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# Differential dose effect of fish oil on inflammation and adipose tissue gene expression in chronic kidney disease patients

Fitsum Guebre-Egziabher M.D., Ph.D. [a,b,c,*], Cyril Debard Ph.D. [a,b], Jocelyne Drai PharmD. [a,b,d], Laure Denis Ph.D. [d,e,f], Sandra Pesenti [b], Jacques Bienvenu Ph.D. [d,e,f], Hubert Vidal Ph.D. [a,b], Martine Laville M.D., Ph.D. [a,b], Denis Fouque M.D., Ph.D. [a,b,c]

[a] Centre de Recherche en Nutrition Humaine Rhône-Alpes, Centre hospitalier universitaire Lyon-Sud, France
[b] INSERM U-1060, Laboratoire CarMeN, Université Lyon-1, INSA de Lyon, Villeurbanne, France
[c] Hospices civils de Lyon, Département de Néphrologie, Hôpital Edouard Herriot, Lyon, France
[d] Hospices civils de Lyon, centre de Biologie, Centre hospitalier universitaire Lyon-Sud, Lyon, France
[e] Université Lyon-1, Faculté de médecine Lyon Sud, France
[f] Inserm U851, Lyon, Gerland

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ABSTRACT

*Objective:* The beneficial effects of ω-3 polyunsaturated fatty acids (PUFAs) in cardiovascular disease are partly attributed to their anti-inflammatory properties. Their potential effect on the adipose tissue of chronic kidney disease (CKD) patients has never been explored.
*Methods:* To determine the metabolic effect of supplementation with two different doses of fish oil (FO), 12 non-dialyzed patients with stage IV/V CKD were randomly allocated to receive 1.8 g or 3.6 g/d of ω-3 PUFA for 10 wk. Metabolic parameters, adipose tissue function, and gene expression were evaluated at baseline and 10 wk.
*Results:* Body weight, fat mass, energy intake, fasting glucose, and insulin were unchanged. The daily intake of 3.6 g of ω-3 PUFA resulted in decreased serum triacylglycerol and increased high-density lipoprotein cholesterol, whereas low-density lipoprotein cholesterol increased with 1.8 g of ω-3 PUFA. Serum adiponectin, leptin, C-reactive protein, and tumor necrosis factor-α were not modified in either group. Interleukin-6 levels tended to decrease with 1.8 g of ω-3 PUFA. Additionally, a subset of inflammation-related genes (*CD68* and *MMP9*) was reduced in subcutaneous adipose tissue in this group. Adiponectin, leptin, and *adipoR2* gene expression were upregulated with 3.6 g of ω-3 PUFA.
*Conclusions:* A moderate dose of FO alters the gene expression profile of adipose tissue to a more antiinflammatory status. Higher doses of FO have a favorable effect on lipid profile and lead to the upregulation of adipokines gene expression suggesting a different dose response to ω-3 PUFA administration in patients with CKD.



## Introduction

Chronic kidney disease (CKD) is associated with high cardiovascular mortality rate [1]. A growing body of evidence suggests that an increased inflammatory state and metabolic disorders are important contributors to the increased cardiovascular disease (CVD) in CKD [2].

Furthermore, adipose tissue has been recognized to contribute to the production of inflammatory factors [3]. Inflammatory alterations in white adipose tissue appear to underly complications of metabolic diseases such as obesity and diabetes mellitus and recent data indicate that this also might be the case in CKD [4,5]. Both animal and human studies show that blood-derived macrophages infiltrate the adipose tissue in obesity and therefore contribute to the inflammatory state of the tissue [6–8]. Abdominal fat deposition is associated with increased inflammation in patients undergoing maintenance haemodialysis [9]. Furthermore, an increased production of

Fitsum Guebre-Egziabher conceived the overall study, was responsible for planning the study, and drafted the manuscript; Denis Fouque participated in the conception of the study and manuscript writing; Jocelyne Drai was responsible for adipokines and plasma fatty-acid profile analysis; Laure Denis and Jacques Bienvenu were responsible for cytokine and CRP analysis; Cyril Debard and Sandra Pesenti for gene expression analysis; Hubert Vidal and Martine Laville for conception of the study and evaluating the results.

The authors have nothing to disclose.

* Corresponding author. Tel.: +33 472110204; fax: +33 472110203.
*E-mail address:* fitsum.guebre-egziabher@chu-lyon.fr (F. Guebre-Egziabher).

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proinflammatory cytokines in adipose tissue of patients with end-stage renal disease has been reported [5].

Currently, there are no therapies for CVD reduction that have been demonstrated to lower the inflammatory state of patients with CKD. Diets enriched with ω-3 PUFA have been associated with a lower incidence of coronary heart disease (CHD), and a reduction of atherosclerotic lesions and inflammatory markers in non-renal patients [10–12].

In rodents, the beneficial effects of ω-3 PUFA on insulin sensitivity and adipose tissue metabolism and gene expression have been reported. The addition of ω-3 PUFAs in the diets of obese diabetic mice helped prevent high-fat diet-induced matrix remodeling, adipocyte enlargement, and inflammation in adipose tissue [13,14].

In humans, 2 mo treatment with 1.8 g ω-3 PUFA reduces adiposity, some atherogenic markers, and adipose tissue inflammation-related genes (matrix metalloprotease *MMP9*, and macrophage surface markers *CD68* and *CD11b*) in women with type 2 diabetes [15]. Furthermore, in elderly patients, 8 wk of high ω-3 consumption increases insulin sensitivity and reduces inflammatory markers [16].

It is well admitted that patients with CKD have lower ω-3 PUFA levels in plasma and cells compared with patients without kidney disease [17]. In patients on hemodialysis, Himmelfarb et al. reported a significant decrease in interleukin (IL)-6 without significant change in C-reactive protein (C-RP) and oxidative stress markers with 8 wk supplementation of docosahexaenoic acid (DHA) and gamma-tocopherol [18].

Given these observations, we hypothesized the following:

- Moderate ω-3 PUFA supplementation may improve plasma inflammatory markers and inflammatory gene expression in the adipose tissue of patients with CKD.
- Increasing the dose of fish oil (FO) may have a greater beneficial effect on inflammation.

## Materials and methods

### *Participants*

Twelve patients with CKD were recruited (6 males and 6 females) from the Department of Nephrology hôpital Edouard Herriot, Lyon, France. The main inclusion criteria were age 18 y to 65 y, glomerular filtration rate <20 mL/min, and body mass index (BMI) <27 kg/m$^2$. Exclusion criteria were the presence of diabetes or inflammatory disease; FO or ω-3 PUFA supplementation in the previous 3 mo; ongoing active illness requiring hospitalization; warfarin, steroid, or antiaggregant agent use; and pregnancy. All participants gave their written consent after being informed of the nature and purpose of the study, as well as possible risks. The protocol was approved by the ethics committee of hospices civils de Lyon and performed according to French legislation (Loi Huriet).

The protocol consists of a 10-wk intervention with FO supplementation. The participants were randomized to moderate FO supplementation (group A; n = 6; 4 males, mean age 50.5 ± 10.8 y, 6 capsules of MaxEPA per day, laboratoire Pierre Fabre Médicament, Boulogne, France) or high FO supplementation (group B; n = 6; 2 males, mean age 50.2 ± 6.7 y, 12 capsules of MaxEPA per day). Each FO was certified to contain a minimum of 180 mg of eicosapentaenoïc acid (EPA) and 120 mg of DHA per capsule.

Participants were requested not to alter their fish consumption during the study and to keep their initial calory intake and nutrient proportions constant throughout the study.

All participants were given written and verbal instructions by a dietetician on how to complete a 3-d (2 weekdays and 1 weekend day) dietary record before the initiation and during the last week of the FO supplementation. PUFA intakes were calculated using food composition tables specific to France (CIQUAL table) [19].

The participants underwent 2 d of testing before and at the end of the intervention. Each participant was referred to the Centre de Recherche of Nutrition Humaine (CRNH) Rhône Alpes, for a comprehensive series of tests following an overnight fast. After anthropometric and body composition measurements, blood was drawn. Resting metabolic rate was measured by indirect calorimetry. Adipose tissue biopsies were performed under local anesthesia.

### *Weight and body composition*

Body weight was recorded with the subject wearing no shoes and light clothing to the nearest 0.1 kg with an electrical scale. The waist to hip ratio was calculated. Body composition was determined with a single-frequency bioelectrical impedance device (Star 50, Spengler, Cachan, France).

Continuous indirect calorimetry was performed with a ventilated hood system (Deltatrac, Datex Instruments Corp., Helsinki, Finland) during 60 min to measure carbohydrate and lipid oxidation rates [20].

### *Laboratory analysis*

Plasma glucose, cholesterol, low-density and high-density lipoproteins, and triglycrerides and albumin were measured using standard automated laboratory techniques.

Plasma insulin was quantified in duplicate by radioimmunoassay (Ins Irma, Kip 1251, MDS Nordion, France). CRP was assessed by immunonephelometry.

Free fatty acids were measured by the colorimetric enzymatic method.

Adiponectin was measured using an immunoassay technique (Quantikine assay, R & D Systems®, Minneapolis, MN, USA) and leptin with an enzyme-linked immunosorbent assay (ELISA) technique (BioVendor® assay, Modrice, Czech Republic). Cytokine profile was investigated using peripheral venous ethylenediaminetetraacetic acid blood samples. Plasma IL-6 and tumor necrosis factor (TNF)-α concentrations were analyzed in duplicate using a quantitative ELISA (Beckman Coulter/Immunotech, Marseille, France; Invitrogen/Biosource, Nivelles, Belgium, respectively), following manufacturer's instructions. Minimum detectable plasma concentrations were 3 pg/mL for IL-6 and for TNF-α.

### *Plasma phospholipid fatty-acid composition*

Lipids were extracted by chloroform methanol (1:1, v/v). The chloroform phase was concentrated under a stream of nitrogen and taken up in chloroform methanol. The lipid classes were separated by thin-layer chromatography on silicagel plates (Merck 5721) using petroleum ether-ethylic ether–acetic acid as developing solvent. The plates were sprayed with bromophenol blue and individual bands of phospholipids and cholesteryl esters were scrapped off into separated tubes. The phospholipids fraction was saponified and transmethylated with sodium methylate, and the cholesteryl ester fraction was methylated with sulphuric acid and dehydrated methanol. The methyl esters of each fraction were removed by hexane and analyzed by gas liquid chromatography on a Fison GC-8000 gas chromatograph (Thermo Separation Products-Les Ullis, France) equiped with a CP-SIL–fused silica capillary column (25 m x 0,25 mm internal diameter coated with 100% cyanopropyl siloxane 88 phase 0,2 μm, Chrompack Les Ullis, France, helium as carrier gas, and a split ratio 1:20).

Initial temperature was 120°C for 1 min then temperature programming was as follow: 20°C/min until 165°C maintained 20 min, 1°C/min until 210°C maintained 1 min, 10°C/min until 250°C maintained 5 min. Identification of the individual methyl ester components was made by frequent comparison with authentic standards and with their retention times. The results were expressed as percentage of the total area of all fatty-acid peaks in each lipid fraction and quantified (mg/L) with the internal Standard added at the beginning of analysis.

### *Adipose tissue biopsies*

Biopsies were performed under local anesthesia. Subcutaneous abdominal white adipose tissue samples were obtained from the peri ombilical level by needle biopsy, as previously described [21]. Tissue samples were immediately frozen in liquid nitrogen and then stored at −80°C for later extraction of RNA.

### *RNA extraction*

Total RNA from subcutaneous tissue was obtained by using the mirVana miRNA Isolation Kit (Applied Biosystems, Courtaboeuf, France) according to the manufacturers recommandations.

### *Quantification of mRNA by using real-time reverse transcription polymerase chain reaction (RT-PCR)*

First-strand cDNAs were synthesized from 250 ng of total RNA in the presence of 100 U of Superscript II (Invitrogen, Eragny, France) and a mixture of random hexamers and oligo (dT) primers (Promega, Charbonnières, France). Real-time RT-PCR was performed using ABsolute QPCR SYBR Green ROX Mix (Abgene, Courtaboeuf, France) with a Rotor-Gene 6000 system (Corbett Life Science, Paris, France). For the purpose of quantification, a standard curve was

systematically generated with six different amounts (150–30,000 molecules/tube) of purified target cDNA cloned in the pGEM plasmid (Promega). Each assay was performed in duplicate. TATA box binding protein (TBP) mRNA levels were measured as internal standard.

### Statistical methods

All values are expressed as mean ± SEM. Paired *t* tests and the Wilcoxon signed- rank tests were performed for comparison between final and baseline values when applicable. Data analysis was undertaken using statview statistical software program (statview version, SAS institute Inc., Cary, NC, USA). Relations between variables were analyzed by the non-parametric Spearman's correlation test and *r* coefficients are provided. *P* values <0.05 were considered statistically significant.

## Results

### Patient compliance

Participants did not report any difficulty or side effects with the FO treatment. Participants' lifestyles were unchanged throughout the study. We evaluated ω-3 fatty acid composition of plasma phospholipids. The level of EPA (C20:5 ω-3) and DHA (C22-6 ω-3) in plasma was significantly increased in both groups, whereas arachidonic acid (AA; C20-4 ω-6) concentration decreased. Accordingly the ratio ω-6/ω-3 significantly decreased in both groups (Table 1). Furthermore, the count of returned FO capsules showed good compliance.

### Baseline characteristics and effects of intervention on dietary control, anthropometry, and nutrient utilization

At baseline, there were no detectable significant differences in clinical and biological variables measured between the two treatment groups.

There was no significant difference between the groups in the daily intake of total energy and nutrients per weight as estimated by 3-d dietary records (Table 2). Body weight, BMI, and percent fat mass measured by bioelectrical impedance remained unchanged in both groups (Table 2). Resting metabolic rate and glucose and lipid oxidation measured by indirect calorimetry were not significantly modified by the intervention either (Table 3).

### Lipid and glucose homeostasis

Fasting plasma glucose and insulin were not significantly influenced by FO treatment. Plasma triacylglycerol was significantly lower after 10 wk in group B (3.6 g of ω-3 PUFA). HDL cholesterol also was significantly increased in this group and remained unchanged in group A, whereas LDL cholesterol increased in the latter. Plasma-free fatty acids remained unchanged in both groups (Table 4).

### Adipokines and inflammatory factors

Interleukin-6 tended to decrease after 10 wk of moderate FO supplementation (group A) without reaching statistical significance ($P = 0.07$). It is noteworthy that baseline plasma IL-6 levels were more elevated in group A than in group B.

Plasma concentrations of leptin, adiponectin, CRP, and TNF-α, were not significantly changed after the treatment in either group (Tables 4 and 5).

### Gene expression analysis in adipose tissue

The gene expression of a subset of adipose tissue secreted proteins was analyzed by real-time RT-PCR in the two treatment groups before and after 10 wk of FO treatment.

There was no significant difference in adipose tissue gene expression between the two groups at baseline. The 12 CKD patients showed a significant positive correlation between percent fat mass and leptin ($r = 0.85$), *CD14* ($r = 0.95$), and *CD68* ($r = 0.70$) gene expression in adipose tissue. Serum leptin level also was correlated with *CD14* ($r = 0.72$) and *CD68* ($r = 0.70$) gene expression (Table 6). There was no significant correlation among adiponectin, IL-6, and CRP plasma levels and inflammatory-related gene expression in adipose tissue (data not shown).

Leptin and adiponectin gene expression (the main adipocyte-secreted hormones) and adiponectin receptor 2 (adipoR2) were significantly increased in group B (3.6 g of ω-3 PUFA), and remained unchanged in the group A (1.8 g of ω-3 PUFA; Fig. 1). A series of genes encoding inflammation-related factors also was analyzed. Among these genes, the matrix metalloprotease *MMP9*, and the macrophage phagocytic activity marker *CD68* were significantly reduced in group A. IL-6 gene expression also was reduced in this latter group, without reaching significance. No significant changes were observed in the monocyte chemotactic protein (MCP-1) in either group (Fig. 2).

## Discussion

This study assessed changes in systemic metabolic and inflammatory markers as well as adipose tissue inflammatory and adipokines gene expression in a 10-wk randomized intervention trial in non-dialyzed patients with CKD. Supplementation with 1.8 g ω-3 PUFA, which is a moderate dose of FO, resulted in an improved inflammatory profile in blood and adipocyte gene expression. These effects were seen despite the significant increase of LDL cholesterol.

**Table 1**
Fatty acid composition of plasma phospholipids at baseline and after 10 wk of fish oil treatment

| Plasma phospholipid fatty acids (%) | Group A (n = 6) | | | Group B (n = 6) | | |
|---|---|---|---|---|---|---|
| | Baseline | Week 10 | % Change from baseline | Baseline | Week 10 | % Change from baseline |
| 20:5 n-3 (EPA) | 0.72 ± 0.40 | 4.05 ± 0.73* | 605.4 ± 352.1 | 0.70 ± 0.27 | 6.95 ± 1.60** | 1011 ± 432 |
| 22:6 n-3 (DHA) | 3.52 ± 0.77 | 6.52 ± 0.78* | 96.1 ± 63.1 | 2.92 ± 0.52 | 6.53 ± 1.06* | 132.8 ± 69.6 |
| 20:4 n-6 | 10.38 ± 1.29 | 8.57 ± 0.63* | −16.82 ± 8.22 | 10.82 ± 1.59 | 8.05 ± 0.55* | −24.3 ± 11.9 |
| Ratio n-6/n-3 | 2.17 ± 0.50 | 1.54 ± 0.15* | −26.7 ± 11.7 | 2.64 ± 0.79 | 0.98 ± 0.20* | −60.7 ± 11.9 |
| n-3 index | 4.23 ± 1.05 | 10.57 ± 0.92** | 162 ± 70 | 3.62 ± 0.77 | 13.48 ± 1.89** | 287 ± 101 |

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid
C20:4 n-6 arachidonic acid.
n-3 index, (20:5 n-3 + 22:6 n-3). Ratio n-6/n-3, (20 :4 n-6 + 22:4 n-6+ 22:5 n-6)/(20:5 n-3 + 22:5 n-3 + 22:6 n-3).
* $P < 0.05$.
** $P < 0.01$.

**Table 2**
Dietary records data; anthropometric measurements at baseline and after 10 wk of fish oil supplementation

| | Group A (n = 6) | | Group B (n = 6) | |
|---|---|---|---|---|
| Energy (kcal/d) | 2013 ± 437 | 1973 ± 441 | 1581 ± 64 | 1703 ± 259 |
| Carbohydrate (g/d) | 234 ± 66 | 216 ± 60 | 164 ± 35 | 180 ± 72 |
| Protein (g/d) | 87 ± 22 | 82 ± 21 | 65 ± 7 | 78 ± 5 |
| Lipids (g/d) | 81 ± 15 | 86 ± 21 | 74 ± 11 | 74 ± 17 |
| *SFA* (g/d) | 30 ± 8 | 35 ± 11 | 27 ± 6 | 29 ± 7 |
| *MUFA* (g/d) | 25 ± 5 | 32 ± 6 | 27 ± 9 | 28 ± 6 |
| *PUFA* (g/d) | 14 ± 5 | 9 ± 3 | 9 ± 2 | 8 ± 4 |
| BMI (kg/m$^2$) | 23.2 ± 0.7 | 23.4 ± 0.6 | 23.4 ± 3.8 | 23.7 ± 1.5 |
| Waist/hip | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.1 | 0.9 ± 0.05 |
| % Fat mass | 25.1 ± 3.4 | 24.4 ± 3.5 | 25.8 ± 2.8 | 25.7 ± 2.9 |

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, poly unsaturated fatty acid; BMI, body mass index

Additionally, supplementation with 3.6 g ω-3 PUFA did not have a dose–response effect on systemic inflammation markers or adipose tissue inflammation-related gene expression despite the improved blood lipid profile and the upregulated adipokine genes expression in adipose tissue.

The effect of 1.8 g EPA plus DHA is mainly characterized by a trend for a decrease in IL-6 and a decreased expression of *MMP9* and *CD68* in adipose tissue. ω-3 PUFAs have been associated with cardiovascular protection even at low doses (<1 g/d) [22]. However, despite the research in this area, some important aspects, such as ideal doses and the mechanisms by which these PUFAs act, are not precisely defined. Although increased adiposity represents a major risk factor for the development of type 2 diabetes and its associated cardiovascular complications, an emerging paradigm supports the view that adipose tissue dysregulation, characterized by impaired lipid storage and inflammatory cytokines production, might play a crucial role in the pathogenesis of insulin resistance and atherosclerosis [23]. Thus, the beneficial effect of FO on risks for CVD could be mediated by a decrease in the inflammatory markers secreted by adipose tissue. In this study, moderate FO treatment significantly reduced or tended to reduce the expression of a cluster of inflammation-related genes in the subcutaneous adipose tissue. Some of these markers such as the metalloprotease (*MMP9*), the macrophage surface (*CD14*), and phagocytic activity (*CD68*), were positively correlated with adiposity (% fat mass) at baseline (Table 6). The result of this study is supported by experimental studies, where ω-3 PUFA supplementation prevents adipose tissue inflammation [14] and matrix remodeling in adipose tissue [13]. Additionally, in postmenopausal women with type 2 diabetes, Kabir et al. have shown that moderate FO treatment (1.8 g EPA + DHA) decreases adiposity and the expression of some inflammation-related genes in adipose tissue [15]. Hence, our observations on adipose tissue gene expression are in line with these previous studies.

**Table 3**
Biochemical measurements and substrate oxidation at baseline and after 10 wk of fish oil supplementation

| | Group A (n = 6) | | Group B (n = 6) | |
|---|---|---|---|---|
| Serum creatinine (μmol/L) | 418 ± 59 | 428.7 ± 73 | 337 ± 42 | 367.2 ± 50 |
| eGFR (ml/min) MDRD | 13.8 ± 2.1 | 14.8 ± 3.1 | 16.0 ± 2.1 | 15.3 ± 2.6 |
| Serum bicarbonate (mmol/L) | 22.8 ± 1.6 | 21.1 ± 0.8 | 24.6 ± 1.3 | 22.6 ± 0.4 |
| Serum albumin (g/L) | 43.3 ± 1.6 | 42.2 ± 1.3 | 41.8 ± 1.6 | 42.3 ± 1.9 |
| Glucose oxidation (mg/kg.min$^{-1}$) | 1.8 ± 0.3 | 1.6 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.3 |
| Lipid oxidation (mg/kg.min$^{-1}$) | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.6 ± 0.1 |
| REE (kcal) | 1396 ± 78 | 1350 ± 189 | 1314 ± 114 | 1296 ± 117 |

eGFR, estimated glomerular filtration rate; REE, resting energy expenditure
Data are expressed as mean ± SEM.

**Table 4**
Lipid and glucose homeostasis and adipokines at baseline and after 10 wk of fish oil treatment

| | Group A (n = 6) | | Group B (n = 6) | |
|---|---|---|---|---|
| | Baseline | Week 10 | Baseline | Week 10 |
| Cholesterol (mmol/L) | 5.1 ± 0.6 | 5.6 ± 0.7 | 4.6 ± 0.3 | 4.8 ± 0.4* |
| HDL (mmol/L) | 1.3 ± 0.2 | 1.4 ± 0.1 | 1.4 ± 0.2 | 1.5 ± 0.2** |
| LDL (mmol/L) | 2.9 ± 0.5 | 3.5 ± 0.7* | 2.5 ± 0.1 | 2.8 ± 0.3 |
| TG (mmol/L) | 2.0 ± 0.4 | 1.7 ± 0.3 | 1.5 ± 0.3 | 1.1 ± 0.2* |
| FFA (mmol/L) | 4.1 ± 1.2 | 4.2 ± 0.9 | 4.6 ± 0.7 | 4.6 ± 1.3 |
| Fasting glucose (mmol/L) | 4.7 ± 0.2 | 4.8 ± 0.2 | 4.5 ± 0.2 | 4.5 ± 0.2 |
| Insulin (pmol/L) | 11.6 ± 1.6 | 13.2+2.7 | 10.6 ± 1.6 | 10.6 ± 2.1 |
| Adiponectin (μg/mL) | 15.4 ± 4.6 | 17.1 ± 4.6 | 19.5 ± 3.0 | 20.8 ± 1.3 |
| Leptin (ng/mL) | 20.3 ± 12.7 | 23.4 ± 14.9 | 19.8 ± 6.6 | 23.8 ± 8.5 |

TG, triacylglycerol; FFA, free fatty acid
Data are expressed as mean ± SEM.
* $P < 0.05$.
** $P < 0.01$ baseline vs 10 wk.

The current study was not able to demonstrate a significant alteration in concentrations of systemic inflammatory factors in response to FO treatment in patients with CKD. In patients with inflammatory conditions, cytokine concentration or production are influenced by EPA and DHA supplementation in a relatively large number of studies [24]. However, some of these studies suggest that local effects at the site of inflammation might be more pronounced than systemic effect [15,25]. Additionally, it has been reported that the effects of PUFA supplementation are mainly detectable when CRP is already elevated at baseline [26,27], which was not the case in our population. In hemodialysis patients with inflammation, Saifullah et al. showed that supplementation with 1.3 g of EPA plus DHA for 12 wk leads to a significant decrease in CRP [28].

An interesting finding of this study is that increasing the dose of FO does not affect inflammation-related gene expression in adipose tissue, despite an improved lipid profile.

In an ex vivo study, a negative but U-shaped dose–response relationship between long chain ω-3 fatty acid intake and IL-6 production by monocytes has been reported in an open uncontrolled study, with a maximum inhibition demonstrated at a supplementary intake of 1 g/d [29]. Wallace et al. studied the same type of dose effect in a controlled, blinded parallel study and found a similar result [30]. The current data from supplementation studies in healthy subjects have failed to reveal a thresfold value for, and dose–response effects on, immunomodulation with EPA and DHA [31]. Inflammatory cytokine concentrations and production are influenced by FO in a

**Table 5**
Plasma inflammatory markers at baseline and after 10 wk of fish oil treatment

| | Group A (n = 6) | | Group B (n = 6) | |
|---|---|---|---|---|
| | Baseline | Week 10 | Baseline | Week 10 |
| CRP (mg/L) | 1.33 ± 0.6 | 1.47 ± 0.4 | 1.46 ± 0.4 | 1.60 ± 0.7 |
| TNF -α (pg/mL) | 27.4 ± 3.3 | 28.4 ± 2.8 | 21.0 ± 3.0 | 22.5 ± 3.3 |
| IL-6 (pg/mL) | 14.7 ± 3.8 | 10.2 ± 3.1 | 8.3 ± 2.0 | 9.3 ± 2.5 |

CRP, C-reactive protein; TNF-a, tumor necrosis factor; IL-6, interleukin 6
Data are expressed as mean ± SEM.

**Table 6**
Correlations between values of adipose tissue gene expression of some adipokines and inflammatory markers in one hand and clinical variables on the other hand

| | Baseline value | Fat mass (%) | Plasma leptin (ng/mL) |
|---|---|---|---|
| mRNA/TBP Baseline value | | 25.5 ± 2.1 | 20.1 ± 6.8 |
| Leptin | 5025.9 ± 1168.2 | 0.85** | 0.82** |
| Adiponectin | 32799.1 ± 5263.1 | | |
| CD68 | 2919.9 ± 508.6 | 0.71* | 0.70* |
| CD14 | 463.5 ± 101.2 | 0.95* | 0.72* |
| MCP-1 | 28.2 ± 4.0 | | |
| MMP9 | 2416.0 ± 666.1 | 0.63* | 0.54 |

CD68, macrophage phagocytic activity marker; CD14, macrophage surface marker; MCP-1, monocyte chemotactic protein; MMP9, metalloprotease 9; TBP, TATA box binding protein
Values are expressed as mean ± SEM.
* $P < 0.05$.
** $P < 0.001$.

relatively large number of studies in patients with inflammatory conditions. Because the designs and experimental conditions used differ between studies, it is not possible to thoroughly investigate the potential presence of dose–response effects. Data allowing direct comparison within the same inflammatory disease are very limited and inconclusive. Approximately 1 g EPA plus DHA per day are recommended for CVD risk reduction on the basis of evidence from large secondary prevention trials, which showed that this intake can reduce cardiovascular mortality by 20% to 30% without significant reductions in triacylglycerol [22]. Therefore, the cardioprotective action of ω-3 fatty acids is thought to occur via multiple benefits, such as antiinflammatory effects secondary to changes in cell membrane properties that affect cell signaling and gene expression. Our data clearly suggest that increasing the intake of FO up to 3.6 g/d has no additional beneficial immunomodulatory effect. However, the results of this study corroborate the effectiveness of pharmacologic doses of ω-3 fatty acids in reducing triacylglycerol concentrations. Published data on the effect of FO treatment on LDL and HDL cholesterol are less consistent. Our results are in line with previous reports in predialysis CKD patients [32,33]. Although these increases in HDL and LDL cholesterol have been reported, the exact mechanisms are not known.

Another interesting finding of this study is the upregulation of adipokines (leptin and adiponectin) and adipoR2 associated with the decrease of triacylglycerol and an increase of HDL. It has been reported that feeding with a diet containing large amounts of FO increases plasma adiponectin concentration in rats [34] and mice [35]. The increased adiponectin gene expression in epididymal fat and the increase of plasma adiponectin is likely mediated by the activation of peroxisome proliferator-activated receptor γ (PPARγ) in mice fed FO in a dose-dependent fashion [35]. In obese human participants, supplementation with 1.8 g of EPA for 3 mo has been reported to decrease triacylglycerol and increase plasma adiponectin levels [36]. Moreover, EPA has been found to increase the expression of PPARγ itself [37]. The favorable effect of FO administration on lipoprotein profile in nondialyzed patients with CKD has been reported by Svensson et al. with an 8-wk administration of high-dose ω-3 PUFA (2.4 g EPA + DHA) [33]. Furthermore, patients with CKD who reported high fish intake had greater ω-3 PUFA content in adipose tissue compared with patients with low fish intake, suggesting a good incorporation of these PUFA in adipose tissue [33]. It is possible that the incorporation of these fatty acids in adipose tissue might modulate the expression of transcription factors and increase the number of differenciated adipocytes able to produce more adiponectin. But these authors were not able to show an effect on CRP [38].

Our study, however, has some limitations. First, the limited number of patients may have prevented us from reaching significance. Second, the duration of the study was relatively short, eventually limiting the detection of systemic effect, but most published data report the effect of 8 wk to 10 wk of FO supplementation. Third, because of the limited amount of fat taken by biopsy, confirmation of gene expression changes at the protein level could not be determined in adipose tissue.

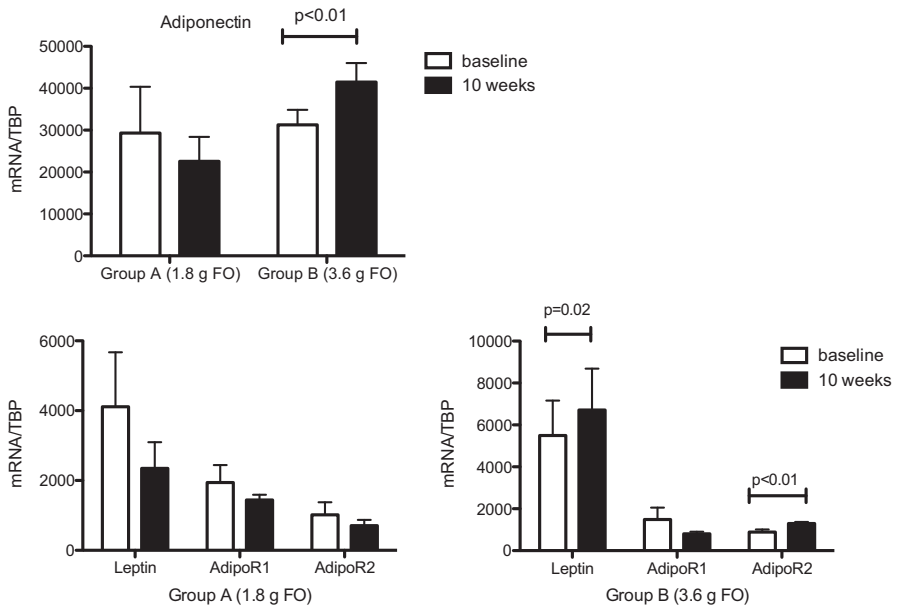


**Fig. 1.** Effect of 10 wk of fish oil treatment on the gene expression of adipokines and adiponectin receptors 1 and 2 in subcutaneous adipose tissue. Data are normalized for TATA box binding protein (TBP) and expressed as mean ± SEM. AdipoR1, adiponectin receptor 1; AdipoR2, adiponectin receptor 2.

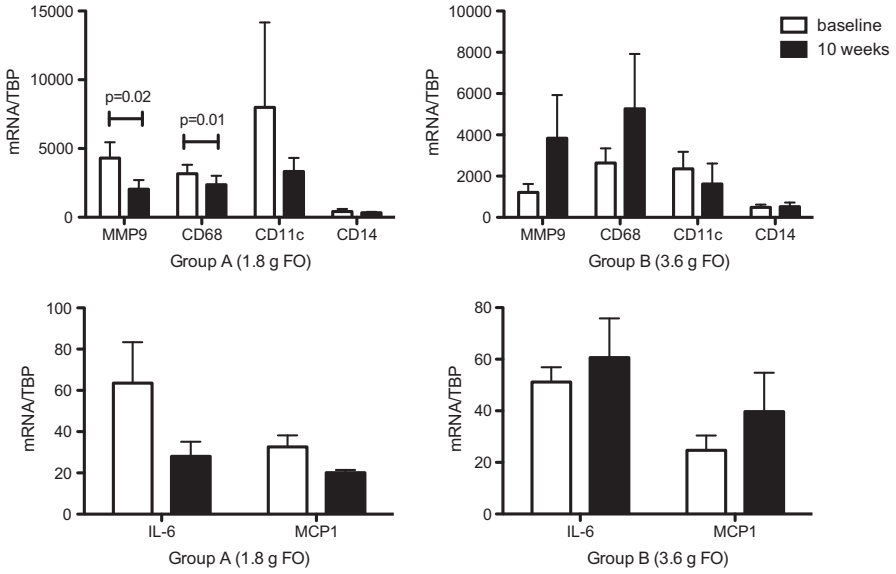


**Fig. 2.** Effect of 10 wk of fish oil treatment on the expression of inflammation related genes in subcutaneous adipose tissue. Data are normalized for TATA box binding protein (TBP) and expressed as mean ± SEM. CD11c, macrophage surface marker; CD14, macrophage surface marker; CD 68, macrophage phagocytic activity marker; IL-6, interleukine 6; MCP-1, monocyte chemotactic protein; MMP9, matrix metallo protease 9.

*Study strengths*

The studied population was homogenous, with similar range of glomerular filtration rate, and with exclusion of diabetic, obese, and also elderly patients, to tentatively reduce the effect of other confounding metabolic factors. These stringent inclusion and exclusion criteria may reduce potential variability in treatment effects. The compliance was excellent and confirmed by fatty-acid composition in plasma. Furthermore, we observed changes in gene expression, whereas body composition was not modified throughout the study in both groups. Finally, this is the first study to report a dose-effect response to ω-3 PUFA that may contribute to explain conflicting data about their antiinflammatory effects.

## Conclusions

Supplementation of a moderate dose of ω-3 PUFA promotes a more antiinflammatory-like adipose tissue gene expression profile, whereas high FO intake has no immunomodulatory effect while upregulating some adipokines gene expression and improving lipid profile.These data suggest a differential dose–response effect of ω-3 PUFA.

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