The effect of isoflavone extract ingestion, as Trinovin, on plasma steroids in normal men

John G. Lewis a,*, Jonathan C. Morris b, Bruce M. Clark b, Peter A. Elder a

a Steroid & Immunobiochemistry Laboratory, Canterbury Health Laboratories, Christchurch, New Zealand
b Department of Chemistry, University of Canterbury, Christchurch, New Zealand

Received 8 May 2000; received in revised form 15 February 2001; accepted 8 February 2001

Abstract

Plasma testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone sulfate, androsterone and epiandrosterone sulfates, cortisol and sex hormone binding globulin were measured in six adult men before and during daily isoflavone extract ingestion (40 mg) in the form of Trinovin tablets. Although modest plasma genistein levels were achieved following three weeks of Trinovin ingestion (106–356 nmol/l) there were no significant changes in most of the analytes tested. However plasma levels of dihydrotestosterone showed an increase that reached significance when combined basal levels were compared to levels following Trinovin treatment. The results suggest that the daily ingestion of isoflavones in the form of Trinovin (1 tablet/day), over a short term, does not alter most plasma steroid levels. We therefore question the value of Trinovin, at the recommended dosage, as offering protective effects against prostate disease by mechanisms involving either significant modulation of plasma steroid or SHBG levels. In contrast the increase in dihydrotestosterone plasma levels could be seen as possibly detrimental. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Isoflavones; Genistein; Biochanin; Daidzein; Formononetin; Steroids; SHBG; 5α-reductase; Dihydrotestosterone; Trinovin

1. Introduction

There is substantial epidemiologic evidence that dietary factors may be a contributing factor to a lower incidence of prostate cancer and benign prostatic hyperplasia seen in oriental compared to western males [1,2]. Isoflavones are a group of implicated dietary components and may be one of the restraining factors inhibiting the promotion and progression of prostatic disease [3,4]. Isoflavones are abundant in different leguminous plants including soy [5] which form a significant part of the Asian diet [6]. They circulate [7] and are excreted at many times the levels found in Europeans consuming their traditional diet [8].

Isoflavones have been shown to exhibit estrogenic properties [9] as well as affect the activity of steroi
dogenic enzymes in vitro. Daidzein and genistein can modulate sex hormone-binding globulin (SHBG) expression [10] and together with biochanin and formononetin can significantly inhibit 5α-reductase and 17β-hydroxysteroid dehydrogenase [11] while other isoflavonoid phytoestrogens have been shown to inhibit aromatase activity [12].

An isoflavone preparation, Trinovin, has been recently introduced which claims to assist the normal function and quality of life in men when the dietary intake of isoflavones is low. Trinovin is a natural isoflavone dietary supplement tablet. Each tablet contains 40 mg isoflavones extracted from red clover and includes genistein, biochanin, daidzein and formononetin. We used the recommended 1 tablet daily dose to investigate plasma steroid levels in normal European men before and during three weeks ingestion. We show that even though plasma genistein levels rise to levels found in oriental males consuming their traditional diet there is no significant change in most plasma steroids tested or SHBG levels. There is however a significant rise in plasma DHT levels when basal levels are compared to the treatment levels. We conclude that if Trinovin offers any prostate protection the mechanism is unlikely to be by modulation of
Table 1
Basal plasma hormone levels (week 1, 0900 h) of the 6 volunteers. All values are nmol/l unless otherwise stated. Method specific normal ranges are shown in parenthesis

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Subject</th>
<th>Cortisol (200–800)</th>
<th>Androstenedione (&lt;6.0)</th>
<th>Testosterone (9.0–38.0)</th>
<th>DHT (pmol/l) (500–2500)</th>
<th>DHEAS (μmol/l) (2.0–12.0)</th>
<th>AoS/epiAoS (μmol/l) (0.5–6.0)</th>
<th>SHBG (9–60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>236</td>
<td>2.1</td>
<td>15.6</td>
<td>734</td>
<td>3.5</td>
<td>1.1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>488</td>
<td>4.9</td>
<td>31.3</td>
<td>1848</td>
<td>11.0</td>
<td>1.0</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>212</td>
<td>2.8</td>
<td>32.3</td>
<td>1800</td>
<td>5.2</td>
<td>3.1</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>272</td>
<td>3.5</td>
<td>23.5</td>
<td>1065</td>
<td>7.4</td>
<td>0.5</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>246</td>
<td>2.0</td>
<td>19.9</td>
<td>1092</td>
<td>7.0</td>
<td>1.5</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>274</td>
<td>4.2</td>
<td>22.5</td>
<td>1499</td>
<td>6.4</td>
<td>0.8</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 shows data for basal plasma hormone levels in the first week of treatment. Each volunteer's hormone levels are listed along with their respective normal ranges. The data is expressed as a percentage of the basal value to allow comparison between individuals as well as between steroids. 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%. Sex hormone-binding globulin was also measured by ELISA as previously described and compares well with the ligand binding method [16]. The SHBG normal range also compares well with others and more recently reported data [17] including that from a large age-related cohort [18]. Analyte levels were expressed as a percentage of the basal value to allow comparison between individuals as well as between steroids. There was no between-assay variation as all samples for a hormone were assayed in the same assay and were all analyzed in duplicate. Within-assay variation for all assays was < 10% over the hormone range of volunteer samples tested. This was previously determined by the mean ± S.D. of 3 quality control samples each analyzed 5 times in duplicate. The 3 quality control samples covered values below the normal range, within the normal range and either the upper limit of the normal range or above the normal range depending on the hormone and covered the range of the volunteer levels in this study.

2. Experimental

2.1. Subjects

Ethical approval was obtained for this study and six consenting normal adult male healthy volunteers (age range 40–53 years) were recruited. The volunteers were all compliant and motivated senior scientific and medical staff. Blood (5 ml) was drawn into EDTA tubes on the same designated study day of the week always at 09:00 h. Weeks 0 and 1 were basal and immediately following blood sampling at week 1, Trinovin (Novogen Laboratories Pty Ltd, North Ryde NSW, Australia) was taken orally by each subject and continued on a daily basis (1 tablet/day) for three weeks until completion of the study. Following the first tablet subsequent daily tablets were taken with breakfast generally between 0730 and 0800 h. Each tablet of Trinovin contains 40 mg of isoflavones extracted from red clover including 40 mg genistein, biochanin, diadzein and formononetin. Further blood samples were drawn at weeks 2 and 4. Plasma was immediately separated and stored in aliquots at −20°C until assayed.

2.2. Hormone assays

Plasma cortisol was measured by a direct ELISA using a monoclonal antibody and immobilized cortisol in which cortisol in plasma samples or standards competes with immobilized steroid for antibody binding sites [13]. Plasma testosterone was similarly measured but following extraction and using a polyclonal testosterone antibody as previously described [14]. 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 dextrancoated 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 androsterone sulfate and epiandrosterone sulfate (AoS/epiAoS) were measured directly in diluted plasma samples by parallel competitive ELISAs as previously described [15]. 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) recognizes 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%. Sex hormone-binding globulin was also measured by ELISA as previously described and compares well with the ligand binding method [16]. The SHBG normal range also compares well with others and more recently reported data [17] including that from a large age-related cohort [18]. Analyte levels were expressed as a percentage of the basal value to allow comparison between individuals as well as between steroids. There was no between-assay variation as all samples for a hormone were assayed in the same assay and were all analyzed in duplicate. Within-assay variation for all assays was < 10% over the hormone range of volunteer samples tested. This was previously determined by the mean ± S.D. of 3 quality control samples each analyzed 5 times in duplicate. The 3 quality control samples covered values below the normal range, within the normal range and either the upper limit of the normal range or above the normal range depending on the hormone and covered the range of the volunteer levels in this study.

2.3. Genistein analysis

Plasma genistein was analyzed in duplicate by HPLC/MS using deuterated genistein [19] as an internal standard. Briefly plasma samples, 1 ml, were spiked with d2,
genistein, 100 pmol, and hydrolyzed overnight at 37°C using β-glucuronidase and sulfatase (363 and 11 units/ml respectively). Following a preliminary hexane extraction to remove lipids plasma was extracted 3 times with 2 ml of diethyl ether and the combined extracts dried and reconstituted in 200 µl methanol for analysis by reverse-phase HPLC/MS [20]. HPLC/MS was carried out using a Micromass LCT mass spectrometer with an electrospray probe coupled to a Waters 2790 liquid chromatograph with a phenomenex C8 column (2 mm i.d. × 150 mm l.). A six-point calibration curve was used from 0 to 600 pmol d$_0$ genistein and 100 pmol d$_2$ genistein. Samples were interpolated on the curve using the d$_0$ [M-1] to d$_2$ [M-1] genistein ratio 269/271. Quality control samples were also run using stripped plasma spiked with either 30 or 300 pmol d$_0$ genistein. Assay variation was always better than 10%.

2.4. Statistical analysis

Statistical analysis was carried out using the paired Student’s t test. The results for each group for a particular analyte were compared against each other and in addition the results for the combined basal groups (weeks 0 and 1) were compared with the combined treatment groups (weeks 2 and 4).

3. Results and discussion

The steroid hormone and SHBG levels for all 6 volunteers at week 1 sampling are shown in Table 1. All values were within the method specific normal ranges. The mean ± S.D. of each analyte level for all 6 volunteers expressed as % of the basal level over the study period is shown in Table 2. The basal level is defined as the mean hormone level of week 0 and week 1 samples, prior to Trinovin ingestion. The percentage of the basal level in sample week 0 and 1 provides a measure of normal variation against which the Trinovin samples can be compared. There are no significant differences between basal plasma levels and Trinovin treatment plasma levels for most of the analytes tested. Apart from DHT the lack of any significant differences between basal levels and levels after one or three weeks of isoflavone extract ingestion, in the form of Trinovin, for any of the steroids tested as well as SHBG is of interest. It suggests that if Trinovin does offer any prostate protection then it is unlikely to involve changes in plasma steroids, apart from DHT, or SHBG. This is in contrast to classic agents such as finasteride that provide prostate protection by inhibiting 5α-reductase [21] as evidenced by dramatic declines in both plasma DHT [22] and androsterone and epitandrosterone sulfates [23].

Even though this study involves a small number of relatively young and healthy volunteers we can conclude that this isoflavone extract, used according to the instructions and at the recommended dose, is not an important inhibitor of 5α-reductase, in vivo. However more importantly the extract could act to increase 5α-reductase activity in vivo as indicated by raised plasma DHT levels following treatment, Table 3. This would seem to be an adverse outcome in terms of offering prostate protection. Others have shown, compared to finasteride, relatively weak inhibition of 5α-reductase activity by isoflavones in vitro [11] and reported lower plasma levels of 5α-reduced metabolites in Japanese compared to US males [24]. It has been suggested that lower 5α-reductase activity may have a role in the reduced incidence of prostate cancer among Japanese men. Whether lower plasma levels of 5α-reduced steroids in Japanese males is the result of a high intake of dietary isoflavones appears unlikely as the isoflavone content of a Trinovin tablet (40 mg) approximates the daily isoflavone intake in the traditional Japanese diet [5,6]. Furthermore plasma genistein levels are initially undetectable and rise following Trinovin (Table 4) to levels similar to those found in subjects consuming soy protein diets [20] and similar to plasma genistein levels found by Adlercreutz et al. [7] in Japanese males consuming their traditional diet. However they are still many fold less than the concentration required to significantly inhibit both 5α-reductase and 17β-hydroxysteroid dehydrogenase, in vitro [11].

The unchanged levels of testosterone and androstenedione during the course of our study also provide evidence that 40 mg Trinovin/day also has little effect on 17β-hydrox-

<table>
<thead>
<tr>
<th>Trinovin</th>
<th>Cortisol</th>
<th>Androstenedione</th>
<th>Testosterone</th>
<th>DHT</th>
<th>DHEAS</th>
<th>AoS/epiAoS</th>
<th>SHBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>–</td>
<td>95.7 ± 13.8</td>
<td>105.3 ± 15.4</td>
<td>100.0 ± 13.2</td>
<td>100.3 ± 17.3</td>
<td>98.2 ± 5.9</td>
<td>105.3 ± 11.6</td>
</tr>
<tr>
<td>Week 1</td>
<td>–</td>
<td>103.6 ± 12.5</td>
<td>94.7 ± 15.4</td>
<td>100.0 ± 13.2</td>
<td>99.7 ± 17.3</td>
<td>101.8 ± 5.9</td>
<td>94.7 ± 34.1</td>
</tr>
<tr>
<td>Week 2</td>
<td>+</td>
<td>103.9 ± 17.0</td>
<td>97.6 ± 21.8</td>
<td>95.5 ± 16.4</td>
<td>110.7 ± 29.7</td>
<td>96.4 ± 8.8</td>
<td>92.7 ± 34.1</td>
</tr>
<tr>
<td>Week 4</td>
<td>+</td>
<td>102.5 ± 7.3</td>
<td>94.8 ± 9.3</td>
<td>100.0 ± 16.6</td>
<td>127.3 ± 25.3</td>
<td>102.0 ± 19.5</td>
<td>106 ± 45.8</td>
</tr>
</tbody>
</table>

Table 2
Mean ± SD of hormone levels over the study expressed as % of basal level. The basal level is the mean of week 0 and week 1 samples.
steroid dehydrogenase activity in vivo. However the possibility still exists that isoflavone exposure over the long term may exert cumulative effects in vivo and a tendency toward an inverse relationship between soy product intake and serum testosterone levels has been reported in Japanese males [24].

Similarly the unaltered SHBG levels during the course of the study provide evidence that SHBG levels are unaffected by Trinovin. It has been shown that the production of SHBG can be increased by exposure of HepG2 cells to high levels of isoflavones in vitro [10] (between 5 and 10 μmol/l) whereas at 0.5 μmol/l there were no significant increases in SHBG levels. Our findings are consistent with this report and appear to contrast with a report of lower plasma SHBG levels in Japanese compared to US males [25].

We have shown that Trinovin ingestion raises plasma genistean to levels similar to those reported in Japanese males consuming soy protein diets. A possible criticism of this study, apart from the small size, is that plasma sampling was carried out a short time after isoflavone ingestion and although moderate genistean levels were achieved at 0900 h they may not be maintained throughout the day. These in turn are likely to depend on variable conversions from Biochanin A. We cannot rule out these possibilities although it has been shown that free genistean levels in plasma reach peak levels 4–6 h after ingestion of 125 g of cooked soya beans [26]. It may be that much higher doses of Trinovin are required to modulate steroid hormone levels. Notwithstanding these constraints we would still question the value of Trinovin, at the recommended dose, as offering protective effects against prostate disease by mechanisms involving either significant modulation of plasma steroids, apart from DHT, or SHBG levels. Other mechanisms of protection against prostate disease by isoflavones in Trinovin at this dosage are still however possible [27]. Rather our data shows that there is a trend toward an increase in plasma DHT levels although there was no correlation between plasma genistean and DHT levels ($r^2 = 0.0875$). The trend toward increased DHT levels could suggest the possibility of an adverse outcome in terms of prostate protection although genistean has recently been shown to exhibit antiandrogenic activity by partially blocking the DHT stimulated production of prostate-specific antigen from the steroid hormone receptor positive BT-474 cell line [28].

### Table 4

<table>
<thead>
<tr>
<th>Week</th>
<th>Trinovin</th>
<th>Plasma genistean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>–</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>210 ± 92</td>
<td>106–356</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>166 ± 50</td>
<td>113–233</td>
</tr>
</tbody>
</table>

References


