Circulating levels of isoflavones and markers of 5α-reductase activity are higher in Japanese compared with New Zealand males: What is the role of circulating steroids in prostate disease?

J.G. Lewis a,*, S. Nakajin b, S. Ohno b, A. Warnock c, C.M. Florkowski a, P.A. Elder a

a Steroid & Immunobiochemistry Laboratory, Canterbury Health Laboratories, P.O. Box 151, Christchurch 8001, New Zealand
b Department of Biochemistry, Hoshi University School of Pharmacy and Pharmaceutical Sciences, Tokyo, Japan
c Waiouru Military Base, Waiouru, New Zealand

Received 17 March 2005; received in revised form 29 April 2005; accepted 10 June 2005
Available online 30 August 2005

Abstract

Epidemiological evidence implicates dietary isoflavone intake as protective against prostate disease. A putative mechanism is attenuated circulating androgen levels in male populations consuming an isoflavone rich diet. We investigated this hypothesis by collecting plasma from 60 Japanese and 60 New Zealand males aged between 21 and 31 years each consuming their traditional diets. We measured plasma testosterone, dihydrotestosterone (DHT), androstenedione, dehydroepiandrosterone sulfate (DHEAS), the combined levels of androsterone sulfate and epiandrosterone sulfate (AoS/epiAoS), sex hormone-binding globulin, and cortisol and corticosteroid-binding globulin as well as the isoflavones genistein and equol. Plasma genistein and equol levels were several times higher in Japanese males as would be expected from an isoflavone rich diet. However, androstenedione, DHEAS, calculated free testosterone and paradoxically markers of 5α-reductase, DHT and AoS/epiAoS were all also significantly higher in Japanese rather than the New Zealand male counterparts. All other comparisons were not significant. Plasma DHT and DHEAS correlated positively with plasma equol and plasma AoS/epiAoS correlated positively with genistein levels. Taken together the results suggest that, rather than reduced levels of steroidogenesis, Japanese males may have increased 5α-reductase activity and possibly altered 17β-hydroxysteroid dehydrogenase activity. Significantly the positive association between isoflavones levels and 5α-steroids is counter-intuitive to isoflavone intake offering prostate protection, unless this is postulated to occur through other mechanisms.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Prostate; Diet; Isoflavones; DHEAS; Androsterone sulfate; 5α-Reductase

1. Introduction

Epidemiological studies indicate a lower incidence of both benign prostatic hyperplasia and prostate cancer in Asian men [1,2]. It has been postulated that this is due to the higher consumption of foods containing isoflavones [3,4], which form a significant part of the Asian diet [5]. Isoflavones are found in different leguminous plants including soy [6] with the most abundant being genistein, glycitein, daidzein and their 7-O-acetylglucosides, 7-O-malonylglucosides, and 7-O-acetylglucosides [7]. They circulate [8] and are excreted at many times the levels found in Europeans consuming their traditional diet [9]. Genistein has been shown to exhibit estrogenic properties [10] and down regulate the expression of estrogen (α and β) and androgen receptors in rats [11] and decrease testosterone levels and inhibit prostate growth but not affect 5α-reductase activity in rats [12]. In mice reduced levels of both circulating testosterone and dihydrotestosterone (DHT) have been observed along with prostate growth inhibition following genistein treatment [13]. It has also been reported to modulate sex hormone-binding globulin expression (SHBG) and act as a potential ligand [14,15]. Genistein has been shown to inhibit 5α-reductase, 17β-hydroxysteroid dehydrogenase and aromatase activity [16–18], in vitro. Reduced 5α-reductase activity has been suggested to have...
a role in the reduced incidence of prostate cancer among Japanese compared to US males where lower plasma levels of 5α-reduced metabolites have been reported [19]. However, there are also conflicting reports of the relationship between soy intake and androgen levels [20,21]. As prostate growth is androgen dependent, via DHT, it is important to investigate the relationship between isoflavones and circulating androgens, particularly testosterone and more importantly its 5α-reduced product DHT. In a limited study we reported a significant increase in plasma DHT levels following short-term isoflavone ingestion [22]. This is counter-intuitive and challenges the concept of isoflavones offering prostate protection by inhibiting 5α-reductase activity thereby lowering DHT and hence its trophic effect on the prostate. Here we extend these studies and compare steroid hormone levels in 60 Japanese and 60 New Zealand males between 21 and 31 years each consuming their traditional diets. We show not only higher plasma genistein levels but paradoxically higher circulating markers of 5α-reductase activity and adrenal androgens in the Japanese population. These findings support our previous conclusion that isoflavones are not important inhibitors of either 5α-reductase or other hydroxysteroid dehydrogenases and suggest that other mechanisms of prostate protection by isoflavones may need to be invoked.

2. Materials and methods

2.1. Subjects

Ethical approval was obtained for this study and blood (5 mL EDTA) was collected between 11:00 and 12:00 h from 60 apparently healthy Japanese males aged 21–31 years and who were determined by interview to be consuming a traditional isoflavone rich Japanese diet. Blood was also collected from 60 apparently healthy New Zealand males at 11:00–12:00 h aged 21–31 years who were determined by interview to be consuming a traditional New Zealand diet, which is generally very low in foods containing isoflavones. Blood sampling was also seasonally adjusted with the Japanese samples collected 6 months prior to the New Zealand series. Plasma was separated and stored at −70°C. The Japanese samples were despatched to New Zealand on dry ice and stored at −70°C until required.

2.2. Hormone assays

Plasma cortisol was measured by a direct enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody and immobilized cortisol in which cortisol in plasma samples or standards competes with immobilized steroid for antibody binding sites [23]. Corticosteroid-binding globulin was measured by ELISA using polyclonal and monoclonal antibodies as described previously [24]. Free cortisol was calculated from the total cortisol and CBG level using the Coolsen’s equation [25]. Plasma testosterone was measured by ELISA following extraction with diethyl ether [26]. Plasma sex hormone-binding globulin was measured by ELISA as described previously [27]. Free testosterone was calculated from the total testosterone and SHBG levels [28]. Androstenedione was measured by “in house” radioimmunoassay following extraction and using 3H-androstenedione and a rabbit polyclonal antibody. Separation of bound from free androstenedione was by dextran-coated charcoal. Plasma dihydrotestosterone (DHT) was measured using the “active” dihydrotestosterone kit (DSL 9600) from Diagnostic Systems Laboratories, Inc, Webster, TX. Plasma dehydroepiandrosterone sulfate (DHEAS) and the combined levels of androstenedione sulfate and epandrosterone sulfate (AoS/epiAoS) were measured directly in diluted plasma samples by parallel competitive ELISAs [29]. Briefly, assays for both DHEAS and AoS/epiAoS used DHEA-thyroglobulin coated microtitre plates and identical DHEAS standards but different monoclonal antibodies on each plate. One antibody (8B11) has high specificity for DHEAS and the other (7G4) recognises DHEAS with exceptional and equal cross-reactivity to AoS and epiAoS. This allows the determination of the combined AoS/epiAoS levels in plasma after correction for the DHEAS contribution. Accuracy of the steroid ELISAs has been previously determined by both recovery experiments and comparison with GC-MS reference sera and found to be between 90 and 110%. Samples were arranged in alternating groups of four in each batch in order to treat the Japanese and New Zealand samples similarly. All samples were assayed in duplicate. Within-assay variation for all assays was <10% over the hormone range of volunteer samples tested and the between assay variation was <13%.

2.3. Isoflavone analysis

Plasma genistein and equol were analyzed using Labmaster time-resolved fluorescence immunosassay kits (Labmaster, Turku, Finland) according to the manufacturers instructions. Briefly plasma (200 µL) was hydrolysed overnight at 37°C by incubation with 200 µL of β-glucuronidase/sulphatase (Sigma G0751; 2 mg/mL) in 0.2 M acetate buffer, pH 5.0. This was followed by two extractions with diethyl ether, each 2.0 mL. Each extract was decanted into a tube following freezing of the aqueous layer in a dry ice/acetone bath. The combined extract was then washed with 1 mL distilled water and the aqueous layer was frozen in a dry ice/acetone bath and the organic layer decanted and evaporated to dryness. The organic extracts were reconstituted and the analysis performed according to the instructions. The plates were read using a BMG Fluostar microplate reader using time-resolved fluorescence parameters and a 3 mm fibre optic cable. Plasma extraction followed by washing the organic extracts served to totally remove any residual EDTA, which is likely to interfere with time-resolved fluoroimmunoassay.
2.4. Statistical analysis

Statistical analysis was carried out using Sigmastat 3.1 software. Comparison between groups was by the unpaired t-test or the Mann–Whitney U-test when data was not adequately normal. Association between isoflavones and steroid analytes were calculated using Spearman’s Rank Correlation coefficient. Steroid and binding protein values were all within the method specific age adjusted reference male ranges. No values were excluded for data analysis.

3. Results and discussion

The mean age ± S.D. for the Japanese and New Zealand male population was 26.6 ± 2.1 and 25.8 ± 2.9 years, respectively. They were not significantly different. Non significant comparisons between Japanese and New Zealand males of plasma steroid hormones and binding protein levels are shown in Table 1. Cortisol, CBG and calculated free cortisol did not differ between Japanese and New Zealand males. Similarly for testosterone and SHBG there were no significant differences although calculated free testosterone reaches significance. Whether a more direct method of free testosterone determination, such as equilibrium dialysis showed the same trend would be of interest, however, the limited supply of plasma prevented this option.

In the absence of body mass indices (BMIs) the similarity of SHBG levels between the Japanese and New Zealand male populations implies that their BMIs are not significantly different as plasma SHBG is a negative and significant correlate of anthropometric measurements [30].

Table 2 shows the comparisons of steroid hormones that were significantly different between Japanese and New Zealand males. Paradoxically dihydrotestosterone is significantly higher in Japanese males and this is reflected by a significantly lower testosterone DHT ratio compared with New Zealand males. The higher levels of DHT in the Japanese population is consistent with our previous study where the short-term ingestion of isoflavone extract from red clover (Trinovin) resulted in an elevation in plasma DHT levels over baseline in normal adult males [22]. Interestingly we also show the combined levels of androsterone and epitandrosterone sulfates are also higher in the Japanese compared to New Zealand males. These sulfates are the major circulating 5α-reduced steroids in circulation [31] and provide additional support that genistein is not an important inhibitor of 5α-reductase in vivo. We have also observed, in a small group of subjects with equivalent mean age, higher plasma levels of AOS/epiAOS in Japanese compared to Finnish males each consuming their traditional diets [Lewis and Adlercreutz, unpublished observations]. This observation also supports the suggestion that genistein does not result in significant 5α-reductase inhibition in vivo. Rather our data could reflect a trend to increased 5α-reductase activity. This could be construed as an adverse outcome in terms of prostate protection. Others have reported lower plasma levels of 5α-reduced metabolites in Japanese compared to US males [32].
but these metabolites are indirect measures of 5α-reductase activity compared with DHT which reflects prostatic 5α-reductase [33] and AoS/epiAoS which reflects systemic 5α-reductase activity [34]. Combining the data showed there was no significant correlation between plasma levels of DHT and AoS/epiAoS. This is in contrast to the situation where plasma DHT and AoS/epiAoS have been shown to correlate in individuals when 5α-reductases are potently inhibited by finasteride [34]. However, we did find a significant and positive correlation of plasma DHT with plasma equol and plasma AoS/epiAoS with plasma genistein levels (Table 3). This could suggest that isoflavones may stimulate rather than inhibit 5α-reductase activity.

Plasma androstenedione was also significantly higher in the Japanese male population compared to the New Zealand male group. This is also reflected by a significantly lower ratio of testosterone:androstenedione ratio in the Japanese group. This could reflect some degree of alteration of 17β-hydroxysteroid dehydrogenase activity, aromatase or 3β-hydroxysteroid dehydrogenase activity by dietary isoflavones in the Japanese population, but testing this hypothesis would required carefully constructed trials. These enzymes have been reported to be inhibited by genistein [35–37], in vitro, at higher concentrations than appears in circulation. Our findings also show the Japanese male population to have higher plasma DHEAS than New Zealand males. This appears to contrast with a reported decline in serum DHEA in prostate cancer patients following 3-months intensive treatment with isoflavones [38] although the doses used were 10-fold higher than found in an isoflavone rich diet.

Plasma genistein levels reported here in Japanese males are similar to previous reports in Japanese consuming their traditional diet [8]. The levels are several fold higher than found in New Zealand males (Table 2). Interestingly we did detect significant levels of genistein in some New Zealand subjects who were all regularly consuming a traditional New Zealand diet. However, with the recent popularity of Asian convenience foods we cannot rule out the possibility that some New Zealand subjects consumed this product(s) prior to blood sampling. It is known that the isolated consumption of product(s) containing soy will rapidly increase plasma genistein levels [39]. Other non-soy foods, which form part of the western diet, may also contain small but significant amounts of genistein [40,41]. We made the assumption that these foods and the isolated ingestion of soy product are less likely to affect steroid levels than sustained consumption of an isoflavone rich diet and for these reasons all data in both groups were included in the analysis. There was no significant correlation of any steroids other than DHT, AoS/epiAoS and DHEAS with isoflavone levels supporting the notion that these isoflavones are not important modulators of steroidogenic activity, other than possibly 5α-reductase.

Our data comparing plasma steroid levels between Japanese and New Zealand males each consuming their traditional diets show the Japanese have higher rather than lower androgen levels. This finding is therefore not consistent with the hypothesis that high isoflavone intake results in reduced levels of circulating androgens, which in turn contribute to an improved degree of prostate protection seen in Japanese males. While isoflavones may be the candidate molecules they could exert their anti-carcinogenic effects by a variety of other mechanisms including estrogenic [10] or anti-estrogenic activity [42]. Genistein has also been shown to inhibit vitamin D hydroxylases, which could increase the responsiveness of prostate cells to the anti-proliferative effects of 1,25-dihydroxyvitamin D [43]. The recent use of microarray analysis has indicated that low concentrations of genistein can modulate differential effects in androgen receptor mediated pathways in the LNCaP prostate cell line [44] and inhibit DHT-induced expression of transcription [45]. More recently it has been reported that equol, a major metabolite of soy based isoflavones, can block the trophic effect of DHT on the rat prostate in vivo by specifically binding circulating DHT, with high affinity, as well as sequestering DHT from the androgen receptor [46]. These findings could provide a novel mechanism for prostate protection despite elevated circulating DHT in the Japanese male population consuming their isoflavone rich diet. Our data showing a positive significant correlation of DHT with equal levels in normal males could offer support to this concept of protection. Not all individuals can metabolize soy isoflavones to form equol and this ability is closely related to a lower incidence of prostate cancer [47]. Whether equol bound DHT compared to free DHT conferred any advantage in a cohort of controls and prostate patients consuming an isoflavone rich diet would be of interest. In our study we also found higher mean levels of plasma equol in Japanese males (Table 1 legend) although this did not reach significance. Analysis of these required non-parametric methods as both equal and genistein were not normally distributed. Interestingly there is a variable frequency of equol producers from dietary isoflavones between different populations [47]. In the current study we have confirmed and extended our previous findings and shown significantly higher plasma 5α-reduced steroids in Japanese males compared with New Zealand males each consuming their traditional diets, which correlate positively with plasma isoflavone levels. The outcome of elevated plasma DHT seems counter-intuitive with the concept of isoflavones offering prostate protection unless this can be postulated to occur through independent mechanisms. This issue however still remains controversial as a very recent study in healthy young men consuming soy protein diets noted a small but significant decline in DHT after 57 days of soy protein diet.

Table 3

<table>
<thead>
<tr>
<th>Compairisons</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHT vs. equal</td>
<td>0.189</td>
<td>0.0396</td>
</tr>
<tr>
<td>AoS/epiAoS vs. genistein</td>
<td>0.190</td>
<td>0.0390</td>
</tr>
<tr>
<td>DHEAS vs. equal</td>
<td>0.227</td>
<td>0.0115</td>
</tr>
</tbody>
</table>

The analytes from Japanese and New Zealand males are analyzed together.
with either a low or high isoflavone content [48]. Interestingly they also found a significant increase in DHEAS following the low but not high isoflavone diet. Whether any 5α-reductase
adrenal steroid sulfates contribute to this rise could merit attention.

Acknowledgement

We thank Associate Professor Chris Frampton for statistical advice.

References


