Nutrition 29 (2013) 730–736

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

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

ARTICLE INFO

Article history:
Received 21 May 2012
Accepted 19 October 2012

Keywords:
ω-3 PUFA
Adipose tissue
Inflammation
Chronic kidney disease
Adipokines

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).

0899-9007/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2012.10.011

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