Well-Characterized Garlic-Derived Materials Are Not Hypolipidemic in APOE*3-Leiden Transgenic Mice

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ABSTRACT Garlic is reported to have beneficial effects on risk factors associated with cardiovascular disease, including normalization of plasma lipid levels. However, numerous studies do not support this beneficial effect of garlic on plasma lipids. This contradiction may result from the use of different garlic-derived materials, experimental designs, and/or animal models. The present study investigated the hypolipidemic effect of garlic-derived materials in APOE*3-Leiden mice, a model well suited for drug and dietary intervention studies of hyperlipidemia. APOE*3-Leiden mice were fed a garlic-derived sulfur-rich compound, either allicin (0.29 g · L drinking water−1) or diallyldisulfide (0.27 g · kg diet−1), or powdered garlic, of either the kwai (42 g · kg diet−1) or morado (42 g · kg diet−1) variety. The amounts of garlic-derived materials supplied allowed free intake of allicin or allicin equivalents (diallyldisulfide, kwai, or morado) at 44 mg · kg body wt−1 · d−1. Mice were fed a nonpurified diet for 4 wk, followed by a Western diet for 8 wk, both supplemented with the garlic-derived materials. These diets had no consistent effect on plasma lipids and did not affect lipoprotein profiles, which are markers for whole-body cholesterol synthesis and intestinal sterol absorption. The current data indicate that the postulated effects of garlic on cardiovascular disease are not caused via modulation of plasma lipid levels. J. Nutr. 134: 1500–1503, 2004.

KEY WORDS: • garlic • lipids • cholesterol synthesis • lipoprotein metabolism • APOE*3-Leiden transgenic mice

Garlic is widely used in preventive cardiovascular medicine. Recent studies report that garlic has beneficial effects on risk factors associated with cardiovascular disease, including modulation of plasma lipid levels (1–3). Confirming these reports, previous studies with rabbits and rats treated with garlic also report a modulation of plasma lipids, caused by a decrease in concentrations of plasma cholesterol and triglycerides and markers of cholesterol synthesis and absorption (4–12). In addition, human studies report a decrease in plasma cholesterol and triglyceride levels after garlic treatment (2,13,14). Studies with cultured rat hepatocytes suggest that the inhibition of cholesterol synthesis at least partly explains the possible hypolipidemic effect of garlic (15,16). Conversely, numerous animal and human studies report that garlic treatment does not affect plasma lipids (1–3,17–19). These conflicting data may, at least in part, be due to the use of different garlic-derived materials. The in vitro, in vivo, and human studies used a wide variety of garlic-derived materials, including water- and lipid-soluble garlic extracts, aged garlic extract, garlic oil, garlic powders, garlic-derived sulfur-containing compounds (i.e., S-alllylcysteine, allicin, and alliin), and a metabolite (i.e., diallyldisulfide). In addition, the garlic-derived materials often were produced using nonstandardized procedures and were poorly chemically characterized. Experimental designs and models differed widely among the studies. Studies were conducted with rats, rabbits, or mice with or without cholesterol feeding; normolipidemic and hyperlipidemic human subjects; and coronary heart disease patients, as well as pregnant subjects. Hence, it is difficult to determine whether garlic truly modulates plasma lipids under conditions relevant to human patients.

The present study evaluated the possible hypolipidemic effects of the garlic-derived sulfur-containing compound allicin, the allicin metabolite diallyldisulfide (DADS), and powdered kwai and morado garlic (2 varieties that are well characterized chemically) in mice, under highly standardized conditions that approximate a human model. Allicin and DADS were selected based on the evidence that they might cause the beneficial effects of garlic on lipid metabolism (Fig. 1) (20,21). Commercially available kwai garlic powder was chosen as a standard because it was used in previous garlic studies (2,3,9,10,14). The European variety morado was selected because it was fertilized with higher levels of sulfur during cultivation. All garlic-derived materials were produced under highly standardized procedures and were well characterized chemically. The experiments were conducted with APOE*3-Leiden transgenic mice, a mouse model of mild hyperlipidemia that allows the titration of plasma lipids to selected levels relevant to conditions in humans by the addition of cholesterol and fat to the diet (22). Furthermore, the effects of dietary intervention with nutritional compounds such as fish oil, cafestol, or stanol esters and of treatment with hypolipidemic drugs such as statins and fibrates on plasma lipid and lipoprotein concentrations in APOE*3-Leiden mice are comparable to those in humans (23–25). APOE*3-Leiden mice were fed a nonpurified diet for 4 wk (basal conditions in which endogenous cholesterol synthesis was not suppressed), followed by a Western diet for 8 wk (mild hyperlipidemic conditions in which endogenous cholesterol synthesis was
and triglyceride levels were measured enzymatically, using commercially available kits (No. C0534 and No. 337-B; Sigma Diagnostics). Size fractionation of the plasma lipoproteins was performed using a Smart system (Pharmacia) as previously described (24). Plasma alanine aminotransferase (ALAT) concentration was measured enzymatically (ALAT test; Roche Diagnostics).

Markers of whole-body cholesterol synthesis and intestinal sterol absorption. Plasma intermediates of cholesterol synthesis (lathosterol, lanosterol, and squalene) were used as markers of whole-body cholesterol synthesis, and plant sterols (campesterol and β-sitosterol) were used as markers of intestinal sterol uptake. Plasma concentrations of these markers were determined as previously described (24).

Statistical analysis. All data are presented as means ± SD. Data were analyzed using the Mann-Whitney U test. Food intake data were analyzed by repeated measures for the nonpurified and Western diet testing periods. Differences were considered significant at values of \( P < 0.05 \).

RESULTS

Garlic-derived materials. The powdered kwai and morado garlic varieties contained similar total amounts of sulfur-rich compounds (5.11 and 5.16% by wt, respectively). Kwai contained 1.72% alliin, with an allicin liberation capacity of 7.2 g·kg⁻¹·d⁻¹, 0.25% (by wt) cholesterol, 0.25% vitamin mix, 2.4% mineral mix, 40.5% sucrose, 15% cocoa butter, 1% corn oil, 10% corn flour, 6.20% cellulose, and 20% casein; Hope Farms). Plasma cholesterol concentration was measured, and the mice were assigned to groups with equal mean plasma cholesterol levels.

The true allicin-equivalent dose was calculated from the food and water intake, body weight, and garlic powder composition data for each group (Table 1). The morado group received a lower experimental dose than the other treatment groups over both diet testing periods. Changes in plasma lipids, lipoproteins, and ALAT were minor and inconsistent. When fed the nonpurified diet, the plasma cholesterol levels of mice in the control group when fed the nonpurified diet were 8.0 ± 0.8 vs. 3.1 ± 0.4 mmol·L⁻¹, \( P = 0.039 \). In addition, morado had a higher γ-glutamyl-S-allylcysteine content, compared with kwai (1.29 vs. 1.36%). However, morado had a higher γ-glutamyl-1-propenylcysteine content, compared with kwai (2.14 vs. 1.31%).

Plasma lipids and lipoprotein distribution. In the present study, the switch from the nonpurified diet to the Western diet increased plasma cholesterol levels in mice (3.1 ± 0.4 vs. 9.3 ± 1.4 mmol·L⁻¹, \( P < 0.001 \), as expected (22). Changes in plasma lipid levels were minor and inconsistent. When fed the nonpurified diet, the plasma cholesterol levels of mice in the control group were 8.0 ± 0.8 vs. 3.1 ± 0.4 mmol·L⁻¹, \( P = 0.004 \). However, this difference vanished when the mice were fed the Western diet (9.2 ± 0.8 vs. 9.3 ± 1.4 mmol·L⁻¹, \( P = 0.894 \)). The DADS group had plasma triglyceride levels 25% lower than those of the control group when fed the nonpurified diet (0.9 ± 0.3 vs. 1.2 ± 0.3 mmol·L⁻¹, \( P = 0.039 \)). Again, this difference vanished when mice were fed the Western diet (1.0 ± 0.4 vs. 1.0 ± 0.4 mmol·L⁻¹, \( P = 1.000 \)). The morado group had plasma triglyceride levels 17% higher than those of the control group when fed the Western diet (1.2 ± 0.3 vs. 1.0 ± 0.4 mmol·L⁻¹, \( P = 0.026 \)). In addition, the lipoprotein distributions were consistent with the measured plasma cholesterol (Fig. 2) and triglyceride levels (data not shown) across both diet testing periods.
All treatment groups had similar plasma ALAT levels in both diet testing periods. In addition, the treatments had no effect on ALAT levels, indicating normal liver function relative to the control group (33 ± 13 vs. 30 ± 1 U·L⁻¹).

**Markers of whole-body cholesterol synthesis and intestinal sterol absorption.** Control levels of the whole-body cholesterol synthesis markers squalene, lanosterol, and lanosterol in mice fed the nonpurified diet were 1.0 ± 0.3, 0.3 ± 0.1, and 0.4 ± 0.1 μmol·mmol plasma cholesterol⁻¹, respectively. Control levels of the intestinal sterol absorption markers β-sitosterol and campesterol were 8.6 ± 0.5 and 31.1 ± 3.3 μmol·mmol plasma cholesterol⁻¹, respectively. As expected, switching the mice from the nonpurified (cholesterol-free) diet to the Western (cholesterol-containing) diet markedly decreased plasma concentrations of squalene, lanosterol, lanosterol, β-sitosterol, and campesterol (by 50, 69, 50, 79, and 97%, respectively). These data indicated that increasing the amount of cholesterol in the diet decreased both endogenous cholesterol synthesis and the uptake of dietary sterols. In addition, plasma concentrations of the synthesis and uptake markers did not differ between the nonpurified and Western diet periods for any treatment group (data not shown). Hence, the allicin, DADS, kwai, and morado dietary treatments did not affect endogenous cholesterol synthesis and intestinal sterol absorption in the current experiment.

**DISCUSSION**

The present study investigated the hypolipidemic effects of the garlic-derived sulfur-containing compound allicin, its metabolite DADS, and 2 powdered garlic varieties (kwai and morado) in APOE*3-Leiden mice. Treatment with these materials had no consistent effect on plasma cholesterol and triglyceride levels in mice first fed a nonpurified diet (basal condition) then switched to a Western diet (mild hypercholesterolemic condition). Lipoprotein profiles and markers of whole-body cholesterol synthesis and intestinal sterol absorption were not affected. Hence, the data indicate that the postulated beneficial effect of garlic on cardiovascular disease is not caused via modulation of plasma lipid levels.

All treatment groups ingested an equal amount of allicin, either in its pure form, in the form of its bioactive metabolite DADS, or in the form of its precursor allin (in powdered kwai and morado garlic), equal to an allicin or allicin-equivalent dose of 30–45 mg·kg body wt⁻¹·d⁻¹ (Table 1). The present study demonstrated that allicin lacks a hypolipidemic effect in all these forms, at least at the maximum dose tolerated by mice with free access to food. The powdered kwai and morado garlic are complex mixtures that also contain the sulfur-rich compounds γ-glutamyl-S-allylcysteine and γ-glutamyl-1-propenylcysteine. γ-Glutamyl-S-allylcysteine inhibits cholesterol synthesis in rat hepatocytes (16). The present study showed that the γ-glutamyl-S-allylcysteine and γ-glutamyl-1-propenylcysteine components of the garlic preparations do not modulate the hypolipidemic effect of allicin at the dosage applied, nor do they have hypolipidemic potential themselves. In addition, the kwai and morado preparations contained no other sulfur-rich or nonsulfur compounds with hypolipidemic properties.

The Western diet used in the present study contained 0.25% cholesterol by weight. Consumption of this low-cholesterol diet itself induces plasma cholesterol levels up to 10 mmol·L⁻¹ in APOE*3-Leiden mice. In contrast, it is difficult to induce hypercholesterolemia in normal mice, rats, and rabbits, and (mild) elevation of plasma cholesterol can be

**FIGURE 2** The effect of dietary garlic-derived sulfur-rich compounds (allicin and diallyldisulfide) and powdered garlic varieties (kwai and morado) on lipoprotein cholesterol concentrations in APOE*3-Leiden mice fed a nonpurified diet for 4 wk and a Western diet for 8 wk. Samples from 12 mice per group were pooled.

**TABLE 1**

Dose of garlic-derived material administered to mice, as calculated prior to dietary treatment, measured after 4 wk of supplemental treatment with a nonpurified diet, and measured after a subsequent 8 wk of supplemental treatment with a Western diet

<table>
<thead>
<tr>
<th>Group</th>
<th>Preparation</th>
<th>Allicin equivalent</th>
<th>Preparation²</th>
<th>Allicin equivalent³</th>
<th>Preparation²</th>
<th>Allicin equivalent³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allicin</td>
<td>44.0</td>
<td>44.0</td>
<td>51.5</td>
<td>51.5</td>
<td>42.8</td>
<td>42.8</td>
</tr>
<tr>
<td>DADS</td>
<td>40.0</td>
<td>44.0</td>
<td>38.4</td>
<td>43.2</td>
<td>33.0</td>
<td>36.6</td>
</tr>
<tr>
<td>Kwai</td>
<td>6300.0</td>
<td>44.1</td>
<td>6638.0</td>
<td>47.7</td>
<td>5760.0</td>
<td>41.4</td>
</tr>
<tr>
<td>Morado</td>
<td>6300.0</td>
<td>44.1</td>
<td>6680.0</td>
<td>36.3</td>
<td>5462.0</td>
<td>29.7</td>
</tr>
</tbody>
</table>

¹ Data assume that each mouse weighs 20 g and consumes 3 mL of water and 3 g of diet daily; 1 mol of allicin is converted to 1 mol of DADS; and kwai and morado garlic powder have a similar allin content and allicin liberation capacity (1.7% by wt and 7 g·kg⁻¹, respectively, per data provided by the manufacturer).
² Data corrected for food intake and body weight after 4 wk (nonpurified diet period) and 12 wk (Western diet period) of experimental treatment.
³ Data corrected for allicin liberation capacity as assayed by HPLC of kwai and morado garlic powder at the end of the experiment (kwai: allin content 1.72%, allicin liberation capacity 7.2 g·kg⁻¹; morado: allin content 1.19%, allicin liberation capacity 5.4 g·kg⁻¹).
induced only by supplementing diets with high concentrations (1 to 2%) of cholesterol. Most hypolipidemic effects of garlic reported in mice, rats, and rabbits were observed under these extreme dietary cholesterol conditions, and the relevance of the outcome of these studies is debatable (4.6–10.12). Whether cholesterol intake is an important determinant of the outcome of a garlic study is subject to speculation. However, in this light it is noteworthy that most human studies, like the present transgenic mouse study, report a negative outcome regarding the effect of garlic on plasma lipid levels.

The postulated hypolipidemic effects of garlic are thought to be mediated via suppression of hepatic cholesterol synthesis (15,16). Cholesterol synthesis in mice (and in rodents in general) can easily be suppressed by dietary cholesterol (24). Also, in the current mouse model, cholesterol synthesis was suppressed by consumption of a diet containing only 0.25% cholesterol (see Results). As mentioned above, most studies with mice, rats, and rabbits that report a hypolipidemic effect of garlic used diets containing 1 to 2% cholesterol. Under these extreme conditions of dietary cholesterol, it seems unlikely that garlic could still induce hypolipidemic effects via suppression of endogenous cholesterol synthesis. Two recent human trials included markers for cholesterol synthesis in their plasma analysis (27,28). Bolstering the present data, these studies report no effect of garlic on these markers (27,28). In addition, they report no effect of garlic on plasma cholesterol.

The present study supports the conclusion that any beneficial effect of garlic on cardiovascular disease is not caused via modulation of plasma lipid levels. Whether garlic might exert other beneficial effects relevant to cardiovascular disease remains an open question. Several animal and human studies report that garlic may lower systolic blood pressure (1–2). However, as with plasma lipids, the literature reports conflicting data regarding blood pressure (3). In the current study, treatment with alliin, DADS, and kwai and morado garlic powder did not affect basal systolic blood pressure (99 ± 3 and 103 ± 8 mmHg for the nonpurified and Western diets, respectively), whereas previous studies reported that APOE*3-Leiden mice do respond to blood-pressure-lowering compounds such as the calcium antagonist amlopidine (unpublished data). Hence, the present data do not support the hypothesis that garlic exerts a beneficial effect on cardiovascular disease via modulation of systolic blood pressure.

In summary, this study found no hypolipidemic effect of garlic components and preparations. Further examination of the possible beneficial effects of garlic in cardiovascular disease by directly determining its effects on the progression of atherosclerotic disease is needed. Studying these effects under highly standardized conditions with a sensitive animal model that has a human-like lipid profile will enhance the relevance of the findings to humans. APOE*3-Leiden transgenic mice would be a helpful tool in this regard.

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LITERATURE CITED