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Myocardial infarction does not affect fatty acid profiles in rats

Gregory C. Shearer¹, Jinghai Chen¹, Yuefeng Chen¹, and William S. Harris¹

¹Cardiovascular Health Research Center, Sanford Research/USD, South Dakota USA

Abstract

Plasma and red blood cell fatty acid (RBC FA) composition have both been proposed as biomarkers for cardiovascular (CV) risk. Since case/control studies using samples obtained after a CV constitute a source of supporting evidence, demonstrating that FA profiles are not affected by a myocardial infarction (MI) would improve our understanding of the usefulness such studies. The primary goal of the present study was to determine the impact of an MI on RBC and whole plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels and to do so with sufficient power to conclude that there was no effect. FA profiles were obtained from rats 24 hours after an MI or a sham-MI and compared to control animals by tests for differences and equivalence. In RBCs, neither DHA nor EPA were changed and were statistically equivalent in control and MI rats, as were a majority of other FAs and FA composite indices; only shingolipid-associated fatty acids had abundances that were changed in either MI or sham-MI animals. In whole plasma 8 of 22 FAs were changed in MI or MI and sham-MI rats, including EPA which was reduced from 2.53 (2.3, 2.8)% to 1.71 (1.4, 2)%; mean (95% CI). In conclusion, the levels of EPA, DHA, and most other FAs in RBCs are unaffected by an MI or by sham surgery, whereas the same cannot be said of plasma. This finding suggests that differences between cases and controls have prognostic implications.

Keywords

Fatty acids; myocardial infarction; eicosapentaenoic acid; docosahexaenoic acid; heart; red blood cell; biomarkers

INTRODUCTION

Plasma and red blood cell (RBC) fatty acid (FA) composition have both been proposed as biomarkers for cardiovascular disease (CVD) risk [1,2]. Not only individual FAs but also composite indices (e.g., the omega-3 index, n-6/n-3 ratios, etc.) may predict CVD events and could therefore theoretically serve as risk markers. One approach to risk marker validation is to conduct a case-control study in which the FA composition of samples taken from patients with disease is compared to that of individuals free of the disease. Previous work from our group has shown that RBC levels of n-3 FAs can distinguish healthy controls from cases admitted to the hospital with an acute coronary syndrome [3]. A major weakness of these types of case-control studies is that the marker of interest (here, RBC FA composition) is always measured after the event has occurred, and thus a physiological response to the event itself

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Corresponding Author: Gregory Shearer, Cardiovascular Health Research Center, Sanford Research/USD, 1100 E 21st St., Sioux Falls, SD 57105, Tel: 605.328.1362, Fax: 605.328.1301, greg.shearer@usd.edu.

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could have altered the marker, making it potentially useful diagnostically, but not prognostically. In other words, measuring the marker could help to diagnose a condition in someone who had already experienced it, but it would not necessarily identify individuals at increased risk for the disease. If it could be shown that the FA profile measured after the event was not affected by the event, then a FA profile measurement could be far more informative, identifying high-risk subjects and allowing preventive measures to be instituted to reduce future risk. The purpose of this study was to determine the extent to which an experimental myocardial infarction (MI) affected the RBC FA composition in a rat model. If it did not, then RBC FA measurements would be more likely to have prognostic value for MI.

An MI was previously reported to not affect RBC membrane omega-3 FA content [4], and to produce only minor changes in platelet membrane [5] and free fatty acid composition [6]. However, these studies were designed to detect *changes* in FA profiles, not to establish *no-effect*, hence the conclusion that an MI did not alter FA composition could simply be a problem of power, not of effect. Since none of these studies reported the power to detect a change, the extent to which one can be confident of a no-effect conclusion cannot be determined. The primary goal of the present study was to determine the impact of an MI on RBC and whole plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels with sufficient power to conclude that there was, indeed, no effect. Two secondary goals were also considered: 1) the impact of an MI on other FA composite and multivariate indexes, and 2) the impact on the FA composition of myocardial tissue.

MATERIALS AND METHODS

Animals & Study Design

Female Sprague Dawley rats (n=31) were obtained from Harlan-Olac at 11 weeks of age. They were kept in 12 hour light/dark rooms and fed a diet that our pilot studies indicated would achieve an omega-3 index (*i.e.*, RBC EPA+DHA) that would be the equivalent to that seen in humans. The diet (Dyets; Bethlehem, PA) contained 6g of menhaden oil and 34g of corn oil per kg of food, and a total of 10% energy from fat (1.4% from menhaden oil). Animals were fed *ad libitum* for 4 weeks, and then were randomized to 1 of 3 treatment groups: myocardial infarction (MI; n=10), Sham MI (n = 10), and no surgical treatment (Control; n = 11). Myocardial infarctions were induced in the MI and Sham MI groups according to the methods outlined previously. Briefly, the animals were anesthetized with Ketamine/Xylazine and prepared under aseptic conditions, intubated and ventilated with a small animal ventilator. The chest was opened at the left 4–5 intercostal space and the heart exposed. Infarction was induced by ligation of the left anterior descending coronary artery 2–3mm below the aortic root with 5-0 suture. Sham-MI rats underwent the identical procedure except the ligature was not tightened, and thus the artery was not occluded [7]. The animals were anesthetized and prepared under aseptic conditions, the chest opened and the heart exposed. Infarction was induced by ligation of the left anterior descending coronary artery. Sham-MI rats underwent the identical procedure except the ligature was not tightened, and thus the artery was not occluded. The study was approved by the Animal Review Committee of the University of South Dakota.

Typically, blood drawn for prospective registries of MI patients is taken 2–3 days after admission (*e.g.*, Block *et al*)[8] to give patients time to stabilize and to administer registry-related questionnaires. To account for the higher metabolic rate of rats, we selected 24 hours post MI for sampling to approximate the same stage in the acute phase response. The animals were anesthetized with isoflurane and exsanguinated by aortic puncture into a syringe containing 0.2 mL 0.5M EDTA. The collected blood was placed in an ice bath and plasma was separated within 1 hour.

In order to assess the effects of an MI on myocardial FA composition, we obtained tissue from both the left (infarcted) and right (non-infarcted) ventricles. Tissue was excised from the posterior, inferior section of the left ventricle distal to where the ligatures were placed, and from the right (non-infarcted) ventricle.

Lipid Extraction from Tissues

Lipids were extracted from tissues using an adaptation of the method of Bligh and Dyer [9]. Briefly, 1 mL chloroform containing 0.05 g/mL BHT and 2 mL methanol were added to approximately 100 mg of myocardium or 150 μ L of plasma and were homogenized for 2 minutes (Ultra Turrax Homogenizer). After 2 minutes, 1 mL chloroform was added and the sample was homogenized for 30 seconds, followed by the addition of 1 mL water and a final 30 second homogenization followed by centrifugal separation of the organic and aqueous layers.

Fatty acid analysis

FAs in RBCs and in the lipid extracts from both plasma and myocardium, were methylated using 250 μ L of 14% BF₃-Methanol (Sigma) at 100°C for 10 min as previously described [10]. FA methyl esters (FAMES) thus generated were extracted by the addition of 250 μ L of water and 250 hexane. A 50 μ L aliquot of the hexane layer was transferred to a GC vial and FAMES were analyzed on a GC2010-FID (Shimadzu Corporation, Columbia, MD) equipped with a 100m capillary column (SP-2560, 0.25 mm internal diameter, 0.2 μ m film thickness from Supelco, Bellefonte, PA).

Data analysis, statistics and calculations

Differences in RBCs and whole plasma FAs were tested using one-way ANOVA followed by Tukey's post test, while myocardial tissues were tested using paired two-way ANOVA followed by post-hoc contrast measures. Three considerations underscored the statistical approach, each primarily driven by the goal of the study, *i.e.*, to test for no difference. Since the goal of most studies is to demonstrate true positives (*i.e.*, a study difference), they are powered to cautiously identify positive results. Here, since the primary hypothesis is that RBC FA composition will not be affected by a MI, we sought to cautiously identify true negatives. For this reason, despite multiple testing of 24 FAs and up to 17 other indices, unadjusted *p*-values <0.05 are reported (*i.e.*, we intentionally did not adjust for multiple testing), and in the myocardial tissue analysis where multiway-ANOVA was used, interactions of *p* < 0.10 were conservatively considered for post-hoc analysis. Second, a pilot study with different time points and dietary *n*-3 FAs abundances was conducted (*n*= 13 rats) in order to design the present study with a 90% chance of detecting a change of ≥ 0.5 units in the omega-3 index at *p*= 0.01. Third, formal equivalence testing was employed to more powerfully estimate the true negatives. Since obvious species and dietary differences exist in the datasets, all differences are expressed after normalization to the control mean (*i.e.* Mean Control FA = 100%). Equivalence testing was performed as described by GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com using the method of Schuirmann [11]. Under normal testing the null hypothesis is expressed as $H_0: \Delta_{FA} = 0$. In equivalence testing, the null hypothesis is $H_0: \Delta_{FA} \geq \chi$, where χ is some threshold defined by the user as an unimportant difference, termed here 'indifference'; and the alternative hypothesis is $H_1 = \Delta_{FA} < \chi$. Here, the 90% confidence interval for the difference between the control and MI rats is calculated. If the CI falls entirely within the zone of indifference, then you can conclude with $\geq 95\%$ confidence that the treatments are essentially equivalent. This means that one of the challenging aspects of equivalence testing is being able to set a threshold for indifference. We derived this threshold from the differences in the differences between ACS

patients and controls (normalized in the same manner as in rats) in the largest comparable study published to date [8,12].

All hypothesis tests were two sided and model assumptions were confirmed. Where indicated, the data were log-transformed to achieve a normal distribution. In cases where model assumptions were not met, non-parametric tests were used when available. JMP 7.0.2 was used for statistical analyses.

RESULTS

Animals

At 24 hours post-intervention, there were no significant differences in weight among groups: 231 (inter-quartile range: 240g, 250), 239g (240, 250) and 233g (240, 240) for the Control, Sham MI and MI rats, respectively. All animals survived the surgical procedure and the median infarct size produced was 359 (375, 306) mm², or roughly 30–50% of left ventricular mass. In humans, this corresponds to a massive heart attack which, if survived, would be likely to progress to congestive heart failure.

Red Blood cell FA composition

Neither MI nor sham treatment had any effect on most RBC FAs, including EPA and DHA (Table 1). The only 2 FAs to differ were lignoceric and nervonic acids, both components of sphingomyelins, which were somewhat elevated in the MI animals; palmitoleic and lignoceric were elevated in the sham-MI group compared to controls and γ -linolenic acid was elevated in MI relative to the sham-MI group. No further changes in individual FA content were detected. Additional summary and composite indexes were tested: none were altered by either MI or sham-MI using the usual statistical tests for differences.

Red blood cell equivalence

We also determined whether the FA composition observed in the MI group was equivalent to that in the Control group (Figure 1). An estimate of an irrelevant difference, or zone of indifference, was made of 17 FAs and 3 composite indices based on the differences previously reported [8,12]. In 5 of 17 FAs, including DHA, the abundance measured in the MI group is unambiguously equivalent to that seen on control rats. Four more FAs, including EPA, were equivalent provided the zone of indifference is 50% of the difference observed in humans between ACS cases and healthy controls, in both cases indicating that it is unlikely that the changes observed in case/control studies could be only due to the MI. In 8 other FAs, the results are ambiguous since the CI for the difference falls in both the range of the difference observed in humans as well as zero. It should be noted that in three cases (palmitic acid, eicosadienoic acid, and arachidonic acid) the difference observed in the MI in rats was either greater or practically identical to that observed in humans. Finally, two of the three composite indices, including the omega-3 index, were equivalent while the monounsaturated FAs result was ambiguous.

Whole plasma FA composition

More substantial shifts in FA composition were noted in whole plasma (Table 2). Eight of twenty-four FAs were altered in MI or Sham MI animals vs. Controls. Ten FAs were altered by either the surgery or the surgery and the MI. In one case, eicosatrienoic acid, the levels in the sham MI group were different from those in the Control group, and for 2 FAs, the levels in MI group different from those in the sham MI and Control groups. For the 5 remaining FAs, levels were the same in the MI and Sham MI groups, but different from Control. Five of twelve composite indexes were also altered, four of them in both the MI and Sham MI groups.

Myocardial Tissue composition

The FA composition of myocardial tissue excised from the area of infarction in the left ventricle is contrasted with that of Sham MI and control groups as well as with non infarcted tissue from the right ventricle (Supplemental Table 1).

DISCUSSION

Here we examined the effect of an MI (or major trauma such as thoracic surgery) on RBC, whole plasma and myocardial FA composition using an animal model. We found no significant effect of either intervention on the RBC FA profile whereas plasma FA composition was changed. Thus, of the two, the RBC is the preferable source of tissue for risk assessment in case/control studies. By designing this study to specifically detect *no difference* rather than *differences*, our results provide a confidence in the resistance of RBC FA composition to acute trauma not afforded by past studies.

Prior to initiating this study we conducted a pilot study in order to assure that a reasonable chance of detecting a change existed and to determine the best conditions under which those differences could be detected. We chose a diet that would result in moderately elevated n-3 FA levels. The average omega-3 index achieved here was 5–6% which is ~2% higher than the mean omega-3 index among Americans [13]. These moderately high levels gave us a greater opportunity to detect a difference in the omega-3 index than a lower, more typical, index would have. The fact that the MIs were produced in healthy animals allowed us to avoid the possibility that another pathologic condition contributed to the FA differences we might have observed, and the inclusion of a sham-MI group (that underwent major surgical trauma with its attendant acute phase response) allowed us to separate potential FA differences resulting from surgical stress *vs.* an MI *per se*.

We used two statistical approaches to test our hypothesis that an MI does not alter the FA composition of RBC membranes. First we used the more common test for differences, employing a low threshold for false-positives ($p < 0.05$; no adjustment for multiple comparisons) in order to best identify FAs differing by treatment. Since we were primarily hypothesizing no difference between the MI and Control groups, we also tested for equivalence (*i.e.*, was the difference less than or equal to what would be considered unimportant). The MI and control groups had conclusively equivalent RBC FA compositions particularly for the FAs of primary interest, the long chain omega-3 FAs, EPA and DHA. Both FAs common in sphingolipids, lignoceric and nervonic acids, were elevated ~40% suggesting a possible change in sphingolipid metabolism. Notably, the effects of an MI on arachidonic acid, palmitic acid, stearic acid, oleic acid and four other minor FAs did not yield a strong conclusion for either equivalence or change and warrant further study. Overall, these data provide compelling evidence for the resilience of the RBC FA to the stress of an MI.

In contrast to the RBC, both the MI and the surgery had an impact on whole plasma FA composition as both individual FAs and composite indexes in both the MI and sham-MI groups differed from the Controls. This is possibly a consequence of altered lipoprotein metabolism associated with acute phase response during recovery from an MI [14]. While DHA was not impacted, EPA was markedly reduced both the MI sham-MI groups, and consequently the omega-3 index was also reduced. Thus in plasma, regardless of whether the changes are due directly to the MI or to secondary consequences of an MI such as trauma, it seems clear that it is not desirable for use as a biomarker after an MI or any other major trauma.

As expected, multiple FAs were also affected in myocardial tissues, primarily in the infarcted tissue. With the exception of linoleate, there was not a clear relationship between the changes

observed in plasma and those observed in myocardial tissue. Linoleic acid in plasma increased to about 20% of total, while it decreased in infarcted myocardium to the same levels.

In conclusion, neither an MI nor surgical trauma affected the RBC omega-3 index whereas this metric was affected in plasma. Hence, RBCs may be used in case-control studies of cardiac events and confidently assumed to reflect pre-MI patterns. Thus, to the extent that the FA patterns differ in cases and controls, RBC FA patterns may have not only diagnostic, but also prognostic, value.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Equivalence of fatty acids

In order to test equivalence the mean difference (90% confidence interval), expressed as a percent of control abundance, between control and MI rats for select fatty acids. Equivalence testing requires an interval of indifference be defined and if the 90% confidence interval (CI) falls within the zone of indifference, you can conclude with 95% confidence that the levels are equivalent. In this case three zones of indifference are proposed at 25% (light grey), 50% (medium grey) and 100% (dark grey) of the case/control difference found in humans [8,12]. For example, if the CI falls in the light grey range, then you can conclude that no difference exists given a zone of indifference that is 50% of the human differences. If the CI falls in dark grey range, the results are ambiguous.

Table 1

Fatty acid profiles from red blood cells^a.

Fatty acid ^b	Structure	MI (n=11)	Sham MI (n=10)	Control (n=10)
Myristic acid	14:0	0.242 (0.21, 0.27)	0.280 (0.25, 0.31)	0.253 (0.22, 0.29)
Palmitic acid ^d	16:0	26.3 (25, 27)	26.0 (26, 26)	26.2 (26, 27)
Palmitoleic acid [*]	16:1n7	0.265 (0.23, 0.30) ^A	0.319 (0.28, 0.36) ^B	0.251 (0.21, 0.29) ^{AB}
<i>trans</i> Palmitoleic acid	16:1n7t	0.0935 (0.081, 0.11)	0.0907 (0.078, 0.10)	0.105 (0.093, 0.12)
Stearic acid	18:0	18.9 (17, 20)	18.2 (17, 20)	20.3 (19, 22)
Oleic acid	18:1n9	7.62 (7.4, 7.9)	7.38 (7.1, 7.7)	7.53 (7.3, 7.8)
<i>trans</i> Oleic acid ^d	18:1t	0.299 (0.27, 0.32)	0.347 (0.29, 0.40)	0.314 (0.28, 0.35)
Linoleic acid (n6)	18:2n6	8.62 (8.1, 9.1)	8.54 (8.0, 9.0)	8.03 (7.5, 8.5)
<i>trans</i> Linoleic acid (n6)	18:2n6t	0.0413 (0.036, 0.046)	0.0339 (0.029, 0.039)	0.0339 (0.029, 0.039)
α -Linolenic acid (n3) ^c	18:3n3	0.042 (0.034, 0.052)	0.0321 (0.026, 0.040)	0.0299 (0.024, 0.037)
γ -Linolenic acid (n6)	18:3n6	0.0909 (0.081, 0.10) ^A	0.111 (0.10, 0.12) ^B	0.0983 (0.087, 0.11) ^{AB}
Eicosenoic acid	20:1n9	0.0697 (0.060, 0.079)	0.0621 (0.052, 0.072)	0.069 (0.059, 0.079)
Eicosadienoic acid (n6)	20:2n6	0.188 (0.17, 0.20)	0.204 (0.19, 0.22)	0.200 (0.18, 0.22)
Eicosatrienoic acid (n6)	20:3n6	0.495 (0.45, 0.55)	0.434 (0.39, 0.48)	0.514 (0.46, 0.57)
Arachidonic acid (n6)	20:4n6	27.0 (26, 28)	27.6 (26, 29)	26.1 (25, 27)
Eicosapentaenoic acid (n3)	20:5n3	0.889 (0.78, 0.99)	0.94 (0.83, 1.0)	0.871 (0.76, 0.98)
Docosatetraenoic acid (n6)	22:4n6	1.49 (1.4, 1.6)	1.52 (1.4, 1.6)	1.58 (1.5, 1.7)
Docosapentaenoic acid (n3)	22:5n3	1.87 (1.8, 2.0)	2.01 (1.9, 2.1)	1.98 (1.9, 2.1)
Docosapentaenoic acid (n6)	22:5n6	0.552 (0.52, 0.58)	0.535 (0.51, 0.56)	0.557 (0.53, 0.59)
Docosahexaenoic acid (n3)	22:6n3	4.8 (4.6, 5.0)	4.86 (4.6, 5.1)	4.68 (4.4, 4.9)
Lignoceric acid [*]	24:0	0.301 (0.27, 0.34) ^A	0.220 (0.18, 0.26) ^B	0.199 (0.16, 0.24) ^B
Nervonic acid ^{c, *}	24:1n9	0.161 (0.14, 0.18) ^A	0.127 (0.10, 0.15) ^{AB}	0.0962 (0.073, 0.12) ^B
Composite Indices				
Omega-3 Index		5.69 (5.4, 6)	5.8 (5.5, 6.1)	5.56 (5.3, 5.9)
Total Saturated		45.1 (44, 47)	44.6 (43, 46)	46.7 (45, 48)
Total Monounsaturated		8.12 (7.8, 8.4)	7.89 (7.6, 8.2)	7.94 (7.7, 8.2)
Total Polyunsaturated ^d		46.1 (45, 48)	46.8 (45, 48)	44.6 (43, 46)
n3		7.61 (7.3, 7.9)	7.84 (7.5, 8.2)	7.56 (7.2, 7.9)
n6 ^d		38.4 (37, 40)	38.9 (37, 41)	37.1 (36, 38)
n3 HUFA		7.56 (7.2, 7.9)	7.8 (7.5, 8.1)	7.53 (7.2, 7.9)
n6 HUFA		29.5 (29, 31)	30.1 (29, 31)	28.7 (28, 30)
Log(n6/n3) ^d		5.06 (4.9, 5.2)	4.98 (4.8, 5.2)	4.91 (4.7, 5.1)
n6H/n3H ^c		3.91 (3.8, 4.0)	3.86 (3.7, 4.0)	3.82 (3.7, 3.9)
n3H/total H ^d		0.204 (0.20, 0.21)	0.206 (0.20, 0.21)	0.208 (0.20, 0.21)
total trans		0.434 (0.40, 0.47)	0.472 (0.44, 0.51)	0.454 (0.42, 0.49)

^a reported as percent of total FA. Mean (95% CI) except where otherwise noted. Tested using one-way ANOVA.^b most common name given. Omega-9, omega-6 and omega-3 FAs are indicated as n9, n6, and n3 respectively.^c Analysis on log-transformed data to achieve normal distribution.^d Failed test for homogeneity. Welch's ANOVA performed for means equal allowing different standard deviations. 95% CI derived without pooled estimate of variance.^{*} p<0.05 for overall ANOVA; Tukey's honest significant difference post hoc test. Treatments not sharing capital letters are significantly different at p<0.05.

Table 2

Fatty acid profiles from whole plasma^a.

Fatty acid ^b	Structure	MI (n=11)	Sham MI (n=10)	Control (n=10)
Myristic acid (n6)	14:0	0.506 (0.42, 0.59)	0.476 (0.39, 0.56)	0.496 (0.41, 0.58)
Palmitic acid *	16:0	16.5 (16, 17) ^A	16.2 (15, 17) ^{AB}	14.9 (14, 16) ^B
Palmitoleic acid *	16:1n7	1.2 (1.0, 1.4) ^A	1.2 (1.0, 1.4) ^A	1.69 (1.4, 2.0) ^B
<i>trans</i> Palmitoleic acid ^d	16:1n7t	0.104 (0.09, 0.12)	0.117 (0.10, 0.13)	0.116 (0.11, 0.12)
Stearic acid	18:0	13.5 (13, 14)	13.6 (13, 14)	13.2 (12, 14)
Oleic acid	18:1n9	14.7 (14, 16)	14.1 (13, 15)	14.2 (13, 16)
<i>trans</i> Oleic acid ^d	18:1t	0.129 (0.10, 0.16)	0.118 (0.093, 0.15)	0.111 (0.088, 0.14)
Linoleic acid (n6) ^{d, *}	18:2n6	19.9 (19, 21) ^A	19.9 (18, 21) ^A	17.6 (16, 19) ^B
<i>trans</i> Linoleic acid (n6) ^e	18:2n6t	0.157 (0.12, 0.18)	0.164 (0.15, 0.19)	0.14 (0.13, 0.17)
α -Linolenic acid (n3)	18:3n3	0.155 (0.13, 0.18)	0.159 (0.13, 0.19)	0.121 (0.092, 0.15)
γ -Linolenic acid (n6) ^{e, *}	18:3n6	0.259 (0.23, 0.35) ^{AB}	0.253 (0.24, 0.28) ^B	0.368 (0.33, 0.41) ^A
Eicosenoic acid ^{c, *}	20:1n9	0.071 (0.061, 0.082) ^{AB}	0.0672 (0.057, 0.079) ^B	0.0909 (0.078, 0.11) ^A
Eicosadienoic acid (n6) ^e	20:2n6	0.128 (0.11, 0.15)	0.123 (0.094, 0.14)	0.128 (0.12, 0.14)
Eicosatrienoic acid (n6) ^{c, d, *}	20:3n6	0.469 (0.35, 0.59)	0.368 (0.34, 0.40)	0.535 (0.49, 0.58)
Aracadic acid (n6) *	20:4n6	22.8 (21, 25) ^A	23.9 (22, 26) ^{AB}	26.5 (25, 28) ^B
Eicosapentaenoic acid (n3) *	20:5n3	1.71 (1.5, 1.9) ^A	1.68 (1.5, 1.9) ^A	2.38 (2.2, 2.6) ^B
Docosatetraenoic acid (n6)	22:4n6	0.319 (0.27, 0.37)	0.294 (0.24, 0.35)	0.279 (0.23, 0.33)
Docosapentaenoic acid (n3)	22:5n3	0.675 (0.62, 0.73)	0.701 (0.65, 0.76)	0.702 (0.65, 0.76)
Docosapentaenoic acid (n6)	22:5n6	0.265 (0.22, 0.31)	0.223 (0.18, 0.27)	0.208 (0.16, 0.25)
Docosahexaenoic acid (n3) ^{c, d}	22:6n3	5.72 (5.4, 6.1)	5.76 (5.1, 6.5)	5.88 (5.5, 6.3)
Lignoceric acid ^{e, *}	24:0	0.283 (0.26, 0.34) ^A	0.325 (0.24, 0.40) ^A	0.229 (0.21, 0.24) ^B
Nervonic acid ^{e, *}	24:1n9	0.137 (0.096, 0.17) ^A	0.133 (0.11, 0.21) ^A	0.0662 (0.061, 0.093) ^B
Composite Indexes				
Omega3 Index ^{d, *}		7.43 (7.1, 7.8) ^A	7.44 (6.7, 8.2) ^A	8.26 (7.8, 8.7) ^B
Total Saturated ^{d, *}		30.8 (30, 31) ^A	30.7 (30, 31) ^A	28.9 (29, 29) ^B
Total Monounsaturated		16.2 (15, 17)	15.6 (14, 17)	16.1 (15, 17)
Total Polyunsaturated		52.5 (51, 54)	53.3 (52, 55)	54.7 (53, 56)
Total n3 ^{d, *}		8.26 (7.9, 8.6) ^A	8.3 (7.5, 9.1) ^A	9.09 (8.6, 9.5) ^B
Total n6		44.2 (43, 46)	45 (44, 46)	45.6 (44, 47)
n3 HUFA ^{d, *}		8.10 (7.7, 8.5) ^A	8.14 (7.3, 9.0) ^A	8.97 (8.5, 9.4) ^B
n6 HUFA *		23.9 (22, 26) ^A	24.8 (23, 27) ^{AB}	27.5 (26, 29) ^B
n6/n3		3.17 (3.1, 3.2)	3.20 (3.1, 3.3)	3.31 (3.2, 3.4)
n6H/n3H ^c		2.94 (2.8, 3.1)	3.05 (2.9, 3.3)	3.06 (2.9, 3.3)
n3H/total H		0.254 (0.24, 0.27)	0.248 (0.24, 0.26)	0.247 (0.24, 0.26)
total trans ^e		0.28 (0.23, 0.30)	0.278 (0.26, 0.31)	0.258 (0.23, 0.3)

^a reported as percent of total FA. Mean (95% CI) except where otherwise noted. Tested using one-way ANOVA.^b most common name given. Omega-9, omega-6 and omega-3 FAs are indicated as n9, n6, and n3 respectively.^c Analysis on log-transformed data to achieve normal distribution.^d Failed test for homogeneity. Welch's ANOVA performed for means equal allowing different standard deviations. 95% CI derived without pooled estimate of variance.^e Failed test for normality or homogeneity even with transformation. Median and inter-quartile range reported. Tested using Kruskal-Wallis one-way ANOVA on ranks followed by Dunn's post hoc test.

* p<0.05 for overall ANOVA; Tukey's honest significant difference post hoc test. Treatments not sharing capital letters are significantly different at p<0.05.