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RESEARCH PAPER

Differences in metabolomic and transcriptomic profiles between responders and non-responders to an n-3 polyunsaturated fatty acids (PUFAs) supplementation

Iwona Rudkowska · Ann-Marie Paradis · Elisabeth Thifault · Pierre Julien · Olivier Barbier · Patrick Couture · Simone Lemieux · Marie-Claude Vohl

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Abstract Studies have demonstrated large within-population heterogeneity in plasma triacylglycerol (TG) response to n-3 PUFA supplementation. The objective of the study was to compare metabolomic and transcriptomic profiles of responders and non-responders of an n-3 PUFA supplementation. Thirty subjects completed a 2-week run-in period followed by a 6-week supplementation with n-3 PUFA (3 g/d). Six subjects did not lower their plasma TG (+9 %) levels (non-responders) and were matched to 6 subjects who lowered TG (−41 %) concentrations (responders) after the n-3 PUFA supplementation. Pre-n-3 PUFA supplementation characteristics did not differ between the non-responders and responders except for plasma glucose concentrations. In responders, changes were observed for plasma hexose concentrations, docosahexaenoic acid, stearoyl-CoA-desaturase-18 ratio, and the extent of saturation of glycerophosphatidylcholine after n-3 PUFA supplementation; however, no change in these parameters was observed in non-responders. Transcriptomic profiles after n-3 PUFA supplementation indicate changes in glycerophospholipid metabolism in both subgroups and sphingolipid metabolism in non-responders. Six key genes in lipid metabolism: fatty acid desaturase 2, phospholipase A2 group IVA, arachidonate 15-lipoxygenase, phosphatidylethanolamine *N*-methyltransferase, monoglyceride lipase, and glycerol-3-phosphate acyltransferase, were expressed in opposing direction between subgroups. In sum, results highlight key differences in lipid metabolism of non-responders compared to responders after an n-3 PUFA supplementation, which may explain the inter-individual variability in plasma TG response.

Keywords Lipidomics · Metabolic pathways · Metabolites · Microarray · Nutrigenomics

Abbreviations

AC	Acylcarnitines
AGPAT	1-Acylglycerol-3-phosphate O-acyltransferases
ALOX15	Arachidonate 15-lipoxygenase
CPT	Cell preparation tube
D5D	Delta5-desaturase
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FDR	False discovery rate
FA	Fatty acid
FADS2	Fatty acid desaturase 2
ELOVL2	Fatty acid elongase
GPAM	Glycerol-3-phosphate acyltransferase
glyPC	Glycerophosphatidylcholines
HDL-C	High-density lipoprotein cholesterol
HIF	Hypoxia-inducible factor
IPA	Ingenuity pathway analysis
LDL-C	Low-density lipoprotein cholesterol
LPL	Lipoprotein lipase
lysoPC	Lysophosphatidylcholines
MGLL	Monoglyceride lipase

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I. Rudkowska · A.-M. Paradis · E. Thifault · P. Couture · S. Lemieux · M.-C. Vohl (✉)
Institute of Nutraceuticals and Functional Foods (INAF), Laval University, Pavillon des Services, 2440, Boulevard Hochelaga, Quebec, QC G1V 0A6, Canada
e-mail: marie-claude.vohl@fsaa.ulaval.ca

I. Rudkowska · P. Julien · O. Barbier · M.-C. Vohl
Endocrinology and Genomics, Laval University Medical Center, Quebec, QC, Canada

MUFA	Monounsaturated FA
NrF2	Nuclear factor (erythroid-derived 2)-like 2
NF-kB	Nuclear factor kB
PLA2	Phospholipase A2
PNPLA2	Patatin-like phospholipase domain containing 2
PBMCs	Peripheral blood mononuclear cells
PPARA	Peroxisome proliferator-activated receptor alpha
PEMT	Phosphatidylethanolamine *N*-methyltransferase
SCD	Stearoyl-CoA desaturase
TC	Total cholesterol
TG	Triacylglycerol
SAM	Significance analysis of microarrays
SM	Sphingomyelins

Introduction

The cardioprotective properties of the fish oil-derived long-chain n-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been demonstrated in numerous studies (Harris 1997). The evidence from those studies indicates that n-3 PUFA have hypo-triglyceridemic, anti-inflammatory, anti-atherogenic, and anti-arrhythmic effects, which are the potential underlying mechanisms for reducing the risk of cardiovascular disease (Harris 1997). Yet, there is well-recognized heterogeneity in triacylglycerol (TG) response to n-3 PUFA supplementation (Madden et al. 2011): for example, 31 % of all volunteers from the Fish Oil Intervention and Genotype (FINGEN) Study showed no reduction in TG after 1.8 g EPA and DHA per day for 8 weeks (Caslake et al. 2008). This heterogeneity in TG response within studies is likely to be at least partly attributable to genetic variability within the study population that may be apparent in metabolic pathways.

The analysis of metabolomic and transcriptomic signatures pre- and post-n-3 PUFA supplementation may provide information on the physiological pathways which are affected by supplementation. Previous studies have demonstrated the effects of nutrient intakes on the specific metabolomic profiles (Suhre et al. 2011; Wittwer et al. 2011). Further, alterations in the metabolome may derive from changes in gene expression levels (Wittwer et al. 2011). Recent studies demonstrate that peripheral blood mononuclear cells (PBMCs) profiling adequately reflect gene expression levels of various tissues (Liew et al. 2006; Rudkowska et al. 2011b). Studies showed that supplementation with n-3 PUFA can alter gene expressions toward a more anti-atherogenic and anti-inflammatory profile (Bouwens et al. 2009; Rudkowska et al. 2013). Overall, the assessment of metabolic and transcriptomic profiles can potentially enlighten us on changes that occur in physiological pathways.

Thus, the objective was to examine whether metabolome and transcriptome analyses can be used to distinguish between subjects who respond and who do not respond to an n-3 PUFA supplementation by lowering plasma TG concentrations.

Subjects and methods

Study population

A total of 254 subjects from the greater Quebec City metropolitan area were recruited to participate in the study. In total, 210 subjects completed the intervention protocol. For the purpose of this large-scale "Omics" study, the first 30 subjects who completed the study were included for the metabolomic and transcriptomic profiling. Further, subjects who completed the study were separated into sub-groups: responders and non-responders. Non-responders are defined by no reduction in plasma TG concentrations after the n-3 PUFA supplementation (change in plasma TG of $\geq$0 mmol/L; TG post-n-3 PUFA minus TG pre-n-3 PUFA supplementation).

Participants had a BMI between 25 and 40 kg/m^2 and were not currently taking any lipid-lowering medications. Subjects were excluded from the study if they had taken n-3 PUFA supplements for at least 6 months prior, used oral hypolipidemic therapy, or had been diagnosed with diabetes, hypertension, hypothyroidism, or other known metabolic disorders such as severe dyslipidemia or coronary heart disease. The experimental protocol was approved by the Ethics Committees of Laval University Hospital Research Center and Laval University. This trial was registered at clinicaltrials.gov as NCT01343342.

Study design and diets

First, subjects followed a run-in period of 2 weeks. Individual dietary instructions were given by a trained dietician to achieve the recommendation from *Canada's Food Guide*. Subjects were asked to follow these dietary recommendations and maintain their body weight stable throughout the protocol. Some specifications were given regarding the n-3 PUFA dietary intake: not exceed 2 fish or seafood servings per week (max 150 g), prefer white flesh fishes instead of fatty fishes (examples were given), and avoid enriched n-3 PUFA dietary products such as some milks, juices, breads, and eggs. Subjects were also asked to limit their alcohol consumption during the protocol;

2 regular drinks per week (approximately 28 grams of alcohol per week) were allowed. In addition, subjects were not allowed to take n-3 PUFA supplements (such as flaxseed), vitamins or natural health products during the protocol.

Second, after the run-in period, each participant received a bottle containing needed *fish oil* capsules for the following 6 weeks. They were invited to take 5 (1 g of fish oil concentrate each) capsules per day (Ocean Nutrition, Nova Scotia, Canada), providing a total of 3 g of n-3 PUFA (including 1.9 g EPA and 1.1 g DHA) per day. For a facilitated digestion, we recommended to take fish oil capsules while eating. Compliance was assessed from the return of bottles. Subjects were asked to report any deviation during the protocol, write down their alcohol and fish consumption as well as the side effects. Before each phase, subjects received detailed written and oral instructions on their diet.

Biochemical parameters

Lipid, glycemic and inflammation markers

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after 12-h overnight fast and 48-h alcohol abstinence. Blood samples were taken to identify and exclude individuals with any metabolic disorders. Afterward, selected participants had blood samples taken at prior and after the n-3 PUFA supplementation period. Plasma was separated by centrifugation (2,500×*g* for 10 min at 4 °C), and samples were aliquoted and frozen for subsequent analyses. Plasma total cholesterol (TC) and TG concentrations were measured using enzymatic assays (Burstein and Samaille 1960). The HDL-C fraction was obtained after the precipitation of very low-density lipoprotein and LDL particles in the infranatant with heparin manganese chloride (Albers et al. 1978). LDL-C was calculated with the Friedewald formula (Friedewald et al. 1972). Fasting insulinemia was measured by radioimmunoassay with polyethylene glycol separation (Desbuquois and Aurbach 1971). Fasting glucose concentrations were enzymatically measured (Richterich and Dauwalder 1971). Apolipoprotein (apo) B100 concentrations were measured in plasma by the rocket immunoelectrophoretic method of Laurell, as previously described (Laurell 1966). Plasma C-reactive protein (CRP) was measured by nephelometry (Prospec equipment Behring) using a sensitive assay, as described previously (Pirro et al. 2001).

Fatty acids analysis

Fatty acid (FA) composition of erythrocyte membranes was determined by gas chromatographic analysis. Membranes of lysed erythrocytes were isolated by centrifugation (21,000×*g*, 15 min) and washed twice with 0.9 % NaCl solution. Cell membranes were resuspended in 200 µL of the NaCl solution and were spiked with phosphatidylcholine C:15 (Avanti Polar Lipids, Alabaster, AL), used as internal standard. Lipids were extracted using a mixture of chloroform–methanol (2:1 v/v) according to a modified Folch method (Shaikh and Downar 1981). Fatty acid profiles were obtained after methylation in methanol/benzene 4:1 (v/v) (Lepage and Roy 1986) and capillary gas chromatography using a temperature gradient on a HP5890 gas chromatograph (Hewlett Packard, Toronto, Canada) equipped with a HP-88 capillary column (100 m × 0.25 mm i.d. × 0.20 µm film thickness; Agilent Technologies, Palo Atto, CA) coupled with a flame ionization detector (FID). Helium was used as carrier gas (split ratio 1:80). FA were identified according to their retention time, using the following standard mixtures as a basis for comparison: the FAME 37 mix (Supelco Inc., Bellefonte, PA) and the GLC-411 fatty acid mix (NuChek Prep Inc, Elysian, MN), as well as the following methylated fatty acids C22:5 w6 (Larodan AB, Malmö, Sweden) and C22:5 w3 (Supelco Inc., Bellefonte, PA). Erythrocyte FA profiles were expressed as the relative percentage areas of total FAs.

Further, FA ratios used as surrogate measures of desaturase indices, including stearoyl-CoA desaturase (SCD), delta5-desaturase (D5D), and fatty acid elongase (ELOVL2), were calculated with the percentages of FAs in erythrocytes. The SCD18 was calculated with the ratio of oleic acid (18:1n-9) on stearic acid (18:0). Second, the D5D was calculated by using arachidonic acid (20:4n-6) on dihomo-gamma-linolenic acid (20:3n-6). The delta6-desaturase (D6D) was not calculated since gamma-linolenic acids (18:3n-6) by linoleic acid (18:2n-6) FA percentages were not detectable. Finally, ELOVL2 was calculated with the ratio of DHA (22:6n-3) on EPA (20:5n-3).

Metabolomic analyses

Metabolite profiling

The targeted metabolite profiling with the Biocrates Absolute IDQ p150 (Biocrates Life Sciences AG, Austria) mass spectrometry method was used to quantify 163 metabolites from four compound classes including: 41 acylcarnitines (ACs) (AC*x*:*y*, where *x* denotes the number of carbons in the side chain and *y* the number of double bonds), 13 amino acids (proteinogenic + ornithine), hexoses (sum of hexoses—90–95 % glucose), and 107 lipids. These lipids are subdivided into 3 different classes; 15 sphingomyelins (SM) and SM-derivatives, 15 lysophosphatidylcholines (lysoPC), and 77 glycerophosphatidylcholines (glyPC). Glycerophospholipids are

further differentiated with respect to the presence of ester (*a*) and ether (*e*) bonds in the glycerol moiety, where two letters (*aa*, *ae*, or *ee*) denote that the first and the second positions of the glycerol unit are bound to a FA residue, while a single letter (*a* or *e*) indicates a bond with only one FA residue; the latter molecular species are usually called lyso-phospholipids. Assays used 10 μL of plasma from each subject pre- and post-n-3 PUFA supplementation. The metabolite profiling was carried out according to the manufacturer's instructions at CHENOMX (Edmonton, AL, Canada). For all analyzed metabolites, the concentrations are reported in μM.

Transcriptomics analyses

Peripheral blood mononuclear cells

Blood samples were collected into an 8-mL Cell Preparation Tube (CPT) (Becton–Dickinson, Oakville, On, Canada) pre- and post-supplementation. Peripheral blood mononuclear cells (PBMCs) were separated by centrifugation (1,500×*g*, 20 min, at room temperature) and washed according to the manufacturer's instructions. Total RNA were extracted with RNeasy Plus Mini Kit (QIAGEN, Mississauga, On, Canada) according to manufacturer's protocol. After spectrophotometric quantification and verification of the total RNA quality via the Agilent 2100 Bioanalyser (Agilent Technologies, Palo Alto, CA, United States), samples were used for microarray analysis. Samples were excluded from additional analysis on microarray chips if they had poor RNA quality (RIN < 8).

Transcriptomic profiling

Two hundred nanograms of total RNA were amplified and labeled using the Illumina TotalPrep RNA Amplification kit (Ambion). cRNA quality was assessed by capillary electrophoresis on Agilent 2100 Bioanalyzer. Expression levels of 48,803 mRNA transcripts, to investigate 37,804 genes, were assessed by the Human-6 v3 Expression BeadChips (Illumina). Hybridization was carried out according to the manufacturer's instructions at the McGill University/Génome Québec Innovation Center (Montreal, Canada).

Analysis of microarray data

These data are based on the "minimum information about a microarray experiment" (MIAME), to ensure that microarray data can be easily interpreted and that results derived from its analysis can be independently verified (Brazma et al. 2001). The microarray analysis was performed using FlexArray software (Blazejczyk et al. 2007). The Lumi algorithm was used to normalize Illumina microarray data. Specifically, expression values were normalized by using Lumi via the Robust Multiarray Average (RAM) algorithm (Bolstad et al. 2003). This step was followed by quantile normalization and $\log_2$ transformation. The samples were then grouped according to time (pre- and post-supplementation) and responsiveness to n-3 PUFA supplementation (responder and non-responders). To assess which transcripts were differentially expressed between conditions examined, we used a Significance Analysis of Microarrays (SAM) algorithm, an adaptation of a *t* test for microarray data, on all probes. In general, the SAM application assigns a score to gene on the basis of changes in gene expression relative to standard deviation of repeated measurements. Then, SAM uses permutations of the repeated measurements to estimate the False Discovery Rate (FDR) (Tusher et al. 2001). A cutoff of $P \leq 0.05$ was used to select the regulated genes. In addition, a fold change cutoff was also computed by FlexArray software to assess the level and the direction of the gene regulation. This fold change is calculated as the absolute ratio of normalized intensities between the mean values of all individual fold change (post-/pre-supplementation). Thus, two cutoff values were used to minimize the chances of false positives. Fold changes at ≥ 1.2, and $P \leq 0.05$ (up-regulated) or fold changes at ≤ 0.8 and $P \leq 0.05$ (down-regulated) were taken from each treatment to determine differentially expressed transcripts and transcript lists were generated.

Biological pathway analyses

Pathway analyses allowed to determine whether genes found to be differentially expressed belong to pre-defined networks more than expected by chance alone and help to add structure to the vast amount of data generated by microarrays. The Ingenuity Pathway Analysis (IPA) system (Ingenuity® Systems, www.ingenuity.com) was used to visualize gene expression data in the context of biological pathways. First, an input file was uploaded in the IPA system: fold changes of all probe sets between pre- and post-n-3 PUFA supplementation and dataset in Core Analysis were created. Further, the core dataset was analyzed using the general settings for IPA system as "Ingenuity knowledge base (genes)" and "considered only molecules and/or relationships where species is humans". Finally, we examined the canonical pathway analysis, in particular, the lipid metabolism category, which allows us to link expression data to clinical endpoints for mechanistic hypothesis generation and identification of putative mechanisms.

The significance value associated with Functional Analysis for a dataset is a measure of the likelihood that the association between a set of Functional Analysis molecules

Table 1 Biochemical parameters of responders and non-responders pre- and post-n-3 PUFA supplementation

	Responders ($n = 6$)			Non-responders ($n = 6$)			P value between resp. and non-resp.	
	Pre-n-3 PUFAs	Post-n-3 PUFAs	P value	Pre-n-3 PUFAs	Post-n-3 PUFAs	P value	Pre-n-3 PUFAs	Delta (post- and pre-n-3 PUFAs)
Gender	2 men (33 %) and 4 women (67 %)			1 man (16 %) and 5 women (83 %)				
Age (years)	40 ± 4 years			30 ± 4 years			NS	
BMI (kg/m^2)	29.9 ± 1.5			30.5 ± 2.7			NS	NS
Triacylglycerols[a] (mmol/L)	1.75 ± 0.47	0.99 ± 0.23	0.0002	1.03 ± 0.15	1.12 ± 0.17	0.045	NS	0.0153
Cholesterol (mmol/L)								
Total	5.00 ± 0.28	5.03 ± 0.42	NS	5.02 ± 0.43	4.88 ± 0.33	NS	NS	NS
LDL	2.76 ± 0.37	3.03 ± 0.42	NS	2.85 ± 0.43	2.57 ± 0.33	NS	NS	0.0443
HDL[a]	1.43 ± 0.16	1.54 ± 0.18	0.0267	1.70 ± 0.11	1.80 ± 0.12	NS	NS	NS
Ratio TC/HDL-C	3.66 ± 0.37	3.39 ± 0.33	0.0001	3.05 ± 0.41	2.79 ± 0.30	0.0018	NS	NS
Apo-B100 (g/L)	0.87 ± 0.08	0.88 ± 0.08	NS	0.81 ± 0.12	0.80 ± 0.08	NS	NS	NS
Ratio LDL/Apo-B	3.18 ± 0.69	3.42 ± 0.64	0.0108	3.51 ± 0.48	3.18 ± 0.48	0.0247	NS	0.0039
Glucose (mmol/L)	5.33 ± 0.19	5.42 ± 0.17	NS	4.77 ± 0.21	4.98 ± 0.29	NS	0.0058	NS
Insulin (pmol/L)	68.80 ± 20.45	90.83 ± 27.54	NS	73.00 ± 8.83	89.17 ± 5.80	NS	NS	NS
CRP[a](mg/L)	2.11 ± 0.95	2.13 ± 1.06	NS	1.20 ± 0.40	1.10 ± 0.40	NS	NS	NS

Data are shown as mean ± SEM

TC total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

[a] P value derived from transformed data

in the experiment and a given process or pathway is due to random chance. Smaller p values ($P \leq 0.05$) indicate statistically significant, non-random associations. The P-value is calculated using the right-tailed Fisher Exact Test with a cutoff of ±1.2. IPA suggests to use p-values as starting points for further investigation; however, pathways with larger p-values ($P > 0.05$) may be biologically relevant even if not statistically significant. In addition, there is no directionality associated with the relationship (i.e. the function cannot be interpreted as being up- or down-regulated).

Statistical analyses

Results are presented as mean ± SEM. Variables not normally distributed were transformed by natural logarithm to normalize their distribution. Outlier values (≥±3SD) were excluded from statistical analyses. Data were analyzed using a paired t test to determine significant changes between pre- and post-supplementation periods for each of the subgroups: responders and non-responders. An unpaired t test was used to establish differences between pre- and post-data between the subgroups. The delta of change was calculated (post-minus pre-supplementation levels), and an unpaired t test was performed to determine differences between responders and non-responders. Second, an analysis of variance model (ANOVA) adjusted for the effects of age, sex, and BMI was carried out on each of the variables. Statistical analyses were performed with SAS statistical software, version 9.1 (SAS Institute Inc, Cary, NC). Statistical significance was defined as $P \leq 0.05$.

Results

First, a comparison of the biochemical parameters pre- and post-n-3 PUFA supplementation for 6 responders and 6 non-responders who were used for the OMICs study is presented in Table 1. In sum, the 6 responders had higher pre-supplementation plasma glucose concentrations compared to the 6 non-responders; yet, all other pre- and post-supplementation parameters were similar in both subgroups. Obviously, the n-3 PUFA supplementation was associated with a decrease in fasting TG concentrations in responders, from 1.75 to 0.99 mmol/L (−0.76 mmol/L, −41 %). In contrast, the non-responders increased their plasma TG from 1.03 to 1.12 mmol/L (0.10 mmol/L, 9 %). In parallel, the LDL-C/apo-B ratio, an indicator of LDL-particle size (Wagner et al. 2002), increased in responders and decreased in non-responders. Similarly, the change (delta value) of plasma TG and LDL-C/apo-B ratio between responders and non-responders were different ($P = 0.0153$ and $P = 0.0039$, respectively). In both, responders and non-responders, there were no differences in TC and LDL-C pre- and post-supplementation; however, there was an increase in HDL-C and a decrease in TC/

Table 2 Replication sample of responders and non-responders pre- and post-n-3 PUFA supplementation

	Responders (n = 60)			Non-responders (n = 60)			P value between resp. and non-resp.	
	Pre-n-3 PUFAs	Post-n-3 PUFAs	P value	Pre-n-3 PUFAs	Post-n-3 PUFAs	P value	Pre-n-3 PUFAs	Delta (post- and pre-n-3 PUFAs)
Gender	26 men (43 %) and 34 women (57 %)			30 men (50 %) and 30 women (50 %)			NS	
Age (years)	32.02 ± 1.11 years			31.1 ± 1.16 years			NS	
BMI (kg/m^2)	29.3 ± 0.5	29.3 ± 0.5	NS	27.8 ± 0.5	28.0 ± 0.5	0.0007	NS	NS
Triacylglycerols[a] (mmol/L)	1.61 ± 0.09	0.99 ± 0.06	<0.0001	1.03 ± 0.06	1.20 ± 0.07	<0.0001	<0.0001	<0.0001
Cholesterol (mmol/L)								
Total	4.94 ± 0.12	4.83 ± 0.13	NS	4.77 ± 0.13	4.87 ± 0.14	NS	NS	0.0264
LDL	2.87 ± 0.11	2.94 ± 0.12	NS	2.79 ± 0.12	2.84 ± 0.13	NS	NS	NS
HDL[a]	1.33 ± 0.05	1.44 ± 0.05	<0.0001	1.50 ± 0.05	1.48 ± 0.05	NS	0.0142	0.0001
Ratio TC/HDL-C	3.90 ± 0.13	3.59 ± 0.14	<0.0001	3.33 ± 0.12	3.48 ± 0.14	0.0054	0.0018	<0.0001
Apo-B 100 (g/L)	0.89 ± 0.03	0.91 ± 0.03	NS	0.83 ± 0.03	0.88 ± 0.03	0.0015	NS	NS
Ratio LDL/Apo-B	3.28 ± 0.06	3.20 ± 0.05	NS	3.36 ± 0.06	3.20 ± 0.07	0.0098	NS	NS
Glucose (mmol/L)[a]	5.15 ± 0.06	5.17 ± 0.06	NS	4.94 ± 0.05	5.04 ± 0.07	NS	0.0155	NS
Insulin (pmol/L)[a,*]	84.93 ± 3.47	80.91 ± 3.35	NS	75.31 ± 3.31	82.70 ± 3.90	0.0456	0.0226	NS
CRP[a](mg/L)	2.78 ± 0.47	2.69 ± 0.40	NS	2.87 ± 0.68	3.06 ± 0.83	NS	NS	NS

Data are shown as mean ± SEM

TC total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

* Subjects excluded for responders (n = 5 for pre-n-3 PUFAs and n = 3 for post-n-3 PUFAs) and non-responders (n = 2 for pre-n-3 PUFAs and n = 4 for post-n-3 PUFAs)

[a] P value derived from transformed data

HDL-C ratio in responders. In addition, the delta of LDL-C levels between responders and non-responders were different ($P = 0.0039$).

Further, results of the biochemical characteristics of the sub-group of 6 responders and 6 non-responders used for the OMICs study were validated in a second group of 60 responders and 60 non-responders from the entire cohort ($n = 210$), as described in Table 2. In sum, the pre-supplementation characteristics of 60 non-responders compared to the 60 best-responders were similar; except that higher plasma TG ($P < 0.0001$), glucose ($P = 0.016$), insulin ($P = 0.023$), and ratio TC/HDL-C ($P = 0.0018$) together with lower HDL-C levels ($P = 0.014$) were observed in responders. Obviously, the plasma TG decreased in responders ($P < 0.0001$) and increased in non-responders ($P < 0.0001$). Further, responders increased HDL-C levels ($P < 0.0001$) and decreased TC/HDL-C ratio ($P < 0.0001$) after n-3 PUFA supplementation. In contrast, non-responders increased their TC/HDL-C ratio ($P = 0.005$) as well as insulin ($P = 0.046$), apoB levels ($P = 0.0015$), and LDL/apo-B ratio ($P = 0.0098$). Finally, changes (delta) pre- to post-n-3 PUFA supplementations were different between the responders and non-responder subgroup for TG ($P < 0.0001$), TC ($P = 0.026$) levels including HDL-C ($P = 0.0001$) and TC/HDL-C ratio ($P < 0.0001$). Further, the same variables were statistically different between groups after further adjustments for age, sex, and BMI (data not shown). In sum, the 6 individuals that are classified as responders and non-responders analyzed in the OMICs sub-study represent adequately the characteristics of the responders and non-responders of the entire cohort.

Fatty acids analysis

The 6-week n-3 PUFA supplementation as expected increased the concentration of total n-3 and decreased total n-6 FA content in erythrocytes in both subgroups (Table 3). In brief, the n-3 PUFA supplementation was associated with a similar increase in total n-3 in erythrocytes in both subgroups, from 7.11 to 10.38 % of total free FA for responders and from 7.00 to 10.21 % of total free FA for non-responders. In the responder subgroup, increases were also observed for EPA, DHA, and n-3/n-6 FAs ratio after n-3 PUFA supplementation. In the non-responder subgroup, an increase was observed for EPA and ratio of n-3/n-6 FAs; however, the change in DHA content in erythrocytes did not reach statistical significance probably due to large inter-individual variability. Further, D5D increased and ELOVL2 decreased in both subgroups. Yet,

Table 3 Erythrocytes fatty acid profiles of responders and non-responders pre- and post-n-3 PUFA supplementation

(% of free FA)	Responders ($n = 6$)			Non-responders ($n = 6$)			*P* value between resp. and non-resp.	
	Pre-n-3 PUFAs	Post-n-3 PUFAs	*P* value	Pre-n-3 PUFAs	Post-n-3 PUFAs	*P* value	Pre-n-3 PUFAs	Delta (post- and pre-n-3 PUFAs)
Stearic acid (18:0)	15.37 ± 0.33	15.42 ± 0.27	NS	15.03 ± 0.34	15.10 ± 0.33	NS	NS	NS
Oleic acid (18:1n-9)	11.93 ± 0.10	11.56 ± 0.25	NS	11.57 ± 0.29	11.46 ± 0.32	NS	NS	NS
Arachidonic acid (20:4n-6)	14.06 ± 0.16	12.87 ± 0.28	0.0118	14.10 ± 0.27	12.87 ± 0.19	0.0004	NS	NS
Dihomo-gamma-linolenic acid (20:3n-6)	2.06 ± 0.26	1.56 ± 0.19	0.0051	1.83 ± 0.07	1.43 ± 0.10	0.0054	NS	NS
Eicosapentanoic acid (20:5n-3)	0.67 ± 0.09	2.33 ± 0.23	<0.0001	0.77 ± 0.12	2.12 ± 0.24	0.0044	NS	NS
Docosahexaenoic acid (22:6n-3)	4.01 ± 0.56	5.05 ± 0.43	0.0059	4.10 ± 0.35	5.36 ± 0.47	NS	NS	NS
Total N-6	29.10 ± 0.71	25.54 ± 0.92	0.0002	29.44 ± 0.48	25.99 ± 0.57	0.0010	NS	NS
Total N-3	7.11 ± 0.66	10.38 ± 0.71	<0.0001	7.00 ± 0.52	10.21 ± 0.73	0.0014	NS	NS
Ratio total N-3/total N-6	0.25 ± 0.03	0.41 ± 0.04	0.0001	0.24 ± 0.02	0.40 ± 0.04	0.0018	NS	NS
Desaturase indices								NS
SCD18 (18:1n-9/18:0)	0.78 ± 0.02	0.75 ± 0.02	0.0153	0.77 ± 0.02	0.76 ± 0.02	NS	NS	NS
FADS1 D5D (20:4n-6/20:3n-6)	7.31 ± 0.78	8.80 ± 0.95	0.0053	7.76 ± 0.29	9.17 ± 0.61	0.0174	NS	NS
ELOVL2 (22:6n-3/20:5n-3)	6.04 ± 0.32	2.20 ± 0.10	<0.0001	5.60 ± 0.48	2.67 ± 0.37	0.0057	NS	NS

Data are shown as mean ± SEM; supplementation is the effect of n-3 PUFA supplementation on parameters

RBC red blood cells, *FA* fatty acid, *EPA* eicosapentanoic acid, *DHA* docosahexaenoic acid

SCD18 decreased in responders and was not changed in non-responders. There were no differences between the delta for FA composition between responders and non-responders.

Changes in metabolites

The results from the entire panel of metabolites analyzed with the Biocrates Absolute IDQ p150 are available in Supplemental file. Briefly, results demonstrate that there was an increase in unsaturated FA in glyPC (Supplemental file: Table A) to a greater extent in responders as compared to non-responders after the n-3 PUFA supplementation period. In addition, the ratio of glyPC aa C40:5/glyPC aa C40:6 in responders and non-responders decreased after n-3 PUFA supplementation (from 0.53 to 0.31 ($P = 0.05$), and from 0.47 to 0.31 ($P = 0.21$), respectively, for responders and non-responders). Additionally, four glycerophospholipids (PC aa C38:4, PC aa C40:4, PC ae C36:1, PC ae C44:4) changed differently in response to n-3 PUFA supplementation and there was a decrease in responders but not in non-responders. Further, results demonstrate no major differences between responders and non-responders for SM (Supplemental file: Table B), lysoPC (Supplemental file: Table C), AC (Supplemental file: Table D), and amino acid (Supplemental file: Table E) concentrations after n-3 PUFA supplementation. Nevertheless, the hexose (Supplemental file: Table F) concentrations decreased in responders' subgroup only after n-3 PUFA supplementation.

Changes in gene expression levels

First, we compared the pre- to post-n- 3 PUFA supplementation gene expression values to adjust for baseline differences since microarrays are more appropriate to distinguish changes in the relative gene expression. In the responder subgroup, 252 transcripts were differentially expressed (38 % (97) down-regulated and 62 % (155) up-regulated) when comparing the pre- to post-levels of gene expression after n-3 PUFA supplementation. In the non-responder subgroup, 1,020 transcripts were differentially expressed (37 % (376) down-regulated and 63 % (644) up-regulated) when comparing the pre- to post-n-3 PUFA supplementation gene expression levels. Finally, only 10 transcripts were changed in both subgroups. Previously, this microarray data were validated with real-time RT-PCR for the entire cohort ($n = 30$ subjects) and demonstrate that results are highly correlated between both methods for (Rudkowska et al. 2013).

Pathway analysis results

We first examined the pathways reported to be affected by an n-3 PUFA supplementation (Bouwens et al. 2009; Rudkowska et al. 2011a, 2012) and found no noteworthy

Table 4 Changes in the expression of genes in PBMCs involved in glycerophospholipid metabolism after n-3 PUFA supplementation in responders and non-responders ($P = 0.02$ and $P = 0.02$, respectively)

Gene symbol	Gene name	Responders ($n = 6$)	Non-responders ($n = 6$)
AGPAT3	1-Acylglycerol-3-phosphate O-acyltransferase 3	1.223	
AGPAT4	1-Acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)	−1.238	
CDIPT	CDP-diacylglycerol–inositol 3-phosphatidyltransferase		1.257
CHAT	Choline O-acetyltransferase	−1.273	
CHKA	Choline kinase alpha	−1.306	
CHKB	Choline kinase beta		−1.565
DGKE	Diacylglycerol kinase, epsilon 64 kDa		−1.454
DGKZ	Diacylglycerol kinase, zeta		−1.256
ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase 2	1.247	−1.418
ETNK1	Ethanolamine kinase 1	−1.214	−1.343
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	−1.422	1.502
MGLL	Monoglyceride lipase	1.547	−1.290
NAPEPLD	N-Acyl phosphatidylethanolamine phospholipase D	−1.357	−1.382
PEMT	Phosphatidylethanolamine N-methyltransferase	1.338	
PHKA1	Phosphorylase kinase, alpha 1 (muscle)	1.222	−1.204
PIGF	Phosphatidylinositol glycan anchor biosynthesis, class F		1.229
PIGO	Phosphatidylinositol glycan anchor biosynthesis, class O	1.279	
PLA2G6	Phospholipase A2, group VI (cytosolic, calcium-independent)	1.378	
PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	−1.203	
PLA2G2C	Phospholipase A2, group IIC		1.396
PLA2G2D	Phospholipase A2, group IID		1.232
PLA2G2F	Phospholipase A2, group IIF	1.294	
PLA2G4A	Phospholipase A2, group IVA (cytosolic, calcium-dependent)		−1.250
PLCB1	Phospholipase C, beta 1 (phosphoinositide-specific)		1.246
PLCD3	Phospholipase C, delta 3		1.340
PLCL1	Phospholipase C-like 1	−1.504	1.450
PPAP2A	Phosphatidic acid phosphatase type 2A	1.336	1.207
PPAP2B	Phosphatidic acid phosphatase type 2B		1.283
PPAP2C	Phosphatidic acid phosphatase type 2C	1.251	−1.462
PPAPDC1A	Phosphatidic acid phosphatase type 2 domain containing 1A	−1.276	

difference between the responder and non-responder subgroups for the oxidative stress response mediated by nuclear factor (erythroid-derived 2)-like 2 (NrF2), mechanism of gene regulation by peroxisome proliferators via peroxisome proliferator-activated receptor alpha (*PPARA*), hypoxia-inducible factor (HIF) signaling, nuclear factor kB (NF-kB) signaling pathway, and oxidative stress (data not shown). From the metabolomic results, the lipid metabolism seems to be the most different between responders and non-responders to an n-3 PUFA supplementation. Therefore, we examined the pathways in the lipid metabolism category and found the main changes in these 4 pathways (*P* value from Fisher Exact Test): glycerophospholipid metabolism ($P = 0.02$ responders; $P = 0.02$ non-responders) (Table 4), sphingolipid metabolism ($P = 0.27$ responders; $P = 0.04$ non-responders) (Table 5), arachidonic acid metabolism ($P = 0.52$ responders; $P = 0.11$ non-responders) (Table 6), and linoleic acid metabolism ($P =$ NS responders; $P = 0.08$ non-responders) (Table 7). Other pathways in the lipid metabolism were not modified.

Discussion

In this study sample of 30 participants, 20 % of subjects did not lower their plasma TG concentrations with a 3 g/d of EPA and DHA for 6 weeks. Further, the 12 individuals who were identified as either responders or non-responders in the sub-study represented adequately the characteristics of the responders and non-responders of the entire cohort. Taken as a whole, responders have a more deteriorated

Table 5 Changes in the expression of genes in PBMCs involved in sphingolipid metabolism after n-3 PUFA supplementation in responders and non-responders ($P = 0.27$ and $P = 0.04$, respectively)

Gene symbol	Gene name	Responders ($n = 6$)	Non-responders ($n = 6$)
ACER2	Alkaline ceramidase 2		1.378
ARSK	Arylsulfatase family, member K	−1.209	
ASAH1	N-Acylsphingosine amidohydrolase (acid ceramidase) 1		−1.281
ASAH2C	N-Acylsphingosine amidohydrolase (non-lysosomal ceramidase) 2C	−1.361	−1.226
ENPP7	Ectonucleotide pyrophosphatase/phosphodiesterase 7	1.212	
FUT7	Fucosyltransferase 7 (alpha (1,3) fucosyltransferase)		−1.608
GBA3	Glucosidase, beta, acid 3 (cytosolic)		−1.419
KDSR	3-Ketodihydrosphingosine reductase	1.244	1.223
PIGO	Phosphatidylinositol glycan anchor biosynthesis, class O	1.279	
PPAP2A	Phosphatidic acid phosphatase type 2A	1.336	1.207
PPAP2B	Phosphatidic acid phosphatase type 2B		1.283
PPAP2C	Phosphatidic acid phosphatase type 2C	1.251	−1.462
PPAPDC1A	Phosphatidic acid phosphatase type 2 domain containing 1A	−1.276	
PTPRJ	Protein tyrosine phosphatase, receptor type, J		−1.241
SGMS1	Sphingomyelin synthase 1		1.244
SGMS2	Sphingomyelin synthase 2		−1.233
SGPP1	Sphingosine-1-phosphate phosphatase 1		−1.271
SPHK1	Sphingosine kinase 1	1.247	
UGT8	UDP glycosyltransferase 8		−1.315

biochemical profiles which are linked to more favorable changes following n-3 PUFA supplementation. FA composition after n-3 PUFA supplementation between responders and non-responders was similar, except for DHA content and SCD18 ratio. Further, changes in glycerophospholipid metabolism were observed in both subgroups and sphingolipid metabolism in non-responders; nevertheless, 6 genes including: fatty acid desaturase 2 (*FADS2*), phospholipase A2 group IVA (*PLA2G4A),* arachidonate 15-lipoxygenase (*ALOX15*), phosphatidylethanolamine *N*-methyltransferase (*PEMT*), monoglyceride lipase (*MGLL),* and glycerol-3-phosphate acyltransferase (*GPAM*) were expressed in opposite direction between responders and non-responders.

Pre-n-3 PUFA supplementation characteristics of responders and non-responders did not differ except for plasma glucose concentrations which were higher in responders. In the responders' subgroup, hexose concentrations decreased without changing plasma glucose or insulin concentrations after n-3 PUFA supplementation. Previous studies examining the effects of an n-3 PUFA supplementation on glucose-insulin homeostasis are unclear (Hartweg et al. 2008). On the other hand, plasma glucose and TG levels are closely related to the metabolic syndrome and previous research has shown that individuals with higher TG levels seem to benefit the most from an n-3 PUFA supplementation (Balk et al. 2006).

After the n-3 PUFA supplementation, the non-responder subgroup did not change DHA concentrations in RBCs as the responders' subgroup increased levels, despite the fact that both groups had a similar compliance in clinical study. Metabolomic data also demonstrate an increase in unsaturated FA to a greater extent in responders than in non-responders after the n-3 PUFA supplementation period. In particular, the ratio of glyPC aa C40:5/glyPC aa C40:6 decreased in responders. The decrease in the ratio of glyPC aa C40:5/glyPC aa C40:6 corresponds to an increase in DHA levels (Altmaier et al. 2011). Further, four glycerophospholipids changed differently in response to an n-3 PUFA supplementation between two groups. Previously, PC aa C38:4 concentration was positively related to "sausage/ham" food group, the PC aa C40:4/PC aa C40:6 ratio was inversely related to "fish" food group, and a higher PC ae C36:1/PC aa C38:4 ratio was related to "flaked oats, muesli, and cornflakes" (Altmaier et al. 2011), which reveal that the responders may have had a healthier FA profile post-n-3 PUFA supplementation. In addition, this is also observed in the transcriptomic profiles of glycerophospholipid metabolism after n-3 PUFA supplementation, which changed to different extents in both subgroups. Overall, FA incorporation in plasma and RBCs were different between responders and non-responders.

Dietary FA intake changes the composition of cellular membrane, which may result in changes in membrane

Table 6 Changes in the expression of genes in PBMCs involved in arachidonic acid metabolism after n-3 PUFA supplementation in responders and non-responders ($P = 0.52$ and $P = 0.11$, respectively)

Gene symbol	Gene name	Responders ($n = 6$)	Non-responders ($n = 6$)
ALOX5	Arachidonate 5-lipoxygenase		−1.229
ALOX15	Arachidonate 15-lipoxygenase	1.216	
ALOX15B	Arachidonate 15-lipoxygenase, type B		1.302
CYP2F1	Cytochrome P450, family 2, subfamily F, polypeptide 1		−1.214
CYP2J2	Cytochrome P450, family 2, subfamily J, polypeptide 2		1.235
CYP4F3	Cytochrome P450, family 4, subfamily F, polypeptide 3	−1.269	−1.545
CYP4F8	Cytochrome P450, family 4, subfamily F, polypeptide 8		−1.326
GGT1	Gamma-glutamyltransferase 1	1.203	−1.313
GGT2	Gamma-glutamyltransferase 2		1.324
GGT3	Gamma-glutamyltransferase 3 pseudogene	−1.298	
GGT7	Gamma-glutamyltransferase 7	−1.307	1.274
GPX1	Glutathione peroxidase 1		−1.261
LCAT	Lecithin-cholesterol acyltransferase		1.225
PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	−1.203	
PLA2G2C	Phospholipase A2, group IIC		1.396
PLA2G2D	Phospholipase A2, group IID		1.232
PLA2G2F	Phospholipase A2, group IIF	1.294	
PLA2G4A	Phospholipase A2, group IVA (cytosolic, calcium-dependent)		−1.250
PLA2G6	Phospholipase A2, group VI (cytosolic, calcium-independent)	1.378	

Table 7 Changes in the expression of genes in PBMCs involved in linoleic acid metabolism after n-3 PUFA supplementation in responders and non-responders (P = NS and $P = 0.08$, respectively)

Gene symbol	Gene name	Responders ($n = 6$)	Non-responders ($n = 6$)
ALOX5	Arachidonate 5-lipoxygenase		−1.229
ALOX15B	Arachidonate 15-lipoxygenase, type B		1.302
CYP2F1	Cytochrome P450, family 2, subfamily F, polypeptide 1		−1.214
CYP2J2	Cytochrome P450, family 2, subfamily J, polypeptide 2		1.235
CYP4F8	Cytochrome P450, family 4, subfamily F, polypeptide 8		−1.326
FADS2	Fatty acid desaturase 2		−1.306
LCAT	Lecithin-cholesterol acyltransferase		1.225
PLA2G2C	Phospholipase A2, group IIC		1.396
PLA2G2D	Phospholipase A2, group IID		1.232
PLA2G4A	Phospholipase A2, group IVA (cytosolic, calcium-dependent)		−1.250

fluidity and permeability along with the functions of the cells (Das 2001; Frisardi et al. 2011; Gieger et al. 2008; Ma et al. 2004). High tissue PUFA concentrations have been shown to have beneficial effects on the metabolic syndrome (Vessby 2003). Since tissue FA composition is influenced not only by dietary fat but also by the endogenous metabolism of FA and genetic variations (Madden et al. 2011; Simopoulos 2010), it is possible that these non-responders carry particular polymorphisms which may influence the ability to modify FA levels in cellular membranes. Examining the transcriptomic data, differences in expression levels of genes that modify FA composition were observed between responders and non-responders after an n-3 PUFA supplementation. Specifically, *FADS2* is decreased in non-responders subgroup potentially indicating that this particular gene, which encodes a desaturase enzyme regulating unsaturation of FAs, is down-regulated to a greater extent by n-3 PUFA supplementation. Previous research has demonstrated that *FADS* gene clusters, especially SNPs from *FADS1* and *FADS2*, are major determinants of plasma DHA concentrations and hence plasma TG levels (Gieger et al. 2008;

Suhre et al. 2011). Further, *PLA2* was changed after n-3 PUFA supplementation, but to different extents in both groups. The *PLA2* represent an important superfamily of enzymes that catalyze the rate limiting hydrolysis of membrane phospholipids. Studies have shown that EPA can be released by the actions of calcium-independent PLA2 (iPLA2; type VI (*PLA2G6*)) and DHA released by cytosolic PLA2 (cPLA2; type IV (*PLA2G4A*)) (Denys et al. 2005; Lassegue and Clempus 2003). Recently, Hartiala et al. (2012) demonstrated that a functional variant (rs12746200) of *PLA2G4A* was associated with a CVD phenotype mediated by dietary PUFAs. Additionally, the expression levels of *ALOX15* increased in responders. The activation of ALOX15 protein has been shown to increase synthesis of DHA (Kenchegowda and Bazan 2010; Wittwer and Hersberger 2007). In addition, both subgroups increased patatin-like phospholipase domain containing 2 (*PNPLA2*), which catalyzes the initial step in TG hydrolysis and may influence quantity of DHA in cytoplasm (Notari et al. 2006). Thus, these differences in expression levels of genes that may influence FA composition in cell membranes should be investigated further.

Additionally, the SCD18 desaturation ratio did not change in non-responders after the n-3 PUFA supplementation. A recent clinical trial showed that fish intake may lower plasma TG by reducing the SCD18 ratio; however, mRNA levels of *SCD* in PBMCs were not altered after intake of fish (Telle-Hansen et al. 2011). In the current study, *SCD* gene expression levels were also not changed. Overall, SCD levels may contribute to plasma TG concentrations and consequently may impact the response to an n-3 PUFA supplementation.

N-3 PUFAs lower plasma TG concentrations by enhancing free FA β-oxidation, increasing catabolism of chylomicrons and very low-density lipoproteins (VLDL), as well as up-regulating the expression of the lipoprotein lipase (*LPL*) (Qi et al. 2008). Metabolomic results demonstrate no major differences in AC levels between responders and non-responders. However, previous results show that an n-3 PUFA supplementation does increase AC concentrations, particularly in men and consequently indicating an increase in β-oxidation rate (Rudkowska et al. 2012). Further, we examined differentially expressed genes in the lipid metabolism, which could influence plasma TG concentrations. First, *PEMT* encodes an enzyme which catalyzes the methylation of phosphatidylethanolamine to form phosphatidylcholine, which is required for hepatic secretion of TG in VLDL. Results show that *PEMT* gene expression was increased in responders only. Additionally, a study showed that *PEMT*-deficient mice also had substantially diminished concentrations of DHA and arachidonic acid in plasma (Watkins et al. 2003). Second, data shows that responders also increased *MGLL* gene after n-3 PUFA supplementation. *MGLL* functions together with hormone-sensitive lipase (*LIPE*) to hydrolyze TG stores. *MGLL* also complements *LPL* in completing for hydrolysis of monoglycerides resulting from the degradation of lipoprotein TG. Third, *GPAM* is known to play a key role in lipid biosynthesis (Wendel et al. 2009), which was decreased in responders while being increased in non-responders. Overall, future studies should investigate on how n-3 PUFA supplementation regulates the transcription of these key genes.

In addition, metabolomic results demonstrate that cholesterol levels changed even if the SM did not differ after n-3 PUFA supplementation; yet, transcriptomic data also indicate a change in SM metabolism in the non-responder subgroup. Since cholesterol concentrations are known to slightly alter after n-3 PUFA supplementation (Balk et al. 2006); n-3 PUFA supplementation has modified cholesterol metabolism in the non-responders to a greater extent.

In conclusion, this study demonstrates that individuals having a more deteriorated metabolic profile before the supplementation respond better to n-3 PUFA supplementation. In addition, differences in FA incorporation as well as TG and cholesterol metabolism together with differences in expression of key genes exit between subjects' responders and non-responders to an n-3 PUFA supplementation.

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References

Albers JJ, Warnick GR, Wiebe D, King P, Steiner P, Smith L, Breckenridge C, Chow A, Kuba K, Weidman S, Arnett H, Wood P, Shlagenhaft A (1978) Multi-laboratory comparison of three heparin-Mn2 + precipitation procedures for estimating cholesterol in high-density lipoprotein. Clin Chem 24:853–856

Altmaier E, Kastenmuller G, Romisch-Margl W, Thorand B, Weinberger KM, Illig T, Adamski J, Doring A, Suhre K (2011) Questionnaire-based self-reported nutrition habits associate with serum metabolism as revealed by quantitative targeted metabolomics. Eur J Epidemiol 26:145–156

Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J (2006) Effects of omega-3 fatty acids on serum markers of

cardiovascular disease risk: a systematic review. Atherosclerosis 189:19–30
Blazejczyk M, Miron M, Nadon R (2007) FlexArray: a statistical data analysis software for gene expression microarrays. Génome Québec, Montreal, Canada. http://genomequebec.mcgill.ca/FlexArray
Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19:185–193
Bouwens M, van de Rest O, Dellschaft N, Bromhaar MG, de Groot LC, Geleijnse JM, Muller M, Afman LA (2009) Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr 90:415–424
Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC, Gaasterland T, Glenisson P, Holstege FC, Kim IF, Markowitz V, Matese JC, Parkinson H, Robinson A, Sarkans U, Schulze-Kremer S, Stewart J, Taylor R, Vilo J, Vingron M (2001) Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet 29:365–371
Burstein M, Samaille J (1960) On a rapid determination of the cholesterol bound to the serum alpha- and beta-lipoproteins. Clin Chim Acta 5:609
Caslake MJ, Miles EA, Kofler BM, Lietz G, Curtis P, Armah CK, Kimber AC, Grew JP, Farrell L, Stannard J, Napper FL, Sala-Vila A, West AL, Mathers JC, Packard C, Williams CM, Calder PC, Minihane AM (2008) Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. Am J Clin Nutr 88:618–629
Das UN (2001) Nutritional factors in the pathobiology of human essential hypertension. Nutrition 17:337–346
Denys A, Hichami A, Khan NA (2005) n-3 PUFAs modulate T-cell activation via protein kinase C-alpha and -epsilon and the NF-kappaB signaling pathway. J Lipid Res 46:752–758
Desbuquois B, Aurbach GD (1971) Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab 33:732–738
Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
Frisardi V, Panza F, Seripa D, Farooqui T, Farooqui AA (2011) Glycerophospholipids and glycerophospholipid-derived lipid mediators: a complex meshwork in Alzheimer's disease pathology. Prog Lipid Res 50:313–330
Gieger C, Geistlinger L, Altmaier E, de Hrabe AM, Kronenberg F, Meitinger T, Mewes HW, Wichmann HE, Weinberger KM, Adamski J, Illig T, Suhre K (2008) Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. PLoS Genet 4:e1000282
Harris WS (1997) n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 65:1645S–1654S
Hartiala J, Gilliam E, Vikman S, Campos H, Allayee H (2012) Association of PLA2G4A with myocardial infarction is modulated by dietary PUFAs. Am J Clin Nutr 95:959–965
Hartweg J, Perera R, Montori V, Dinneen S, Neil HA, Farmer A (2008) Omega-3 polyunsaturated fatty acids (PUFA) for type 2 diabetes mellitus. Cochrane Database Syst Rev CD003205
Kenchegowda S, Bazan HE (2010) Significance of lipid mediators in corneal injury and repair. J Lipid Res 51:879–891
Lassegue B, Clempus RE (2003) Vascular NAD(P)H oxidases: specific features, expression, and regulation. Am J Physiol Regul Integr Comp Physiol 285:R277–R297
Laurell CB (1966) Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 15:45–52
Lepage G, Roy CC (1986) Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 27:114–120
Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA (2006) The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. J Lab Clin Med 147:126–132
Ma DW, Seo J, Switzer KC, Fan YY, McMurray DN, Lupton JR, Chapkin RS (2004) n-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. J Nutr Biochem 15:700–706
Madden J, Williams CM, Calder PC, Lietz G, Miles EA, Cordell H, Mathers JC, Minihane AM (2011) The impact of common gene variants on the response of biomarkers of cardiovascular disease (CVD) risk to increased fish oil fatty acids intakes. Annu Rev Nutr 31:203–234
Notari L, Baladron V, Aroca-Aguilar JD, Balko N, Heredia R, Meyer C, Notario PM, Saravanamuthu S, Nueda ML, Sanchez–Sanchez F, Escribano J, Laborda J, Becerra SP (2006) Identification of a lipase-linked cell membrane receptor for pigment epithelium-derived factor. J Biol Chem 281:38022–38037
Pirro M, Bergeron J, Dagenais GR et al (2001) Age and duration of follow-up as modulators of the risk for ischemic heart disease associated with high plasma C-reactive protein levels in men. Arch Intern Med 161:2474–2480
Qi K, Fan C, Jiang J, Zhu H, Jiao H, Meng Q, Deckelbaum RJ (2008) Omega-3 fatty acid containing diets decrease plasma triglyceride concentrations in mice by reducing endogenous triglyceride synthesis and enhancing the blood clearance of triglyceride-rich particles. Clin Nutr 27:424–430
Richterich R, Dauwalder H (1971) Determination of plasma glucose by hexokinase-glucose-6-phosphate dehydrogenase method. Schweiz Med Wochenschr 101:615–618
Rudkowska I, Ponton A, Jacques H, Lavigne C, Holub BJ, Marette A, Vohl MC (2011a) Effects of a supplementation of n-3 polyunsaturated fatty acids with or without fish gelatin on gene expression in peripheral blood mononuclear cells in obese, insulin-resistant subjects. J Nutrigenet Nutrigenomics 4:192–202
Rudkowska I, Raymond C, Ponton A, Jacques H, Lavigne C, Holub BJ, Marette A, Vohl MC (2011b) Validation of the use of peripheral blood mononuclear cells as surrogate model for skeletal muscle tissue in nutrigenomic studies. OMICS 15:1–7
Rudkowska I, Paradis AM, Thifault E, Julien P, Tchernof A, Couture P, Lemieux S, Barbier O, Vohl MC (2013) Transcriptomic and metabolomic signatures of an n-3 polyunsaturated fatty acids supplementation in a normolipidemic/normocholesterolemic Caucasian population. J Nutr Biochem 24:54–61
Shaikh NA, Downar E (1981) Time course of changes in porcine myocardial phospholipid levels during ischemia. A reassessment of the lysolipid hypothesis. Circ Res 49:316–325
Simopoulos AP (2010) Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk. Exp Biol Med (Maywood) 235:785–795
Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wagele B, Altmaier E, Deloukas P, Erdmann J, Grundberg E, Hammond CJ, de Angelis MH, Kastenmuller G, Kottgen A, Kronenberg F, Mangino M, Meisinger C, Meitinger T, Mewes HW, Milburn MV, Prehn C, Raffler J, Ried JS, Romisch-Margl W, Samani NJ, Small KS, Wichmann HE, Zhai G, Illig T, Spector TD, Adamski J, Soranzo N, Gieger C (2011) Human metabolic individuality in biomedical and pharmaceutical research. Nature 477:54–60
Telle-Hansen VH, Larsen LN, Hostmark AT, Molin M, Dahl L, Almendingen K, Ulven SM (2011) Daily intake of cod or salmon for 2 weeks decreases the 18:1n-9/18:0 ratio and serum Triacylglycerols in healthy subjects. Lipids

Tusher VG, Tibshirani R, Chu G (2001) Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci USA 98:5116–5121

Vessby B (2003) Dietary fat, fatty acid composition in plasma and the metabolic syndrome. Curr Opin Lipidol 14:15–19

Wagner AM, Jorba O, Rigla M, Alonso E, Ordonez-Llanos J, Perez A (2002) LDL-cholesterol/apolipoprotein B ratio is a good predictor of LDL phenotype B in type 2 diabetes. Acta Diabetol 39:215–220

Watkins SM, Zhu X, Zeisel SH (2003) Phosphatidylethanolamine-N-methyltransferase activity and dietary choline regulate liver-plasma lipid flux and essential fatty acid metabolism in mice. J Nutr 133:3386–3391

Wendel AA, Lewin TM, Coleman RA (2009) Glycerol-3-phosphate acyltransferases: rate limiting enzymes of triacylglycerol biosynthesis. Biochim Biophys Acta 1791:501–506

Wittwer J, Hersberger M (2007) The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins Leukot Essent Fatty Acids 77:67–77

Wittwer J, Rubio-Aliaga I, Hoeft B, Bendik I, Weber P, Daniel H (2011) Nutrigenomics in human intervention studies: current status, lessons learned and future perspectives. Mol Nutr Food Res 55:341–358