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The effect of camelina oil on vascular function in essential hypertensive patients with metabolic syndrome: A randomized, placebo-controlled, double-blind study

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Data described in the manuscript, code book, and analytic code will be made available upon request to the corresponding author.

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Running head: Camelina oil in hypertension with metabolic syndrome

ABBREVIATIONS

AA: arachidonic acid

Aix: augmentation index

ALA: alpha-linolenic acid

DGLA: dihomo-gamma-linolenic acid

DHA: docosahexaenoic acid

DPA: docosapentaenoic acid

EDA: eicosadienoic acid

EPA: eicosapentaenoic acid

FMD: flow-mediated dilatation

GA: gondoic acid

GTN: glyceryl trinitrate

HOMA-IR: homeostatic model assessment for insulin resistance

hsCRP: high-sensitivity C-reactive protein

IMT: intima-media thickness

LA: linoleic acid

MDRD: modification of diet in renal disease

PUFAs: polyunsaturated fatty acids

PWV: pulse wave velocity

RBC: red blood cells

SFA: saturated fatty acids

TBARS: thiobarbituric acid reactive substances

TEI: total energy intake

ABSTRACT

1 **Background.** The effects of a dietary supplementation with the vegetable omega-3 α -
2 linolenic acid (ALA) on cardiovascular homeostasis are unclear. In this context, it would be
3 interesting to assess the effects of camelina oil.

4 **Objective.** This study aimed to assess the cardiovascular and metabolic effects of camelina
5 oil in hypertensive patients with metabolic syndrome.

6 **Methods.** In a double-blind placebo-controlled randomized study, treated essential
7 hypertensive patients with metabolic syndrome received during 6 months either cyclodextrin-
8 complexed camelina oil containing ≈ 1.5 g ALA/day (n=40), or an isocaloric placebo (n=41),
9 consisting in the same quantity of cyclodextrins and wheat starch. Anthropometric data,
10 plasma lipids, glycemia, insulinemia, creatininemia, thiobarbituric acid reactive substances,
11 high-sensitivity C-reactive protein, and n-3, n-6 and n-9 fatty acids in erythrocyte membranes
12 were measured. Peripheral and central blood pressures, arterial stiffness, carotid intima-media
13 thickness and brachial artery endothelium-dependent flow-mediated dilatation and
14 endothelium-independent dilatation were assessed.

15 **Results.** Compared to placebo, camelina oil increased ALA (mean \pm SD: 0 ± 0.04 vs.
16 $0.08\pm 0.06\%$, $P<0.001$), its elongation product eicosapentaenoic acid (EPA; 0 ± 0.5 vs.
17 $0.16\pm 0.65\%$, $P<0.05$), and the n-9 gondoic acid (0 ± 0.04 vs. $0.08\pm 0.04\%$, $P<0.001$). No
18 between-group difference was observed for cardiovascular parameters. However, changes in
19 flow-mediated dilatation were associated with the magnitude of changes in EPA ($r=0.26$,
20 $P=0.03$). Compared to placebo, camelina oil increased fasting glycemia (-0.2 ± 0.6 vs. 0.3 ± 0.5
21 mmol/L, $P<0.001$) and homeostatic model assessment for insulin resistance (HOMA-IR; -
22 0.8 ± 2.5 vs. 0.5 ± 0.9 , $P<0.01$) index, without affecting plasma lipids, or inflammatory and
23 oxidative stress markers. Changes in HOMA-IR index were correlated with the magnitude of

24 changes in gondoic acid ($r=0.32$, $P<0.01$). Nutritional intake remained similar between
25 groups.

26 **Conclusion.** ALA supplementation with camelina oil did not improve vascular function but
27 adversely affected glucose metabolism in hypertensive patients with metabolic syndrome
28 Whether this adverse effect on insulin sensitivity is related to gondoic acid enrichment,
29 remains to be elucidated.

30 **Keywords:** Camelina oil, alpha-linolenic acid, vascular function, essential hypertension,
31 metabolic syndrome

32

33 INTRODUCTION

34 Metabolic syndrome represents a cluster of metabolic abnormalities, which develops during
35 the chronic exposure to a positive energy balance resulting from physical inactivity and
36 excess caloric intake. Metabolic syndrome is highly prevalent in essential hypertensive
37 patients potentiating vascular endothelial dysfunction and worsening their cardiovascular
38 prognosis (1-3).

39 The typical Western diet associates increased saturated fat intake together with a high
40 consumption of n-6 fatty acids relative to n-3 fatty acids (4). An excess in n-6 fatty acids
41 gives rise to pro-inflammatory metabolites while reducing the conversion of n-3 fatty acids to
42 cardiovascular protective mediators (4). An increasing number of studies suggests that marine
43 n-3 long-chain polyunsaturated fatty acids (PUFAs), namely eicosapentaenoic acid (EPA;
44 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), exert a beneficial effect on human
45 health and in particular reduce the cardiovascular risk (5-7). However, dietary supplements
46 are heavily based on fish oil which represents an organoleptic hurdle and raises environmental
47 concerns with the depletion of fatty fish stock combined with contamination by xenobiotics.

48 In this context, vegetable oils, rich in the n-3 fatty acid alpha-linolenic acid (ALA; 18:3 n-3),
49 have been approved by food safety authorities and are increasingly used in human food.
50 However, evidence for direct biological effects of ALA is scarce and its elongation to
51 bioactive EPA and even more to DHA remains elusive (5,8). In addition, the effect of ALA
52 on cardiovascular prognosis remains uncertain (5,7,8). Furthermore, although the effects of
53 flaxseed oil have been largely investigated (9), no study has evaluated the cardiovascular
54 effects of *Camelina Sativa* oil, which is used as a dietary supplement due to its high ALA
55 content. Interestingly, although the reported effects of dietary ALA intake on metabolic and
56 inflammatory markers are in general disappointing (10,11), *Camelina* oil supplementation
57 may be effective to prevent alterations in serum lipid profile (12,13). Whether the intake of

58 camelina oil, the composition of which differs from that of flaxseed oil by its high content of
59 omega-9 fatty acids in particular gondoic acid (20:1 n-9) (14), is associated with specific
60 cardiometabolic effects remains to be evaluated.

61 In this context, the aim of the present study was to carefully assess the vascular effects of
62 ALA supplementation with camelina oil in essential hypertensive patients with metabolic
63 syndrome. The principal objective was to show that camelina oil improves endothelial
64 function, as measured by flow-mediated vasodilatation (FMD) of the brachial artery induced
65 by forearm ischemia. Secondary objectives were to evaluate the effects of camelina oil on
66 peripheral and central hemodynamics, arterial function and dimension as well as on metabolic
67 homeostasis.

68

69 **SUBJECTS AND METHODS**

70 **Study Design and Ethical Statement**

71 A 6-month randomized, double-blind, placebo-controlled study was conducted to evaluate the
72 safety and efficacy of camelina oil supplementation in essential hypertensive patients with
73 metabolic syndrome. This multi-center study was conducted in the Departments of
74 Pharmacology of Rouen University Hospital and of Georges Pompidou European Hospital,
75 the Polyvalent-Nephrology Department of Manhès Hospital and the Department of Vascular
76 Medicine in Toulouse University Hospital, all in France. The study was approved by the local
77 Ethics Committee (Committee for the Protection of Persons of Normandy), and all
78 participants gave written informed consent. The study was conducted in accordance with the
79 Principles of Good Clinical Practice and the Declaration of Helsinki and was registered at
80 <https://clinicaltrials.gov> under the unique identifier NCT01566188.

81

82 **Population**

83 Patients with essential hypertension and metabolic syndrome were recruited by general
84 practitioners of local research networks and hospital hypertension centers. Eligible patients
85 were aged 18 to 70 years, with drugs-treated essential hypertension and at least 2 other
86 components of the metabolic syndrome defined in the National Cholesterol Education
87 Program (NCEP) Adult Treatment Panel (ATP) III (15), *i.e* increased waist circumference
88 (>102 cm for men, >88 cm for women); elevated triglycerides (≥ 150 mg/dl); low HDL
89 cholesterol (<40 mg/dl in men, <50 mg/dl in women); and/or impaired fasting glucose (≥ 110
90 mg/dl). Main exclusion criteria were secondary hypertension, treated diabetes or fasting
91 glycemia >126 mg/dl, chronic kidney disease or estimated glomerular filtration rate by
92 modification of diet in renal disease (MDRD) <60 ml/min, history of coronary artery disease,
93 peripheral vascular disease, stroke, myocardial infarction or heart failure, aspartate

94 aminotransferase or alanine aminotransferase levels ≥ 3 times the upper limit of normal; and
95 other clinically significant abnormal laboratory test results.

96

97 **Treatments**

98 Eligible participants were randomized to receive during 6 months either β cyclodextrin-
99 complexed camelina oil or an isocaloric placebo. Camelina oil was obtained from Phytocos
100 SAS[®] (Grosseuvre, France), and contained in particular 30% ALA and 15% of the omega-6
101 linoleic acid (LA; 18:2 n-6) (Fatty acid oil composition is given in Supplementary Table 1).
102 Camelina oil was combined with cyclodextrins using a patented manufacturing process (PCT/
103 FR2898817A1). Cyclodextrins are cyclic oligosaccharides produced from starch by
104 enzymatic conversion and are commonly used in the food industry pharmaceutical industries.
105 Camelina oil and cyclodextrins in a ratio of 37%/63% were mixed in a planetary
106 mixer. Demineralized water was added to obtain a powder of inclusion complex that was then
107 dried in the oven. The peroxide value, induction time, bacteriology counts were controlled
108 before the batch release. The advantage of this combination is twofold. First, cyclodextrin
109 allows the transformation of camelina oil into a powder which facilitates its administration
110 and masks its taste and odor in comparison with soft capsules. Second, cyclodextrin is a
111 dietary fiber that facilitates the absorption of n-3 fatty acids in the intestine and may reduce
112 the absorption of saturated fatty acids (SFA) and cholesterol (16,17). Previous results
113 obtained in rats have shown that this formulation increases plasma concentrations of ALA,
114 EPA and to a lesser extent DHA (18). The placebo consisted of a mixture of cyclodextrin, in a
115 quantity equivalent to that present in the active treatment, and wheat starch, making it
116 possible to obtain a powder identical in organoleptic terms to that obtained from camelina oil
117 and a comparable caloric intake. In addition, it does not contain any PUFAs of the n-3 or n-6
118 type. The treatment consisted of 25 ml of a powder containing 5.2 g of camelina oil (1.5 g of

119 ALA, or 6 g of wheat starch with 9 g of cyclodextrin) added to yoghurt, a dairy dessert or half
120 a glass of water, in two intakes per day during a meal. For precise measurement, patients were
121 provided with specific plastic measuring spoons to control the quantity of powder used.

122 The oil/cyclodextrin and starch/cyclodextrin powders were prepared, packaged in airtight
123 tanks and labelled in accordance with the randomization list and in compliance with French
124 regulatory authorities by the Department of Pharmacy of Rouen University Hospital and sent
125 to the pharmacies of each investigation center.

126

127 **Investigations and follow-up visits**

128 Participants were seen at baseline and at 6 months (180 ± 9 days) after treatment initiation for
129 arterial and biological measurements. In addition, two follow-up visits were performed 2
130 months (60 ± 3 days) and four months (120 ± 6 days) after treatment initiation to ensure
131 treatment compliance and tolerance.

132 Measurements were performed by assessors blinded to the treatment, in the morning while
133 overnight-fasted subjects were in a quiet air-conditioned room (22°C to 24°C). Subjects were
134 asked to refrain from smoking since the previous evening. Participant's height, weight and
135 waist circumference were measured. Blood sampling was performed allowing the
136 measurements of plasma lipids, glycemia, insulinemia, creatininemia, high-sensitivity C-
137 reactive protein (hsCRP), adiponectinemia, levels of thiobarbituric acid reactive substances
138 (TBARS), as well as the quantification of major n-3, n-6 and n-9 fatty acids and SFA in red
139 blood cell (RBC) membranes by gas chromatography as previously described (19).

140 After a 15-minute rest in supine position, brachial blood pressures and heart rate were
141 measured using validated brachial cuff oscillometric devices. Then, carotid artery pressure
142 was recorded non-invasively by applanation tonometry (SphygmoCor, AtCor Medical) and
143 calibrated as previously described (20,21). Briefly, carotid pressure waveforms were analyzed

144 by the SphygmoCor software to identify the time to the shoulder of the first and second
145 pressure wave components during systole. The pressure at the shoulder of the first component
146 was identified as P1 height (forward pressure wave), and the pressure difference between this
147 point and the maximal pressure during systole (augmentation: AP) was identified as the
148 reflected pressure wave. Cardiovascular coupling was characterized by the augmentation
149 index (AIx), representing the effect of the arterial wave reflections on the magnitude of
150 central pressures, and defined as the ratio of augmentation to carotid pulse pressure:
151 $AIx=(AP/PP) \times 100$, where PP is pulse pressure (20,21). Carotid-to-femoral pulse wave
152 velocity (PWV), as indicator of aortic stiffness, was determined by simultaneous
153 measurement of pressure waves at the carotid and femoral arteries with pressure transducers
154 (Complior SP, Artech-Medical or SphygmoCor depending on the investigation centers)
155 (20,21). Internal diameter, far wall intima-media thickness (IMT) and stroke change in
156 diameter were measured, 1 cm beneath the bifurcation, on the right common carotid artery by
157 multiarray high-resolution echotracking (ArtLab System, Esaote Pie Medical) (20,21). The
158 cross-sectional distensibility coefficient was estimated from the variations in arterial cross-
159 sectional area (ΔA) and blood pressure (ΔP), assuming the lumen to be circular, as $DC=\Delta A/A$
160 $\cdot \Delta P$ where A is the diastolic lumen area, ΔA is the stroke change in lumen area, and ΔP is the
161 central pulse pressure obtained with applanation tonometry (20,21).

162 Endothelial function, the primary objective, was measured from brachial artery diameter and
163 blood flow, recorded on the right arm using echotracking and Doppler (ArtLab System)
164 during endothelium-dependent flow- FMD (21). Briefly, an arterial occlusion cuff placed on
165 the forearm was inflated for 5 minutes at 50 mmHg above systolic blood pressure followed by
166 deflation to induce reactive hyperemia. Brachial diameter and flow were measured
167 continuously by Esaote ultrasound scanners, coupled with a 10-MHz linear probe attached to
168 a stereotactic arm (21). After return to basal artery diameter and flow, the brachial

169 endothelium-independent dilatation in response to the nitric oxide donor glyceryl trinitrate
170 (GTN spray: 0.3 mg) was measured (21).

171 Furthermore, lifestyle recommendations were given to all patients at the end of the first
172 exploration visit in accordance with the French National Nutrition and Health Program
173 Score,. Patients also filled a nutritional questionnaire was given at the end of each exploration
174 visit to assess the changes in total energy and major nutrient intake during the study period
175 (22).

176

177 **Statistical analysis**

178 *Sample size calculation*

179 The primary endpoint of the study was the difference in brachial artery FMD between the
180 camelina oil and placebo groups. Using the reproducibility of FMD, calculated in 8 healthy
181 subjects explored at 48-h intervals (mean difference: -0.2%, SD: 1.9%), a target sample size
182 of 40 participants in each group was needed to demonstrate a significant difference between
183 groups for a change in FMD of 1.2% with 80% power and a 5% significance level. This effect
184 size represents an expected improvement in FMD of 20-25% which is half of the mean FMD
185 increase associated with intake of n-3 fatty acids, including ALA supplementation with
186 flaxseed oil and/or walnuts (23-25).

187

188 *Analysis*

189 Values are presented as mean \pm SD. Data normality was assessed by the Shapiro-Wilk test.
190 Chi-square test was used to compare categorical variables and the unpaired 2-sample t test or
191 Wilcoxon's rank-sum test in case of non-normality were used to compare baseline continuous
192 variables.

193 The effects of camelina oil and placebo on primary endpoint and all other measured biological
194 and functional parameters was compared using mixed ANOVA with time as within-subjects
195 factor (before and after 6-month treatment) and treatment group as between-subjects factor
196 (placebo or camelina oil) followed by Bonferroni's multiple comparison tests to assess the
197 time effect in each treatment group. Pearson correlation analyses were performed to assess in
198 the whole study population the relationships between changes in relevant clinical and
199 biological parameters, and major changes in RBC fatty acid composition. Analysis was
200 performed using GraphPad Prism software 8.0.2. A 2-sided $P \leq 0.05$ was considered
201 statistically significant.

202

203 **RESULTS**

204 **Population**

205 Figure 1 shows the flow chart of the study. Ninety-five patients were included into the study
206 (Rouen: n=46, Paris: n=42, Toulouse: n=5, Manhès: n=2). Among them, 45 were randomized
207 to receive the placebo and 50 received camelina oil. After randomization, eleven patients
208 withdrew from the study prematurely for personal reasons (n=7) or treatment intolerance
209 (n=4). In addition, three patients who did not collect their treatment from hospital pharmacies
210 were excluded from the study. Finally, the data of 40 patients in the camelina oil group and
211 41 patients in the placebo group were available for analysis. There was no difference between
212 the placebo and camelina oil groups for baseline clinical characteristics including age, blood
213 pressure values, antihypertensive treatments and the number of metabolic syndrome
214 components (Table 1). Antihypertensive and lipid-lowering agents remained stable
215 throughout the study (data not shown).

216

217 **Treatment tolerance**

218 No serious adverse events occurred during the study. The adverse events reported mainly
219 corresponded to low-to-moderate intensity digestive disorders such as abdominal pain,
220 bloating, flatulence or diarrhea or constipation (Supplementary Table 2). These digestive
221 disorders were observed regardless of the treatment group, according to previous results
222 obtained after administration of cyclodextrins (26).

223

224 **Nutritional status**

225 Analysis of available nutritional questionnaires at baseline showed a slightly higher total
226 energy intake (TEI), compared with the French recommendations but without difference
227 between groups (Table 2) (27). The macronutrient profile was characterized by a

228 carbohydrate intake lower than expected (36-39% TEI) and a protein intake higher than
229 expected (20% TEI), whereas lipid intake was within the recommended range (37-39% TEI).
230 The fat intake predominantly concerned SFA (13-15% TEI) but also monounsaturated fatty
231 acids (14-15% TEI), while intake of PUFAs was low (6% TEI). In addition, there was an
232 imbalance in the estimated n-6/n-3 ratio in favor of n-6. No change in the nutrient profile,
233 including cholesterol and alcohol intake, was identified at the end of the 6-month
234 supplementation in patients supplemented with either placebo or camelina oil, including
235 cholesterol and alcohol intakes.

236

237 **Fatty acid content in RBC membranes**

238 Figure 2 shows major metabolic pathways and changes in saturated, n-3, n-6 and n-9 fatty
239 acid content in RBC membranes of patients treated with camelina oil or placebo. No
240 significant difference was observed between groups at baseline. As expected, ALA levels
241 increased in patients supplemented with camelina oil compared to patients receiving placebo
242 (mean change: $0.08 \pm 0.06\%$ vs. 0 ± 0.04 , $P < 0.001$), supporting a good compliance with
243 treatment. In addition, partial elongation of ALA to longer n-3 PUFAs is shown by an
244 increase in levels of EPA ($0.16 \pm 0.65\%$ vs. 0 ± 0.5 , $P < 0.05$) and n-3 docosapentaenoic acid (n-3
245 DPA or clupanodonic acid; 22:5 n-3) but not in DHA. Regarding n-6 fatty acids, despite its
246 presence in camelina oil, there was no change in linoleic acid (LA) levels in RBC membranes.
247 In fact, only eicosadienoic acid (EDA, 20:2 n-6) levels, the LA elongation product, were
248 higher in patients supplemented with camelina oil compared to placebo. Dihomo-gamma-
249 linolenic acid (DGLA, 20:3 n-6) levels were not altered but arachidonic acid (AA, 20:4 n-6)
250 levels were lower in patients supplemented with camelina oil. As a result, there was an
251 increase in total n-3 fatty acids without a significant change in n-6 fatty acids, leading to a

252 decrease in the n-6/n-3 ratio in patients supplemented with camelina oil compared to placebo
253 (Supplementary Figure S1A-C).

254 Furthermore, the oleic acid levels in RBC membranes did not change over time but there was
255 an increase in levels of GA ($0.08\pm 0.04\%$ vs, 0 ± 0.04 , $P<0.001$) and nervonic acid (24:1 w-9)
256 in patients supplemented with camelina oil compared to placebo. Finally, as expected from
257 the absence of change in the nutritional status and from camelina oil composition, there was
258 no between-group difference in the SFA content of RBC membranes.

259

260 ***Systemic hemodynamics, carotid artery geometry and function***

261 Baseline parameters were similar between groups. Brachial and carotid artery pressures, IAx
262 and aortic-to-femoral PWV did not change during the 6-month treatment with camelina oil or
263 placebo (Table 3). In addition, carotid artery diastolic diameter, IMT, stroke change in
264 diameter and distensibility were similar between groups (Table 3).

265

266 ***Brachial artery endothelial function***

267 Baseline parameters were similar between groups. Resting blood brachial artery blood flow
268 and diameter did not change during the 6-month treatment with camelina oil or placebo
269 (Supplementary Table 3). No between-group difference was observed for brachial artery
270 FMD or for post-ischemic hyperemia, and endothelium-independent dilatation in response to
271 GTN was similar (Figures 3A-C).

272

273 ***Glucose and lipid homeostasis, inflammation and oxidative stress markers***

274 Baseline parameters were similar between groups. BMI but not waist circumference tended to
275 be higher in patients supplemented with camelina (Figure 4A-B). In addition, fasting blood
276 glucose levels were higher in patients receiving camelina oil and lower in patients treated with

277 placebo and, with a significant difference between groups (Figure 4C; mean change: 0.3 ± 0.5
278 vs. -0.2 ± 0.6 mmol/L, $P<0.001$). Plasma insulin levels were not significantly different between
279 groups, leading to an unexpected worsening of the homeostatic model assessment for insulin
280 resistance (HOMA-IR) index in patients receiving camelina oil compared to placebo (0.5 ± 0.9
281 vs. -0.8 ± 2.5 , $P<0.01$) (Figure 4D-E). Accordingly, plasma adiponectin levels did not vary in a
282 similar way in patients supplemented with camelina oil compared to placebo but the decrease
283 with camelina oil was not statistically significant (Figure 4F). There was no difference
284 between groups for total, LDL and HDL cholesterol or for triglyceride levels (Figure 4E-J).
285 Finally, levels of hsCRP, the marker of inflammation, did not vary in a similar way in patients
286 receiving camelina oil compared to placebo but the decrease with camelina oil was not
287 statistically significant (Figure 4K). There was no difference between groups for TBARs, the
288 marker of oxidative stress (Figure 4L).

289

290 ***Correlations between changes in brachial artery FMD, HOMA index and changes in RBC***
291 ***fatty acid content***

292 To explore the relationship between fatty acids, vascular function and glucose homeostasis,
293 Pearson correlation analyses were performed in the whole population (Supplementary Figure
294 2). Changes in the levels of major fatty acids in RBC affected by camelina oil
295 supplementation *i.e.*, ALA, EPA, EDA, AA and GA, were well correlated ($r=0.24$ to 0.63 ;
296 $P<0.05$). Changes in brachial artery FMD were only significantly associated with changes in
297 the levels of EPA in RBC ($r=0.26$, $P=0.03$) while changes in HOMA index were correlated
298 with ALA ($r=0.28$, $P=0.024$), EDA ($r=0.34$, $P=0.004$) and GA ($r=0.32$, $P=0.007$) enrichment.

299

300

301 DISCUSSION

302 This study was designed to carefully investigate the effect of long-term supplementation with
303 camelina oil, which is an ALA-rich oil, on the cardiovascular homeostasis of hypertensive
304 patients with metabolic syndrome. Despite antihypertensive and lipid-lowering treatments,
305 cardiovascular risk is high in these patients as shown by their increase in aortic stiffness and
306 decrease in brachial artery FMD (28,29). In addition, dietary questionnaires suggest that
307 these patients had a shift of n-6/n-3 ratio from ~10:1 to the optimal 2:1 (30). As expected,
308 almost all patients in the camelina oil group had an increase in ALA content in RBC
309 membrane and we can confirm a partial elongation of ALA to EPA and n-3 DPA but not
310 DHA (12). In fact, high level of ALA may inhibit the formation of DHA precursors (24:5 n-3
311 to 24:6 n-3) by saturating Δ^6 -desaturase activity needed for its conversion to EPA (31). Of
312 note, an elongation of ALA was not observed in some supplementation studies, which could
313 be related to higher baseline levels of long-chain n-3 PUFAs (32). Regarding the n-6 series,
314 probably due to LA content in camelina oil, its elongation product EDA increased in patients
315 supplemented with camelina oil. However, conversely, AA and DTA decreased in patients
316 supplemented with camelina oil. Again, this may be due to the high level of ALA that
317 competes with LA for endogenous conversion to long-chain PUFAs (33). Accordingly, there
318 was a significant inverse relationship between changes in ALA and AA in the overall study
319 population. Furthermore, camelina oil contains n-9 fatty acids and an increase in GA, and its
320 elongation product nervonic acid, in RBC was observed in supplemented patients.

321 In this context, we observed that camelina oil did not affect peripheral and central blood
322 pressures or arterial geometry and stiffness. While meta-analyses of randomized controlled
323 trials suggest that long-chain n-3 PUFAs slightly lower blood pressure, in particular in
324 untreated hypertensive subjects (34,35), the effect of ALA supplementation remains less
325 investigated. In fact, the FLAX-PAD study, showed that a daily intake of 30 g of milled

326 flaxseed during 6 months lowered peripheral but also central blood pressures in patients with
327 peripheral artery disease (32-34). This beneficial effect of flaxseed oil was confirmed in a
328 meta-analysis (39) but, as observed in the present work, a large clinical trial did not show a
329 significant effect of ALA supplementation on blood pressure in high-risk patients (40).
330 Similarly, it was suggested that long-chain n-3 PUFAs may reduce arterial stiffness (41) and
331 remodeling (42) but evidence of a beneficial effect of ALA at this level is more limited (43-
332 46). In absence of change in hyperemic stimulus and in endothelium-independent dilatation in
333 response to GTN, we were not able to demonstrate a significant improvement of brachial
334 artery FMD in patients supplemented with camelina oil compared with placebo although
335 changes in FMD parallel those of EPA. A larger number of subjects could have demonstrated
336 a beneficial effect of camelina oil on endothelial vasomotor function, but the substantial lost-
337 to-follow-up rate observed of approximately 15% may have contributed to limiting the power
338 of the study. The latter is putatively related to the duration of the study with a 6-month
339 treatment period that is at least twice that usually reported in the literature (23) but also to
340 gastrointestinal disorders related to the cyclodextrin fiber effect (26). Many clinical trials have
341 assessed the effect of omega-3 fatty acids supplementation on endothelial function, with
342 conflicting results, including those concerning ALA (23-25,47-49). The beneficial effect of n-
343 3 PUFAs on vascular function appear to increase with the quantity administered, weaker
344 patient health status, lower baseline levels of these fatty acids in cell membranes, but also
345 probably with the ability to produce protective n-3-derived oxylipins (23,38,50,51). The
346 conversion of EPA to vasodilator epoxyeicosatetraenoic acids but especially during
347 inflammation to resolving oxylipins, like resolvins or maresins may contribute to the
348 improved inflammatory status of patients supplemented with camelina oil, as illustrated by
349 the decrease in hsCRP (51). In contrast, despite its high content in tocopherols, oxidative

350 stress appeared not modified in patients supplemented with camelina oil, as suggested by the
351 absence of change in TBARS plasma levels.

352 Furthermore, the beneficial effects of camelina oil on vascular function may have been
353 limited by its deleterious effect on metabolic homeostasis, illustrated by the increase in
354 particular HOMA-IR and, although less marked, the decrease in adiponectin levels. In
355 contrast, one previous randomized open clinical trial showed no effect of camelina oil on
356 fasting or post load blood glucose or insulin concentrations in patients with impaired glucose
357 tolerance (13). Because the supplementation period with camelina oil was half that of ours,
358 this suggests that long-term camelina oil intake may be needed to alter glucose metabolism. In
359 the absence of change in nutrient intake and in the presence of an anti-inflammatory action,
360 the mechanism leading to the unexpected worsening of insulin resistance in patients
361 supplemented with camelina oil remains to be elucidated. On the one hand, correlation
362 analyses showed significant positive relationships between changes in HOMA-IR and ALA or
363 EDA. However, a meta-analysis of randomized supplementation studies did not show clear
364 evidence for a deleterious effect of n-3 or n-6 fatty acids on glucose homeostasis, except a
365 slight but non-significant increase in baseline insulin levels for ALA (52,53). On the other
366 hand, a putative deleterious effect of the increase in long-chain n-9 PUFAs, especially GA,
367 should be explored. In fact, changes in GA levels were also correlated with HOMA-IR and
368 such a deleterious effect has never been reported with other ALA-rich regimens, especially
369 flaxseed oil and walnuts that do not contain a high proportion of GA (9,47,53). An increased
370 conversion of oleic acid to GA was observed in female diabetic rats but the significance of
371 this increase remained elusive (54). Corroborating our results, low but significant positive
372 correlations were found between BMI, fasting glucose levels and HOMA-IR, and individual
373 n-9 fatty acid concentrations in erythrocytes, including GA, in participants of The
374 Ludwigshafen Risk and Cardiovascular Health Study, demonstrating the negative prognostic

375 value of the increase in n-9 levels (55). Furthermore, we could not confirm the results of
376 previous works showing a beneficial effect of camelina oil on lipid homeostasis but half of
377 the patients included in the present study had already received lipid-lowering agents (12,13).
378 This double-blind randomized, placebo-controlled study demonstrates that ALA
379 supplementation with camelina oil did not improve vascular function in hypertensive patients
380 with metabolic syndrome. Whether the lack of beneficial effect of ALA supplementation on
381 vascular function was due to the adverse effect on insulin sensitivity, putatively related to
382 gondoic acid enrichment, remains to be elucidated. Whether a beneficial effect was limited by
383 the unexpected worsening of insulin resistance putatively related to n-9 enrichment, especially
384 in GA, remains to be elucidated. Importantly, this work supports the need for high-quality
385 clinical trials to carefully assess the effects of dietary supplements, in particular camelina oil
386 given its increasing use in human food.

387

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395 collected the data; ND, FB, MS and AC contributed to treatment production. AM, AFC, AB,
396 JA, BP, ABR, PB and RJ: recruited and followed-up patients. JB analyzed the data and wrote
397 the manuscript; PB and RJ: made critical revision of the manuscript. All authors: read and
398 approved the final manuscript.

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400 **CONFLICTS OF INTEREST**

401 None

402

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Table 1. Baseline characteristics of hypertensive patients with metabolic syndrome randomized to camelina oil or placebo group

Parameters	Placebo (n=41)	Camelina oil (n=40)	<i>t-value</i>	<i>P=</i>
Age, years	55.0±7.5	57.7±8.4	ND	0.10
Male, n (%)	27 (65)	28 (70)	ND	0.81
BMI, kg/m ²	30.3±4.2	30.0±4.0	ND	0.91
Waist circumference, cm	107±11	108±10	ND	0.98
Systolic blood pressure, mm Hg	134±12	130±10	ND	0.30
Diastolic blood pressure, mm Hg	82±9	81±9	0.93	0.35
Heart rate, bpm	72±9	74±10	1.2	0.23
Treatments n (%)				
ACEI/ARB	34 (83)	30 (75)	ND	0.65
Beta-blockers	11 (27)	9 (23)	ND	0.80
Calcium channel blockers	14 (34)	14 (35)	ND	>0.99
Diuretics	13 (32)	12 (30)	ND	>0.99
Statins	17 (41)	21 (53)	ND	0.32
Smoking (never/past/current), n	22/13/6	20/15/5	ND	0.85
Total cholesterol, mg/dl	201±36	201±43	0.08	0.94
LDL cholesterol, mg/dl	122±37	119±36	0.33	0.74
HDL cholesterol, mg/dl	46±11	47±16	ND	0.88
Triglycerides, mg/dl	165±71	180±78	ND	0.49
Fasting glycemia, mg/dl	105±13	105±12	0.15	0.88
Metabolic syndrome components (3/4/5), n	23/13/5	22/16/2	ND	0.45
Estimated GFR by MDRD, ml/min	88±15	87±18	ND	0.57

Values are means±SD or patient numbers (percentages). Data normality was assessed by the Shapiro-Wilk test. Chi-square test was used to compare categorical variables and the unpaired 2-sample t test or Wilcoxon's rank-sum test in case of non-normality was used to compare continuous variables. t-values are given for normally-distributed parameters ACEI/ARB: angiotensin-converting enzyme inhibitors/ angiotensin receptor blockers, GFR: glomerular filtration rate, MDRD: modification of diet in renal disease. ND: not determined.

Table 2. Changes in the nutritional status of hypertensive patients with metabolic syndrome supplemented with camelina oil or placebo during 6 months.

Parameters	Group	Baseline	6-month treatment	Mean difference [95% CI]	<i>f-value</i>	<i>P=</i>
Total energy intake, kcal/day	Placebo	2320±894	2216±1041	75 [-242, 391]	0.22	0.64
	Camelina oil	2444±858	2410±885			
Proteins, g/day	Placebo	119±55	112±59	6 [-15, 27]	0.30	0.59
	Camelina oil	128±65	119±49			
Carbohydrates, g/day	Placebo	205±83	206±103	3 [-30, 36]	0.032	0.86
	Camelina oil	229±85	237±93			
Lipids, g/day	Placebo	102±51	93±51	5 [-14, 24]	0.31	0.58
	Camelina oil	102±41	96±42			
Saturated fatty acids, g/day	Placebo	39±21	35±21	3 [-5, 10]	0.50	0.48
	Camelina oil	39±17	37±18			
MUFAs, g/day	Placebo	41±21	37±21	2 [-7, 10]	0.17	0.68
	Camelina oil	40±17	38±17			
PUFAs, g/day	Placebo	15±8	14±7	1 [-2, 4]	0.21	0.65
	Camelina oil	15±6	15±7			
Linoleic acid (18:2 n-6), g/day	Placebo	12±7	11±6	1 [-4, 4]	0.40	0.53
	Camelina oil	11±5	12±6			
Alpha-linolenic acid (18:3 n-3), g/day	Placebo	1.2±0.5	1.1±0.5	0.1 [-0.2, 0.3]	0.16	0.69
	Camelina oil	1.2±0.5	1.2±0.6			
18:2 n-6/18:3 n-3	Placebo	9.8±2.8	10.5±3.7	0 [-1.6, 1.7]	0.002	0.97
	Camelina oil	10.0±3.0	11.0±3.9			
Cholesterol, mg/day	Placebo	437±198	400±233	22 [-63, 108]	0.28	0.60
	Camelina oil	469±269	424±229			
Alcohol, mg/day	Placebo	15±16	15±15	-2 [-8, 4]	0.34	0.57
	Camelina oil	14±19	18±20			

Values are means±SD. Given *P* values are the treatment group – time interaction effect assessed using mixed ANOVA with time as within-subjects factor and treatment group as between-subjects factor (n=36 patients per group at baseline, n=30 for placebo and n=26 for camelina oil at 6 months; degree of freedom=49). MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids.

Table 3. Changes in systemic hemodynamics, carotid artery geometry and function of hypertensive patients with metabolic syndrome supplemented with placebo or camelina oil during 6 months.

Parameters	Group	Baseline	6-month treatment	Mean difference [95% CI]	<i>f</i> -value	<i>P</i> =
Brachial systolic blood pressure, mm Hg	Placebo	134±12	134±13	0 [-5, 6]	0.008	0.93
	Camelina oil	134±10	134±14			
Brachial diastolic blood pressure, mm Hg	Placebo	79±9	79±9	1 [-2, 4]	0.51	0.48
	Camelina oil	81±9	82±10			
Heart rate, bpm	Placebo	69±11	68±10	1 [-2, 3]	0.20	0.65
	Camelina oil	66±7	66±8			
Carotid systolic blood pressure, mm Hg	Placebo	124±13	124±14	2 [-4, 8]	0.40	0.53
	Camelina oil	126±12	127±15			
Carotid pulse pressure, mm Hg	Placebo	45±11	45±11	0 [-4, 5]	0.02	0.89
	Camelina oil	45±8	45±11			
Carotid augmentation index, %	Placebo	7.0±14.5	10.4±13.8	-0.1 [-4.8, 4.6]	0.001	0.97
	Camelina oil	12.0±15.1	14.6±15.2			
Carotid-to-femoral PWV, m/s	Placebo	10.2±1.8	10.4±1.9	-0.1 [-0.7, 0.5]	0.15	0.70
	Camelina oil	10.6±1.7	10.7±2.0			
Carotid diastolic diameter, mm	Placebo	7.24±0.75	7.33±0.70	-0.07 [-0.29, 0.15]	0.44	0.51
	Camelina oil	7.25±0.83	7.26±0.77			
Carotid IMT, µm	Placebo	605±109	612±120	8 [-27, 43]	0.21	0.65
	Camelina oil	616±110	631±108			
Carotid stroke change in diameter, mm	Placebo	342±120	337±112	7 [-30, 45]	0.15	0.70
	Camelina oil	329±105	332±97			
Carotid distensibility, mm Hg ⁻¹	Placebo	19.3±8.1	19.1±7.5	1.0 [-2.5, 4.4]	0.31	0.58
	Camelina oil	18.5±8.0	19.0±9.6			

Values are means±SD. Given *P* values are the treatment group – time interaction effect. assessed using mixed ANOVA with time as within-subjects factor and treatment group as between-subjects factor (n=39-40 patients for camelina oil and placebo at each time point; degree of freedom=76-78). IMT: intima-media thickness, PWV: pulse wave velocity.

LEGENDS OF FIGURES

Figure 1

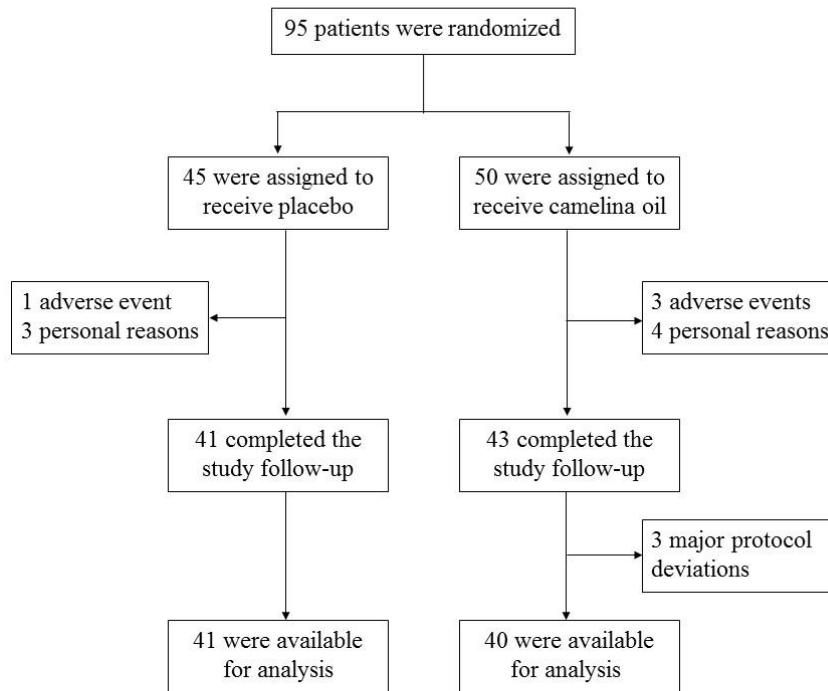


Figure 1. Flow chart of the study

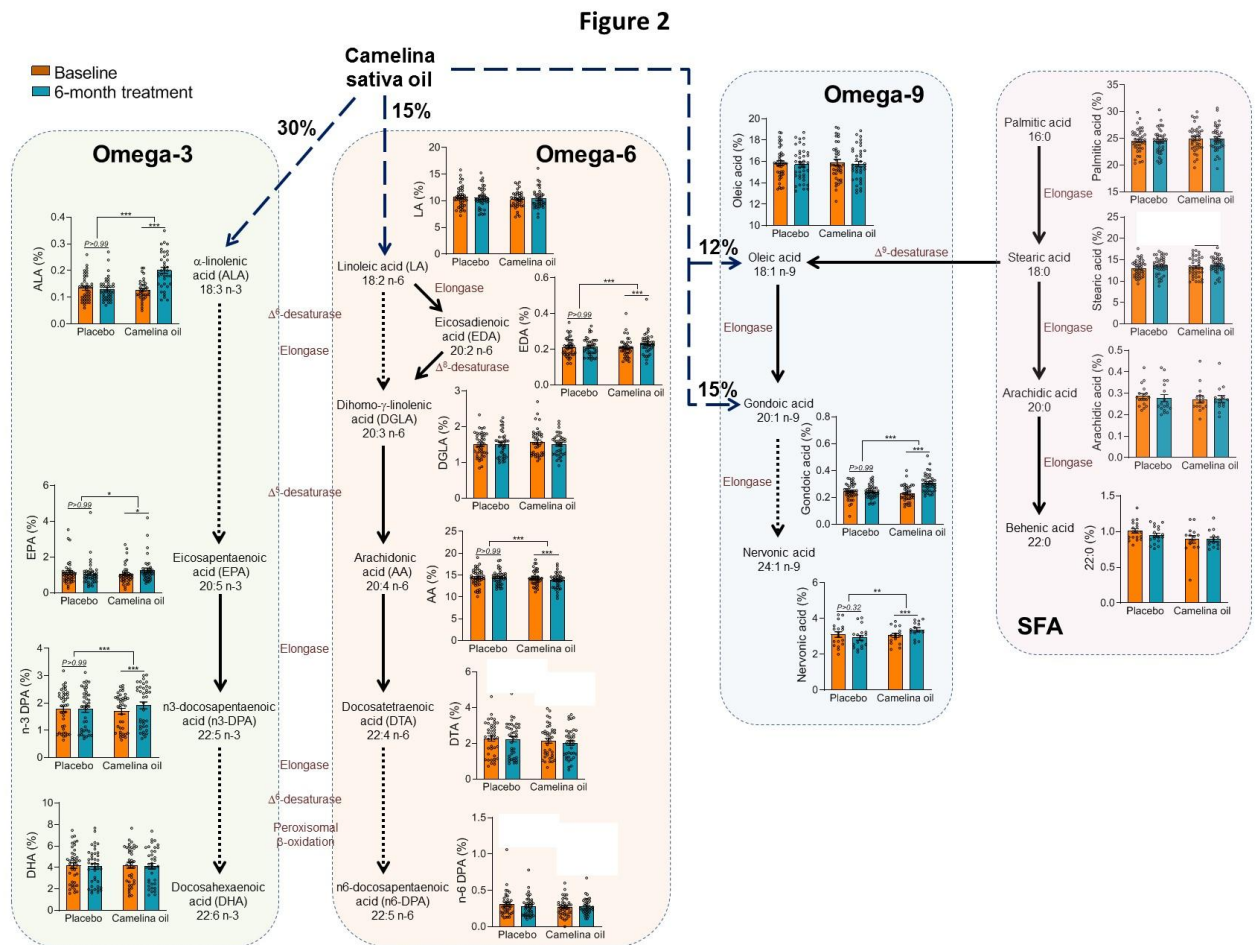


Figure 2. Metabolic pathways and changes in n-3, n-6 and n-9 fatty acids and in saturated fatty acids (SFA) quantified in red blood cell membranes of hypertensive patients with metabolic syndrome supplemented with camelina or placebo during 6 months. Major fatty acids present in camelina oil are given as percentages (see Supplementary Table 1 for full fatty acid composition of camelina oil). Results are given as percentage of total fatty acids. Means \pm SEM are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. P values were obtained using mixed ANOVA with time as within-subjects factor and treatment group as between-subjects factor followed by Bonferroni's multiple comparison tests to assess the time effect in each treatment group ($n = 39$ – 41 patients for placebo and $n = 34$ – 39 for camelina oil at each time point except for nervonic acid, arachidic acid and behenic acid with $n = 18$ for placebo and $n = 14$ – 15 for camelina oil).

Figure 3

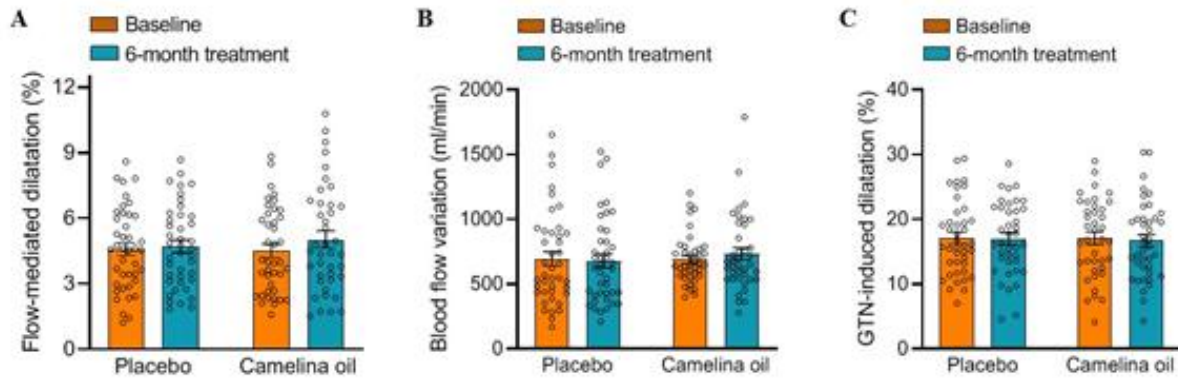


Figure 3. Changes in brachial artery endothelium-dependent flow-mediated dilatation (A), reactive hyperemia (B) and endothelium-independent dilatation to glyceryl trinitrate (GTN; C) in hypertensive patients with metabolic syndrome supplemented with camelina oil or placebo during 6 months. Means \pm SEM are shown. *P* values were obtained using mixed ANOVA with time as within-subjects factor and treatment group as between-subjects factor (n=40-41 patients for placebo and n=39-40 for camelina oil at each time point).

Figure 4

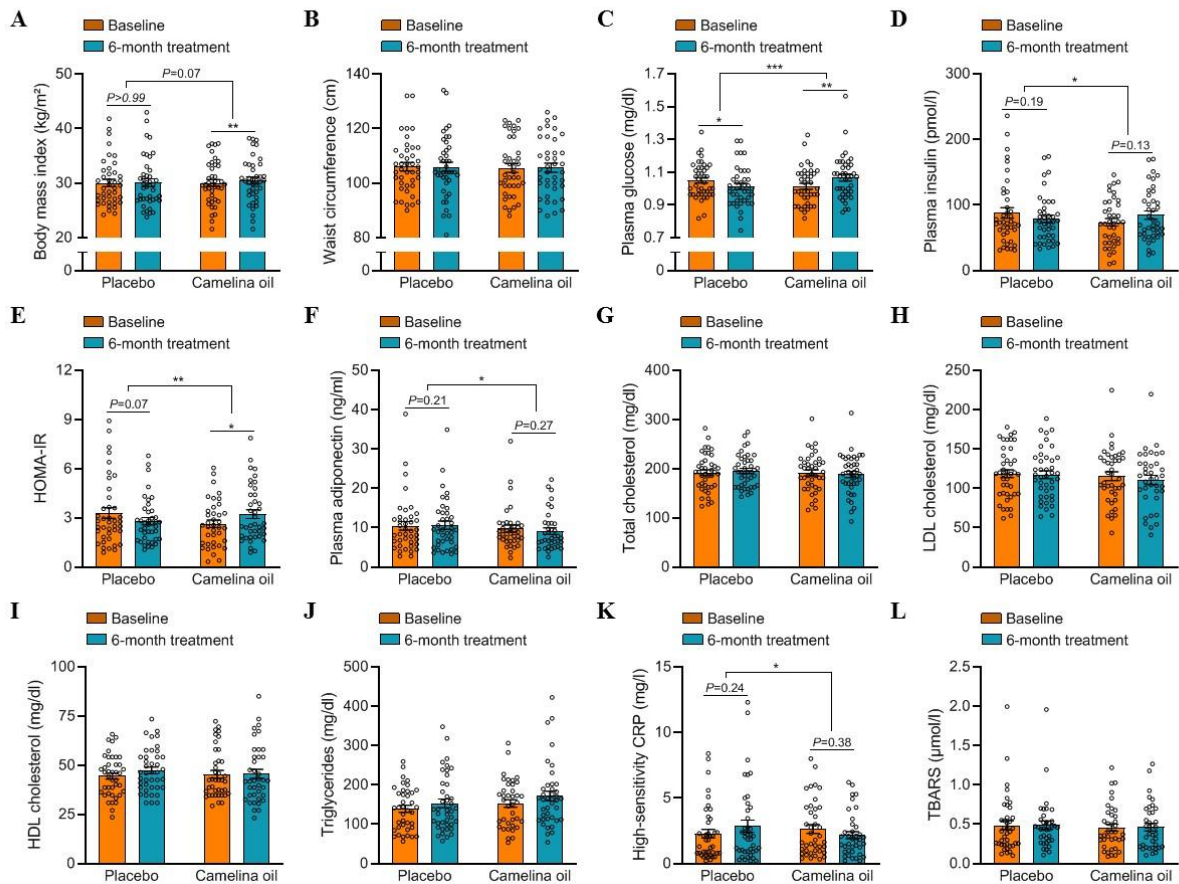


Figure 4. Changes in BMI (A), waist circumference (B), fasting blood glucose (C) plasma insulin (D), homeostatic model assessment for insulin resistance (HOMA-IR) index (E), plasma adiponectin (F), total (G), LDL (H) and HDL cholesterol (I), triglycerides (J), high-sensitivity C-reactive protein (CRP; K) and thiobarbituric acid reactive substances (TBARS; L) in hypertensive patients with metabolic syndrome supplemented with camelina oil or placebo during 6 months. Means \pm SEM are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. P values were obtained using mixed ANOVA with time as within-subjects factor and treatment group as between-subjects factor followed by Bonferroni's multiple comparison tests to assess the time effect in each treatment group (n=37-41 patients for placebo and n=37-40 for camelina oil at each time point).