

Effects of the flavonol quercetin and α -linolenic acid on *n*-3 PUFA status in metabolically healthy men and women: a randomised, double-blinded, placebo-controlled, crossover trial

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Abstract

Increased dietary intake and tissue status of the long-chain *n*-3 PUFA, EPA and DHA, is associated with cardiovascular benefits. Epidemiological and animal studies suggest that concomitant nutritive intake of flavonoids may increase the conversion of α -linolenic acid (ALA) to longer-chain *n*-3 fatty acids EPA and DHA. We investigated the effects of increased ALA intake on fatty acid composition of serum phospholipids and erythrocytes in metabolically healthy men and women and whether fatty acid profiles and ALA conversion were affected by regular quercetin intake or sex. Subjects (*n* 74) were randomised to receive at least 3.3 g/d ALA with either 190 mg/d quercetin (ALA+quercetin) or placebo (ALA+placebo) in a double-blinded, placebo-controlled, crossover trial with 8-week intervention periods separated by an 8-week washout period. A total of seven subjects dropped out for personal reasons. Data from the remaining sixty-seven subjects (thirty-four males and thirty-three females) were included in the analysis. Both interventions significantly increased serum phospholipid ALA (ALA+placebo: +69.3%; ALA+quercetin: +55.8%) and EPA (ALA+placebo: +37.3%; ALA+quercetin: +25.5%). ALA+quercetin slightly decreased DHA concentration by 9.3%. Erythrocyte ALA and EPA significantly increased with both interventions, whereas DHA decreased. Fatty acid composition did not differ between sexes. We found no effect of quercetin. Intake of 3.6 g/d ALA over an 8-week period resulted in increased ALA and EPA, but not DHA, in serum phospholipids and erythrocytes. Neither quercetin supplementation nor sex affected the increment of ALA and relative proportions of *n*-3 PUFA in serum phospholipids and erythrocytes.

Key words: α -Linolenic acid: Quercetin: *n*-3 Long-chain PUFA: Sex differences

Epidemiological and clinical studies indicate that high intake of *n*-3 PUFA may lower the risk for CVD, and studies strongly suggest that *n*-3 PUFA reduces the incidence of cardiovascular outcomes and all-cause-mortality^(1–3). Most of these cardioprotective effects are attributed to the marine long-chain *n*-3 PUFA, EPA (C20:5 *n*-3) and DHA (C22:6 *n*-3)⁽⁴⁾. Studies on the effects of plant-derived α -linolenic acid (ALA; C18:3 *n*-3) on CVD risk have revealed inconsistent results^(5–8).

ALA is essential for humans because they cannot synthesise it *de novo*⁽⁹⁾. Major dietary sources of ALA include vegetable oils, such as rapeseed and linseed oils, seeds and nuts^(8,10,11). ALA serves as a precursor molecule and can be converted into longer-chain *n*-3 fatty acids, including EPA and DHA. These reactions are catalysed by an enzymatic system involving a series of elongation

and desaturation steps. Besides the elongases, the key enzymes are Δ^5 - and Δ^6 -desaturases⁽⁹⁾. However, conversion efficiency in human adults is low, with approximately 5% of ALA converted to EPA and even less (<1%) to DHA^(4,11,12).

The regulation of PUFA metabolism is not fully understood, although genetic variations, dietary components, nutrient-related hormonal regulation and substrate competition may alter endogenous fatty acid levels⁽¹³⁾. For example, Tang *et al.*⁽¹⁴⁾ demonstrated that gene expression regulation of desaturases involves transcription factors such as PPAR α .

Data from epidemiological and animal studies suggest that the conversion of ALA to EPA and DHA is increased through simultaneous nutritive intake of flavonoids^(15–17). Quercetin (3,3',4',5,7-pentahydroxyflavone), one of the predominant flavonoids in

Abbreviations: ALA, α -linolenic acid; DPA, docosapentaenoic acid.

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human nutrition, is ubiquitously distributed in (edible) plants and is one of the most potent antioxidants of plant origin⁽¹⁸⁾. In addition, experimental studies with quercetin demonstrated that this polyphenol interacts with PPAR α ^(19,20). Thus, we hypothesised as one potential mechanism that quercetin may influence the activity of Δ^6 -desaturases and thus increase the fatty acid conversion rates.

In addition, stable isotope tracer studies indicate that the conversion of ALA to DHA is more efficient in females^(21–25), which may be explained by greater expression and activity of enzymes (i.e. Δ^6 -desaturases) and higher oestrogen levels in women⁽²⁶⁾. These findings in short-term studies using stable isotopes were confirmed by Childs *et al.*⁽²⁷⁾, who showed sex-related differences in a long-term intervention with dietary ALA in humans.

To the best of our knowledge, no previous intervention study has examined the effects of ALA and quercetin supplementation on conversion of ALA to long-chain PUFA in humans. Therefore, we conducted a controlled dietary intervention study to investigate, for the first time, whether conversion of nutritive ALA is increased by simultaneous intake of quercetin. Study variables include fatty acid profiles of serum phospholipids and erythrocytes. A second aim was to investigate potential sex-related differences in ALA conversion and *n*-3 PUFA status following ALA supplementation in metabolically healthy, young men and women.

Methods

Subjects

Of 250 volunteers (all of whom were university students), 140 attended a screening that included physical assessments (body height and weight, resting blood pressure, heart rate, waist and hip circumference), clinical assessments (liver function, serum lipids and lipoproteins, glucose and haematology), medical history and a dietary questionnaire (3 d food record). Inclusion criteria were non-smoking status, age 19–35 years and BMI 19–25 kg/m². Exclusion criteria included fasting serum TAG ≥ 2.26 mmol/l (≥ 200 mg/dl), fasting serum LDL-cholesterol ≥ 4.14 mmol/l (≥ 160 mg/dl), metabolic or endocrine diseases, malabsorption syndromes, pregnancy and lactation, alcohol abuse, dietary supplements (e.g. polyphenols, fish oil fatty acids or vitamin E) and restrictive dietary requirements. Volunteers with no daily use of vegetable oil and spread in their habitual diet were also excluded.

In all, seventy-four metabolically healthy subjects (thirty-seven women and thirty-seven men) were included in the study. Of them, seven subjects dropped out for personal reasons. Data from the remaining sixty-seven subjects (thirty-three women and thirty-four men) completing the entire study were included in the analysis (per-protocol evaluation). Participant flow from initial screening to final analysis is shown in Fig. 1. Baseline characteristics are presented in Table 1.

The study protocol was explained in detail to the participants, and all of them gave written consent prior to the beginning of the study. The protocol was in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethical Committee of the Medical Faculty of the Rheinische Friedrich-Wilhelms-Universität Bonn, Germany. The trial was registered

at www.germanctr.de/ and <http://apps.who.int/trialsearch/asDRKS00005076>.

The subjects were instructed to maintain their habitual diet, physical activity levels, lifestyle and body weight throughout the study. Subjects using contraceptives (thirty women) or thyroid drugs (*n* 4) were asked to continue taking their medication throughout the study.

Study design and dietary intervention

The study was conducted in a double-blinded, randomised, placebo-controlled, crossover trial, with two 8-week intervention periods separated by an 8-week washout period (Fig. 2). During the intervention periods, participants ingested at least 3.3 g/d ALA. Subjects were asked to replace their normal margarine or butter with a commercially available rapeseed oil-based margarine (ALA content, 7.7% of total fatty acids; fat content of margarine, 80%, Goldina; Ostthüringer Nahrungsmittelwerk Gera GmbH) and their habitual vegetable oil with refined rapeseed oil (ALA content, 8.8% of total fatty acids; Brökelmann+Co Ölmühle GmbH+Co.). To reach the daily ALA amount, participants ingested at least 30 g of rapeseed oil and 25 g of rapeseed oil-based margarine. All subjects received a dosage spoon with a 10 g capacity to calculate their consumption of margarine and oil. To monitor compliance, participants documented daily oil and margarine ingestion in the study diary. The study margarine and oil was provided two times per week and were incorporated into the usual diet of the participants. In addition, all participants were offered bread, buns and stirred cakes that contained rapeseed oil and rapeseed oil-based margarine, which were formulated especially for this study to encourage consumption of the required amount of ALA. Other dietary sources of *n*-3 fatty acids such as fatty fish, *n*-3 fatty acid-enriched foods, fish oil capsules, nuts and seeds were not allowed. For this purpose, study participants were given a detailed list of foods rich in *n*-3 fatty acids.

In addition to the ALA intervention, all participants were supplemented with quercetin (*verum*) or placebo in the form of hard gelatin capsules. Subjects were instructed to take one capsule with each principal meal for a total of three capsules per day. Capsules were produced at the Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany. Quercetin capsules were filled with onion skin extract powder (152 mg per capsule), whereas placebo capsules contained mannitol (approximately 170 mg per capsule). The onion skin extract powder (*Allium cepa* L; Rudolf Wild GmbH & Co. KG) contained 45.5% quercetin, and each capsule contained 63.5 mg of quercetin. The hard gelatin capsules (Coni-Snap[®] size 3) were supplied by Capsugel, and quercetin and placebo capsules were identical in shape and taste. Capsules were filled using a Dott Bonapace semi-automatic capsule-filling machine. Quality was checked by determining the homogeneity of weight distributions in a sample of twenty random capsules from each batch, and the microbiological burden of the capsules was determined after manufacture and before release. The blisters containing twenty capsules of quercetin or placebo were packed into study boxes. The primary investigators, study personnel and participants were



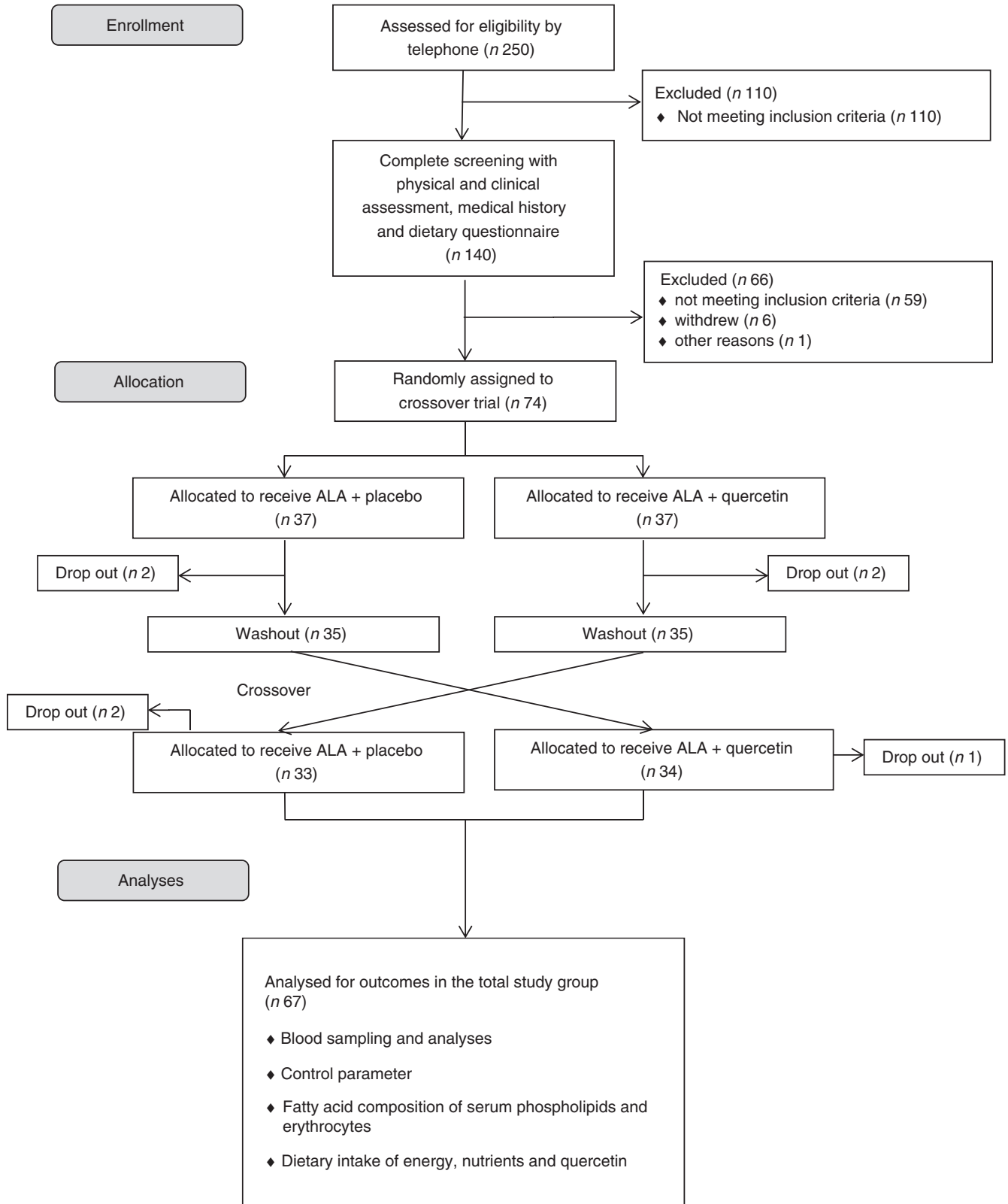


Fig. 1. Flow diagram of participants. ALA, α -linolenic acid.

blinded to the treatment. A quercetin dose of 190 mg/d was selected to represent approximately fifteen times the estimated mean daily quercetin intake in Germany⁽²⁸⁾ and other European

populations⁽²⁹⁾. The bioavailability of quercetin from onion skin extract and similar quercetin dosages were examined in two previous studies by our research group^(30,31).

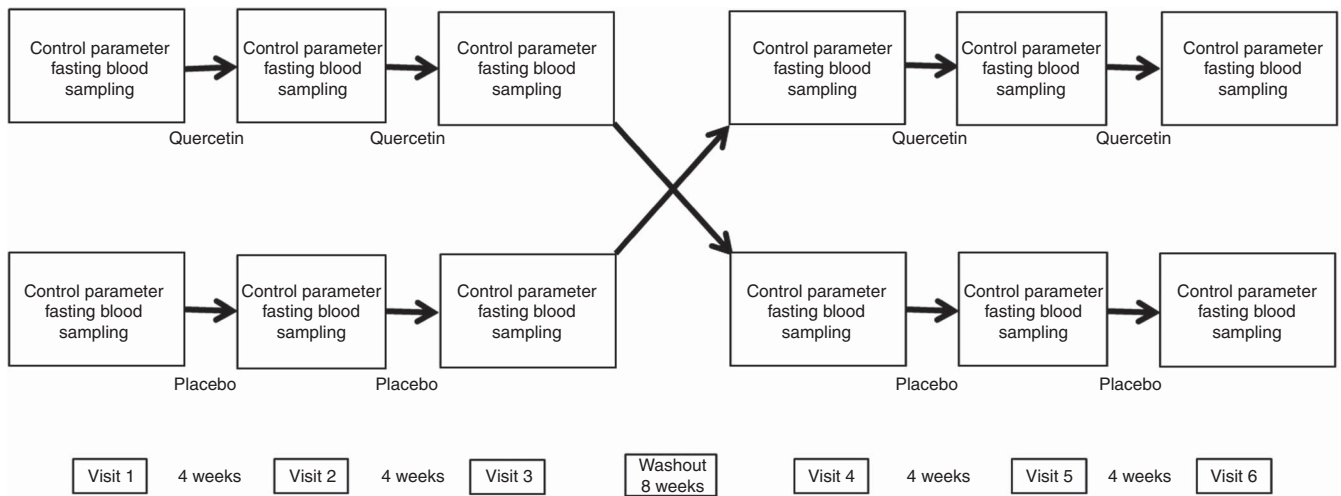


Fig. 2. Study design. Participants (n 67) were randomly assigned to consume α -linolenic acid (ALA)+placebo or ALA+quercetin and consumed the assigned capsules for 8 weeks. After an 8-week washout period, they crossed over to the alternate treatment.

Subjects were assigned to quercetin or placebo treatment using a block-wise randomisation scheme. Separate computer-generated randomisation schedules for men and women were created to achieve an equal distribution of men and women in each intervention group. Randomisation, allocation to one of the capsule treatments and capsule handling were managed by an independent researcher (B. A.). Study boxes with capsule blisters were distributed on days 0 and 28 of each intervention period at a 20% surplus. Leftovers and empty blisters were collected on days 28 and 56. Compliance was observed by counting capsules at the end of the study and instructing study participants to document capsule consumption, side effects, physical activity and other relevant observations in the study diary. In addition, we measured plasma quercetin concentrations at the beginning and end of the intervention periods (see below).

Subjects 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. Each of the 3 d food records represented food and beverage intake on 2 weekdays and 1 weekend day and was used to calculate the habitual dietary intake of energy, nutrients, long-chain *n*-3 fatty acids and quercetin.

Sample size was calculated on the basis of an expected increase in serum phospholipid EPA. The power calculation revealed that fifty-one subjects had to complete the study to achieve 80% power with a significance of $P < 0.05$ to detect a change of 6% between the quercetin and placebo treatments. Assuming a 30% dropout rate, we aimed to randomly assign seventy-four study participants.

Venous blood sampling and anthropometric measurements were conducted during six study visits (at the beginning, 4-week point and at the end of each intervention period). For each visit, study participants were advised to come to the study centre in the early morning after an overnight fast.

Measurements

Anthropometrics. Body height was determined on a stadiometer to the nearest 0.1 cm. Body weight was recorded to the

nearest 100 g. Waist circumference was measured midway between the lowest rib and the iliac crest with the subject at minimal respiration. Hip circumference was measured at the height of the greater trochanters. Body composition was assessed by bioelectrical impedance analysis (Nutriguard-M, Multi Frequency Phase-Sensitive Bioelectrical Impedance Analyzer; Data Input). Fat-free mass was calculated according to Sun *et al.*⁽³²⁾, and fat mass was calculated by subtracting fat-free mass from body weight.

Blood sample processing and analysis

Venous blood samples were collected at all visits between 07.00 and 09.30 hours under standardised conditions after an overnight fast. The subjects abstained from alcoholic beverages for 24 h prior to 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 and plasma samples from one subject were analysed in the same assay run (except for sample analyses in fresh samples, see below).

Clinical safety parameters and haematologic measurements.

Clinical safety and haematological 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. Haematological parameters, including erythrocytes, leucocytes, thrombocytes, Hb, haematocrit, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration and mean platelet volume, were determined using fluorescence flow cytometry. The sodium lauryl sulfate (SLS) Hb detection method was



performed using cyanide-free SLS, and the DC sheath flow detection method was performed using a Sysmex XE-5000 analyzer (Sysmex). Serum alanine transaminase, aspartate transaminase and γ -glutamyl-transpeptidase were determined using bichromatic kinetic methods, which are adaptations of the methodology recommended by the International Federation of Clinical Chemistry, with a Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics).

Serum lipid parameters, high-sensitivity C-reactive protein and sex hormones. Serum concentration of total cholesterol was measured using polychromatic endpoint, whereas serum concentrations of LDL-cholesterol, HDL-cholesterol, TAG, total bilirubin and uric acid, and plasma concentration of glucose were measured using bichromatic endpoint with a Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics). Serum concentration of urea was measured using VIS-photometry with a Dimension Vista 1500 analyzer. Serum concentrations of apo B and A1, albumin and high-sensitivity C-reactive protein were determined using nephelometric methods with a Dimension Vista 1500 analyzer. Serum concentrations of Na and K were analysed using the indirect potentiometric V-LYTE multisensory technology method with a Dimension Vista 1500 analyzer. Serum concentrations of progesterone, testosterone, interstitial cell-stimulating hormone and follicle-stimulating hormone were determined by chemiluminescence immunoassay with a Siemens Immulite, and of oestradiol with a Dimension Vista 1500 analyzer (Siemens Healthcare Diagnostics).

Fatty acid composition of serum phospholipids. As a short-term marker for fatty acid changes, we determined the fatty acid composition of serum phospholipids in duplicate by GC (model 3900; Varian GmbH; flame ionisation detector). After Folch extraction⁽³³⁾ was performed on serum samples, the phospholipid fraction was separated using a silica TLC plate in a solvent mixture of petroleum ether and acetic acid (17:3 by volume)⁽³⁴⁾. After scraping off the phospholipid band under UV light, the phospholipid fraction was methylated by transesterification with methanol-HCl (25:1 by volume) and incubated at 95°C for 4 h. The fatty acid methyl esters were extracted with petroleum ether, dissolved in heptane and injected into the GC. Peaks of interest were identified by comparing with authentic fatty acid methyl ester standards (1,2-diepentadecanoyl-sn-glycero-3-phosphatidylcholine; Larodan Fine Chemical). Selected fatty acids were expressed as a percentage of the total area by dividing the integrated area under the peak by the total area of all fatty acids. Fatty acids were also determined quantitatively from the internal standard and expressed as $\mu\text{mol/l}$. The intra-assay CV were <10%.

Fatty acid composition of erythrocytes. As a long-term marker for fatty acid changes, we determined the fatty acid composition of erythrocytes. Erythrocytes were washed three times with ice-cold isotonic saline to remove the buffy coat, and 1 ml of saline and 20 μl of butylhydroxytoluene (0.01%) in methanol were added to avoid oxidation. Erythrocytes were

stored in a N₂ atmosphere at -80°C until analysis. Erythrocytes were thawed and haemolysed by adding 1 ml to 1 ml of ice-cold water. Prior to extraction, 340 mg of NaCl was added. The lipid fraction was extracted using methanol-chloroform (1:2 by volume) according to a modified Folch method⁽³³⁾, methylated using methanol-HCl (25:1 by volume) and incubated at 95°C for 4 h. The resulting fatty acid methyl esters were extracted with petroleum ether, dissolved in heptane and injected into the GC. Analyses were conducted in duplicate. The intra-assay CV were <10%.

Evaluation of 3 d food records

Self-reported intakes of energy, macronutrients, dietary fibre and antioxidant pro-/vitamins were calculated using the computer-based nutrient calculation program EBISpro (University of Hohenheim) based on the German Nutrient Database Bundeslebensmittelschlüssel (Max Rubner-Institute). Quercetin intake was estimated using the US Department of Agriculture flavonoid database⁽³⁵⁾.

Statistical analyses

All statistical analyses were performed using IBM SPSS statistical software (SPSS version 21; IBM Corporation). The distribution of variables was analysed 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 the 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 *v.* endpoint) and intergroup (changes during quercetin *v.* changes during placebo treatment) comparisons of normally distributed data were performed using paired Student's *t* tests.

We calculated the treatment difference and examined sex-related differences 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. We also performed repeated-measures ANOVA (RM-ANOVA) for the ALA, EPA and DHA content in the phospholipid fractions. The fixed factors were treatment (two levels: quercetin and placebo) and time of measurement (three levels: weeks 0, 4 and 8 of the intervention periods). If the residuals were not normally distributed, RM-ANOVA were conducted with log-transformed variables. In all cases, a value of $P \leq 0.05$ (two-sided) was considered statistically significant. Unless otherwise indicated, descriptive data are presented as arithmetic means and standard deviations. A test for carry-over effects according to Kenward & Jones⁽³⁶⁾ was used. No carry-over effects between the two intervention periods could be observed. All analyses are based on a per-protocol basis. Complete data sets were available for all sixty-seven participants. Pearson's correlation coefficients were used to assess relationships between ALA, EPA and DHA in erythrocytes and serum phospholipids (calculated as a percent of total fatty acids) at baseline (week 0) and during the intervention.

Results

Subject characteristics at screening

Characteristics of the participants at screening are presented in Table 1. We observed sex-related differences in body height, body weight, waist circumference, systolic blood pressure, HDL-cholesterol and uric acid (Table 1).

Compliance, dietary intake and consumption of rapeseed oil and margarine

Count of returned capsules indicated almost full compliance (94.4 (SD 5.8) and 95.6 (SD 5.0)% for ALA+quercetin and ALA+placebo consumption, respectively). Compliance with quercetin supplementation was objectively confirmed by a marked increase in fasting plasma concentration of quercetin from 30.4 to 494.7 nmol/l (1660.6%; mean change +464.2 nmol/l; $P \leq 0.0001$) and of total flavonols from 50.2 to 570.0 nmol/l (1065.2%; mean change, +519.9 nmol/l; $P \leq 0.0001$).

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, antioxidant vitamins (e.g. vitamin E, vitamin C), dietary fibre and quercetin during the intervention periods (data not shown). The 3 d dietary records were not significantly different at screening and during the washout periods. Thus, we calculated the habitual diet by averaging the data from both of these records. The percentage of energy derived from protein, carbohydrates and fat, as well as fatty acid intake, differed significantly between the habitual diet and the diet during the interventions (Table 2). As expected, subjects increased consumption of ALA and avoided other sources of long-chain PUFA during the intervention periods.

The mean estimated daily intake of rapeseed oil was 23.7 (SD 7.9) and 23.3 (SD 8.1) g for the ALA+placebo and ALA+quercetin interventions, respectively. Mean estimated daily intake of margarine was 24.2 (SD 9.8) and 24.1 (SD 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 (SD 0.8) g and did not differ between intervention groups. Total ALA intake (which included the contribution from the remainder of the diet) was 2.16% (5.4 g/d) and 2.17% (5.4 g/d) of energy for ALA+placebo and ALA+quercetin interventions, respectively. The reported daily energy and nutrient intakes during the intervention periods are presented in Table 2.

Body weight, waist and hip circumference, body composition and safety parameters

Body weight, waist and hip circumference, relative fat mass and fat-free mass remained constant throughout the study (data not shown). Biomarkers of liver and renal function (ALT, AST, GGT, bilirubin, albumin, creatinine, urea and uric acid), haematology, serum electrolytes and sex hormones were within normal ranges at all time points and were not different between the groups (data not shown).

Fatty acid composition of serum phospholipids

Fatty acid composition was not different between the two groups at baseline. ALA supplementation of a duration of 8 weeks led to 69.3 and 55.8% increases in serum phospholipid ALA concentration in the ALA+placebo and ALA+quercetin interventions, respectively (both $P \leq 0.0001$; Table 3). Moreover, EPA concentration increased with both interventions (37.3% for ALA+placebo; $P=0.001$ and 25.5% for ALA+quercetin; $P=0.048$), and the increases in ALA and EPA concentration were similar between the interventions. DHA concentration decreased by 9.3% with ALA+quercetin ($P=0.0002$) and was significantly different from the decrease in the ALA+placebo group ($P=0.021$). However, the pattern of change in ALA, EPA and DHA concentration differed over the

Table 1. Subject characteristics and blood parameters at screening (Mean values and standard deviations)

	Total (n 67)		Women (n 33)		Men (n 34)		Women v. men (P)*
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	24.6	3.9	24.1	2.8	25.0	4.8	0.930
Body height (cm)	174.1	8.8	168.4	6.2	179.7	7.3	≤ 0.0001
Body weight (kg)	67.7	10.9	60.3	7.0	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
Hip circumference (cm)	89.9	5.4	88.7	5.2	91.1	5.5	0.077
Waist:hip ratio	0.89	0.06	0.88	0.06	0.91	0.05	0.085
Systolic BP (mmHg)	130.9	14.6	123.9	12.8	137.7	13.0	≤ 0.0001
Diastolic BP (mmHg)	84.1	9.5	83.2	10.6	85.0	8.4	0.286
Heart rate (min)	72.1	13.8	75.3	13.8	69.1	13.3	0.132
Serum TAG (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
Uric acid (μmol/l)	241.55	72.78	187.19	38.90	292.70	58.74	≤ 0.0001

BP, blood pressure.

* Unpaired Student's *t* test or Mann–Whitney *U* test.

Table 2. Reported daily energy and nutrient intakes in healthy men and women during the 8-week supplementation with α -linoleic acid (ALA) + placebo or ALA + quercetin (Mean values and standard deviations)

	Habitual diet		ALA + placebo (n 67)		ALA + placebo v. habitual diet (P)†	ALA + quercetin (n 67)		ALA + quercetin v. habitual diet (P)†
	Mean	SD	Mean	SD		Mean	SD	
Total energy (MJ/d)‡	9.28	1.94	9.80	1.81	0.074	9.79	2.12	≤0.0001
Protein (En%)	16.1	3.0	14.2	2.5	≤0.0001	14.0	2.7	≤0.0001
Carbohydrates (En%)	41.9	5.9	40.0	4.9	0.004	39.4	5.6	≤0.0001
Total fat (En%)	39.5	5.8	43.6	4.7	≤0.0001	44.9	5.5	≤0.0001
SFA (En%)	16.0	3.5	13.4	2.7	≤0.0001	13.6	2.9	≤0.0001
MUFA (En%)	13.9	2.3	19.6	2.6	≤0.0001	20.4	3.0	≤0.0001
PUFA (En%)	6.8	2.2	8.7	1.4	≤0.0001	8.9	1.4	≤0.0001
<i>n</i> -6 PUFA (En%)	5.78	1.85	6.47	1.10	0.001	6.68	1.10	≤0.0001
18:2 (<i>n</i> -6) (g/d)	13.76	6.28	16.07	4.22	≤0.0001	16.46	4.30	≤0.0001
20:4 (<i>n</i> -6) (g/d)	0.14	0.09	0.11	0.07	0.031	0.15	0.14	0.681
<i>n</i> -3 PUFA (En%)	1.03	0.59	2.22	0.43	≤0.0001	2.24	0.42	≤0.0001
18:3 (<i>n</i> -3) (g/d)	2.21	1.47	5.41	1.40	≤0.0001	5.39	1.34	≤0.0001
20:5 (<i>n</i> -3) (g/d)	0.06	0.09	0.02	0.05	0.003	0.03	0.08	0.003
22:6 (<i>n</i> -3) (g/d)	0.17	0.20	0.09	0.10	≤0.0001	0.11	0.18	0.023
<i>n</i> -6: <i>n</i> -3 PUFA ratio	6.29	2.31	2.97	0.48	≤0.0001	3.04	0.53	≤0.0001
LA:ALA ratio	6.9	2.5	3.0	0.5	≤0.0001	3.1	0.5	≤0.0001
Dietary fibre (g/MJ)	2.66	0.96	2.42	0.73	0.004	2.38	0.77	≤0.0001
Quercetin (mg/d)	7.5	5.7	8.7	6.3	0.036	7.7	5.0	0.580
Vitamin E (mg/MJ)§	1.61	0.46	2.32	0.38	≤0.0001	2.42	0.43	≤0.0001

En%, energy percent; LA, linoleic acid.

† Unpaired Student's *t* test or Mann-Whitney *U* test.

‡ To convert to kcal/d multiply by 239.

§ α -Tocopherol equivalents.

course of the intervention. ALA was only significantly increased over the first 4 weeks in both groups (both $P \leq 0.0001$, Fig. 3), and the EPA increase was only significant during the first 4 weeks in the ALA + placebo group ($P = 0.005$) (Fig. 4). DHA decreased significantly over the first 4 weeks in the ALA + quercetin group ($P = 0.0001$) (Fig. 5). Overall, we observed no sex-related differences in changes in ALA, EPA and DHA concentration in serum phospholipid fatty acids from week 0 to week 8. We observed a significant decrease in the *n*-6 fatty acid, arachidonic acid (AA), in the ALA + quercetin group ($P = 0.003$).

Similarly, the percentage distribution of ALA ($P \leq 0.0001$ for both treatments) and EPA ($P \leq 0.0001$ for ALA + placebo and $P = 0.007$ for ALA + quercetin) increased significantly (Table 3), whereas the percentage distribution of DHA decreased in the ALA + quercetin group ($P = 0.016$). Percentage stearidonic acid decreased ($P = 0.011$ for ALA + placebo and $P = 0.0002$ for ALA + quercetin) and percentage docosapentaenoic acid (DPA) increased with both interventions ($P = 0.006$ for ALA + placebo and $P = 0.0001$ for ALA + quercetin), although no significant intergroup effects were observed.

Serum phospholipid fatty acid profiles were not different between men and women at baseline, after the two treatments, or over the course of the treatment (change from week 0 to week 8) (data not presented).

Fatty acid composition of erythrocytes

After 8 weeks, erythrocyte ALA content increased by 48.6% ($P \leq 0.0001$) with the ALA + placebo intervention and by 45.8% ($P \leq 0.0001$) with the ALA + quercetin intervention (Table 4). EPA increased by 12.7% in the ALA + quercetin group

($P = 0.008$). DHA decreased by 2.9% with both treatments ($P = 0.005$ for ALA + placebo and $P = 0.001$ for ALA + quercetin). DPA increased by 3.8% in the ALA + placebo group ($P = 0.003$) and by 4.5% in the ALA + quercetin group ($P \leq 0.0001$) (Table 4). However, intergroup comparisons did not show an effect of adding quercetin to ALA, and neither treatment significantly affected the content of (*n*-6) fatty acids linoleic acid (LA) and AA in erythrocytes (Table 4). Erythrocyte fatty acid profiles were not different between men and women at baseline, after the two treatments, or over the course of the treatment (from week 0 to week 8) (data not presented).

Correlations

Baseline erythrocyte content of ALA, EPA and DHA was significantly correlated with baseline percentage fatty acid content in serum phospholipids (for ALA + placebo: ALA, $r = 0.59$, $P \leq 0.0001$; EPA, $r = 0.54$, $P \leq 0.0001$; DHA, $r = 0.76$, $P \leq 0.0001$ and for ALA + quercetin: ALA, $r = 0.56$, $P \leq 0.0001$; EPA, $r = 0.67$, $P \leq 0.0001$; DHA, $r = 0.79$, $P \leq 0.0001$). In addition, fatty acid changes in erythrocytes were significantly correlated with changes in serum phospholipids (ALA + placebo: ALA, $r = 0.61$, $P \leq 0.0001$; EPA, $r = 0.44$, $P = 0.0002$; DHA, $r = 0.55$, $P \leq 0.0001$ and ALA + quercetin: ALA, $r = 0.50$, $P \leq 0.0001$; EPA, $r = 0.35$, $P = 0.004$; DHA, $r = 0.39$, $P = 0.001$). The increase in erythrocyte EPA content was negatively correlated with erythrocyte EPA content at baseline (ALA + placebo: $r = -0.343$, $P = 0.004$ and ALA + quercetin: $r = -0.495$, $P \leq 0.0001$). Thus, the erythrocyte EPA content at baseline and the change in erythrocyte EPA content were related, as individuals with lower baseline

Table 3. Fatty acid composition of serum phospholipids (in $\mu\text{mol/l}$ and % total fatty acids) in metabolically healthy men and women during 8-week supplementation with α -linoleic acid (ALA) + placebo or ALA + quercetin* (Mean values and standard deviations)

	ALA + placebo (n 67)							ALA + quercetin (n 67)							Treatment difference†		
	Baseline		Endpoint		Mean change		P value intragroup comparison	Baseline		Endpoint		Mean change		P value intragroup comparison			P value intergroup comparison
	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD				
Fatty acids ($\mu\text{mol/l}$)																	
Total SFA	1978.25	418.90	1924.07	416.03	-54.18	318.46	0.168	2074.54	507.55	1895.07	419.92	-179.47	410.63	0.001	0.040	-125.29	489.15
16:0	1341.00	307.98	1275.90	317.53	-65.10	223.54	0.020	1410.49	377.74	1250.00	321.93	-160.50	287.03	≤ 0.0001	0.026	-95.40	342.84
18:0	487.23	103.72	497.22	92.62	9.99	88.68	0.360	505.86	119.03	497.88	100.83	-7.98	118.83	0.764	0.226	-17.97	137.71
Total MUFA	413.26	102.32	424.14	95.19	+10.89	71.08	0.159	440.16	109.93	423.19	99.41	-16.97	96.58	0.273	0.023	-27.86	114.73
18:1 (n-9)	296.59	76.06	301.20	68.21	+4.61	56.86	0.509	312.14	83.39	297.87	74.54	-14.27	77.84	0.138	0.106	-18.88	94.27
Total PUFA	1796.41	398.03	1744.99	382.36	-51.42	282.72	0.141	1852.46	430.75	1732.46	369.45	-120.00	349.99	0.007	0.188	-68.58	422.40
Total (n-6) PUFA	1581.17	335.17	1520.43	322.50	-60.75	254.25	0.055	1630.01	373.17	1521.09	312.42	-108.92	319.66	0.007	0.306	-48.18	382.17
18:2 (n-6)	943.96	215.68	913.28	188.51	-30.68	177.22	0.161	957.47	216.06	918.32	188.47	-39.09	212.06	0.136	0.795	-8.41	264.17
20:4 (n-6)	468.38	122.00	453.59	120.11	-14.79	80.78	0.139	493.09	147.54	455.00	121.06	-38.08	101.77	0.003	0.124	-23.30	122.29
Total (n-3) PUFA	215.23	82.85	224.56	78.09	+9.33	49.83	0.130	222.44	81.03	211.37	74.99	-11.07	57.70	0.121	0.031	-20.40	75.99
18:3 (n-3)	14.02	9.07	19.51	8.13	+5.50	7.67	≤ 0.0001	13.80	7.87	18.21	8.24	+4.41	7.28	≤ 0.0001	0.341	-1.08	9.24
20:5 (n-3)	37.29	19.26	45.80	20.58	+8.52	20.68	0.001	41.37	26.05	45.02	20.97	+3.65	23.43	0.048	0.179	-4.87	34.83
22:6 (n-3)	161.85	64.33	156.77	62.85	-5.09	34.27	0.229	165.15	60.89	145.78	57.56	-19.37	40.54	0.0002	0.021	-14.29	49.41
% Total fatty acids																	
Total SFA	45.30	1.16	44.99	1.34	-0.31	1.02	0.014	45.44	1.00	43.50	1.88	-1.94	1.59	≤ 0.0001	≤ 0.0001	-1.62	1.81
16:0	29.84	1.70	28.89	2.03	-0.94	1.29	≤ 0.0001	29.96	1.75	28.33	2.60	-1.63	2.06	≤ 0.0001	0.019	-0.69	2.30
18:0	11.64	1.16	12.19	1.42	0.56	0.91	≤ 0.0001	11.63	1.25	12.32	1.30	0.68	1.07	≤ 0.0001	0.372	0.13	1.17
Total MUFA	14.97	1.53	15.73	1.44	+0.76	1.15	≤ 0.0001	15.15	1.57	15.78	1.52	+0.63	1.48	0.001	0.592	-0.13	2.03
18:1 (n-9)	10.57	1.42	10.98	1.30	+0.42	1.05	0.002	10.66	1.51	10.92	1.35	+0.26	1.40	0.133	0.490	-0.16	1.86
Total PUFA	39.64	1.47	39.24	1.47	-0.40	1.26	0.011	39.41	1.63	39.69	1.98	+0.27	1.95	0.254	0.016	+0.68	2.25
Total (n-6) PUFA	34.72	1.60	34.01	1.50	-0.71	1.57	0.0005	34.31	1.85	34.46	2.18	+0.15	2.29	0.595	0.010	0.85	2.65
18:2 (n-6)	21.62	2.04	21.40	1.87	-0.22	2.05	0.391	21.10	2.23	21.77	2.48	+0.67	2.39	0.025	0.022	+0.89	3.11
20:4 (n-6)	9.27	1.67	9.11	1.51	-0.16	1.12	0.246	9.32	1.61	9.26	1.56	-0.06	0.88	0.598	0.536	0.10	1.36
Total (n-3) PUFA	4.80	0.92	5.12	0.91	+0.32	0.83	0.002	4.96	1.08	5.12	0.93	+0.15	0.91	0.174	0.223	-0.17	1.12
18:3 (n-3)	0.29	0.15	0.43	0.13	+0.13	0.14	≤ 0.0001	0.28	0.13	0.40	0.13	+0.12	0.14	≤ 0.0001	0.510	-0.01	0.18
18:4 (n-3)	0.09	0.03	0.08	0.03	-0.01	0.03	0.011	0.09	0.04	0.08	0.03	-0.02	0.03	0.0002	0.155	-0.01	0.04
20:5 (n-3)	0.77	0.32	0.99	0.42	+0.21	0.42	≤ 0.0001	0.83	0.45	0.98	0.40	+0.15	0.44	0.007	0.500	-0.07	0.60
22:5 (n-3)	0.73	0.22	0.78	0.23	+0.04	0.13	0.006	0.74	0.23	0.81	0.24	+0.06	0.12	0.0001	0.408	+0.02	0.17
22:6 (n-3)	2.86	0.68	2.79	0.68	-0.07	0.50	0.224	2.97	0.78	2.80	0.66	-0.17	0.56	0.016	0.214	-0.10	0.63

* 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 - baseline)) - (ALA + placebo (endpoint - baseline)).

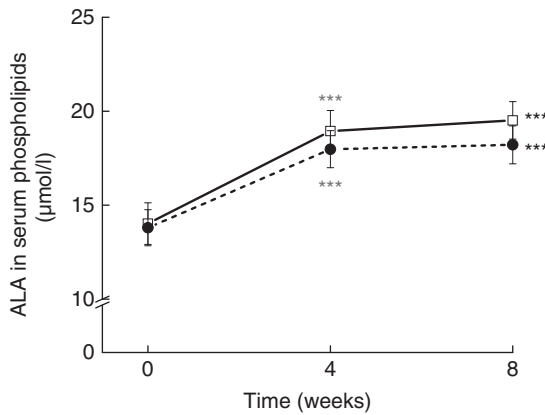


Fig. 3. Time curves of α -linolenic acid (ALA) concentration in serum phospholipids in metabolically healthy men and women before, after 4-week and after 8-week supplementation with --●--, ALA + quercetin (190 mg/d) and --□--, ALA + placebo (n 67). Values are means, with their standard errors represented by vertical bars. *** Mean value was significantly different from that at baseline ($P \leq 0.0001$; intragroup comparisons).

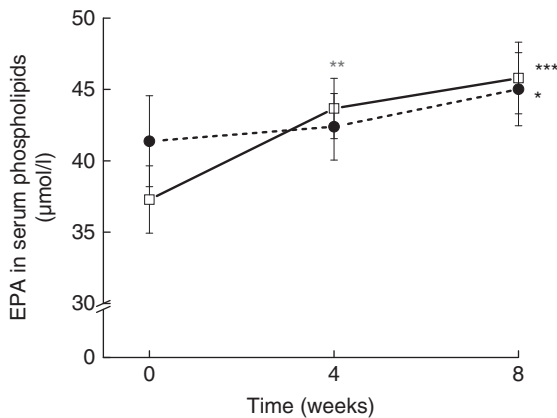


Fig. 4. Time curves of EPA concentration in serum phospholipids in metabolically healthy men and women before, after 4-week and after 8-week supplementation with --●--, α -linolenic acid (ALA) + quercetin (190 mg/d) or --□--, ALA + placebo (n 67). Values are means, with their standard errors represented by vertical bars. Mean value was significantly different from that at baseline: * $P = 0.048$, ** $P = 0.005$, *** $P = 0.001$ (intragroup comparisons).

EPA contents demonstrated greater increases in erythrocyte EPA with ALA supplementation (Fig. 6).

Discussion

The aim of this double-blinded, placebo-controlled, crossover intervention study in metabolically healthy men and women was to investigate whether conversion of ALA, analysed by changes in fatty acid profiles of serum phospholipids and erythrocytes, is influenced by simultaneous intake of quercetin. A second aim was to investigate potential sex-related differences in ALA conversion and n -3 PUFA status following ALA intake. We showed that increased dietary ALA significantly increased ALA, EPA and DPA, but not DHA, in serum phospholipids and erythrocytes. Changes in the fatty acid composition of erythrocytes, as indicated by relative proportions of ALA, EPA, DPA and DHA, correlated with fatty acid changes in

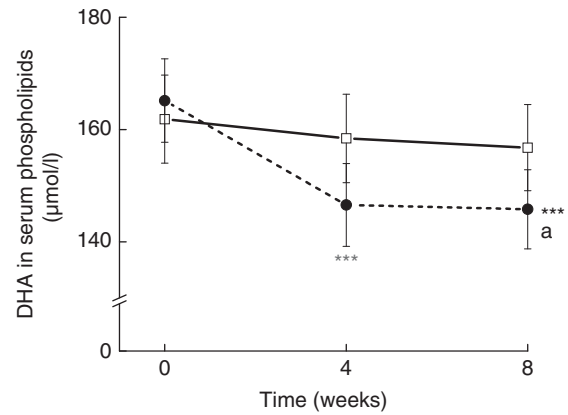


Fig. 5. Time curves of DHA concentration in serum phospholipids in metabolically healthy men and women before, after 4-week and after 8-week supplementation with --●--, α -linolenic acid (ALA) + quercetin (190 mg/d) or --□--, ALA + placebo (n 67). Values are means, with their standard errors represented by vertical bars. *** Mean value was significantly different from that at baseline ($P \leq 0.0001$; intragroup comparisons). † Changes in DHA were significantly different between ALA + placebo and ALA + quercetin ($P = 0.021$; intergroup comparison).

serum phospholipids. Contrary to our hypothesis, neither quercetin supplementation nor sex influenced the conversion of ALA to long-chain n -3 fatty acids.

Changes in fatty acid profiles

The intervention significantly increased ALA content in serum phospholipids and erythrocytes, which objectively indicated good compliance and effective incorporation of the ingested n -3 fatty acid. The content of EPA and DPA also increased in the serum phospholipids and erythrocytes, indicating the occurrence of metabolic conversion of ALA to EPA and DPA through a series of elongation and desaturation reactions. The increase in EPA and DPA occurred with a daily dose of 3.6 g of ALA, which represented approximately three times the estimated daily dietary ALA intake (approximately 0.5–1.7 g) in most European countries and the USA^(10,37,38). We demonstrated that this high dose was easily achieved with regular consumption of ALA-rich rapeseed oil and margarines and did not require dietary supplements.

The significant increase in ALA, EPA and DPA, but not DHA, following ALA intervention is in agreement with earlier ALA intervention studies^(25,39–45). The lack of increase in DHA in serum phospholipids and erythrocytes may be because the ALA dose (3.6 g/d) was too low to exert a significant increase in DHA. Although ALA is an essential fatty acid for humans, >60% and up to 85% undergoes β -oxidation and is used as an energy and carbon source^(9,38,46). Thus, only a relatively small amount of the ingested ALA undergoes elongation and desaturation and is used for n -3 long-chain PUFA synthesis^(47,48). Barceló-Coblijn *et al.*⁽⁴⁹⁾ noted that 2.4–3.6 g/d ALA for 12 weeks increased EPA, but not DHA, content in erythrocytes, and they concluded that fatty acid composition of erythrocytes is influenced in a dose- and time-dependent manner. Results of other studies also confirm that the DHA content in plasma phospholipids does not change with ALA intakes of up to 20 g/d^(37,41,43), suggesting that

Table 4. Fatty acid composition of erythrocytes (% total fatty acids) in metabolically healthy men and women during 8-week supplementation with α -linoleic acid (ALA) + placebo or ALA + quercetin*

% Total fatty acids	ALA + placebo (n 67)							ALA + quercetin (n 67)							Treatment difference†		
	Baseline		Endpoint		Mean change		P value intragroup comparison	Baseline		Endpoint		Mean change		P value intragroup comparison	P value intergroup comparison	Mean	SD
	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD				
Total SFA	47.11	3.08	47.25	3.10	+0.14	1.36	0.735	46.82	1.19	48.24	3.76	+1.42	3.26	0.310	0.440	+1.28	4.09
16:0	27.23	1.60	26.52	1.42	-0.71	1.17	≤0.0001	27.67	2.12	26.62	1.64	-1.05	1.55	≤0.0001	0.192	-0.34	1.76
18:0	12.85	1.07	13.44	1.59	0.59	1.07	≤0.0001	12.86	1.18	13.29	1.34	0.43	1.42	0.001	0.262	-0.17	1.89
Total MUFA	20.00	1.08	20.56	1.34	+0.56	1.07	0.003	20.12	1.20	20.47	1.26	+0.62	1.14	0.002	0.695	+0.05	1.35
18:1 (n-9)	14.02	0.94	14.23	1.08	+0.22	0.77	0.024	13.91	1.01	14.31	1.02	+0.40	0.82	0.0002	0.045	+0.18	0.98
Total PUFA	33.12	1.51	32.41	1.31	-0.71	1.62	0.001	32.62	1.44	32.37	1.77	-0.25	1.44	0.376	0.112	+0.46	2.12
Total (n-6) PUFA	26.33	1.58	25.84	1.19	-0.49	1.43	0.013	25.80	2.01	25.82	1.57	+0.01	1.50	0.592	0.043	+0.50	1.87
18:2 (n-6)	11.40	1.06	11.41	0.93	+0.01	0.81	0.947	11.21	1.24	11.34	1.01	+0.14	1.03	0.280	0.249	+0.13	1.27
20:4 (n-6)	12.56	1.21	12.41	1.01	-0.15	0.91	0.192	12.46	1.41	12.44	1.23	-0.02	0.85	0.878	0.374	+0.13	1.19
Total (n-3) PUFA	6.59	1.19	6.55	0.74	-0.05	1.00	0.585	6.39	0.83	6.43	0.74	+0.04	0.46	0.400	0.964	+0.09	1.17
18:3 (n-3)	0.19	0.07	0.26	0.08	+0.07	0.07	≤0.0001	0.19	0.08	0.26	0.07	+0.07	0.06	≤0.0001	0.467	-0.01	0.08
18:4 (n-3)	0.09	0.08	0.09	0.06	+0.00	0.09	0.310	0.08	0.05	0.08	0.04	-0.01	0.05	0.037	0.061	-0.01	0.10
20:5 (n-3)	0.61	0.27	0.64	0.28	+0.03	0.22	0.092	0.60	0.31	0.64	0.27	+0.04	0.20	0.008	0.463	+0.01	0.32
22:5 (n-3)	1.99	0.34	2.05	0.32	+0.06	0.16	0.003	1.95	0.32	2.03	0.33	+0.08	0.15	≤0.0001	0.300	+0.02	0.23
22:6 (n-3)	3.55	0.79	3.42	0.75	-0.13	0.37	0.005	3.57	0.87	3.44	0.79	-0.14	0.33	0.001	0.923	-0.01	0.50

* 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 - baseline)) - (ALA + placebo (endpoint - baseline)).

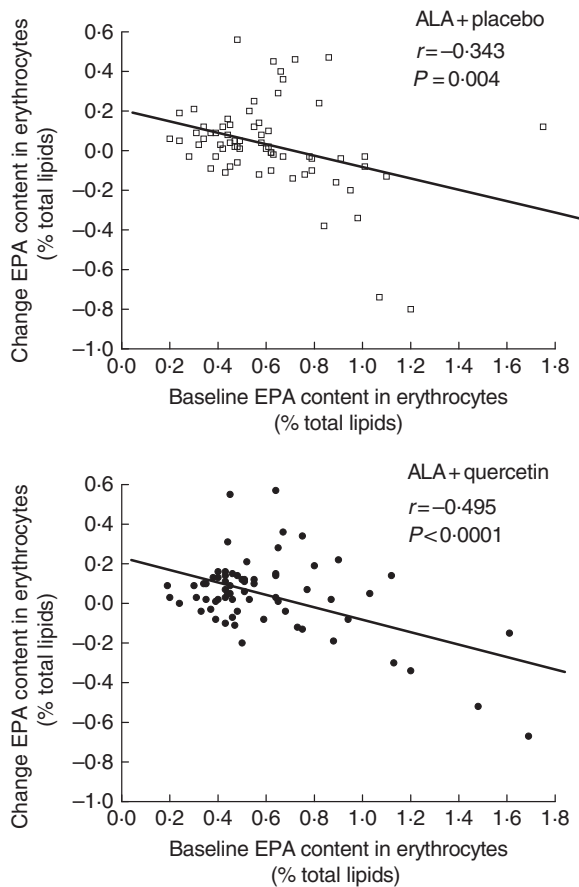


Fig. 6. Relationship between baseline EPA content in erythrocytes and change after 8 weeks, ●, α -linolenic acid (ALA)+quercetin (190 mg/d) or, □, ALA+placebo supplementation in metabolically healthy men and women (n 67). r , Pearson's rank correlation coefficient.

the lack of DHA increase in our study was not due to an inadequate dose of ALA.

An equally important point is that the intervention period of 8 weeks may have been too short to increase DHA content in erythrocytes. Because synthesis of DHA involves more steps than synthesis of EPA⁽³⁷⁾, assessing the effect on DHA content likely requires longer duration of ALA supplementation. Accordingly, some studies suggest that DHA blood measures increase with long-term ALA supplementation (52 weeks)⁽⁵⁰⁾.

Other studies have also shown a decreased proportion of DHA after an increase in dietary ALA intake^(51,52). DHA may also be retroconverted, causing ALA and C24:6 (n -3) to compete for Δ^6 -desaturase and resulting in EPA accumulation and limited synthesis and accumulation of DHA. This mechanism has been postulated in Hep-G2 phospholipids and previous human trials⁽⁵³⁻⁵⁷⁾. Studies estimate that 0.5–12% of DHA is retroconverted to EPA^(12,58). Moreover, in a few cases, the declines in EPA/DHA status might be declared by the exclusion of long-chain n -3 PUFA sources in the habitual diet of the participants.

Effect of quercetin supplementation

In the present study, quercetin did not significantly increase EPA in serum phospholipids and erythrocytes when compared

with placebo, indicating that quercetin did not affect the conversion of ALA. Moderate wine drinking has been associated with increased levels of EPA and DHA in plasma fatty acids in epidemiological studies^(15,59,60), and a cohort study shows a significant association between erythrocyte levels of EPA and DHA in wine drinkers but not in beer or spirit drinkers⁽¹⁶⁾, which suggests that components in wine only, not ethanol, affect the metabolism of n -3 PUFA. Animal studies show that consumption of flavonoids increases conversion of ALA, further supporting the hypothesis that ethanol does not play a major role in the association between wine drinking and increased n -3 PUFA metabolism^(17,61,62). Quercetin, ubiquitous in plants, is among the most potent antioxidants of plant origin and a predominant flavonoid in human nutrition⁽¹⁸⁾. Flavonoids exert biological effects by modulation of mammalian cell signaling pathways⁽⁶³⁻⁶⁵⁾, and experimental studies demonstrate that quercetin interacts with PPAR α ^(19,20), which may be a mechanism by which it influences Δ^6 -desaturase activity and conversion of ALA. Wu *et al.*⁽⁶⁶⁾ reported that dietary consumption of ALA with the polyphenol curcumin increases EPA and DHA content in the rat liver and brain due to elevated activity of the required enzymes (i.e. Δ^6 -desaturase). The reason why quercetin supplementation did not modulate the (n -3) PUFA elongation-desaturation pathway to increase EPA and DHA content in our study is unclear, although the relatively low bioavailability of quercetin in humans is a possible explanation. We demonstrated that, despite good compliance to quercetin supplementation, the plasma levels of quercetin were low (about 0.5 $\mu\text{mol/l}$)^(30,31,67). In contrast to our study, the applied doses of flavonoids in cell culture and animal studies were higher (e.g. about 500 mg/d)⁽⁶²⁾. Thus, possibly a pharmacological dose is needed to exert similar effects in humans.

Influence of sex

We found no significant sex-related differences in serum phospholipid and erythrocyte fatty acid profiles at baseline, after the two treatments, or over the course of the intervention. Stable isotope tracer studies show that the conversion of ALA to EPA and DHA is greater in young women of child-bearing age than in men of similar age^(12,21-25,68). Childs *et al.*⁽²⁷⁾ confirmed these findings in a re-examination of their data. The greater capacity of ALA to DHA conversion among women of child-bearing age may be an evolutionary adaption to ensure an adequate supply of DHA for the fetus^(37,69,70). Regulation of n -3 fatty acid metabolism and the conversion rates of ALA may be influenced by oestrogen^(26,27,44), as the DHA concentration in maternal blood is significantly elevated during the third trimester of pregnancy when the circulating levels of oestrogen are highest^(69,71). In addition, erythrocyte DHA content is greater in women taking an oestradiol-based contraceptive pill than in women not taking oral contraceptives^(72,73). In the present study, we could not find any significant differences in contraceptive *v.* non-contraceptive users in ALA conversion. Oestrogen may regulate the expression and activity of desaturases and elongases through PPAR α ⁽²⁶⁾. However, Sibbons *et al.*⁽⁷⁴⁾ showed that physiological concentrations of progesterone caused increased mRNA expression of the key enzymes for ALA



conversion in Hep-G2 cells, while oestrogen and testosterone had no significant effect. The reasons for the lack of sex-related differences in the present study are unclear. Decsi & Kennedy⁽⁶⁸⁾ postulated that sex explains only about 2% of the variability of plasma phospholipid DHA values. The regulation of PUFA metabolism is complex and includes genetic variations, dietary components and substrate competition⁽¹³⁾, whereby the lack of sex-related difference in the present study can be explained by heterogeneity and variance in PUFA metabolism.

Strengths and limitations

The major strengths of our study are the double-blinded, placebo-controlled, crossover design, relatively large sample size, low dropout rate and high compliance with the treatments. Furthermore, we examined the fatty acid composition of serum phospholipids and erythrocytes to depict short- and long-term changes, respectively, with the intervention. In addition, we examined both percentage fatty acid profiles and absolute concentrations of fatty acids in serum phospholipids. A potential limitation is that we did not measure Δ^6 -desaturase activity. Thus, we cannot confirm our hypothesis that increased conversion of ALA is due to increased Δ^6 -desaturase activity. Methods to measure Δ^6 -desaturase activity are complex and limited. The enzyme activity can be determined by taking subcutaneous adipose tissue or liver biopsies or estimations using product:substrate ratios of individual fatty acids. However, Sjögren *et al.*⁽⁷⁵⁾ showed that desaturation indices for Δ^6 -desaturase do not correlate with mRNA expression of the enzyme determined from biopsies. Another potential limitation might be the interference of the habitual diet of the participants. We included only subjects with sporadic intake of long-chain *n*-3 PUFA sources. However, the change in habitual diet, as a potential confounder, may account for some of the declines in EPA/DHA status.

Conclusion

The present study showed that daily supplementation with 3.6 g/d ALA over 8 weeks improved *n*-3 long-chain PUFA status in metabolically healthy subjects. ALA supplementation increased ALA, EPA and DPA, but not DHA, in serum phospholipids and erythrocytes. The conversion of ALA was not affected by concomitant ingestion of a supra-nutritional dose of 190 mg/d quercetin. In addition, we did not find sex-specific differences in the responses to ALA intervention.

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C. B., P. S. and S. E. designed the study. B. Z. was the medical advisor. C. B. and S. E. recruited the subjects and performed the study. B. A. performed the blinding procedure. P. L. produced the capsules. C. B. performed the statistical calculations with help from R. F. and S. E. C. B., S. W., R. F., B. Z. and S. E. participated in

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