

## Effects of replacing meat with soyabean in the diet on sex hormone concentrations in healthy adult males

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A randomised crossover dietary intervention study was performed to evaluate the effects of replacing meat protein in the diet with a soyabean product, tofu, on blood concentrations of testosterone, dihydrotestosterone, androstenediol glucuronide, oestradiol, sex hormone-binding globulin (SHBG), and the free androgen index (total testosterone concentration/SHBG concentration  $\times$  100; FAI). Forty-two healthy adult males aged 35–62 years were studied. Diets were isoenergetic, with either 150 g lean meat or 290 g tofu daily providing an equivalent amount of macronutrients, with only the source of protein differing between the two diets. Each diet lasted for 4 weeks, with a 2-week interval between interventions. Fasting blood samples were taken between 07.00 and 09.30 hours. Urinary excretion of genistein and daidzein was significantly higher after the tofu diet ( $P < 0.001$ ). Blood concentrations of sex hormones did not differ after the two diets, but the mean testosterone:oestradiol value was 10 % higher ( $P = 0.06$ ) after the meat diet. SHBG was 3 % higher ( $P = 0.07$ ), whereas the FAI was 7 % lower ( $P = 0.06$ ), after the tofu diet compared with the meat diet. There was a significant correlation between the difference in SHBG and testosterone:oestradiol and weight change. Adjusting for weight change revealed SHBG to be 8.8 % higher on the tofu diet (mean difference 3 (95 % CI 0.7, 5.2) nmol/l;  $P = 0.01$ ) and testosterone:oestradiol to be significantly lower,  $P = 0.049$ ). Thus, replacement of meat protein with soyabean protein, as tofu, may have a minor effect on biologically-active sex hormones, which could influence prostate cancer risk. However, other factors or mechanisms may also be responsible for the different incidence rates in men on different diets.

### Meat: Soyabean: Sex hormones

Prostate disease is being increasingly diagnosed in many developed countries. Benign enlargement of the prostate may cause urethral obstruction, discomfort and urinary problems, while prostate cancer has become a major cause of death among older men in 'Westernised' populations (Muir *et al.* 1991). However, the precise aetiological factors have not been clearly established.

Both genetic and environmental factors have been implicated in prostate carcinogenesis (Meikle & Stanish, 1982; Whittemore *et al.* 1995). Although latent prostate tumours have a similar incidence throughout the world (Akazaki & Stemmerman, 1973; Breslow *et al.* 1977), international comparisons of invasive prostate cancer rates suggest that lifestyle factors, including the diet, may contribute to the progression of the disease (Armstrong & Doll, 1975; Zaridze *et al.* 1985; Muir *et al.* 1991). The rates of prostate cancer increase among men who migrate from a

country of low incidence rates to one with high incidence rates (Shimizu *et al.* 1991; Yu *et al.* 1991; Grulich *et al.* 1995) and this increase could be due to their adoption of a Western-style diet.

The consumption of animal products, especially the intake of fat (Slattery *et al.* 1990; Giovannucci *et al.* 1993), red meat (Talamini *et al.* 1986; Giovannucci *et al.* 1993) and some dairy products (Talamini *et al.* 1986; Snowdon, 1988), has been associated with an increased risk for prostate cancer. In contrast, prostate cancer rates are low in most Asian countries, where soyabean products are frequently consumed, and it has been proposed that lower disease rates might be due, at least in part, to protective effects of soyabean consumption (Adlercreutz, 1990) and of other dietary and lifestyle factors.

Prostate disease increases with age, and is considered to be dependent on sex hormones, particularly androgens

**Abbreviations:** adiol-G, androstenediol glucuronide; DHT, dihydrotestosterone; FAI, free androgen index; SHBG, sex hormone-binding globulin.

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(Noble, 1977; Zumoff *et al.* 1982). In the prostate, testosterone is acted upon by the enzyme 5 $\alpha$ -reductase and converted to dihydrotestosterone (DHT), a more potent androgen that regulates intraprostatic metabolism (Coffey, 1986). Increased exposure of the prostate to DHT is associated with increased tissue growth (Meikle *et al.* 1980). DHT is metabolised to androstenediol and these two androgens have been measured in plasma (Kinouchi & Horton, 1974; Horton *et al.* 1982). Although 5 $\alpha$ -reductase is a tissue enzyme, its activity has been assessed indirectly through the measurement of serum androstenediol glucuronide (adiol-G; Horton *et al.* 1982). The balance between androgens and oestrogens has also been suggested as a possible influence on prostatic function and on the development of prostate disease (Hammond, 1978; Partin *et al.* 1991; Wilding, 1995). Both the biological activity and the metabolic clearance of these hormones are strongly influenced by sex hormone-binding globulin (SHBG), which reversibly binds the steroid hormones with different affinities (Burke & Anderson, 1972; Anderson, 1974; Dunn *et al.* 1981). It is the small proportion of unbound sex hormones in the circulation that is biologically active (Mahoudeau *et al.* 1971).

The role of sex hormone metabolism on prostate disease has been explored in a few cross-sectional and clinical investigations. Lower adiol-G concentrations in Asian men than in Caucasian and African-American men have been reported (Lookingbill *et al.* 1991; Ross *et al.* 1992), suggesting that variations in 5 $\alpha$ -reductase activity may explain some differences in prostate cancer rates in populations at different risk for the disease. Pharmacological inhibition of 5 $\alpha$ -reductase enzyme using the drug finasteride has resulted in symptomatic relief and reduction of the prostatic volume in patients with benign prostatic hypertrophy (Gormley *et al.* 1992), and the role of finasteride in the prevention of prostate cancer is also being investigated (Brawley *et al.* 1994). Certain dietary constituents may mediate prostate cancer growth via hormonal (Hutchinson, 1976) and other mechanisms. Hence, any dietary component that influences the amount of biologically-available androgens within the prostate gland is of interest.

Soyabean and processed soyabean products contain isoflavones such as genistein and daidzin (Coward *et al.* 1993), phyto-oestrogens that possess weak oestrogenic properties (Shutt & Cox, 1972). An *in vitro* study has demonstrated that genistein inhibits the growth of prostate tumour cells (Peterson & Barnes, 1993). The finding that genistein may also inhibit 5 $\alpha$ -reductase activity *in vitro* (Evans *et al.* 1995) suggests that soyabean consumption might explain some differences in 5 $\alpha$ -reductase activity between populations, as measured by serum concentrations of adiol-G (Lookingbill *et al.* 1991; Ross *et al.* 1992), and the subsequent differences in prostate cancer rates.

A vegetarian diet has been associated with increased circulating levels of SHBG (Belanger *et al.* 1989; Key *et al.* 1990). In addition, *in vitro* data suggest that genistein may increase hepatic SHBG production (Mousavi & Adlercreutz, 1993; Loukovaara *et al.* 1995). Any effects of dietary constituents on SHBG levels are likely to affect sex hormone metabolism through its influence on hormone

binding and clearance. A small number of intervention studies have reported effects of various nutrients on endogenous sex hormone levels in men. Decreased intake of fat (Hamalainen *et al.* 1984; Dorgan *et al.* 1996), increased dietary fibre (Dorgan *et al.* 1996) and changes to a vegetarian diet (Raben *et al.* 1992) have resulted in lower plasma androgen levels. Previous diet-intervention studies have involved simultaneous changes in various dietary components, and there is little information available on the specific effects of soyabean consumption on sex hormone concentrations in healthy adult males.

In this intervention study, meat protein in the diet of free-living male volunteers was replaced with protein from a soyabean product, tofu, to investigate whether soyabean consumption may influence endogenous levels of sex hormones and their binding protein in such a way as to reduce overall androgenic activity.

### Subjects and methods

Healthy omnivorous Caucasian males between the ages of 35 and 62 years were recruited through local contacts and newspaper advertisements. Individuals with symptoms or previous diagnosis of prostate disease or other illnesses, with alcohol intakes exceeding 10 % total daily energy intake, or those taking long-term medications that might affect sex hormone concentrations, were not included. Athletes who train regularly for competitive sport, and obese men with a BMI >35 kg/m<sup>2</sup>, were also excluded from the study. The study protocol was approved by the Deakin University Ethics Committee, and written informed consent was obtained from all the participants.

A randomised crossover design was used. Before the start of the study, participants completed a 7 d weighed-food record on their usual diets. Each participant was then randomly assigned to one of the two diets for a period of 4 weeks, subsequently resuming the usual diet for 2 weeks as a 'wash-out period' before being assigned to the other diet for a further 4 weeks. The two diets provided similar amounts of energy, protein, carbohydrate, fat, dietary fibre and alcohol, differing only in the source of dietary protein. The meat diet included 150 g lean red meat/d (raw weight, but eaten as cooked product). The tofu diet contained 290 g tofu/d (raw weight), containing about 35 g soyabean protein, in specially-prepared meals and biscuits, and subjects were allowed not more than one serving of chicken or fish per week. Additional amounts of butter (5 g), animal fat (lard; 5 g) and olive oil (8 ml) were used to compensate for the lower fat content of tofu compared with meat and to minimise differences in intake of total, saturated, monounsaturated and polyunsaturated fat during the two diets. Tofu was provided and purchased from a single source (Blue Lotus Foods, Kilsyth, Victoria, Australia). Other aspects of the diet were kept as constant as possible, and subjects were also asked to keep their physical activity pattern as similar as possible during the two diets. Subjects were contacted at least weekly to be given support and advice for any difficulties encountered in order to improve compliance.

Each subject completed a 7 d weighed-diet record during the last week of each diet period. Diet records were

analysed using the software System for Online Dietary Analysis version 5 (SODA, Cottesloe, WA, Australia) that used updated nutrient composition data for Australian foods.

Height and weight of each participant was measured and BMI calculated before and after each diet period. Venous blood samples were collected before the study and on two occasions 3 d apart at the end of each diet after an overnight fast between 07.00 and 09.30 hours at a uniform time for each subject to minimise variation in hormone concentrations due to diurnal rhythms. Serum was separated at 4°C and stored at -80°C for later analysis. Commercial radioimmunoassay (RIA) kits were used to measure serum concentrations of total testosterone (Medgenix, Biosource, Belgium), DHT, adiol-G (Diagnostics Systems Laboratories Inc, TX, USA), and oestradiol (Spectria, Orion Diagnostica, Espoo, Finland). Serum SHBG was analysed by immunoradiometric assay (Spectria). All samples from the same subject were analysed in a single batch. The intra-assay CV were less than 6 % for testosterone and SHBG, and less than 10 % for DHT, adiol-G and oestradiol. The free androgen index (total testosterone concentration/SHBG concentration × 100; FAI) and testosterone:oestradiol were calculated. Samples from eleven subjects were assayed for cholesteryl ester-fatty acids using GC after extraction from serum according to the method described by Sinclair *et al.* (1987).

A 24 h urine collection was completed at the end of each diet period and portions were immediately frozen at -20°C. Genistein and daidzein were isolated by reverse-phase HPLC (Shimadzu system LC10A; Shimadzu Scientific Instruments Pty Ltd, Melbourne, Australia) after deconjugation using glucuronidase (Sigma Chemical Company, St Louis, MO, USA) and extraction with diethyl ether following a method developed by Eldridge (1982). The urinary creatinine concentration was measured using a Hitachi 704 autoanalyser (Hitachi Ltd, Tokyo, Japan).

Sample size was calculated to provide an 80 % power of detecting a 10 % difference in hormone levels. Statistical analyses were performed using the Statistical Package for the Social Sciences version 7.5 (SPSS Inc. Chicago, IL, USA). Mean hormone values from two separate blood samples collected at the end of each diet were used for analysis, as these values were not significantly different. Non-normally-distributed data were log transformed. The general linear model was used to compare hormone results at the end of the two diet periods, taking carry-over effects into consideration (Fleiss, 1986). As there were no carry-over effects for any of the variables, this factor was disregarded in the analysis of the diet effects. Mean results for each diet were compared using the paired *t* test for normally-distributed data or the Wilcoxon signed-rank test for data that were not normally distributed after log transformation. As there was a significant correlation between weight change and SHBG ( $r = 0.36$ ), the paired difference was adjusted using multiple linear regression.

## Results

Of the forty-five subjects enrolled in the study, three were excluded before data analysis as they were unable to

**Table 1.** Dietary intakes of healthy adult males when receiving the lean meat and tofu diets<sup>‡</sup>

(Values are means and standard deviations for forty-two subjects)

	Lean-meat diet		Tofu diet	
	Mean	SD	Mean	SD
Energy (MJ)	9.6	0.3	9.5	0.3
Protein (g)	96.7	3.1	93.2	2.9
Fat (g)	84.5	3.4	81.9	3.4
SF (g)	33.5	1.4	31.8	1.5
MF (g)	31.0	1.4	29.2	1.3
PF (g)	12.7	0.7	13.2	0.7
P:S	0.4	0.1	0.42*	0.2
Carbohydrate (g)	262.1	56.7	260.9	64.6
Cholesterol (mg)	253.2	11.2	189.5†††	16.3
Fibre (g)	28.0	1.4	26.6	1.2
Alcohol (g)	16.9	21.4	17.9	21.0
Protein (% energy)	17.2	0.3	16.9	0.3
Carbohydrate (% energy)	43.7	0.8	44.0	0.8
Fat (% energy)	32.4	0.8	31.9	0.8
SF (% energy)	12.9	0.5	12.4	0.4
MF (% energy)	11.8	0.3	11.5	0.4
PF (% energy)	4.8	0.2	5.1	0.2
Alcohol (% energy)	4.9	0.8	5.4	0.9

SF, saturated fat; MF, monounsaturated fat; PF, polyunsaturated fat; P:S polyunsaturated:saturated fat.

Mean value was significantly different from that for lean-meat diet (paired *t* test): \* $P = 0.047$ .

Mean value was significantly different from that for lean-meat diet (paired *t* test): ††† $P < 0.001$ .

‡ For details of diets, see p. 558.

complete the prescribed diets. Thus, data were analysed on forty-two men, of mean age 45.7 (SD 7.6) years and mean BMI 26.2 (SD 3.3) kg/m<sup>2</sup> at baseline. The mean intakes of energy, protein, carbohydrate, total and saturated fat, monounsaturated fat, polyunsaturated fat, fibre and alcohol did not differ during the meat and tofu diets (Table 1). The tofu contained 0.29 mg genistin/g and 0.12 mg diadzin/g. The mean body weights of the subjects after the meat and tofu diets were 83.1 (SD 10.8) kg and 83.5 (SD 11.3) kg respectively ( $P = 0.05$ ).

Urinary concentrations of genistein and daidzein are shown in Table 2. Urine samples were available for thirty-one subjects and all showed greatly increased excretion on the tofu diet. The serum cholesteryl ester-fatty acid concentrations for individual saturated and monounsaturated fatty acids did not differ after the two diets.

Hormone concentrations after the two diets are shown in Table 3. Serum testosterone, DHT, adiol-G and oestradiol did not differ significantly after the two diets. SHBG was

**Table 2.** Urinary concentrations of genistein and daidzein for healthy adult males receiving the lean-meat and tofu diets<sup>†</sup>

(Values are means and standard deviations for thirty-one subjects)

	Lean-meat diet		Tofu diet	
	Mean	SD	Mean	SD
Genistein:ng/μmol creatinine	12.4	11.2	201.8***	195.9
μmol/24 h	0.3	0.5	2.9***	3.0
Daidzein:ng/μmol creatinine	20.3	23.4	401.3***	254.0
μmol/24 h	0.5	1.0	6.1***	5.4

Mean values were significantly different from those for the lean-meat diet (paired *t* test): \*\*\* $P < 0.001$ .

† For details of diets and procedures, see p. 558.

**Table 3.** Serum hormone values for healthy adult males after 4 weeks on the lean-meat and tofu diets\*  
(Values are means and 95 % CI for forty-two subjects)

	Lean-meat diet		Tofu diet		Tofu diet – lean meat diet		
	Mean	95 % CI	Mean	95 % CI	Difference‡	95 % CI	P value
Testosterone (nmol/l)	15.4	13.7, 17.1	15.5	13.8, 17.1	0.1	–0.6, 0.8	0.94§
Dihydrotestosterone (nmol/l)	1.08	0.93, 1.24	1.13	0.96, 1.31	0.05	–0.02, 0.12	0.22
Androstenediol glucuronide (nmol/l)	16.6	14.4, 18.8	17.9	15.4, 20.4	1.3	0.09, 2.4	0.08
Sex hormone-binding globulin (SHBG; nmol/l)	33.2	28.6, 37.7	34.3	29.6, 38.9	1.1	–0.08, 2.3	0.07¶
Adjusted value†					3.0	0.7, 5.2	0.01
Free androgen index (total testosterone/SHBG×100)	50.0	44.8, 55.2	46.7	42.0, 51.5	–3.3	–6.8, 0.2	0.06¶
Oestradiol (pmol/l)	69.6	60.6, 78.6	74.2	65.6, 82.9	4.6	–3.0, 12.2	0.13
Testosterone:oestradiol	248.9	210.4, 287.5	223.3	194.6, 252.0	–25.6	–51.0, 0.3	0.06
Adjusted value†					–28.0	–53.0, –2.6	0.049

\* For details of diets and procedures, see p. 558.

† Values after adjusting for weight change using multiple linear regression.

‡ Mean difference of untransformed results.

§ Difference = 0, based on Wilcoxon signed-ranks test.

|| Difference = 0, based on paired *t* test of log-transformed values.

¶ Difference = 0, based on paired *t* test of untransformed values.

3 % higher after the tofu diet than after the lean meat diet ( $P = 0.07$ ), and the FAI was 7 % lower on the tofu diet ( $P = 0.06$ ). The mean serum oestradiol concentrations did not differ significantly after the two diets, although the testosterone:oestradiol tended to be higher on the meat diet ( $P = 0.06$ ). There was a significant correlation between the difference in SHBG and testosterone:oestradiol and weight change. The paired difference adjusted for weight change by multiple linear regression showed the mean SHBG to be 8.8 % higher on the tofu, and testosterone:oestradiol to be lower.

## Discussion

The present study was designed to replace dietary protein from meat with soyabean protein, while keeping the intake of other nutrients as similar as possible. Tofu was chosen, as it is easy to prepare and is adaptable to many recipes. Previous diet-intervention studies that investigated the effects of soyabean intake on hormonal responses involved female subjects (Cassidy *et al.* 1994; Petrakis *et al.* 1996). To our knowledge, this is the first intervention study reporting the effects of a soyabean diet on sex hormone levels and 5 $\alpha$ -reductase activity in men. Urinary concentrations of the isoflavones genistein and daidzein increased significantly after the tofu diet, and the values we obtained were within the range of reported values in Japanese men consuming a traditional diet (Adlercreutz *et al.* 1991), who are considered to be at low risk for prostate disease.

The results of the present study suggest that overall androgen activity, as measured by increased SHBG and lowered testosterone:oestrogen, may be lowered by dietary isoflavones. Other studies have reported changes in SHBG concentrations in response to dietary changes (Anderson *et al.* 1987; Reed *et al.* 1987; Key *et al.* 1990; Gates *et al.* 1996). However, the effects of other dietary factors, BMI, physical activity and other lifestyle characteristics on SHBG could not be excluded (Gates *et al.* 1996). The present study attempted to ensure that macronutrients and physical activity levels were constant throughout the study period by intensive contact with the participants. However,

we found a small but significant ( $P = 0.05$ ) difference in body weight on the two diets, and the results were adjusted for weight change when appropriate.

Although serum oestradiol did not differ on the two diets, the lower testosterone:oestradiol after the tofu diet is consistent with our hypothesis that a soyabean diet may result in lower androgen activity and is likely to be related to the increase in SHBG. Since SHBG has greater binding affinity to testosterone than to oestrogen (Dunn *et al.* 1981), an increase in circulating SHBG may alter the balance between unbound androgens and oestrogens in favour of oestrogen (Burke & Anderson, 1972; Anderson, 1974). Serum levels of testosterone and its metabolites did not differ significantly between the two diets. The FAI had a slight tendency to be lower on the tofu diet; however, as FAI was calculated and is very sensitive to the SHBG measure, the actual changes in free hormone levels are not certain. We thus found no conclusive evidence that short-term soyabean intake has an influence on the circulating concentrations of androgens and the activity of 5 $\alpha$ -reductase, as measured by serum andiol-G. It is possible that homeostatic mechanisms may have caused attenuation of any differences in circulating hormone levels in response to the diet changes. Furthermore, questions have been raised as to whether serum androgen levels accurately reflect hormone metabolism in prostate tissue (Rittmaster *et al.* 1993; Hsing, 1996) and our findings do not exclude the possibility of an effect of soyabean intake on hormone metabolism within the prostate.

Since sex hormones and SHBG may be sensitive to weight changes, the interpretation of our results was confounded by the small change in mean body weight (0.4 kg). The changes were not consistent with the self-reported energy intakes, which showed no major difference between the two diets. Small variations in body weight are common over several months, and some variation in energy intake and/or physical activity occurred in a few subjects, with the greatest weight alteration after changing from one diet to another. Energy intakes of all subjects, evaluated using the method described by Goldberg *et al.* (1991), suggested the possibility of under-reporting of energy

intakes in a number of subjects during both diets, but no evidence that this differed between the two diets. However, the increase in urinary phyto-oestrogen excretion on the tofu diet reflects a marked increase in soyabean intake; regular feedback during the study from the highly-motivated volunteers indicates that all subjects accomplished a major change from a meat- to a soyabean-based diet with few other differences; and the cholesteryl ester-fatty acids support overall compliance with the planned diets.

The biological significance of the slight reduction in the total androgen activity that results from higher SHBG concentrations in response to a soyabean diet, and whether soyabean intake over a span of many years may affect sex hormone metabolism, requires further study. The findings from a prospective study of sex hormone levels and prostate cancer risk suggest that increased levels of testosterone and low SHBG, even within normal endogenous ranges, are associated with a higher risk of prostate cancer (Gann *et al.* 1996), as are low levels of oestradiol. In that study there was a 35 % difference in prostate cancer risk in the groups with a median SHBG of 17.6 and 25.2 nmol/l.

Asian populations start to consume soyabean products at a young age, as a regular feature of the diet. Minor hormonal changes resulting from long-term soyabean intake may confer protection against prostate cancer, in combination with other dietary or lifestyle factors. However, *in vitro* studies have suggested direct inhibitory effects of genistein on tumour growth through other mechanisms (Akiyama *et al.* 1987; Wei *et al.* 1993; Fotsis *et al.* 1995), and such effects could explain an effect of soyabean independent of hormonal influences. In reality, Asian diets differ from Western diets in more than just soyabean consumption in that they generally contain less fat and include a higher intake of cereals, vegetables and fish (Gates *et al.* 1996). A combination of these factors may provide long-term protection; this area warrants further epidemiological research, larger intervention studies and *in vitro* investigation of possible mechanisms of action.

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