RESEARCH ARTICLE

PPARα L162V polymorphism alters the potential of n-3 fatty acids to increase lipoprotein lipase activity

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Omega-3 fatty acids (FAs) may accelerate plasma triglyceride (TG) clearance by altering lipoprotein lipase (LPL) activity. Yet, the ability of n-3 FAs to increase LPL activity is dependent on transcription factors such as peroxisome proliferator-activated receptor alpha (PPARα). The objective was to examine the effects of n-3 FAs on LPL activity considering the occurrence of *PPARα* L162V polymorphism. First, 14 pairs of men either L162 homozygotes or carriers of the V162 allele were supplemented with n-3 FAs. Second, transient transfections in HepG2 cells, for the L162- and V162-PPARα variants with the peroxisome proliferator-response element from the human *LPL* gene, were transactivated with n-3 FAs. *In vivo* results demonstrate that the LPL activity increased non-significantly by 14.4% in L162 homozygotes compared with 6.6% in carriers of the *PPARα*-V162 allele, after n-3 FA supplementation. Additionally, the L162 homozygotes tended towards an inverse correlation between LPL activities and plasma TG levels. Conversely, carriers of the V162 allele showed no such relationship. *In vitro* data demonstrates that transcription rates of *LPL* tended to be higher for the L162-PPARα than V162-PPARα after n-3 FAs activation. Overall, these results indicate that n-3 FA supplementation increases the transcription rate of *LPL* to a greater extent in L162-PPARα than V162-PPARα.

Keywords:
Docosahexaenoic acid / Eicosapentanoic acid / Lipoprotein lipase / PPARα polymorphism / Triglycerides

1 Introduction

Omega-3 fatty acids (FAs), including eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to reduce postprandial triglyceride (TG) concentrations in numerous studies [1, 2]. Studies have established that the mechanism by which n-3 FAs reduce TG in humans is partially due to inhibition of hepatic VLDL secretion rates [3]. Yet, evidence also suggests that n-3 FAs exert effects on the removal of TG-rich lipoproteins through stimulation of lipoprotein lipase (LPL), a TG hydrolase present on the capillary endothelium of various tissues [4]. Low levels of LPL activity are associated with premature atherosclerosis and accelerated progression of atherogenesis [5].

Peroxisome proliferator-activated receptor alpha (PPARα) has been shown to regulate the expression of genes encoding for proteins involved in lipid and lipoprotein homeostasis [6]. For example, treatment of human subjects with PPARα agonists – such as fibrates – is associated with an increase in post-heparin LPL activity, suggesting that stimulation of plasma TG clearance by PPARα agonists can be attributed to enhanced LPL activity [7–9].

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Abbreviations: apo, apolipoprotein; **DHA,** docosahexaenoic acid; **EPA,** eicosapentanoic acid; **FA,** fatty acid; **HDL-C,** HDL-cholesterol; **HTGL,** hepatic triglyceride lipase; **LDL-C,** LDL-cholesterol; **LPL,** lipoprotein lipase; **PPARα,** peroxisome proliferator-activated receptor alpha; **RBC,** red blood cells; **TC,** total cholesterol; **TG,** triglyceride

Researchers have observed that carriers of the V162 allele of the *PPAR*α gene have higher concentrations of serum TG, total cholesterol (TC), LDL-cholesterol (LDL-C), apolipoprotein (apo) B and apo C-III compared with L162 homozygotes [10–15]. Since the *PPAR*α L162V is a polymorphism located in the DNA binding domain of PPARα, the variant receptor presents differential ligand-mediated activation [13, 16]. The best candidates for endogenous PPARα ligands are PUFA such as linoleic acid, DHA and EPA. After adjusting for n-3 PUFA intake, epidemiological studies have demonstrated an unfavorable effect of the *PPAR*α L162V polymorphism on metabolic factors [14, 17]. Also, a nutritional intervention recognized differences in lipid parameters in carriers of the V162 allele *versus* L162 homozygotes after modification of dietary polyunsaturated to saturated fat ratio [18].

Given that n-3 PUFAs are natural ligands of PPARα [19], the hypothesis of the present study is that carriers of the *PPAR*α-V162 allele increase LPL activity to a less significant degree after an n-3 PUFA supplementation compared with L162 homozygotes. Therefore, the main objective of this study was to investigate LPL activity levels, in subjects' carrying the *PPAR*α-V162 allele *versus* L162 homozygotes, after n-3 PUFA supplementation and to validate the *in vivo* finding in an *in vitro* transfection setting.

2 Materials and methods

2.1 *In vivo* study

2.1.1 Subjects

One hundred and fifteen Caucasian male subjects, aged 18–55 years, were recruited using flyers and newspaper advertisements in the greater Québec City metropolitan area. 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 hypertension, diabetes, severe dyslipidemia, or coronary heart disease. Finally, 14 carriers of the *PPAR*α-V162 allele were matched to 14 L162 homozygotes according to age (0.36 ± 2.41 years) and BMI ($0.70 \pm 0.55\,kg/m^2$). The experimental protocol was approved by the ethics committees of Laval University Hospital Research Center and Laval University.

2.1.2 Study design and diets

Twenty-eight subjects followed a run-in period of 2 wk. Individual dietary instructions were given by a trained dietician to achieve the National Cholesterol Education Program Step 1 diet guidelines [20]. 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: to not exceed two fish or seafood servings per week (max 200 g), preferred white flesh fishes instead of fatty fishes (examples were given), and to 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; two regular drinks per week were allowed. In addition, subjects were not allowed to take n-3 PUFA supplements, vitamins, or natural health products during the protocol.

After the 2-wk run-in, each participant received a bottle containing all needed fish oil capsules for the following 6 wk and were invited to take 5 (1 g oil each) capsules per day (Ocean Nutrition, Nova Scotia, Canada), providing a total of 3 g of n-3 (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.

2.1.3 Plasma lipid determination

Blood samples were collected from an antecubital vein into vacutainer tubes containing EDTA after a 12-h overnight fast and 48-h alcohol abstinence. Blood samples were taken to determine the *PPAR*α L162V genotype of each participant and analysed to identify and exclude from study individuals with any metabolic disorders. Afterward, selected participants had blood samplings at the pre and post n-3 PUFA supplementation periods. Plasma was separated by centrifugation ($2500 \times g$ for 10 min at 4°C) and samples were portioned and frozen for subsequent measurements. Plasma TC and TG concentrations were measured using enzymatic assays [21, 22]. The HDL-cholesterol fraction was obtained after precipitation of VLDL and LDL particles in the infranatant with heparin manganese chloride [23]. The LDL-C was calculated with the Friedewald formula [24].

2.1.4 Genotype determination

Genetic analyses were performed on genomic DNA isolated from human leukocytes. The *PPAR*α L162V polymorphism was determined by the PCR-restriction fragment length polymorphism method, as previously described [25].

2.1.5 FAs analysis

The n-3 FA concentration in red blood cell (RBC) membranes was measured using a gas chromatograph (HP 5890 gas chromatograph; Hewlett Packard, Toronto, ON,

Canada) and a capillary column DB-23 (30 m × 0.25-mm diameter × 0.25-μm film thickness; Agilent Technologies, Palo Alto, CA, USA) with nitrogen as the carrier gas. Erythrocyte FA profiles were expressed as the relative percentage areas of total FAs.

2.1.6 LPL activity

Plasma postheparin (60 IU/kg body weight) LPL and hepatic triglyceride lipase (HTGL) activities were measured after a 12-h overnight fast. LPL and HTGL activities were determined in postheparin plasma after pre-incubation with SDS, as previously described by Watson *et al.* [25]. Activities were expressed as micromoles of free FAs released per milliliter of plasma per hour.

2.1.7 Statistical analysis

Results are presented as mean ± SD. Subjects included 14 L162 homozygotes, 13 L162/V162 heterozygotes, and 1 V162 homozygotes. Carriers of the V162 allele were combined for all analyses. Statistical analyses were performed with SAS statistical software, version 9.1 (SAS Institute, Cary, NC). Statistical analyses were conducted using the PROC MIXED procedure from SAS on the metabolic variables, *via* the effect of genotype (L162 homozygotes *versus* V162 carriers), the effect of the n-3 PUFA supplementation (pre-supplementation *versus* post-supplementation) and the interaction effect between genotype and n-3 PUFA supplementation. Data were checked for normality of residuals using the Shapiro-Wilk test. If an abnormal distribution was detected, the data were $\log_{10}$ transformed before analyses. Pearson's correlation coefficients were computed to examine the strength and direction of a linear relationship between two variables. Statistical significance was defined as $p \leq 0.05$.

2.2 *In vitro* study

2.2.1 Plasmid construction

The wild-type L162-*PPAR* expression plasmid (pSG5-hPPARα vector) was a kind gift from Professor B. Staels (Unité INSERM 545, Institut Pasteur de Lille, France). The pSG5-mRXRα plasmid was described previously [26]. The V162-*PPAR*α expression plasmid was derived from the wild-type, through site-directed mutagenesis (QuickChange site-directed mutagenesis kit, Stratagene, La Jolla, CA, USA) using the 5′-CGATTTCACAAGTGC**G**TTTC-TGTCGGGATG-3′ oligonucleotide (the nucleotide in boldface type denotes the C→G change mutated base). Variant cDNAs were directly sequenced to confirm that no spurious base changes have been introduced during the procedure. The LPL-PPRE reporter plasmid was obtained by cloning 3 copies of the dimerized 5′-CGTCTGCCCTTTCCC-CCTCTTCTC-3′ oligonucleotide (underlined nucleotides identify the PPAR response element) in the thymidine kinase-driven reporter plasmid (TK-pGL3) [26, 27].

2.2.2 Transfection assays

PPARα is expressed predominantly in liver and kidney which are target tissues for the peroxisome proliferators [28]. Therefore, human hepatoma HepG2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented by 10% fetal bovine serum, 1% of streptomycin penicillin, 1% of sodium pyruvate and 1% of glutamine. HepG2 cells were plated at a density of 75×10^3 cells/well of 24-well plates and were transfected using the ExGen reagent (Invitrogen, Burlington, Canada) with 50 ng of the LPL-PPRE reporter plasmid, 10 ng of the *PPAR*α (wild-type or mutant) and *RXR* expression plasmids and 30 ng of the *pRL-NULL* expression vector for 6 h at 37°C. All samples were complemented with pBS-SK+plasmid (Stratagene) to an identical amount (500 ng/well). Similar experiments were performed with a negative control consisting of the empty TK-pGL3-basic plasmid (Promega). After transfection, cells were cultured in DMEM supplemented by 0.2% fetal bovine serum for 24 h to strengthen cell membrane before addition of FAs. Afterwards, cells were transactivated for 24 h in absence or presence of ciprofibrate (positive control), EPA, DHA or a mixture of EPA:DHA (Sigma-Aldrich, Oakville, ON, Canada) in concentrations varying between 1–10 μM to reflect biological plasma or RBC concentration of FAs [29]. Each FA was dissolved by serial dilution in solvent DMSO, as recommended by manufacture, and then added to DMEM. Cells were treated with either solvent (DMSO), 0.01% final concentration), or treatments with ciprofibrate (250 μM dissolved in DMSO), EPA (pure EPA was dissolved by serial dilution at 1 μM, 5 μM, and 10 μM in DMSO), DHA (pure DHA dissolved by serial dilution at 1 μM, 5 μM, and 10 μM in DMSO), or mixtures of EPA and DHA (pure EPA and DHA dissolved and mix at the following concentrations 5:5 μM, 15:5 μM, and 5:15 μM in DMSO). The luciferase activities were quantified with a luminometer (Bertholus, LB956V) and expressed as fold induction over control (TKpGL3 transfected and DMSO-treated cells). The assays were performed in triplicates. The experiment was conducted in duplicate.

2.2.3 Data analysis

Firefly luciferase activities were normalized with the corresponding Renilla luciferase reporter activity as internal control. Fold induction was calculated by taking the control DMSO (Sigma-Aldrich) as baseline.

3 Results

3.1 *In vivo* study

Twenty-eight healthy young males were recruited and completed the study. The subjects' baseline characteristics for L162 homozygotes ($n = 14$) and V162 carriers ($n = 14$) are age: 39.1 ± 11.4 years and 39.1 ± 11.3 years; weight: 86.6 ± 17.6 kg and 79.9 ± 7.4 kg; BMI: 26.5 ± 3.2 kg/m^2 and 25.7 ± 2.8 kg/m^2; and waist circumference: 97.4 ± 13.1 cm and 92.4 ± 6.6 cm, respectively. No differences were observed between the genotype groups for anthropometric indices and plasma lipoprotein/lipid concentrations.

Metabolic parameters are described in Table 1 with the independent effects of the genotype, the n-3 PUFA supplementation and interaction between genotype and n-3 PUFA. Results from blood lipids and dietary intake analysis have been previously published and are discussed elsewhere [30]. Briefly, the n-3 PUFA supplementation was associated with a similar decrease in fasting TG levels in both genotypes, from 1.41 to 1.17 mmol/L (−11.4%, percentage mean of the individual differences) for the L162 homozygotes and from 1.22 to 1.01 mmol/L (−13.1%, percentage mean of the individual differences) for carriers of the *PPAR*α-V162 allele. There were no differences seen between the baseline and endpoint results of TC, LDL-C, and HDL-cholesterol in either allele after n-3 PUFA supplementation.

In addition, as expected, the 6-wk n-3 PUFA supplementation increased the level of total n-3, EPA and DHA content in RBCs, demonstrating the compliance of subjects to the supplementation regimen (Table 1). In brief, the n-3 PUFA supplementation was associated with a similar increase in total n-3 in RBCs in both genotypes, from 8.81 to 10.65% of total free FA for L162 homozygotes (25.3%, percentage mean of the individual differences) and from 8.56 to 11.31% of total free FA for carriers of the *PPAR*α-V162 (32.7%, percentage mean of the individual differences). Comparable and significant increases were also observed for the EPA and DHA subcomponents after the n-3 PUFA supplementation in both genotype groups.

An increase in postheparin plasma LPL activity in L162 homozygotes (14.4%, percentage mean of the individual differences) and carriers of the *PPAR*α-V162 allele (6.6%, percentage mean of the individual differences) was noted after n-3 PUFA supplementation (Table 1). Large intra-individual variations in LPL activity may have excluded the possibility of reaching a statistically significant increase after n-3 PUFA supplementation. In addition, a significant effect was observed on plasma postheparin HTGL activity after n-3 PUFA supplementation, the extent of the increase being similar in genotype groups.

Correlation coefficients were computed to examine the relationship between postheparin LPL activities and plasma TG levels. Pre-supplementation data followed a similar direction in L162 homozyotes and carriers of the *PPAR*α-V162 allele (−0.49, $p = 0.07$; −0.42, $p = 0.13$, respectively), as demonstrated in Fig. 1, panel A. In addition, the L162 homozygote subgroup showed a tendency for an inverse relationship (−0.46, $p = 0.10$) between post n-3 PUFA LPL activities and plasma TG levels, as demonstrated in Fig. 1, panel B. Conversely, carriers of the *PPAR*α-V162 allele showed no such relationship (0.31, $p = 0.28$) between post n-3 PUFA LPL activities and plasma TG levels. No

Table 1. Metabolic variables before and post 6-week n-3 PUFA supplementation according to the PPARα L162V polymorphism

	L162 homozygotes ($n = 14$)		V162 carriers ($n = 14$)		*p* value		
	Pre n-3 PUFAs	Post n-3 PUFAs	Pre n-3 PUFAs	Post n-3 PUFAs	Geno.	Suppl.	Inter.
Cholesterol (mmol/L)							
Total	4.92 ± 1.2	4.85 ± 1.2	4.61 ± 1.0	4.73 ± 1.0	0.59	0.76	0.19
LDL	3.23 ± 1.1	3.27 ± 1.0	2.95 ± 0.9	3.13 ± 0.8	0.56	0.10	0.29
HDL	1.05 ± 0.3	1.04 ± 0.2	1.10 ± 0.3	1.13 ± 0.3	0.50	0.59	0.33
Triglycerides (mmol)	1.41 ± 0.8	1.17 ± 0.6	1.22 ± 0.7	1.01 ± 0.6	0.46	0.03	0.83
Ratio TC/HDL	5.05 ± 1.8	4.90 ± 1.6	4.11 ± 1.8	4.45 ± 1.4	0.25	0.67	0.30
RBC N-3 (% of free FA)							
Total N-3	8.81 ± 1.8	10.65 ± 2.3	8.56 ± 0.8	11.31 ± 1.0	0.81	<0.0001	0.36
EPA[a)]	0.84 ± 0.4	2.13 ± 0.7	0.82 ± 0.2	2.34 ± 0.4	0.17	<0.0001	0.77
DHA	5.04 ± 1.3	5.45 ± 1.3	4.92 ± 0.8	5.71 ± 0.7	0.81	0.02	0.47
LPL[a)] (μmole free FA/mL.h)	5.28 ± 2.9	5.37 ± 2.1	5.83 ± 2.3	5.89 ± 1.9	0.30	0.54	0.57
HTGL (μmole free FA/mL.h)	20.02 ± 6.9	22.16 ± 6.3	20.84 ± 4.9	21.50 ± 5.0	0.97	0.04	0.28

Data are shown as mean ± SD; PPAR is for the effect of the genotype on parameters; supplementation is the effect of n-3 PUFA supplementation on parameters; interaction is the effect of the supplementation (n-3 PUFA) with genotype (Geno) on parameters.

a) *p* value derived from log transformed data. Abbreviations: total cholesterol (TC); high density lipoprotein-cholesterol (HDL-C); red blood cells (RBC); fatty acid (FA); eicosapentanoic acid (EPA); docosahexaenoic acid (DHA); lipoprotein lipase (LPL); hepatic triglyceride lipase (HTGL).

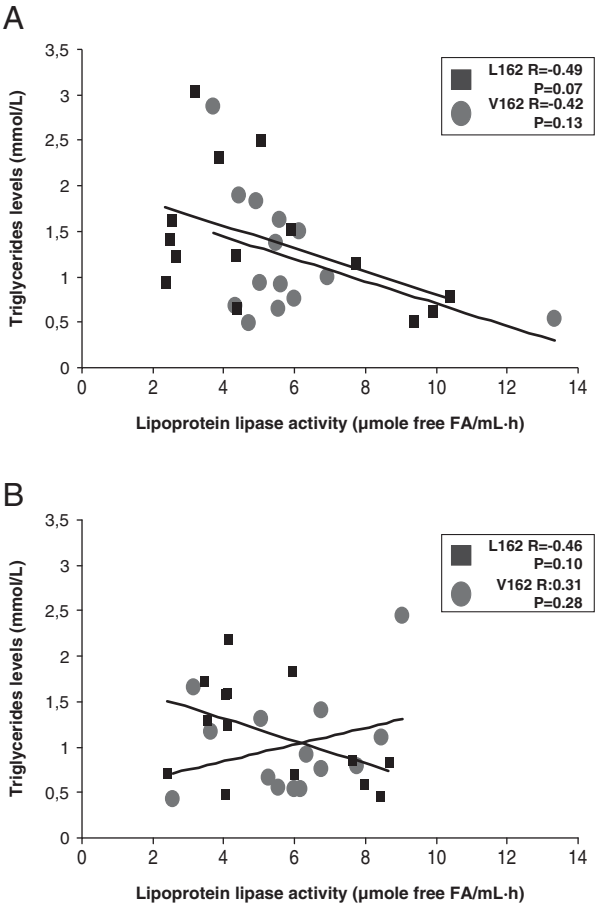
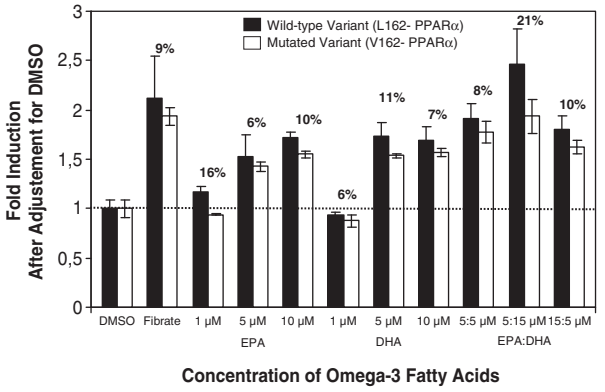


Figure 1. Correlation between triglyceride levels and lipoprotein lipase activity in subjects L162 homozygotes and V162 carriers before n-3 PUFA supplementation (panel A) and post n-3 PUFA supplementation (panel B). Panel A: Before n-3 PUFA supplementation. Panel B: Post n-3 PUFA supplementation.

relationship was seen between HTGL and TG in any of the genotype groups (data not shown).

3.2 *In vitro* study

Transient transfection assays in HepG2 human hepatoma cells were performed to compare L162-PPARα to V162-PPARα transcriptional activation by EPA and/or DHA. In sum, two independent transient transfection assays were performed with similar results for transcriptional activity. As expected from previous reports [27, 31], the present study confirms that n-3 PUFAs act as PPARα activators, and that activation of this nuclear receptor with fibrates, EPA, and/or DHA activate the PPAR response element located at −169/−157 bp of the human LPL gene (Fig. 2). In addition, additive effects of mixtures of EPA and DHA were established for both the V162-PPARα and the L162-PPARα (Fig. 2). However, the transcriptional level of V162-PPARα variant was consistently lower by 6 to 21%



Mean ± SD
Percentage difference between variants
Two independent experiments were done with similar results

Figure 2. Transcriptional activity of L162-PPARα and V162-PPARα in HepG2 cells supplemented with EPA, DHA, and with mixtures of EPA:DHA. Three copies of the LPL-PPRE were cloned upstream of the thymidine kinase (TK) minimal promoter-driven luciferase reporter (TKpGL3). The resulting construct (100 ng) was co-transfected with the pRL-NULL plasmid (30 ng) in HepG2 cells in presence of pSG5-hPPARα and pSG5-mRXRα (10 ng). Cells were subsequently treated or not with Ciprofibrate (250 µM) or increasing concentrations of EPA, DHA or EPA:DHA for 24 h. Values were normalized to internal Renilla luciferase activity as described in Section 2 and expressed as fold-induction relative to the control (TK-pGL3) set at 1. Values are representative of two independent experiments realized in triplicates.

than the L162-PPARα variant after n-3 PUFA activation (Fig. 2).

4 Discussion

The present *in vivo* and *in vitro* studies have provided data supporting the notion that the *PPARα* L162V polymorphism influences the level of LPL activity, which may impact TG levels and perhaps explain the large individual variability of the TG response to n-3 PUFA supplementation. The results demonstrate that L162 homozygotes have a greater ability to increase *LPL* transcription, and hence LPL activity, than carriers of the *PPARα*-V162 allele after n-3 PUFA supplementation.

Numerous studies have demonstrated that n-3 PUFAs lower TG levels in a dose-dependent manner, with the TG lowering being proportional to baseline levels. In the current study, TG levels were lowered to a similar extent by 17% after n-3 PUFA supplementation in both *PPARα* genotype groups. Similarly, in trials of subjects with high TG levels taking n-3 PUFAs in dosages of 3.4–4 g/day, TG levels decreased by 16 to 45% [2]. Yet, the large variability in response to treatment compared to previous studies is possibly due to the dose or ratio of n-3 PUFAs, the duration of study, the health status, diet and other confounding factors. Further, the present study demonstrates that there was a high compliance of subjects consuming n-3 PUFA supplements as witnessed by the high enrichment of RBCs

in total n-3 FAs. Overall, the n-3 PUFAs were consumed and absorbed by subjects, resulting in substantial TG lowering.

No significant increase in LPL activity was demonstrated in either *PPARα* genotype group in response to n-3 PUFA supplementation. There are conflicting observations on the effect of n-3 PUFAs on LPL activity. Similarly to the current study, some studies did not find any influence of n-3 FAs on postheparin LPL activity in humans [32–36], whereas others showed that n-3 FAs increased the activity of postheparin LPL [37, 38]. In an animal study, there was no effect of fish oil *versus* beef tallow oil on postheparin plasma LPL activity in rats [39]. Therefore, these data are in accordance with previous studies, where n-3 PUFA supplementation does not automatically increase significantly plasma LPL activity. Yet, it is possible that the role of a small but constant elevation in postheparin LPL activity may contribute to TG lowering.

Notwithstanding the modest effects of *PPARα* genotype and supplementation on LPL activity, TG levels had a tendency to be inversely correlated to LPL activity (−0.46, $p = 0.10$) in L162 homozygotes, the latter possibly reflecting an increase in the expression of the *LPL* gene following an n-3 PUFA supplementation. Previously, Scanu *et al.*, [40] demonstrated similar results: postheparin LPL activity was correlated inversely with fasting TG (−0.53, $p < 0.05$) in hypertriglyceridemic subjects after a n-3 PUFA supplementation for 1 month. However, no such correlation (0.31, $p = 0.28$) was found in carriers of the *PPARα*-V162 allele, as TG levels did not display any relationship with LPL activity. These results indicate that the modulation of plasma TG may be influenced by the *PPARα* L162V polymorphism that influences the response to n-3 FA. Yet, the magnitude of the genetic influence is not precisely known since it is possible that many other polymorphisms or haplotypes as well as environmental factors may influence TG metabolism and contribute to the development of hypertriglyceridemia.

Some limitations of the present human study do exist. First, the sample size of the clinical trial may have been insufficiently large to detect significant changes in LPL activity in subjects with the *PPARα*-V162 allele compared with controls. In addition, the subjects recruited displayed in general a healthy metabolic profile; therefore, less significant increase in LPL activity may have been observed. Second, a mechanism other than an increase of LPL activity may have also contributed to the beneficial effect of n-3 PUFA supplementation on plasma TG levels. Given that the stimulation of LPL production by PPARα agonists occurs in parallel with a decrease in hepatic production of apo C-III, this potential combination may contribute to the hypolipidemic action of the n-3 PUFAs [41]. Even if these current limitations are present, the potential of differences in LPL activity between genotypes is plausible.

The trends exposed in the *in vivo* data were further investigated in an *in vitro* setting to determine whether a difference between genotypes does in fact exist. The direct influence of the *PPARα* L162V polymorphism on transcriptional levels of the *LPL* gene was therefore examined in human hepatoma HepG2 cells. The results confirm a proportional increase in transcriptional activity in the PPAR responsive region of the LPL promoter with the supplementation of EPA, DHA or combination of EPA: DHA to reach physiological levels of these n-3 PUFAs. Since most fish oils contain more EPA than DHA, it was thought that EPA contributes to the suppression of postprandial hypertriglyceridemia. However, a few studies showed that DHA was equally effective in reducing plasma TG [42, 43]. Yet, in the current study the individual FAs contributed to a similar extent to the increase in transcriptional levels of *LPL*. Interestingly, EPA and DHA exerted an additive effect on transcriptional activation compared with individual FAs. Therefore, the higher transcriptional level of *LPL* may not be due only to the amount of the individual FAs but also to their metabolic interaction.

Consistently, the V162-PPARα variant showed a lower *LPL* transcriptional response (6–21%) than the L162-PPARα variant in response to all doses and combinations of n-3 PUFAs. This is in line with previous data indicating that carriers of the *PPARα*-V162 allele have a more atherogenic lipid profile [14, 18]. The present data demonstrates that the V162-PPARα variant may not be as efficient in the activation of transcription parameters, which supports the human trial results where no relationship between the LPL activity and TG lowering was seen in carriers of the *PPARα*-V162 allele after n-3 PUFA supplementation. Overall, carriers of the *PPARα*-V162 allele may demonstrate lower potential to increase transcription rate of certain genes, including *LPL*, after n-3 PUFA supplementation.

As a whole, the results demonstrate that the effect of n-3 PUFAs on plasma TG clearance appears to be at least partially mediated by changes in LPL activity in L162 homozygotes but not in carriers of the *PPARα*-V162 allele. Yet, no clinical or observational studies with the *PPARα* L162V polymorphism have measured LPL activity or expression. Therefore, further studies need to be conducted to confirm if there exists physiological difference in LPL activity and expression of *LPL* gene in individuals with or without *PPARα* L162V polymorphism and their relation to n-3 PUFA supplementation.

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The authors have declared no conflict of interest.

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