Do dietary phytochemicals with cytochrome P-450 enzyme-inducing activity increase high-density-lipoprotein concentrations in humans?1–3

M Nazeem Nanjee, Hans Verhagen, Geert van Poppel, Cathy JM Rompelberg, Peter J van Bladeren, and Norman E Miller

ABSTRACT Low plasma concentrations of high-density lipoprotein (HDL) are associated with increased risk of coronary heart disease. Several drugs that induce the microsomal cytochrome P-450-dependent enzyme system in liver and intestine, the sites of HDL apolipoprotein (apo) A-I and A-II synthesis, raise plasma HDL concentrations in humans. To test the hypothesis that phytochemicals with cytochrome P-450–inducing activity may also increase plasma HDL concentrations, two controlled dietary trials were undertaken in healthy nonsmoking males aged 20–28 y. One study examined the effect of replacing 300 g glucosinolate-free vegetables with 300 g Brussels sprouts/d for 3 wk. The other study examined the effects of 150 mg eugenol/d in capsule form, using a double-blind, placebo-controlled crossover design. There were no significant increases in plasma apo A-I, apo A-II, HDL cholesterol, or HDL phospholipids. These results suggest that dietary phytochemicals that induce members of the cytochrome P-450 system do not necessarily raise plasma HDL concentrations in humans, but do not exclude the possibility that some phytochemicals may have such an effect. Am J Clin Nutr 1996;64:706–11.

KEY WORDS Plasma lipids, high-density lipoproteins, diet, phytochemicals, cytochrome P-450, indoles, alkylbenzenes, cruciferous vegetables, eugenol, apolipoproteins, glucosinolates

INTRODUCTION Plasma high-density lipoproteins (HDLs) are a heterogeneous family of particles of different sizes and composition. Their principal protein component, apolipoprotein (apo) A-I, is synthesized in the liver and small intestine (1). Low plasma HDL cholesterol is a major risk factor for the development of coronary heart disease (CHD) (2–6). A 0.026-mmol/L (1-mg/dL) change in plasma HDL cholesterol is associated with a 2–3% change in CHD incidence (5). Evidence that this reflects an antiatherogenic effect of HDL has been provided by gene transfer (7–9) and pharmacologic (10, 11) and HDL infusion (12, 13) experiments in animals. This may reflect the central role that HDLs play in the transport of cholesterol from peripheral tissues (14, 15), or their effects on platelet function (16), fibrinolysis (17), or the oxidative modification of low-density lipoproteins (LDLs) (18).

Diets high in total fat (and low in carbohydrates) (19), cholesterol (20), or alcohol (21) all increase plasma HDL cholesterol. Diets rich in trans fatty acids lower plasma HDL cholesterol (22). Long-chain cis fatty acids (monounsaturated, polyunsaturated, and saturated) have only a small effect, except in diets of extreme composition (23–26). Dietary fiber and soy protein also have little or no effect (27, 28). However, the effects of other phytochemicals have not been studied and the overall contribution of diet to the determination of plasma HDL concentrations is uncertain.

Several metabolic processes contribute to the regulation of plasma HDL concentration (1, 15). One of these processes is the production rate of apo A-I (29–32). The normal control of apo A-I gene expression in the liver and intestine is not yet clear. However, it is of interest that several drugs that are inducers of members of the cytochrome P-450 enzyme system in liver, such as phenobarbital, phenytoin, and carbamazepine, all increase plasma HDL concentrations in humans by up to 75% (33–38). Much smaller increases produced by gemfibrozil or niacin contributed to a lower incidence of CHD (39) or to reduced progression of coronary atherosclerosis (40) in long-term clinical trials. In epilepsics taking anticonvulsants, HDL cholesterol was positively correlated with the cytochrome P-450 content of liver biopsies (41). Such patients may also have a 30% reduction in CHD mortality (42). In rats, phenobarbital increased hepatic apo A-I mRNA abundance severalfold (43). Such findings have raised the possibility that dietary phytochemicals that induce the cytochrome P-450 system might also raise plasma HDL concentrations.

Cruciferous vegetables (eg, cabbage, Brussels sprouts, and broccoli) are rich in glucosinolates, which after ingestion are metabolized to indoles that induce certain isoenzymes of the...
cytochrome P-450 system in liver and intestine (44–47). Feeding these indoles to rodents increased the metabolism of chemical carcinogens and altered the susceptibility of the animals to the development of tumors (45, 47, 48). Consumption of a diet rich in Brussels sprouts and cabbage increased the oxidative metabolism of phenacetin and antipyrine in humans (49). More recently, oral administration of eugenol (4-allyl-1-hydroxy-2-methoxybenzene)—the major component of oil of cloves and also present in cinnamon, basil, nutmeg, and other plants—was shown to induce cytochrome P-450-dependent dealkylation reactions in rat liver microsomes (50) and to prevent reduction of the hepatic cytochrome P-450 content produced by carbon tetrachloride (51). In none of these studies were measurements made of plasma lipids. To explore the possibility that dietary phytochemicals with cytochrome P-450-inducing activity may increase plasma HDL concentrations, we studied the effects of Brussels sprouts and eugenol in humans.

SUBJECTS AND METHODS
Clinical procedures
The Brussels sprouts and eugenol studies were approved by the TNO Medical Ethical Committee.

Brussels sprouts study
Ten healthy nonsmoking males were studied (Table 1). All had normal liver function as determined by routine clinical chemistry analysis. All standardized meals were prepared and eaten in a university hospital. During the first 3 wk of the study all subjects avoided glucosinolate-containing foods and consumed on each day a standardized meal containing 300 g glucosinolate-free vegetables (endives, French beans, peas, beets, fava beans, and/or chicory; no onions or garlic). During a subsequent 3-wk intervention period, five of the subjects were randomly allocated to continue on the glucosinolate-free diet (group 1), whereas the other five subjects (group 2) consumed 300 g cooked Brussels sprouts/d (replacing the 300 g of the glucosinolate-free vegetables). The Brussels sprouts were from the same batch as that used by Boggaerts et al (52). One kilogram contained the following glucosinolates: 2.15 mmol sinigrin, 0.38 mmol glucobrassicin, 0.04 mmol neoglucobrassicin, 0.62 mmol progoitrin, and 0.19 mmol gluconapin. Throughout the study the subjects were allowed only one alcoholic drink per day, and abstained from any medication.

Blood was collected at the end of each 3-wk period. There were no dropouts.

Eugenol study
A double-blind, placebo-controlled crossover design was used. A different group of 10 healthy, nonsmoking males participated (Table 1). All had normal liver function as determined by routine clinical chemistry. Each was given a dietary exclusion list, which included cloves, cinnamon, basil, nutmeg, roasted and grilled meats, cruciferous vegetables, and vitamin supplements (53). Eugenol [CAS (chemical abstracts) registration no. 97–53-0; food grade] was obtained from Sigma Chemical Company (St Louis). Capsules containing 50 mg eugenol (dissolved in dried starch) and placebo capsules containing dried starch alone were prepared within 1 wk of the study. Subjects were randomly allocated to receive one eugenol capsule three times daily (at 0800, 1600, and 2300) or one placebo capsule three times daily, for 7 d (five subjects per group). Capsules were dispensed in bottles containing three drops of menthe piperita aetereolium to disguise the smell of eugenol. The second week was a washout period, during which no capsules were taken. During the third week each subject took the alternative capsules. Subjects were not permitted alcohol or medications. Blood samples were collected at the end of the eugenol and placebo periods. There were no dropouts.

Laboratory measurements
All assays were made without knowledge of the source of the plasma samples. Plasma concentrations of total cholesterol, triacylglycerols, and choline-containing phospholipids (54) were measured by enzymatic microcolorimetry. Plasma HDL cholesterol and phospholipids were quantified after precipitation of apo B-containing lipoproteins with polyethylene glycol (PEG) 6000 (55). Plasma LDL cholesterol was calculated from plasma total cholesterol, triacylglycerols, and HDL cholesterol (56). Plasma concentrations of apo A-I, apo A-II (the second most abundant HDL protein), and apo B (the major LDL protein) were measured by immunoelectrophoresis using gels containing 0.1% (by vol) Tween 20 (Sigma Chemical Co) to ensure complete exposure of cryptic epitopes. Polyclonal antiseras, raised in goats to human apos, were purchased from International Immunology Corp (Murrieta, CA).

Statistical analyses
In the Brussels sprouts study, analysis of variance was used. Within each group of subjects, mean plasma concentrations at the end of the first 3 wk were compared with those at the end of the second 3 wk by using the paired t test. In addition, the mean within-subject changes in concentrations in the two groups were compared by Students’ t test. In the eugenol study, concentrations at the end of the placebo period were compared with those at the end of the eugenol period by using the paired t test. A P value < 0.05 was considered significant.

RESULTS
No adverse effects were observed after routine clinical chemical measures of thyroid, kidney, or liver function (alanine and aspartate aminotransferases, lactate dehydrogenase, alkaline phosphatase, and γ-glutamyltransferase). Body weights

TABLE 1
Clinical characteristics of subjects in the Brussels sprouts and eugenol studies

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<tr>
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<th>Brussels sprouts</th>
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<tr>
<td></td>
<td>Group 1 (n = 5)</td>
<td>Group 2 (n = 5)</td>
<td>Eugenol (n = 10)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>24.6 ± 2.7</td>
<td>24.0 ± 2.5</td>
<td>22.5 ± 2.3</td>
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<tr>
<td>Body weight (kg)</td>
<td>76.0 ± 7.4</td>
<td>81.4 ± 7.6</td>
<td>75.9 ± 6.8</td>
</tr>
<tr>
<td>Quetelet index (kg/m²)</td>
<td>22.8 ± 1.9</td>
<td>22.9 ± 1.3</td>
<td>22.7 ± 1.8</td>
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<tr>
<td>Plasma cholesterol (mmol/L)</td>
<td>3.4 ± 0.9</td>
<td>4.0 ± 0.8</td>
<td>4.1 ± 0.4</td>
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<tr>
<td>Plasma triacylglycerol (mmol/L)</td>
<td>0.6 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
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* x ± SD.
measured in the clinic did not change significantly. Plasma lipid and apo concentrations in the Brussels sprouts study are summarized in Table 2; there were no significant effects. Mean changes in plasma HDL-cholesterol, apo A-I, and LDL-cholesterol concentrations and their 95% CIs, respectively, were as follows: 3.1% (−9.6, 15.7), −1.4% (−15.4, 12.6), and 10.7% (−5.1, 26.5). Results obtained in the eugenol study are presented in Table 3. Again there were no significant effects of treatment. Mean changes in plasma HDL-cholesterol, apo A-I, and LDL-cholesterol concentrations and their 95% CIs, respectively, were as follows: 4.8% (−6.2, 15.8), 3.3% (−9.7, 13.1), and 3.2% (−15.9, 22.3).

**DISCUSSION**

The two studies described in this paper are the first attempts to test the hypothesis that dietary phytochemicals with known cytochrome P-450 enzyme-inducing activity in liver or intestine increase plasma HDL concentrations in humans. Two interventions were studied. The effects of cruciferous vegetables, known to be rich in glucosinolates, were investigated by substituting 300 g Brussels sprouts for an equivalent amount of other vegetables in a glucosinolate-free diet. A control group, studied in parallel, consumed the glucosinolate-free diet. Although there were some differences in mean baseline (ie, control period) concentrations between the subjects who went on to the Brussels sprouts diet and those who continued on the glucosinolate-free diet, these were not large and none were significant. It is unlikely, therefore, that the failure of the Brussels sprouts diet to increase the concentration of any one of the four measured HDL components was a false-negative outcome resulting from imperfect matching of the two groups of subjects.

In the Brussels sprouts study the subjects were given standardized meals differing only in vegetable content. Therefore, energy and micronutrient intakes were constant. In both studies there were limitations on alcohol consumption, and body weights remained essentially constant. In the eugenol study the volunteers were asked to adhere to their habitual diets and were given a dietary exclusion list of substances containing significant amounts of eugenol or other cytochrome P-450 inducers. The possibility that a significant HDL-raising effect of eugenol was obscured by a coincident change in fat consumption can be discounted because the HDL concentration is affected only by extreme alterations in dietary fatty acids (19, 23–26). Furthermore, any reductions of saturated fat or cholesterol consumption, or increases in polyunsaturated fats, would have lowered LDL-cholesterol and apo B concentrations (19, 20, 23–25), and no such changes occurred.

The question arises as to whether the interventions were given in sufficient quantity or for long enough for effects on cytochrome P-450-dependent enzyme activities to be manifest. Note that in the Brussels sprouts study each subject in group 2 ingested 6.3 kg sprouts during the 3-wk period: a substantial intake, and probably more than a free-living subject could be expected to include in their habitual diet. The dose of eugenol that we used (150 mg/d) is close to the recommended acceptable upper limit of daily intake (2.5 mg/kg body wt), as recommended by the Joint FAO/WHO Expert Committee on Food Additives (57), and is >200 times the estimated mean daily per capita consumption (58). Therefore, it could be ar-

<table>
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<td>Plasma lipid and apolipoprotein (apo) concentrations in the Brussels sprouts study&lt;sup&gt;1&lt;/sup&gt;</td>
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<th>Group 1</th>
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<tr>
<td></td>
<td>First period</td>
<td>Second period</td>
<td>First period</td>
<td>Second period</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>3.40 ± 0.40</td>
<td>3.50 ± 0.36</td>
<td>4.03 ± 0.35</td>
<td>4.36 ± 0.30</td>
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<td>Triacylglycerols (mmol/L)</td>
<td>0.62 ± 0.11</td>
<td>0.79 ± 0.16</td>
<td>1.04 ± 0.07</td>
<td>1.15 ± 0.18</td>
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<td>Phospholipids (mmol/L)</td>
<td>2.29 ± 0.14</td>
<td>2.37 ± 0.10</td>
<td>2.54 ± 0.16</td>
<td>2.74 ± 0.10</td>
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<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.23 ± 0.19</td>
<td>1.29 ± 0.16</td>
<td>1.05 ± 0.07</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>HDL phospholipids (mmol/L)</td>
<td>1.18 ± 0.12</td>
<td>1.19 ± 0.09</td>
<td>1.11 ± 0.08</td>
<td>1.10 ± 0.06</td>
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<td>LDL cholesterol (mmol/L)</td>
<td>1.89 ± 0.48</td>
<td>1.86 ± 0.44</td>
<td>2.51 ± 0.30</td>
<td>2.77 ± 0.32</td>
</tr>
<tr>
<td>Apo B (μmol/L)</td>
<td>1.31 ± 0.25</td>
<td>1.18 ± 0.27</td>
<td>1.71 ± 0.10</td>
<td>1.50 ± 0.14</td>
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<tr>
<td>Apo A-I (μmol/L)</td>
<td>51 ± 2.6</td>
<td>52 ± 4.3</td>
<td>47 ± 1.2</td>
<td>47 ± 3.3</td>
</tr>
<tr>
<td>Apo A-II (μmol/L)</td>
<td>20 ± 0.8</td>
<td>21 ± 0.9</td>
<td>20 ± 1.6</td>
<td>22 ± 1.2</td>
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<sup>1</sup> × SEM; n = 5/group. To convert results to mg/dL, divide by 0.0259 for cholesterol, 0.0113 for triacylglycerol, 0.0129 for phospholipids, 0.0182 for apo B, 0.357 for apo A-I, and 0.588 for apo A-II.
gued that dietary changes of greater magnitude would have provided results of little relevance to CHD prevention in the community.

It seems unlikely that substantial increases in plasma HDL concentrations would have emerged if the dietary interventions had been continued longer because no trends were observed and both the cytochrome P-450 enzyme system and apo A-I gene expression usually respond rapidly to chemical inducers. Thus, phenobarbital increased cytochrome P-450 enzymes in cultured hepatocytes five- to eightfold in 72–96 h (59, 60). In rats, hepatic apo A-I mRNA abundance was elevated 10-fold within 16 h of a single dose of phenobarbital (43). A single dose of indole-3-carbinol to rats increased cytochrome P-450 1A mRNA in liver fourfold in 16 h (61). In mice, several cytochrome P-450–dependent reactions increased after administration of indole-3-carbinol for 1 wk (62). Eugenol increased cytochrome P-450–dependent reactions in rats by more than twofold within 10 d (50). Franceschini et al (35) observed increases in plasma HDL cholesterol of 18% and 42% when healthy humans were given phenytoin for 2 and 4 wk, respectively. Feeding indole-3-carbinol to humans for 1 wk increased the hydroxylation of estradiol by 56% (63), and Pantuck et al (49) observed increases in oxidative drug metabolism in humans after only 7 d of a diet containing 150 g Brussels sprouts and 100 g cabbage. Thus, although the possibility of a small effect of Brussels sprouts or eugenol on plasma HDL during long-term consumption cannot be discounted, any major effects of potential value for coronary disease prevention have probably been excluded by our results.

Based on the known within-subject variances of plasma lipid and apo concentrations (64), our Brussels sprouts trial could have detected an 8.5–μmol/L (24 mg/dL) increase in plasma apo A-I concentrations with an α of 0.05 and a power of 0.95. The corresponding figure for plasma HDL cholesterol is 0.175 mmol/L (6.8 mg/dL). Such an effect would be predicted to reduce CHD incidence by ∼15% (5). The larger eugenol trial could have detected changes in concentration of lesser magnitude (HDL cholesterol, 0.125 mmol/L; apo A-I, 6.0 μmol/L). Thus, our study groups were large enough to have detected effects of potential significance for CHD prevention.

It should be emphasized, however, that our conclusion that neither Brussels sprouts nor eugenol are likely to be of value for raising plasma HDL concentration should not be extrapolated to other dietary components that induce cytochrome P-450 enzymes. The cytochrome P-450 system includes a large family of isoenzymes with different substrate specificities, the different members of which show great variations in their inducibility by different xenobiotics (59–61, 65–67). The isoenzymes that affect apo A-I synthesis rate have not been identified, and may not be included among those that are induced by indoles or eugenol. For this reason there would have been little value in measuring other indexes of cytochrome P-450 induction (eg, antipyrine clearance) in our subjects. It is also noteworthy that indoles can inhibit some isoenzymes of the system (62) and that cruciferous vegetables are also rich in thiocyanates that can have a similar effect (68). Furthermore, indole-3-carbinol has been shown to stimulate the hydroxylation of androstenedione, testosterone, and estradiol (62, 63, 65, 67, 69), which might have modified any direct effect on plasma HDL concentrations via the known effects of gonadal steroids on apo A-I metabolism and hepatic lipase activity (70). For these reasons it remains possible that other cytochrome P-450–inducing phytochemicals might have significant HDL-elevating activity, and further work in this area is warranted.

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