Effect of quercetin on traits of the metabolic syndrome, endothelial function and inflammation in men with different APOE isoforms


Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Physiology and Biochemistry of Nutrition, Hermann-Weigmann-Str. 1, D-24103 Kiel, Germany

Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Physiology and Biochemistry of Nutrition, Haid-und-Neu-Str. 9, D-76131 Karlsruhe, Germany

Institute of Human Nutrition and Food Science, Hermann-Rodewald-Str. 6, D-24098 Kiel, Germany

Center of Clinical Research, tecura GmbH, Kiel, Germany

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Abstract

Background and aims: The polyphenol quercetin may prevent cardiovascular diseases due to its vasorelaxant and anti-oxidative properties. We investigated the effects of quercetin on risk factors of atherosclerosis, biomarkers of inflammation and oxidative stress, depending on the apolipoprotein E (APOE) genotype.

Methods and results: In a double-blind crossover study 49 healthy male subjects with APOE genotype 3/3 (n = 19), 3/4 (n = 22) and 4/4 (n = 8) consumed 150 mg/d quercetin or placebo for 8 weeks each, intermitted by a three-week washout phase. After each intervention, endothelial function, anthropometry, metabolic and inflammatory parameters were measured in the fasting and postprandial state following a standardized lipid-rich meal.

Endothelial function was not changed. In all subjects combined, quercetin significantly decreased waist circumference (P = 0.004) and postprandial systolic blood pressure (P = 0.044). Postprandial triacylglycerol concentrations were significantly decreased and HDL-cholesterol concentrations increased after quercetin as compared to placebo consumption (P = 0.025). Quercetin also moderately increased levels of TNFα (P = 0.024). There
was a significant gene—diet interaction for waist circumference and for body mass index (BMI).

Conclusions: Quercetin supplementation improved some risk factors of cardiovascular disease, yet exerted slightly pro-inflammatory effects. Genotype-dependent effects were seen only on waist circumference and BMI. © 2011 Elsevier B.V. All rights reserved.

Introduction

There is some evidence that the ingestion of flavonoids may be inversely correlated with the incidence of cardiovascular diseases and related risk factors [1]. Quercetin—a major dietary flavonoid—occurs naturally in many plant foods, mainly in onions, broccoli, green cabbage and apples, and in lower concentrations in black tea and red wine. Quercetin may act through various anti-inflammatory mechanisms, e.g. regulation of the expression of cellular adhesion molecules and of the secretion of pro-inflammatory cytokines and chemokines [2,3]. Quercetin reduced also blood pressure both in human and animal studies [4–6] and inhibited the platelet activation pathway in man [7]. It improved endothelial function in rat models of hypertension [8]. Both short- and long-term black tea consumption improved endothelial function in patients with existing coronary artery disease [9].

The multifunctional and polymorphic protein apolipoprotein E (APOE ) plays a key role in the metabolism of plasma lipids and affects the development of atherosclerosis. There are three isoforms designated E2, E3, and E4 in which carriers of the APOE4 allele are at a higher coronary risk [10]. APOE4 has arginine instead of cysteine at position 112 and thus one SH-group less than APOE3. This may contribute to impaired antioxidant activity. The fact that the anti-oxidative capacity of APOE4 is lower compared to the other isoforms could render them more susceptible to cardiovascular disease [11]. Furthermore APOE4 is associated with larger VLDL and potentially atherogenic remnant size triacylglycerol-rich lipoproteins (TRL), whereas APOE3 preferentially binds to HDL particles [12] which have anti-inflammatory and antiatherogenic properties. Dysfunction of either the coronary or peripheral vascular endothelium constitutes an early and independent predictor of cardiovascular events [1,13]. The APOE4 allele was associated with impaired endothelium-dependent arterial dilation in the early stage of type 2 diabetes mellitus [14].

Both normolipidemic and hyperlipidemic subjects with the APOE4 genotype showed a stronger postprandial triacylglycerol response than respective subjects with APOE3/3 [15]. The protective effect of dietary compounds may be better visible in the postprandial state as well [9]. Furthermore, potentially atherogenic and pro-inflammatory remnant lipoproteins that may contribute to endothelial dysfunction are generated in the postprandial state [16]. Anti-oxidants such as ascorbic acid can enhance endothelial function [17]. Since quercetin consumption was expected to exert anti-oxidative effects the impact of an 8-week quercetin intervention on endothelial function was investigated. In addition, metabolic, oxidative and inflammatory parameters were assessed. Carriers of the APOE4 allele were compared to APOE3 homozygotes from a prospectively genotyped study group in a double-blind randomized controlled clinical trial, both in the fasting and in part in the postprandial state, following a lipid-rich meal.

Methods

Study population

Forty-nine subjects (age 48–68 years) were recruited from the population-based cohort Metabolic Intervention Cohort Kiel (MICK, n = 1508) characterized previously [18]. Major exclusion criteria were known disorders that affect the digestion and metabolism of food components, and established diabetes mellitus (fasting glucose levels >6.9 mmol/L after repeated determination). Further exclusion criteria were intake of lipid lowering and anti-hypertensive drugs, hormones, and other drugs with an impact on gastrointestinal motility, absorption or metabolism of nutrients. Eight APOE4/4 homozygotes were matched with 20 APOE3/3 homozygotes and 22 APOE3/4 heterozygotes. Right at onset two subjects with APOE3/3 and one with APOE3/4 dropped out of the study, because of a herniated vertebral disc, inability to swallow the capsules and night sweat. Only the latter condition may be related to treatment. They were replaced by other APOE3/3 and 3/4 genotype carriers. The study was approved by the local Ethics Advisory Committee and carried out according to the Helsinki declaration. Participants gave written informed consent prior to the study.

Study design

The study was single-centre, double-blind, randomized, crossover, placebo-controlled, and performed between May 2007 and May 2008. Two 8-week treatment periods were separated by a 3-week washout period. Subjects were randomly assigned to receive a total of either 150 mg quercetin dihydrate (Voigt Global Distribution Inc., Lawrence, KS, USA) or placebo respectively, provided in six capsules per day, which were consumed with the three principal meals, two capsules per meal. Verum and placebo capsules were identical in shape and taste. Compliance was checked by counting returned capsules. It was considered sufficient if >85% of the capsules were consumed. All participants achieved this goal. Participants were instructed not to change their eating habits and physical activity routine, and not to use any dietary supplements of vitamins, minerals, or oil preparations.

Anthropometric parameters and blood pressure were recorded following standard operation procedures according to WHO and Deutsche Hochdruckliga, respectively and
blood samples were collected at baseline (day zero) and after every 8-week intervention. Fasting blood samples were collected between 0700 and 0900. A potato soup (as previously described [19]) containing 60 g sunflower oleic oil (Henry Lamotte Oils GmbH, Bremen, Germany), was then consumed within 15 min. After the meal blood samples were collected repeatedly, after 30 and 60 min and then hourly over a period of 8 h for the measurement of post-prandial triacylglycerol concentration, and over a period of 5 h for glucose and insulin concentrations. Plasma or serum aliquots were stored at –20 °C or –80 °C until analysis, depending on assay requirements.

Endothelial function

Vascular endothelial function was assessed at a separate visit two days after anthropometry and blood sampling, at the start of the study and after every 8-week intervention, by reactive hyperemia with finger plethysmographic methodology (Reactive Hyperemia peripheral arterial tonometry, PAT) using the Endo-PAT2000 system (Itamar, Caesarea, Israel), as described previously [20]. PAT index was determined in the fasting state and 4 h after consuming the meal described above. PAT index was calculated as the ratio of the average amplitude of the PAT signal post occlusion divided by the average baseline amplitude, and corrected to the non-occluded control arm. Low PAT readings predict adverse cardiovascular events [20] and show a significant association with adiposity and other cardiovascular risk factors [21]. PAT measurement has been approved by the FDA.

Biochemical analyses

Routine biochemical parameters were analyzed in an accredited laboratory (University Hospital Kiel). Glucose, total (TC), high density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) cholesterol, and triacylglycerol concentrations for HDL-C, LDL-C, fasting triacylglycerols and glucose. In all subjects. Statistical analysis was performed with SPSS (SPSS for windows, Release 18, LEAD Technologies Inc.). Data are given as means ± SEM.

Results

Characteristics of study subjects at baseline

Baseline body weight, body mass index (BMI) and waist circumference did not differ between APOE genotype groups (Table 1). The different genotypes showed similar concentrations for HDL-C, LDL-C, fasting triacylglycerols and glucose. In APOE4 carriers, TC concentration tended to be higher than in APOE3/3 homozygotes (P = 0.051). Fasting PAT indices were significantly higher in the APOE4 group (P < 0.01), suggesting better endothelial function, but postprandial endothelial function did not differ between APOE isoforms. Postprandial PAT correlated with fasting PAT indices (r = 0.366; P < 0.01), but neither fasting nor postprandial PAT correlated with the respective systolic (SBP) or diastolic blood pressure (DBP). CRP concentration was significantly lower in the APOE4 compared to the E3/3 group (P < 0.05).

Anthropometry

Quercetin treatment as compared to placebo decreased waist circumference by 0.63 cm (P < 0.01) in all subjects. A significant diet-by-genotype interaction effect was
Data are expressed as mean (Fig. 1, Table 2). The postprandial glucose increase was also significantly lower following quercetin treatment (slightly but non-significantly attenuated following placebo). The AUC over 8 h was not lower in quercetin treatment (P = 0.085) (Fig. 1, Table 2). The postprandial glucose increase was also slightly but non-significantly attenuated following quercetin treatment (P = 0.066). None of these changes differed by genotype. Other lipid parameters, fasting glucose and insulin concentrations and postprandial insulin remained largely unchanged. Independently of the intervention, the APOE4 genotype was associated with significantly higher TC (P = 0.05) and non-significantly higher LDL-C concentrations (P = 0.075).

**Lipid peroxidation and inflammation**

Quercetin increased TNFα concentration by 0.11 pg/ml (P < 0.05) and decreased total GSH concentration in erythrocytes non-significantly (P = 0.067). Other inflammatory parameters (s-E-Selectin, s-VCAM, s-ICAM, oxLDL, hs-CRP) and the urinary isoprostane 8-iso-PGF2α were not changed.

**Plasma quercetin**

Quercetin concentration in plasma was increased from 121.9 ± 7.5 to 193.8 ± 20.4 nmol/L following quercetin as compared to placebo consumption (n = 49, P < 0.01). There was no genotype-dependent difference of responsiveness (data not shown), as observed in another recent quercetin supplementation trial [6].

**Discussion**

Recently, the cardiovascular effects of quercetin were investigated in a group of men and women with a high cardio-metabolic risk phenotype where the APOE polymorphism was retrospectively genotyped [6]. In this study we prospectively genotyped members of the MICK cohort and matched different APOE genotypes for similar body shape, weight and age. Our male study subjects were of normal weight and without signs of the metabolic syndrome.
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Area under the curve of the triacylglycerol response during the first 4 h postprandially. Means ± SEM; *p < 0.05.

**Figure 1** (A) Fasting and postprandial triacylglycerol concentrations following 8-week placebo or quercetin supplementation. (B) Area under the curve of the triacylglycerol response during the first 4 h postprandially.

**Table 2** Fasting and postprandial parameters after 8-week placebo and quercetin supplementation according to the APOE polymorphism.

<table>
<thead>
<tr>
<th></th>
<th>APOE3/3 (n = 19)</th>
<th>APOE4 (n = 30)</th>
<th>p intervention</th>
<th>p genotype</th>
<th>p interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>Placebo</td>
<td>26.3 ± 0.5</td>
<td>Quercetin</td>
<td>26.1 ± 0.5</td>
<td>0.320</td>
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<tr>
<td>Body weight (kg)</td>
<td>Placebo</td>
<td>84.7 ± 1.8</td>
<td>Quercetin</td>
<td>84.2 ± 1.7</td>
<td>0.319</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>Placebo</td>
<td>100.1 ± 1.6</td>
<td>Quercetin</td>
<td>98.4 ± 1.4</td>
<td>0.004</td>
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<td>Fasting parameters</td>
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<td></td>
</tr>
<tr>
<td>Endo-PAT</td>
<td>Placebo</td>
<td>1.95 ± 0.19</td>
<td>Quercetin</td>
<td>1.96 ± 0.19</td>
<td>0.821</td>
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<tr>
<td>SBP (mmHg)</td>
<td>Placebo</td>
<td>135.8 ± 3.6</td>
<td>Quercetin</td>
<td>133.4 ± 3.9</td>
<td>0.094</td>
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<td>DBP (mmHg)</td>
<td>Placebo</td>
<td>82.7 ± 1.3</td>
<td>Quercetin</td>
<td>81.7 ± 2.1</td>
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<td>Glucose (mmol/L)</td>
<td>Placebo</td>
<td>5.74 ± 0.13</td>
<td>Quercetin</td>
<td>5.64 ± 0.12</td>
<td>0.535</td>
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<td>Insulin (pmol/L)</td>
<td>Placebo</td>
<td>109.0 ± 12.5</td>
<td>Quercetin</td>
<td>103.5 ± 9.8</td>
<td>0.228</td>
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<td>HOMA-IR</td>
<td>Placebo</td>
<td>3.94 ± 0.53</td>
<td>Quercetin</td>
<td>3.66 ± 0.42</td>
<td>0.228</td>
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<td>Triacylglycerols (mmol/L)</td>
<td>Placebo</td>
<td>1.45 ± 0.11</td>
<td>Quercetin</td>
<td>1.42 ± 0.12</td>
<td>0.138</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>Placebo</td>
<td>5.22 ± 0.25</td>
<td>Quercetin</td>
<td>5.34 ± 0.19</td>
<td>0.205</td>
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<td>HDL-cholesterol (mmol/L)</td>
<td>Placebo</td>
<td>1.23 ± 0.07</td>
<td>Quercetin</td>
<td>1.31 ± 0.09</td>
<td>0.025</td>
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<td>LDL-cholesterol (mmol/L)</td>
<td>Placebo</td>
<td>3.25 ± 0.19</td>
<td>Quercetin</td>
<td>3.33 ± 0.15</td>
<td>0.400</td>
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<tr>
<td>s-E-Selectin (ng/mL)</td>
<td>Placebo</td>
<td>48.2 ± 6.1</td>
<td>Quercetin</td>
<td>49.8 ± 6.4</td>
<td>0.188</td>
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<td>s-VCAM (ng/mL)</td>
<td>Placebo</td>
<td>829.5 ± 41.3</td>
<td>Quercetin</td>
<td>837.9 ± 43.8</td>
<td>0.827</td>
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<td>s-ICAM (ng/mL)</td>
<td>Placebo</td>
<td>239.8 ± 13.4</td>
<td>Quercetin</td>
<td>242.1 ± 12.9</td>
<td>0.324</td>
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<td>oxLDL (U/L)</td>
<td>Placebo</td>
<td>81.1 ± 5.1</td>
<td>Quercetin</td>
<td>80.3 ± 4.1</td>
<td>0.681</td>
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<td>GSH (nmol/mL erythrocytes)</td>
<td>Placebo</td>
<td>1499 ± 71</td>
<td>Quercetin</td>
<td>1415 ± 66</td>
<td>0.067</td>
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<tr>
<td>CRP (mg/L)</td>
<td>Placebo</td>
<td>3.30 ± 0.97</td>
<td>Quercetin</td>
<td>4.07 ± 1.41</td>
<td>0.174</td>
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<tr>
<td>TNFα (pg/mL)</td>
<td>Placebo</td>
<td>1.76 ± 0.15</td>
<td>Quercetin</td>
<td>1.98 ± 0.17</td>
<td>0.204</td>
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<tr>
<td>8-iso-PGF2α (pg/mg creatinine)</td>
<td>Placebo</td>
<td>191.5 ± 164.3</td>
<td>Quercetin</td>
<td>143.6 ± 81.2</td>
<td>0.400</td>
</tr>
</tbody>
</table>

Bold indicates P-values less than 0.05.

**PAT** = peripheral arterial tonometry; **SBP** = systolic blood pressure; **DBP** = diastolic blood pressure; **HOMA-IR** = homeostasis model assessment of insulin resistance; **CRP** = C-reactive protein.

Data are expressed as mean ± SEM; p for **RM-ANOVA**.
except for slightly increased SBP. Endothelial function appeared normal [21]. At entry APOE4 subjects had just marginally higher TC concentration (which was confirmed during the intervention period) and only trends toward higher LDL-C and triacylglycerol concentrations, opposite to what might have been expected [6,10]. CRP concentrations were lower in APOE4 than APOE3/3 subjects, as observed before in a large population-based study. Those authors concluded that the serum CRP is independently determined by the common genetic polymorphisms within the APOE gene [23]. The nature of this unexpected association between APOE genotype and CRP is not clear.

Quercetin treatment tended to decrease fasting SBP and significantly decreased postprandial SBP by 5.7 mmHg, confirming previous observations in humans [4,6]. While Egert et al. [6] observed an improvement only in APOE3 homozygous subjects, both APOE3/3 and APOE4 subjects benefited in our study (Table 2). Despite favorable changes in postprandial SBP, endothelial function was not significantly improved, neither the fasting nor the postprandial values. This may be due to the fact that study participants showed fairly normal PAT indices to start with [21]. Fitting with this outcome endothelium-derived adhesion molecules E-selectin, ICAM and VCAM as well as oxLDL were not affected by quercetin administration. Contrary to expectations baseline endothelial function was better in APOE4 as compared to APOE3/3 genotype, but following placebo treatment the difference was no longer significant. In animal models quercetin improved endothelial function under hypertoncise conditions only [8]. Furthermore quercetin decreased SBP, and improved dyslipidemia and insulin sensitivity only in obese Zucker rats, but not in lean rats [5].

Again baseline PAT indices did not correlate with blood pressure, emphasizing that factors regulating endothelial function differ at least in part from those regulating blood pressure.

In this study, quercetin did not change fasting, but decreased the postprandial triacylglycerol concentrations. This may result from a quercetin-induced reduction of fatty acid and triacylglycerol synthesis in the liver [24]. In some previous human studies quercetin or a quercetin-rich grape concentrate decreased triacylglycerol concentrations [25,26], but not in other studies [4,27,28]. In this study quercetin increased also HDL-C, as observed before for a grape concentrate [28]. Triacylglycerol concentrations are usually inversely related to HDL-C. TC and LDL-C were not decreased, consistent with two [4,27] but contrary to other previous reports [26,28]. None of these effects were APOE genotype-dependent, while Egert et al. [6] found adverse effects of quercetin on HDL-C and the LDL-C/HDL-C ratio only in APOE4 subjects, and adverse effects on apolipoprotein A-I only in APOE3 subjects. Their study subjects were obese, of a wide age range and both sexes [6] which might have resulted in gender or age bias.

The only adverse effect of quercetin in our study was an increased TNFα concentration, again with no difference by APOE genotype. But concentrations were very low under placebo and quercetin treatment alike. All other inflammatory parameters determined were, however, not significantly changed. In other human intervention studies of shorter duration quercetin improved only some of the inflammatory markers [6] or none at all [29]. Nevertheless quercetin supplementation tended to decrease total glutathione, fitting with a previous observation in vitro [30]. Quercetin did not affect the concentration of 8-iso-PGF2α, an established parameter of lipid peroxidation, again with no difference by APOE genotype. This indicates that APOE3 is not superior to APOE4 in its anti-oxidative capacity.

Quercetin moderately but significantly reduced BMI, body weight and waist circumference in APOE3/3 but not in APOE4 subjects. No such effects have been reported before in humans. But in lean and obese rats quercetin attenuated weight gain, which was attributed to its anti-inflammatory effects on adipose tissue [5]. Other mechanisms may also be operative, like anti-adipogenic effects through the modulation of the AMPK, ERK and JNK signaling pathways [31].

The impact of quercetin effects on lipid concentrations in humans seem to be variable and it is not clear to what extent genetic background, diet or lifestyle habits may contribute. Our study in healthy, normal-weight men did not confirm the hypothesis of a beneficial impact of quercetin on endothelial function. Our data, however, provide evidence of moderate beneficial effects on blood pressure and lipidemia, and show for the first time that the postprandial state may emphasize quercetin effects that are less clear under fasting conditions. There were genotype-dependent effects on BMI and waist circumference which could not be explained by anti-inflammatory actions of quercetin. Quercetin exerted even slightly pro-inflammatory effects, independent of genotype.

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