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# Effect of alpha-linolenic acid in combination with the flavonol quercetin on markers of cardiovascular disease risk in healthy, non-obese adults: A randomized, double-blinded placebo-controlled crossover trial

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ABSTRACT

*Objectives:* Alpha-linolenic acid (ALA) and quercetin are characteristic compounds in plant-based diets. Cardioprotective effects have been described for both substances, although a possible benefit of combining ALA and quercetin has not, to our knowledge, been evaluated yet. The aim of this study was to investigate the potential independent and additive effects of ALA and quercetin on blood pressure (BP) and lipid and glucose metabolism, as well as on biomarkers of inflammation, oxidative stress, and antioxidant status in healthy, non-obese men and women. Another aim was to examine whether chronic supplementation of supranutritional doses of quercetin would result in an accumulation of plasma quercetin concentration over time.
*Methods:* In a double-blinded, placebo-controlled crossover trial, healthy volunteers were randomized to receive 3.6 g/d ALA plus 190 mg/d quercetin or placebo for 8 wk. Data from 67 individuals (34 men, 33 women, mean age: 24.6 y) were assessed.
*Results:* Plasma quercetin, tamarixetin, isorhamnetin, and kaempferol increased significantly from baseline to study end with ALA + quercetin but not with ALA + placebo. No significant effect on office systolic BP, mean 24 h ambulatory BP (ABP), or mean daytime ABP was seen in either study group. Both interventions significantly decreased total cholesterol, low-density lipoprotein cholesterol, non–high-density lipoprotein cholesterol, and apolipoprotein B to a similar extent. No effect on high-density lipoprotein cholesterol, apolipoprotein A1, glucose, uric acid, oxidized low-density lipoprotein, C-reactive protein, or lipid-adjusted retinol, α-tocopherol, or β-carotene was seen in either group.
*Conclusion:* Although dietary supplements of 3.6 g/d ALA over an 8-wk period improved lipid profiles in healthy adults, antioxidative and oxidative status, inflammation, and BP remained unchanged. No evidence was seen for an additive or synergistic effect of ALA plus quercetin on markers of cardiovascular disease risk.


## Introduction

Previous studies have demonstrated the protective effect of marine ω-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) against cardiovascular diseases (CVDs) [1–5]. Specifically, EPA and DHA are seen to improve vascular endothelial function and reduced serum triacylglycerols (TGs), arterial blood pressure (BP), and inflammation [6–8]. Additionally, epidemiologic studies have shown an inverse

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association between tissue or blood levels of EPA and DHA and cardiovascular events [9,10]. It is unknown whether alpha-linolenic acid (ALA), which is abundant in plant foods such as vegetables oils (e.g., linseed [flaxseed] oil and canola [rapeseed] oil), also has a significant preventive effect. Dietary ALA can be desaturated and elongated to form long-chain ω-3 PUFAs in humans, but the extent to which this occurs, and whether ALA has physiologic effects independent of its role as a precursor for long-chain ω-3 PUFAs, are unclear [11–13]. We have previously demonstrated that dietary ALA significantly reduced serum TGs in metabolically healthy participants [8]. In patients with metabolic syndrome traits, a high-ALA hypoenergetic diet has been associated with greater decreases in serum TGs, diastolic BP (DBP), and serum YKL-40 than a low-ALA control diet [14]. Additionally, there is evidence from epidemiologic and clinical trials that ALA can attenuate inflammation [15].

Quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the predominant flavonoids and one of the most potent antioxidants of plant origin [16]. It is ubiquitously distributed in edible plants, with rich sources being onions, kale, unpeeled apples, berries, citrus fruits, and tea (*Camellia sinensis*) [17]. The mean dietary intake in Western populations is estimated to be ~10 to 30 mg/d [17–19]. Epidemiologic studies, together with data from animal models and some clinical trials, suggest a role for flavonoids, particularly quercetin, in the prevention of CVD and other age-related chronic diseases [20–24]. We have previously shown that supranutritional doses of quercetin significantly reduce systolic BP (SBP) [25–27] and plasma-oxidized low-density lipoprotein (LDL) [25] in overweight to obese patients, as well as altering human monocyte gene expression [28]. By contrast, biomarkers of endothelial function and systemic inflammation were not affected by quercetin [25,29,30].

ALA and quercetin are characteristic compounds in plant-based cardioprotective diets, but to our knowledge, there has been no clinical investigation of the combined effectiveness of ALA and quercetin on CVD risk factors in humans. Therefore, the aim of the present study was to investigate the potential independent and additive effects of ALA and quercetin on BP and lipid and glucose metabolism, as well as on biomarkers of inflammation, oxidative stress, and antioxidant status in healthy, non-obese men and women. Another aim was to examine whether chronic supplementation of supranutritional doses of quercetin will result in an accumulation of plasma quercetin concentration over time. The study was initially designed to investigate the effects of ALA and quercetin supplementation on conversion of ALA to long-chain PUFAs, and the results of these primary analyses were published previously [31]; the results presented here represent secondary analyses of data from this group of patients.

## Methods

### *Participants*

Details of the study design, interventions, and participant recruitment, enrollment, and randomization have been previously described [31]. In brief, the study recruited non-smoking, non-obese, healthy volunteers 19 to 35 y of age. The main exclusion criteria were fasting serum TGs ≥2.26 mmol/L (≥200 mg/dL), fasting serum LDL cholesterol (LDL-C) ≥4.14 mmol/L (≥160 mg/dL), metabolic or endocrine diseases, malabsorption syndromes, pregnancy and lactation, alcohol abuse, consumption of dietary supplements (e.g., polyphenols, long-chain [ω-3] PUFA, or vitamin E), and restrictive dietary requirements [31]. Initial screening included physical assessments (e.g., height and weight, resting BP, and waist circumference), clinical assessments (liver function, serum lipids and lipoproteins, glucose, and hematology), medical history, and completion of a dietary questionnaire.

Seventy-four metabolically healthy individuals (37 women, 37 men) were included in the study. Seven participants dropped out for personal reasons. Data from the remaining 67 participants (33 women, 34 men) who completed the entire intervention were included in the analysis [31].

The study was conducted according to the guidelines laid down in the 1964 Declaration of Helsinki and its later amendments, and all procedures involving human participants were approved by the ethical committee of the Medical Faculty of the Rheinische Friedrich-Wilhelms-Universität Bonn, Germany. Written informed consent was obtained from all participants. The trial was registered at www.germanctr.de/ and http://apps.who.int/trialsearch/as DRKS00005076. The participants were instructed to maintain their usual diet, physical activity levels, lifestyle, and body weight throughout the study [31].

### *Study design*

This was a double-blinded, randomized, placebo-controlled crossover trial, with two 8-wk intervention periods separated by an 8-wk washout. During the intervention periods, participants ingested ≥3.3 g/d ALA. Participants were asked to replace their normal margarine or butter with a commercially available rapeseed oil–based margarine (Goldina, Ostthüringer Nahrungsmittelwerk Gera GmbH; ALA content, 7.7% of total fatty acids, fat content of margarine 80%); usual vegetable oil was replaced by refined rapeseed oil (Brökelmann+Co Ölmühle GmbH + Co; ALA content, 8.8% of total fatty acids). To reach the daily ALA amount, participants ingested ≥30 g of rapeseed oil and 25 g of rapeseed oil–based margarine. All participants received a 10-g dosage spoon to calculate their consumption of margarine and oil. To monitor compliance, they documented daily oil and margarine ingestion in a study diary. The study margarine and oil was provided twice per week and was incorporated into the usual diet. Additionally, all participants were offered bread, buns, and cakes that contained rapeseed oil and rapeseed oil–based margarine. Other dietary sources of ω-3 fatty acids such as fatty fish, ω-3 fatty acid– enriched foods, fish oil capsules, nuts, and seeds were not permitted [31].

In addition to the ALA intervention, all participants supplemented quercetin (verum) or placebo in the form of hard gelatin capsules. Participants were instructed to take one capsule with each main meal (three capsules per day). Quercetin capsules contained onion skin extract powder; placebo capsules contained mannitol. The quercetin content of the onion skin extract powder (*Allium cepa* L, Rudolf Wild GmbH & Co. KG) was 45.5%; the bioavailability of quercetin from onion skin extract was described previously [32,33]. Participants were assigned to placebo or quercetin treatment by blocked randomization procedure, as described previously [31]. The primary investigators, study personnel, and participants were blinded to the treatment.

Compliance was evaluated by counting capsules at the end of the study and instructing study participants to document capsule consumption, adverse effects, physical activity, and other relevant observations in the study diary. Additionally, plasma flavonol concentrations were measured at the beginning of the treatment period, at 4 wk, and at the end of the 8-wk intervention period.

Participants were advised to keep 3-d food records at the beginning and end of both intervention periods, as well as at the time of screening and during the washout period. The dietary records were used to calculate the normal dietary intake of energy, nutrients, long-chain ω-3 fatty acids, and quercetin.

Venous blood sampling and anthropometric measurements (weight, waist circumference, and body composition) were conducted during six study visits (at the beginning of the study period, at week 4, and at the end of each intervention period). For each visit, participants were advised to come to the study center in the early morning after fasting overnight [31].

### *Blood pressure measurements*

Office BP measurements were conducted at weeks 0, 4, and 8 and obtained using an automatic BP measurement device (boso carat professional) under standardized conditions [34,35]. Each participant sat quietly for 5 to 10 min, after which time their arm was placed at heart level and SBP and DBP were measured at least twice in 3- to 5-min intervals. If BP measurements varied by 10 mm Hg, an additional measurement was performed. The accumulated measurements were then averaged to determine overall SBP and DBP. The mean arterial pressure (MAP) was calculated as (DBP + 1/3 [SBP − DBP]).

In a subset of 52 volunteers, 24-h ambulatory BP (ABP) monitoring was conducted at the beginning and end of the intervention periods (weeks 0 and 8). Recordings of 24-h ABP were taken every 15 min between 0600 and 2200 (daytime) and every 30 min between 2200 and 0600 (nighttime) using an ABP monitor (Spacelabs: Monitor type 90207, boso: Monitor type TM-2430 PC 2) on the nondominant arm. On the day of measurement, participants were instructed to maintain their usual activity levels and to refrain from strenuous exercise. The following BP parameters were assessed: 24-h, daytime and nighttime SBP, DBP, MAP, and the nocturnal dip in SBP and DBP.

### *Blood sampling and analysis*

Venous blood samples were collected at all study visits between 0700 and 0930 under standardized conditions after an overnight fast. Participants abstained from alcoholic beverages for 24 h before visits and were advised not to engage in strenuous exercise the day before sampling. The last capsule was taken in the evening before blood sampling. Blood was drawn into tubes containing EDTA, lithium heparin, fluoride, or a coagulation activator (Sarstedt). Plasma and serum were obtained by centrifugation at 3000*g* for 15 min at 6°C. The aliquots

of plasma and serum were immediately frozen in cryovials and stored at −80°C until analysis. All laboratory measurements were performed without knowledge of the treatment.

*Serum lipid parameters, plasma glucose, and C-reactive protein*

Serum concentration of total cholesterol (TC) was measured using polychromatic endpoint measurement, whereas serum concentrations of LDL-C, HDL-C, TGs, and uric acid, and plasma concentration of glucose were measured using bichromatic endpoint measurement with a Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Serum non–HDL-C was calculated by subtracting HDL from the TC value. Serum concentrations of apolipoprotein (apo)B, apoA1, and high-sensitivity C-reactive protein (CRP) were determined using nephelometric methods with a Dimension Vista 1500 analyzer. All these parameters were assayed from fresh samples within 4 h of sampling at the Central Laboratory of the Institute for Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, as described previously [31].

*Plasma retinol, α-tocopherol, ß-carotene and oxidized LDL*

For determination of retinol, α-tocopherol, and β-carotene, plasma samples were deproteinized by the addition of ethanol (containing apocarotenal as the internal standard, 5 μmol/L). Fat-soluble vitamins were extracted with *n*-hexane and analyzed using normal-phase high-performance liquid chromatography (HPLC; column, Nucleosil 100-5 CN, 250 × 4.0 nm, Macherey-Nagel, Düren, Germany) and ultraviolet detection (292 nm). Plasma oxidized LDL (ox-LDL) (Immundiagnostik, Bensheim, Germany) was determined in duplicate using commercially available enzyme-linked immunoassay kits according to the manufacturer's instructions and quality controls.

*Plasma flavonol concentrations*

Analyses of plasma concentrations of quercetin, its monomethylated derivatives tamarixetin (4′-*O*-methyl quercetin) and isorhamnetin (3′-*O*-methyl quercetin) and the dehydroxylated quercetin metabolite kaempferol were carried out using HPLC with fluorescence detection as described previously [27,36]. All samples were treated enzymatically with β-glucuronidase/sulphatase before the extraction of the flavonols. Total plasma flavonols were calculated as follows:

$$\textit{total}\ \text{flavonols}\ (\text{nmol/L}) = \text{quercetin}\ (\text{nmol/L}) + \text{kaempferol}\ (\text{nmol/L}) + \text{isorhamnetin}\ (\text{nmol/L}) + \text{tamarixetin}\ (\text{nmol/L}).$$

*Statistical analyses*

All statistical analyses were performed using IBM SPSS statistical software package (SPSS version 21; IBM Corporation, Armonk, NY, USA). The distribution of variables was analyzed by checking normal plots of the data, and Kolmogorov–Smirnov and Shapiro–Wilk tests were performed to test for normality. Differences between sexes at screening were tested using the unpaired Student's *t* test if data were normally distributed and by Mann–Whitney U test if data were not normally distributed. Baseline values were compared between groups using paired Student's *t* tests or Wilcoxon signed-rank tests. Intragroup (baseline versus endpoint) and intergroup (changes during ALA + quercetin versus changes during ALA + placebo treatment) comparisons of normally distributed data were performed using paired Student's *t* tests. We also performed repeated-measures analysis of variance (RM-ANOVA) for the concentrations of plasma quercetin, isorhamnetin, tamarixetin, kaempferol, and total flavonols. The fixed factors were treatment (two levels: ALA + quercetin and ALA + placebo) and time of measurement (three levels: weeks 0, 4, and 8 of the intervention periods). If the residuals were not normally distributed, RM-ANOVAs were conducted with log-transformed variables. We calculated the treatment difference between the groups using unpaired Student's *t* tests. Intra- and intergroup data that were not normally distributed were compared using Wilcoxon signed-rank tests. In all cases, a $P \leq 0.05$ (two-sided) was considered statistically significant. Unless otherwise indicated, descriptive data are presented as arithmetical mean ± SD. All analyses are based on a per-protocol basis. Pearson's correlation coefficient was used for relationships between different variables.

## Results

*Participant characteristics and compliance*

Table 1 presents characteristics of the participants at screening. Differences with respect to body height, body weight, waist circumference, SBP, HDL-C, and uric acid were observed between male and female participants [31].

Count of returned capsules indicated a high level of compliance (94.4% ± 5.8% and 95.6% ± 5% for the ALA + quercetin and ALA + placebo treatments, respectively) [31]. Compliance with quercetin supplementation was confirmed by a marked increase (1660.6%) in fasting plasma quercetin concentration from baseline to study end (mean change: +464 ± 331 nmol/L; $P \leq 0.0001$, Fig. 1a). Additionally, plasma concentrations of kaempferol, isorhamnetin, tamarixetin and total flavonols increased significantly from baseline to study end after quercetin but not after placebo supplementation (Fig. 1b–e). Time-response analysis showed that quercetin supplementation caused a significant, rapid increase in overnight-fasted plasma quercetin from baseline to week 4; levels continued to increase at a slower rate to study end but did not reach statistical significance ($P = 0.073$ for comparison week 8 versus week 4; Fig. 1a). Similar response was observed for isorhamnetin, tamarixetin, and total flavonols (Fig. 1b–e).

Analyses of 3-d dietary records indicated no significant intra- and intergroup differences in mean daily intakes of energy, protein, carbohydrates, total fat, fatty acids, cholesterol, antioxidants (e.g., vitamins E and C), dietary fiber, and quercetin during the intervention periods (data not shown). Mean estimated daily intake of rapeseed oil was 23.7 ± 7.9 and 23.3 ± 8.1 g for the ALA + placebo and ALA + quercetin interventions, respectively.

**Table 1**
Characteristics and blood parameters of participants at screening*

| | Total (N = 67) | Women (n = 33) | Men (n = 34) | Women vs men (*P*-value)† |
|---|---|---|---|---|
| Age (y) | 24.6 ± 3.9 | 24.1 ± 2.8 | 25 ± 4.8 | 0.930 |
| Body height (cm) | 174 ± 8.8 | 168 ± 6.2 | 180 ± 7.3 | ≤0.0001 |
| Body weight (kg) | 67.7 ± 10.9 | 60.3 ± 7 | 74.9 ± 8.9 | ≤0.0001 |
| BMI (kg/m$^2$) | 22.2 ± 2.3 | 21.3 ± 2.2 | 23.2 ± 2.1 | 0.001 |
| Waist circumference (cm) | 80.6 ± 6.8 | 78.3 ± 6.5 | 82.9 ± 6.4 | 0.002 |
| Systolic BP (mm Hg) | 130.9 ± 14.6 | 123.9 ± 12.8 | 137.7 ± 13 | ≤0.0001 |
| Diastolic BP (mm Hg) | 84.1 ± 9.5 | 83.2 ± 10.6 | 85.0 ± 8.4 | 0.286 |
| Serum triacylglycerols (mmol/L) | 1.09 ± 0.60 | 1.14 ± 0.46 | 1.04 ± 0.71 | 0.051 |
| Serum total cholesterol (mmol/L) | 4.59 ± 0.80 | 4.75 ± 0.69 | 4.44 ± 0.87 | 0.117 |
| Serum HDL-cholesterol (mmol/L) | 1.61 ± 0.42 | 1.77 ± 0.44 | 1.45 ± 0.33 | 0.001 |
| Serum LDL-cholesterol (mmol/L) | 2.60 ± 0.66 | 2.56 ± 0.64 | 2.63 ± 0.70 | 0.693 |
| Plasma glucose (mmol/L) | 4.58 ± 0.41 | 4.53 ± 0.35 | 4.64 ± 0.45 | 0.293 |
| Serum uric acid (μmol/L) | 242 ± 73 | 187 ± 39 | 293 ± 59 | ≤0.0001 |

BMI, body mass index; BP, blood pressure.
Adapted from [31].
*Data shown as mean ± SD.
†Unpaired Student's *t* test or Mann–Whitney U test.

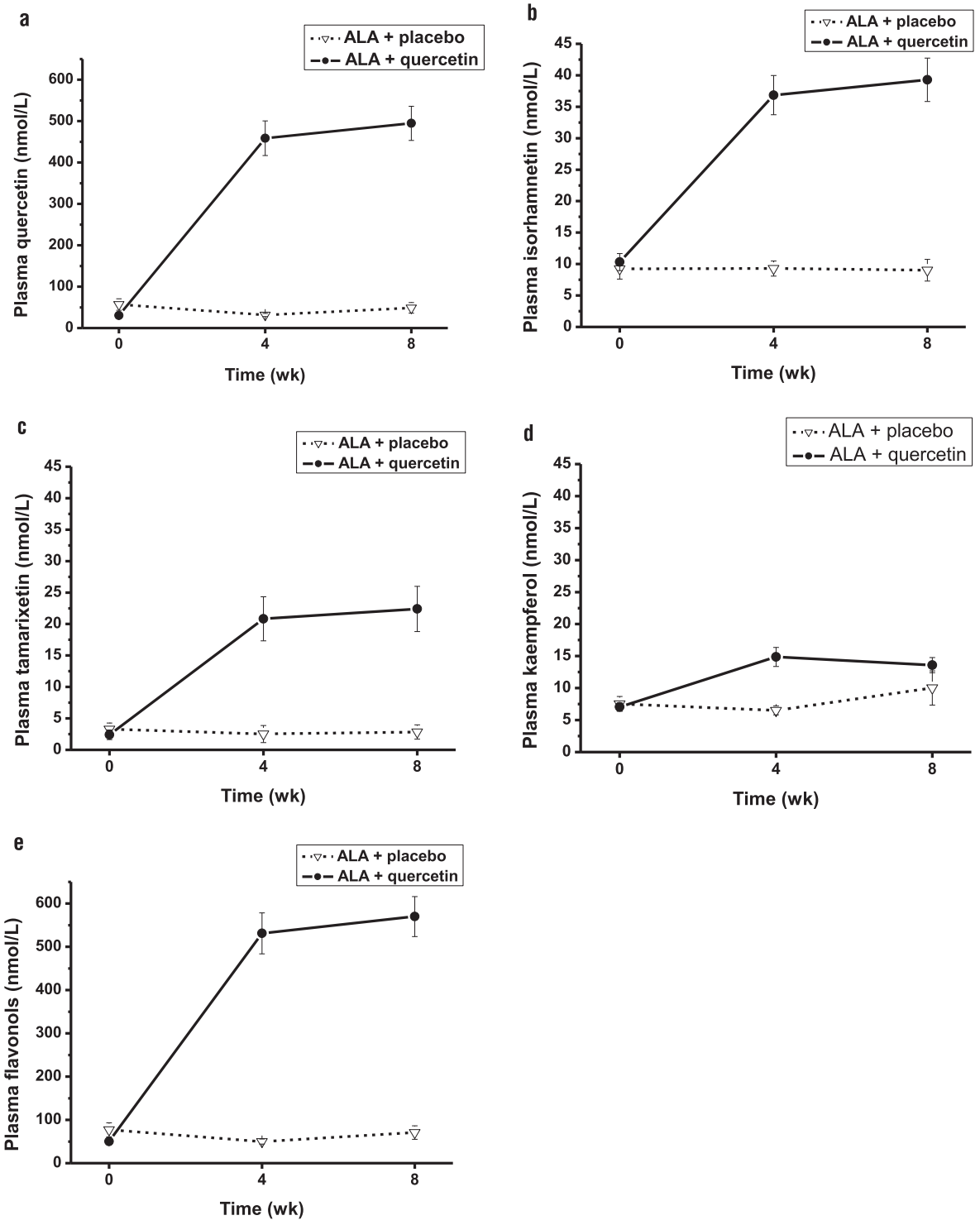


**Fig. 1.** Plasma concentration of quercetin (a), isorhamnetin (b), tamarixethin (c), kaempferol (d), and total flavonols (e) in healthy non-obese men and women during 8-wk supplementation with ALA plus placebo or ALA plus quercetin. Values represent mean ± SEM. Plasma concentrations differ significantly over time between the two treatment groups ($P < 0.0001$ for effect of time, and $P < 0.0001$ for the time × treatment interaction using repeated-measures analysis of variance). ALA, alpha-linolenic acid.

Mean estimated daily intake of margarine was 24.2 ± 9.8 and 24.1 ± 8.9 g for the ALA + placebo and ALA + quercetin interventions, respectively. The mean daily ALA intake from rapeseed oil and margarine was 3.6 ± 0.8 g and did not differ between intervention groups. Total ALA intake (which included the contribution from the remainder of the diet) was 5.4 ± 1.34 (2.2% of total energy) and 5.4 ± 1.40 g/d for ALA + quercetin and ALA + placebo interventions, respectively [31]. Compliance with ALA intake was confirmed by a marked increase in ALA content of serum phospholipids and erythrocytes [31]. The intervention did not significantly affect body weight, waist circumference, relative fat mass or fat-free mass [31].

*Blood pressure monitoring*

No significant changes in office SBP were seen in either intervention (Table 2). A significant decrease in office DBP (−3 ± 6.3 mm Hg; $P \leq 0.001$) and MAP (−2.5 ± 6.2 mm Hg; $P = 0.001$) was seen in the ALA + quercetin group only. However, these changes did not differ significantly from the ALA + placebo treatment (Table 2). As men had significantly higher SBP than women at screening (Table 1), we also analyzed office SBP in this subgroup only. Neither intervention significantly affected office SBP in men (data not shown).

No significant changes in mean 24-h and mean daytime ABP parameters (SBP and DBP, MAP) were seen in either group (Table 2). Additionally, no effect on nocturnal dip in SBP and DBP was seen. In the ALA + placebo group, but not in the ALA + quercetin group, we observed a small increase in mean nighttime SBP (+2.0 ± 6.9 mm Hg, $P = 0.045$; intergroup comparison, $P = 0.706$; Table 2).

*Serum lipids, plasma glucose, plasma ox-LDL, plasma/serum antioxidants, and serum CRP*

A significant decrease in serum TGs was observed in the ALA + quercetin group, but not in the ALA + placebo group (Table 3). Both interventions significantly decreased serum TC, LDL-C, non−HDL-C, and apoB from baseline to study end to a similar extent (Table 3). The baseline fasting serum LDL-C concentrations and the change in serum LDL-C concentrations were related, with those individuals with higher baseline LDL-C concentrations demonstrating greater reductions in serum LDL-C in response to the interventions (ALA + quercetin, $r = -0.489$, $P < 0.0001$; ALA + placebo, $r = -0.305$, $P = 0.015$; Fig. 2). Similarly, baseline serum apoB concentrations and decreases in apoB from baseline to end were related (ALA + quercetin, $r = -0.371$, $P = 0.002$; ALA + placebo, $r = -0.335$, $P = 0.006$). Neither ALA + quercetin nor ALA + placebo significantly affected serum HDL-C, apoA1, plasma glucose, serum uric acid, plasma ox-LDL, or serum high-sensitivity CRP (Table 3). Additionally, lipid-adjusted plasma retinol, α-tocopherol, and β-carotene were not significantly affected by either intervention (Table 3). Fasting plasma concentrations of vitamins E and A and β-carotene were within normal concentration ranges in all participants (α-tocopherol, 12–48 μmol/L; retinol, 0.7–2.8 μmol/L; β-carotene, 0.28–2.3 μmol/L). There were no significant associations between ox-LDL concentrations and lipoprotein-lipid profile variables (TC, LDL-C, HDL-C, and TGs) between ox-LDL and plasma/serum antioxidants or between ox-LDL concentrations and high sensitivity CRP (data not shown).

## Discussion

Results from the present study demonstrated that an 8-wk intervention with ALA with or without quercetin in healthy nonobese adults resulted in significant improvements in fasting serum LDL-C, non−HDL-C, and apoB, whereas HDL-C and apoA1 remained unchanged. Antioxidative and oxidative status, inflammation, and mean 24-h ABP remained unchanged in both groups. These data did not support our original hypothesis, that combined application of ALA plus quercetin may provide an additive or synergistic improvement in markers of cardiometabolic risk. The ALA dosage of 3.6 g/d was selected to provide approximately three times the estimated dietary ALA intake in most European countries and the United States [31,37] and was readily achieved with regular consumption of rapeseed oil and rapeseed oil−based margarine. The quercetin dosage of 190 mg/d represented ~15 times the estimated mean daily quercetin intake in Germany [27,38] and other European populations [19].

Both treatments decreased fasting LDL-C, non−HDL-C, and apoB by 8%, 10%, and 2%, respectively, with greater reductions in individuals with higher baseline concentrations. Interestingly, this effect occurred in normolipidemic healthy participants (LDL-C < 4.14 mmol/L). Additionally, ALA + quercetin decreased TG levels, although this effect did not differ significantly from the placebo group. Published reports on the effects of ALA or quercetin on serum lipids are scarce and data are inconsistent. In previous human studies, we reported no significant effects of ALA (4 and 6 g/d) on TC and LDL-C in healthy participants [8,39]. By contrast, Kratz et al. [40] demonstrated a decrease in TC and LDL-C during a rapeseed oil−based diet (7 g/d ALA) in healthy individuals, confirming the observations we report here. Goyens and Mensink [41] found a significant decrease in LDL-C and apoB concentrations during an ALA-rich diet (1.1 En% ALA) compared with a control diet in healthy participants. The underlying mechanisms by which ALA effects lipid metabolism is not well understood, but studies indicate that ALA has effects on serum lipids similar to those of linoleic acid, its ω-6 fatty acid counterpart. For example, earlier dietary intervention studies showed that dietary ALA was as effective as linoleic acid in lowering blood cholesterol in healthy men [42,43] and in patients with mild hypertension [44].

The effects of quercetin on lipid profiles have recently been summarized in two meta-analyses of randomized controlled trials [45,46]. Sahebkar [45] found a significant decrease in TGs at pharmacologic doses (≥500 mg/d), but no significant effects on LDL-C and HDL-C. The proposed lipid-lowering mechanisms of quercetin include an increase in fecal cholesterol and bile-acid excretion and inhibition of de novo TG synthesis leading to reduced VLDL-TG concentrations [45,47]. In a second meta-analysis [46], chronic supplementation with flavonols (primarily quercetin) was associated with a decrease in TGs as well as TC and LDL-C. However, subgroup analysis revealed no significant effects in healthy individuals or in those with normal baseline levels for any of the blood lipids [46]. In the present study, quercetin supplementation did not enhance the effects of the ALA intervention, possibly due to the significant effect of the ALA intervention, the non-pharmacologic quercetin dose, and the normolipidemic nature of the study population.

In contrast to our previous findings in overweight and obese participants with metabolic syndrome traits [25,27], ALA + quercetin did not reduce office SBP or 24-h ABP profiles. Only a small decrease in DBP (−3 mm Hg) was seen in the ALA + quercetin group, which did not differ from results seen with ALA + placebo. Few studies have determined the effects of ALA on BP, and results are inconsistent, showing no effects [48] or a decrease in SBP and DBP [49]. By contrast, a recent meta-analysis of seven placebo-controlled trials (the majority of which involved normotensive or prehypertensive individuals) showed significant reductions both in SBP (−3 mm Hg, 95% confidence interval [CI], −5.75 to −0.33; $P = 0.028$) and DBP (−2.63 mm Hg, 95% CI, −3.26 to −2.01; $P < 0.001$) after quercetin supplementation [50]. When studies were categorized according to the quercetin dose, a significant reduction in SBP and DBP was seen in randomized controlled trials using quercetin doses ≥500 mg/d; no significant effects were seen with doses <500 mg/d [50]. The mechanisms by which quercetin exerts these effects are not completely understood, although it is most likely to arise from modulation of different types of cell signaling and gene expression. Hypotheses tested in different experimental and clinical trials include lowering of oxidative stress, interference with the renin−angiotensin system, and improvement of endothelial function [51]. The fact that no BP-lowering effect was observed

**Table 2**
Office and 24-h ambulatory blood pressure in healthy non-obese men and women during 8-wk supplementation with ALA + placebo or ALA+quercetin[*,†]

| | ALA + placebo (n = 67) | | | *P*-value intragroup comparison | ALA + quercetin (n = 67) | | | *P*-value intragroup comparison | *P*-value intergroup comparison | Treatment difference (mean and 95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | Endpoint | Mean change (mean and 95% CI) | | Baseline | Endpoint | Mean change (mean and 95% CI) | | | |
| Office BP | | | | | | | | | | |
| SBP (mm Hg) | 126.1 ± 12.2 | 124.7 ± 11.8 | −1.4 (−3.5/0.8) | 0.206 | 126.0 ± 11.1 | 124.4 ± 12.8 | −1.6 (−3.7/0.5) | 0.129 | 0.880 | −0.2 (−3.5/3.0) |
| DBP (mm Hg) | 79.1 ± 8.1 | 78.2 ± 7.8 | −1.0 (−2.4/0.5) | 0.193 | 79.7 ± 7.5 | 76.7 ± 8.0 | −3.0 (−4.5/−1.5) | ≤0.001 | 0.052 | −2.0 (−4.1/0.0) |
| MAP (mmHg) | 94.8 ± 8.7 | 93.7 ± 8.5 | −1.1 (−2.6/0.4) | 0.141 | 95.1 ± 7.9 | 92.6 ± 8.9 | −2.5 (−4.1/−1.0) | 0.001 | 0.171 | −1.4 (−3.5/0.6) |
| | ALA + placebo (n = 52) | | | *P*-value intragroup comparison | ALA+quercetin (n = 52) | | | *P*-value intragroup comparison | *P*-value intergroup comparison | Treatment difference (mean and 95% CI) |
| | Baseline | Endpoint | Mean change (mean and 95% CI) | | Baseline | Endpoint | Mean change (mean and 95% CI) | | | |
| 24-h ABP | | | | | | | | | | |
| SBP (mm Hg) | 122.9 ± 8.6 | 124 ± 9.1 | 1.1 (−0.5/2.8) | 0.173 | 123 ± 7.5 | 123.1 ± 8.3 | 0.1 (−1.0/1.2) | 0.854 | 0.250 | −1 (−2.8/0.8) |
| DBP (mm Hg) | 72.9 ± 6.2 | 73.5 ± 6.1 | 0.5 (−0.6/1.7) | 0.360 | 72.9 ± 4.9 | 73.2 ± 5.6 | 0.3 (−0.5/1.1) | 0.476 | 0.704 | −0.3 (−1.7/1.1) |
| MAP (mm Hg) | 89.6 ± 6.2 | 90.3 ± 6.4 | 0.6 (−0.7/2.0) | 0.336 | 89.7 ± 5 | 89.9 ± 5.7 | 0.2 (−0.7/1.1) | 0.629 | 0.568 | −0.4 (−1.9/1.1) |
| Day-time (0600–2200) | | | | | | | | | | |
| SBP (mm Hg) | 125.4 ± 9 | 126.3 ± 9.5 | 0.9 (−1/2.8) | 0.327 | 125.5 ± 7.8 | 125.4 ± 8.7 | −0.2 (−1.5/1.2) | 0.825 | 0.330 | −1.1 (−3.3/1.1) |
| DBP (mm Hg) | 75.5 ± 6.5 | 76.1 ± 6.6 | 0.6 (−0.7/2) | 0.351 | 75.6 ± 5.2 | 75.7 ± 6.1 | 0.1 (−0.9/1.2) | 0.835 | 0.550 | −0.5 (−2.3/1.2) |
| MAP (mm Hg) | 92.0 ± 6.6 | 92.7 ± 6.9 | 0.7 (−0.8/2.2) | 0.353 | 92.3 ± 5.3 | 92.1 ± 6 | −0.1 (−1.2/1) | 0.844 | 0.390 | −0.8 (−2.7/1.1) |
| Nocturnal dip in SBP (%) | 9.4 ± 5 | 8.5 ± 6.1 | −0.9 (−2.6/0.7) | 0.246 | 9.3 ± 5.5 | 8.3 ± 6.7 | −1 (−3.3/1.3) | 0.374 | 0.955 | −0.1 (−3.0/2.9) |
| Nocturnal dip in DBP (%) | 15.1 ± 7.8 | 15.6 ± 8.5 | 0.5 (−1.9/2.9) | 0.660 | 15.9 ± 7.3 | 15.1 ± 8.3 | −0.8 (−3.2/1.7) | 0.533 | 0.511 | −1.3 (−5.2/2.6) |
| Night-time (2200–0600) | | | | | | | | | | |
| SBP (mm Hg) | 113.8 ± 9.2 | 115.8 ± 10.3 | 2 (0.0/4) | 0.045 | 114± 9.4 | 115.4 ± 10.3 | 1.4 (−1/3.9) | 0.249 | 0.706 | −0.6 (−3.7/2.5) |
| DBP (mm Hg) | 64.1 ± 7.3 | 64 ± 6.9 | −0.1 (−1.7/1.5) | 0.887 | 63.5 ± 6.2 | 64.4 ± 6.7 | 0.9 (−0.4/2.3) | 0.172 | 0.394 | 1.1 (−1.4/3.5) |
| MAP (mm Hg) | 81.1 ± 7.4 | 81.6 ± 7.3 | 0.4 (−1.2/2.1) | 0.587 | 80.5 ± 6.5 | 81.9 ± 7.1 | 1.3 (−0.2/3) | 0.097 | 0.491 | 0.9 (−1.7/3.5) |

ABP, ambulatory blood pressure; BP, blood pressure; CBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure.
*Data represent mean ± SD. For mean change and treatment difference, mean and 95% CI are given.
†The two groups did not differ significantly with regard to any of the variables at baseline (paired Student's *t* tests or Wilcoxon signed-rank tests). Treatment difference was calculated by [ALA+quercetin (endpoint minus baseline)] minus [ALA+placebo (endpoint minus baseline)].

**Table 3**
Fasting serum lipids, serum apolipoprotein A1 and B, plasma glucose, plasma antioxidants and plasma ox-LDL in healthy non-obese men and women during 8-wk supplementation with ALA + placebo or ALA + quercetin[*,†]

| | ALA + placebo (n = 67) | | | *P*-value intragroup comparison | ALA+quercetin (n = 67) | | | *P*-value intragroup comparison | *P*-value intergroup comparison | Treatment difference (mean and 95% CI) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | Endpoint | Mean change (mean and 95% CI) | | Baseline | Endpoint | Mean change (mean and 95% CI) | | | |
| Serum triacylglycerols (mmol/L) | 1.11 ± 0.56 | 1.11 ± 0.60 | 0.01 (−0.09/0.11) | 0.738 | 1.24 ± 1.04 | 1.02 ± 0.47 | −0.21 (−0.45/0.04) | 0.024 | 0.093 | −0.22 (−0.49/0.06) |
| Serum total cholesterol (mmol/L) | 4.59 ± 0.71 | 4.33 ± 0.78 | −0.26 (−0.38/−0.14) | ≤0.001 | 4.68 ± 0.99 | 4.35 ± 0.83 | −0.32 (−0.46/−0.19) | ≤0.0001 | 0.484 | −0.06 (−0.25/0.12) |
| Serum HDL-cholesterol (mmol/L) | 1.60 ± 0.41 | 1.59 ± 0.43 | −0.01 (−0.06/0.03) | 0.627 | 1.59 ± 0.45 | 1.60 ± 0.41 | 0.02 (−0.04/0.08) | 0.555 | 0.485 | 0.03 (−0.05/0.11) |
| Serum LDL-cholesterol (mmol/L) | 2.52 ± 0.66 | 2.33 ± 0.65 | −0.18 (−0.27/−0.10) | ≤0.0001 | 2.62 ± 0.77 | 2.39 ± 0.67 | −0.23 (−0.33/−0.14) | ≤0.0001 | 0.436 | −0.05 (−0.16/0.07) |
| Serum non-HDL-cholesterol (mmol/L) | 2.99 ± 0,76 | 2.74 ± 0,77 | −0.25 (−0.36/−0.13) | ≤0.0001 | 3.09 ± 0.98 | 2.75 ± 0.77 | −3.34 (−0.47/−0.21) | ≤0.0001 | 0.291 | −0.09 (−0.27/0.08) |
| Serum apoA1 (g/L) | 1.67 ± 0.31 | 1.63 ± 0.33 | −0.03 (−0.07/0.00) | ≤0.0001 | 1.67 ± 0.34 | 1.65 ± 0.32 | −0.03 (−0.07/0.02) | ≤0.0001 | 0.863 | 0.01 (−0.05/0.06) |
| Serum apoB (g/L) | 0.78 ± 0.19 | 0.71 ± 0.18 | −0.07 (−0.10/−0.04) | 0.070 | 0.80 ± 0.21 | 0.73 ± 0.19 | −0.07 (−0.09/−0.04) | 0.236 | 0.788 | 0.00 (−0.03/0.04) |
| Plasma glucose (mmol/L) | 4.45 ± 0.40 | 4.54 ± 0.43 | 0.09 (−0.01/0.19) | 0.084 | 4.50 ± 0.48 | 4.52 ± 0.41 | 0.02 (−0.07/0.11) | 0.666 | 0.230 | −0.07 (−0.18/0,04) |
| Serum high sensitivity CRP (mg/L) | 1.32 ± 1.74 | 1.78 ± 3.54 | 0.45 (−0.29/1.20) | 0.423 | 1.23 ± 1.70 | 1.34 ± 1.79 | 0.11 (−0.32/0.55) | 0.798 | 0.695 | −0.34 (−1.22/0.55) |
| Plasma retinol (μmol/L) | 1.57 ± 0.47 | 1.48 ± 0.42 | −0.09 (−0.16/−0.01) | 0.021 | 1.60 ± 0.48 | 1.51 ± 0.46 | −0.09 (−0.16/−0.02) | 0.008 | 0.979 | −0.00 (−0.10/0.09) |
| Plasma retinol (μmol/g)[‡] | 0.59 ± 0.18 | 0.58 ± 0.18 | −0.01 (−0.05/0.03) | 0.693 | 0.58 ± 0.18 | 0.60 ± 0.19 | 0.02 (−0.01/0.05) | 0.173 | 0.226 | 0.03 (−0.02/0.07) |
| Plasma α-tocopherol (μmol/L) | 24.80 ± 6.14 | 23.40 ± 6.09 | −1.40 (−2.39/−0.41) | 0.006 | 25.70 ± 7.73 | 23.40 ± 5.75 | −2.30 (−3.35/−1.24) | ≤0.0001 | 0.218 | −0.90 (−2.34/0.54) |
| Plasma α-tocopherol (μmol/g)[‡] | 9.14 ± 1.91 | 8.99 ± 1.83 | −0.15 (−0.56/0.26) | 0.466 | 9.11 ± 1.75 | 9.16 ± 1.57 | 0.05 (−0.28/0.39) | 0.757 | 0.455 | 0.20 (−0.34/0.75) |
| Plasma β-carotene (μmol/L) | 1.17 ± 1.19 | 1.10 ± 1.06 | −0.07 (−0−15/0.00) | 0.019 | 1.21 ± 1.12 | 1.06 ± 0.87 | −0.15 (−0.26/−0.35) | 0.002 | 0.417 | −0.07 (−0.22/0.07) |
| Plasma ß−carotene (μmol/g)[‡] | 0.44 ± 0.45 | 0.44 ± 0.43 | −0.01 (−0.04/0.02) | 0.592 | 0.43 ± 0.34 | 0.43 ± 0.33 | −0.01 (0.04/0.02) | 0.595 | 0.993 | −0.00 (−0.06/0.06) |
| Plasma ox-LDL (ng/mL) | 236 ± 280 | 228 ± 281 | −8 (−25/9) | 0.359 | 212 ± 204 | 209 ± 220 | −2 (−18/13) | 0.732 | 0.600 | 6 (−15/26) |
| Serum uric acid (μmol/L) | 246 ± 74 | 249 ± 71 | 3 (−5/11) | 0.427 | 249 ± 69 | 244 ± 66 | −5 (−12/2) | 0.151 | 0.102 | −8 (−18/2) |

ox-LDL, oxidized low-density lipoprotein.
*Data represent mean ± SD. For mean change and treatment difference, mean and 95% CI are given.
†The two groups did not differ significantly with regard to any of the variables at baseline (paired Student's *t* tests or Wilcoxon signed-rank tests).
‡Adjusted for total lipids (μmol/g TL). Treatment difference was calculated by [ALA + quercetin (endpoint minus baseline)] minus [ALA + placebo (endpoint minus baseline)].

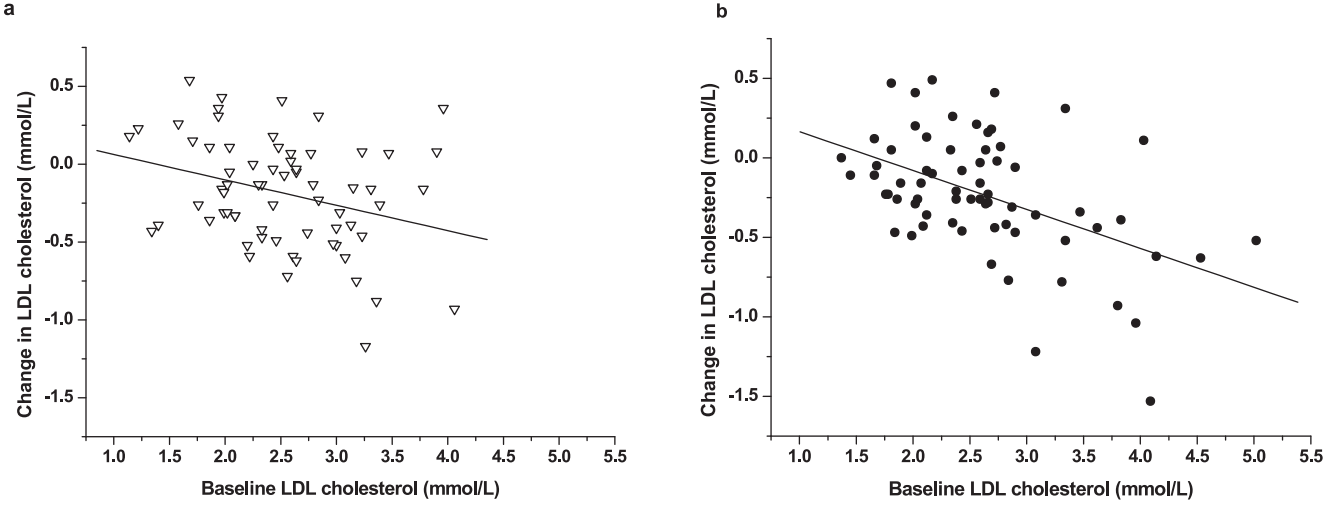


**Fig. 2.** Relationship between baseline serum LDL-cholesterol concentration and the change in concentration in healthy non-obese men and women during 8-wk supplementation with ALA + placebo (A) or ALA + quercetin (B). *r*, Pearson's correlation coefficient. (ALA + placebo, $r = -0.305$, $P = 0.015$ and ALA + quercetin, $r = -0.489$, $P < 0.0001$). ALA, alpha-linolenic acid; LDL, low-density lipoprotein.

in the present study may be explained by insufficient quercetin dosing (190 mg/d) and the normal baseline BP level of the study population, possibly allowing little room for improvement.

Although we observed elevated concentrations of quercetin in the plasma during ALA + quercetin treatment, we did not see changes in the oxidant/antioxidant status (plasma levels of ox-LDL, retinol, α-tocopherol, β-carotene, and serum uric acid) compared with placebo. This finding is in accordance with data obtained in other human intervention trials examining the potential effects of quercetin supplementation on antioxidant/oxidant biomarkers [52–54]. There are several possible explanations for this observation. First, plasma concentrations of quercetin during supplementation of ~0.5 μmol/L may have been too low to improve the plasma antioxidant status. Second, participants in the present study were young, metabolically healthy, and had an adequate dietary intake of antioxidants. Additionally, they did not smoke or undertake excessive physical exercise. Therefore, serum concentrations of HDL-C and plasma antioxidants were high and further improvement as a result of quercetin dosing may have been unlikely.

There are limited available data on the effects of ALA on biomarkers of oxidant/antioxidant biomarkers in humans. Omega-3 PUFAs such as ALA are prone to oxidation, which may increase in vivo oxidation [39,55]. We have previously compared the individual effects of ALA (4.4 g/d), EPA (2.2 g/d), and DHA (2.3 g/d) supplemented for 6 wk on antioxidant and oxidant biomarkers in healthy individuals. The ω-3 fatty acids were administered with margarines, which were enriched either with ALA, EPA, or DHA ethyl ester and stabilized with natural mixed tocopherols [56]. Plasma uric acid and antioxidative capacity showed no significant changes in any of the groups. Plasma concentrations of lipid peroxidation product MDA significantly increased with the EPA and DHA intervention, but not with the ALA intervention, suggesting that increased consumption of antioxidants may have occurred during the intake of EPA and DHA, but not during ALA [55]. In the present study, ALA was supplemented by using commercial rapeseed oil and rapeseed oil–based margarine. Both contained adequate amounts of vitamin E to ensure technological and physiological protection against lipid peroxidation. In accordance with our previous findings [39,55], we conclude that regular dietary intake of ALA of up to ~5 g/d does not exert adverse effects with regard to oxidant/antioxidant biomarkers when provided in the context of a diet with an adequate intake of antioxidants.

Neither treatment significantly affected high-sensitivity CRP, a marker of systemic (low-grade) inflammation. However, for both, ALA and quercetin antiinflammatory effects have been described in cell culture and/or animal model studies [57–59]. Human intervention studies that investigated the effects of ALA [60–64] or quercetin [25,27,30,65] on CRP levels show inconsistent results. An explanation for the lack of effect on serum CRP seen in the present study may be the health status of the study population (i.e., overweight/obese individuals and those with inflammatory diseases were excluded) and the low CRP levels seen at baseline.

In accordance with previous human studies [25,27,52], regular intake of supranutritional doses of quercetin increased fasting plasma quercetin concentrations and resulted in concentrations in the low micromolar range (~0.5 μmol/L). During quercetin treatment, we also detected low concentrations of the methylated metabolites isorhamnetin and tamarixetin. This finding is also consistent with previous studies [27,33,52,66]. However, in contrast to mice and rats, in which nearly 50% of the absorbed quercetin is methylated to isorhamnetin [67,68], the proportion of methylated derivatives in human plasma is relatively low (10%–20% of total plasma flavonols). Because the elimination half-life of quercetin calculated from previous plasma kinetic studies is relatively long (range: 7–46 h) [33,52,69,70], we hypothesized that quercetin and/or its metabolites may accumulate in plasma (and possibly also in cells and tissues) if consumed regularly for 8 wk. Our data indicate that a steady-state plasma concentration of quercetin of ~0.5 μmol/L was reached before or at least after 4 wk of supplementation of 190 mg/d quercetin. Earlier quercetin supplementation studies in humans also have reported final quercetin concentrations in this range after supplementation of supranutritional doses of 150 to 162 mg/d [25,27,52]. Jin et al. [71] investigated the variable plasma quercetin response to 12-wk quercetin supplementation. The dose–response increase in plasma quercetin for pharmacologic doses of 500 and 1000 mg/d was achieved within the first month and maintained for the rest of the study, as was also seen in the present study.

The key strengths of the present study was its double-blinded, placebo-controlled crossover design and its relatively large sample size, as well as the homogenous nature of the study population, low dropout rate, and high level of compliance. However, the study was originally designed to investigate the effects of quercetin supplementation on the conversion of ALA to long-chain PUFAs [31] and therefore the exploratory nature of these secondary analyses could be considered as a limitation. Additionally, it is important to consider that the present study population was metabolically healthy, and the results may not be representative of other populations. In the present study, we examined the combined effects of a macronutrient (ALA) and a phytochemical (quercetin). Relative to the simplicity of conducting flavonol or EPA + DHA supplement studies, ALA examinations are more complicated because ALA is incorporated via foods, which changes the composition of the test diet. In the present study, we used rapeseed oil and a rapeseed oil–based margarine to supplement the diet with ALA. This intervention, however, also led to an unavoidable decrease in saturated fatty acids and an increase in monounsaturated fatty acids compared to participant's normal diet [31]. It is, therefore, difficult to differentiate the effects of the changes in total fatty acid profile of the diet from the individual effects of ALA; this is a potential complication of all human ALA studies.

## Conclusion

Daily supplementation with 3.6 g ALA over an 8-wk period improved lipid profiles in metabolically healthy non-obese men and women. By contrast, parameters of antioxidative and oxidative status, inflammation, and mean 24-h ABP remained unaffected by daily ALA + quercetin or ALA + placebo treatment. Contrary to our hypotheses, we found no evidence of an additive or synergistic effect of combined ALA plus a supranutritional dose of quercetin on CVD risk markers.

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