Isoflavonoids are a class of flavonoids that are derived in the human diet mainly from soybean-based foods. The major dietary isoflavonoids, genistein and daidzein, have estrogen-like activity and are classed as phytoestrogens. Because estrogens can lower serum LDL cholesterol and raise HDL cholesterol, the objective of this study was to determine if isoflavonoids could improve serum lipids in healthy subjects. Forty-six men and 13 postmenopausal women not receiving hormone replacement therapy completed a randomized, double-blind, placebo-controlled trial of two-way parallel design and 8 wk duration. One tablet containing 55 mg of isoavonoids (predominantly in the form of genistein) or one placebo tablet was taken daily with the evening meal. Subjects maintained their usual diet and physical activity, which were unchanged throughout the intervention. Measurement of isoavonoids and their metabolites in 24-h urine samples provided an assessment of compliance and of isoavonoid metabolism. Serum total, LDL, HDL and HDL subclass cholesterol, triglycerides and lipoprotein (a) were assessed at baseline and during the last week of intervention. After adjustment for baseline values, no significant differences in postintervention serum lipid and lipoprotein (a) concentrations between groups were identified. Further adjustment for age, gender and weight change did not alter the results. In addition, changes in urinary isoavonoids were not significantly correlated with changes in serum lipids and lipoprotein (a). Therefore, this study does not support the hypothesis that isoavonoid phytoestrogens can improve the serum lipids, at least in subjects with average serum cholesterol concentrations. J. Nutr. 128: 728–732, 1998.
vonoid supplementation on serum lipids in humans therefore remain unclear.

In this study, healthy men and postmenopausal women with serum lipid concentrations approximating the mean of the general population were recruited. The objective was to determine if dietary supplementation with purified isoflavonoids, at an intake level achievable by dietary means, could improve serum lipids in this group.

**SUBJECTS AND METHODS**

**Subjects.** Fifty-nine healthy subjects, 46 men and 13 postmenopausal women not receiving hormone replacement therapy, aged between 35 and 69 y, were recruited from the general population. There were 22 men and 7 women in the placebo group (mean age 57.0 y), and 24 men and 6 women in the isoflavonoid group (mean age 54.3 y). Subjects excluded from the study were current smokers or ex-smokers who had stopped smoking for less than 6 mo; those whose usual alcohol intake exceeded four standard drinks per day (>40 g alcohol/d); diabetics on medication; those with any history of heart, liver or renal disease; those with systolic blood pressure >125 mm Hg; those taking medication for dyslipidemia or hypertension or any medication that might influence serum lipids concentrations or blood pressure; and vegetarians or individuals whose usual diet included more than one soy-containing meal per week. The project was approved by the Royal Perth Hospital Ethics Committee, and all participants gave informed written consent.

**Experimental design.** The study was a randomized, double-blind, placebo-controlled trial of two-way parallel design and 8 wk duration. Baseline assessments were performed in the last 2 wk of a 4-wk run-in phase; randomization performed by a third party observer occurred at the end of run-in phase. Subjects were assigned to take either one tablet containing 55 mg of isoflavonoids or one placebo tablet daily with their evening meal, throughout the intervention. The isoflavonoid tablets provided by Novogen (North Ryde, Sydney, Australia) were derived from subterranean clover (Trifolium subterraneum) and contained the following (per tablet in mg): 16 biochanin A, 30 genistein, 8 formononetin and 1 daidzein. Compliance was assessed by tablet counts at 2-wk intervals and by measurement of isoflavonoids in 24-h urine samples at baseline and postintervention.

**Food intake and body weight assessment.** Usual food intake, alcohol consumption and physical activity were assessed by questionnaire at baseline and monitored by a dietician throughout the intervention to ensure minimal changes of these potential confounders throughout the study. Food intake was assessed by 24-h diet records completed on two week days and one day of the weekend. Instruction was given on the use of the Salter digital food scales (Tonbridge, England) and the recording of all foods and beverages consumed over a 24-h period on a standardized food record form to ensure accuracy of the food records. Food intake data were analysed using Xyris, a nutrient analysis program based on NUTTAB 1993 food composition tables (Xyris, Brisbane, Australia). Body weight was measured at baseline, at the end of intervention and at 2-wk intervals during the supplementation period.

**Biochemical analyses.** During the last week of baseline and the final week of intervention, blood was taken from each subject after an overnight fast. Serum lipids and other biochemical measurements were performed in the Department of Clinical Biochemistry at Royal Perth Hospital. Total cholesterol and triglycerides were analyzed enzymatically on a Roche Diagnostics Cobas Mira analyzer (Basel, Switzerland) with the use of reagents from Trace Scientific (Perth, Australia). HDL cholesterol was analyzed on a heparin-manganese chloride supernatant, HDL3 cholesterol was measured by a single precipitation procedure (Gidez et al. 1982) and HDL2 cholesterol was calculated by difference (total HDL cholesterol − HDL3 cholesterol). The Friedewald formula (Friedewald et al. 1972), converted to SI units, was used to calculate LDL cholesterol. Lipoprotein (a) was measured in serum by using a latex-enhanced nephelometric method. Measurements were performed on the Behring Nephelometer (Behring Werke, Marburg, Germany) by using the Behring N-Latex Lp(a) method (Behring Diagnostics, Sydney, Australia). DAKO rabbit anti-human antibodies (DAKO Corporation, Carpitera, CA) were used instead of Behring antibodies.

A 24-h urine collection was performed at baseline and during the final week of intervention. Urinary isoflavonoids, including biochanin A, genistein, formononetin, daidzein, and the daidzein metabolites equol and O-desmethylangolensin (O-DMA) were measured by using HPLC according to the method of Franke et al. (1995).

**Statistics.** Statistical analyses were performed using SPSS software (Chicago IL). The independent-samples t-test was used to compare means of baseline values. Levene’s test for equality of variances was used to compare means of baseline values. Results are presented as means ± SEM. Pearson’s correlation coefficient (r) was used to determine the degree and direction of association between two variables. ANOVA was used to compare postintervention differences between active and placebo groups after adjustment for baseline values. Adjustment for co-variates such as age, gender and weight change was also performed with the use of this technique.

**RESULTS**

**Weight and dietary intake.** There was no significant difference between placebo and isoflavonoid groups in mean weight at baseline (84.1 ± 2.2 vs. 82.9 ± 2.5 kg, respectively), and no significant difference between groups in postintervention weight after adjustment for baseline values. In addition, there

| Table 1
| Baseline and postintervention daily energy and macronutrient intakes for subjects in the placebo and isoflavonoid groups |
|-------------------|-------------------|
| Characteristic    | Placebo           | Isoflavonoid      |
|                   | Baseline          | Post              | Baseline          | Post              |
| Energy, kJ        | 9640 ± 433        | 9366 ± 523        | 9405 ± 473        | 9278 ± 504        |
| Protein, g        | 102 ± 4           | 99 ± 5            | 98 ± 5            | 95 ± 5            |
| Total fat, g      | 80 ± 4            | 76 ± 6            | 79 ± 5            | 85 ± 6            |
| Saturated, g      | 32 ± 2            | 31 ± 3            | 31 ± 3            | 33 ± 3            |
| Monounsaturated, g| 29 ± 2            | 27 ± 2            | 29 ± 2            | 32 ± 2            |
| Polyunsaturated, g| 13 ± 1            | 12 ± 1            | 13 ± 1            | 13 ± 1            |
| Cholesterol, mg   | 342 ± 37          | 284 ± 24          | 274 ± 25          | 289 ± 28          |
| Carbohydrate, g   | 267 ± 14          | 259 ± 15          | 264 ± 15          | 253 ± 14          |
| Sugar, g          | 117 ± 6           | 114 ± 8           | 112 ± 9           | 104 ± 9           |
| Starch, g         | 147 ± 10          | 143 ± 9           | 148 ± 9           | 146 ± 9           |
| Fiber, g          | 28 ± 2            | 30 ± 2            | 28 ± 2            | 26 ± 2            |
| Alcohol, g        | 17 ± 4            | 19 ± 5            | 14 ± 4            | 10 ± 3            |

1 Values are means ± SEM. For placebo group, n = 29; for isoflavonoid group, n = 30.
were no differences in total energy or macronutrient intakes between placebo and isoflavonoid groups at baseline and no differences between groups in the postintervention daily energy and nutrient intake after adjustment for baseline values. The baseline and postintervention energy and macronutrient intakes for the two groups are presented in Table 1.

### Isoflavonoids

Unadjusted baseline and postintervention 24-h excretion of isoflavonoids and metabolites is presented in Table 2. After adjustment for baseline values, there were significantly greater urinary concentrations of biochanin A (P < 0.05), genistein (P < 0.0001), formononetin (P < 0.05), daidzein (P < 0.0001) and O-DMA (P < 0.01) in the isoflavonoid group compared with the placebo group. The magnitude of the difference was larger for genistein and daidzein than for other compounds.

### Lipids and lipoproteins

The unadjusted baseline and postintervention lipid and Lp(a) concentrations are presented in Table 3. After adjustment for baseline values, there were no significant differences between placebo and isoflavonoid groups in postintervention lipid and Lp(a) concentrations. Further adjustment for age, weight change and change in nutrient intakes did not alter the outcome. Analyses of baseline adjusted postintervention differences in serum lipids and Lp(a) performed in subjects with baseline serum total cholesterol concentrations above the 50th percentile (5.3 mmol/L) also showed no differences between groups. Correlations between changes in urinary isoflavonoids or metabolites and serum lipid and Lp(a) concentrations within the isoflavonoid group did not show any significant associations.

### DISCUSSION

Dietary supplementation with 55 mg/d of isoflavonoids did not significantly alter serum lipid and Lp(a) concentrations. An estrogen-like effect of isoflavonoids was the primary proposed mechanism for lipid lowering. Therefore subjects with low circulating estrogen concentrations, men and postmenopausal women not receiving hormone replacement therapy, were recruited.

An isoflavonoid intake of 55 mg/d was proposed to have effects on serum lipid concentrations similar to those of estrogen. Concentrations of estrogen that have been shown to improve serum lipids have been equivalent to about 1.5–2 mg/d of estradiol (Tikkanen 1996). Genistein and daidzein have estrogenic activity (Setchell and Adlercreutz 1988), which may be of the order of 1000–10,000 times less than that of estradiol (Messina et al. 1994). However, in populations with high intakes of isoflavonoids, such as the Japanese, plasma concentrations of isoflavonoid phytoestrogens (Adlercreutz et al. 1993) may be around 1000–10,000 times that of circulating concentrations of estradiol 17-β (Genuith 1986) in men and postmenopausal women.

An isoflavonoid intake of 55 mg/d is also representative of an upper level of intake achievable by dietary means. The Japanese population, who consume large amounts of soy products, are estimated to have a mean isoflavonoid intake of ~30 mg/d (Messina 1995), including about 20 mg/d genistein. A greater part of the isoflavonoid content of the active supplement (46 mg) was genistein and biochanin A (which is converted directly to genistein). Our conclusions must therefore be limited to possible effects of genistein.

When ingested by humans, isoflavonoids undergo acidic and enzymatic hydrolysis and demethylation to yield the aglycones, genistein and daidzein. Biochanin A is converted to genistein and formononetin is converted to daidzein. Genistein and daidzein may then be further metabolized by gut flora (Setchell and Adlercreutz 1988). The metabolic products of genistein metabolism in humans have not been clearly demonstrated. The two main products of daidzein metabolism appear to be equol and O-DMA (Kelly et al. 1993). The isoflavonoids and their metabolites are unlikely to be further metabolized once they have been absorbed. A more detailed discussion of isoflavonoid metabolism is given by Joannou et al. (1995) and Kurzer and Xu (1997).

Greater urinary excretions of genistein and daidzein were observed in the isoflavonoid group: 1.22 and 2.53 mg/d, respectively. However, these increases were considerably less than the increase in the intake of biochanin A plus genistein (46 mg/d) and formononetin plus daidzein (9 mg/d). The subjects taking the active tablet therefore excreted 11% of the daily increase in genistein plus biochanin A, and 28% of the daily increase in daidzein plus formononetin. These results are similar to those of Xu et al. (1994) who found that the bioavailability for genistein was 9%, and for daidzein was 21%.
There was considerable variability in urinary isoflavonoid concentrations in the isoflavonoid group. This result suggests that there was variability in isoflavonoid metabolism and absorption between individuals, which is consistent with previous findings (Morton et al. 1994). However, we found no significant correlations between changes in urinary isoflavonoids and serum lipids and Lp(a) concentrations in the isoflavonoid group.

Although we found no effect of isoflavonoids on serum lipids in a healthy population, the results do not eliminate the possibility of a hypolipidemic effect of isoflavonoids in hypercholesterolemic subjects. Studies that have shown decreases in serum LDL cholesterol with soy protein in humans have generally involved hypercholesterolemic subjects (Descovich et al. 1980, Gaddi et al. 1987 and 1991, Verrillo et al. 1985). In a meta-analysis of human trials with a soy protein intervention focusing on lipid effects, the factor most strongly associated with decreases in LDL cholesterol concentrations was the initial cholesterol concentration of the study population (Anderson et al. 1995). In our study, analyses of postintervention lipid differences in those subjects with higher \( (>5.3 \text{ mmol/L}) \) baseline serum total cholesterol concentrations did not demonstrate any significant differences between groups. However, it remains possible that if isoflavonoids have a cholesterol-lowering effect, little or no activity may be seen in normocholesterolemic subjects.

In conclusion, supplementation with \( \sim 55 \text{ mg of isoflavonoids per day does not significantly alter serum lipid or Lp(a) concentrations in healthy middle-aged subjects with serum lipid concentrations approximating the mean of the general population. Because a large percentage of the supplement used was in the form of genistein, the results indicate that at least this isoflavonoid does not influence serum lipids and Lp(a) significantly. These results do not support the hypothesis that isoflavonoid phytoestrogens lower LDL cholesterol and Lp(a) and raise HDL cholesterol in subjects with average serum lipid concentrations.\

ACKNOWLEDGMENTS

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LITERATURE CITED


| Table 3 |

Effects of an 8-wk intervention, involving dietary supplementation with isoflavonoids (55 mg/d) or placebo, on lipid and lipoprotein (a) concentrations | Placebo | Isoflavonoid |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol/L</td>
<td>Baseline</td>
<td>Post</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.07 ± 0.15</td>
<td>5.07 ± 0.15</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.24 ± 0.12</td>
<td>3.34 ± 0.13</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.21 ± 0.05</td>
<td>1.20 ± 0.05</td>
</tr>
<tr>
<td>HDL2 Cholesterol</td>
<td>0.53 ± 0.04</td>
<td>0.46 ± 0.03</td>
</tr>
<tr>
<td>HDL3 Cholesterol</td>
<td>0.68 ± 0.03</td>
<td>0.74 ± 0.03</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.26 ± 0.11</td>
<td>1.18 ± 0.10</td>
</tr>
<tr>
<td>Lp(a) mg/L</td>
<td>163 ± 46</td>
<td>163 ± 44</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. For placebo group, \( n = 28 \); for isoflavonoid group, \( n = 30 \).
2 Baseline and postintervention values are shown for subjects in the placebo and isoflavonoid groups.
3 \( P < 0.05 \) for difference between placebo and isoflavonoid groups in baseline HDL cholesterol concentrations.
4 \( P < 0.05 \) for difference between placebo and isoflavonoid groups in baseline triglyceride concentrations.
5 Lp(a) (mg/L).


