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Research paper

# Eicosapentaenoic acid modulates fatty acid metabolism and inflammation in *Psammomys obesus*

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ABSTRACT

The desert gerbil, *Psammomys obesus*, is a unique polygenic animal model of metabolic syndrome (insulin resistance, obesity and type 2 diabetes), and these pathological conditions resemble to those in human beings. In this study, the animals were fed *ad libitum* either a natural diet (ND) which contained desertic halophile plants or a standard laboratory diet (STD) or a diet which contained eicosapentaenoic acid (EPA), hence, termed as EPA diet (EPAD). In EPAD, 50% of total lipid content was replaced by EPA oil. By employing real-time PCR, we assessed liver expression of key genes involved in fatty acid metabolism such as PPAR-α, SREBP-1c, LXR-α and CHREBP. We also studied the expression of two inflammatory genes, i.e., TNF-α and IL-1β, in liver and adipose tissue of these animals. The STD, considered to be a high caloric diet for this animal, triggered insulin resistance and high lipid levels, along with high hepatic SREBP-1c, LXR-α and CHREBP mRNA expression. TNF-α and IL-1β mRNA were also high in liver of STD fed animals. Feeding EPAD improved plasma glucose, insulin and triacylglycerol levels along with hepatic lipid composition. These observations suggest that EPA exerts beneficial effects in *P. obesus*.



## 1. Introduction

The last decades have been the witness of tremendous rise in global prevalence of obesity [1]. Obesity causes dyslipidemia, which associated with insulin resistance, generates ectopic lipid accumulation, leading to initiation and aggravation of type 2 diabetes (T2D), liver cirrhosis and cardiovascular disease [1].

The desert gerbil, *Psammomys obesus*, develops obesity, and then, T2D which are very similar to human pathologies as far as their installation and progression are concerned [2]. Indeed, this animal never develops T2D in its natural habitat because of its low calorie diet, composed mainly of halophile plants [2]. However, once transferred to laboratory conditions, this animal develops insulin resistance which rapidly turns into metabolic syndrome and T2D. In addition to its low physical activity, the standard laboratory chow brings high calories to this animal [3]. The development of obesity in this animal model is accompanied with low liver glucose-6-phosphate hydrolysis which contributes to insulin resistance with high lipogenic activity [2]. Insulin resistance in *P. obesus* is also associated with increased adipose tissue lipoprotein lipase (LPL) activity, thus contributing to high plasma trigacylgycerol (TAG) levels and increased fat storage [3]. Furthermore, the development of insulin resistance and diabetes in *P. obesus* has been shown to be accompanied by an increase in hepatic AMPK phosphorylation and mRNA levels of key lipogenic enzymes like stearoyl-CoA desaturase-1 (SCD1), mitochondrial glycerol-3-phosphate acyltransferase (mGPAT) and fatty acid synthase [4].

*Abbreviations:* ALT, alanine transaminase; AST, aspartate transaminase; CHREBP, carbohydrate response element binding protein; T2D, type 2 diabetes; EPA, eicosapentaenoic acid; IL-1β, interleukin 1β; LXR-α, liver X receptor α; LPL, lipoprotein lipase; MUFA, mono-unsaturated fatty acid; NEFA, non-esterified fatty acids; PPAR-α, peroxisome proliferator-activated receptor α; PPAR-γ, peroxisome proliferator-activated receptor γ; PUFAs, polyunsaturated fatty acids; $SCD1i_1$, stearoyl-CoA desaturase 1 index-1; $SCD1i_2$, stearoyl-CoA desaturase 1 index-2; SFA, saturated fatty acids; SREBP-1c, sterol regulatory element binding protein-1c; TAG, triacylglycerol; TC, total cholesterol; TNF-α, tumor necrosis factor α.

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It has been proposed that chronic excess of dietary n-6 fatty acids coupled to deficiency of n-3 fatty acids promotes obesity [5]. Moreover, several reports have shown beneficial effects of fish oils, containing n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in T2D in rodents and human beings [6]. The n-3 PUFAs exert protective effects, in part, by controlling synthesis and oxidation of saturated and monounsaturated fatty acids, thus, lowering hepatic fat contents and improving blood lipid profiles [7]. Besides, due to their anti-inflammatory properties, n-3 PUFAs could influence hepatic metabolism in context of insulin resistance and obesity. So far, no study has directly investigated the role of n-3 PUFAs, particularly EPA, in liver fatty acid metabolism and inflammation in *P. obesus*. The aim of present study is to assess whether dietary n-3 PUFAs, substituting n-6 PUFAs, trigger anti-inflammatory responses that would exert beneficial effects, at the transcriptional level, in *P. obesus*. Hence, we measured the expression of mRNA, encoding genes which regulate liver lipogenesis, fatty acid oxidation and tissue inflammation, and also determined liver fat contents and fatty acid composition in *P. obesus*, fed a diet containing n-3 fatty acids.

## 2. Materials and methods

### 2.1. Animals and diets

Male desert gerbils, *P. obesus,* trapped in southwest of Algeria (30°7 north latitude and 2°10 west longitude), were maintained as previously described [8]. During 15 days of acclimatization to laboratory conditions, the animals were fed on their natural halophile plant (*Salsola foetida*) which belongs to Chenopodiacae family. Detailed plant composition is presented in Table 1. This plant represents a low energy diet (1.67 kJ/g of fresh plant), thus providing 84±4 kJ/day/animal.

Later on, the animals were randomly separated into three groups: first group − animals ($n = 10$) were maintained on the same natural diet (ND); second group − animals ($n = 10$) were fed on a standard laboratory diet (STD) containing sunflower oil, rich in n-6 fatty acids; and third group − animals ($n = 10$) were fed on STD wherein 50% of sunflower oil was substituted by n-3 EPA (EPA-70), termed as EPA diet (EPAD) (Table 1). All animals of experimental groups had free access to food and saline water (0.9% of NaCl). To ovoid oxidation, EPA oil was aliquoted in glass bottles under a stream of nitrogen. Both oils were kept at 4 °C. The STD and EPAD contained equal amounts of sucrose, cellulose, casein, lipids, salt mixture, vitamin mixture, and DL-methionine (Table 1). These ingredients were purchased from Sigma (USA). As described and used previously [9], both STD and EPAD were iso-caloric and provided 13 kJ/g, and their compositions are presented in Table 1.

**Table 1**
Composition of the diets (% of diet).

| Diet composition | ND | STD | EPAD |
|---|---|---|---|
| Starch | – | 58.7 | 58.7 |
| Proteins | 3.5 | – | – |
| Casein | – | 20 | 20 |
| Lipids | 0.4 | – | – |
| Sunflower oil | – | 5 | 2.5 |
| Omegavie EPA 70 | – | – | 2.5 |
| Carbohydrates | 2.5 | – | – |
| Sucrose | – | 5 | 5 |
| Cellulose | 6 | 5 | 5 |
| Minerals | 6.8 | 4 | 4 |
| Vitamins | – | 2 | 2 |
| DL-methionine | – | 0.3 | 0.3 |
| Water | 80.8 | – | – |
| **Energy (kJ/g)** | 1.67 | 13 | 13 |

Sunflower oil (Fleurial, Cevital, Algeria) contained the following: C16:0 (6%), C18:0 (3.5%), C18:1 (27.5%), C18:2 (62%), n-3 PUFAs (1.5%) and α-tocopherol (3%). EPA-70 ethyl ester (Omegavie, Qualitysilver®Ice, France) contained the following: EPA (70%), other n-3 PUFAs (5%), other fatty acids (22%) and α-tocopherol (3%). Uneaten food was daily discarded. After 12 weeks of experiments, the animals were anesthetized by ketamine (50 mg/kg, El-Kendi, Algeria) and diazepam (5 mg/kg, Saidal, Algeria), and sacrificed by cervical dislocation. Blood was collected in heparinized tubes, and tissues (liver and epididymal adipose tissue) were quickly removed, weighed and conserved in liquid nitrogen or fixed in a suitable medium. All experimental procedures were approved by Algerian Institutional Animal Care Committee which belongs to National Administration of Algerian Higher Education and Scientific Research (Algiers).

### 2.2. Biochemical analysis

Plasma glucose and lipids (triacylglycerols and total cholesterol) were measured by a spectrophotometric method (Spinreact kit, Spain). Plasma insulin was determined by ELISA kit (EMD Millipore, USA) where rat insulin was used as standard. Non esterified fatty acids (NEFA) were measured by a colorimetric method (Wako Chemicals, Germany). Plasma aspartate transaminases (AST) and alanine transaminase (ALT) activities were measured by a spectrophotometric method, using Cobas Mira automatic analyzer.

### 2.3. Liver lipids, fatty acid composition and SCD1 index

Hepatic lipids were extracted as described elsewhere [10]. Hepatic TAG and cholesterol were measured in lipid extracts by using commercial colorimetric kit (Sodioba, USA).

The lipid extract was dried under a stream of nitrogen in teflon tubes, saponified and transmethylated at 80 °C for 20 min with 14% boron trifluoride/methanol. Fatty acid methyl esters were then extracted in the presence of 2 ml of hexane and separated by gas liquid chromatography (Packard, USA), equipped with flame ionization detector set at 240 °C and a 30-m capillary glass column coated with carbowax 20 M (Applied Science Labs, USA). C17:0 methyl ester was used as an internal standard. Analysis of fatty acid peaks was achieved with reference to retention time of authentic standards (Nu-Chek-Prep, USA) by using Delsi Enica 31 (Delsi Nermag, France).

SCD1 is an enzyme that catalyzes the desaturation of palmitate (16:0) to palmitoleate (16:1), and stearate (18:0) to oleate (18:1). As it was not possible to measure directly its activity, we deduced its activity by calculating ratio between product and precursor [11]. Hence, two indices were calculated, i.e., SCD1 index-1 ($SCD1i_1$) by the ratio of C16:1/C16:0 and SCD1 index-2 ($SCD1i_2$) by the ratio of C18:1/C18:0.

### 2.4. Detection of mRNA by real-time PCR (RT-PCR)

Total RNA from liver and epididymal adipose tissue was isolated by Trizol reagent (Invitrogen, USA). Quality of isolated RNA was determined using denaturing agarose gel electrophoresis. RNA was quantified by determining its UV absorbance at 260 nm. 500 ng of total RNA was reverse transcribed with iScript cDNA synthesis kit according to manufacturer's instructions (Bio-Rad, France). RT-PCR was performed on iCycler iQ real-time detection system, and amplification was undertaken by using SYBR Green detection. Primers against genes of interest were taken from previous published studies [12–14] as described in Table 2.