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A low omega-6 polyunsaturated fatty acid (n-6 PUFA) diet increases omega-3 (n-3) long chain PUFA status in plasma phospholipids in humans

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31

SUMMARY

This study aimed to determine the effect of reducing the dietary linoleic acid (LA) intake from ~5% to <2.5% energy (%E) on n-3 long chain PUFA (LCPUFA) status in humans. Thirty-six participants followed a <2.5%E LA diet for 4 weeks. Nutrient intakes were estimated from diet diaries and blood samples were collected for assessment of fatty acid composition in plasma and erythrocyte phospholipids. LA intakes were reduced from 4.6%E to 2%E during the low LA intervention ($P<0.001$) while n-3 LCPUFA intakes were unchanged. LA and total n-6 PUFA content of plasma and erythrocyte phospholipids were significantly reduced after the low LA diet phase ($P<0.001$). The n-3 LCPUFA content was significantly increased in plasma phospholipids after the low LA diet compared to baseline (6.22% vs 5.53%, $P<0.001$). These data demonstrate that reducing LA intake for 4 weeks increases n-3 LCPUFA status in humans in the absence of increased n-3 LCPUFA intake.

45

INTRODUCTION

46 The fatty acid composition of the typical diet of Western countries, including Australia, has
47 undergone a substantial shift over the past half-century. This shift has been driven primarily
48 by the replacement of animal fats with plant-based oils and spreads in cooking, baking and
49 processed food and has resulted in a significant decrease in the per capita intake of saturated
50 fats and a three-fold increase in the intake of the omega-6 polyunsaturated fatty acid (n-6
51 PUFA), linoleic acid (LA) [1, 2].

52

53 The health impacts of this marked change in dietary fatty acid composition are unclear,
54 however concerns have been raised that this substantial increase in LA intake could have
55 negative impacts on cardiovascular and metabolic health [3, 4]. This suggestion is based on
56 the biochemical properties of the n-6 PUFA, since the long-chain derivative of LA,
57 arachidonic acid (AA), gives rise to pro-inflammatory and pro-thrombotic compounds [5],
58 and the fact that high circulating concentrations of n-6 PUFA have been implicated in an
59 increased risk of inflammatory and allergic conditions in epidemiological studies [6]. While
60 data from humans directly linking increased LA intake and disease are lacking, data from the
61 Sydney heart study recently published by Ramsden and colleagues provided the first evidence
62 from a randomized controlled trial that a dietary intervention in which saturated fats were
63 replaced by concentrated sources of LA (as opposed to a mixture of n-6 and n-3 PUFA) was
64 associated with an increased risk of death from coronary heart disease, raising renewed
65 concerns about the impact of n-6 PUFA on human health [7].

66

67 LA competes with the short-chain n-3 PUFA alpha-linolenic acid (ALA) for the enzymes
68 required for conversion to their respective long-chain derivatives and for incorporation into
69 the plasma membrane [8]. Consequently, high LA diets may limit the capacity of increases in

70 dietary n-3 PUFA intake to improve n-3 PUFA status [9]. Given that several bioactive
71 mediators derived from n-3 LCPUFA are less potent in their inflammatory actions than their
72 AA-derived analogs [10], and others actually have potent inflammation-resolving [11] or
73 neuroprotective properties [12], this competition has the potential to contribute to negative
74 health outcomes.

75

76 Current strategies to improve n-3 LCPUFA status of the population have focused almost
77 exclusively on increasing dietary n-3 LCPUFA intake, via increased consumption of oily fish
78 or through fish oil supplementation [13]. However, many Australians struggle to achieve
79 regular fish intake [14] and dwindling marine resources have raised questions about the long-
80 term sustainability of this approach [15]. Given the competition that exists between n-6 and
81 n-3, it has been suggested that lowering the LA content of the diet has the potential to both
82 limit the production of n-6 derived pro-inflammatory mediators and enhance the biological
83 efficacy of n-3 LCPUFA consumed in the diet. However, the ubiquity of LA in the food
84 supply, particularly in pre-prepared and take-away foods, makes any attempt to reduce the n-
85 6 PUFA intake of free-living humans challenging.

86

87 We previously designed a low n-6 PUFA diet, in which we reduced the LA content of the diet
88 from ~5% to ~2%E by replacing standard plant-based oils and spreads with low n-6 PUFA
89 alternatives (Macadamia oil and butter), and limiting intake of processed and take-away
90 foods which utilize high n-6 PUFA oils [16]. Importantly, we showed that the reduction in
91 LA intake could be achieved while still adhering to the national dietary recommendations
92 (The Australian Guide to Healthy Eating (AGHE) guidelines) [17] and maintaining a
93 saturated fat intake of less than 10%E [16]. The aim of the present study was to determine
94 whether following this low LA diet for a 4 week period would result in reduced n-6 PUFA

95 and increased n-3 LCPUFA content in plasma and erythrocyte phospholipids in healthy
96 human subjects.

97

98 ## PATIENTS AND METHODS

99 ### Participants

100 Participants were recruited using email advertisements and flyers, and interested individuals
101 were screened by research staff for eligibility. The inclusion criteria were: BMI <35kg/m^2
102 and weight stable, aged 18-65yrs, able to eat >5 meals at home per week and not regularly
103 consuming more than 2-3 fish meals per week. Exclusion criteria included taking high
104 potency fish oil supplements (>3g/day EPA/DHA), gastric mal-absorption, vegetarian or
105 vegan diet, pregnant or breastfeeding. All participants gave informed consent. This study was
106 approved by the Human Research Ethics Committees of the University of Adelaide and
107 University of South Australia.

108

109 ### Study design

110 This was an open-label clinical trial which consisted of a 2 week control phase, during which
111 participants were instructed to continue their habitual dietary intake, followed by 4 weeks on
112 the low LA diet. No details of the low LA diet were provided at enrolment in order to
113 minimize the potential for this to influence dietary choices during the 2 week control phase.
114 Importantly this study design allowed for each subject to act as their own control which is an
115 important consideration in free-living intervention dietary studies given the potential for
116 considerable variability in dietary intakes between individuals. Baseline demographic, dietary
117 and medical information was collected at enrolment.

118

119 ### Clinic Appointments

120 All participants attended clinic appointments at enrolment, after the 2 week control phase and
121 at the completion of the 4 week dietary intervention. All appointments were conducted
122 between 7:30am and 9:00am after an overnight fast of at least 12 hours. During the clinic
123 appointments, weight and height were measured with participants in light clothing and
124 without shoes, using a digital weighing scale (SALTER, Victoria, Australia) and a
125 stadiometer (SECA, New South Wales, Australia) respectively and Body Mass index
126 calculated (weight/height^2). Fasting venous blood samples (8 ml) were collected at each
127 clinic appointment. After collection, blood samples were centrifuged for 15 mins at 3500xg at
128 4ºC to separate plasma and erythrocytes for subsequent analysis of fatty acids.
129
130 <u>Dietary Information</u>
131 All participants were asked to maintain a 3-day weighed food diary each week during both
132 the control and dietary intervention periods. Instructions on completing the diary were
133 provided at enrolment, and participants were instructed to record their dietary intake for 2
134 week days and 1 weekend day in each week of the study. Electronic kitchen scales (SALTER
135 1021, Victoria, Australia) and standard metric measuring cups (Décor Cook® Measuring
136 Cups/Spoons, Victoria, Australia) were provided to participants to assist them in completing
137 the weighed food diary.
138
139 <u>Low LA diet</u>
140 The low LA diet was based on the diet previously designed by our group [16] and aimed to
141 achieve an LA intake of ~2%E whilst maintaining saturated fat intake at <10%E and not
142 altering the intake of n-3 LCPUFA. Participants were provided with Macadamia oil
143 (Suncoast Gold Vitality™ Macadamia oil, 1.24g LA/100g) and butter (Western Star™ Butter,
144 1.60g LA/100g) and were instructed to use these in place of their usual oils and spreads in

food preparation and cooking. Participants were also provided with a list of specific food types and brands to be avoided during the low LA diet phase (based on a cut-off level of <1g LA/100g and/or <1g LA/serving size of food product). To facilitate compliance, participants were provided with written materials identifying low LA alternatives for commonly consumed foods to assist them in making appropriate food choices when eating out or purchasing take away foods. A list of low LA recipes was also provided to participants. These resources were adapted from those produced for participants in a previous low n-6 PUFA trial [18]. All participants were contacted by telephone, email or social networking media every 1-2 weeks for ongoing support and to monitor compliance with weighed food diaries and participants were encouraged to contact study staff at any time during the trial with any questions or concerns.

Dietary analyses

The diet diaries were analyzed for energy intake, macronutrient composition, and fatty acid content including LA, ALA and total n-3 LCPUFA, using the FoodWorks Program (FoodWorks 7 Professional Student, Xyris Software), which uses the latest AUSNUT 2007 food composition tables. These were developed as part of the National Children's Nutrition and Physical Activity Survey, which contains nutrient values for more than 4200 foods, beverages and supplements. The n-3 LCPUFA content of foods was obtained from the AUSNUT 2007 and Australian RMIT Fatty Acids database within the FoodWorks program and expressed as grams/day and percentage of total energy intake (%E). In cases where information on the LA and ALA content of specific foods was not available on the FoodWorks databases, the LA content of these foods was either estimated based on similar foods, or manually calculated based on the food's main fat source.

170 Fatty Acid Analyses

171 The fatty acid composition of plasma and erythrocyte phospholipids was analysed using gas
172 chromatography as described in detail previously [19]. Briefly, total lipids were extracted
173 from plasma and erythrocytes with chloroform/isopropanol and chloroform/methanol (2:1
174 v/v) [20]. Thin layer chromatography (TLC) on silica gel plates (Silica gel 60H; Merck,
175 Darmstadt, Germany) was then used to separate the phospholipids from total lipid extracts.
176 The lipid classes were visualised under UV light and the phospholipids were transferred into
177 a vial containing 1% (v/v) sulphuric acid (H_2SO_4) in methanol. All phospholipids were
178 transesterified with 1% (v/v) H_2SO_4 in methanol at 70°C for 3 hours. After the samples were
179 cooled, the resulting fatty acid methyl esters (FAME) were extracted in n-heptane and
180 transferred into 2ml chromatography vials containing 1 to 2 grains of anhydrous sodium
181 sulphate (Na_2SO_4). FAME were then separated and quantified by gas chromatography (GC)
182 (Hewlett-Packard 6890, California, United States of America) using techniques described in
183 detail elsewhere [21]. For statistical purposes, fatty acids at concentrations lower than the
184 limit of detection (0.05%) were allocated a set value of 0.025% (half the limit of detection).
185 All solvents used for extraction and separation contained 0.005% (w/v) of the antioxidant,
186 butylated hydroxyanisol (BHA). All solvents used in this study were purchased from Ajax
187 Firechem Pty Ltd (Auckland, NZ) or Chem-Supply (South Australia, Australia) and were of
188 analytical grade. Unless specified otherwise, other chemicals and reagents were purchased
189 from Sigma-Aldrich (Missouri, United States of America).

190

191 Statistical analysis

192 All data are presented as median and inter quartile range (IQR). Due to the small sample size
193 and non-normal distribution of a number of dietary intake variables and erythrocyte/plasma
194 fatty acids, data were analysed using non-parametric tests. Dietary intake, plasma and

195 erythrocyte phospholipids fatty acid composition data at baseline and after 4 weeks on the
196 low LA diet were compared using the Wilcoxon signed-rank test for matched pairs. A P value
197 of <0.05 was considered statistically significant.

198

199 RESULTS

200 Subjects

201 Thirty-six healthy participants (male=12, female=24) were recruited to the study. Of these,
202 three subjects withdrew from the study during the 2 week control phase before the low LA
203 PUFA diet commenced, due either to an inability to provide a blood sample (n=1) or because
204 of increased work commitments (n=2). A total of 33 participants completed the study,
205 including one participant whose results were excluded due to incomplete dietary records
206 being maintained by the participant. Therefore, the results from the remaining 32 participants
207 were included in the final analysis. The baseline characteristics of the participants are shown
208 in Table 1. There was no change in body weight or BMI after the 4 weeks on the low LA diet
209 compared to weight at baseline (data not shown).

210

211 Dietary data

212 The mean dietary intakes of energy, macronutrients, n-6 and n-3 PUFA during the 2 week
213 control phase and during the 4-week dietary intervention are presented in Table 2.
214 Participants consumed significantly less total energy and total fat during the low LA diet
215 phase compared to baseline ($P<0.05$). There was no significant difference, however, in the
216 intake of carbohydrate or protein in either g/day or as a percentage of total energy between
217 baseline and the low LA diet phase.

218

219 Dietary LA intake decreased from 4.6%E at baseline to 2%E during the low LA diet period
220 (P<0.001). Dietary ALA intake also decreased significantly during the low LA intervention
221 compared to baseline (P<0.001). There was a small but significant increase in the intake of
222 saturated fat as a percentage of energy during the low LA diet phase compared to baseline
223 (P<0.05). Importantly, there was no change in n-3 LCPUFA intake during the low LA
224 intervention (Table 2).

225

226 Plasma phospholipid fatty acid profile

227 Median plasma phospholipid fatty acids at baseline and following 4 weeks on the low LA
228 diet are presented in Table 3. After 4 weeks on the low LA diet, there was a significant
229 decrease in both LA and total n-6 PUFA content of plasma phospholipids compared to the
230 control period (P<0.001), but no change in their AA content. There was no change in the
231 saturated fat content of the plasma phospholipids after the low LA intervention. The plasma
232 phospholipid content of EPA (P<0.01), DPA (P<0.05), DHA (P<0.001) and total n-3
233 LCPUFA (P<0.001) were all significantly increased after 4 weeks on the low LA diet (Table
234 3). There was no difference in the ALA content of the plasma phospholipids after 4 weeks on
235 the low LA diet (Table 3).

236

237 Erythrocyte phospholipid fatty acid profile

238 Median erythrocyte phospholipid fatty acids at baseline and following 4 weeks on the low LA
239 diet are presented in Table 4. Consistent with the plasma phospholipids, after 4 weeks on the
240 low LA diet, there was a significant decrease in both LA and total n-6 PUFA content of
241 erythrocyte phospholipids compared to the control phase (P<0.001), but no change in AA
242 content. However, in contrast to the plasma phospholipids, there was no difference in
243 erythrocyte phospholipid concentrations of the n-3 LCPUFA, EPA, DPA or DHA following

244 the low LA dietary intervention (Table 4). Stearic acid (18:0) content in the erythrocyte
245 phospholipids increased from 11.4% at baseline to 11.9% ($P<0.05$) after 4 weeks on the low
246 LA diet.
247

248 ## DISCUSSION

249 This study has demonstrated that it is feasible to reduce LA intake in the diet from 4.6%E to
250 2%E in free-living humans. This is only the second study to demonstrate that n-6 PUFA
251 intakes can be reduced to this extent in an outpatient setting, and the first in which the
252 intervention was based on a combination of dietary advice and provision of study oils and
253 fats, without providing additional study foods. The observed diet-induced reduction in the
254 LA content of plasma and erythrocyte phospholipids without an accompanying decrease in
255 AA content in either blood fraction in the present trial is consistent with previous findings
256 [18, 22, 23]. Collectively, these results suggest that any positive effects of reduced LA intake
257 on inflammatory pathways are unlikely to be mediated by a reduction in AA. It is possible,
258 however, that lowering LA intake for a 4 week period was insufficient to elicit changes in
259 AA content and that AA content would have decreased had the diet been continued for a
260 more extended period.

261 Lowering the LA content of the diet for a 4 week period did, however, significantly increase
262 the EPA, DPA and DHA content in plasma, but not erythrocyte phospholipids. Importantly,
263 this occurred in the absence of any increase in n-3 LCPUFA intake, and a reduction in the
264 dietary intake of ALA. The finding of an increase in n-3 LCPUFA status after following a
265 low n-6 LA diet is in agreement with the results of MacIntosh and colleagues who provided
266 participants with a low n-6 PUFA diet for 12 weeks[18], and consistent with the hypothesis
267 that high n-6 diets can limit uptake of dietary n-3 LCPUFA into tissues. The finding that

268 plasma phospholipid n-3 LCPUFA increased despite a significant reduction in dietary n-3
269 ALA suggests that enhanced incorporation of dietary n-3 PUFA/n-3 LCPUFA, rather than
270 increased conversion from n-3 ALA, likely played the dominant role in increasing n-3
271 LCPUFA status. This interpretation is supported by the finding that the ALA content of the
272 plasma and erythrocyte phospholipids were not decreased following the 4 weeks on the low
273 LA diet, despite the significant reduction in dietary ALA intake.

274 In contrast to the effects in plasma, there was no change in EPA, DPA or DHA content of
275 erythrocyte phospholipids after 4 weeks on the low LA diet in this study. This is consistent
276 with the results of two previous 4-week Australian trials [22, 23], but differs from that of
277 Macintosh and colleagues, who reported a 51% increase in EPA and 19% increase in DHA
278 content in erythrocyte phospholipids following a low n-6 diet for 12 weeks [18]. Since
279 erythrocytes have a longer half-life than plasma phospholipids, a 4 week period of dietary LA
280 lowering may be insufficient to produce any changes in tissue n-3 status in erythrocyte
281 phospholipids [24]. However, the difference in trial duration cannot completely explain the
282 results, since MacIntosh et al found substantial increases in EPA and DHA content in
283 erythrocytes after only 4 weeks that were similar in magnitude to those at 12 weeks
284 (Ramsden, unpublished data). Two key differences between the present study and the study
285 conducted by MacIntosh et al may help explain these results; the Macintosh trial was
286 conducted in a US population which had much higher LA intakes at baseline compared to our
287 Australian population (7.4%E vs. 4.6%E), and the MacIntosh trial maintained baseline
288 dietary n-3 ALA throughout the intervention phase while n-3 ALA intake decreased
289 significantly in the present trial. As a result, reducing LA intake to approximately 2 %E in the
290 US trial would be expected to produce a proportionally greater increase in n-3 LCPUFA
291 status due to a more pronounced reduction in competition for esterification into membrane
292 phospholipids and conversion of n-3 ALA to EPA and DHA.

293 The unintentional decrease in ALA intake in our trial was likely due to the fact that many key
294 dietary sources of ALA (canola oil, nuts and seeds) are also relatively high in LA and were
295 not permitted during the low LA dietary intervention. In addition, the macadamia oil and
296 butter that were used as substitutes for standard vegetable oils and spreads contain very low
297 levels of ALA. This was avoided in the MacIntosh trial by providing a small amount of
298 ground flaxseed to participants during the low n-6 dietary intervention in order to maintain
299 average US ALA intake despite a reduction in high LA vegetable oils that are also major
300 sources of n-3 ALA (e.g. canola, soy) [18].

301 Limitations

302 As with the majority of dietary studies, the accuracy of dietary recording is a potential
303 limitation in the current trial. While weighed food records are generally regarded as one of
304 the higher quality methods of dietary recording, the very act of asking people to record their
305 dietary intake has the potential to alter their dietary choices, both as a result of social
306 desirability bias and potential to favour foods that are easier to weigh/record [25]. However,
307 the significant reduction in LA and total n-6 PUFA content of both the plasma and
308 erythrocyte phospholipids following 4 weeks on the low LA diet provides evidence of good
309 compliance with the diet regimen. The decrease in energy intake and small, but significant,
310 increase in the intake of saturated fat as a percentage of energy during the low LA diet is not
311 unexpected given the restrictions on take-away/processed foods and intake of plant/vegetable
312 based oils, however the potential impact of these changes would need to be considered if a
313 low n-6 LA diet were to be maintained longer term. This study was also designed to have
314 each individual act as their own control, there was no parallel control group. The benefit of
315 this design is that it eliminates the variation between individuals especially in regard to
316 dietary intake. However, not having a control group is a limitation of this study and larger

317 randomised controlled trials with a control group need to be conducted in future
318 investigations.
319

320 Clearly, the magnitude of change in n-3 LCPUFA status in this 4-week trial is much less than
321 achieved by directly supplementing the diet with n-3 LCPUFA. Therefore, the potential for
322 low LA diets to provide a substitute for n-3 LCPUFA supplementation to raise n-3 LCPUFA
323 status may be limited. However, recent evidence suggests that high n-6 LA intakes have the
324 potential to produce negative health effects through the production of bioactive lipid
325 mediators derived from n-6 LA (e.g. hydroperoxy- and hydroxy-fatty acids), i.e. independent
326 of n-3 LCPUFA status [26-28]. It is therefore possible that low n-6 LA diets may be
327 associated with clinical benefits in spite of relatively modest effects on n-3 LCPUFA status.
328 For example, the bioactive n-6 LA metabolite leukotoxin is reported to contribute to tissue
329 damage [29, 30], and the n-6 LA metabolites 9- and 13-HODE are reported to have pro-
330 nociceptive properties [31, 32]. Indeed, Ramsden and colleagues recently reported that a
331 combined high n-3 plus low n-6 (H3-L6) dietary intervention reduced the severity of
332 symptoms in chronic headache sufferers [33]. However, the relative importance of lowering
333 dietary n-6 LA and increasing dietary n-3 PUFAs in producing the anti-nociceptive effects is
334 not yet clear. In addition, the issue of sustainability of n-3 LCPUFA supplementation from
335 marine sources has been called into question, and so there is a need to critically examine
336 alternative strategies for maintaining/increasing n-3 LCPUFA status in the population. Thus,
337 low n-6 PUFA diets may indeed have an important role to play in human health.
338

339 ## CONCLUSIONS

340 We have demonstrated that it is possible to reduce dietary LA intake to ~2%E in a free-living
341 human population with no provision of foodstuffs apart from a highly monounsaturated oil

342 (macadamia oil) and butter, and without causing an increase in total fat intake. The low LA
343 diet reduced LA and total n-6 PUFA content of plasma and erythrocyte phospholipids.
344 Importantly, the low LA diet also increased the n-3 LCPUFA content of the plasma
345 phospholipids, in the absence of any increase in n-3 LCPUFA intake, almost certainly due to
346 higher incorporation of dietary n-3 LCPUFA. These data support the hypothesis that short-
347 term reductions in dietary LA intake have the potential to improve n-3 LCPUFA status, albeit
348 modestly, without a need to increase dietary n-3 LCPUFA intake. We speculate that the
349 magnitude of the increase in n-3 LCPUFA status would be greater by maintaining or
350 increasing dietary ALA content and/or extending the intervention period beyond 4 weeks,
351 and this remains an important avenue for further studies. In addition, it will be important to
352 determine whether the biochemical changes identified in response to the low LA diet provide
353 clinical benefits, particularly in conditions associated with increased bioactive derivatives of
354 n-6 LA.
355

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360 manuscript.

361

362 CONFLICT OF INTEREST

363 The authors have no conflicts to declare.

364

365

Table 1: Baseline characteristics of participants

	Sample population
Total number of participants (N)	32
Gender	10 males; 22 females
Age (years)	Range:19-62; Mean ± SD: 31.5 ± 12.3
BMI (kg/m^2)	Range: 17.4-34.7; Mean ± SD: 23.5 ± 3.6

Table 2:

Dietary daily intake of energy, macronutrients and fatty acids during the control and low LA diet phase (median interquartile range)

	Control Phase	Low LA diet
Total energy (kJ)	7852 (6509 - 9566)	6734 (5637 - 8374)*
Protein (g)	82.0 (69.2 - 99.1)	81.4 (67.3 - 99.9)
Carbohydrate (g)	205.7 (165.1 - 243.6)	196.7 (138.4 - 242.8)
Total fat (g)	63.0 (51.3 - 83.8)	51.5 (42.1 - 64.5)*
Monounsaturated fat (%E)	10.81(9.54-14.38)	11.76(9.23-14.33)
Saturated fat (%E)	10.8 (8.1 - 12.7)	11.5 (9.5 - 13.8)*
n-3 LCPUFA (mg)	235.0 (132.5 - 365.0)	215.0 (150.0 - 415.0)
n-3 LCPUFA (%E)	0.12% (0.07 - 0.20)	0.15% (0.08 - 0.17)
LA (g)	8.5 (6.6 - 13.6)	4.2 (9.5 - 13.8)*
LA (%E)	4.6 (3.5 - 5.3)	2.0 (1.7 - 2.6)*
ALA (g)	1.23 (0.81 - 1.68)	0.57 (0.45 - 0.81)*
ALA (%E)	0.53 (0.44 - 0.81)	0.31 (0.27 - 0.38)*

ALA, α-linolenic acid; LA, linoleic acid; * $P<0.05$

Table 3: Plasma phospholipid fatty acid profile at baseline and after 4 weeks on the low LA diet (median interquartile range).

All results are expressed as a percentage of the total fatty acid in plasma phospholipids.

		Baseline	Week 4
Monounsaturated fatty acids	Total Mono	13.54 (12.66–14.60)	14.48 (13.13–15.39)
	Oleic (18:1n-9)	10.15 (9.57–11.45)	10.98 (9.6–11.67)
Saturated fatty acids	Palmitic acid (16:0)	27.5 (26.9 – 28.1)	27.7 (26.7 – 28.7)
	Stearic acid (18:0)	13.7 (12.9 – 14.1)	13.5 (12.7 – 14.1)
n-6 PUFA	LA (18:2n-6)	21.0 (19.2 – 22.5)	18.6 (17.3 – 21.2)***
	DGLA (20:3n-6)	3.05(2.64-3.44)	3.20(2.61-3.80)
	AA (20:4n-6)	9.7 (8.6 – 11.0)	9.9 (9.5 – 11.3)
	Total n-6 PUFA	35.1 (33.5 – 36.5)	33.7 (31.8 – 35.0)***
n-3 PUFA	ALA (18:3n-3)	0.17 (0.14 – 0.23)	0.19 (0.15 – 0.22)
	EPA (20:5n-3)	0.87 (0.80 – 1.18)	1.09 (0.83 – 1.34)**
	DPA (22:5n-3)	0.88 (0.79 – 1.01)	0.95 (0.78 – 1.12)*
	DHA (22:6n-3)	3.47 (3.01 – 3.90)	3.77 (3.33 – 4.26)**
	Total n-3 PUFA	5.53 (5.04 – 6.56)	6.22 (5.57 – 6.69)***

LA, linoleic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapantaenoic acid; DPA, docosapantaenoic acid; DHA, docosahexaenoic acid; * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 4: Erythrocyte phospholipid fatty acid profile at baseline and after 4 weeks on the low LA diet (median interquartile range).

All results are expressed as a percentage of the total fatty acid in erythrocyte phospholipids.

		Baseline	Week 4
Monounsaturated fatty acids	Total Mono		
	Oleic (18:1n-9)		
	Stearic acid (18:0)	11.4 (10.9 – 12.3)	11.9 (11.2 – 12.6)*
n-6 PUFA	LA (18:2n-6)	10.6 (9.71 – 11.3)	9.41 (8.78 – 10.5)***
	DGLA (20:3n-6)		
	AA (20:4n-6)	13.4 (12.8 – 14.1)	13.3 (12.7 – 14.0)
	Total n-6 PUFA	29.3 (28.7 – 30.9)	27.9 (27.2 – 28.7)***
n-3 PUFA	ALA (18:3n-3)	0.14 (0.13 – 0.15)	0.12 (0.11 – 0.15)
	EPA (20:5n-3)	0.76 (0.67 – 0.95)	0.82 (0.67 – 0.99)
	DPA (22:5n-3)	2.43 (2.19 – 2.66)	2.40 (2.11 – 2.61)
	DHA (22:6n-3)	4.67 (4.07 – 5.50)	4.64 (4.12 – 5.22)
	Total n-3 PUFA	8.20 (7.67 – 8.87)	8.01 (7.60 – 8.66)

LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapantaenoic acid; DPA, docosapantaenoic acid; DHA, docosahexaenoic acid;

*P<0.05,**P<0.01,***P<0.001

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