Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in humans

Maud N Vissers, Peter L Zock, Gert W Meijer, and Martijn B Katan

ABSTRACT

Background: Intake of unsaponifiable compounds from edible oils, such as plant sterols, can lower serum cholesterol concentrations in humans. However, little is known about effects of other chemically related unsaponifiables in edible oils, such as triterpene alcohols.

Objective: We studied the effects of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on cholesterol concentrations in healthy, normolipemic volunteers.

Design: Twenty-eight men and 32 women consumed 29 g/d of 3 margarines for 3 wk each on a crossover, double-blind basis. A margarine based on sunflower oil was used as the control. Concentrations of plant sterols from rice bran oil or triterpene alcohols from sheanut oil were added to make 2 experimental margarines with the same fatty acid composition as the control margarine.

Results: Intake of 2.1 g plant sterols/d from rice bran oil decreased total cholesterol by 0.19 mmol/L (95% CI: −0.31, −0.07 mmol/L) and LDL cholesterol by 0.20 mmol/L (95% CI: −0.30, −0.10 mmol/L). HDL-cholesterol and triacylglycerol concentrations in healthy humans to define the structural elements responsible for such effects.

Conclusions: We found that 2.1 g plant sterols/d from rice bran oil lowered serum total cholesterol by 5% and LDL cholesterol by 4% in normolipemic humans, whereas triterpene alcohols from sheanut oil did not significantly affect lipoprotein concentrations in all subjects combined.

INTRODUCTION

Plant sterols, or phytosterols, are minor constituents of vegetable oils present in the unsaponifiable fraction. Large doses of plant sterols inhibit cholesterol absorption in humans and cause a modest decrease in serum cholesterol concentration (1–3). The most common plant sterols in the human diet are the 4-desmethylsterols β-sitosterol, campesterol, and stigmasterol, which are found in edible vegetable oils, such as corn, soybean, and rapeseed (canola) oil. Like cholesterol, these plant sterols are unsaturated with a double bond at carbon 5 (Figure 1). Plant sterols can be converted into stanols by hydrogenation; β-sitosterol is transformed into its saturated counterpart sitostanol. Stanols occur rarely in nature. The cholesterol-lowering effect of β-sitosterol and sitostanol has been well established (4, 5). Sterols with other structures may vary in their potential to reduce plasma cholesterol concentrations.

Plant sterols are synthesized from squalene; one of the first intermediate products is cycloartenol, a 4,4-dimethylsterol (Figure 1; 6). Rice bran oil contains these 4,4-dimethylsterols, such as cycloartenol and 24-methylene cycloartanol, as ferulic acid esters of 4-desmethylsterols, such as β-sitosterol and campesterol, which are the end products of plant sterol synthesis from squalene. The results of animal and some human studies suggest that rice bran oil may reduce plasma cholesterol concentrations (8–10).

Another class of unsaponifiables is the triterpene alcohols. Strictly speaking, triterpene alcohols are not plant sterols, but there are similarities in their structures (Figure 1). Sheanut oil contains ~8% unsaponifiable material, which is a mixture of fatty acid and cinnamic acid esters of such triterpene alcohols as α-amyrine, butyrospermol, lupeol, and β-amyrine (11). Little is known about the effect of sheanut oil on cholesterol concentrations (5).

We examined the effects of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in healthy humans to define the structural elements responsible for such effects.

SUBJECTS AND METHODS

Subjects

Subjects were recruited via publicity in local newspapers and posters in university buildings and dormitories. We carefully
explained the study protocol to the subjects before they gave their written, informed consent. The study protocol was approved by the Medical Ethical Committee of Wageningen University.

Subjects were eligible if they were >17 y of age, did not use drugs known to affect concentrations of serum lipoproteins, and were not pregnant, lactating, or following a prescribed diet. Volunteers filled out a medical questionnaire that was reviewed by an independent physician. Persons with a history of gastrointestinal, liver, or kidney disease were excluded, as were those with glucosuria, proteinuria, anemia, or a serum concentration of total cholesterol >7.5 mmol/L or of fasting triacylglycerol >2.3 mmol/L. Thirty-two women and 28 men aged 18–59 y were enrolled in the study.

Design and treatment

The 9-wk study was double-blind with multiple crossovers. Subjects consumed 29 g/d of 3 margarines for 3 wk each. The subjects were stratified according to age and sex and then randomly allotted to 1 of the 6 possible treatment sequences. A commercially available diet margarine (Flora; Van den Bergh Foods, Purfleet, United Kingdom) was used as the control margarine and served as the base for preparing the 2 experimental margarines (Unilever Research, Vlaardingen, Netherlands). Concentrates of plant sterols from rice bran oil or triterpene alcohols were added so that each margarine had the same fatty acid composition (Table 1). The margarines were provided in tubs that contained on average 28.9 g. The subjects consumed the contents of 1 tub over 1 d. They were not allowed to use the margarine for baking or frying; they usually consumed it as a spread on bread for breakfast, lunch, and in-between meals or added it to prepared hot meals. The mean intake of total plant sterols (in free sterol equivalents) was 0.06 g/d from the control margarine, 2.1 g/d from the rice bran oil margarine, and 2.6 g/d from the sheanut oil margarine (as triterpene alcohols).

The subjects were asked to maintain their usual diet and lifestyle. All subjects kept daily records of illness and deviations from the protocol and they returned empty margarine tubs. Ninety-six percent of the tubs were returned empty; diaries kept by the subjects and anonymous questionnaires administered after the trial showed that 99.5% of the scheduled amount of the control margarine, 97.3% of the rice bran oil margarine, and 99.1% of the sheanut oil margarine was consumed. To check adherence independently, we added 285 μmol lithium chloride to each 30 g margarine and determined plasma lithium concentrations (by inductively coupled plasma mass spectrometry, model Elan 6000 spectrometer; Perkin-Elmer Corp, Norwalk, CT). Mean plasma lithium concentration increased to 4.6 ± 1.3 μmol/L, which was 5 times the baseline concentration of 0.9 ± 0.3 μmol/L in similar subjects who did not consume added lithium chloride (12). This confirmed adherence to the protocol.

In each 3-wk period, intakes of energy, fatty acids, and cholesterol were estimated by a 24-h recall. Intake was similar for each treatment, although cholesterol intake was slightly higher in the period in which the control margarine was consumed (Table 2). Body weights were measured on day 18 of each 3-wk period. Mean body weight did not differ significantly between treatments: 70.4 ± 9.4 kg with the control margarine, 70.2 ± 9.2 kg with the rice bran oil margarine, and 70.1 ± 9.1 kg with the sheanut oil margarine.

Blood sampling

Two venous blood samples were taken from subjects after an overnight fast at the end of each 3-wk period, one on day 18 and another on day 21. Serum was obtained by centrifugation at
TABLE 1
Compositions of the experimental margarines

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Rice bran</th>
<th>Sheanut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat phase (fat + sterols + other esters)</td>
<td>70</td>
<td>82</td>
<td>86</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>70</td>
<td>71</td>
<td>75</td>
</tr>
<tr>
<td>Saturated</td>
<td>16</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>15</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>40</td>
<td>38</td>
<td>35</td>
</tr>
<tr>
<td>Total plant sterol</td>
<td>0.2</td>
<td>7.3</td>
<td>0.4</td>
</tr>
<tr>
<td>4,4’-Dimethylsters</td>
<td>&lt;0.1</td>
<td>3.4</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Monomethylsters</td>
<td>&lt;0.1</td>
<td>0.4</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>4-Desmethylsters</td>
<td>0.2</td>
<td>3.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Triterpene alcohols</td>
<td>—</td>
<td>—</td>
<td>8.9</td>
</tr>
<tr>
<td>Other unknown sterol-related compounds</td>
<td>—</td>
<td>1.2</td>
<td>—</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>—</td>
<td>2.9</td>
<td>—</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>—</td>
<td>—</td>
<td>2.1</td>
</tr>
<tr>
<td>Water phase</td>
<td>30</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Lithium chloride</td>
<td>997</td>
<td>859</td>
<td>999</td>
</tr>
</tbody>
</table>

1 Mean of 3 batches.  
2 Amounts as free sterol or triterpene alcohol equivalents.  
3 µmol/100 g.

1187 × g for 10 min at 4°C and stored at −80°C. Samples were enzymatically analyzed for total and HDL cholesterol and triacylglycerol (13–15). The mean bias for control samples provided by the Centers for Disease Control and Prevention in Atlanta was −1% for total and HDL cholesterol and 10% for triacylglycerol. The within-run CV ranged from 0.5% to 1.1%. LDL-cholesterol concentrations were calculated (16). For each subject, the serum lipoprotein values on days 18 and 21 of each period were averaged before statistical analysis.

Margarine analyses

The fatty acid compositions of the 3 margarines were analyzed by methanalysis of the fatty acids in a sample of margarine extracted with a methanolic NaOH solution using boron trifluoride as catalyst. The methyl esters were extracted with hexane and analyzed by gas chromatograph (Hewlett Packard, Wilmington, DE) with a CP Wax 58 column (Chrompack, Middelburg, Netherlands). Analysis of plant sterols and triterpene alcohols was done by saponifying the margarine with KOH and extracting the unsaponifiable part into diisopropylether. After extraction and derivatization with 1% TMCS (trimethylsilyl chloride) in BSTFA [N,O-bis(trimethylsilyl) trifluoroacetamide] and N,N-dimethylformamide, the fractions were analyzed on a gas chromatograph (Hewlett Packard) by means of splitless injection with hydrogen as carrier gas. In the case of the 4,4'-dimethylsters, the response factor compared with cholesterol was 0.87 on the basis of 5 concentrations of cycloartenol. For this reason, we also analyzed the plant sterols by using on-column injection with hydrogen as carrier gas and with helium as carrier gas. The response factors were 0.91 for hydrogen and 0.98 for helium as carrier gas. We therefore used the data from the on-column injection with helium as carrier gas.

Statistical analyses

The data were analyzed by two-factor, repeated-measures analysis of variance with interaction by using the general linear models (GLM) subprogram of SAS (17). Because the interaction between sex and margarine was significant for total and LDL cholesterol, the analysis was also performed for men and women separately. Tukey’s procedure was used for pair-wise comparisons of the margarines and for calculation of 95% CIs of the differences in plasma lipoprotein concentrations between 2 margarines.

RESULTS

All 60 subjects completed the study. None of the subjects used medications that could have affected the results. Three subjects became ill during the period in which they consumed the rice bran oil margarine. Excluding the data of these subjects from the analyses did not alter the results. We therefore present results of analyses that included all subjects. Because there was a significant interaction between sex and margarine for total cholesterol and LDL cholesterol, we also present the results of men and women separately.

Serum lipids and lipoproteins

In all subjects combined, rice bran oil margarine decreased total cholesterol by 0.19 mmol/L (95% CI: −0.31, −0.07 mmol/L) and LDL cholesterol by 0.20 mmol/L (95% CI: −0.30, −0.10 mmol/L) compared with the control margarine (Table 3). Of the 60 subjects, 44 showed lower concentrations and 15 showed higher concentrations of LDL cholesterol with rice bran oil margarine than with control margarine (Figure 2). Rice bran oil margarine did not affect HDL-cholesterol or triacylglycerol concentrations (Table 3).

TABLE 2
Dietary intake of energy, fatty acids, and cholesterol of the subjects while consuming the 3 study margarines

<table>
<thead>
<tr>
<th></th>
<th>Margarine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Energy (MJ/d)</td>
<td>11.8 ± 3.9</td>
</tr>
<tr>
<td>(kcal/d)</td>
<td>2820 ± 941.6</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>34.9</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>12.1</td>
</tr>
<tr>
<td>Monounsaturated fatty acids</td>
<td>11.2</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>9.2</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>225.9 ± 190.2</td>
</tr>
</tbody>
</table>

1 Each subject consumed each margarine for 3 wk in random order. Values are based on one 24-h recall per person in each 3-wk period. n = 60.
2 Mean ± SD.
TABLE 3
Serum lipid and lipoprotein cholesterol concentrations at the end of the 3 margarine periods and the differences between experimental and control margarine

<table>
<thead>
<tr>
<th>Lipid and lipoprotein values</th>
<th>Control</th>
<th>Rice bran oil</th>
<th>Sheanut oil</th>
<th>Differences from control margarine (95% CI)</th>
</tr>
</thead>
</table>
| Total cholesterol
| Men | 4.15 ± 0.89<sup>1</sup> | 4.01 ± 0.93 | 4.01 ± 0.80 | −0.14 (−0.31, 0.03) |
| Women | 4.32 ± 0.62 | 4.08 ± 0.67<sup>1</sup> | 4.35 ± 0.75 | −0.24 (−0.41, −0.07) |
| All | 4.24 ± 0.76 | 4.05 ± 0.80<sup>1</sup> | 4.19 ± 0.79 | −0.19 (−0.31, −0.07) |
| HDL cholesterol
| Men | 1.37 ± 0.30 | 1.35 ± 0.31 | 1.32 ± 0.30 | −0.02 (−0.08, 0.04) |
| Women | 1.64 ± 0.38 | 1.64 ± 0.41 | 1.65 ± 0.40 | 0.00 (−0.06, 0.07) |
| All | 1.51 ± 0.37 | 1.50 ± 0.39 | 1.50 ± 0.39 | −0.01 (−0.05, 0.03) |
| LDL cholesterol<sup>2</sup>
| Men | 2.42 ± 0.70 | 2.27 ± 0.71<sup>4</sup> | 2.29 ± 0.60<sup>4</sup> | −0.15 (−0.28, −0.03) |
| Women | 2.30 ± 0.47 | 2.06 ± 0.48<sup>4</sup> | 2.30 ± 0.59 | −0.24 (−0.39, −0.10) |
| All | 2.36 ± 0.59 | 2.16 ± 0.60<sup>4</sup> | 2.29 ± 0.59 | −0.20 (−0.30, −0.10) |
| Triacylglycerol
| Men | 0.77 ± 0.39 | 0.86 ± 0.54 | 0.88 ± 0.53 | 0.09 (−0.06, 0.24) |
| Women | 0.84 ± 0.47 | 0.83 ± 0.42 | 0.87 ± 0.44 | −0.01 (−0.08, 0.07) |
| All | 0.81 ± 0.43 | 0.85 ± 0.48 | 0.85 ± 0.48 | 0.04 (−0.04, 0.12) |

<sup>1</sup>All participants (28 men and 32 women) were included in the analysis. They consumed each margarine in random order for 3 wk each. To convert total, HDL-, and LDL-cholesterol values to mg/dL, multiply by 38.67. To convert triacylglycerol values to mg/dL, multiply by 88.54.

<sup>2</sup>Significant interaction between sex and margarine, P = 0.03.

<sup>3</sup>T ± SD.

<sup>4</sup>Significantly different from the control margarine, P < 0.05 (adjusted with Tukey’s procedure for multiple comparisons).

<sup>5</sup>Significantly different from sheanut oil margarine, P < 0.05 (adjusted with Tukey’s procedure for multiple comparisons).

Sheanut oil margarine did not affect total, LDL-, or HDL-cholesterol or triacylglycerol concentrations compared with the control margarine in all subjects combined (Table 3). Thirty-three subjects had lower concentrations and 25 had higher concentrations of LDL cholesterol with sheanut oil margarine than with the control margarine (Figure 2). In men, sheanut oil margarine decreased LDL cholesterol by 0.13 mmol/L (95% CI: −0.26, −0.01 mmol/L) and nonsignificantly decreased total cholesterol by 0.14 mmol/L (95% CI: −0.31, 0.03 mmol/L) (Table 3).

DISCUSSION
The results of this study show that 2.1 g plant sterols/d from rice bran oil lowers serum total cholesterol by 5% and LDL cholesterol by 9% in normolipemic subjects whereas triterpene alcohols from sheanut oil have no or much smaller effects. Earlier studies on rice bran oil could not disentangle the effects of the fatty acids from those of plant sterols (18–21). In these studies, intake of rice bran oil sterols was 4–9 times lower than in our study, and these studies addressed the consumption of complete oils, not their plant sterols per se. One other study investigated the effects of concentrated rice bran oil sterols in humans and found—in contrast with our study—no significant effect on cholesterol concentrations in normo- and mildly hypercholesterolemic subjects (5).

Our study cannot directly answer the question of whether 4,4'-dimethylsterols alone affect cholesterol concentrations; half of the plant sterols supplied by the rice bran oil margarine were 4,4'-dimethyl sterols (1.0 g/d) and half were 4-desmethyl sterols (1.0 g/d, mainly β-sitosterol and campesterol). The rice bran oil margarine in the study of Weststrate and Meijer (5), who found no effect on cholesterol concentrations, supplied mainly 4,4'-dimethyl sterols such as cycloartenol and 24-methylene cycloartenol (1.1 g/d) and much less 4-desmethyl sterols, including β-sitosterol (0.5 g/d) (5). This suggests that 4,4'-dimethyl sterols may have no effect on cholesterol concentrations. Any effect of rice bran oil sterols is therefore probably due to 4-desmethyl sterols. This notion is also supported by the studies of Hendriks et al (22) and Sierksma et al (23), in which 0.8 g 4-desmethyl sterols/d from soybean oil lowered cholesterol concentrations by 4.9% and 3.8%, respectively. In our study, 1.0 g 4-desmethyl sterols/d decreased cholesterol concentrations by 5%, the same extent as observed in these 2 studies. Also, in rats a combination of the 4,4'-dimethyl sterol cycloartenol and the 4-desmethyl sterol β-sitosterol did not lower cholesterol concentrations more than did β-sitosterol alone (24). Thus, any effect of rice bran oil sterols on cholesterol is probably due to the 4-desmethyl sterols, with 4,4'-dimethyl sterols having no or a much smaller effect. However, this issue can be settled only in a study that directly tests the effects of 4,4'-dimethyl sterols.

Plant sterols probably decrease plasma cholesterol concentrations by inhibiting cholesterol absorption in the small intestine (1, 25–27). The 4-desmethyl sterol β-sitosterol and cholesterol are more similar in structure than are the 4,4'-dimethyl sterols and cholesterol. The 4,4'-dimethyl sterols have 2 extra methyl groups compared with cholesterol (Figure 1) and is absorbed less than is cholesterol. Heinemann et al (28) suggested that increasing the side chain substitution of cholesterol decreases its absorbability in humans. For example, ß-sitosterol has a side chain substitution of an ethyl group compared with cholesterol (Figure 1) and is absorbed less than is cholesterol. Heinemann et al also indicated an inverse relation between the absorbability of plant sterols and their efficiency in inhibiting cholesterol absorption. Compared with cholesterol, the 4-desmethyl sterol cycloartenol does not have a side chain substitution but has an additional double bond at carbon 24, and 24-
methylene cycloartenanol has a methylene group at carbon 24. Hence, these 4,4′-dimethylsterols might be more absorbable and therefore less effective in inhibiting cholesterol absorption than the 4-desmethylsterol β-sitosterol. No data are available in the literature with respect to the absorption of the 4,4′-dimethylsterols in humans. However, in rats, the absorption rate of cycloartenol was 4-fold higher than that of β-sitosterol (24). Thus, the differential effects of 4-desmethylsterols and 4,4′-dimethylsterols on serum cholesterol concentrations may be explained by several structural differences.

In our study, intakes of 4-desmethylsterols and 4,4′-dimethylsterols were 5–10-fold higher than in a normal diet, and potential adverse effects of such relatively high intakes need to be considered carefully. In patients with homozygous sitosterolemia, a high percentage of β-sitosterol is absorbed from the intestine; this is believed to account for the plant sterol accumulation in plasma and early atherosclerosis in such patients (29). Homozygous sitosterolemia is an extremely rare condition, but heterozygotes occur more frequently and such heterozygotes might theoretically hyperabsorb plant sterols. One study found that plasma plant sterol concentrations in heterozygous subjects were 2–3 times higher than in control subjects and 10–20 times lower than in homozygous subjects (30). Two other studies (31, 32) found normal plasma concentrations in heterozygous subjects. These data suggest that there is no reason to expect important adverse effects of foods rich in plant sterols in heterozygous subjects. Weststrate and Meijer (5) showed that the intake of 1.5 g β-sitosterol/d and 0.8 g campesterol/d compared with a control (<0.1 g/d) increased plasma β-sitosterol and campesterol concentrations from 3.3 to 4.6 mg/L and from 7.0 to 12.1 mg/L, respectively (5). These concentrations are within the range of normal values (33). Thus, adverse effects of high intakes of 4-desmethylsterols such as β-sitosterol and campesterol seem unlikely, but long-term observational data are still desirable to confirm the safety of high intakes, especially for subjects who carry the sitosterolemia gene or genes.

We did not analyze plasma plant sterol concentrations in our study, and we are not aware of other data with respect to the absorption of the 4,4′-dimethylsterols, such as cycloartenol, in humans. If cycloartenol is as highly absorbed in humans as in rats, it could be a reason to avoid fortification of food with rice bran sterols because these are rich in 4,4′-dimethylsterols.

Sheanut oil margarine did not significantly affect lipoprotein and triacylglycerol concentrations in all subjects combined. This is in line with the results of Weststrate and Meijer (5). The unsaponifiables of sheanut oil consist of triterpene alcohols and minor amounts of 4-desmethylsterols and 4,4′-dimethylsterols (11). Like 4,4′-dimethylsterols, triterpene alcohols have 2 extra methyl groups at carbon 4 compared with 4-desmethylsterols. Also, in triterpene alcohols the cyclopentane ring specific for sterols is absent; instead, triterpene alcohols have 2 cyclohexane rings (ring D; Figure 1). A sterol structure with a cyclopentane ring might be a minimum requirement for inhibiting cholesterol absorption in the intestine. This may explain why triterpene alcohols do not lower serum cholesterol concentrations.

In our study, there was a significant interaction between sex and margarine for total and LDL cholesterol. Sheanut oil margarine slightly lowered total and LDL-cholesterol concentrations in men but not in women. However, the sex-specific analyses were not planned a priori and the difference between men and women was not very large. Therefore, this finding may have been due to chance. Only one postmenopausal woman, aged 59 y, participated in our study and omitting her data did not change the conclusions (data not shown). Therefore, our data refer to premenopausal women.

Only normocholesterolemic subjects participated in our study, and we cannot answer the question of what the effect would have been in hypercholesterolemic subjects. If we calculated the treatment effect for tertiles on the basis of the study entry value for total cholesterol concentration, the response in the low tertile (range: 3.15–4.16 mmol/L) was −0.16 ± 0.3 mmol/L, in the mid tertile (range: 4.17–7.71 mmol/L) was −0.32 ± 0.3 mmol/L, and in the highest tertile (range: 7.74–7.32 mmol/L) was −0.10 ± 0.5 mmol/L. There were no significant differences between tertiles. We therefore estimate that the effect of plant sterols from rice bran oil is similar in normo- and hypercholesterolemic subjects. However, this issue can be settled only in a study that directly tests the effect of plant sterols from rice bran oil in hypercholesterolemic subjects.

In summary, plant sterols from rice bran oil lowered serum total and LDL-cholesterol concentrations in normolipemic humans, whereas triterpene alcohols from sheanut oil did not affect the average cholesterol concentration in all subjects combined. The effect of rice bran oil sterols on serum cholesterol concentrations is probably due to the 4-desmethylsterol β-sitosterol and not to 4,4′-dimethylsterols such as cycloartenol and 24-methylene cycloartenanol. Because rice bran oil contains mainly 4,4′-dimethylsterols and less 4-desmethylsterols, it might not be an efficient dietary source of cholesterol-lowering plant sterols.
We thank Frans Schouten and Marleen van Nuenen for the lithium analysis, Els Siebelink for taking the 24-h recalls, Peter van de Bovenkamp for the analyses of the margarines, all the laboratory technicians for analyzing the blood samples, and Unilever Research Vlaardingen for the preparation of the special margarines. We also thank the volunteers for their enthusiastic participation.

REFERENCES