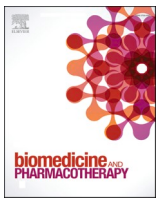
Biomedicine & Pharmacotherapy 164 (2023) 114945

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha



Preventive nutritional supplementation with furan fatty acid in a DIO mouse model increases muscle mass and reduces metabolic disorders

Laetitia Dore [a], Erwann Durand [b,c], Béatrice Bonafos [a], Jatuporn Chaiyut [f], Laurent Vaysse [d,e], Siriluck Liengprayoon [f], Sylvie Gaillet [a], Laurence Pessemesse [a], Karen Lambert [g], Christelle Bertrand-Gaday [a], Charles Coudray [a], Ariane Sultan [h], François Casas [a,i,*], Christine Feillet-Coudray [a,1,*]

[a] DMEM, Univ Montpellier, INRAE, Montpellier, France
[b] CIRAD, UMR Qualisud, 34398 Montpellier, France
[c] Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de La Réunion, Montpellier, France
[d] CIRAD, UPR BioWooEB, 34398 Montpellier, France
[e] BioWooEB, Univ Montpellier, CIRAD, Montpellier, France
[f] Kasetsart agricultural and agro-industrial product improvement institute, Kasetsart University, Bangkok, Thailand
[g] INSERM U1046, Montpellier, France
[h] Service Diabète-Nutrition, Université Montpellier, PHYMEDEXP, Montpellier, France

ARTICLE INFO

Key words:
Furan fatty acids
Obesity
Insulin sensitivity
Skeletal muscle
Liver
Mitochondria

ABSTRACT

The increase in obesity has become a major global health problem and is associated with numerous metabolic dysfunctions. Furan fatty acids (FuFAs) are minor lipids present in our diet. Recently we showed that FuFA-F2 extracted from *Hevea brasiliensis* latex stimulates muscle anabolism in mice in vitro and in vivo, mimicking in part physical activity. While skeletal muscle is essential for energy metabolism and is the predominant site of insulin-mediated glucose uptake in the post prandial state, our results suggested that FuFA-F2 could have favorable effects against obesity. The aim of this work was therefore to study whether a preventive nutritional supplementation with FuFA-F2 (40 mg or 110 mg/day/kg of body weight) in a diet-induced obesity (DIO) mouse model may have beneficial effects against obesity and liver and skeletal muscle metabolic dysfunction. We showed that 12 weeks of FuFA-F2 supplementation in DIO mice decreased fat mass, increased lean mass and restored normal energy expenditure. In addition, we found that FuFA-F2 improved insulin sensitivity. We revealed that FuFA-F2 increased muscle mass but had no effect on mitochondrial function and oxidative stress in skeletal muscle. Furthermore, we observed that FuFA-F2 supplementation reduced liver steatosis without impact on mitochondrial function and oxidative stress in liver. Our findings demonstrated for the first time that a preventive nutritional supplementation with a furan fatty acid in DIO mice reduced metabolic disorders and was able to mimic partly the positive effects of physical activity. This study highlights that nutritional FuFA-F2 supplementation could be an effective approach to treat obesity and metabolic syndrome.

1. Introduction

The increase in obesity has become a major global health problem [1] and is associated with numerous metabolic dysfunctions including insulin resistance and inflammation. Obesity contributes strongly to the risk of developing metabolic syndrome [2]. Metabolic syndrome is also associated with an increased risk of cardiovascular disease and cerebrovascular disease (CVD) [3]. Due to its mass and function, skeletal muscle is essential for energy metabolism and is the predominant site of insulin-mediated glucose [4]. Maintaining its function is therefore central to limit the risk of developing a metabolic syndrome.

Furan fatty acids (FuFAs) are minor fatty acids present in small quantities in food and are characterized by a furan ring in the carboxyalkyl chain [5]. FuFAs are synthesized in plants [6] and

* Corresponding authors.
E-mail addresses: francois.casas@inrae.fr (F. Casas), christine.coudray@inrae.fr (C. Feillet-Coudray).
[i] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2023.114945
Received 27 March 2023; Received in revised form 25 May 2023; Accepted 26 May 2023
Available online 30 May 2023

microorganisms [7,8]. After ingestion, they are also found in animals and humans [5]. Fish is a particularly important food source of FuFAs, due to its high consumption of algae and marine microorganisms rich in FuFAs [9,10], and FuFAs represents up to 1% of the fatty acids found in commercial fish oils [11]. Interestingly, these fatty acids, in the form of FuFA-F2 (10,13-epoxy-11-methyl-octadecan-10,12-dienoic acid), are particularly abundant in the latex of PB235 clone of *Hevea brasiliensis* latex used to produce the natural rubber [12,13]. FuFAs have various biological effects that highlight that they are valuable bioactive components for human health [14]. In particular, due to the electron-rich furan ring, FuFAs have antioxidant properties [15–17]. In the presence of ROS, its ring will open and form an unstable and highly reactive dioxoene [18]. However, due to the lack of commercially available FuFAs, only a few studies have been performed to test their biological function. *In vitro*, FuFAs increase the expression of adiponectin, an adipokine known for its anti-inflammatory and insulin sensitive effects in 3T3-L1 adipocyte cells [19]. *In vivo*, in a rat model of arthritis, FuFAs have a greater anti-inflammatory activity than eicosapentaenoic acid (EPA) [20]. Furthermore, many works indicate that the ω-3 PUFAs contained in fish may contribute to the prevention of CVD [21,22]. However, few studies suggest that it is the FuFAs present in fish that may be involved in the beneficial effect of fish consumption on CVD [10]. In addition, in vitro studies show that FuFAs inhibit platelet aggregation induced by arachidonic acid [23], reduce the oxidation of low-density lipoproteins (LDL) [16] and inhibit the progression of non-enzymatic lipid peroxidation [24]. Thus, after consumption of fish, FuFAs, incorporated into blood phospholipids and in intimate contact with endothelial cells where LDL oxidation occurs [25], would protect against the development of atherosclerosis. Very recently, using FuFA-F2 extracted from *Hevea brasiliensis* latex, we demonstrated that this furan fatty acid increased protein content in C2C12 myotubes in vitro (Pelletier et al., in revision) [26]. In addition, we showed that oral gavage with FuFA-F2 for 3 weeks was sufficient to increase muscle mass and promote a more oxidative muscle metabolism in C57Bl6 mice. Our findings demonstrated for the first time that FuFA-F2 stimulates muscle anabolism in mice in vitro and in vivo, mimicking in part physical activity [26].

Obesity is associated with numerous metabolic dysfunctions. While liver is a key organ that performs a wide range of biochemical functions necessary for the metabolic homeostasis of the entire body, skeletal muscle is essential for energy metabolism and is the predominant site of insulin-mediated glucose uptake in the post prandial state [4]. Our previous data demonstrating that FuFA-F2 promotes muscle anabolism suggest that it could be beneficial in patients suffering from obesity and insulin resistance [26]. The aim of this study is to determine whether preventive nutritional supplementation with FuFA-F2 could slow down the development of metabolic disorders on an obesogenic diet-fed mice model.

2. Materials and Methods

2.1. FuFA-F2 purification

Fresh latex from the PB235 *Hevea brasiliensis* clone was collected from a plantation of Visahakit Thai Rubber Co., Ltd., Chanthaburi, Thailand. Lipid extraction was performed by adding freshly harvested latex dropwise into continuously stirred ethyl acetate (Univar, Illinois, USA) with a ratio latex:solvent of 1:5 (v/v). Coagulum made mainly of insoluble polyisoprene was removed by filtration and the extract was let decanted in separating funnel overnight. The upper fraction of lipid-containing ethyl acetate was collected and evaporated. Total lipid extract was further purified using flash chromatography (Combiflash EZ Prep, Teledyne ISOC, USA). Lipid extract (9 g of lipids dissolved in hexane) was loaded onto a 750 g column containing 300 g of silica gel 60 (0.040–0.063 mm, Merck). Mobile phase was made of 2.4 l of hexane: diethyl ether (90:10, v/v) at the flow rate of 50 ml/min and the collection volume was 50 ml/fraction. The separation quality was checked by spotting an aliquot of each fraction on a thin layer chromatography plate following the method described for neutral lipid by Liengprayoon et al. [27]. The selected fractions containing only Trifuranoylglycerols (TGF) were saponified and the purity of the produced free FuFA-F2 was analyzed by GC-FID on methyl ester derivatives as described previously [27].

2.2. Animals and diets

Forty 6-week-old male C57BL/6 J mice (Janvier Labs), weighing about 22 g, were housed (2 per cage) under conditions of constant temperature (22 °C +/−1 °C), humidity (40–50%) and a standard dark cycle (19.30–07.30 h). The mice were randomized, according to their initial weight, into four groups of ten animals and fed for 12 weeks with one of the four following semi-purified diets: (1) control diet, (2) HFHS diet, (3) HFHS + FuFA-F2 (0.048% FuFA-F2/kg diet), (4) HFHS + FuFA-F2 (0.127% FuFA-F2/kg diet). The detailed composition of the diets is given in the supplementary Table 1. The control diet contains 5% lipids as a mixture of rapeseed oil, high oleic sunflower oil, sunflower oil and linseed oil (oil mixture of Carrefour), the HFHS diets contain 25% lipids (5% of a mixture of rapeseed oil, high oleic sunflower oil, sunflower oil and linseed oil and 20% of lard) and 30% sucrose. The FuFA-F2 was incorporated into the diet after dissolution in the combined table oils (oil mixture of Carrefour). For the first dose of FuFA-F2 this corresponds for a mouse to an intake of about 40 mg/day/kg of mice and for the second dose of FuFA-F2 to an ingestion of 110 mg/day/kg. Throughout the study, mice were given free access to tap water and to their respective diets. Mice body weight was followed weekly and food consumption was determined every 2 days (during the week) or 3 days (the weekend). A detailed scheme of the study design is provided in Fig. 1.

2.3. Body composition and metabolic analyses

Mice whole-body composition (fat and lean masses) was measured every 2 weeks using an EchoMRI-700 whole-body composition analyzer, according to the manufacturer's instructions. Oxygen consumption and carbon dioxide production were measured using a Comprehensive Lab Animal Monitoring System (CLAMS) (Columbus Instruments). Mice were housed in individual cage inside a controlled cabinet. Mice were acclimatized individually in metabolic cages at 22 °C with ad libitum access to food and water for 24-h, prior to a 24-h period of automated recordings. Sampled air from individual cages was passed through sensors to determine O2 and CO2 content. The respiratory exchange ratio (RER) was calculated as the volume of CO2 vs. volume of O2 (VCO2/VO2) ratio [28].

2.4. Oral Glucose Tolerance Test (OGTT) and Insulin Tolerance Test (ITT)

Following an overnight fasting (13 h), mice were administrated glucose solution (2 g/kg) by oral gavage, and blood samples were collected from the tail vein at the indicated times for glucose and insulin determination. Insulin tolerance test were performed the last weeks of the study. ITT was assessed after 2 h fasting by administration of human insulin (0.75 U/kg) by intraperitoneal injection and blood glucose levels were monitored. Blood samples were collected from the tail vein at the indicated times. Glycemia was measured using a OneTouch Verio glucometer (Lifescan).

2.5. Sampling and routine biochemical analyses

At the end of the experiment, the animals were anaesthetized and blood from 13 h-fasted mice was collected from the inferior vena cava and distributed into heparinized tubes. Plasma was collected and stored at −80 °C until analysis. After cervical dislocation of mice, liver and muscles were removed, rinsed with 0.9% NaCl, weighed, cut into several

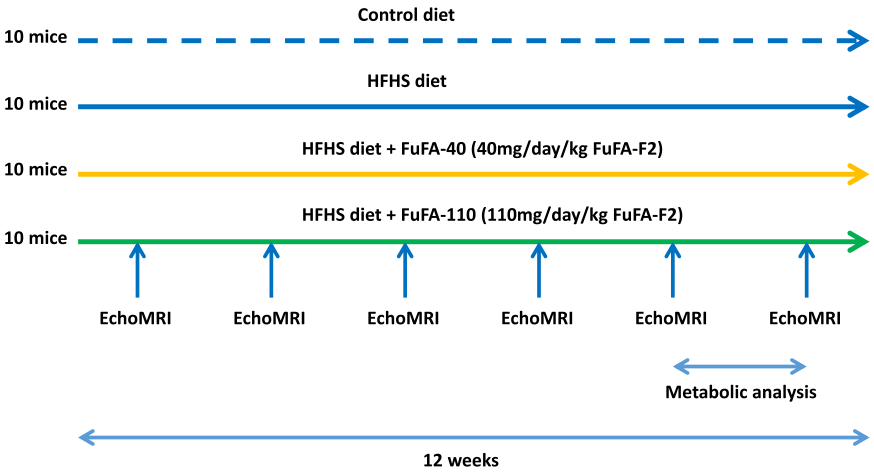


Fig. 1. Design of the study. Forty 6-week-old male C57BL/6 were fed for 12 weeks a control diet, a HFHS diet, a HFHS diet+FuFA-40 (0.048% FuFA-F2/kg diet) or a HFHS diet+FuFA-110 (0.127% FuFA-F2/kg diet). Whole-body composition was measured every 2 weeks using an EchoMRI-700 analyzer. Mice oxygen consumption and carbon dioxide production were measured using a Comprehensive Lab Animal Monitoring System the last weeks of the study. Oral glucose tolerance test and insulin tolerance test were performed the last weeks of the study.

parts, when necessary, plunged into liquid nitrogen and then kept at −80 °C until analysis. Plasma levels of glucose, total cholesterol, triglycerides and free fatty acids as well as enzymatic activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured at the ANEXPLO analysis platform (CHU RANGUEIL-BP 84225, France). Plasma levels of insulin and leptin were quantified with ELISA kits (Merck Millipore and Crystal Chem respectively).

2.6. Neutral lipids measurement

Liver or muscle samples were homogenized in NaCl solution (9 g/L) and Triton X-100 (0.1%) and free fatty acids, triglycerides and total cholesterol levels were quantified on the tissue homogenate by enzymatic methods (Wako-NEFA-C kit; Cholesterol CHOD-PAP SOBIODA kit and triglycerides LQ SOBIODA kit) [29].

2.7. Plasma quantification of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF)

Plasma levels of CMPF were quantified with ELISA kit (MyBiosource), according to manufacturer instruction.

2.8. Histological study

After euthanasia by cervical dislocation, liver samples and the tibialis anterior muscles were collected. Livers were immediately fixed in 4% paraformaldehyde at 4 °C for 24 h then embedded in paraffin. Tissue sections were cut at a thickness of 4 μm and stained with Hematoxylin & Eosin (H&E). Tibialis anterior muscle were freshly frozen in Tissue-Tek, and then stored at −80 °C. For immunostaining, the sections (10 μm thickness) were permeabilized 30 min in PBS, 20% horse serum, 0.1% triton at RT, and incubated for 1 h at 37 C with the anti-laminin (1/200, rabbit polyclonal, Sigma L9393) in a solution of PBS, 1% BSA. Sections were washed in PBS 1X for 10 min 3 times and incubated with the secondary antibody (Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488) for 1 h at 37 C. Sections were washed in PBS 1 × 2 times for 10 min, incubated 30 s with DAPI, and washed once in PBS for 10 min and mounted with Aqueous Mounting Medium PermaFluor™. For the morphometric analysis, muscle sections were scanned using a NanoZoomer (Hamamatsu Photonics) with a 20X objective. Image J free software was used to analyze and quantify the pictures for each entire area.

2.9. Tissue oxidative stress status and mitochondrial enzymatic activities

Long-established oxidative stress parameters (TBARS (Thiobarbituric Acid Reactive Susbtances), -SH, GSH (glutathione), catalase, SOD and MnSOD (superoxide dismutase), GPX (glutathione peroxidase)), citrate synthase and different mitochondrial respiratory complex activities were measured in liver and muscle as previously reported [30,31].

2.10. Protein isolation and western blotting analysis

Frozen liver or muscle samples were homogenized using an Ultra Turax homogenizer. Protein levels were assessed by Western blotting. Total proteins were measured using the Bio-Rad protein assay according to the manufacturer's instructions. Signals were revealed using a ClarityTM Western ECL Substrate Kit, and proteins were visualized by enhanced chemiluminescence using the ChemiDoc Touch Imaging System and quantified with Image Lab. Touch Software (version 5.2.1).

2.11. Antibodies

Primary antibodies used include: anti-GLUT2 (1/1000, Abcam ab54460); anti-GLUT4 (1/2000, Abcam ab654); anti-p-ACC (1/1000, Abcam ab92809), anti-FAS (1/1000, Cell signaling 3180); anti-PGC1α (1/1000, Abcam 54481); anti-CPT1 (1/1000, Abcam ab137550); Anti-α-Tubulin (1/6000, Sigma T5160); Anti-beta-Actin (1/400, Santa Cruz sc81178).

2.12. Statistical analyses

All results are presented as means ± SD, or as percentages. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test. The limit of statistical significance was set at $p < 0.05$. The means with different letters were significantly different. Statistical analyses were performed using the StatView program.

3. Results

3.1. In DIO mice, FuFA-F2 decreased fat mass, increased lean mass and restored normal energy expenditure

As expected, final body weight and fat mass were increased with 12 weeks of HFHS diet compared to controls (Fig. 2 A), although moderately. Interestingly, FuFA-F2 supplementation with the highest dose (110 mg/day/kg) induced a significant decrease in fat mass (9.2 g for HFHS vs 6.9 g for FuFA-110, $p < 0.05$) and increase in lean mass (22.4 g for HFHS vs 23.6 g for FuFA-110, $p < 0.05$). The lower dose of FuFA-F2 (40 mg/day/kg) induced less marked effects that did not reach statistical significance (Fig. 2 A) (supplementary Figure 1). We recorded no specific effect of FuFA-110 on food intake or leptin secretion compared

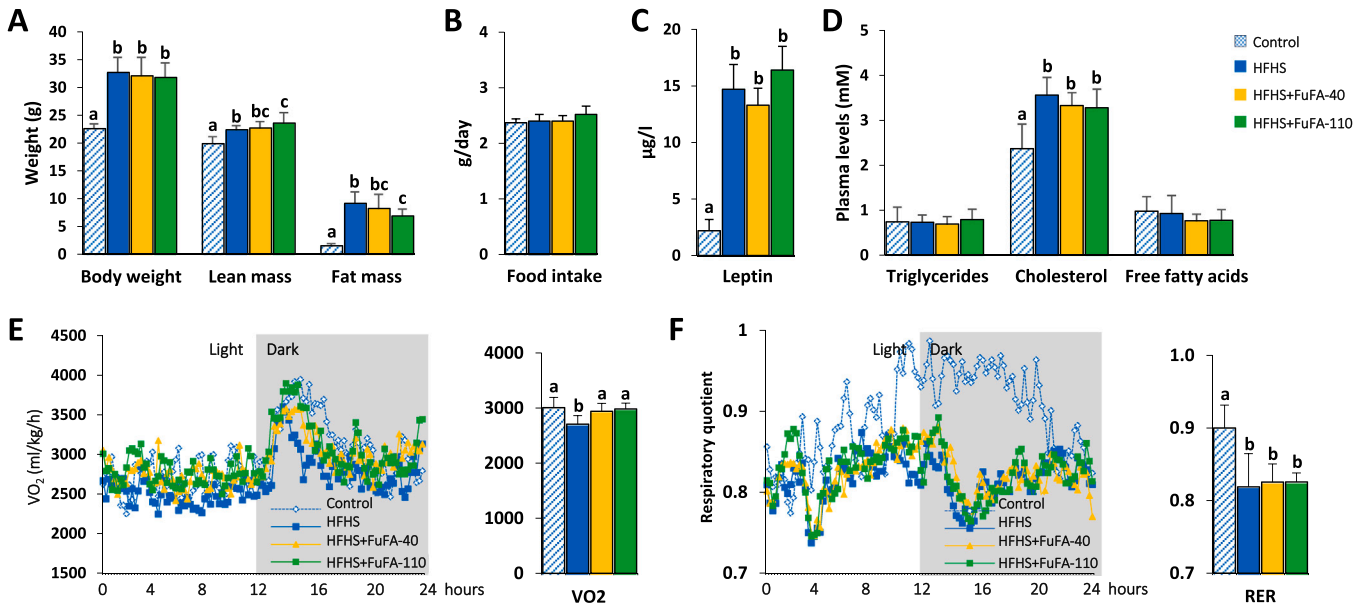


Fig. 2. Mice characteristics. (A) Body weight, lean and fat mass. (B) Food intake. (C) Plasma leptin. (D) Plasma cholesterol triglycerides and free fatty acids. (E) Oxygen consumption in mice (VO2). (F) Respiratory exchange ratio (RER) in mice. For each mouse, VO2 and VCO2 were measured 111 times in about 24 h. The respiratory exchange ratio (RER) was calculated as the volume of CO2 vs. volume of oxygen (VCO2/VO2) ratio. Mean VO2 and RER values are mean of 8 mice for each group considering all the cycles. For all other parameters, n = 10 animals per group. Results were expressed as means ± SD. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test. The limit of statistical significance was set at $p < 0.05$. The means with different letters were significantly different.

to the DIO mice (Fig 2BC). In addition, while plasma cholesterol levels were significantly increased with HFHS diet compared to controls, no specific effect of FuFA-F2 was observed on plasma triglycerides, cholesterol and free fatty acids levels (Fig. 2D).

We next investigated the basal metabolism, oxygen consumption and carbon dioxide production using Oxymax-CLAMS system. We found that oxygen consumption reflecting metabolic expenditure was decreased in DIO mice compared to controls (3007 ml O_2/kg/h for control vs 2707 ml O_2/kg/h for HFHS, $p < 0.0001$) (Fig. 2E). In addition, we observed that FuFA-F2 supplementation restored normal energy expenditure whatever the doses (2940 ml O_2/kg/h for FUFA-40 and 2983 ml O_2/kg/h for FuFA-110) (Fig. 2E). Moreover, analysis of RER revealed that as expected HFHS diet favored the oxidation of lipids, which was reflected by a lower RER value compared to controls

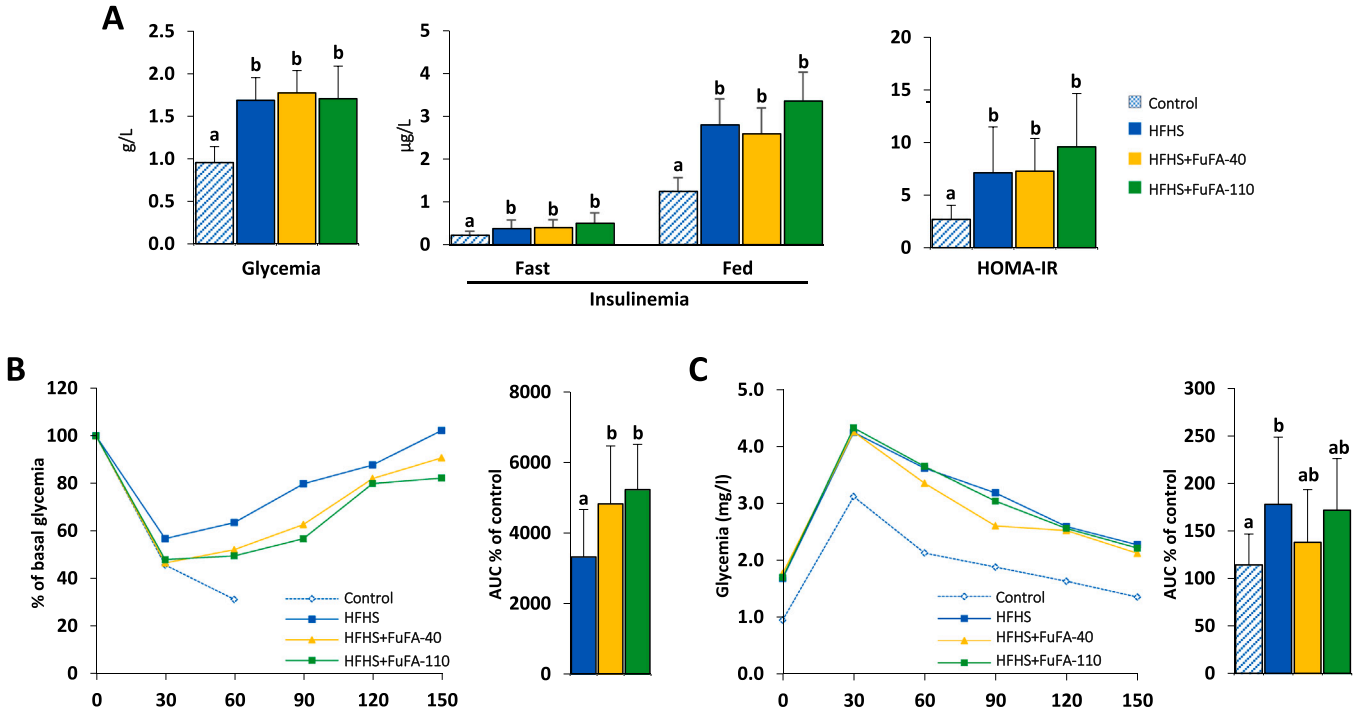


Fig. 3. Glucose homeostasis parameters. (A) Glycemia in fast state, plasma insulin level in fast and fed state and HOMA-IR. (B) Insulin tolerance test. (C) Oral glucose tolerance test. Results were expressed as means ± S.D., n = 8–10 animals per group. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test. The limit of statistical significance was set at $p < 0.05$. The means with different letters were significantly different.

(Fig. 2 F). FuFA-F2 supplementation no further impacted RER compared to HFHS group (Fig. 2 F).

3.2. In DIO mice, FuFA-F2 improved insulin sensitivity

In fasted state we showed that plasma glucose, insulin levels and HOMA-IR index were increased in mice fed with the HFHS diet without effects of FuFA-F2 supplementation (Fig. 3A). It is important to note that in mice these values do not correspond to pathological values [32]. Next, we perform the insulin tolerance test. In control animals, insulin caused an excessive drop in blood glucose levels and it was even necessary to interrupt the insulin tolerance test and inject glucose 30 min after insulin injection. Indeed, their glycemia levels has reached the lower limit set in the ethical guidelines (Fig. 3B). Interestingly, we found that FuFA-F2 supplementation increased insulin sensitivity compared to DIO mice, whatever the dose of FuFA-F2 (respectively to HFHS group +45% for FuFA-40 and +57% for FuFA-110, $p < 0.05$) (Fig. 3B). According to OGTT, DIO mice were glucose intolerant (Fig. 3B). In DIO mice supplemented with FuFA-F2 we also observed an increase in the area under the curve. However, and unlike the DIO mice, this difference was not significant compared to the control group, suggesting that FuFA-F2 slightly improved glucose tolerance (Fig. 3C).

3.3. In DIO mice, FuFA-F2 increased muscle mass

We found that 3 months of food supplementation with FuFA-F2 increased quadriceps weight (the heavier skeletal muscle in mice) (185 mg for HFHS group vs respectively 199 mg for FuFA1 and 211 mg for FuFA2, $p < 0.05$) and tended to increase gastrocnemius weight ($p < 0.07$) compared to HFHS group (Fig. 4 A), in a way that seems dose-dependent. In addition, muscle cholesterol, triglycerides and free fatty acids content remained unchanged whatever the diet (Fig. 4B). Next, we examined the cross-sectional area of the tibialis muscle although the weight of this small postural muscle did not appear to be affected by FuFA-F2 supplementation (supplemental Fig. 2). However, this muscle is the most practical for analyzing the whole surface. Morphometric analysis of tibialis indicated that neither the HFHS diet nor FuFA-F2 supplementation had any impact on total fiber number and mean cross-sectional area (CSA) (Fig. 4C-D). Furthermore, analysis of the number of fibers in each range of area also showed no difference whatever the groups (Fig. 4E). In addition, skeletal muscle expression of glucose transporter GLUT4 (a key player in the insulin-induced glucose uptake by muscle), PGC1α (a master regulator of mitochondrial activity), FAS (fatty acid synthase, an enzyme that catalyzes fatty acid synthesis), phosphorylated form of ACC (Acetyl-CoA-carboxylase, involved in the biosynthesis of fatty acids) and CPT1 (carnitine acyltransferase 1, an essential enzyme in the beta-oxidation of long chain fatty acids) was studied by western-blot. Except for the expression of FAS which

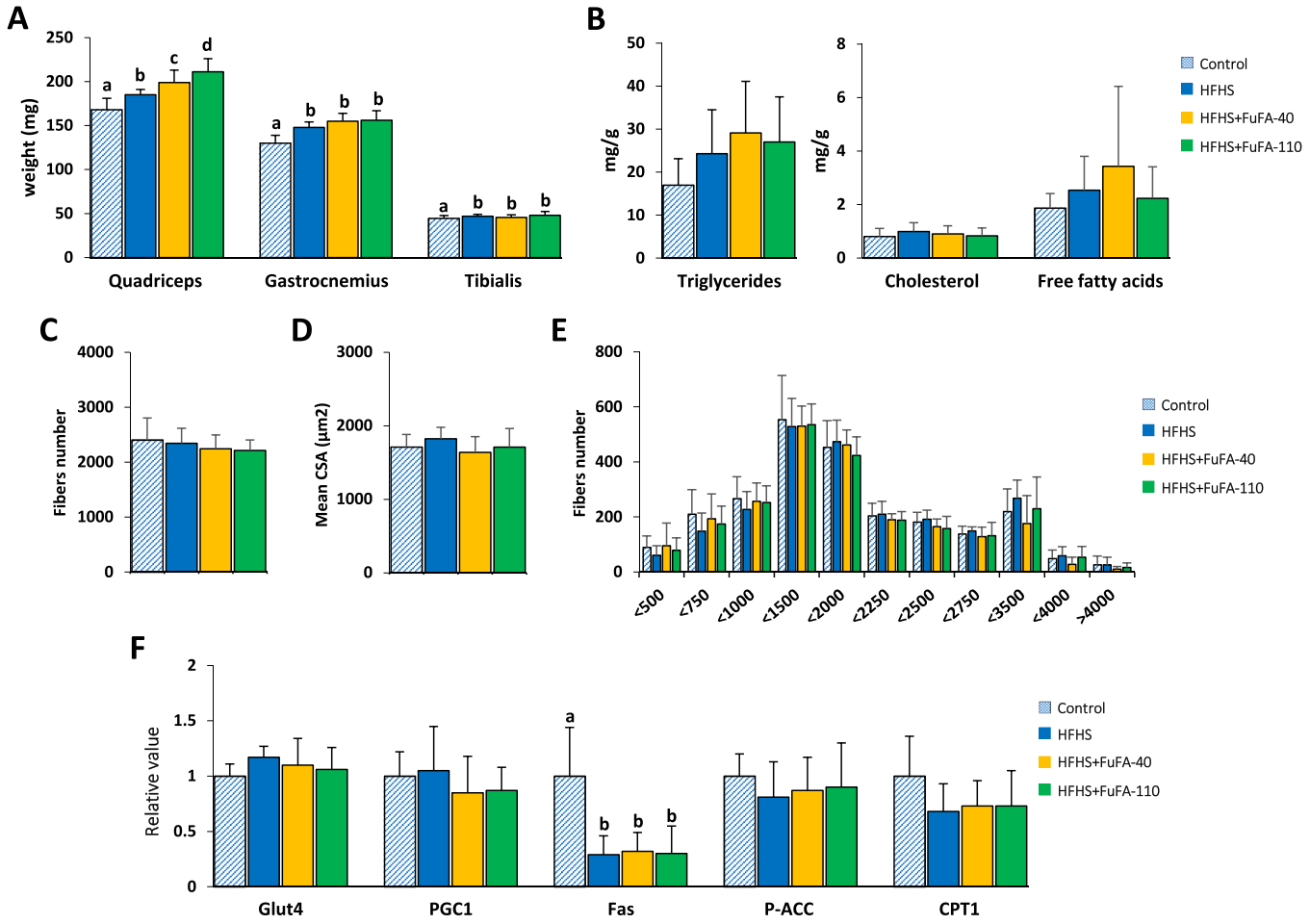


Fig. 4. Muscle phenotype. (A) Quadriceps, gastrocnemius and tibialis muscles weight. (B) Quadriceps muscle content of cholesterol triglycerides and free fatty acids measured on tissue homogenate by enzymatic method. (C) Total fibers number. (D) Mean fibers area (μm^2). (E) Fiber size distribution in tibialis muscles. (F) Relative Quadriceps muscle protein expression of key players in glucose and lipogenesis homeostasis. Results were expressed as means ± S.D., n = 10 animals per group. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test. The limit of statistical significance was set at $p < 0.05$. The means with different letters were significantly different.

decreased with the HFHS diet, none of these proteins were altered by either the diet or by FuFA-F2 supplementation (Fig. 4 F).

3.4. In DIO mice, FuFA-F2 had no effect on mitochondrial function and oxidative stress in skeletal muscle

We further measured the mitochondrial respiratory chain activities that determine the metabolic properties of skeletal muscle. Citrate synthase activity (CS), frequently used as a marker of the number of mitochondria, remained unchanged whatever the groups (Fig. 5 A). In addition, the activities of mitochondrial respiratory chain complexes were also not modified (Fig. 5B). 3-Hydroxyacyl-CoA dehydrogenase (beta-HAD) is an enzyme involved in the mitochondrial β oxidation. While it increased in the HFHS group, we observed that FuFA-F2 supplementation reduced its expression to a level similar to the control group (Fig. 5 C). Regarding oxidative stress, we found an increase of TBARS levels on DIO mice. Interestingly, we showed that this level decreased in mice supplemented with the lowest dose of FUFA-F2 and tended to decrease with the highest dose (Fig. 5D). However, HFHS diet or FuFA-F2 supplementation had no effect on others parameters such as -SH levels, GSH content and antioxidant enzyme activities (catalase, SOD and GPx) (Fig. 5D-F).

3.5. In DIO mice, FuFA-F2 reduced liver steatosis

We found that liver weight and liver cholesterol level were increased by the HFHS diet (Fig. 6AC). However, plasma ALAT and ASAT activities (Alanine and Aspartate aminotransferase), biochemical markers of hepatic fibrosis, were not modified regardless of the diet (Fig. 6B). Interestingly, whereas we observed a significant increase of liver triglycerides in DIO mice, we found that FuFA-F2 supplementation decreased triglyceride levels and even restored normal levels with the highest dose (Fig. 6 C). Free fatty acid levels remained unchanged whatever the groups (Fig. 6 C). Furthermore, in line with triglyceride analysis, histologic analysis revealed the presence of some lipid micro droplets in liver of DIO mice compared to controls (Fig. 6D). However, these micro droplets were almost absent in FuFA-F2 supplemented animals. These data indicate that FuFA-F2 supplementation prevent or slow down liver steatosis induced by HFHS diet. Liver gene expression of key players in glucose and lipogenesis homeostasis revealed no difference whatever the groups excepted for PGC1α which decreased with FuFA-F2 supplementation (Fig. 6E).

3.6. In DIO mice, FuFA-F2 had no effect on mitochondrial function and oxidative stress in liver

We next investigated the mitochondrial function and oxidative stress parameters in liver. We found a slight decrease of the activities of citrate synthase and mitochondrial respiratory chain complexes with the HFHS diet. However, FuFA-F2 supplementation had no influence (Fig. 7AB). In addition, beta-HAD remained unchanged (Fig. 7 C). Regarding oxidative stress, HFHS diet or FuFA-F2 supplementation had no effect on -SH levels, GSH content and antioxidant enzyme activities (catalase, SOD and GPx) (Fig. 7D-F) while we observed a slight decrease level of TBARS (a marker of lipid peroxidation) with FuFA-F2 supplementation (Fig. 7 C). This set of data indicates that preventive FuFA-F2 supplementation had no effect on mitochondrial function and oxidative stress in liver.

3.7. In DIO mice, FuFA-F2 had no effect on plasma CMPF concentrations

3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) is one of the major endogenous metabolites of furan fatty acids and omega 3 fatty acids [33,34]. Interestingly, Prentice et al. [35] showed that administration of CMPF to mice before or after high-fat diet feeding improved insulin sensitivity, increased beta-oxidation and reduced hepatic steatosis. In order to verify whether the different health benefits of FuFA-F2 supplementation could be related to an increase in CMPF, we measured plasma CMPF concentrations in mice. However, our results showed that FuFA-F2 supplementation did not induce any increase in CMPF in the plasma of mice (Fig. 8). These data indicate that the health benefits of FuFA-F2 are not related to an increase in plasma CMPF levels.

4. Discussion

The aim of this study was to determine whether 12 weeks of preventive nutritional supplementation with FuFA-F2 could prevent the development of metabolic disorders on an obesogenic diet-fed mice model. In this study we tested 2 different doses which corresponded to approximately 40 mg and 110 mg/day/kg of body weight of FuFA-F2.

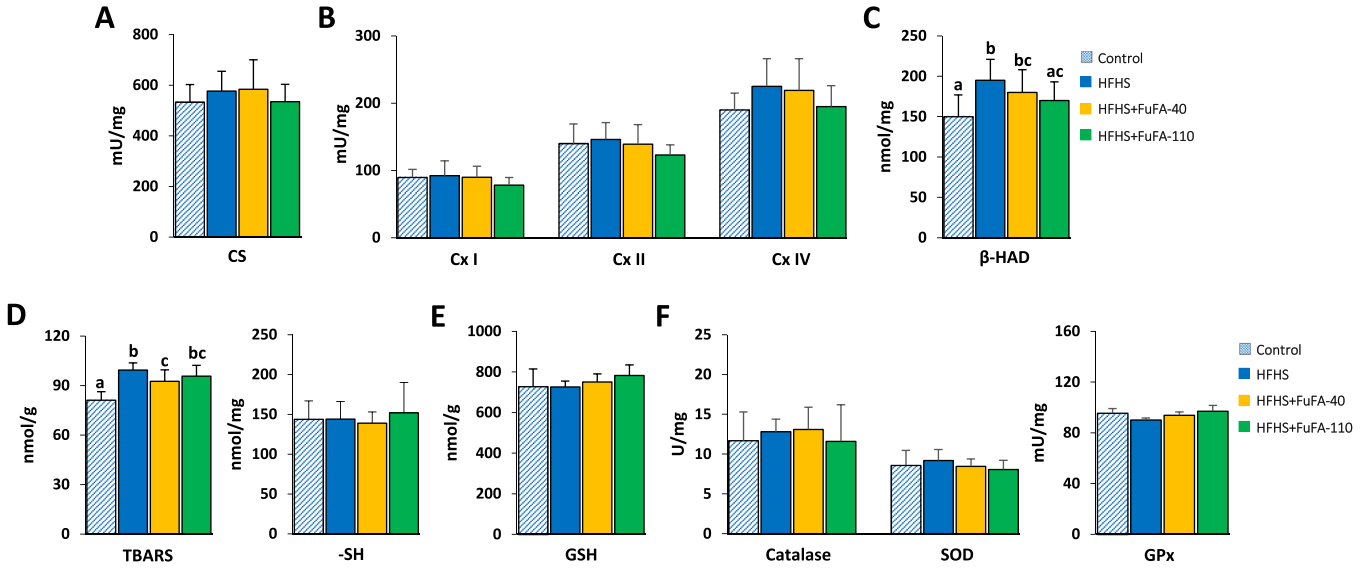


Fig. 5. Mitochondrial activity and muscle oxidative stress parameters. (A) Citrate synthase activity. (B) Mitochondrial respiratory chain activities. (C) β-HAD activity. (D) TBARS and –SH values. (E) GSH content. (F) Catalase, SOD and GPx activities. Results were expressed as means ± S.D., n = 10 animals per group. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test. The limit of statistical significance was set at $p < 0.05$. The means with different letters were significantly different.

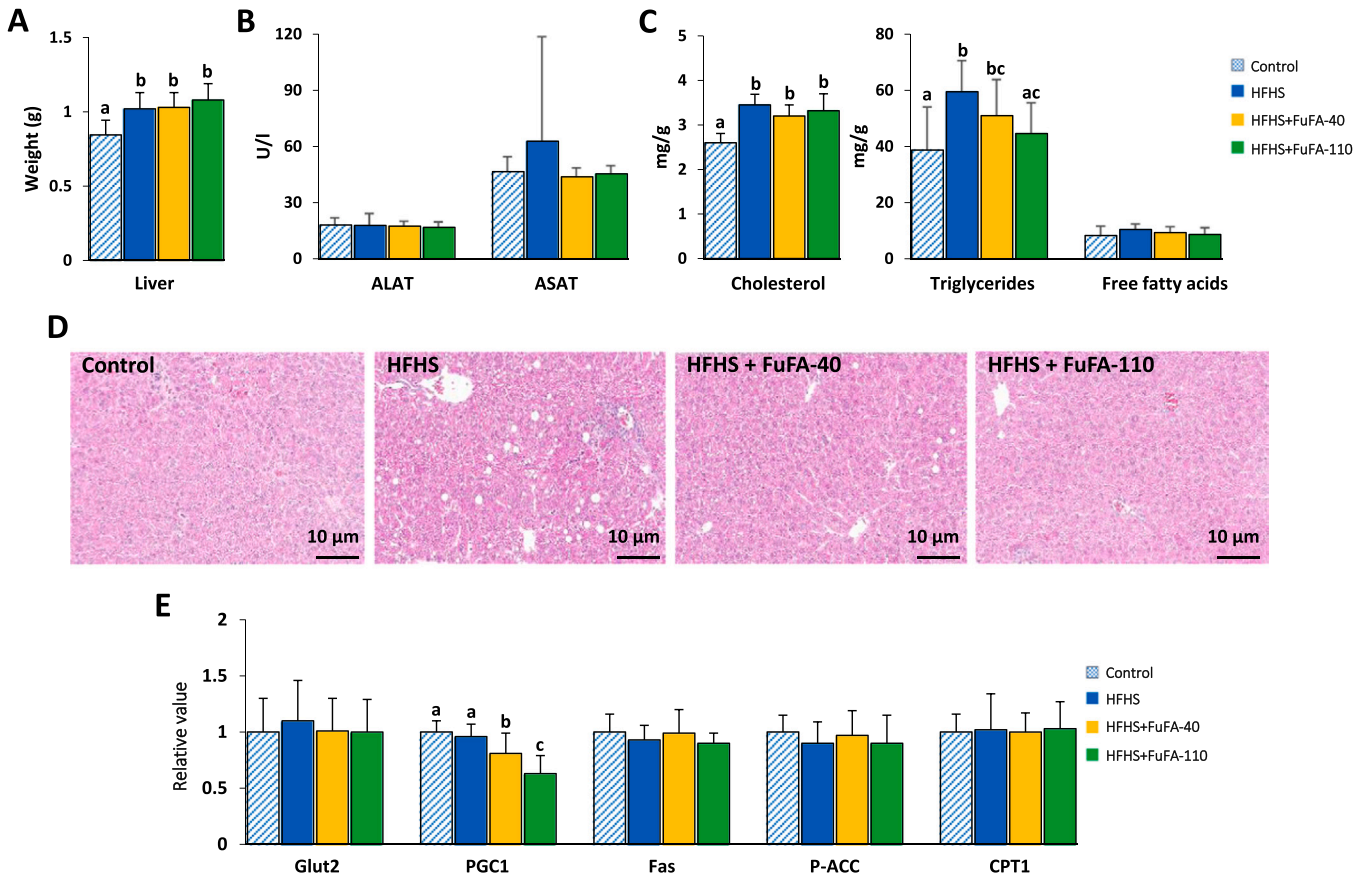


Fig. 6. Liver phenotype. (A) Liver weight after 12 weeks of diet. (B) Plasma ALAT and ASAT activities. (C) Hepatic content of cholesterol triglycerides and free fatty acids. H&E staining of liver, pictures are representative from each group. Scale bar, 10 µm. (E) Relative Liver protein expression of key players in glucose and lipogenesis homeostasis. Results were expressed as means ± S.D., n = 10 animals per group. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test. The limit of statistical significance was set at $p < 0.05$. The means with different letters were significantly different.

There are different forms of furan fatty acids. The furan fatty acid used in this study is extracted from *Hevea brasiliensis* and is of the F2 form (10,13-epoxy-11-methyl-octadecan-10,12-dienoic acid). In fish, FuFAs are found mainly as F6 form (12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid) with content from 1 to 300 µg/g [36]. In dairy products, FuFAs are found mainly as F3 (1-(3,4-dimethyl-5-propyl-furan-2-yl) undecanoic acid) and F6 form with content from 10 to 450 µg/g [37]. In food plants, FuFAs are found mainly as F3 and F6 forms with content from 4 to 700 µg/g dry weight [38]. In plant oil, FuFAs are found mainly as F3 and F6 forms with content from 1 to 250 µg/g of oil [38]. In soybean oil, furan fatty acids are found as F2 and F3 forms [39]. Thus, the FuFA-F2 that we used in this study is a fatty acid that can be found in the food of plant origin.

After 12 weeks of this western-style diet, our mice became moderately obese [40]. Nevertheless, in the DIO mice we observed that the animals ended up with a weight of 32 g including more than 10 g of fat mass. Moreover, indirect calorimetry experiments revealed a classic decrease in daily energy expenditure in these mice [28]. In addition, ITT and OGTT experiments showed that mice fed the HFHS diet developed insulin resistance and glucose intolerance compared to the control group. However, even though the fasting blood glucose and insulin values increased significantly in the HFHS group, these did not reach values that are considered pathological in mice (diabetes is defined with fasting glucose levels >2,5 g/l and prediabetes were defined as between 2 and 2,5 g/l) [32]. On the other hand, our data indicated that this moderate obesity was sufficient to induce hepatic steatosis as evidenced by the level of triglycerides in the liver and histological analyses. This set of data clearly indicates that DIO mice suffer from metabolic syndrome.

Compared to DIO mice, we observed that preventive nutritional supplementation with FuFA-F2 did not prevent weight gain. However, body composition analysis showed a reduction in fat mass gain and a parallel increase in lean mass corresponding to muscle mass gain. This favorable change in body composition most likely explains the restoration of normal energy expenditure in these mice. Further, our results showed that FuFA-F2 supplemented mice were not glucose intolerant and were more insulin sensitive compared to DIO mice. These results highlight the positive effect of FuFA-F2 on carbohydrate metabolism. It can be hypothesized that the lack of difference between fasting blood glucose and insulin values in the FuFA-F2 groups compared to the HFHS group is due to the fact that these values, although elevated, are not pathological [32]. Interestingly, our data showed that FuFA-F2 supplementation was sufficient to prevent the development of hepatic steatosis as observed in DIO mice. The decrease in fatty acid content in the liver induced by FuFA-F2 supplementation was probably related to the favorable change in body composition of the animals and to the increase in energy metabolism. At the molecular level, we did not observe any modification of FAS and β-HAD known to regulate lipid metabolism. However, we found a decrease in the expression of PGC-1α, which is a major regulator of gluconeogenesis [41,42]. Interestingly, it has been previously shown that a reduction in the expression of PGC-1α was responsible for a decrease in hepatic steatosis (decrease in hyperglycemia and lipid accumulation) in mice treated with PPARδ agonist (GW0742)[43,44]. This result suggests that the decrease of PGC-1α induced by FuFA-F2 could be accompanied by an inhibition of gluconeogenesis and lipogenesis and favor fatty acid oxidation of TG. In line with this hypothesis, we find a highly significant correlation between

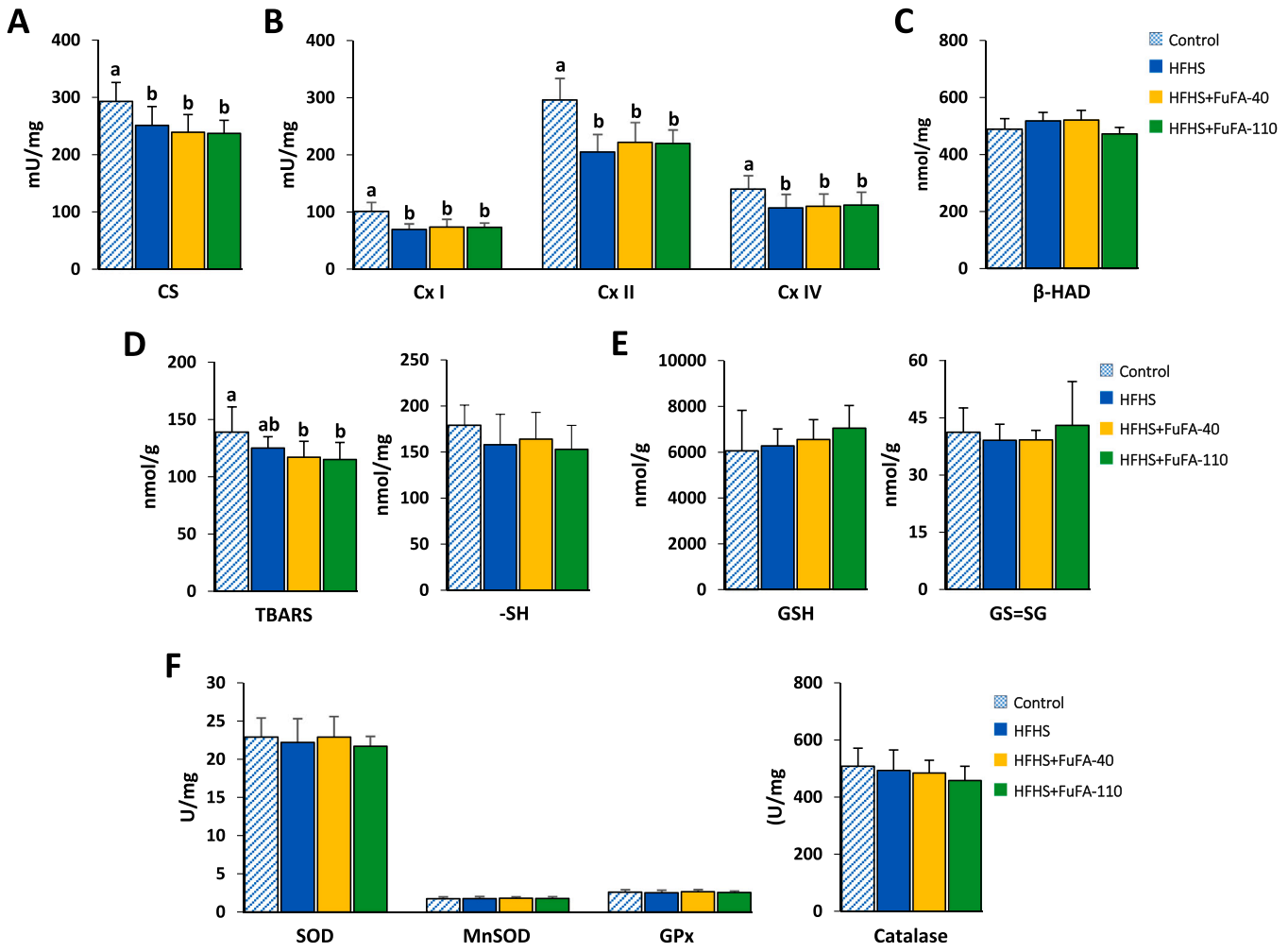


Fig. 7. Mitochondrial activity and liver oxidative stress parameters. (A) Citrate synthase activity. (B) Mitochondrial respiratory chain activities. (C) β-HAD activity. (D) TBARS and –SH values. (E) GSH and GS=SG content. (F) SOD, MnSOD, GPx activities and Catalase activity. Results were expressed as means ± S.D., n = 10 animals per group. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test. The limit of statistical significance was set at $p < 0.05$. The means with different letters were significantly different.

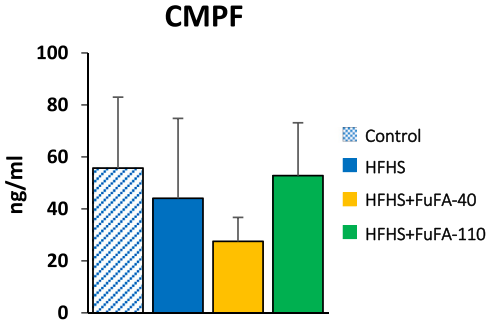


Fig. 8. Plasma CMPF concentrations. Results were expressed as means ± S.D., n = 10 animals per group. Groups were tested for the effects of diet by a one-way ANOVA test, followed up by a Fisher's Least Significant Difference test.

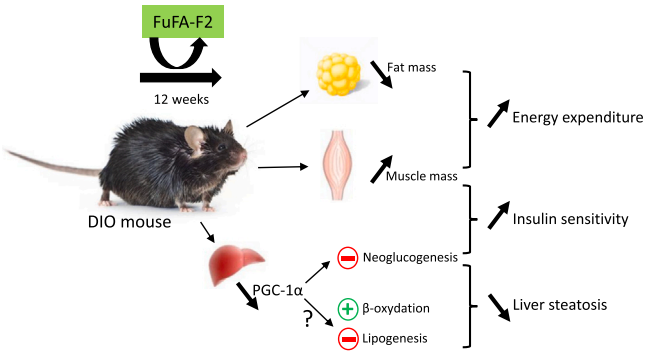


Fig. 9. Influence of a preventive FuFA-F2 supplementation on DIO mice.

PGC-1α expression and hepatic TG levels (Supplemental Fig. 3). However, the exact role of FuFA-F2 on gluconeogenesis and β-oxidation remains to be confirmed. This set of data reveals that preventive FuFA-F2 supplementation largely prevents the development of metabolic disorders in mice fed an obesogenic diet (Fig. 9).

In our previous study, we showed that 3 weeks of oral gavage in C57Bl6 mice with FuFA-F2 at a concentration of 20 mg/day/kl was sufficient to induce an increase in muscle mass in these animals [26]. However, in this work we have privileged a nutritional approach and we have chosen to test two different higher doses of FuFA-F2 (40 mg and 110 mg/day/kg of body weight) as they are integrated in the food matrix. The use of these two different doses of FuFA-F2 provided interesting data. Thus, whatever the dose, identical results were obtained on resting energy expenditure, glucose tolerance and insulin sensitivity. On the other hand, we observed dose-dependent effects on fat mass, lean mass,

quadriceps weight and reduction of hepatic steatosis. These very promising results also suggest that there is still much to be studied about the type of FuFA-F2 supplementation (gavage or nutritional), their concentration and their duration.

Physical activity is known to improve metabolic syndrome [45–47]. The results obtained in this study show that FuFA-F2 supplementation alone is able to reproduce, at least in part, the positive effects of physical activity, namely an increase of muscle mass, an increase of resting energy metabolism and an improve sensitivity to insulin. The prescription of an adapted physical activity is in expansion in the management of obese patients although it is not always obvious because of many factors such as a degraded body image, a higher energy cost, a painful joint mobilization and a higher risk of injury by loss of balance. FuFA-F2 supplementation, by promoting muscle mass gain at the expense of fat mass, could also facilitate the resumption of physical activity and thus increase the motivation of obese patients to regain active mobility and autonomy in their daily lives.

CMPF is one of the major endogenous metabolites of furan fatty acids and omega 3 fatty acids [33,34]. In addition, Prentice et al. [35] showed that administration of CMPF to mice improved insulin sensitivity and reduced hepatic steatosis. These data suggested that the different health benefits of FuFA-F2 supplementation might be related to an increase in CMPF. However, our results showing that FuFA-F2 supplementation does not impact plasma CMPF levels, rules out this hypothesis.

In this study, we showed different positive health effects of preventive nutritional supplementation with FuFA-F2 in a DIO mouse model. However, we only obtained a moderate obesity, which did not allow us to see possible effects of FuFA-F2 on diabetes. Moreover, it would be interesting to study the effect of curative FuFA-F2 supplementation on already obese animals. Finally, the limited amount of FuFA-F2 at our disposal did not allow us to study the impact of this bioactive fatty acid on mice of both sexes.

Last, the health benefits of FuFA-F2 on metabolic disorders highlighted in this study also suggest that furan fatty acids might be an interesting biomarker for the risk of developing metabolic syndrome. In this respect, the determination of furan fatty acids in the blood of cohort of obese patients suffering from obesity and metabolic syndromes could be of interest.

In conclusion, we have shown for the first time that preventive nutritional supplementation with FuFA-F2 in DIO mice increased muscle mass and reduced the development of metabolic disorders. FuFA-F2 supplementation was associated with a more favorable body composition, an improved resting energy expenditure, a better insulin sensitivity, and no hepatic steatosis development. In addition, this study also suggests that FuFA-F2 alone is able to reproduce, at least in part, the positive effects of physical activity. Our results highlighted that FuFA-F2, a new biosourced molecule derived from the latex of *Hevea brasiliensis* tree, produced in an environmentally sustainable way, may be an effective approach to treat obesity and metabolic syndrome.

Animals and Ethics Statement

Our institution guidelines for the care and use of laboratory animals were observed and all experimental procedures were approved by the local ethical committee in Montpellier, France (Reference APAFIS#31900–2021060417205673_V2).

Funding

This work was supported by the French National Research Institute for Agriculture, Food and Environment (INRAE), by the French Lipid Nutrition Group and by the by the Key Initiative Food & Health of MUSE (Montpellier University of Excellence). Laetitia Dore was funded by Agence Régionale de Santé Occitanie.

CRediT authorship contribution statement

L.D., E.D., B.B., J.C., L.V., S.L., L.P., K.L., C.B-G., and F.C acquisition of data. E.D., S.G., C.C., A.S., F.C., and C.F-C. analysis and interpretation of data. C.F-C. and F.C. conception and design, wrote the paper. All the authors have read and approved the final version of this manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

We thank Mélanie Macart from the animal staff for daily animal care. We thank Pierre Delobel for these helpful suggestions for morphometric analysis of muscle sections. The authors also wish to thank the Metamus DMeM facility (https://doi.org/10.15454/WYR2–8706), which belongs to the Montpellier animal facilities network (RAM, Biocampus) for technical support and expertise for metabolism phenotyping, and Metamontp platform supported by the European fund for the regional development of Occitanie (FEDER). The authors also thank the “Réseau d’Histologie Expérimentale de Montpellier” (RHEM) histology facility for processing our liver samples and Genotoul Anexplo for biochemical analysis of blood samples.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114945.

References

[1] L.M. Jaacks, et al., The obesity transition: stages of the global epidemic, Lancet Diabetes Endocrinol. 7 (3) (2019) 231–240.
[2] I. Lingvay, et al., Obesity management as a primary treatment goal for type 2 diabetes: time to reframe the conversation, Lancet 399 (10322) (2022) 394–405.
[3] M.J. Guembe, et al., Risk for cardiovascular disease associated with metabolic syndrome and its components: a 13-year prospective study in the RIVANA cohort, Cardiovasc Diabetol. 19 (1) (2020) 195.
[4] R.A. DeFronzo, D. Tripathy, Skeletal muscle insulin resistance is the primary defect in type 2 diabetes, Diabetes Care 32 (2009) S157–S163.
[5] L. Xu, et al., Furan fatty acids - beneficial or harmful to health? Prog. Lipid Res 68 (2017) 119–137.
[6] A. Batna, J. Scheinkonig, G. Spiteller, The occurrence of furan fatty acids in Isochrysis sp. and Phaeodactylum tricornutum, Biochim Biophys. Acta 1166 (2–3) (1993) 171–176.
[7] R.A.S. Lemke, et al., A bacterial biosynthetic pathway for methylated furan fatty acids, J. Biol. Chem. 295 (29) (2020) 9786–9801.
[8] N. Shirasaka, K. Nishi, S. Shimizu, Biosynthesis of furan fatty acids (F-acids) by a marine bacterium, Shewanella putrefaciens, Biochim Biophys. Acta 1346 (3) (1997) 253–260.
[9] K. Hannemann, et al., The common occurrence of furan fatty acids in plants, Lipids 24 (4) (1989) 296–298.
[10] G. Spiteller, Furan fatty acids: occurrence, synthesis, and reactions. Are furan fatty acids responsible for the cardioprotective effects of a fish diet? Lipids 40 (8) (2005) 755–771.
[11] C.M. Scrimgeour, Quantitative analysis of furanoid fatty acids in crude and refined cod liver oil, J. Am. Oil Chem. Soc. 54 (5) (1977) 210–211.
[12] H. Hasma, A. Subramaniam, The occurrence of a furanoid fatty acid inHevea brasiliensis latex, Lipids 13 (12) (1978) 905–907.
[13] S. Liengprayoon, et al., Glycolipid composition of Hevea brasiliensis latex, Phytochemistry 72 (14–15) (2011) 1902–1913.
[14] K. Alvarado, et al., Potential beneficial effects of furan fatty acids, bioactive food lipids, Cah. De. Nutr. Et. De. Diet. 56 (2) (2021) 117–125.
[15] M.H. Masuchi Buscato, et al., Furan fatty acids in enriched omega-3 fish oil: Oxidation kinetics with and without added monomethyl furan fatty acid as potential natural antioxidant, Food Chem. 327 (2020), 127087.

[16] Y. Okada, M. Kaneko, H. Okajima, Hydroxyl radical scavenging activity of naturally occurring furan fatty acids, Biol. Pharm. Bull. 19 (12) (1996) 1607–1610.
[17] Y. Okada, et al., Inhibitory effects of naturally occurring furan fatty acids on hemolysis of erythrocytes induced by singlet oxygen, Yakugaku Zasshi 110 (9) (1990) 665–672.
[18] A. Batna, G. Spiteller, Oxidation of furan fatty acids by soybean lipoxygenase-1 in the presence of linoleic acid, Chem. Phys. Lipids 70 (2) (1994) 179–185.
[19] J. Lauvai, et al., The furan fatty acid 9M5 acts as a partial ligand to peroxisome proliferator-activated receptor gamma and enhances adipogenesis in 3T3-L1 preadipocytes, Lipids 54 (5) (2019) 277–288.
[20] T. Wakimoto, et al., Furan fatty acid as an anti-inflammatory component from the green-lipped mussel Perna canaliculus, Proc. Natl. Acad. Sci. USA 108 (42) (2011) 17533–17537.
[21] A. Alhassan, et al., Consumption of fish and vascular risk factors: a systematic review and meta-analysis of intervention studies, Atherosclerosis 266 (2017) 87–94.
[22] D. Mozaffarian, J.H. Wu, Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events, J. Am. Coll. Cardiol. 58 (20) (2011) 2047–2067.
[23] G. Graff, et al., Inhibition of blood-platelet aggregation by dioxo-ene compounds, Biochim. Et. Biophys. Acta 799 (2) (1984) 143–150.
[24] C.T. Fuchs, G. Spiteller, 9-(3,4-dimethyl-5-pentyl-furan-2-yl) nonanoic acid and 9-(3,4-dimethyl-5-propyl-furan-2-yl) nonanoic acid: new naturally occurring peroxidase inhibitors, Z. Fur Naturforsch. C. -a J. Biosci. 54 (11) (1999) 932–936.
[25] V. Puchta, G. Spiteller, H. Weidinger, F-Acids - a new component of the phospholipids of human-blood, Liebigs Ann. Der Chem. 1 (1988) 25–28.
[26] Pelletier F, D.E., Chaiyut J., Bronstein C., Pessemesse L., Vaysse L., Liengprayoon S., Gaillet S., Brioche T., Bertrand-Gaday C., Coudray C., Sultan A., Feillet-Coudray C* and Casas F*., *Furan fatty acid extracted from Hevea brasiliensis latex increases muscle mass in mice.* in revision, 2023.
[27] S. Liengprayoon, et al., Lipid compositions of latex and sheet rubber from Hevea brasiliensis depend on clonal origin, Eur. J. Lipid Sci. Technol. 115 (9) (2013) 1021–1031.
[28] P.M. Marvyn, et al., Data on oxygen consumption rate, respiratory exchange ratio, and movement in C57BL/6J female mice on the third day of consuming a high-fat diet, Data Brief. 7 (2016) 472–475.
[29] C. Coudray, et al., A mitochondrial-targeted ubiquinone modulates muscle lipid profile and improves mitochondrial respiration in obesogenic diet-fed rats, Br. J. Nutr. 115 (7) (2016) 1155–1166.
[30] M. Benlebna, et al., Long-term intake of 9-PAHPA or 9-OAHPA modulates favorably the basal metabolism and exerts an insulin sensitizing effect in obesogenic diet-fed mice, Eur. J. Nutr. (2020).
[31] M. Benlebna, et al., Long-term high intake of 9-PAHPA or 9-OAHPA increases basal metabolism and insulin sensitivity but disrupts liver homeostasis in healthy mice, J. Nutr. Biochem 79 (2020), 108361.
[32] R.J. Fajardo, et al., A review of rodent models of type 2 diabetic skeletal fragility, J. Bone Min. Res 29 (5) (2014) 1025–1040.
[33] G. Liu, et al., Pure omega 3 polyunsaturated fatty acids (EPA, DPA or DHA) are associated with increased plasma levels of 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid (CMPF) in a short-term study in women, in: Food Funct, 11, 2020, pp. 2058–2066.
[34] T.B. Wahl HG, H.M. Liebich, The effect of dietary fish oil supplementation on the concentration of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid in human blood and urine, J. High. Resolut. Chromatogr. 15 (12) (1992) 815–818.
[35] K.J. Prentice, et al., CMPF, a metabolite formed upon prescription omega-3-acid ethyl ester supplementation, prevents and reverses steatosis, EBioMedicine 27 (2018) 200–213.
[36] D. Pacetti, et al., Effect of spawning on furan fatty acid profile of edible muscle and organ tissues from sardine (Sardina pilchardus) and anchovy (Engraulis encrasicolus), J. Agric. Food Chem. 61 (16) (2013) 3969–3977.
[37] C. Wendlinger, W. Vetter, High concentrations of furan fatty acids in organic butter samples from the German market, J. Agric. Food Chem. 62 (34) (2014) 8740–8744.
[38] I. Mawlong, M.S. Sujith Kumar, D. Singh, Furan fatty acids: their role in plant systems, Phytochem. Rev. 15 (1) (2016) 121–127.
[39] F. Müller, M. Hogg, W. Vetter, Valuable furan fatty acids in soybeans and soy products, Eur. Food Res. Technol. 246 (7) (2020) 1383–1392.
[40] E.D.M. de Moura, et al., Diet-induced obesity in animal models: points to consider and influence on metabolic markers, Diabetol. Metab. Syndr. 13 (1) (2021) 32.
[41] S. Herzig, et al., CREB regulates hepatic gluconeogenesis through the coactivator PGC-1, Nature 413 (6852) (2001) 179–183.
[42] P. Puigserver, et al., Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction, Nature 423 (6939) (2003) 550–555.
[43] S.H. Koo, et al., PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3, Nat. Med 10 (5) (2004) 530–534.
[44] M.Y. Lee, et al., Peroxisome proliferator-activated receptor delta agonist attenuates hepatic steatosis by anti-inflammatory mechanism, Exp. Mol. Med 44 (10) (2012) 578–585.
[45] P.A.G.A. Committee, , 2008, in: Physical activity guidelines advisory committee report, 2008, US Department of Health and Human Services, Washington, DC, 2008, pp. A1–H14.
[46] S. Bertrais, et al., Sedentary behaviors, physical activity, and metabolic syndrome in middle-aged French subjects, Obes. Res 13 (5) (2005) 936–944.
[47] E.S. Ford, et al., Sedentary behavior, physical activity, and the metabolic syndrome among U.S. adults, Obes. Res 13 (3) (2005) 608–614.