ORIGINAL PAPER

Omega-3 fatty acids regulate gene expression levels differently in subjects carrying the *PPARα* L162V polymorphism

Iwona Rudkowska · Christophe Garenc ·
Patrick Couture · Marie-Claude Vohl

Abstract Omega-3 fatty acids (FAs) are natural ligands of the peroxisome proliferator-activated receptor-α (PPARα), a nuclear receptor that modulates expression levels of genes involved in lipid metabolism. The L162V polymorphism of the *PPARα* gene is associated with a deteriorated metabolic profile. We postulate that subjects carrying the *PPARα*-V162 allele exhibit differences in the expression of *PPARα* and its target genes after incubation with omega-3 FAs compared with L162 homozygotes. Peripheral blood monocytes from six men carrying the *PPARα*-V162 allele paired for age and for body mass index with six L162 homozygotes were differentiated into macrophages and activated with eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or mixtures of EPA:DHA. Data demonstrates that gene expression levels of *PPARα* and apolipoprotein AI (*APOA1*) were significantly lower for carriers of the *PPARα*-V162 allele compared to L162 homozygotes after the addition of DHA and a mixture of EPA:DHA. Additionally, lipoprotein lipase (*LPL*) gene expression displayed a tendency to be lower in the *PPARα* L162V polymorphism subgroup after the addition of a mixture of EPA:DHA. Consequently, individuals carrying the *PPARα*-V162 allele may demonstrate inferior improvements in their lipid profile due to alterations in gene expression rates in response to omega-3 FA supplementation.

Introduction

Omega-3 fatty acids (FAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are associated with cardiovascular benefits [2, 11]. The effects of omega-3 FAs on lipid metabolism are likely mediated by changes in gene expression. More specifically, FAs and their derivatives are natural ligands of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), which heterodimerize with the retinoid × receptor (R×R) before triggering the expression of target genes [13]. Particular target genes include lipoprotein lipase (*LPL*) [15], a central enzyme in triglyceride metabolism, and *apolipoprotein AI* (*APOAI*) [34], a key structural element in high-density lipoproteins (HDL). Overall, omega-3 FAs bind to *PPARα* and consequently have the potential to lower plasma triglyceride (TG) levels and increase HDL-cholesterol (HDL-C) levels.

In addition, numerous studies revealed associations between the *PPARα* L162V polymorphism and changes in the serum concentrations of lipids/lipoproteins [8, 21, 29–32]. Indeed, the *PPARα* L162V polymorphism alone or in interaction with dietary fat intake was shown to be associated with components of the metabolic syndrome [21, 29]. An intervention study recognized differences in lipid parameters in carriers of the *PPARα*-V162 allele versus L162 homozygotes after modification of dietary polyunsaturated to saturated fat ratio [16]. Furthermore, the *PPARα* L162V polymorphism has been also shown to have an effect on transactivation efficiency in vitro after

I. Rudkowska · C. Garenc · P. Couture · M.-C. Vohl
Lipid Research Center, CHUL Research Center,
The Nutraceuticals and Functional Foods Institute (INAF),
Quebec, Canada

M.-C. Vohl (✉)
Lipid Research Center, CHUQ-CHUL,
2705 Laurier Blvd, TR-93, Quebec, QC G1V 4G2, Canada
e-mail: marie-claude.vohl@crchul.ulaval.ca

activation by a synthetic ligand, such as fibrates [8, 25]. Additionally, a recent study demonstrated differential effects of the *PPARα* L162V polymorphism on transcriptional rate with physiological concentrations of natural ligands- omega-3 FAs [24].

Yet, it is uncertain whether subjects who carry the *PPARα*-V162 allele compared to L162 homozygotes have altered expression of *PPARα, APOA1* and *LPL* after omega-3 FA supplementation. Since *PPARα* has been identified in macrophages to control lipid metabolism [20], we hypothesize that monocytes-derived macrophages from subjects who carry the *PPARα*-V162 allele exhibit differences in the expression of key genes involved in lipid metabolism when incubated with omega-3 FAs compared with macrophages from L162 homozygotes.

Methods

Subjects

Twelve Caucasian male subjects, including six carriers of the *PPARα*-V162 allele matched according to age and body mass index (BMI) to six L162 homozygotes, participated in the study. Subjects were excluded from the study if they had taken omega-3 FA supplements for at least 6 months prior to the study, used oral hypolipidemic therapy, or had been diagnosed with diabetes, hypertension, hypothyroidism, or other known metabolic disorders. They gave written consent to participate in this study, which was approved by the ethics committee of Laval University Hospital Research Center.

Anthropometric and blood pressure measurements

Body weight, height and waist circumference were measured according to the procedures recommended by the Airlie Conference [5] and were taken at screening. BMI was calculated as weight per meter squared (kg/m^2). Resting blood pressure measurements were performed after a 5 min rest in a sitting position, Phases I and V of Korotkoff sounds being respectively used for systolic (SBP) and diastolic (DBP) blood pressures.

Dietary intake determination

A validated food-frequency questionnaire (FFQ) was administered to the participants by a registered dietician [10]. This FFQ is based on typical food items available in Quebec and contains 91 items: 27 items had between 1 and 3 sub-questions. The subjects were asked how often they consumed each item per day, per week, per month, or none at all during the last month. Several examples of portion

size and food models were provided for a better estimation of real serving consumed by the subject. Nutrient intakes from the FFQ were compiled with Nutrition Data System for Research (NDS-R) software version 4.03 (Nutrition Coordination Center, University of Minnesota, Minneapolis, USA).

Plasma lipids determination

Blood samples (65 ml) were collected from an antecubital vein into vacutainer tubes containing EDTA after a 12 h overnight fast and 48 h alcohol abstinence. Blood sample was taken to determine the PPARα-L162V genotype of each participant and to measure blood lipid levels. Plasma was separated by centrifugation (2,500×g for 10 min at 4°C). Plasma total cholesterol (TC) and TG concentrations were measured using enzymatic assays [4, 14]. The HDL-C fraction was obtained after precipitation of low-density lipoprotein (LDL) particles in the infranatant with heparin manganese chloride [1]. The LDL-cholesterol (LDL-C) was calculated with the Friedewald formula [9].

Genotype determination

Genetic analyses were performed on genomic DNA isolated from human leukocytes. The *PPARα* L162V polymorphism was determined by the polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method, as previously described [33].

Cell culture

Peripheral blood monocytes were isolated by Ficoll-Paque density gradient centrifugation (GE Healthcare) [6]. Briefly, heparinized blood was diluted with HBSS (Fisher), layered over Ficoll-Paque and centrifuged for 30 min at 600×g at room temperature. The buffy coat was collected, pelleted at 300×g for 10 min at room temperature, washed and re-suspended in RPMI 1640 media with 10% FBS (Fisher), 1% Hepes (Fisher), 1% sodium pyruvate (MP biomedical), 1% penicillin/streptomycine (Invitrogen), and 0.5% glucose (Sigma-Aldrich) to allow selection of monocytes by adhesion. Monocyte-derived macrophages were cultured for 10 days in a humidified incubator at 37°C and 5% CO$_2$. Afterwards, cells were incubated for 48 h in absence or presence of omega-3 FAs at concentrations reflecting biological plasma or red blood cells concentration [12]. In addition, we observed that higher dose of omega-3 FAs cause higher mortality of cells probably due to increase membrane fragility; thus, may potentially impact final absolute gene expression rates (unpublished data). For macrophage treatments, serum free medium/ MSFM (Burlington, ON, Canada) was used to minimize

interference of FAs found in bovine serum [3]. Cells were treated with either the serum free medium/MSFM alone (control), the vehicle (DMSO, 0.01% final concentration) alone or the treatments of EPA or/and DHA (Sigma-Aldrich). Briefly, pure EPA or DHA was dissolved by serial dilution to 10 μM in DMSO. For mixtures of 5:5 μM, 15:5 μM and 5:15 μM EPA:DHA, the dissolved individual omega-3 FAs at appropriate concentrations were mixed together.

RNA isolation

On day 12 of culture, macrophage total RNA was extracted using the RNeasy Mini Kit (QIAGEN) according to the manufacturer's instructions and stored at −80°C. After spectrophotometric quantification and verification of the total RNA quality via the Agilent 2100 Bioanalyser (Agilent Technologies, Palo Alto, CA, USA), cDNA was generated using the High Capacity cDNA Archive Kit (Applied Biosystems) as described in the protocol.

Real-time RT-PCR

cDNA was mixed with TaqMan Universal PCR Master Mix (Applied Biosystems) and a gene-specific primer and probe mixture (predeveloped TaqMan Gene Expression Assays; Applied Biosystems) in a final volume of 20 μl. The assays used were as follows: Hs00173425_m1 (*LPL*), Hs00231882_m1 (*PPARα*), Hs00163641_m1 (*APOA1*), and β-actin as the house keeping gene Hs99999903_m1 (*ACTB*). All assays used the same fluorescent reporter probe (FAM™ dye-labeled) and thus each combination treatment and gene was analyzed in individual wells on a 96-well plate. All samples were run in duplicate on an Applied Biosystem 7500 Real Time PCR System (Applied Biosystems) using the following thermal cycling profile: 50°C (2 min), 95°C (10 min), followed by 40 steps of 95°C for 15 s and 60°C for 60 s. The RT-PCR results were imported into Microsoft Excel and the average value of duplicate C_t values calculated. Data were adjusted for the endogenous control (ACTB) and then expressed as percentage of differences relative to control (serum free medium/MSFM) set to 100%.

Statistical analyses

Data are shown as mean ± SD. All data were tested for normal distribution with the Shapiro-Wilk test for normality. Statistical analyses were conducted using Student's *t* test. Paired *t* tests were used to test within-group differences using SAS 9.1. A two-tailed value of $P \leq 0.05$ was considered as significant.

Results

Subjects characteristics

The subjects' characteristics at screening are presented in Table 1 according to L162V genotype. There were a total of six subjects carrying the *PPARα*-V162 allele matched for age and BMI to 6 L162 homozygotes. The mean difference between genotype groups was 1.7 ± 3.1 years and 1.8 ± 3.9 kg/m^2. There were no significant differences between genotype groups for anthropometrical indices, blood pressures and plasma lipid concentrations.

Energy, macro and micro-nutrient intakes were similar between genotype groups. As shown in Table 2, L162 homozygotes had a significantly higher percentage of fat and saturated fat in the diet compared to carriers of the *PPARα*-V162 allele. All other macronutrients were consumed to a similar percentage in both groups.

Gene expression

Rates of gene expression levels of *PPARα*, *APOA1* and *LPL* between alleles were not different after the addition of DMSO (data not shown). Yet, after the addition of omega-3 FAs, significant differences between subgroups were observed. First, expression of *PPARα* gene in human macrophages of L162 homozygotes homozygotes tends to increase following incubation with 10 μM EPA and 10 μM DHA (Fig. 1). However, the expression of *PPARα* in macrophages from carriers of the *PPARα*-V162 allele was consistently lowered by 1–4% in comparison to L162 homozygotes. Furthermore, there was a significant difference in the expression of *PPARα* between the carriers of the *PPARα*-V162 allele and the L162 homozygotes subgroup following incubation with 10 μM DHA ($P = 0.04$) and 10:5 μM EPA:DHA ($P = 0.04$).

Secondly, similar trends were seen for the expression of *APOA1* in macrophages (Fig. 2). More specifically, the addition of EPA or DHA increased the expression of *APOA1* in L162 homozygotes. Again, the expression of *APOA1* of L162 homozygotes compared to carriers of the *PPARα*-V162 allele was 4, 5, 1, 4 and 2% greater with 10 μM EPA, 10 μM DHA, 5:5 μM EPA:DHA, 10:5 μM EPA:DHA and 5:10 μM EPA:DHA, respectively. In addition, there was a significant difference between subgroups after the incubation with 10 μM DHA ($P = 0.02$) and 10:5 μM EPA:DHA ($P = 0.01$).

Thirdly, *LPL* expression increased in L162 homozygotes after the addition of EPA, DHA or mixture 5:5 EPA:DHA compared to controls (Fig. 3). Once more, the expression of *LPL* had a tendency to be consistently lower in carriers of the *PPARα*-V162 allele than in L162 homozygotes following incubation with 10 μM EPA, 10 μM DHA, 5:5 μM

Table 1 Baseline characteristics of study participants according to the PPARα L162V polymorphism matched for age and BMI

	L162 homozygotes ($n = 6$)	V162 carriers ($n = 6$)
Age (years)	35.5 ± 11.2	37.2 ± 11.7
Weight (kg)	77.3 ± 11.5	83.4 ± 9.8
Body mass index (kg/m^2)	23.8 ± 3.4	25.6 ± 3.8
Waist circumference (cm)	87.5 ± 8.4	92.3 ± 8.4
Systolic blood pressure (mmHg)	122.0 ± 12.1	118.8 ± 9.1
Diastolic blood pressure (mmHg)	70.0 ± 9.1	73.3 ± 6.9
Cholesterol		
Total (mmol/L)	4.3 ± 1.1	4.6 ± 0.8
LDL (mmol/L)	2.5 ± 1.1	2.5 ± 0.9
HDL (mmol/L)	1.3 ± 0.3	1.3 ± 0.4
Ratio total cholesterol/HDL	3.5 ± 1.2	3.4 ± 0.9
Triglycerides (mmol/L)	1.1 ± 0.3	0.9 ± 0.3
ApoB 100 (g/l)	0.8 ± 0.3	0.9 ± 0.2

Data are shown as mean $\pm$ SD. Statistical analysis was performed using a Student's t test. No significant differences between both groups were identified

Table 2 Daily energy and nutrient intake from food frequency questionnaire of study participants according to the PPARα L162V polymorphism matched for age and BMI

	L162 homozygotes ($n = 6$)	V162 carriers ($n = 6$)
Energy, kcal/day	$3,231 \pm 518$	$3,083 \pm 684$
Lipids, g/day (%E)	126 ± 28 (35%[a])	107 ± 24 (31%[b])
SFA, g/day (%E)	44 ± 10 (12%[a])	35 ± 7.1 (10%[b])
PUFA, g/day (%E)	20 ± 6.8 (5.6%)	19 ± 5.0 (5.6%)
C18:2, g/day (%E)	17 ± 6.0 (4.7%)	16 ± 4.7 (4.7%)
C20:4, g/day (%E)	0.3 ± 0.1 (0.1%)	0.3 ± 0.1 (0.1%)
C18:3, g/day (%E)	2.7 ± 1.0 (0.7%)	1.9 ± 0.5 (0.6%)
EPA, mg/day	110 ± 50	230 ± 160
DHA, mg/day	220 ± 90	420 ± 270
MUFA, g/day (%E)	52 ± 12 (14%)	44 ± 12 (13%)
TFA, g/day (%E)	5.6 ± 2.2 (1.3%)	4.5 ± 1.3 (1.5%)
Cholesterol, mg/day	485 ± 189	454 ± 94
Carbohydrates, g/day (%E)	380 ± 66 (47%)	386 ± 112 (50%)
Proteins, g/day (%E)	139 ± 21 (17%)	139 ± 30 (18%)
Alcohol, g/day (%E)	11 ± 3.7 (2.4%)	11 ± 7.2 (2.6%)
Total dietary fiber, g/day	26 ± 4.5	29 ± 5.7

Data are shown as mean $\pm$ SD; *SFA* saturated fatty acids, *PUFA* polyunsaturated fatty acids, *C18:2* linoleic acid, *C20:4* arachidonic acid, *C18:3* α-linolenic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *MUFA* monounsaturated fatty acids, *TFA* trans fatty acids. Statistical analysis was performed using a Student's t test

[a, b] Means in the same row with different script letters are significantly different, $P < 0.05$

EPA:DHA, and 10:5 μM EPA:DHA. The expression of *LPL* tended to be different between the two subgroups after the addition of 10:5 μM EPA:DHA ($P = 0.10$). Overall, the expression of *PPARα* and target genes is consistently lower in carriers of the *PPARα*-V162 allele than in L162 homozygotes.

Discussion

Results from the current study indicate that there are disparities in gene expression levels in macrophages from carriers of the *PPARα*-V162 allele and L162 homozygotes incubated with omega-3 FAs, which suggest functional differences between genotypes. Data shows differences in *PPARα* and *APOA1* gene expression levels following incubation with DHA and mixtures of EPA:DHA, between carriers of the *PPARα*-V162 allele and L162 homozygotes. A similar trend is seen for *LPL* expression following addition of a mixture of EPA:DHA. In sum, the study clearly support the hypothesis that a gene-nutrient interaction between the *PPARα* L162V polymorphism and omega-3 FAs exists.
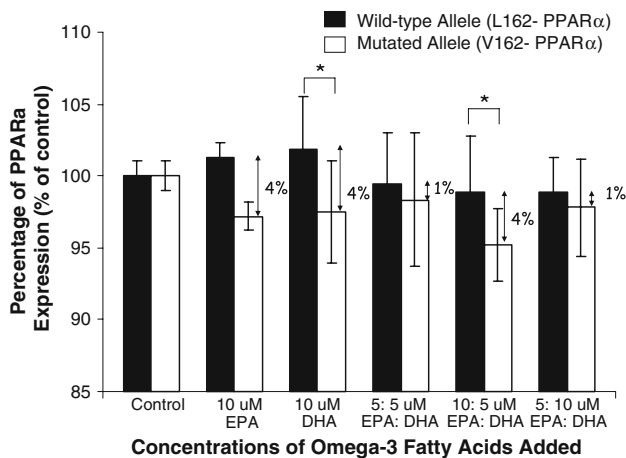
Fig. 1 Expression of *PPARα* in macrophage of subjects who carry *PPARα*-V162 (*n* = 6) allele compared to L162 homozygotes (*n* = 6) supplemented EPA, DHA, and mixtures of EPA: DHA. Duplicates of real-time RT-PCR were performed for each sample. Values were normalized with a housekeeping gene, *b-actin*, as described in "Methods". Then, treated samples were expressed as percentage of gene expression of control sample and compared between alleles. Statistical analysis was performed using a Student's *t* test. Data was not normally distributed; therefore, was transformed in LOG values for statistical analysis. *Asterisk* describes a statistically significantly different between wild-type and mutated allele (*P* ≤ 0.05)
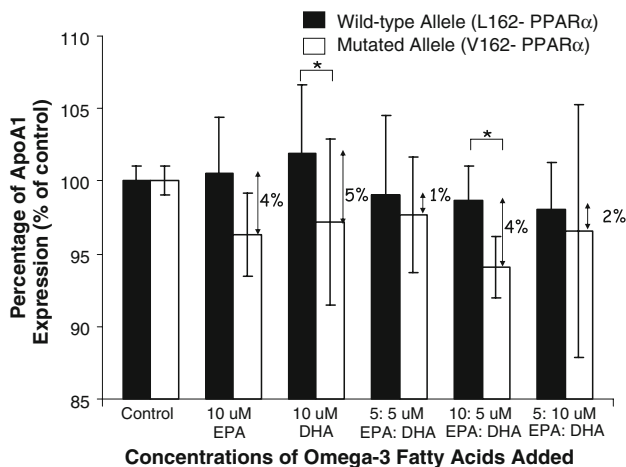

Fig. 3 Expression of *LPL* in macrophage of subjects who carry *PPARα*-V162 (*n* = 6) allele compared to L162 homozygotes (*n* = 6) supplemented EPA, DHA, and mixtures of EPA: DHA. Duplicates of real-time RT-PCR were performed for each sample. Values were normalized with a housekeeping gene, *b-actin*, as described in "Methods". Then, treated samples were expressed as percentage of gene expression of control sample and compared between alleles. Statistical analysis was performed using a Student's *t* test. Data was not normally distributed; therefore, was transformed in LOG values for statistical analysis. *Hash* describes a tendency to be different between wild-type and mutated allele (*P* ≤ 0.10)

Fig. 2 Expression of *ApoA1* in macrophage of subjects who carry *PPARα*-V162 (*n* = 6) allele compared to L162 homozygotes (*n* = 6) supplemented EPA, DHA, and mixtures of EPA: DHA. Duplicates of real-time RT-PCR were performed for each sample. Values were normalized with a housekeeping gene, *b-actin*, as described in "Methods". Then, treated samples were expressed as percentage of gene expression of control sample and compared between alleles. Statistical analysis was performed using a Student's *t* test. Data was not normally distributed; therefore, was transformed in LOG values for statistical analysis. *Asterisk* describes a statistically significantly different between wild-type and mutated allele (*P* ≤ 0.05)

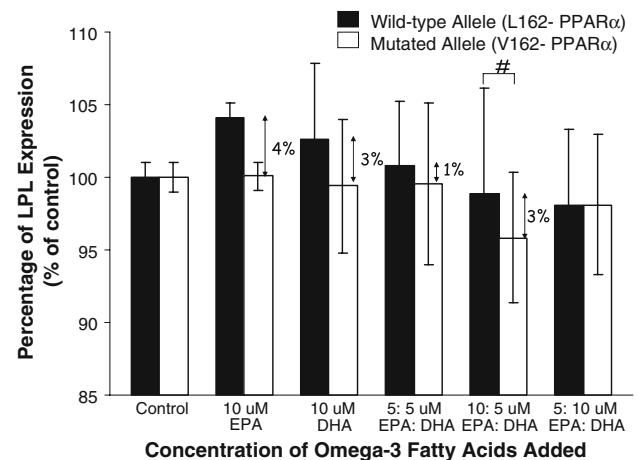First, numerous in vivo and in vitro studies have shown that *PPARα* is activated by FAs [19, 26]. *PPARα* responds to changes in both exogenous dietary fat and newly synthesized fat by triggering the expression of multiple enzymes involved in fatty acyl CoA formation and hydrolysis, FA elongation and desaturation, and FA oxidation [17]. However, certain FAs are more effective activators of *PPARα* than others. For example, adding FAs that are normally abundant in cells, such as oleic acid, has little effect on intracellular nonesterified FAs quantity and does not induce *PPARα* activity [17, 18]. In contrast, the addition of a FA that is typically a minor component of the cell, such as DHA, alters the nonesterified FAs fraction and significantly activates *PPARα* and triggers the expression of target genes [17, 18]. In this study, both EPA and DHA at physiological doses were able to modestly increase *PPARα* gene expression in L162 homozygotes, but not in carriers of the *PPARα*-V162 allele. Results also demonstrate differences between the alleles in the expressions of *PPARα* after the addition of DHA or EPA:DHA. A previous in vitro study demonstrated that at low synthetic ligand levels, the activity of PPAR-V162 isoform was lower than the wild-type isoform; however, at high synthetic ligand levels, the activity of the polymorphic receptor was higher than the wild-type [25]. Therefore, a physiological dose of omega-3 FAs was added to macrophages to represent the average values of plasma or red blood cell enrichment with omega-3 FAs that may be found after an omega-3 FAs supplementation in individuals instead of a supra-physiological dose. Further, if the mutation would be located in the ligand binding domain, it would have been suggested

that the polymorphism interferes with the ligand recognition; however, the L162V polymorphism is located in the DNA binding domain, thus, the mutation is thought to have an impact on the receptor's ability to bind DNA [25]. Generally, carriers of the PPARα-V162 allele maybe more at risk of chronic metabolic diseases, in particular if they consume low intake of omega-3 FAs, due to insufficient control of PPARα.

Second, PPARα stimulates the expression of a large set of genes involved in lipid metabolism, thus there is an interest in examining these particular genes. PPARα has been shown to regulate lipid metabolism through the control of the expression of genes encoding for apolipoproteins. Studies in transgenic mice and rabbits have demonstrated that overexpression of the human APOAI gene results in increased plasma HDL and apoAI concentrations, which provide protection against atherogenesis [7, 22, 28]. Further, synthetic ligands such as fibrates, have shown to upregulate the expression of APOAI in humans studies [34]. Additionally, human studies have also shown that fibrates lower the risk of cardiovascular diseases by raising HDL-C and lowering TG [23]. The current study shows that EPA and DHA were able to increase APOA1 gene expression beyond control samples in the L162 homozygous subgroup. The PPARα-V162 allele subgroup, did not reach the same level of APOA1 expression particularly after DHA or EPA:DHA treatment. This data may provide an explanation in regards to previous epidemiological and clinical studies where carriers of the PPARα-V162 allele demonstrated lower HDL-C and apoA1 levels [8, 16, 30, 32]. Overall, the low plasma levels of HDL-C and apoA1 described in previous studies may be due to a diminished expression of APOA1 gene in carriers of the PPARα-V162 allele seen after controlling for omega-3 FA intake.

Thirdly, previous studies have demonstrated that PPARα ligands such as FAs can upregulate the LPL expression [15]. In the current study, omega-3 FAs were capable to increase LPL gene expression levels. Macrophages of non-carriers of the PPARα L162V polymorphism demonstrated a tendency towards higher LPL expression after the addition of EPA:DHA mixture compared to carriers of the PPARα-V162 allele. Yet, the difference in LPL gene expression level did not reach statistical difference between the subgroups possibly due to the low number of subjects upon which the present study was based. Since, previous observational studies have shown that carriers of the PPARα L162V polymorphism have demonstrated differences in plasma TG [21, 27, 32] and apoCIII levels [27], further investigation warranted to give a plausible rational for the involvement of the PPARα L162V polymorphism in the interindividual variability observed in plasma TG levels.

In conclusion, even if sample size of the study was small, all genes under study displayed a tendency to be expressed at a lower level in macrophages of individuals carrying the PPARα-V162 allele versus L162 homozygotes, which may explain the interindividual variability of lipid response in the efficacy of omega-3 FA supplementation. The current study is in accordance with results of our previous in vitro study [24], where in vitro transfection of the mutated allele demonstrated lower transcriptional rate compared to wild-type allele. The current study confirms that these differences between PPARα-V162 allele carriers and L162 homozygotes translate to physiological differences in gene expression rates of human macrophages, and thus provides further mechanistic evidence for functional differences between genotypes. Even small but consistent differences in gene expression may have substantial changes in energy and lipid metabolism over time. In addition, multiple genes may exhibit small differences within the pathway, suggesting more systematic effects. Therefore, the impact of omega-3 FAs on set of genes versus individual genes may provide further understanding of the impact of PPARα L162V polymorphism on the metabolic response to omega-3 FAs.

References

1. 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-Mn^{2+} precipitation procedures for estimating cholesterol in high-density lipoprotein. Clin Chem 24:853–856
2. 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
3. Barbier O, Torra IP, Duguay Y, Blanquart C, Fruchart JC, Glineur C, Staels B (2002) Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol 22:717–726
4. 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
5. Callaway CW (1988) CWBC Standardization of anthropometric measurements. The Airlie (VA) Consensus Conference. Human Kinetics Publishers, Champaign
6. Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, Fruchart JC, Chapman J, Najib J, Staels B (1998) Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. J Biol Chem 273:25573–25580

7. Duverger N, Kruth H, Emmanuel F, Caillaud JM, Viglietta C, Castro G, Tailleux A, Fievet C, Fruchart JC, Houdebine LM, Denefle P (1996) Inhibition of atherosclerosis development in cholesterol-fed human apolipoprotein A-I-transgenic rabbits. Circulation 94:713–717

8. Flavell DM, Pineda TI, Jamshidi Y, Evans D, Diamond JR, Elkeles RS, Bujac SR, Miller G, Talmud PJ, Staels B, Humphries SE (2000) Variation in the PPARalpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. Diabetologia 43:673–680

9. 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

10. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S (2004) Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. Nutr J 3:13

11. Harris WS (1997) n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 65:1645S–1654S

12. Jungling E, Kammermeier H (1988) A one-vial method for routine extraction and quantification of free fatty acids in blood and tissue by HPLC. Anal Biochem 171:150–157

13. Kersten S (2008) Peroxisome proliferator activated receptors and lipoprotein metabolism. PPAR Res 2008:132960

14. McNamara JR, Schaefer EJ (1987) Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. Clin Chim Acta 166:1–8

15. Michaud SE, Renier G (2001) Direct regulatory effect of fatty acids on macrophage lipoprotein lipase: potential role of PPARs. Diabetes 50:660–666

16. Paradis AM, Fontaine-Bisson B, Bosse Y, Robitaille J, Lemieux S, Jacques H, Lamarche B, Tchernof A, Couture P, Vohl MC (2005) The peroxisome proliferator-activated receptor alpha Leu162Val polymorphism influences the metabolic response to a dietary intervention altering fatty acid proportions in healthy men. Am J Clin Nutr 81:523–530

17. Pawar A, Botolin D, Mangelsdorf DJ, Jump DB (2003) The role of liver X receptor-alpha in the fatty acid regulation of hepatic gene expression. J Biol Chem 278:40736–40743

18. Pawar A, Jump DB (2003) Unsaturated fatty acid regulation of peroxisome proliferator-activated receptor alpha activity in rat primary hepatocytes. J Biol Chem 278:35931–35939

19. Reddy JK, Mannaerts GP (1994) Peroxisomal lipid metabolism. Annu Rev Nutr 14:343–370

20. Rigamonti E, Chinetti-Gbaguidi G, Staels B (2008) Regulation of macrophage functions by PPAR-alpha, PPAR-gamma, and LXRs in mice and men. Arterioscler Thromb Vasc Biol 28:1050–1059

21. Robitaille J, Brouillette C, Houde A, Lemieux S, Perusse L, Tchernof A, Gaudet D, Vohl MC (2004) Association between the PPARalpha-L162V polymorphism and components of the metabolic syndrome. J Hum Genet 49:482–489

22. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM (1991) Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature 353:265–267

23. Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, Faas FH, Linares E, Schaefer EJ, Schectman G, Wilt TJ, Wittes J (1999) Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. N Engl J Med 341:410–418

24. Rudkowska I, Verreault M, Barbier O, Vohl MC (2009) Differences in transcriptional activation by the two allelic (L162V polymorphic) variants of PPARalpha after omega-3 fatty acids treatment. PPAR Res 2009:369602

25. Sapone A, Peters JM, Sakai S, Tomita S, Papiha SS, Dai R, Friedman FK, Gonzalez FJ (2000) The human peroxisome proliferator-activated receptor alpha gene: identification and functional characterization of two natural allelic variants. Pharmacogenetics 10:321–333

26. Schoonjans K, Staels B, Auwerx J (1996) The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta 1302:93–109

27. Shin MJ, Kanaya AM, Krauss RM (2008) Polymorphisms in the peroxisome proliferator activated receptor alpha gene are associated with levels of apolipoprotein CIII and triglyceride in African-Americans but not Caucasians. Atherosclerosis 198:313–319

28. Singaraja RR, Bocher V, James ER, Clee SM, Zhang LH, Leavitt BR, Tan B, Brooks-Wilson A, Kwok A, Bissada N, Yang YZ, Liu G, Tafuri SR, Fievet C, Wellington CL, Staels B, Hayden MR (2001) Human ABCA1 BAC transgenic mice show increased high density lipoprotein cholesterol and ApoAI-dependent efflux stimulated by an internal promoter containing liver X receptor response elements in intron 1. J Biol Chem 276:33969–33979

29. Tai ES, Corella D, Demissie S, Cupples LA, Coltell O, Schaefer EJ, Tucker KL, Ordovas JM (2005) Polyunsaturated fatty acids interact with the PPARA-L162 V polymorphism to affect plasma triglyceride and apolipoprotein C-III concentrations in the Framingham Heart Study. J Nutr 135:397–403

30. Tai ES, Demissie S, Cupples LA, Corella D, Wilson PW, Schaefer EJ, Ordovas JM (2002) Association between the PPARA L162 V polymorphism and plasma lipid levels: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 22:805–810

31. Tanaka T, Ordovas JM, Delgado-Lista J, Perez-Jimenez F, Marin C, Perez-Martinez P, Gomez P, Lopez-Miranda J (2007) Peroxisome proliferator-activated receptor alpha polymorphisms and postprandial lipemia in healthy men. J Lipid Res 48:1402–1408

32. Uthurralt J, Gordish-Dressman H, Bradbury M, Tesi-Rocha C, Devaney J, Harmon B, Reeves EK, Brandoli C, Hansen BC, Seip RL, Thompson PD, Price TB, Angelopoulos TJ, Clarkson PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Gordon PM, Hoffman EP (2007) PPARalpha L162V underlies variation in serum triglycerides and subcutaneous fat volume in young males. BMC Med Genet 8:55

33. Vohl MC, Lepage P, Gaudet D, Brewer CG, Betard C, Perron P, Houde G, Cellier C, Faith JM, Despres JP, Morgan K, Hudson TJ (2000) Molecular scanning of the human PPARa gene: association of the L162v mutation with hyperapobetalipoproteinemia. J Lipid Res 41:945–952

34. Vu-Dac N, Schoonjans K, Laine B, Fruchart JC, Auwerx J, Staels B (1994) Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. J Biol Chem 269:31012–31018