EFFECTS OF ISOFLAVONE DIETARY SUPPLEMENTATION ON CARDIOVASCULAR RISK FACTORS IN TYPE 2 DIABETES.

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Short running title: Isoflavones in type 2 diabetes.
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A diet supplemented with soy protein and isoflavones has been shown to reduce cardiovascular risk factors in postmenopausal women with type 2 diabetes. However, it remains unclear which component is responsible for these effects. Our aim was to determine whether the addition of isoflavones alone modify cardiovascular risk markers in this group of patients.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in women in developed countries(1) and women with diabetes are four times more likely to die from CVD than men(2). Amongst others, postmenopausal oestrogen depletion, greater insulin resistance and dyslipidemia(3,4) may contribute to their high risk of accelerated CVD.

Modification of lifestyle is important to reduce CVD risk factors and delay progression of type 2 diabetes associated complications. In particular, the addition of oral supplements, such as soy products as part of a healthy diet, have attracted recent interest because their beneficial effects on the lipid profiles(5-10). However, scant information is available on the effects of soy in individuals with type 2 diabetes(11-13), who are at higher risk due to hyperlipidemia, lower HDL level and abnormalities in LDL/lipoprotein composition(14). It also remains unclear whether a beneficial effect can be attributed to the soy protein or isoflavones.

We have shown that soy protein combined with isoflavones can improve glycemic control, insulin resistance and lipids in patients with type 2 diabetes(11). Therefore, our aim was to determine if this effect was due to the isoflavone component alone.

RESEARCH DESIGN AND METHODS

Study design

This was a randomized, double blind, placebo controlled, cross over study with a four week wash out period separating the placebo and active phases (12 weeks each). Subjects provided informed consent. Randomization was performed using a random number generator.

Subjects

Thirty two Caucasian postmenopausal women with diet controlled type 2 diabetes (according to WHO criteria(15)) and amenorrhoea (more than one year) were recruited. Exclusion criteria were: breast/uterine cancer; uncontrolled hypothyroidism, treatment with oral hypoglycaemic agents, insulin, oestrogens or statins initiated less than 4 months before the trial.

Baseline characteristics are included in table1. Six subjects withdrew from the study: subject 1(S1) required a cholecystectomy, S2 a coronary angioplasty, S3 had an acute attack of polymyalgia rheumatica requiring steroids, S4-6 were unable to comply with study requirements.

Intervention

The soy preparation (Essential Nutrition, U.K) contained 132 mg of isoflavones (genistein 53%, daidzein 37% and glycitein 10%). It was devoid of soluble fiber. The placebo was an identical tablet of microcrystalline cellulose. Compliance was monitored by counting returned medication.

Study measurements

Venous blood samples were collected at each visit after a 12 hour overnight fast. HbA1c, glucose and lipid levels were measured using standard methods. LDL cholesterol was calculated using the...
Friedewald equation and insulin resistance using the HOMA-IR method(16).

**Statistical analysis**
Mean percentage changes obtained at the end of isoflavone treatment were compared to those at the end of the placebo phase using the paired Student’s t test if they followed a Gaussian distribution or the Wilcoxon’s signed-rank test if those changes violated the assumption of normality when tested using the Kolmogorov-Smirnov test (CRP and HOMA-IR data). The period effect (calculated by comparing the mean difference between placebo and isoflavone treatment in the group starting on placebo with the group starting on isoflavones) and the carry-over effect (comparing baseline values for each treatment group) were tested using the Student’s t-test. The results were considered statistically significant if the two-tailed P value was <0.05. Statistical analysis was performed using SPSS (version 15). P values are included in table 1.

**RESULTS**
A total of 26 patients completed the study. No period or carryover effects were detected. Both study preparations were well tolerated with >90% compliance.

**Effects on glycemic control/other effects**
There were no significant differences detected in glucose, HbA1c, HOMA-IR, total cholesterol, triglycerides, HDL, LDL levels between isoflavone and placebo phases. Likewise, no significant differences were seen in BMI, blood pressure or CRP between treatment and placebo phases.

**DISCUSSION**
This study showed that soy isoflavones alone do not confer significant cardiovascular protection or positive effects on glycemic control in this group of patients. This is in accord with studies with red clover isoflavones in diabetes that observed no change of plasma lipoproteins or glycated haemoglobin, although basal endothelial function(17) and arterial compliance(18) did alter. However, it is possible that effects mediated by the isoflavones are too modest to be detected over the 3 month study period.

Epidemiological studies(19,20) have indicated that there is no significant association between the standard western dietary intake of isoflavones (0.369 – 0.770 mg/day) and the reduction of cardiovascular events or lipid levels in different cohorts of postmenopausal women over prolonged periods of time(4-6 years). Our subjects received more than 150 times this amount, yet no significant changes were observed over a 3 month period. The dose of isoflavones given was the same we used with 30g soy protein which reduced these cardiovascular and glycemic parameters within the same time frame(11). In addition, supplementation with isoflavones alone (40-150mg/day) in subjects without diabetes showed that there was no change in lipid profile in peri/postmenopausal women, both healthy and mildly hypercholesterolemic (18,21,22).

Conversely, isolated soy protein has been shown to reduce total cholesterol (9.3%), LDL cholesterol(12.9%) and triglycerides (10.5%)(23). The FDA recommends that 25g soy protein/day may reduce CVD(24) since beneficial effects have also been observed with different combinations of soy protein and isoflavones in healthy or mildly hypercholesterolaemic postmenopausal/perimenopausal women (9,10,25) and in men/postmenopausal women with type 2 diabetes(11,12).

In conclusion, isoflavones alone did not alter CVD markers over a 3 month period. This suggests that either the soy protein component alone or a synergistic effect between the protein with the isoflavones may be responsible for any CVD changes.
References


Table 1. Subjects characteristics and effects on cardiovascular risk at the start of the trial and after three months of treatment (mean ± SD),% change. % change in each group were compared to obtain the p value for the between treatment difference.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Isoflavones</th>
<th>Between-treatment difference (p value)</th>
<th>Period Effect (p value)</th>
<th>Cross Over Effect (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>31 ± 6.4</td>
<td>30.7 ± 5.5</td>
<td>0.01 (-0.55 to 0.59)</td>
<td>p=0.97</td>
<td>p=0.1</td>
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<td><strong>Three months</strong></td>
<td>30.7 ± 5.5</td>
<td>30.7 ± 5.5</td>
<td>0.02 (-0.69 to 0.75)</td>
<td>p=0.35</td>
<td>p=0.5</td>
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<tr>
<td><strong>% change</strong></td>
<td></td>
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<tr>
<td><strong>BMI (Kg/m²)</strong></td>
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<td></td>
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<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>133 ± 15</td>
<td>137 ± 16</td>
<td>4.26 (-1.28 to 9.8)</td>
<td>p=0.35</td>
<td>p=0.5</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>75 ± 10</td>
<td>76 ± 8</td>
<td>2.79 (-2.99 to 8.57)</td>
<td>p=0.38</td>
<td>p=0.5</td>
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<tr>
<td><strong>HbA1c (%)</strong></td>
<td>6.7 ± 0.6</td>
<td>6.8 ± 0.7</td>
<td>1.00 (-0.20 to 2.2)</td>
<td>p=0.58</td>
<td>p=0.5</td>
</tr>
<tr>
<td><strong>Gluc (mmol/L)</strong></td>
<td>7.0 ± 1.4</td>
<td>6.9 ± 1.3</td>
<td>-0.34 (-3.6 to 2.9)</td>
<td>p=0.59</td>
<td>p=0.1</td>
</tr>
<tr>
<td><strong>Insul. (µU/ml)</strong></td>
<td>14.1±10</td>
<td>13 ± 6.9</td>
<td>-0.39 (-12 to 11.2)</td>
<td>p=0.15</td>
<td>p=0.4</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>4.6±4.5</td>
<td>4.5 ± 2.5</td>
<td>-4.49 (-15.58 to 6.6)</td>
<td>p=0.24</td>
<td>p=0.5</td>
</tr>
<tr>
<td><strong>Chol (mmol/L)</strong></td>
<td>5.4 ± 1</td>
<td>5.4 ± 0.9</td>
<td>2.14 (-7.54 to 3.25)</td>
<td>p=0.96</td>
<td>p=0.3</td>
</tr>
<tr>
<td><strong>LDL (mmol/L)</strong></td>
<td>3.4 ± 0.9</td>
<td>3.4 ± 0.8</td>
<td>3.6 (-10.5 to 3.1)</td>
<td>p=0.97</td>
<td>p=0.2</td>
</tr>
<tr>
<td><strong>HDL (mmol/L)</strong></td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>0.07 (-3.69 to 4.4)</td>
<td>p=0.93</td>
<td>p=0.8</td>
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<td><strong>TG (mmol/L)</strong></td>
<td>1.8 ± 0.8</td>
<td>1.7 ± 0.7</td>
<td>-1.16 (-16.2 to -7.9)</td>
<td>p=0.74</td>
<td>p=0.8</td>
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<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>5.1 ± 6.7</td>
<td>6.4 ± 10.1</td>
<td>24.4 (-46.5 to 2.21)</td>
<td>p=0.40</td>
<td>p=0.3</td>
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</tbody>
</table>

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Gluc, plasma glucose; Insul., Insulin; HOMA-IR, HOMA-Insulin resistance; Chol, total cholesterol; TG, triglycerides; LDL, low density lipoproteins; HDL, high density lipoproteins; CRP, C-reactive protein; SD: standard deviation, CI, confidence interval