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Title: Diets high in n-3 fatty acids are associated with lower arterial stiffness in patients with rheumatoid arthritis: a latent profile analysis

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Abstract

Supplementation with n-3 fatty acids can influence inflammation and markers of arterial stiffness which are increased in patients with rheumatoid arthritis (RA). However, it is unknown whether specific patterns of dietary fatty acid intake are similarly associated. In a longitudinal study, 86 RA patients reported their dietary intake and had arterial stiffness measured using the augmentation index (AIx) at baseline and 8 months. Latent profile analysis (LPA) was performed to characterise patterns of fatty acid intake using 16 major fatty acids. Models for 2-6 profiles were compared using the Akaike and Bayesian Information Criterion. Associations between AIx and the profiles were adjusted for age, gender, disease activity, fish oil supplementation, medications, physical activity and socioeconomic status. LPA identified 5 distinct profiles. Profile-1 subjects (n=7) reported significantly higher intake of palmitoleic acid (16:1), arachidonic acid (20:4N-6); eicosapentaenoic acid (20:5N-3), docosahexaenoic acid (22:6N3), and docosapentaenoic acid (22:5N3) ($p<0.001$ for each) than profiles 2 (n=14), 3 (n=19), 4 (n=23) and 5 (n=23), and significantly higher grilled and tinned fish consumption. The AIx varied significantly across the 5 profiles (p=0.023); subjects in profile 1 had a significantly lower AIx than those in profile 3 (β= -7.2%; 95%CI= -11.5 to -2.9; p=0.001) who had the lowest reported intake of n-3 fatty acids. Fish oil supplementation was also independently associated with lower AIx (β= -4.15%; 95%CI= -6.73 to -1.56; p=0.002). A diet characterised by a higher reported intake of N-3's, palmitoleic acid (16:1) and arachidonic acid (20:4N6) is associated with a lower AIx in RA patients.

Introduction

Patients with rheumatoid arthritis (RA) are known to have increased systemic inflammation and arterial stiffness [1; 2], a process now thought to be a significant contributor in the development of atherosclerosis and cardiovascular disease (CVD) [3; 4]. TNF-alpha antagonists have been shown to provide a small but significant reduction in aortic stiffness, as measured by the augmentation index (AIx) using pulse wave analysis, in RA patients [4]. This suggests that pharmacological and, possibly, non-pharmacological interventions might exert beneficial effects on markers of arterial stiffness, with a consequent reduction in CVD risk in this patient group.

Dietary fatty acids are a major dietary component and known to influence the cascade of pro- and anti-inflammatory prostaglandins [5]. In patients with RA, fish oil supplementation has reduced inflammatory biomarkers [6], disease activity [7] and treatment failure [8]. In smokers [9] and individuals with the metabolic syndrome [10] fish oil supplementation has reduced markers of systemic inflammation, such as interleukin-6 and TNF-alpha in parallel with reductions in arterial stiffness. Whether or not certain patterns of fatty acid intake in the diet are associated with reduced inflammation and central and peripheral arterial wave reflection is however more difficult to assess. A wide variety of food sources contain high levels of one or more of the major fatty acids [11], of which a large number are associated with CVD [12] and each ranges widely between individual's diets [13]. Attempting to determine the independent associations between each of these fatty acids and measures of arterial stiffness with the use of multivariate regression models is problematic due multi-collinearity between many of the fatty acids. In addition, many other known and unknown factors in the diet besides fatty acids can influence arterial stiffness, creating the potential for residual confounding.

Latent profile analysis (LPA), a special form of latent class analysis (LCA), is an objective (model-based) data reduction technique that enables the identification of underlying patterns within data, with each pattern defined by virtue of having similar values across a set of observed continuous and/or categorical variables [14]. LCA can also be thought of as being a "person-centred" rather than a "variable-centred" approach, since the focus of the analysis is on identifying groups of individuals with a pattern of similar values, rather than identifying the effects of each individual variable. LCA thus captures the heterogeneity within and between groups and can identify the most parsimonious number of distinct patterns in the data via formal measures of model fit [15].

In this study we aimed to identify and describe the unique patterns of dietary fatty acid intake existing within a representative sample of RA patients that we have previously studied [16]. We then examined which, if any of these patterns were associated with the AIx, a marker of arterial stiffness and wave reflection, and whether or not there were separate and additive effects of n-3 fish oil supplementation. In individuals aged 50 and over, aortic arterial wave reflection measured using the AIx has been found to be a more sensitive marker of arterial stiffening than pulse wave velocity [17].

2. Methods

2. 1 Study design

We conducted a longitudinal study with repeated measurements of reported dietary intake and AIx at baseline and at 8 months follow-up. LPA was used to determine the major patterns of dietary fatty acid intake. LPA is a particular form of LCA which can be used to identify hidden groups from a large number of continuous (LPA) or categorical (LCA) observed variables [14]. The latent (hidden) variable that is formed when using LCA or LPA is categorical and clusters subjects (or other units) into a small number groups (i.e. classes or profiles). Our primary interest was to determine the strength of the relationship between these groups (defined by different patterns of dietary fatty acid intake) and the AIx. Each fatty acid was measured as a continuous variable in units of grams consumed per day (g/day). We also assessed the independent association between use of fish oil supplements and the AIx.

2.2 Patient recruitment and Ethical approval

We studied a consecutive series of patients with stable RA, aged ≥18 years, recruited from the outpatient clinics of the Rheumatology Department at Flinders Medical Centre and the Repatriation General Hospital, in the Southern Health Local Heath Network, Adelaide, Australia. RA was diagnosed according to the 1987 American College of Rheumatology or the 2010 American College of Rheumatology/European League Against Rheumatism criteria [18]. Exclusion criteria were atrial fibrillation, active cancer or current treatment with anti-cancer drugs, heart failure and cognitive impairment. The study (registered in the Australian New Zealand Clinical Trials Registry with the registration number ACTRN12616001366448)

was approved by the Southern Adelaide Clinical Human Research Ethics Committee (Ethics Approval Number: 76.14). Each participant gave written consent before entering the study in accordance with the Declaration of Helsinki.

2.3 Augmentation index

AIx (corrected at a heart rate of 75 beats per minute) was measured using Pulse Wave Analysis (PWA, SphygmoCor version 7.1, AtCor Medical, Sydney, Australia) [19]. Measurement of the AIx was performed at baseline and 8 months.

2.4 Dietary Fatty acids

Data on reported dietary intake was collected at baseline and 8 months. Nutrient intake from the diet including fatty acid intake was determined using the validated Dietary Questionnaire for Epidemiological Studies version 2 (DQES v2) [20; 21; 22]. The DQES v2 is a modification of a food frequency questionnaire (FFQ) that was developed by Cancer Council Victoria in the late 1980s to measure dietary intake of people taking part in the Melbourne Collaborative Cohort Study (MCCS). The DQES v2 comprises a food list of 74 items grouped into 4 food categories; 1. Cereal foods, sweets and snacks, 2. Dairy products, meats and fish, 3. Fruit, 4. Vegetables. Each item has 10 frequency response options ranging from 'Never' to '3 or more times per day' and a section covering intake of 6 types of alcoholic beverage with 10 frequency response options ranging from 'never' to 'every day'. The questionnaire also contains 4 questions that relate to portion size (with regards to potatoes, steak, vegetables, and meat or vegetable casserole). Responses to these questions are used to calculate a single portion size factor (PSF) indicating whether on average a person eats median size serves (PSF=1), more than the median (PSF > 1), or less than the median (PSF < 1). The PSF is used to scale the standard portion size for different foods up or down, from which energy intake and nutrient intakes can be calculated for each food frequency item. The questionnaire asks subjects to describe their intake of food over the preceding 12 months according to food type and quantity. The data collected by DQES v2 is used to calculate nutrient intakes. The calculation of the majority of the nutrients is based on Australian nutrient composition data from NUTTAB95. Calculation of the intakes of individual fatty acids are based on a data set obtained from Professor Neil Mann, RMIT University, and now used in the FoodWorks nutrient analysis software. The results from the software analysis provides results that include

information on total energy intake (kJ/day), saturated fatty acid intake (g/day), monounsaturated fatty acid intake (g/day), polyunsaturated fatty acid intake (g/day) and the intake of 30 different fatty acids (g/day) including the 16 major fatty acids included in the latent profile analysis described in detail below.

2.5 Clinical and demographic characteristics

The following data were collected from patient interviews, medical questionnaires, clinical notes, and hospital administrative databases: age, gender, medical and medication history, weight, height, body mass index (BMI), the 28-joint disease activity score (DAS28) [23], time spent doing physical activity, level of education, marital status, health insurance status and income. The DAS28 was measured using high sensitivity C-reactive protein measured at each time point and erythrocyte sedimentation rate, and swollen and tender joint count taken from patient notes at the same time-point. Clinic peripheral systolic (SBP) and diastolic (DBP) BP were measured in the morning, in a quiet environment at room temperature, using the clinically validated automatic BP monitor (model UA-767PC) (AND Medical, Sydney, Australia) according to current guidelines [24]. High sensitivity C-reactive protein (CRP) was measured in serum by latex-enhanced immunoturbidimetry on an automated Modular PPE Analyzer using instrument conditions and reagents supplied by the manufacturer (Roche Diagnostics) [25].

2.6 Statistical analysis

Descriptive statistics were used to describe the baseline characteristics of the subjects using either mean and SD for normally distributed continuous variables, median and inter-quartile range for non-normally distributed continuous variables and frequency (percentage) for categorical variables. Correlations between individual fatty acids were estimated using Pearson's r correlation coefficient. LPA was performed to identify the pattern of dietary fatty acid intake using the estimated dietary intakes (in grams/day) of 16 different major fatty acids (Table 2) obtained from the FFQs and listed as major fatty acids in a recent guide to dietary reference intakes [28]. The LPA was performed using the mean fatty acid intakes recorded across the 2 visits for each subject, rather than for each visit separately. There were several reasons for choosing this approach. First, the relatively high within-subject variability of fatty acid intake across the 2 visits (mean ICC=0.51 between baseline and 8 months) indicated a

relatively high degree of measurement error of the true average intake. Second, there were problems with convergence for some of the models when using separate visit data. Third, averaging the data for the 2 visits meant that each subject was classified with a single latent profile which assists interpretation of the results. In addition, since intake of fatty acids was based on g/day, we used energy adjusted intakes for analysis using the nutrient residual method [29]. Briefly, we performed a linear regression of each mean fatty acid on mean daily energy intake and used the resulting residuals as the new variables for the LPA. Models were estimated for between 2 and 6 latent profiles, and for each model the profile membership of each subject was decided based their highest (posterior) predicted probability of profile membership. Model fit was based on the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC). The selected model was based on both model fit (lowest AIC and BIC), and consideration of the number of subjects assigned to each profile (a minimum of 5 subjects) since 5% of subjects per profile is a good rule of thumb [30]. Line plots of the standardised energy adjusted fatty acid intake for the 16 FA's were used to identify the main fatty acids characterising each profile. Standardisation of the fatty acids i.e. calculating a z-score, was performed to ease interpretation since absolute fatty acid intakes vary considerably across the range of fatty acids. Mean differences in the 16 FAs across the profiles were compared using linear regression with additional adjustment for energy intake [29]. We compared the AIx across latent profiles using linear mixed effects univariate and multivariate regression with adjustment for age, gender, BMI, visit (baseline or 8 months), DAS28, use of ibuprofen, use of folic acid, use of methotrexate, energy intake, hours of physical activity per week, income, level of education, marital status, and private health insurance status. The subject was included in the model as a random intercept. We also compared SBP, DBP and $\log_{10}$-transformed CRP across latent profiles using the same approach. In each of these models we assessed the overall significance of the profile variable using a Wald test that compared models with and without the profile variable. Finally for comparison purposes, we assessed the association between AIx and each individual fatty acid using the same univariate and multivariate mixed effects regression with the same adjustment as above.

Analysis was performed using STATA (StataCorp, version 15.1, USA) and R software (for figures). The LPA was performed using the STATA "gsem" command for generalised structural equation modelling, for which latent class analysis was incorporated in version 15. A type 1 error rate of α=0.05 was considered statistically significant for all regression

analyses. Based on a Bonferroni correction, a type 1 error rate of p=0.001 was considered significant when comparing the 16 individual fatty acid intakes across profiles.

1. Results

1. Clinical and demographic characteristics and fatty acid intake

A total of 86 subjects were recruited and measures of diet and AIx were recorded at baseline and after 8 months. The baseline characteristics of the subjects are described in Table 1. The median (IQR) age of the subjects was 64 (56-69) and 63 (73.3%) were female. The reported mean fatty acid intake (g/day) and correlations between the fatty acids are described in Table 2. There was a high correlation between the saturated fatty acids, between the mono- and poly-unsaturated fats (palmitoleic acid (16:1), oleic acid (18:1) and linoleic acid (18:2)) and between the n-3 fatty acids.

2. Latent profile analysis

All 86 subjects completed dietary assessments at baseline and 79 subjects completed dietary assessment at 8 months. All LPA analyses with 2, 3, 4, 5 and 6 specified latent profiles converged successfully and the AIC and BIC statistics for these are described in Table 3. Based on the lowest BIC, the optimal number of classes was 5. The mean probability of accurately assigned profile membership ranged from 0.94 for profile 4 to 1.00 for profile 1 (Table 4) indicating a high degree of certainty that each individual was assigned to the correct dietary fatty acid pattern.

3. *Fatty acid intakes and food frequencies*

Figure 1 and Table 5 describe the standardised mean fatty acid intake according to each of the 5 dietary patterns. There were overall significant differences (p<0.001) across the 5 patterns for 15 of the 16 fatty acids (all except 18:2 n-6 Trans) (Table 5). In particular, profiles 2, 3, 4 and 5 were significantly lower than profile 1 for palmitoleic acid (16:1), eicosapentaenoic acid (20:5-n3), docosahexaenoic acid (22:6n-3) (p<0.001) and docosapentaenoic acid (22:5 n-3) (p<0.001 for each except; p=0.007 for 16:1 profile 3 vs profile 1) (Figure 1). Profile 1 had the highest reported intake for each of these fatty acids and profile 3 generally the lowest reported intake. Figure 2 displays each individual's fatty acid intake together with the mean

reported intake for the 5 profiles. The mean reported intake for fish, meats and the frequency distribution of the "usual fat spread used on bread" according to the 5 profiles are described in Table 6. There were no significant differences across profiles for fried fish (p=0.08). However, reported intake of grilled fish, tinned fish and total fish were significantly higher amongst subjects in profile 1 (p<0.001 for each). Profile 1 had the highest reported intake for each, whilst profile 3 had the lowest reported intake for each. There were no significant differences across the 5 profiles for each of beef, veal, chicken, lamb, pork, bacon, ham, beef salami, sausages or total meat (p>0.05 for each). There was a significant difference across the 5 profiles in the main choice of fat spread used on bread (p<0.001). The most common spread for profiles 1 and 5 was "Butter" (28.6% and 53.3% respectively) whilst the most common spread for profiles 2, 3 and 4 was "None", "None" and "Poly margarine" (36.0%, 42.1% and 34.9% respectively).

4. Multilevel regression for AIx75, CRP and clinic BP on latent profiles

A total of n=165 observations were included in the mixed effects linear regression analysis for AIx with 79 subjects providing data at both baseline and 8 months and a further 7 subjects providing data at baseline alone. There were 5 subjects that did not have AIx measured at either baseline or 8 months and were therefore excluded from the analysis for AIx.

In univariate analysis, there was an overall non-significant difference (p=0.600) across profiles in the mean AIx (Table 7A). However, after adjustment for age, gender, BMI, time (baseline or 8 months), DAS28, ibuprofen, folic acid, methotrexate, physical activity, income, education, health insurance status, marital status and fish oil, the mean AIx was significantly different across profiles overall (p=0.023). In particular, subjects in profile 1 (high in n-3's, 16:1 and 20:4) had lower AIx than subjects in profile 3 (β= -7.2%, 95% CI= -11.5 to -2.9; p=0.001) (Table 7A). In addition, fish oil supplementation was an independent predictor of lower AIx (β=-4.15, 95% CI= -6.73 to -1.56; p=0.002). In contrast, older age (p=0.028), female gender (<0.001), and higher BMI (0.021) were independent predictors of a higher AIx (Table 7A).

In both univariate and multivariate analysis, there was no overall significant difference in log-transformed CRP across the 4 profiles (p=0.99 and p=0.89 respectively) (Table 7B). However, higher DAS28 was independently associated with higher CRP (p<0.001) (Table 7B).

In multivariate analysis of clinic blood pressure, there was no overall difference between profiles for either SBP (p=0.197) or DBP (p=0.308).

5. Multilevel regression for AIx75 on individual fatty acids

Table 7C shows the results of the univariate and multivariate mixed effects regression for AIx and each of the 16 individual fatty acids. There were no significant associations for any of the fatty acids in either the univariate or multivariate analysis.

Discussion

In this study we used LPA to identify 5 underlying patterns of dietary fatty acid intake that were present within a population of patients with RA. Together with multivariate regression analysis, the LPA methodology allowed us to associate patterns of reported dietary fatty acid intake with the AIx measured using pulse wave analysis. Such associations were not present when fatty acids were assessed individually suggesting that the aggregated information obtained from an LPA approach was better able to recover relevant information on fatty acid intake. Subjects with diets classified as being within fatty-acid profile 1, characterised by a high level of n-3 fatty acids, palmitoleic acid (16:1) and arachidonic acid (20:4 n-6), had a significantly lower AIx than subjects within profiles 2 and 3, both of which had lower levels of n-3 fatty acids. Profile 2 also had lower levels of palmitic acid (16:0), palmitoleic acid (16:1) and oleic acid (18:1) than profile 1, and profile 5 had higher levels of all saturated fatty acids, and lower levels of palmitoleic acid (16:1) and linoleic acid (18:2 n-6). Profile 4 was the only group with higher levels of *trans* fatty acids. The adjusted difference in AIx between groups 1 and 3 was 7.18%, much larger than the mean 1.48% difference recently observed in a meta-analysis of RCT's for patients with RA using TNF-alpha antagonists [4]. When comparing food consumption across the 5 profiles, profile 1 subjects had a similar reported mean meat intake compared to other profiles, but had much higher levels of grilled and tinned fish consumption. There were also small but significant differences in their usual choice of spreads for bread, with profile 1 having a higher use of monounsaturated margarines (21% versus 4% overall), and moderate but non-significantly higher levels of chicken and egg consumption.

In addition to observing a lower AIx for subjects with overall higher fish intake, there was an independent and additive effect of n-3 supplementation with the AIx. This supports the findings of higher reported n-3 intake from the diet being associated with lower AIx, and also

suggests that a dose-response n-3 intake may exist. A reduction in arterial wave reflection following n-3 supplementation has been observed in smokers [9], but there was no change in AIx with 12 weeks of 4g/day of n-3 supplementation daily amongst both young and older healthy subjects [31]. Similarly, there was no association between serum n-3 fatty acids and arterial wave reflection in healthy Japanese men [32]. Together these studies and our study suggest that the benefits of n-3 supplementation in preventing increased arterial stiffening and wave reflection may be most apparent in individuals at greater CVD risk.

Inflammation is known to contribute to arterial stiffening both directly [3; 33], and indirectly, via aging, diabetes and CVD [34], and n-3 fatty acids can reduce inflammation [6; 9; 10]. However, we observed no association between CRP and the AIx. It is therefore possible that the differences we observed in AIx between the 5 groups was mediated directly via other CVD protective effects of fish oil [35] rather than indirectly via their effects on inflammation. In particular, fish oil is known to influence the production of nitric oxide, improving endothelial function and vasodilatation, as well as lowering triglycerides, remnant lipoproteins and platelets [36].

In addition to being high in n-3 fatty acids, profile 1 was also characterised by higher levels of palmitoleic acid (16:1) and arachidonic acid (20:4 n-6). Whilst fish intake contributes to both of these, particularly palmitoleic acid (16:1), the major contributors to arachidonic acid (20:4 n-6) in the diet in the general population are chicken (26.9%), eggs (17.8%), beef (7.3%), sausage and bacon (6.7%) and fish (5.8%) [37]. There were no significant differences between profiles in regards to the mean reported intake of chicken, eggs, beef and sausage, although all were slightly higher in profile 1 compared to other profiles, and in particular chicken and eggs. We can conclude therefore that in addition to fish, a higher consumption of eggs and chicken would have partly contributed towards a higher level of arachidonic acid (20:4 n-6) for profile 1. Arachidonic acid (20:4 n-6) is an essential fatty acid and precursor for the production of inflammatory prostaglandins, and together with the N-3 fatty acids, helps modulate inflammation [5]. There is still debate as to whether or not a higher intake of n-6 fatty acids in general is beneficial or detrimental in regards to reducing risk for CVD [38]. Whilst the American Heart Association has previously given advice to consume at least 5-10% of energy as n-6 polyunsaturated fatty acids in order to reduce heart disease [39], much of the evidence for this advice was based on intervention trials that increased the intake of linoleic acid, the predominant n-6 fatty acid in the Western diet. However, many of the interventions for these trials also increased consumption of n-3 fatty acids (both alpha-

linolenic acid and/or marine n-3 fatty acids, and also restricted the intake of trans-fatty acids) [38]. In addition, too little attention was paid to the individual fatty acids within the n-6 intake, assuming instead that their effects could be considered equivalent. A call was therefore made for greater focus on specific fatty acids and absolute amounts/concentrations rather than on fatty acid classes and ratios [38]. Given that our study demonstrated reduced arterial stiffness amongst a group of individuals with a significantly higher reported intake of arachidonic acid (20:4 n-6), it provides at least some reassurance as to the safety of such a diet, perhaps with the caution that it should be followed in conjunction with a high marine n-3 fatty acid intake. Strengths of this study included the use of LPA which allowed us to identify the main patterns of reported dietary fatty acid intake, and the use of these as a single dietary exposure variable rather than many individual fatty acids either alone or together. When we assessed the individual fatty acids alone, we found no associations with the AIx. Grouping subjects according to their dietary fatty acid patterns also allowed us to explore which foods were consumed differently across the 5 groups and thereby which were most likely to be responsible for the separate fatty acid patterns. Identifying the specific food groups that contribute to the dietary patterns is also important to help provide practical dietary recommendations. Our dataset was also well described and this allowed us to adjust our analysis for important confounders including age, gender, BMI, various medications, socioeconomic status, and physical activity that each might have been associated with diet quality as well as underlying cardiovascular disease. Except for age, gender and BMI, these variables were not significantly associated with the AIx and did not therefore substantively alter our conclusions. Finally, we adjusted for energy intake to ensure that differences in absolute fatty acid intake (g/day) between the profiles was not simply a result of differences in total food consumption.

Limitations to our study include its observational nature and cross sectional design which therefore precludes making causal inferences. It is possible for example, that subjects with higher arterial wave reflection may have known they were at a higher risk of CVD and may have altered their diet to one that included more fish and/or n-3 supplements. However the possibility of reverse causality is low given that much of the population in general, including those with increased CVD risk, consume less fish than recommended [40], despite its known health benefits. The prevalence of high fish consumption in our study population was also similar to those aged ≥ 65 years in the Australian general population [40]. There is also the possibility that our results were subject to residual confounding by factors that may influence both diet and arterial wave reflection. A healthier diet, and in particular one that is high in n-3

fatty acids, is often a marker of a healthier lifestyle in general, including higher levels of exercise and lower bodyweight. We therefore adjusted for a wide number of possible confounders including exercise, BMI, gender, and several well-established measures of socioeconomic status including level of education, income and marital status. However, our exercise measure only captured the time spent doing physical activity and not intensity. As such, it may not have completely removed the influence of each individual's physical activity on arterial stiffness. In addition, a diet high in n-3 fatty acids might also be higher in other nutrients that themselves can influence arterial function and stiffness including flavonoids [41] and Vitamin D [42] respectively, with the n-3 fatty acid profile perhaps therefore acting partly as a marker for these nutrients. A further limitation was the reliance on self-reported food intake for the estimation of fatty acid intake rather than the use of measured food records or tissue data (plasma or rood blood cells). The latter would provide a more accurate assessment of fatty acid intake from the diet in the short and medium term respectively, and would also not be subject to memory bias. However, the collection of dietary data at repeated time-points rather than a single time-point has previously been shown to increase explanatory power [43] indicating a reduction in measurement error. In addition, the use of food frequency questionnaires has been shown to give reasonably valid measures of fatty acid intake [44]. Our food frequency questionnaire did not query the type or quantity of oils or creams used in cooking information, which might potentially have had a large impact on fatty acid intake and accurate profile classification. Finally, whilst our results were more informative than those obtained from univariate and multivariate regression for each fatty acid, we did not compare our findings with other clustering techniques such as k-means clustering. Such approaches may also be useful in identifying similar dietary behaviours, albeit without objective guidance from model based statistics including the AIC and BIC.

In conclusion, the use of latent profile analysis successfully identified 5 main patterns of dietary fatty acid intake amongst subjects with RA using the nutrient data obtained from repeat dietary food intake questionnaires. Subjects with a dietary fatty acid profile characterised by a high reported intake of N-3's, palmitoleic acid (16:1) and arachidonic acid (20:4 n-6) and reflecting a significantly higher reported fish intake, had significantly lower AIx than subjects with the lowest reported intake of N-3 fatty acids. This was independent of a similar association with fish-oil. Together these findings suggest that n-3 fatty acids are the predominant fatty acids associated with reduced arterial wave reflection. By adhering to a diet high in fish with the possible addition of n-3 supplements, RA patients might help slow the

356 progression of arterial aging and the consequent increase in CVD risk. However, this
357 hypothesis needs to be further investigated in interventional studies.

358

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Conflicts of Interest

None

Authorship

RW formulated the research question, assisted in study design, analysed the data, performed the first draft of the manuscript and finalised the manuscript. **LB** helped formulate the research question, carried out the study, analysed the food frequency questionnaires, and read and approved the manuscript. **ES** formulated the overall study, assisted in study design, supervised the collection of data, was in charge of patient recruitment, read and revised the manuscript. **IDS** assisted with analysis of the data, assisted with the literature review, read and revised the manuscript. **JH** assisted with the research questions, study design, drafting of the manuscript and final manuscript review. **AM** was responsible for the overall study design, project supervision, responsible for data collection for the augmentation index, assisted with drafting of the manuscript and final manuscript approval.

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Table 1: Baseline clinical characteristics of the study population (n=86)

	All subjects (n=86)	Profile 1 (n=7)	Profile 2 (n=14)	Profile 3 (n=19)	Profile 4 (n=23)	Profile 5 (n=23)	p-value[1]
Age (years), median(IQR)	64 (56-69)	61 (52-68)	69 (64-73)	64 (58-69)	61 (51-68)	64 (56-75)	0.52
Male/Female, n (%)	23/63 (27/73)	1/6 (14/86)	5/9 (36/64)	5/14 (26/74)	6/17 (26/74)	6/17 (26/74)	0.92
BMI (kg/m^2), median(IQR)	26.6 (23.1-31.1)	27.7 (23.7-39.5)	23.4 (21.5-27.2)	28.8 (23.9-32.0)	26.0 (23.1-31.1)	27.0 (24.9-30.5)	0.30
DAS28, mean±SD	3.1±1.2	3.0±1.3	3.1±1.1	3.4±1.5	2.8±1.4	3.0±0.9	0.62
C-reactive protein (mg/L), median(IQR)	2.1 (0.8-4.9)	3.7 (0.2-12.0)	1.8 (1.5-6.9)	2.0 (0.6-4.8)	2.1 (1.0-4.7)	2.5 (0.8-4.2)	0.72
Clinic SBP (mmHg), median(IQR)	125 (115-134)	115 (108-125)	122 (112-143)	129 (120-138)	127 (115-134)	125 (114-137)	0.32
Clinic DBP (mmHg), mean±SD	75±11	72±5	71±17	78±9	74±11	77±10	0.38
AIx75 (%), mean±SD	28.7±6.7	24.3±9.4	29.2±7.6	29.1±7.0	29.7±5.2	28.5±6.5	0.47
Methotrexate use, n (%)	56 (65.1)	4 (57.1)	11 (78.6)	10 (52.6)	16 (69.6)	15 (65.2)	0.59
Prednisolone use, n (%)	30 (34.9)	2 (28.6)	6 (42.9)	7 (36.8)	9 (39.1)	6 (26.1)	0.85
Ibuprofen use, n (%)	7 (8.1)	0 (0.0)	1 (7.1)	2 (10.5)	2 (8.7)	2 (8.7)	1.00
Folic acid use, n (%)	46 (53.5)	4 (57.1)	10 (71.4)	8 (42.1)	13 (56.5)	11 (47.8)	0.54
Fish Oil use, n (%)	26 (30.2)	3 (42.9)	5 (35.7)	7 (36.8)	7 (30.4)	4 (17.4)	0.54
Level of education, n (%)							
Primary school	2 (2.3)	0 (0.0)	0 (0.0)	1 (5.3)	12 (52.2)	16 (69.6)	0.34
Secondary school	53 (61.6)	5 (71.4)	11 (78.6)	7 (36.8)	5 (21.7)	3 (13.0)	
Bachelor degree	14 (16.3)	2 (28.6)	3 (21.4)	5 (26.3)	3 (13.0)	1 (4.35)	
Above bachelor degree	5 (5.8)	0 (0.0)	0 (0.0)	2 (10.5)	2 (8.7)	1 (4.35)	
Vocational education	10 (11.6)	0 (0.0)	0 (0.0)	4 (21.0)	1 (4.3)	1 (4.35)	
None	2 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	1 (4.35)	
Income, n (%)							
AUD$0-AUD$24,999	44 (51.2)	3 (42.9)	8 (57.1)	5 (26.3)	12 (52.2)	16 (69.6)	0.31

AUD$25,000-AUD$49,999	20 (23.3)	2 (28.6)	2 (14.3)	8 (42.1)	5 (21.7)	3 (13.0)	
AUD$50,000-AUD$74,999	13 (15.1)	1 (14.3)	3 (21.4)	5 (26.3)	3 (13.0)	1 (4.35)	
AUD$75,000-AUD$99,999	5 (5.8)	1 (14.3)	1 (7.1)	0 (0.0)	2 (8.7)	1 (4.35)	
AUD$100,000-AUD$124,999	3 (3.5)	0 (0.0)	0 (0.0)	1 (5.3)	1 (4.35)	1 (4.35)	
AUD$150,000-AUD$174,999	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.35)	
Physical activity (min/week), median (IQR)	180 (60-360)	180 (60-360)	180 (60-420)	180 (60-480)	180 (90-360)	240 (30-360)	0.48
Private Health Insurance, Yes (%)	49 (57.0)	5 (71.4)	9 (64.3)	11 (57.9)	13 (56.5)	11 (47.8)	0.81
Marital status, n (%)							
Married	55 (64.0)	3 (42.9)	8 (57.1)	13 (68.4)	15 (65.2)	16 (69.6)	0.15
Divorced/Separated	11 (12.8)	1 (14.3)	1 (7.1)	4 (21.0)	5 (21.7)	0 (0.0)	
Widowed	12 (14.0)	2 (28.6)	3 (21.4)	1 (5.3)	3 (13.0)	3 (13.0)	
Never married	1 (14.3)	1 (14.3)	2 (14.3)	1 (5.3)	0 (0.0)	4 (17.8)	
Energy intake (kJ/day), median(IQR)	5,596 (4,079-7,973)	6,651 (5,192-9,610)	6,162 (4,752-8,229)	5,034 (4,214-6,529)	5,079 (4,050-6,486)	5,900 (4,899-8,228)	0.39
Total fat (g/day), median(IQR)	53.1 (37.5-79.6)	93.8 (59.8-115.5)	43.9 (24.0-79.6)	44.0 (29.3-62.0)	53.3 (32.7-81.4)	67.0 (42.9-85.9)	0.08
Monounsaturated fats (g/day), median(IQR)	18.0 (12.5-29.1)	36.9 (18.2-46.2)	14.8 (8.5-28.1)	16.0 (11.3-23.0)	18.9 (12.1-29.1)	23.3 (14.5-28.1)	0.006
Saturated fats (g/day), median(IQR)	23.0 (14.0-33.7)	28.5 (27.2-38.2)	16.1 (9.7-27.9)	15.8 (11.7-24.1)	21.7 (11.8-30.2)	30.6 (19.7-40.3)	0.246
Polyunsaturated fats (g/day), median(IQR)	7.7 (5.0-11.7)	15.0 (5.5-22.5)	7.6 (3.4-11.9)	6.5 (4.5-8.6)	8.3 (7.0-13.6)	6.9 (4.4-9.9)	0.025

[1]P-value for difference between profiles using ANOVA (normal distributions), test of medians (asymmetric distributions) or Fishers Exact (categorical).

Table 2A: Mean dietary fatty acid intake and Pearson's r correlations amongst the 16 dietary fatty acids (n=86).

	Dietary Fatty acid	Mean±SD (g/day)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	8:0 Caprylic acid	0.30±0.18	1.00														
2	10:0 Caproic acid	0.50±0.31	**0.97**	1.00													
3	12:0 Lauric acid	1.32±0.81	**0.97**	**0.89**	1.00												
4	14:0 Myristic acid	2.77±1.57	**0.91**	**0.96**	**0.86**	1.00											
5	16:0 Palmitic acid	13.75±6.60	**0.88**	**0.88**	**0.86**	**0.94**	1.00										
6	18:0 Stearic acid	7.09±3.87	**0.90**	**0.88**	**0.87**	**0.92**	**0.98**	1.00									
7	16:1 Palmitoleic acid	1.50±0.71	**0.61**	**0.63**	**0.57**	**0.74**	**0.88**	**0.82**	1.00								
8	18:1 Oleic acid	21.01±9.64	**0.79**	**0.78**	**0.78**	**0.84**	**0.97**	**0.94**	**0.90**	1.00							
9	18:2 Linoleic acid	7.22±3.39	**0.50**	**0.45**	**0.52**	**0.46**	**0.64**	**0.58**	**0.58**	**0.76**	1.00						
10	18:1; trans	0.45±0.38	**0.59**	**0.61**	**0.61**	**0.68**	**0.61**	**0.59**	**0.34**	**0.54**	**0.50**	1.00					
11	18:2; trans	0.006±0.010	0.12	0.10	0.12	0.04	0.09	0.05	0.01	0.10	0.23	0.36	1.00				
12	20:4 n-6 (AA)	0.065±0.045	0.21	**0.23**	0.18	**0.35**	**0.43**	**0.35**	**0.64**	**0.49**	**0.34**	0.02	-0.08	1.00			
13	18:3 n-3 (LA)	0.79±0.38	0.73	**0.70**	**0.75**	**0.74**	**0.87**	**0.84**	**0.76**	**0.91**	**0.82**	**0.64**	**0.22**	**0.37**	1.00		
14	20:5 n-3 (EPA)	0.139±0.169	-0.02	-0.02	-0.01	0.08	0.14	0.05	**0.38**	0.20	0.21	-0.12	-0.10	**0.85**	0.17	1.00	
15	22:6 n-3 (DHA)	0.287±0.332	-0.01	-0.02	-0.01	0.09	0.15	0.06	**0.40**	**0.22**	**0.22**	-0.12	-0.10	**0.86**	**1.00**	**0.99**	1.00
16	22:5 n-3 (DPA)	0.048±0.050	0.04	0.04	0.03	0.14	0.21	0.13	**0.46**	**0.28**	**0.25**	-0.09	-0.11	**0.90**	**0.22**	**0.98**	**0.99**

Bold indicates correlations which are statistically significant ($p<0.05$). AA=Arachidonic acid, LA=alpha-linolenic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, DPA=docosapentaenoic acid.

Table 3: Model fit statistics and profile membership distribution

			Number of subjects in each profile					
Model	**AIC**	**BIC**	**Profile 1**	**Profile 2**	**Profile 3**	**Profile 4**	**Profile 5**	**Profile 6**
1-profile	674.0	752.5	86					
2-profile	442.3	562.6	47	39				
3-profile	173.5	338.0	7	47	32			
4-profile	121.0	329.6	7	32	24	23		
5-profile	44.8	292.7	7	14	19	23	23	
6-profile	57.7	335.0	6	15	19	22	21	3

Table 4: Mean posterior probabilities associated with profile membership in the 5-profile LCA model

		Probability of membership in each profile				
Assigned fatty acid profile	**n**	**Profile-1**	**Profile-2**	**Profile-3**	**Profile-4**	**Profile-5**
Profile-1	7	**1.00**	0.00	0.00	0.00	0.00
Profile-2	14	0.00	**0.99**	0.01	0.00	0.00
Profile-3	19	0.00	0.03	**0.95**	0.02	0.00
Profile-4	23	0.00	0.01	0.02	**0.94**	0.03
Profile-5	23	0.00	0.00	0.00	0.02	**0.98**

Table 5: Energy adjusted mean standardised fatty acid intakes according to latent profile

			Profile 1 (n=7)	Profile 2 (n=14)	Profile 3 (n=19)	Profile 4 (n=23)	Profile 5 (n=23)	
		n	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p-value[1]
1	8:0 Caprylic acid	86	-1.11±0.86	-0.54±0.55	-0.62±0.52	**0.01±0.49**	**1.17±0.82**	<0.001
2	10:0 Caproic acid	86	-1.05±1.14	-0.37±0.64	-0.68±0.59	**-0.03±0.53**	**1.14±0.72**	<0.001
3	12:0 Lauric acid	86	-1.11±0.95	-0.64±0.41	-0.55±0.50	**0.12±0.61**	**1.06±0.91**	<0.001
4	14:0 Myristic acid	86	-0.62±1.45	-0.69±0.80	-0.58±0.51	-0.01±0.60	**1.10±0.60**	<0.001
5	16:0 Palmitic acid	86	-0.20±1.35	**-1.37±0.63**	-0.34±0.46	0.23±0.49	**0.94±0.65**	<0.001
6	18:0 Stearic acid	86	-0.65±1.32	-1.15±0.69	-0.39±0.47	0.29±0.55	**0.93±0.71**	<0.001
7	18:1 Trans	86	-0.77±1.25	0.07±0.78	-0.69±0.45	**0.52±0.86**	0.24±1.10	0.001
8	18:2 n-6 Trans	86	-0.38±0.78	0.18±1.29	-0.18±0.59	0.28±1.19	-0.13±0.91	0.38
9	16:1 Palmitoleic acid	86	1.44±1.22	**-1.05±0.65**	0.48±0.69	**-0.20±0.63**	**0.01±0.90**	<0.001
10	18:1 Oleic acid	86	0.69±1.74	**-1.47±0.61**	-0.05±0.62	0.45±0.53	0.27±0.65	<0.001
11	18:2 Linoleic acid	86	0.66±1.75	-0.12±1.20	-0.25±0.51	0.68±0.76	**-0.61±0.55**	<0.001
12	18:3 n-3 (LA)	86	0.37±1.04	-0.68±0.69	-0.58±0.30	0.86±1.22	-0.08±0.58	<0.001
13	20:4 n-6 (AA)	86	2.53±1.20	**-0.66±0.43**	**0.44±0.37**	**-0.42±0.56**	**-0.31±0.41**	<0.001
14	20:5 n-3 (EPA)	86	2.62±1.61	**-0.43±0.39**	**0.23±0.50**	**-0.39±0.32**	**-0.34±0.36**	<0.001
15	22:6 n-3 (DHA)	86	2.63±1.60	**-0.44±0.39**	**0.25±0.49**	**-0.39±0.32**	**-0.35±0.34**	<0.001
16	22:5 n-3 (DPA)	86	2.60±1.68	**-0.43±0.43**	**0.25±0.37**	**-0.39±0.35**	**-0.34±0.39**	<0.001

Values in bold indicate statistical significance (p=0.001) compared to Profile 1. [1]P-value across the 5 profiles. AA=Arachidonic acid, LA=alpha-linolenic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, DPA=docosapentaenoic acid.

Table 6. Selected food frequencies across the 2 visits by latent profile, n=86 subjects and n=165 records

	Scale	Profile 1 (Mean±SD)	Profile 2 (Mean±SD)	Profile 3 (Mean±SD)	Profile 4 (Mean±SD)	Profile 5 (Mean±SD)	p-value[1]
Fish fried	1 to 10	2.92±2.02	1.76±0.97	2.03±1.40	1.93±1.08	2.31±1.24	0.17
Fish tinned	1 to 10	7.00±1.84	**3.00±1.71**	**4.34±1.81**	**2.74±1.35**	**3.18±1.20**	<0.001
Fish grilled	1 to 10	5.71±2.40	**3.36±1.78**	4.21±1.12	**3.12±1.48**	**3.40±1.27**	<0.001
Total fish	3 to 30	15.64±4.24	**8.12±3.22**	**10.58±2.72**	**7.79±2.33**	**8.89±2.40**	<0.001
Beef	1 to 10	4.86±2.03	3.36±1.32	3.71±1.45	3.88±1.73	3.82±1.59	0.34
Veal	1 to 10	1.86±2.18	1.88±1.09	1.66±1.26	1.84±1.23	2.42±1.39	0.43
Chicken	1 to 10	4.93±2.16	4.20±1.63	5.00±1.43	4.23±1.72	4.60±1.14	0.29
Lamb	1 to 10	3.36±2.02	2.68±1.28	3.32±1.86	3.40±1.55	3.76±1.49	0.31
Pork	1 to 10	2.79±2.15	2.32±1.80	3.00±1.77	2.16±1.34	2.84±1.33	0.44
Bacon	1 to 10	3.64±2.13	2.28±1.31	2.76±1.44	2.33±1.13	3.16±1.51	0.08
Ham	1 to 10	3.29±2.05	2.60±1.44	2.95±1.52	3.12±1.52	3.96±1.48	0.06
Beef Salami	1 to 10	2.71±2.05	2.08±1.22	2.47±1.87	1.84±1.21	3.00±1.80	0.10
Sausages	1 to 10	2.64±2.24	1.80±0.96	2.08±1.19	2.58±1.65	2.64±1.19	0.27
Total meat	9 to 90	30.1±15.9	23.2±8.1	26.95±7.5	25.4±8.6	30.2±6.7	0.14
Eggs per week	1 to 5	3.57±1.34	2.80±0.0.58	3.47±0.86	2.72±1.10	2.93±0.89	0.005
Nuts	1 to 10	7.00±2.25	3.72±2.42	4.84±2.54	4.58±2.54	**3.11±2.07**	<0.001
Peanut Butter	1 to 10	2.21±1.58	2.24±1.96	2.18±1.77	2.19±1.72	1.76±1.30	0.826
Usual spread on							

bread (choice of one)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**p-value[2]**
None	2 (14.3)	9 (36.0)	16 (42.1)	6 (13.9)	2 (4.4)	<0.001
Margarine	2 (14.3)	5 (20.0)	2 (5.3)	10 (23.3)	3 (6.7)	0.064
Poly Margarine	3 (21.4)	5 (20.0)	9 (23.7)	15 (34.9)	9 (20.0)	0.550
Mono Marg	3 (21.4)	0 (0.0)	0 (0.0)	3 (7.0)	1 (2.2)	0.011
Marg blends	0 (0.0)	0 (0.0)	6 (15.8)	3 (7.0)	6 (13.3)	0.136
Butter	4 (28.6)	6 (24.0)	5 (13.2)	6 (13.9)	24 (53.3)	<0.001
All categories	14 (100.0)	25 (100.0)	38 (100.0)	43 (100.0)	45 (100.0)	

[1]Using mixed effects regression with adjustment for energy intake. [2]Using Fishers Exact. Values in bold indicate statistical significance (p=0.001) compared to Profile 1.

Table 7A: Univariate and multivariate mixed effects regression analysis for the Augmentation index (n=81 subjects, 140 records).

	Univariate analysis		**Multivariate analysis**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value[1]**
Latent profile		0.60		**0.023[2]**
Profile 1	-4.25 (-9.9, 1.4)	0.14	**-7.18 (-11.5, -2.9)**	**0.001**
Profile 2	-2.18 (-6.78, 2.43)	0.35	-1.32 (-5.38, 2.75)	0.526
Profile 3	Reference	-	Reference	-
Profile 4	-0.49 (-4.49, 3.52)	0.81	-2.19 (-5.41, 1.02)	0.182
Profile 5	-1.15 (-5.24, 2.94)	0.58	-2.62 (-6.10, 0.86)	0.139
Age (years)	**0.117 (0.010, 0.225)**	**0.032**	**0.118 (0.012, 0.224)**	**0.028**
Gender (Female vs Male)	**6.50 (3.48, 9.51)**	**<0.001**	**9.19 (6.13, -12.25)**	**<0.001**
Body mass index (kg/m^2)	0.221 (-0.009, 0.450)	0.021	**0.238 (0.036, 0.440)**	**0.021**
Health Insurance (Yes)	1.015 (-1.35, 3.38)	0.40	**2.89 (0.64, 5.13)**	**0.012**
Fish oil supplementation	-2.254 (-4.983, 0.475)	0.106	**-4.15 (-6.73, -1.56)**	**0.002**

DAS28; Disease activity score for 28 joints. [1]Using mixed effects linear regression that included the listed variables and also use of prednisolone, use of ibuprofen, use of folic acid, use of methotrexate, visit (baseline versus 8 months), energy intake, hours of physical activity, marital status, income and education. [2]Overall association for latent profile.

Table 7B: Univariate and multivariate mixed effects regression analysis for log_{10}-transformed CRP (n=86 subjects, 155 records).

	Univariate analysis		**Multivariate analysis**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value[1]**
Latent profile				0.89[2]
Profile 1	0.10 (-0.39, 0.59)	0.69	0.12 (-0.30, 0.54)	0.57
Profile 2	0.11 (-0.29, 0.51)	0.60	-0.04 (-0.43, 0.34)	0.82
Profile 3	Reference	-	Reference	-
Profile 4	0.06 (-0.29, 0.41)	0.73	0.10 (-0.20, 0.40)	0.53
Profile 5	0.07 (-0.28, 0.42)	0.71	0.02 (-0.31, 0.35)	0.90
Age (years)	0.004 (-0.005, 0.014)	0.354	-0.003 (-0.014, 0.007)	0.51
Gender (Female vs Male)	-0.26 (-0.54, 0.01)	0.057	-0.24 (-0.53, -0.03)	0.08
DAS28	**0.17 (0.09, 0.25)**	**<0.001**	**0.22 (0.12, 0.31)**	**<0.001**
Marital status				
Married	Reference	-	Reference	-
Divorced/Separated	-0.16 (-0.51, 0.18)	0.35	-0.30 (-0.61, 0.01)	0.06
Widowed	0.24 (-0.09, 0.58)	0.16	0.31 (-0.04, 0.67)	0.08
Never Married	-0.17 (-0.57, 0.24)	0.42	-0.21 (-0.59, 0.17)	0.28

DAS28; Disease activity score for 28 joints. [1]Using mixed effects linear regression that included the listed variables and also BMI, use of prednisolone, use of ibuprofen, use of folic acid, use of methotrexate, visit (baseline versus 8 months), use of fish oil, energy intake, hours of physical activity, health insurance status, income, and level of education. [2]Overall association for latent profile.

Table 7C: Univariate and multivariate mixed effects regression analysis for the Augmentation index and individual fatty acids (n=81 subjects, 140 records).

	Univariate analysis		**Multivariate analysis**	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value[1]**
8:0 Caprylic acid	-4.95 (-12.8, 2.9)	0.216	-3.61 (-15.5, 8.3)	0.552
10:0 Caproic acid	-2.86 (-7.45, 1.74)	0.223	-1.84 (-5.89, 5.21)	0.609
12:0 Lauric acid	-1.01 (-2.78, 0.75)	0.259	-0.80 (-3.22, 1.61)	0.515
14:0 Myristic acid	-0.59 (-1.50, 0.31)	0.199	-0.45, (-2.01, 1.10)	0.567
16:0 Palmitic acid	-0.12 (-0.34, 0.09)	0.257	0.03 (-0.51, 0.57)	0.911
18:0 Stearic acid	-0.19 (-0.56, 0.18)	0.314	0.08 (-0.61, 0.77)	0.820
16:1 Palmitoleic acid	-0.98 (-2.99, 1.03)	0.338	1.56 (-1.81, 4.94)	0.364
18:1 Oleic acid	-0.08 (-0.23, 0.06)	0.257	-0.01 (-0.35, 0.34)	0.967
18:2 Linoleic acid	-0.25 (-0.67, 0.17)	0.237	-0.28 (-0.83, 0.26)	0.307
18:1; trans	-2.09 (-5.87, 1.69)	0.278	-4.00 (-8.14, 0.14)	0.059
18:2; trans	23.4 (-121, 168)	0.751	38.9 (-85.6, 162)	0.537
20:4 n-6 (AA)	-20.2 (-50.9, 10.5)	0.197	-23.4 (-53.20, 6.39)	0.124
18:3 n-3 (LA)	-2.16 (-5.92, 1.61)	0.261	-4.43 (-11.2, 2.30)	0.197
20:5 n-3 (EPA)	-5.15 (-13.4, 3.07)	0.219	-6.09 (-12.9, 0.76)	0.082
22:6 n-3 (DHA)	-2.64 (-6.81, 1.52)	0.214	-3.18 (-6.67, 0.31)	0.074

[1]With adjustment for age, gender, DAS28, marital status, BMI, use of prednisolone, use of ibuprofen, use of folic acid, use of methotrexate, visit (baseline versus 8 months), use of fish oil, energy intake, hours of physical activity, health insurance status, income, and level of education.

Figure 1 title: Standardised energy-adjusted[1] mean fatty acid intake by latent profile membership (n=86).

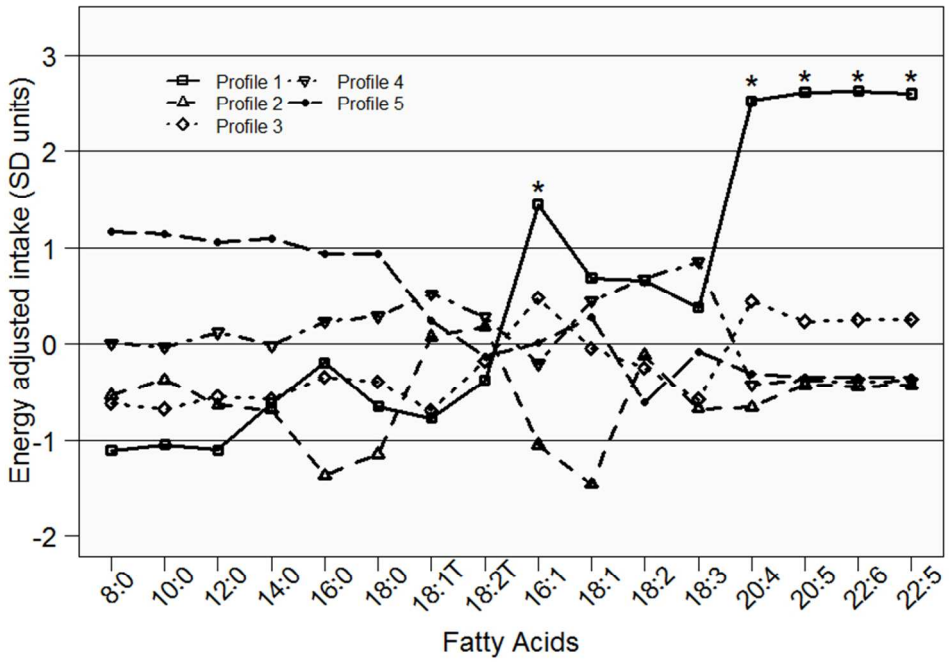
Figure 1 legend

[1]Using separate multivariate linear regression models for each fatty acid regressed on energy intake. * Indicates significantly different to profiles 2, 3, 4 and 5 ($p<0.001$ for each except $p=0.007$ for 16:1 profile 3 vs profile 1).

Figure 2 title: Fatty-acid intakes for each individual and mean intake for each profile (n=86).

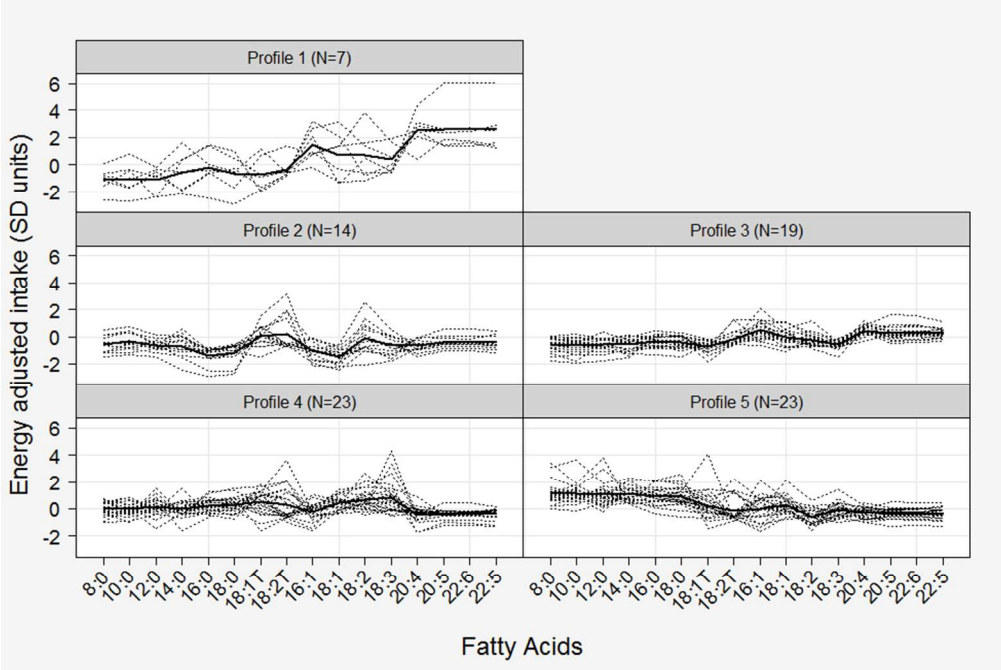
Figure 2 legend

Dotted lines indicate individuals and thicker continuous line indicates mean intake for the profile.



1Using separate multivariate linear regression models for each fatty acid regressed on energy intake. * Indicates significantly different to profiles 2, 3, 4 and 5 ($p<0.001$ for each except $p=0.007$ for 16:1 profile 3 vs profile 1).

229x173mm (96 x 96 DPI)



Dotted lines indicate individuals and thicker continuous line indicates mean intake for the profile.

257x173mm (96 x 96 DPI)