Absence of an atheroprotective effect of the garlic powder printanor in APOE*3-Leiden transgenic mice

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Received 10 March 2004; received in revised form 21 June 2004; accepted 22 July 2004
Available online 11 September 2004

Abstract
Numerous animal studies have reported that garlic can protect against atherosclerosis. However, a comparable number of studies do not support this observation. This contradiction may result from differences in study design, use of different animal models, and use of different garlic formulations and preparations. Here, we investigated the effect of the chemically well-characterized and production-controlled garlic powder printanor on atherosclerosis in the APOE*3-Leiden transgenic mouse, a mouse model well suited for evaluating anti-atherosclerotic properties of drugs and food components under human-like conditions. APOE*3-Leiden mice were fed a Western diet supplemented with either 5 or 50 g kg⁻¹ printanor. As a reference, the commercially available fermented garlic kyolic was included (1.6 g kg⁻¹ diet). Treatment with printanor demonstrated reduced body weight, coinciding with increased feces production and fecal fatty acids excretion. Printanor and kyolic treatment did not affect plasma lipids, markers of inflammation (serum amyloid A, serum-soluble intercellular adhesion molecule-1, and blood-leukocytes tumor necrosis factor-α) production) and vascular activation (plasma von Willebrand factor). As analyzed after 28 weeks of treatment, printanor and kyolic did not affect atherosclerotic lesion type, area or composition. Under conditions relevant to the human situation, the well-characterized and production-controlled garlic powder printanor does not display hypolipidemic, anti-inflammatory or anti-atherosclerotic properties.

Keywords: Garlic; Body weight; Inflammation; Atherosclerosis; APOE*3-Leiden transgenic mice

1. Introduction
Garlic has been reported to have beneficial effects on risk factors associated with cardiovascular disease (CVD) including lowering of plasma lipids, systolic blood pressure, decrease of pro-inflammatory cytokines production and reduction of platelet activation state [1–7]. Through these actions garlic is thought to protect the vessel wall from progressive atherosclerotic disease and consequently CVD [1–4]. Numerous animal studies have been performed showing that garlic indeed has beneficial effects on risk factors associated with CVD [1,2]. However, a comparable number of animal studies do not support these observations [1,2]. Limited number of animal studies focused on the direct effect of garlic on atherosclerotic development. The latter studies included rabbit and mouse studies that showed a garlic reducing effect on atherosclerotic lesion area [8–11]. In contrast, pig and rat studies showed that garlic did not lower atherosclerotic lesion area [12,13]. These conflicting data may be ascribed to the use of the different animal models, as well as to the use of different garlic-derived materials, ranging from raw gar-
lic and garlic powders (with variation in production conditions and chemical composition) to isolated sulfur-containing compounds. In addition, to induce an atherogenic lipoprotein profile, previous animal studies were performed using diets containing a varying and high amount of cholesterol (1–2%, w/w), and one can question the relevance of the outcome of these studies for the human situation. Hence, it remains difficult to conclude whether garlic truly has an atheroprotective effect under conditions relevant to the human situation.

In the present study, we evaluated the anti-atherosclerotic properties of a chemically well-characterized and production-controlled garlic powder derived from the garlic variety printanor. This high-quality garlic powder is produced under high sulfur-fertilization levels during the cultivation, consists of high levels of well-defined bioactive sulfur-containing compounds including allini, S-glutamyl-S-allylcysteine and γ-glutamyl-1-propenylcysteine [14]. Printanor has been produced as a part of European Union research program entitled “Garlic and Health” carried out by a consortium of 15 independent research groups from six countries. The main goals of the “Garlic and Health” project are to further understand and improve the production of active compounds by garlic and to further understand the role of garlic as diet and as therapy in promoting and sustaining health and preventing cancer and importantly cardiovascular diseases. The anti-atherosclerotic properties of printanor were investigated in APOE*3-Leiden transgenic mice. These mice carry a rare genetic variant of APOE, the APOE*3-Leiden, which is associated with a dominantly inherited familial hyperlipoproteinemia (FH) in humans. Their lipoprotein profile is very similar to the FD patients in which elevated triglycerides, lipoproteins, markers of inflammation (serum C-reactive protein, lipoprotein(a), lipopolysaccharide-induced tumor necrosis factor-α (TNFα) production by blood-derived monocytes) and vascular activation (plasma von Willebrand factor (vWF)) are detected [9]. In addition, printanor and kyloric treatment did not affect atherogenic lesion type, area, and composition at the level of the aortic root.

2. Materials and methods

2.1. Mice and experimental diets

Five-month-old female APOE*3-Leiden transgenic mice backcrossed into a C57Black6J background were used [18]. Fifty-eight mice were fed a semi-synthetic Western diet containing 0.2% cholesterol, 15% cocoa butter, 40.5% sucrose, 1% corn oil, 20% casein, 6.2% cellulose, and 2% choline chloride (supplied by Hope Farms, Woerden, The Netherlands) for 3 weeks. Thereafter, mice were assigned to four different treatment groups (n = 14–15 mice per group) with equal average of plasma cholesterol levels. One control group continued receiving the Western diet, two experimental groups received the Western diet supplemented with the French garlic powder printanor (supplied by INRA, Dijon, France) at a concentration of 5 g kg

−1 (0.5%) and 50 g kg

−1 (5%) diet, and one group received the Western diet supplemented with the fermented garlic kyloric (kyloric reserve, Wakunaga, Mission Viejo, CA, USA) at a concentration of 1.6 g kyloric kg

−1 diet. To mask the possible taste of the incorporated garlic-derived material sugar lumps containing alliin (33.3 g kg

−1 diet) were supplemented to the diets. Printanor and kyloric were analyzed by standard high-performance liquid chromatography (HPLC) procedure for content of garlic sulfur-containing compounds (alliin, S-glutamyl-S-allylcysteine and γ-glutamyl-1-propenylcysteine) [14]. All (experimental) diets and water were given ad libitum. The institutional committee on animal welfare of TNO approved all animal experiments.

2.2. Body temperature and total fecal fatty acids composition

Body temperature was measured using a rectal probe for mice (RET-3, Physiostemp instruments, Clifton, NJ, USA).

Feces were collected during a 3-day period per cage (n = 3–4 mice per cage), lyophilized and weighed. To extract fatty acids from a pool of dried feces, 15 mg of dried feces were treated with 1 mL of alkaline methanol, as previously described for bile acids measurement, using pentadecanoic acid as internal standard [19]. After treatment, the tubes were cooled to 4 °C and the fatty acids were extracted with 5 mL of 6% potassium carbonate after centrifuging for 10 min at 14,000 × g. The fatty acids were separated on a gas chromatograph (GC) (Varian, Middelburg, The Netherlands) in a gas chromatograph (GC) (Varian, Middelburg, The Netherlands) equipped with a flame ionization detector. The injector and the flame ionization detector were
kept at 270 °C. The column temperature was programmed from 170–210 °C. Quantification was based on the area ratio of the individual fatty acid to the internal standard [19].

2.3. Blood analysis

After a 4 h fasting period from 7:00 to 11:00, blood samples were collected from each individual mouse by tail bleeding for isolation of EDTA-plasma or serum. Total plasma cholesterol and triglyceride levels were measured enzymatically, using commercially available kits (No. C0534 and No. 337-B, Sigma Diagnostics, Deisenhofen, Germany). Size fractionation of plasma lipoproteins was performed using a Smart-system (Pharmacia, Uppsala, Sweden) as previously described [16].

Plasma alanine aminotransferase (ALAT) was measured enzymatically per mouse (ALAT test, Roche Diagnostics, Mannheim, Germany).

Serum amyloid A (SAA), soluble intercellular adhesion molecule-1 (soluble ICAM1), and plasma vWF were measured in EDTA-coated vials. Blood was diluted 25 molecule-1 (soluble ICAM1), and plasma vWF were measured enzymatically per mouse (ALA T test, Roche Diagnostics, Mannheim, Germany). Size fractionation of plasma lipoproteins was performed using a Smart-system (Pharmacia, Uppsala, Sweden) as previously described [16].

Plasma alanine aminotransferase (ALAT) was measured enzymatically per mouse (ALAT test, Roche Diagnostics, Mannheim, Germany).

2.4. Tumor necrosis factor-α (TNFα) production assay

Whole blood was obtained by tail bleeding and collected in EDTA-coated vials. Blood was diluted 25 times in RPMI medium supplemented with 1-glutamine, penicillin, and streptomycin, (RPMI 1640, BioWhittaker, Verviers, Belgium), which contained varying concentrations of lipopolysaccharide (LPS, Re 595, List Biological Laboratories, Campbell, CA). Following incubation overnight at 37 °C, 50 μL of the supernatant was analyzed for TNFs content by ELISA (TNFα, BD-Biosciences Pharmingen, San Diego, CA, USA) [21].

2.5. Atherosclerosis analysis

Hearts and aortas were fixed in phosphate-buffered 4%-formaldehyde, dehydrated, and embedded in paraffin. Hearts were cross-sectioned (5-μm) throughout the entire aortic root area. Per mouse, four sections with 30-μm intervals were used for quantification of atherosclerotic lesion area [22]. Sections were routinely stained with hematoxylin-phloxine-saffron (HPS). Lesion area was determined using Leica Qwin image analysis software (EIS, Asbury NJ) [22]. The number of leukocytes attached to the endothelium was counted in the same four HPS-stained sections with 30-μm intervals also used for the quantification of atherosclerotic lesion area [23].

2.6. Statistical analysis

All data are represented as mean ± S.D. Data were analyzed using the Mann–Whitney U-test. Food intake data was analyzed with general linear model-repeated measures. p-values less than 0.05 were regarded as statistically significant.

3. Results

3.1. Tolerance of experimental diets

Printanol and kyolic were analyzed for the content of garlic sulfur-containing compounds (alliin, γ-glutamyl-S-allylcysteine and γ-glutamyl-1-propenylcysteine) by high-performance liquid chromatography analysis. Printanol and kyolic contained a comparable amount of alliin (1.61% versus 1.48%, w/w) and γ-glutamyl-S-allylcysteine (0.44% versus 0.54%, w/w). However, printanol had a four-fold higher content of γ-glutamyl-1-propenylcysteine as compared with kyolic (1.27% versus 0.33%, w/w). Thereby, printanol garlic powder contained approximately 30% more of these bioactive sulfur-rich compounds when compared with kyolic (3.32% versus 2.35%, w/w).

All experimental diets were well tolerated and no differences were observed in food intake for mice that consumed experimental garlic containing diet or a control diet. All groups of mice appeared healthy and gained body weight to the same extent during the 28 weeks of the study. However, from week 12 on, the 5% printanol-treated mice had a significantly lower body weight gain (Table 1), resulting in a significant reduction in body weight as compared with controls (23.1 ± 2.4 g versus 24.9 ± 2.3 g, p = 0.007 for week 28). The 0.5% printanol-dose-treated mice also displayed a (non-significant) lower body weight gain (Table 1), resulting in a significant reduction in body weight as compared with controls (23.1 ± 2.3 g versus 24.9 ± 2.4 g, p = 0.026 for week 28).

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food intake (g/mouse/day)</th>
<th>Body weight gain (g)</th>
<th>Total dry feces production (g feces/100 g mouse/day)</th>
<th>Total fatty acid excretion in feces (g/g feces)</th>
<th>Total fatty acid excretion (g/100 g mouse/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.8 ± 0.1</td>
<td>6.2 ± 0.1</td>
<td>1.5 ± 0.3</td>
<td>0.027 ± 0.006</td>
<td>0.037 ± 0.007</td>
</tr>
<tr>
<td>0.5% Printanol</td>
<td>2.9 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>1.7 ± 0.4</td>
<td>0.032 ± 0.008</td>
<td>0.052 ± 0.012</td>
</tr>
<tr>
<td>5% Printanol</td>
<td>2.8 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>1.9 ± 0.5</td>
<td>0.029 ± 0.008</td>
<td>0.053 ± 0.013</td>
</tr>
<tr>
<td>Kyolic</td>
<td>2.9 ± 0.1</td>
<td>6.3 ± 2.6</td>
<td>1.7 ± 0.4</td>
<td>0.023 ± 0.007</td>
<td>0.034 ± 0.013</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. for 14–15 mice per group. *p < 0.05 as compared with the control group.

Fig. 1. Plasma lipoprotein distribution was determined 28 weeks after garlic dietary treatment. Lipoproteins were size-fractionated by FPLC (1 pool, n = 14–15 mice per group), cholesterol (closed squares) and triglycerides (open squares) content of the individual fractions was determined.

20 and 24.6 ± 2.3 g versus 26.6 ± 3.6 g, p = 0.041 for week 28) from week 20 till the end of the experiment (week 28).

Printanor-treated mice had a body temperature comparable to control mice (Control, 38.3 ± 0.2 °C; 0.5% printanor, 38.3 ± 0.2 °C; 5% printanor, 38.3 ± 0.2 °C), indicating that the lower body weight observed in these groups is possibly not due to higher mouse metabolic activity. However, the 5% printanor-treated group did show a significantly (p = 0.006) 21% higher excretion of dry feces as compared with control (Table 1). Since the fatty acid composition of the dry feces was not affected by printanor, the net waste of fecal fatty acids was significantly increased both in the high and in the low doses of printanor group (Table 1), indicating an increased fecal fatty acids excretion for the printanor (both doses)-treated groups. However, these results do not completely explain the 21% higher excretion of dry feces for the 5% printanor-treated group. Possibly, other compounds are being excreted. Kyolic-treated mice had body weight, body temperature, dry feces production and fecal fatty acid excretion comparable to controls.

3.2. Plasma lipids and alanine aminotransferase

Feeding APOE*3-Leiden mice a Western diet resulted in hyperlipidemia with plasma cholesterol levels ranging from 10–13 mmol/L and plasma triglyceride levels of 1.0–1.5 mmol/L. Fast-performance liquid chromatography (FPLC) analysis revealed that plasma cholesterol and triglycerides were mainly found in the VLDL/LDL-sized lipoprotein fractions and no differences were found between printanor, kyolic or control groups (Fig. 1). During 28 weeks of experiment, printanor (both doses) and kyolic treatment did not affect plasma cholesterol, triglycerides or lipoproteins. Hence, cholesterol (and triglyceride) exposure in the garlic-treated groups is comparable with control group (Table 2).

Printanor (both doses) and kyolic did not affect plasma alanine aminotransferase (ALAT) levels during the study (Control, 112 ± 7 U/L; 0.5% printanor, 93 ± 5 U/L; 5% printanor, 107 ± 6 U/L; kyolic, 118 ± 2 U/L for week 28), indicating a comparable liver function for all experimental groups.

3.3. Inflammation-related parameters

As previously shown, markers of inflammation (serum amyloid A and serum-soluble intercellular adhesion molecule-1) and vascular activation (plasma vWF) are good and sensitive markers to determine the level of inflammation or vascular activation in APOE*3-Leiden mice [17]. Printanor (both doses) and kyolic had no significant reducing effect on serum amyloid A (SAA), serum-soluble intercellular adhesion molecule-1 (ICAM1), and plasma vWF levels, indicating that treatment with these garlic powders did not modulate the inflammatory and/or vascular activation status in these mice under Western diet feeding conditions (Table 3).

We also determined the modulatory effects of printanor (and kyolic) on ex vivo TNFα production by blood-derived monocytes following stimulation with LPS. As illustrated in Fig. 2, blood isolated from control APOE*3-Leiden mice fed a high-cholesterol diet (1% cholesterol and 0.5% cholate-containing diet) produced large amounts of TNFs upon LPS stimulation, significantly higher than mice under reg-

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
<th>Cholesterol exposure (mmol/L weeks)</th>
<th>Triglycerides exposure (mmol/L weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.4 ± 2.5</td>
<td>1.0 ± 0.3</td>
<td>346.3 ± 40.5</td>
<td>40.4 ± 8.9</td>
</tr>
<tr>
<td>0.5% Printanor</td>
<td>12.7 ± 2.5</td>
<td>1.0 ± 0.3</td>
<td>359.8 ± 47.0</td>
<td>39.7 ± 6.8</td>
</tr>
<tr>
<td>5% Printanor</td>
<td>12.6 ± 4.4</td>
<td>0.9 ± 0.2</td>
<td>379.1 ± 47.3</td>
<td>40.5 ± 5.6</td>
</tr>
<tr>
<td>Kyolic</td>
<td>10.3 ± 2.1</td>
<td>0.9 ± 0.3</td>
<td>321.3 ± 32.2</td>
<td>37.4 ± 7.9</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. for 14–15 mice per group. *p < 0.05 as compared with the control group.
Lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF-alpha) production by blood-derived monocytes was determined for control, 0.5% printanor-, 5% printanor-, and kyolic-treated groups (n = six mice per group) in APOE*3-Leiden mice at week 26. In addition, we included six mice on regular chow diet (negative control) and six mice on 0.5% cholate-containing high-cholesterol diet (positive control). Significant differences as compared with control on Western diet are indicated ∗p < 0.05.

Blood drawn from Western diet fed mice produced significantly more TNFs following LPS (concentrations 2.3, 9.4, 75, and 150 ng mL⁻¹ of LPS) stimulation as compared with blood drawn from mice fed a regular chow diet. Although this assay is sufficient to detect a modulatory effect of diet with respect to inflammation, we did not detect any effect of printanor (both doses) or kyolic on LPS-induced blood TNF-alpha production.

3.4. Atherosclerosis analysis

Printanor (both doses) and kyolic treatment did not result in any reducing effect on risk factors associated with cardiovascular disease, i.e. plasma cholesterol, cholesterol exposure, and markers of the level of inflammation. We next determined if printanor and kyolic treatment affects atherosclerosis development in APOE*3-Leiden mice. To this end, we evaluated the atherosclerotic lesion type and quantified atherosclerotic lesion area in the aortic root after 28 weeks of treatment under Western diet conditions Table 3. For all experimental groups, atherosclerotic lesions were found in the aortic intima, ranging from mild foam cell (Type I–II lesions) to fibrous plaques with a cap and a lipid core with some cholesterol clefts and extracellular lipid (Type IV lesions). In our study, qualitative assessment of atherosclerotic lesions type showed that 0.5% printanor or kyolic treatment had comparable percentage of mild (Type I–III) and severe (Type IV–V) lesions when compared with control mice (mild lesions: 75 ± 12%, 72 ± 18%, and 81 ± 10%; severe lesions: 25 ± 10%, 28 ± 7%, and 19 ± 5% for control, 0.5% printanor, and kyolic, respectively) (Fig. 3A). The 5% printanor-treated group had a tendency towards a higher percentage of severe lesions as compared with the control group; however, this did not reach statistical significance (38 ± 17% versus 25 ± 10%, p = 0.073) (Fig. 3A). Quantitative assessment of atherosclerotic lesions area revealed that 0.5% printanor- and kyolic-treated groups had an atherosclerotic lesion area comparable to the control-treated group (Control, 42.3 ± 35.6 × 10³ μm²; 0.5% printanor, 43.5 ± 34.1 × 10³ μm²; kyolic, 30.5 ± 27.7 × 10³ μm²) (Fig. 3B). Although the 5% printanor-treated group had a tendency towards a higher lesion area as compared with the control group (79.9 ± 65.6 × 10³ μm² versus 42.3 ± 35.6 × 10³ μm², p = 0.227), this did not reach statistical significance (Fig. 3B). Printanor (both doses) and kyolic had comparable number of leukocytes attached to the endothelium of the atherosclerotic aortic root (Fig. 3C). In addition, gross visual inspection of HPS-stained sections yielded no differences in saffron-positive collagen, macrophage or smooth muscle content of the lesions of the garlic-treated mice. Hence, printanor (both doses) and kyolic did not (significantly) affect atherosclerosis lesion type, area, or composition.

Table 3

<table>
<thead>
<tr>
<th>Condition</th>
<th>SAA (μg/mL)</th>
<th>Soluble ICAM1 (ng/mL)</th>
<th>vWF (µg/gPP- rad⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.7 ± 6.4</td>
<td>33.5 ± 3.7</td>
<td>1540 ± 90.8</td>
</tr>
<tr>
<td>0.5% Printanor</td>
<td>6.5 ± 5.6</td>
<td>33.5 ± 3.7</td>
<td>1540 ± 90.8</td>
</tr>
<tr>
<td>5% Printanor</td>
<td>4.3 ± 3.0</td>
<td>35.7 ± 5.1</td>
<td>1424 ± 88.6</td>
</tr>
<tr>
<td>Kyolic</td>
<td>6.4 ± 5.4</td>
<td>34.7 ± 5.3</td>
<td>1242 ± 62.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. for 14–15 mice per group. ∗p < 0.05 as compared with control group.

4. Discussion

In the present study, we evaluated the anti-atherosclerotic properties of a chemically well-characterized and production-
controlled garlic powder printanor in APOE*3-Leiden transgenic mice. Printanor treatment did not affect plasma lipids, markers of general inflammation and vessel wall activation, and atherosclerotic lesion type, area or composition. Hence, under conditions relevant to the human situation, garlic powder printanor does not display hypolipidemic, anti-inflammatory or anti-atherosclerotic properties.

Treatment of mice with printanor resulted in a reduction in body weight. Loss of body weight was independent of food intake, and coincided with increased feces production and fecal fatty acid excretion. The disposals of fatty acids, i.e. energy via feces can at least partly explain why the printanor-treated mice gain less body weight. How printanor and its constituents affect fecal fatty acid disposal is subject to speculation, but this may be at the level of printanor-mediated inhibition of food absorption including free fatty acids. Interestingly, Elkayam et al. also observed reduced body weight in rats when feeding the garlic-active compound allicin, suggesting that garlic-active compounds are responsible for the weight reduction [24]. As in our study, reduced body weight gain was independent of food intake and independent of any toxic side effects of alllicin. Via an unknown mechanism, the garlic supplementation to food may be effective in weight control in obesity. Interestingly, since the effects were already observed at low amounts (i.e. printanor 0.5%), this may have implications for the human situation.

Previously, we demonstrated that garlic and garlic sulfur-containing compounds do not display any hypolipidemic properties as evaluated in detail in APOE*3-Leiden mice [25], these data were confirmed in the present study (Fig. 1, Table 2). In vitro studies, using endothelial cells showed that high concentrations of garlic sulfur-containing compounds (S-allylcysteine, aged garlic extract, and garlic powder extracts) decrease cytokine production (interleukin (IL)-1β, IL-6, and TNFα), indicating that garlic may have anti-inflammatory properties [5-7]. In the present study, we observe that printanor and kyolic does not affect serum amyloid A, intercellular adhesion molecule-1, lipopolysaccharide-induced tumor necrosis factor-α production by blood-derived monocytes, or plasma von Willebrand factor. Hence, in contrast to the in vitro data, our in vivo data suggest that printanor and kyolic, and possibly garlic in general, do not display anti-inflammatory properties. The reason for this discrepancy may be the use of unrealistic high concentrations of garlic-derived materials in the in vitro studies, which are not achieved in vivo even at a dosage of 50 g kg⁻¹ (5%) garlic powders in the diet.

In the present study we included kyolic (1.6 g kg⁻¹) as reference, since it was shown to exert an anti-atherogenic effect at least in rabbits (64% less fatty streak-like lesions) [10]. In our hands, kyolic treatment at the same dose had no effect on atherosclerosis (p = 0.477, n = 15 mice). One major difference between the two studies is the type of animal model i.e. rabbits versus APOE*3-Leiden transgenic mice and the type of lesion, i.e. fibro fatty lesions (rabbits) versus human-like lesions (APOE*3-Leiden mice). More importantly, to get the rabbits hypercholesterolemic, the diet was supplemented with a high amount of cholesterol (i.e. 1% w/w). In contrast, APOE*3-Leiden mice easily obtain plasma cholesterol levels of 10-13 mmol/L, when the diet is supplemented with only 0.2% (w/w) cholesterol. We have no mechanistic explanation for the difference in response to kyolic between rabbits and APOE*3-Leiden transgenic mice. However, one can ques-
tion the relevance of the findings of the kyolic rabbit study for the human situation, since a human diet contains far less cholesterol.

In conclusion, this study shows an absence of hypolipidemic, anti-inflammatory and anti-atherogenic effect of the well-characterized and production-controlled garlic powder prantin in a humanized animal model. As such we conclude that prantin has no beneficial effect on CVD. Several studies have been performed showing that garlic may have beneficial effects on risk factors associated with CVD [1,2]. However, numerous studies, including ours, do not support these observations [1,2]. Recently, a meta-analysis of the epidemiologic literature on the association between garlic consumption and risk of various cancers has shown the presence of publication bias in this field [26]. Since there is a possibility of publication bias for the current garlic and CVD topics, we propose detailed and extensive meta-analysis of the literature on the association between garlic and CVD. Such analysis might help to clarify whether garlic truly has beneficial effects in cardiovascular disease in humans.

Acknowledgements

This work was supported by a European Union research project QLK1-CT-1999-498 and by the fellowship of the Royal Netherlands Academy of Arts and Sciences attributed to Dr. B.J.M. van Vlijmen. We thank all the people involved in the EU project “Garlic and Health” for their collaboration and Ria van den Hoogen for technical assistance.

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