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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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ABSTRACT

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 of plasma phospholipids was significantly increased 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.

1. Introduction

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

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

LA competes with the short-chain n-3 PUFA alpha-linolenic acid (ALA) for the enzymes required for conversion to their respective long-chain derivatives and for incorporation into the plasma membrane [8]. Consequently, high LA diets may limit the capacity of increases in dietary n-3 PUFA intake to improve n-3 PUFA status [9]. Given that several bioactive mediators derived from n-3 LCPUFA are less potent in their inflammatory actions than their AA-derived analogues [10], and others actually have potent inflammation-resolving [11] or neuroprotective properties [12], this competition has the potential to contribute to negative health outcomes.

Current strategies to improve n-3 LCPUFA status of the population have focused almost exclusively on increasing dietary n-3

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LCPUFA intake, via increased consumption of oily fish or through fish oil supplementation [13]. However, many individuals struggle to achieve regular fish intake [14] and dwindling marine resources have raised concerns regarding the long-term sustainability of this approach [15]. The competition that exists between n-6 and n-3 has led to suggestions that lowering the LA content of the diet has the potential to both limit the production of n-6 derived pro-inflammatory mediators and enhance the biological efficacy of n-3 LCPUFA consumed in the diet. However, the ubiquity of LA in the food supply, particularly in pre-prepared and take-away foods, makes any attempt to reduce the n-6 PUFA intake of free-living humans challenging.

We previously designed a low n-6 PUFA diet, in which we reduced the LA content of the diet from ~5% to ~2%E by replacing standard plant-based oils and spreads with low n-6 PUFA alternatives (Macadamia oil and butter), and limiting intake of processed and take-away foods which utilize high n-6 PUFA oils [16]. Importantly, we showed that the reduction in LA intake could be achieved while still adhering to the national dietary recommendations (The Australian Guide to Healthy Eating (AGHE) guidelines) [17] and maintaining a saturated fat intake of less than 10%E [16]. The aim of the present study was to determine whether following this low LA diet for a 4 week period would result in reduced n-6 PUFA and increased n-3 LCPUFA content in plasma and erythrocyte phospholipids in healthy human subjects.

2. Patients and methods

2.1. Participants

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

2.2. Study design

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

2.3. Clinic appointments

All participants attended clinic appointments at enrolment, after the 2 week control phase and at the completion of the 4 week dietary intervention. All appointments were conducted between 7:30 am and 9:00 am after an overnight fast of at least 12 h. During the clinic appointments, weight and height were measured with participants in light clothing and without shoes, using a digital weighing scale (SALTER, Victoria, Australia) and a stadiometer (SECA, New South Wales, Australia) respectively and Body Mass index calculated (weight/height^2). Fasting venous blood samples (8 ml) were collected at each clinic appointment. After collection, blood samples were centrifuged for 15 mins at 3500*g* at 4 °C to separate plasma and erythrocytes for subsequent analysis of fatty acids.

2.4. Dietary information

All participants were asked to maintain a 3-day weighed food diary each week during both the control and dietary intervention periods. Instructions on completing the diary were provided at enrolment, and participants were instructed to record their dietary intake for 2 week days and 1 weekend day in each week of the study. Electronic kitchen scales (SALTER 1021, Victoria, Australia) and standard metric measuring cups (Décor Cook® Measuring Cups/Spoons, Victoria, Australia) were provided to participants to assist them in completing the weighed food diary.

2.5. Low LA diet

The low LA diet was based on the diet previously designed by our group [16] and aimed to achieve an LA intake of < 2.5%E whilst maintaining saturated fat intake at < 10%E and not altering the intake of n-3 LCPUFA. Participants were provided with Macadamia oil (Suncoast Gold Vitality™ Macadamia oil, 1.24 g LA/100 g) and butter (Western Star™ Butter, 1.60 g LA/100 g) 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 < 1 g LA/100 g and/or < 1 g 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 dining at restaurants 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.

2.6. Dietary analyses

The diet diaries were analysed for energy intake, macronutrient composition, and fatty acid content including LA, ALA and total n-3 LCPUFA, using the FoodWorks programme (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 programme 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.

2.7. Fatty acid analyses

The fatty acid composition of plasma and erythrocyte phospholipids was analysed using gas chromatography as described in