Effect of a phytoestrogen food supplement on reproductive health in normal males

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A B S T R A C T

Animal studies and human intervention trials have demonstrated the cancer chemopreventive properties of plant phytoestrogens, and phytoestrogen supplements are now widely available 'over-the-counter'. However, consumption of phytoestrogen-rich diets can cause impaired fertility and reproductive tract disorders in some animals and the apparent decline in human sperm quality over recent decades may be related to increased exposure to environmental endocrine disruptors. The present study determines the effects of a short-term phytoestrogen supplement on semen quality and serum sex steroid and gonadotrophin levels in human males. Healthy volunteers took a supplement containing 40 mg of isoflavones daily for 2 months and donated blood and semen samples monthly for 2 months before and 4 months after supplementation. Semen samples were analysed for ejaculate volume, sperm concentration, total sperm count, motility and morphology. Blood samples were analysed for sex hormone and gonadotrophin levels and phytoestrogen concentrations, and testicular volume was measured using an orchidometer. The phytoestrogen supplement increased plasma genistein and daidzein concentrations to approx. 1 μM and 0.5 μM respectively; yet, there was no observable effect on endocrine measurements, testicular volume or semen parameters over the study period. This is the first study to examine the effects of a phytoestrogen supplement on reproductive health in males. We conclude that the phytoestrogen dose consumed had no effect on semen quality.

INTRODUCTION

Plant phytoestrogens bind weakly to oestrogen receptors α and β and are able to induce weak oestrogenic and anti-oestrogenic actions in mammalian tissues [1]. Some phytoestrogenic compounds are also reported to have cancer chemoprotective properties via a number of non-receptor-related mechanisms, including inhibition of protein tyrosine kinases [2], impaired DNA topoisomerase activities and protection against DNA damage [3,4], and altered sex hormone metabolism and bioavailability [5–8]. However, phytoestrogens given in high doses or at critical stages of development in rodents can result in severe reproductive tract disorders [9–11], and temporary infertility syndromes in domestic animals have been related to high phytoestrogen consumption in grazing [12].

There has been concern that the apparent decline in sperm quality over recent decades (for a review see [13]) might be related to increased exposure to environmental endocrine disruptors [14,15]. In addition, human intervention trials with phytoestrogen-based supplements in pre- and post-menopausal women have indicated oestrogenic actions, which may be adverse to health

Key words: endocrine disruptors, fertility, isoflavones, phytoestrogens, reproductive health.

Abbreviations: CASA, computer-assisted sperm assessment; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

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[16,17]. There have been no reports to date regarding the effects of plant oestrogens on reproductive health in males. The present study examines the effects of a short-term phytoestrogen supplement on semen quality, as well as sex hormone and gonadotrophin levels, in young healthy males.

MATERIALS AND METHODS

Materials

Subjects received 500 mg tablets consisting of a standardized soy extract containing 40 mg, in total, of the isoflavones genistein, daidzein and glycitein in a standard excipient mixture. (Regen™; Novogen Limited, North Ryde, New South Wales, Australia.)

Subjects and experimental design

The study design was an open, within-subject assessment of the effects of a food supplement (Regen™) containing phytoestrogens on the reproductive system in adult males. A sample size of 12 was calculated to give 95% power to detect a 20% change in parameters at a level of 5% significance. Fifteen non-vegetarian individuals, on no prescribed medication, were recruited from an ongoing Medical Research Council research programme into the cell biology of human spermatozoa. The study was approved by the Lothian Health Paediatrics and Reproductive Medicine Research Ethics sub-committee, and each subject gave written informed consent. All were aged between 18–35 years, of good general health and with no significant medical reproductive history. They had good reproductive health on physical examination, normal semen quality by conventional assessment [17a], and normal serum levels of oestradiol, testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Volunteers attended for assessment at monthly intervals, twice before being given the supplement, twice during supplement and three times post-supplement. They were asked to take one tablet daily of a soy extract containing 40 mg phytoestrogens for 2 months. On each visit, reproductive health was assessed by an experienced clinician and a blood sample was taken by venepuncture. Testicular volume was determined by the same clinician throughout, using an orchidometer. Subjects were also asked to produce a semen sample by masturbation, which was examined for volume, sperm count, sperm motility and morphology and sperm movement, using computer-assisted sperm assessment (CASA). CASA analysis was undertaken in accordance with European Society for Human Reproduction and Embryology guidelines where relevant.

One subject withdrew from the study midway through the supplementation period and so was excluded from the final analysis, three subjects did not attend for the final post-supplementation assessment.

Semen analysis

All semen samples were analysed in the same laboratory according to the protocol of the World Health Organisation [17a,17b] and, before entry to the study, subjects had produced at least two semen samples within the normal reference range defined for our population (ejaculate volume > 1 ml, sperm concentration $> 20 \times 10^6$/ml and overall motility $> 40\%$). Samples were collected by masturbation into sterile plastic containers and the time from ejaculation to analysis was recorded and used as a co-variate in the statistical analysis. Subjects were instructed to abstain from ejaculation for 2 or 3 days before producing the sample.

Semen samples were allowed to liquefy at 37 °C for 30 min before analysis, which was always within 90 min of ejaculation. The volume was measured by weight, assuming 1 ml to weigh 1 g. An aliquot of semen was diluted 1:20 with sperm-diluting fluid (50 g Na_2HCO_3 in 10 ml of 35% formaldehyde/litre of water). The number of spermatozoa was counted using a Neubauer Improved haemocytometer at $\times 400$ magnification (Ortholux; Leitz, Wetzlar, Germany). Motility was examined at $\times 400$ magnification under phase-contrast illumination. At least 100 sperm were examined and motility was expressed as the proportion of sperm showing evidence of movement [WHO grades: a (rapid progressive motility), b (slow or sluggish progressive motility), and c (non-progressive motility)]. Morphology was examined at $\times 400$ magnification, on a wet preparation, using phase-contrast optics and was expressed as a percentage of morphologically normal sperm. In addition, attributes of sperm movement were determined using a commercially available computer-assisted image-analysis system. (HTM-IVOS Software version 10.8; Hamilton-Thorn, 181 Elliott Street, Suite 505, Beverly, MA 01915, U.S.A.).

Hormone and phytoestrogen analysis

Blood was centrifuged at 1500 ***g*** for 15 min and serum or plasma was frozen at -20 °C until use. Serum hormones were assayed by immunoassay [18–21].

Phytoestrogens were prepared for analysis using the method of Morton et al. [22], with the following modifications: the *Helix pomatia* extract was not purified in order to minimize enzyme de-activation, DEAE–Sephadex was used to fractionate the samples and *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide was used for derivatization. Internal standards daidzein-d_3, genistein-d_4 and equol-d_4 were purchased from the Adlercreutz group (University of Helsinki, Finland). Phytoestrogens were determined by isotope dilution GLC–MS, in the selected ion monitoring mode, using an HP 5973 detector in conjunction with an HP 6890 series gas chromatograph equipped with a capillary column (0.25 μm BP-1; 0.22 mm $\times$ 12 m), with helium as the carrier gas.

Statistical analysis

Data were analysed by ANOVA using the Genstat for Windows Statistics Package. This program adjusted for co-variates, which were alcohol, smoking, technician, season effect and abstinence (technician was not a co-variate in the analysis of testicular volume, which was carried out by the same clinician, or in the CASA analysis). The results are presented as group means from the raw data, i.e. before correction for co-variance, and as means $\pm$ S.E.M. from the ANOVA output after correction for co-variance.

RESULTS

Mean plasma concentrations of genistein and daidzein increased to approx. 1 μM and 0.5 μM respectively in subjects during supplementation (Figure 1). Seven of the 14 subjects metabolized daidzein to the metabolite, equol, which reached an average plasma concentration of 0.15 μM (results not shown).

Testicular volume was not significantly affected by the supplement when season was included as a co-variate ($P = 0.10$); however, when season was removed from the ANOVA there was a significant difference between the months ($P < 0.001$). As the majority of the volunteers were sampled in the same season, a treatment effect should not be excluded. Nevertheless, since the testicular volume was still higher than baseline several months post-supplement, a seasonal effect seems more plausible.

There was no change in oestradiol, testosterone, FSH or LH concentrations throughout the study (Table 1). Similarly, the supplement had no significant effect on ejaculate volume, sperm concentration, count or motility (Table 2). This was supported by a lack of effect on sperm movement using CASA (results not shown). The data show an apparent effect on sperm morphology in month 7; however, this can be explained by a change in the reporting criteria in the WHO guidelines at the beginning of 1999 [17b]. With the inclusion of new strict reporting criteria, the reference scale changed and a value > 10 was considered to be good morphology. Adjustment for co-variates had a large effect for sperm count in months 1 and 7, because there was a large coefficient of variation (93%), and imbalance over technicians and seasons was greatest in these months.

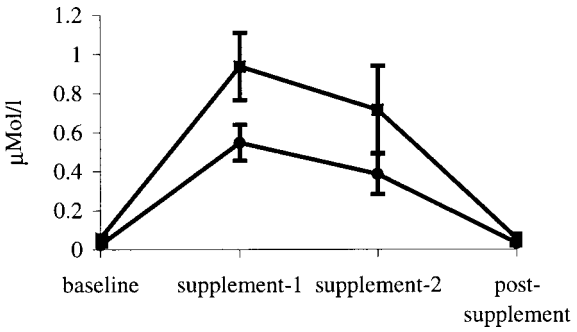

Figure 1 Plasma genistein (■) and daidzein (●) concentrations (μmol/l)

Data are shown as group means $\pm$ S.E.M. The baseline sample was taken 1 month before supplementation, supplement-1 and 2 were taken after 1 and 2 months of supplementation respectively, and the post-supplement sample was taken 1 month post-supplementation.

DISCUSSION

There is very little in literature on the effects of phytoestrogens in males. In theory, exposure to high concentrations of any exogenous oestrogen could cause alterations in gonadotrophin levels and in the function of the reproductive system. Indeed, sexual impotence was reported in males occupationally exposed to synthetic oestrogens in the pharmaceutical industry [23,24], and diethylstilbestrol exposure can cause reproductive dysfunction [24,25]. Furthermore, recent reports suggest that 'environmental oestrogens' may be responsible for the apparent decline in semen quality [15]. If such a link between exogenous endocrine disrupters and the decline in male fertility exists, the fetal–prepubertal period and Sertoli cell development would be of critical importance [15,26]. Plant phytoestrogens are a class of 'environmental oestrogens', with some compounds, including genistein and daidzein, having significant hormonal

Table 1 Blood gonadotrophin and reproductive hormone levels

Results are shown as group means. *P* value and S.E.M. are from the ANOVA output.

Month of study...	1	2	3	4	5	6	7		
	Pre-supplement		During supplement		Post-supplement			*P* value	S.E.M.
Oestradiol (pg/ml)	38.21	35.21	36.24	36.26	33.38	35.62	34.01	0.71	2.02
FSH (units/l)	4.51	4.44	4.59	4.51	4.40	4.49	4.38	0.89	0.12
LH (units/l)	3.27	3.68	3.40	3.50	3.96	3.92	3.40	0.33	0.25
Testosterone (ng/ml)	8.94	8.72	8.07	7.93	7.57	7.56	7.86	0.17	0.43

Table 2 Semen quality data

Results are shown as the group means from the raw, uncorrected data as well as means ± S.E.M. from the ANOVA output after correction for co-variates. Motility and morphology are expressed as a percentage of motile or morphologically normal sperm. Testicular volume is estimated in ml.

Month of study...	1	2	3	4	5	6	7		
	Pre-supplement		During supplement		Post-supplement			*P* value	S.E.M.
Ejaculate volume (ml)									
Raw data	4.32	4.76	4.4	4.4	4.49	4.06	3.72		
Adjusted means	5.05	4.52	4.40	4.37	4.36	4.23	3.04	0.21	0.48
Sperm concentration ($\times 10^{-6}$/ml)									
Raw data	76	82	67	82	81	93	66		
Adjusted means	54	73	71	85	82	103	80	0.96	23.1
Total sperm count ($\times 10^{-6}$)									
Raw data	415	510	280	305	335	409	267		
Adjusted means	575	596	312	271	281	341	81	0.41	158.3
Sperm motility (%)									
Raw data	53	75	72	76	71	73	76		
Adjusted means	68	70	67	72	68	73	79	0.85	4.43
Sperm morphology (%)									
Raw data	33.9	36.2	35.9	38.8	39.0	36.4	11.9		
Adjusted means	25.2	45.7	37.8	36.5	36.4	36.5	14.2	0.01	4.94
Testicular volume (ml)									
Raw data	32.7	34.2	35.2	39.5	41.5	38.9	40.8		
Adjusted means	33.3	34.4	35.8	39.4	41.6	36.5	39.2	0.10	2.19

potency in *in vitro* systems [27]. In male rats, neonatal exposure to genistein reduced LH secretion and plasma testosterone concentrations in adulthood [25], and genistein exposure in adult mice caused decreases in testicular and serum testosterone concentrations as well as pituitary LH content and prostate weight [9]. However, doses administered to experimental animals may be up to 10-fold higher than the equivalent average human consumption [28] and, in the present study, a daily food supplement containing 40 mg of genistein and daidzein over a 2 month period had no effect on gonadotrophin or sex hormone concentrations or on semen quality. Similarly, flaxseed consumption for 6 weeks did not change plasma testosterone or sex hormone binding globulin concentrations in men [29]. This lack of effect on the endocrine system in men is in contrast to several intervention trials in premenopausal women. A soy supplement suppressed mid-cyle peaks of LH and FSH, increased plasma oestradiol levels and had a stimulatory effect on breast tissue, as assessed by an elevated number of proliferating epithelial cells and appearance of hyperplastic epithelial cells and an increased number of progesterone receptors [16,17,30]. This endocrine disruption was not observed in postmenopausal women [16,31], suggesting that phytoestrogen actions are determined, in part, by the concentrations of endogenous oestrogens.

Despite the numerous reports of phytoestrogen-rich diets causing adverse effects in animals (reviewed by Rickard and Thompson [32]), there are relatively few reports of phytoestrogens having adverse effects in humans. An increased risk of autoimmune thyroid disease was observed in infants consuming soy infant formula, and attributed to inhibition of the thyroid peroxidase system by isoflavones [33–35]. However, there have been no long-term studies in which phytoestrogen effects, either beneficial or adverse, have been rigorously studied. This may be due to the fact that phytoestrogen-rich diets have been the staple in Eastern populations for hundreds of years with no documented toxic effects.

The results of the present study led to the conclusion that the isoflavone dosage (40 mg) in the supplement, which is similar to the amounts consumed in many Eastern nations [36], had no effect on gonadotrophin or sex hormone levels or on semen quality. However, the studies in experimental animals suggest that exposure to phytoestrogens in the developmental period is not advisable [32]. Moreover, the results of our study do not exclude the possibility that supplementation at a higher dose or over a more prolonged period would produce modifications in male reproductive health.

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