Equol status and blood lipid profile in hyperlipidemia after consumption of diets containing soy foods¹–⁴

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ABSTRACT

Background: Recent analyses have challenged the effectiveness of soy foods as part of a cardiovascular risk reduction diet.

Objective: The objective of the study was to show whether equol status determines the effectiveness of soy foods to lower LDL cholesterol and to raise HDL cholesterol.

Design: Eighty-five hypercholesterolemic men and postmenopausal women (42 men, 43 women) participated in 1 of 3 studies that represented a range of soy interventions and that followed the same general protocol at a Canadian university hospital research center. Soy foods were provided for 1 mo at doses of 30–52 g/d for the 3 studies as follows: 1) soy foods with either high-normal (73 mg/d) or low (10 mg/d) isoflavones, 2) soy foods with or without a prebiotic to enhance colonic fermentation (10 g polyfructans/d), or 3) soy foods with a low-carbohydrate diet (26% carbohydrate). Studies 1 and 2 were randomized controlled crossover trials, and study 3 was a parallel study.

Results: The separation of the group into equol producers (n = 30) and nonproducers (n = 55) showed similar reductions from baseline in LDL cholesterol (~9.3 ± 2.5% and ~11.1 ± 1.6%, respectively; P = 0.834), with preservation of HDL cholesterol and apolipoprotein A-I only in equol producers compared with reductions in nonproducers (HDL cholesterol: +0.9 ± 2.7% compared with ~4.3 ± 1.1%, P = 0.006; apolipoprotein A-I: −1.0 ± 1.1% compared with −4.7 ± 1.0%; P = 0.011). The amount of urinary equol excreted did not relate to the changes in blood lipids.

Conclusions: Soy foods reduced serum LDL cholesterol equally in both equol producers and nonproducers. However, in equol producers, ~35% of our study population, soy consumption had the added cardiovascular benefit of maintaining higher HDL-cholesterol concentrations than those seen in equol nonproducers. This trial was registered at clinicaltrials.gov as NCT00877825 (study 1), NCT00516594 (study 2), and NCT00256516 (study 3). Am J Clin Nutr 2012;95:564–71.

INTRODUCTION

Soy protein isoflavone content has been implicated as the reason for the cholesterol-lowering property of soy foods. However, many studies have failed to confirm an effect of soy isoflavones when isolated from soy proteins (1), and concern has been expressed that it may be only in those who convert the isoflavone daidzein to equol in whom the effect is seen (2). Thus, a study with 69% of participants as equol producers as opposed to 30% (which is the more usual percentage) (3), which provided soy germ pasta naturally enriched with isoflavones, with little soy protein, resulted in a relatively large reduction in LDL cholesterol of 8.6%; the improvement in LDL cholesterol was more pronounced in equol producers than in nonproducers. Although the isoflavone composition (aglycones to glycosides) in this study was different from that found in soy foods, these findings have rekindled interest in the concept that isoflavones (ie, the phytoestrogens in soy), especially equol produced from the colonic microbial biotransformation of the soy isoflavone daidzein, might be one of the factors responsible for the reduction in LDL cholesterol in subjects consuming soy foods.

Earlier studies had suggested that equol producers tended to eat higher carbohydrate diets (4, 5) and that addition of carbohydrate to fecal cultures in the presence of daidzein, the equol precursor, resulted in greatly increased production of equol (6, 7). Data such as these suggest the possibility that equol production

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may not be a fixed characteristic of an individual but may be subject to dietary manipulations which increase colonic microbial fermentation and hence biotransformation of daidzein to equol. The importance of equol is that it is the most estrogenic of the isoflavones and might act as an SERM\(^5\), such as tamoxifen. In turn, SERMs such as tamoxifen and, by extension, equol, have been shown to result in lower serum cholesterol concentrations (8).

We therefore assessed the equol status of subjects who had taken part in our 3 most recently published studies (9–11) in relation to the effects of soy foods on LDL cholesterol to determine whether equol status was a determinant of LDL-cholesterol reduction in this population. The 3 studies represented the effects of soy given as part of a range of cholesterol-lowering diets.

**SUBJECTS AND METHODS**

**Participants**

Men and postmenopausal women with hyperlipidemia were recruited from patients attending the Risk Factor Modification Centre, St Michael’s Hospital (Toronto, Canada) and from newspaper advertisements. A total of 85 subjects [42 men, 43 women; mean ± SD BMI (in kg/m\(^2\)): 26.9 ± 3.7; age: 59.9 ± 8.9 y] completed one of three 1-mo soy studies in which 15–26 g/1000 kcal soy was consumed. Forty-one subjects (23 men, 18 women) participated in study 1 (high- and low-soy isoflavone study), 22 subjects (10 men, 12 women) participated in study 2 (prebiotic study), and 22 subjects (9 men, 13 women) participated in study 3 (Eco-Atkins study). In study 2, 23 subjects completed the study; one subject chose not to complete the urine collection for determination of equol status. The eligibility criteria for study 1 and 2 were identical, except for the exclusion of those with a BMI >38 in study 1 and >32 in study 2. The minimum LDL-cholesterol cutoff at recruitment was >4.1 mmol/L in studies 1 and 2. However, in study 3, the LDL-cholesterol criterion was >3.4 mmol/L. Therefore, all participants had mild to moderately elevated LDL-cholesterol concentrations at recruitment. Overweight participants were specifically recruited for study 3 for the weight-loss component of the intervention; as a result, the baseline BMI in study 3 was higher than that in studies 1 and 2. Otherwise, there were no differences in age, sex, and baseline LDL cholesterol between the 3 studies at baseline. None of the participants had a history of cardiovascular disease, untreated hypertension (blood pressure >140/90 mm Hg), diabetes, or renal or liver disease; and none were taking medications known to influence serum lipid concentrations, apart from 3 participants who were receiving stable doses of statins throughout the study (Table 1) (9–11). A detailed description of other medications at baseline and changes during the respective substudies has been previously described in greater detail (9–11).

The ethics committees of the University of Toronto and St Michael’s Hospital and the Therapeutic Products Directorate of Health Canada (only for study 3) approved the 3 substudies. The study clinical trial registration numbers (at clinicaltrials.gov) are NCT00877825 (study 1), NCT00516594 (study 2), and NCT00256516 (study 3). Written informed consent was obtained from all participants.

**Study protocol**

All participants completed 1 of the 3 soy studies. Dietary periods within each study were 4 wk in duration in which 30–52 g soy protein/d containing 10–73 mg isoflavones/d (expressed as aglycone equivalents) were provided as soy foods (eg, in the form of beverage, tofu, deli slices, burgers, hot dogs). Treatments were separated by a minimum 2-wk washout period. Both studies 1 and 2 were randomized controlled crossover designs, and study 3 was a parallel study. Details of the study design and measurements are reported elsewhere (9–11). At each visit, fasting body weights, waist circumference, and blood pressure were measured. Blood samples were obtained after 12-h overnight fasts at 2-wk intervals. Twenty-four-hour urine collections were obtained at the end of each treatment in the 3 studies.

**Diets**

The 4-wk study diets were weight-maintaining in studies 1 and 2; study 3 was a weight-reducing diet, with foods provided at 60%...
of estimated caloric requirement. In studies 1 and 2, subjects consumed a self-selected background diet that conformed to a National Cholesterol Education Program Adult Treatment Panel III diet (<7% of energy from saturated fat and <200 mg dietary cholesterol/d) and were provided with soy foods (eg, in the form of milk, tofu, deli slices, burgers, hot dogs). In study 3, food was provided in the metabolically controlled diets. The weight-reducing test diet (Eco-Atkins) included soy foods similar to those in studies 1 and 2 but was low in carbohydrate (26% of energy) and high in protein (31%) and fat (43%) and was also high in gluten, nuts, and monounsaturated fat (11). Mean soy protein intakes for the 3 diets ranged from 30 to 52 g/d (study 1, 50 or 52 g/d; study 2, 30 g/d; study 3, 35 g/d). Studies 1 and 2 included 2 soy phases. In study 1, the soy isoflavone content was either high-normal (73 mg total isoflavones/d) or low (10 mg total isoflavones/d). The high and low isoflavone soy foods were made from either alcohol- or nonalcohol-washed soy protein isolate or from tofu that was made from soybeans selected for their very high or very low isoflavone content (9). In study 2, either a dose of 10 g prebiotic polyfructan/d (Synergy 1; Orafti Group) or a control sugar (maltodextrin) was given with the soy (60.5 mg total isoflavones/d). All subjects who completed the soy containing treatments were included in the current analysis.

Compliance with the soy-containing treatments for each of the 3 studies was good. In study 1, intake of the prescribed soy foods was 97% for the low-isoflavone soy phase and 98% for the high-isoflavone soy phase (9). In study 2, compliance for the soy protein foods was 94% and 95% for the soy foods with a prebiotic and soy foods without, respectively (10). In study 3, compliance as a percentage of total calories prescribed was 95% (11).

Analyses

Serum was analyzed according to the Lipid Research Clinics protocol (12) for total cholesterol, triglycerides, and HDL cholesterol, after dextran sulfate-magnesium chloride precipitation (Bayer Technicon RA1000; Bayer Health Care) (13) or by detergent solubilization and measurement of HDL cholesterol (Roche Hitachi 917; Roche Diagnostics) in the J Alick Little Lipid Research Laboratory. LDL cholesterol was calculated by using the method of Friedewald et al (14) in mmol/L [LDL cholesterol = total cholesterol – (triglycerides/2.2 + HDL cholesterol)].

Urinary metabolites of isoflavones (genistein, daidzein, glycitein, equol, and ODMA) were measured by means of a previously established method, with modification in the trimethylsilylated derivatization procedure for isoflavones, in duplicate by gas chromatography–mass spectrometry (15, 16). In brief, isoflavones were extracted from samples by using solid-phase extraction, β-glucuronidase hydrolysis, and another solid-phase extraction. One hundred microliters of internal standard solution (5α-androstane-3β, 17β-diol, 50 μg methanol/mL) was added to the above-mentioned sample and dried under nitrogen flow. A total of 300 μL pyridine and 100 μL N,O-Bis(trimethylsilyl)acetamide were then added to the dried samples and incubated in room temperature for 1 h. After the reaction, the excess silylating agent and pyridine were then removed under nitrogen gas flow because they are volatile, and the trimethylsilylated samples left in the vial (residue) were dissolved in 100 μL hexane. One microliter of the aliquot was used for injection for the gas chromatography–mass spectrometry analysis.

Equol producers and nonproducers were determined on the basis of urinary equol concentrations. The following criteria for equol production were used: 1) having a minimal concentration of 1000 nmol equl/d (2, 4) and 2) having a log10-transformed urinary equol:daidzein ratio of > -1.75 (17). The CV for equol was <11% for the same day analysis and 26% for between-days analysis. Samples were analyzed in duplicate within the same batch for the same individual. However, in study 1 there were insufficient urine volumes to analyze the samples in duplicate.

Dietary isoflavone concentrations were measured as the 3 aglycones (genistein, daidzein, and glycitein) in study supplements as previously described (9, 10). Individual soy foods in the high- and low-soy isoflavone study were analyzed individually for isoflavones, and the total isoflavones consumed was derived from the product of the individual food and its isoflavone content as consumed in a 2000-kcal diet (9).

Diets were assessed for macronutrients, fatty acids, cholesterol, and fiber by using a computer program based on the USDA database (18).

Statistical analysis

The results were expressed as means ± SEs. All analyses were conducted by using SAS (version 9.2; SAS Institute) (19). Differences between groups in baseline measurements were assessed by 2-sample t test (2-tailed). The potential for carryover effect in the 2 crossover studies (studies 1 and 2) was assessed. There was no significant sequence or carryover effect for LDL and HDL cholesterol in either of these 2 studies. Three participants were receiving a consistent dose of statins during the interventions. The exclusion of these 3 individuals in the analysis did not change the results. Therefore, the 3 individuals were retained in the analysis.

Differences in outcomes between equol producers and nonproducers were assessed by using ANCOVA with mean change from baseline as the response variable; with study, equol status, and study × equol status interaction as the main effects; and with baseline as a covariate, except when percentage changes from baseline were assessed. Outcomes with only end values were analyzed by using a similar model without the use of baseline as a covariate.

Linear associations of interest were determined a priori and assessed by using Pearson’s product-moment correlation. Equol excretion on the soy treatments for equol producers was related to changes in serum lipids, lipid ratios, apolipoproteins, and apo B: apo A-I ratio.

RESULTS

Baseline characteristics of both equol producers and nonproducers were similar (Table 1), with the exception of lower CRP concentrations in equol producers than in nonproducers. Furthermore, there were no significant differences either at baseline or in mean change in the dietary macronutrient profile between equol producers and nonproducers (Table 2).

Lipids

The assessment of differences dependent on equol excretion status using the data from all 85 subjects in the 3 studies indicated significant reductions in LDL cholesterol in both equol producers and in nonproducers (−0.43 ± 0.11 compared with −0.54 ± 0.08
mmol/L, respectively). Similarly, a reduction in apo B was also seen in both equol producers and nonproducers (Table 3). However, a significant reduction in HDL cholesterol was seen in nonproducers, which was not seen in equol producers (−0.07 ± 0.02 compared with 0.0 ± 0.03 mmol/L, respectively; \( P = 0.036 \)). A similar pattern was also seen for apo A-I (−0.08 ± 0.02 compared with −0.02 ± 0.02 g/L, respectively; \( P = 0.010 \) (Table 3). The percentage changes from baseline are reported in Figure 1. There was no difference in response to soy related to equol status for any other lipid, lipoprotein, or apolipoprotein measurement.

### TABLE 2
Nutrient profiles at baseline and during soy treatments (\( n = 85 \))

<table>
<thead>
<tr>
<th></th>
<th>Equol producers (( n = 30 ))</th>
<th>Equol nonproducers (( n = 55 ))</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>Week 0 1751.5 ± 107.4</td>
<td>Week 0 1760.6 ± 86.4</td>
<td>0.404</td>
</tr>
<tr>
<td>Percentage of total energy (%)</td>
<td>Week 0 1724.0 ± 65.5</td>
<td>Week 0 1648.2 ± 59.5</td>
<td>0.040</td>
</tr>
<tr>
<td>Protein</td>
<td>18.5 ± 0.5</td>
<td>22.7 ± 0.8</td>
<td>0.030</td>
</tr>
<tr>
<td>Vegetable protein</td>
<td>7.2 ± 0.4</td>
<td>20.8 ± 1.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Soy protein</td>
<td>0.3 ± 0.1</td>
<td>9.1 ± 0.5</td>
<td>0.257</td>
</tr>
<tr>
<td>Available carbohydrate</td>
<td>54.0 ± 1.4</td>
<td>52.0 ± 2.7</td>
<td>0.857</td>
</tr>
<tr>
<td>Fat</td>
<td>26.0 ± 1.3</td>
<td>24.3 ± 2.1</td>
<td>0.571</td>
</tr>
<tr>
<td>Saturated</td>
<td>7.7 ± 0.6</td>
<td>5.0 ± 0.3</td>
<td>0.458</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>10.3 ± 0.7</td>
<td>11.1 ± 1.5</td>
<td>0.727</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>5.3 ± 0.2</td>
<td>6.5 ± 0.4</td>
<td>0.928</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1.5 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>0.942</td>
</tr>
<tr>
<td>Dietary fiber (g/1000 kcal)</td>
<td>16.2 ± 0.9</td>
<td>18.7 ± 1.2</td>
<td>0.961</td>
</tr>
<tr>
<td>Dietary cholesterol (mg/1000 kcal)</td>
<td>104.8 ± 8.9</td>
<td>34.2 ± 4.7</td>
<td>0.077</td>
</tr>
</tbody>
</table>

\( ^1 \) All values are means ± SEs. There were no significant differences at baseline in dietary macronutrient profile between equol producers and nonproducers. Mean treatment refers to the mean of values for all weeks measured after baseline.

\( ^2 \) \( P \) values represent differences from baseline between equol producers and nonproducers assessed by using ANCOVA, with mean change from baseline as the response variable; study, equol status, and study × equol status interaction as the main effects; and baseline as a covariate.

\( ^3 \) To convert apolipoprotein A-I and apolipoprotein B to mg/dL, multiply by 100.

\( ^4 \) To convert total cholesterol, LDL cholesterol, and HDL cholesterol to mg/dL, divide by 0.0259; to convert triglycerides to mg/dL, divide by 0.0113.

### TABLE 3
Effect of body weight, blood lipids, apolipoproteins, blood pressure, and C-reactive protein in equol producers and nonproducers on soy treatments (\( n = 85 \))

<table>
<thead>
<tr>
<th></th>
<th>Equol producers (( n = 30 ))</th>
<th>Equol nonproducers (( n = 55 ))</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>Week 0 73.6 ± 2.4</td>
<td>Week 0 75.8 ± 1.9</td>
<td>0.909</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 ± 0.7</td>
<td>27.3 ± 0.5</td>
<td>0.946</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.7 ± 2.0</td>
<td>92.5 ± 1.5</td>
<td>0.962</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>Total 6.6 ± 0.14</td>
<td>6.60 ± 0.13</td>
<td>0.401</td>
</tr>
<tr>
<td></td>
<td>LDL cholesterol 4.36 ± 0.11</td>
<td>4.49 ± 0.10</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol 1.29 ± 0.07</td>
<td>1.25 ± 0.05</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Triglycerides 1.99 ± 0.19</td>
<td>1.91 ± 0.14</td>
<td>0.890</td>
</tr>
<tr>
<td>Ratios</td>
<td>Total cholesterol:HDL cholesterol 5.37 ± 0.24</td>
<td>5.57 ± 0.17</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>LDL cholesterol:HDL cholesterol 3.58 ± 0.18</td>
<td>3.80 ± 0.13</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td>Apolipoproteins (g/L)²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>1.63 ± 0.04</td>
<td>1.61 ± 0.04</td>
<td>0.010</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>1.40 ± 0.04</td>
<td>1.28 ± 0.04</td>
<td>0.743</td>
</tr>
<tr>
<td>Apolipoprotein B:apolipoprotein A-I</td>
<td>0.88 ± 0.03</td>
<td>0.90 ± 0.03</td>
<td>0.232</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>2.35 ± 0.51</td>
<td>2.61 ± 0.36</td>
<td>0.223</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>Systolic 120.2 ± 2.4</td>
<td>127.2 ± 2.0</td>
<td>0.417</td>
</tr>
<tr>
<td></td>
<td>Diastolic 75.7 ± 1.4</td>
<td>78.2 ± 1.0</td>
<td>0.630</td>
</tr>
</tbody>
</table>

\( ^1 \) All values are means ± SEs. There were no significant differences at baseline between equol producers and nonproducers. Mean treatment refers to the mean of values for all weeks measured after baseline.

\( ^2 \) \( P \) values represent differences from baseline between equol producers and nonproducers assessed by using ANCOVA, with mean change from baseline as the response variable; study, equol status, and study × equol status interaction as the main effects; and baseline as a covariate.
Body weight, blood pressure, and CRP

No significant differences were observed in changes in body weight, waist circumference, and CRP between equol producers and nonproducers (Table 3). Both equol producers and nonproducers had significant reductions from baseline in systolic blood pressure, but these changes were not different between the 2 groups (Table 3). The reduction in diastolic blood pressure reached significance only in equol nonproducers, but again, this reduction was not significantly different from that in equol producers.

Urinary isoflavones

The mean 24-h urinary isoflavone output of equol producers and nonproducers was similar (Figure 2), with the exception of equol and ODMA. The equol output was 1334 ± 255 nmol/mmol creatinine in equol producers compared with 16 ± 3 nmol/mmol creatinine in equol nonproducers (P < 0.001), and ODMA, as the alternate metabolite of daidzein, showed the inverse effect, with a lower value in equol producers (915 ± 180 nmol/mmol creatine) than in nonproducers (1575 ± 181 nmol/mmol creatine) (P = 0.034).

Correlations

In equol producers, there was no association between urinary equol excretion (nmol/mmol creatinine) and changes in serum lipids, lipid ratios, apolipoproteins, or the apo A-I ratio.

DISCUSSION

This study is the first to our knowledge to show that soy consumption resulted in a similar LDL-cholesterol reduction in equol producers and nonproducers, but that only in equol producers were the concentrations of HDL cholesterol and apo A-I preserved.

The mean LDL-cholesterol reduction in the 3 studies was large due to inclusions of cointerventions in study 3, and some reduction in HDL cholesterol might therefore have been expected (20). However, only in the equol nonproducers was this reduction in HDL cholesterol observed, whereas in equol producers the concentration of HDL cholesterol was preserved despite the relatively large reduction in LDL cholesterol (11). Data from these studies were not used to calculate the overall effect of soy on LDL cholesterol because the objective of the current study was to determine the effect of equol status on the changes in LDL cholesterol.
The effect related to equol status (26).

The HDL-cholesterol-improving effect of equol may be due to a greater estrogenic action than its precursor daidzein and its metabolite ODMA, another metabolite of daidzein (27, 28). Mammalian estrogen and estrogen therapy have also been shown to alter the cholesterol-lowering effect of dietary components in- dependent of soy intake.

A further study that used a similar probiotic approach, but with the probiotic given as yogurt containing 10^8 colony forming units of each of Lactobacillus acidophilus, Bifidobacterium bifidus, and Lactobacillus GG per 100-g serving, showed a greater reduction in cholesterol on soy in those receiving the probiotic supplement (25) and a more consistent effect with both greater total cholesterol and LDL-cholesterol reductions with soy when a resistant starch supplement (bread containing a total of 16–20 g resistant starch) was provided to increase colonic microbial fermentation (prebiotic) (25). In our study, the subgroup who received prebiotic (oligofructose-enriched inulin) showed a potentiation of the soy cholesterol-lowering effect supporting the possible use of coadministration of a prebiotic with soy (10). However, we also failed to show either an increase in urinary equol excretion in response to the prebiotic (data not shown) or a difference in LDL-cholesterol reduction between equol producers and nonproducers.

The HDL-cholesterol-improving effect of equol may be due to a greater estrogenic action than its precursor daidzein and its metabolite ODMA, another metabolite of daidzein (27, 28). Mammalian estrogen and estrogen therapy have also been shown to alter cholesterol and apolipoprotein metabolism and appear to increase HDL cholesterol and reduce LDL cholesterol (29, 30). SERMs, such as toremifene, have actions similar to 17β-estradiol in raising HDL cholesterol (8). Whereas, other SERMs, such as tamoxifen, only show an LDL-cholesterol-lowering effect (8). SERMs therefore differ in their effects on HDL cholesterol and LDL cholesterol as illustrated by the current study.

There are few lifestyle strategies available to raise HDL cholesterol other than exercise, weight reduction, intake of monounsaturated fat, and moderate alcohol intake (31), which makes this action of soy foods of particular interest. In each of the 3 studies included in the current analysis, participants were asked to maintain their level of physical activity during the study. Furthermore, participants in studies 2 and 3 were also asked to abstain from alcohol during the study period. No differences were observed in alcohol intake and changes in body weight between the dietary soy interventions in each of the studies nor between equol producers and nonproducers (Tables 2 and 3).

A similar LDL-cholesterol reduction was observed in equol producers and nonproducers in the current analysis. However, the combination of the studies may still have been underpowered to detect a difference in LDL cholesterol on the basis of equol status. On the basis of the observed treatment difference and the SD, 946 individuals would be needed to detect a significant difference in LDL cholesterol.

No significant changes in blood pressure were observed between the equol producers and nonproducers, although both groups had improvements. It is possible that the 4-wk duration of the dietary interventions may not have been long enough to see significant blood pressure changes in equol producers and nonproducers. However, it is worthwhile to note that all participants had normal blood pressures at baseline, which may have limited the amount of improvement observed with the dietary soy interventions.

In the current studies, 35% of the subjects were defined as equol producers, which is consistent with the 30–40% estimation from the literature for Western populations (4, 5, 21–24, 32–36). This is in contrast to a higher frequency of equol producers among other populations studied (eg, Asian, Italian), which may be related to the type of soy foods consumed because marked differences in isoflavone composition exist between Western and Asian soy foods (37). The majority of isoflavones naturally found in soy foods (eg, soy milk, tofu) are in the β-glucoside form (conjugated) unless the food has been fermented (eg, tempeh, miso), in which the isoflavones are found as hydrolyzed aglycones (unconjugated). It has been hypothesized that absorption of the aglycone form is faster than that of glycosides and thus may be more easily converted to equol than can glycosides (38). No differences in baseline macronutrient profile were observed between equol producers and nonproducers in the current analysis, a similar finding to some (33), but not all, studies. The dietary data, therefore, did not explain the difference between equol producers and nonproducers.

Future studies are needed to confirm the current observation that equol producers and nonproducers have different HDL-cholesterol, but not LDL-cholesterol, responses to soy intake. Such studies would be designed with HDL cholesterol as the primary outcome, and equol status of the study participants should be determined at recruitment. The advantages will be 2-fold; this will allow prospective determination of changes in equol status as a result of the intervention and the distribution of equol producers and nonproducers would be adequately represented in the study design. Last, longer-term studies are needed to determine whether the effects are sustained, diminished, or enhanced with long-term consumption of soy foods as part of a cholesterol-lowering diet.

We conclude that equol status improves the effect of soy food consumption on cardiovascular risk by maintaining HDL cholesterol. Whether soy is also more effective in reducing LDL cholesterol in equol producers is less easy to determine and was not observed in the present study in which attempts to increase equol synthesis failed and urinary equol output did not appear to relate to the LDL-cholesterol-lowering effect of soy. On the other hand, equol status was associated with an increase in HDL-cholesterol concentration in producers compared with nonproducers in relation to soy consumption and, together with the
LDL-cholesterol reduction seen in both equol producers and nonproducers, this supports the use of soy foods as part of the cardiovascular risk reduction diet.

The authors’ responsibilities were as follows—JMWW and DJAJ: study concept and design; JMWW, AM, ZL, and CH: acquisition of data; JMWW, CWCK, ZL, C-JJ, RGJ, PBP, APR, AV, and DJAJ: analysis and interpretation of data; JMWW: drafting of manuscript and study supervision; JMWW, CWCK, AM, ZL, EV, CH, C-JJ, RGJ, PBP, APR, VV, WS, and DJAJ: critical revision of the manuscript for important intellectual content; EV: statistical analysis; DJAJ: obtained funding; JMWW, CWCK, ZL, C-JJ, RGJ, PBP, APR, and VV: administrative, technical, and material support; and DJAJ, JMWW, and EV: full access to all of the data in the study and responsibility for the integrity of the data and the accuracy of the data analysis. None of the funding organizations or sponsors played any role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript. DJAJ reported serving on the Scientific Advisory Board of Unilever, Sanitarium Company, California Strawberry Commission, Loblaw Supermarket, Herbal Life International, Nutritional Fundamental for Health, Pacific Health Laboratories, Metagenics, Bayer Consumer Care, Orafti, Dean Foods, Kellogg’s, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Pulse Canada, Saskatchewan Pulse Growers, and the Canola Council of Canada; he received honoraria for scientific advice from the Almond Board of California, International Tree Nut Council Nutrition Research and Education Foundation, Barilla, Unilever Canada, Sola, Oldways, Kellogg’s, Quaker Oats, Procter & Gamble, Coca-Cola, NuVal Griffin Hospital, Abbott, Canola Council of Canada, Dean Foods, California Strawberry Commission, Haine Celestial, and the Alpro Foundation; he was on the speakers’ panel for the Almond Board of California; he received research grants from Loblaw Brands Ltd, Unilever, Barilla, Almond Board of California, Sola, Haine Celestial, Sanitarium Company, Orafti, International Tree Nut Council, and the Peanut Institute; and he received travel support to meetings from the Almond Board of California, Unilever, Alpro Foundation, and the International Tree Nut Council. DJAJ’s wife is a director of Glycemic Index Institute, Toronto, Canada, and his sister, Caroline Brydson, contributed to the diet booklet used in the study, which may in the future be expanded to book form for the general public. CWCK reported being on speakers’ bureaus for Almond Board of California, Sola, and Unilever and received research grants from CIHR, Unilever, Sola, Loblaw Brands Ltd, International Tree Nut Council, and the Almond Board of California. EV has received partial salary funding from research grants provided by Unilever, Loblaws, and the Almond Board of California. None of the other authors had any disclosures to report.

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