Effect of altering dietary n-6:n-3 PUFA ratio on cardiovascular risk measures in patients treated with statins: a pilot study

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Abstract

Increasing dietary n-3 PUFA decreases the risk of CHD. Since n-6 PUFA compete with n-3 PUFA for common metabolic enzymes, the n-6:n-3 ratio intake rather than the n-3 PUFA intake levels per se may be critical. We aimed to examine whether altering the n-6:n-3 ratio affects cardiovascular risk factors in hypercholesterolaemic patients on lipid management with statins. Adhering to a randomised, crossover study design, patients on statins (n 11) were placed on one of two dietary interventions (Diet high-ratio (HR) – n-6:n-3 = 30:1 or Diet low-ratio (LR) – n-6:n-3 = 17:1) for 4 weeks followed after an 8-week washout period by the alternate diet. Foods enriched with n-3 or n-6 PUFA were delivered to each patient, who were given clear guidance on consumption expectations for the study. Measures of lipid profile, blood pressure and vascular function were determined. Diet LR significantly reduced body weight, LDL-cholesterol, high-sensitivity C-reactive protein, blood pressure and the apoA-1:apoB ratio. While Diet HR trended towards a similar cardioprotective profile, most of the parameters examined did not reach statistical significance. A direct comparison between diets demonstrated no significant superiority of Diet LR over Diet HR. These results suggest that a dietary intervention focused on n-6 and n-3 fatty acids may improve cardiovascular risk factors in patients over and above standard lipid management, but there is no significant advantage of a low n-6:n-3 ratio diet when compared to a high-ratio diet.

Key words: n-6 PUFA, n-3 PUFA: Cardiovascular risk factors: Diet

Dietary n-3 PUFA reduce the risk of incident cardiovascular events(4). Postulated mechanisms underlying this association include improved vascular health and lipid profile(2), α-Linolenic acid, the predominant plant-derived n-3 PUFA, has been shown to decrease lipids and improve endothelial function in hypercholesterolaemic subjects(5,6). As n-6 PUFA are important modulators for the biological activities of n-3 PUFA(5), it has been hypothesised that the dietary ratio of n-6:n-3 PUFA may be more important than, or provide additional information beyond that derived from, the dietary intake of n-3 PUFA alone(6). While the ideal n-6:n-3 PUFA ratio has been estimated as 1:1(5,6), modern Western diets are characterised by a high n-6 PUFA intake and a relatively low n-3 PUFA intake so that the n-6:n-3 PUFA ratio is high (approximately 10:1). n-6:n-3 PUFA ratios as high as 24:1 have been documented in Israeli populations(7) and unsubstantiated reports of ratios as high as 33:1 to 38:1 have been described for South Asian populations(8,9). To our knowledge, there are currently no randomised controlled trials comparing dietary low and high n-6:n-3 PUFA ratios with respect to cardiovascular health.

Dietary long-chain n-3 PUFA lower circulating TAG levels. This is most marked at high doses(9) and is in contrast to the statin group of drugs which potently reduce LDL-cholesterol, but only moderately decrease TAG(10). As such, patients treated with statins for hypercholesterolaemia may potentially benefit from a diet rich in n-3 PUFA and perhaps even more so from a diet with a low n-6:n-3 PUFA ratio. We examined whether dietary n-6:n-3 PUFA ratio influences vascular health and lipid profiles and particularly whether it is able to do so in therapeutically treated hypercholesterolaemic subjects, thus potentially supporting its adjunct use in the clinical management of these patients.

The present study examined the hypothesis that a low dietary n-6:n-3 PUFA ratio can further improve cardiovascular risk profile and vascular health in hypercholesterolaemic patients on standard clinical care.

Abbreviations: HR, high-ratio; hsCRP, high-sensitivity C-reactive protein; LR, low-ratio.

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Experimental methods

Subjects

Participants were recruited from the Lipid Clinic at the Department of Cardiovascular Medicine (Heart Centre), The Alfred Hospital, Melbourne, Australia. Potential participants were identified at the clinic and follow-up screening for suitability and participation willingness took place either by phone or in person. Inclusion criteria were hypercholesterolaemic patients aged 18–60 years, who had been receiving statins as cholesterol-lowering therapy for at least 3 months before the commencement of the study. Exclusion criteria included a history of clinically relevant atheroma, cancer, renal failure, liver disease, current or ex-smokers (less than 6 months since cessation), diabetes, BMI $\geq 30$ kg/m$^2$, an inability to participate in the examinations due to incapacitation, an inability to complete the dietary interventions due to medical, cultural, religious factors or food allergies or intolerances.

$n$-$6$-$n$-$3$ high- and low-ratio diets

Test diets were modified from high-PUFA diets previously developed by L. Brazionis and C. Itsiopoulos, St Vincent’s Hospital, Melbourne, Australia. The fatty acid content of each diet was determined via analysis using the fatty acid tables of FoodWorks 2009 Premium Edition, version 6 (Xyris Software).

The two isoenergetic test diets were supplied in full to the participants as a 7 day menu (see Table 1) repeated over 4 weeks. The study was a randomised crossover design where each participant acted as their own control. Participants were randomised to the order of the test diets using block randomisation (random permuted blocks). This ensured that each participant acted as their own control. Participants were instructed to complete a 7 day diet diary for each diet along with dietary assessments. Dietary intake was adjusted proportionately to energy requirement and the participants were asked to maintain their usual body weight throughout the study.

Each intervention lasted 4 weeks with an 8 week washout between diets. A 4-week intervention with a diet rich in $n$-$3$ fatty acids has previously been demonstrated to be of sufficient duration to improve lipids and vascular function in healthy subjects$^{11,12}$. Participants visited the Heart Centre at the beginning and conclusion of each diet for a series of tests. All participants were instructed to abstain from caffeine-containing beverages on the day of the study as well as to fast overnight before the study day.

Each protocol was conducted in a clinical laboratory under quiet, thermo-regulated ($22^\circ$C) conditions. Subjects were rested in a comfortable supine position throughout the course of the study. There was no set order for vascular assessments and a minimum of 15 min separated the vascular assessments.

Participants were instructed to complete a 7 day diet diary for the total duration of each 4-week intervention. They were provided with a daily list of all prescribed foods, the majority of which were supplied as weighed portions. Then, they were advised by a trained dietitian on how to complete the diary, including being instructed to take note of each food as it was consumed.

### Study design

As stated previously, the study was a randomised crossover design where each patient was assigned to both diets in a random order. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Alfred Hospital Institute Ethics Committee (Ethics no. 139/07).

Written informed consent was obtained from all subjects.

| Table 1. Details of the food provisions supplied to each participant (8.2 MJ/d) |
|-------------------------|-------------------------|-------------------------|
| Diet high-ratio         | Diet low-ratio          |
| Multigrain bread        | 1 loaf/week             | 1 loaf/week             |
| Nuttelex margarine      | 500 g                   | 500 g                   |
| Eggs                    | 60g x 6                 | 60g x 6                 |
| Safflower oil           | 300 ml                  | 300 ml                  |
| Lamb/lamb chops         | 120g (2 serves/week)    | 120g (2 serves/week)    |
| Pork fillet             | 150g (1 serve/week)     | 150g (1 serve/week)     |
| Turkey breast           | 120g (2 slices/week)    | 120g (2 slices/week)    |
| Roast beef              | 100g (4 slices/week)    | 100g (4 slices/week)    |
| Banana bread            | 60g (2 serves/week)     | 60g (2 serves/week)     |
| Ham                     | 100g (3 slices/week)    | 100g (3 slices/week)    |
| Chicken breast          | 290g (2 serves/week)    | 290g (2 serves/week)    |
| $n$-$6$-Enriched pasta sauce | 300 ml (1 serve/week) | 300 ml (1 serve/week) |
| Chicken and vegetable soup | 300 ml (2 serves/week) | 300 ml (2 serves/week) |
| Macadamia nuts          | 250g (4 serves/week)    | 250g (4 serves/week)    |

(1) Diet HR: 8% of dietary energy from PUFA; $n$-$6$-$n$-$3$ ratio approximately 30:1.

(2) Diet LR: 8% of dietary energy from PUFA; $n$-$6$-$n$-$3$ ratio approximately 1:7:1.

Except for sources of polyunsaturated fat, the two diets consisted of similar foods, and thus had similar quantities of total fat, carbohydrates and protein. The diets were isoenergetic, but varied in the $n$-$6$-$n$-$3$ PUFA ratio (Table 2). Dietary intake was adjusted proportionately to energy requirement and the participants were asked to maintain their usual body weight throughout the study.
was eaten, to highlight any uneaten prescribed foods and to record any additional food or drink consumed, which was used to assess dietary compliance. A measure of compliance was obtained by determining the number of times each food was fully consumed compared to the number of times it was supplied in the test diet.

**Anthropometry**

Height, weight and waist circumference were measured by standard techniques. Body weight was measured to the nearest 0.1 kg, with the subjects wearing light clothing and no shoes. Height was measured to the nearest 0.01 m. Waist circumference was measured as the narrowest circumference between the lower rib margin and anterior superior iliac crest.

**Resting blood pressure**

Systolic and diastolic blood pressure was measured while seated and at rest, using an automated oscillometric blood pressure monitor (SureSignsVS3 Monitor; Philips).

**Arterial stiffness**

**Systemic arterial compliance.** Systemic arterial compliance was derived from the measurements of aortic flow velocity with continuous-wave Doppler velocimetry (Multi-Dopplex MDI; Huntleigh Technology) and simultaneous right carotid pressure, measured by applanation tonometry (SPT-301; Millar Instruments). Brachial arterial blood pressure was measured simultaneously by an automated oscillometric blood pressure monitor (SureSignsVS3 Monitor; Philips) to permit calibration of the carotid arterial pressure contour with brachial mean and diastolic blood pressure and to derive carotid systolic blood pressure. Aortic volume flow was obtained by multiplication of the mean velocity flow by the left ventricular outflow tract area, measured by two-dimensional echocardiography (IU22 Ultrasound; Philips).

**Arterial compliance and distensibility.** Brachial artery distensibility (elasticity) and compliance (buffering capacity) were measured using non-invasive high-resolution ultrasound (IU22 Ultrasound; Philips). Briefly, the brachial artery of the non-dominant arm is imaged above the antecubital fossa in the longitudinal plane, using a high-frequency linear array transducer (15–10 MHz). Following this, two B-mode loops were recorded every 2 min with the simultaneous recording of blood pressure. Then, a line perpendicular to the arterial wall was chosen and captured in M-mode (three consecutive cardiac cycles every 2 min). These devices can accurately measure diameter (D) and stroke change in diameter (Dd). Assuming that during the heart cycle arteries predominantly change in diameter, cross-sectional compliance and distensibility coefficient could be calculated. Cross-sectional compliance is the change in cross-sectional area of the vessel (DA) per unit of pressure. Similarly, distensibility coefficient is defined as the relative change in the cross-sectional area of the vessel (DA/D) per unit of pressure.

**Blood samples**

Blood (10 ml) was collected in an EDTA vacutainer tube for the measurement of total cholesterol, HDL-cholesterol, LDL-cholesterol, TAG, glucose, apoA-1 and B and high-sensitivity C-reactive protein (hsCRP). These measurements were performed on a COBAS Integras 400 Plus Analyzer (Roche). The intra-assay CV was 8% for TAG, 1% for apoA-1, 2% for apoB, 8% for hsCRP, 8% for total cholesterol, 8% for HDL-cholesterol and 3% for glucose.

**Statistical analysis**

SPSS version 17 (SPSS Inc.) was used for all data analyses. The effects of a given diet on pre- vs. post-outcome measures were examined using two-tailed paired Student’s t test. Changes in outcome measures between the two test-diets were also examined using a general linear model with time and diet as within-subject factors and sequence (the order in which the diets were consumed) as a between-subjects factor. No significant sequence effect (P>0.10) was observed for any of the variables of interest. All data are presented as median and interquartile range. All the data were considered significant at P<0.05.

**Results**

A total of eleven participants (six males), aged 47 years (range 30–57 years) with average BMI of 24.9 kg/m² completed the
study. All were on statins; six treated with atorvastatin, three with simvastatin and two with rosuvastatin, and it was apparent that their LDL-cholesterol and total cholesterol were well controlled upon entry into the study (Table 3). Of the five female participants, two were post-menopausal, neither of whom received hormone replacement therapy for the duration of the study. There were no significant differences in responses to diets between the sexes (P > 0.10). From the diet diaries, nine of the eleven patients were 100% compliant with both dietary arms. One participant was 70% compliant on Diet LR (with 100% compliance on Diet HR); the other participant demonstrated 60% compliance on Diet HR. Achieved dietary ratios of n-6:n-3 PUFA were 37:1:1 and 1:2:1 for the Diet HR and Diet LR, respectively (Table 2). There were no differences in lipid levels, cardiovascular risk factors or vascular measures at baseline between the two dietary interventions (P > 0.10 for all comparisons, Tables 3 and 4).

Patient weight, blood pressure and plasma measures

Changes in body weight, systolic and diastolic blood pressure, plasma lipid, glucose, hsCRP and apoA-1 and B levels following both dietary interventions are shown in Table 3. Body weight, systolic and diastolic blood pressure, LDL-cholesterol, hsCRP, and apoA-1 and B were all significantly altered following Diet LR. Following Diet HR, only hsCRP and apoA-1 and B levels were significantly decreased. However, a direct comparison of Diet LR v. Diet HR (A before and after diet comparisons) showed that neither diet was advantageous over the other for these parameters.

Vascular health

When measures of vascular health were calculated (Table 4), neither diet influenced the distensibility coefficient, cross-sectional compliance or pressure strain elastic modulus.

Discussion

The present study shows that a diet with a low n-6:n-3 PUFA ratio does not result in improved cardiovascular profile or vascular health when compared with a high n-6:n-3 PUFA ratio diet in subjects treated with statins. Rather, both diets had beneficial effects including favourable effects on total as well as LDL-cholesterol, systolic and diastolic blood pressure, hsCRP levels and the apoA-1:B ratio.

The aim of the study was to investigate whether the ratio of dietary n-6:n-3 PUFA improves the efficiency of delivered cardiovascular benefits. The putative cardiovascular benefits of the dietary n-6:n-3 PUFA ratio arise from n-6 and n-3 fatty acids sharing the same pools of enzymes, cyclo-oxygenase and lipoxygenase, and going through the same oxidation pathways while being metabolised. It is conceivable therefore that a lower consumption of n-6 PUFA, and hence a lower n-6:n-3 PUFA ratio, may allow a higher efficacy for n-3 PUFA, already well described in its own right to have positive cardioprotective effects (5,6), since there would conceivably be less competition for the metabolic breakdown of n-3 fatty acids sharing the same pools of enzymes, cyclo-oxygenase and lipoxygenase, and going through the same oxidation pathways while being metabolised. It is conceivable therefore
acids. In the present study, participants demonstrated favourable lipid and blood pressure outcomes post Diet LR and not post Diet HR, although a direct comparison of both these diets showed no significant benefit of one diet over the other. Guebre-Egziabher et al.\textsuperscript{(12)} examined the effects of lowering $n$-$6:n$-$3$ ratio on lipids and inflammation with a 10-week dietary intervention in a small population of healthy subjects. They reported significant improvement in lipids and inflammatory markers and decreased $n$-$6:n$-$3$ ratio in these subjects, although the study was limited by the absence of a control group\textsuperscript{(12)}.

Statins are the lipid-lowering medication of choice in people with elevated LDL-cholesterol levels. While effective, these patients still have residual risk for CVD compared to those who have a healthy cholesterol profile\textsuperscript{(8)}. While previous work has shown that prescription of $n$-3 fatty acids can improve non-HDL-cholesterol levels over and above simvastatin\textsuperscript{(13)}, no previous study has looked at the efficacy of $n$-$3$ food intake in this context or examined the $n$-$6:n$-$3$ ratio.

In the present study, Diet LR demonstrated a significant improvement of both total and LDL-cholesterol levels, while Diet HR also trended towards an improvement albeit not one that reached significance ($P = 0.06$ in both instances). Both diets improved hsCRP levels, which have been demonstrated to be an independent biomarker for the development of CVD\textsuperscript{(14)}. This suggests that a nutritional focus on improved $n$-$6$ and $n$-$3$ intake has benefits over ‘standard’ Australian dietary habits. We did not, however, demonstrate that manipulating the ratios of these PUFA provided any advantage.

The weight loss with Diet LR, while being statistically significant, was of only minor clinical significance (less than 1 kg), in contrast to the change in LDL-cholesterol of approximately 15%, which is of clinical importance. Furthermore, weight loss is generally not associated with improvements in LDL-cholesterol\textsuperscript{(15)}. Taken together, these suggest that it is unlikely that the reductions in LDL-cholesterol resulting from these dietary interventions are due to concurrent weight loss. It remains unknown whether the changes in LDL-cholesterol would be more or less marked in untreated hypercholesterolaemic subjects or normocholesterolaemic subjects.

We showed no effect of either diet on plasma TAG or glucose levels. The controversy surrounding the association of plasma TAG and cardiovascular disease has recently been summarised in detail\textsuperscript{(16)}. Nonetheless, our findings suggest that in patients treated with statins, which reduce TAG by approximately 10–20%, dietary intervention aimed at altering the $n$-$6:n$-$3$ PUFA ratio is unlikely to improve TAG levels further.

ApoA-1, the predominant component of HDL-cholesterol, promotes cholesterol efflux from tissues. ApoB is the major apo of LDL-cholesterol, which is responsible for cholesterol transport to tissues. Both of these apo have been implicated in atherosclerosis. Unexpectedly, we saw significant reductions in both apoA-1 and B after consumption of both diets. The INTERHEART study (a global study of risk factors in acute myocardial infarction) published in 2008 has found that the apoA-1:apoB ratio is more effective at predicting heart attack risk, in patients who have had an acute

### Table 4. Dietary $n$-$6:n$-$3$ ratio and measures of arterial compliance and distensibility (Medians and interquartile ranges (IQR))

<table>
<thead>
<tr>
<th></th>
<th>Diet LR (low $n$-$6:n$-$3$ ratio)</th>
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<th>Diet HR (high $n$-$6:n$-$3$ ratio)</th>
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<tr>
<td></td>
<td>0 weeks</td>
<td>4 weeks</td>
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<td>Median</td>
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<tr>
<td>Distensibility coefficient (10$^{-3}$kPa)</td>
<td>8.3</td>
<td>3.11</td>
<td>5.5</td>
<td>1.1</td>
<td>0.11</td>
<td>2.9</td>
<td>0.54</td>
<td>2.9</td>
</tr>
<tr>
<td>Cross-sectional compliance (mm$^2$/kPa)</td>
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<tr>
<td>Pressure strain elastic modulus (kPa)</td>
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<td>222.3</td>
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LR, low ratio; HR, high ratio.
myocardial infarction, than either the apo-B or apo-A measure alone (17). ApoA-1:apoB ratio was significantly improved with Diet LR but not with Diet HR. A direct comparison of the two diets, however, showed no significant superiority of Diet LR compared with Diet HR.

Another finding of significance in the present study is the decrease in systolic and diastolic blood pressure after Diet LR. Although this was not observed post-Diet HR, the same magnitude of decrease was observed so that a direct comparison of the two diets showed no significant difference. A study has demonstrated that purified DHA capsules managed to reduce ambulatory blood pressure, more effectively than EPA in mildly hyperlipidaemic men and suggested that DHA is the principal n-3 fatty acid responsible in the blood pressure-lowering effect (18). Therefore, the present study is remarkable in that the drop in blood pressure was achieved with DHA levels ranging between 0·03 and 1·38 g over a short period of time.

In summary, the improvements in lipid profile and BP observed in response to both diets may have reduced our power to observe a difference between the diets. Nonetheless, our results indicate that a dietary intervention focused on n-6 and n-3 PUFA may improve cardiovascular risk factors in patients with hypercholesterolaemia over and above standard lipid management, which if maintained in the long term would be consistent with substantial reductions in the risk of CVD events.

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