Bloemen RJ, Koedam JA, van Neck JW. Human insulin-like growth factor (IGF) binding protein-1 inhibits IGF-I-stimulated body growth but stimulates growth of the kidney in snell dwarf mice. Endocrinology. 2000;141:1493–9.
28. de Vries BB, Robinson H, Stolte-Dijkstra I, Tjon Pian Gi CV, Dijkstra PF, van Doorn J, Halley DJ, Oostra BA, Turner G, Niermeijer MF. General overgrowth in the fragile X syndrome: variability in the phenotypic expression of the FMR1 gene mutation. J Med Genet. 1995; 32:764–9.
29. Supko JG, Phillips LR. High-performance liquid chromatographic assay for genistein in biological fluids. J Chromatogr B Biomed Appl. 1995; 666:157–67.
30. Busby MG, Jeffcoat AR, Bloedon LT, Koch MA, Black T, Dix KJ, Heizer WD, Thomas BF, Hill JM, et al. Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men. Am J Clin Nutr. 2002;75:126–36.
31. Brouwers E, L'homme R, Al Maharik N, Lapcik O, Hampl R, Wahala K, Mikola H, Adlercreutz H. Time-resolved fluoroimmunoassay for equol in plasma and urine. J Steroid Biochem Mol Biol. 2003;84: 577–88.
32. Hills M, Armitage P. The two-period cross-over clinical trial. Br J Clin Pharmacol. 1979;8:7–20.
33. Campbell MJ, Woodside JV, Honour JW, Morton MS, Leathem AJ. Effect of red clover-derived isoflavone supplementation on insulin-like growth factor, lipid and antioxidant status in healthy female volunteers: a pilot study. Eur J Clin Nutr. 2004;58:173–9.
34. Beck V, Rohr U, Jungbauer A. Phytoestrogens derived from red clover: an alternative to estrogen replacement therapy? J Steroid Biochem Mol Biol. 2005;94:499–518.
35. Tsunoda N, Pomeroy S, Nestel P. Absorption in humans of isoflavones from soy and red clover is similar. J Nutr. 2002;132:2199–201.
36. Atkinson C, Frankenfeld CL, Lampe JW. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. Exp Biol Med (Maywood). 2005;230:155–70.
37. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. J Nutr. 1999;129: 1628–35.
38. Aukema HM, Housini I. Dietary soy protein effects on disease and IGF-I in male and female Han:SPRD-cy rats. Kidney Int. 2001;59:52–61.

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