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# Reduction in the desaturation capacity of the liver in mice subjected to high fat diet: Relation to LCPUFA depletion in liver and extrahepatic tissues

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ABSTRACT

α-Linolenic (ALA) and linoleic (LA) acids are precursors of long chain polyunsaturated fatty acids (LCPUFAs), FAs with important biochemical and physiological functions. In this process, desaturation reactions catalyzed by Δ5- and Δ6-desaturase play a major role, enzymes that are subjected to hormonal and dietary regulation. The aim of this study was to assess the influence of a high fat diet (HFD) on activity of liver Δ5 and Δ6 desaturases, in relation to LCPUFA composition in liver and extrahepatic tissues. Male C57BL/6J mice received control diet (CD) (10% fat, 20% protein and 70% carbohydrate) or high fat diet (HFD) (60% fat, 20% protein, and 20% carbohydrate) for 12 weeks. After this time, blood and liver samples were taken for metabolic, morphologic, inflammatory, oxidative stress and desaturase activity assessment, besides FA phospholipid analysis in erythrocytes, heart, adipose tissue and brain. HFD significantly increased hepatic total fat, triacylglycerides and free FA content with macrovesicular steatosis and oxidative stress enhancement, concomitantly with higher fasting serum glucose and insulin levels, HOMA, and serum cholesterol, triacylglycerols, TNF-α, and IL-6. Diminution in liver Δ5- and Δ6-desaturase activities and LCPUFA depletion were induced by HFD, the later finding being also observed in extrahepatic tissues. In conclusion, HFD-induced reduction in the bioavailability of liver LCPUFA is associated with defective desaturation of ALA and LA, with Δ5- and Δ6-desaturase activities being correlated with insulin resistance development. Data analyzed point to the liver as a major organ responsible for extrahepatic LCPUFA homeostasis, which is markedly deranged by HFD.


## 1. Introduction

α-Linolenic acid (C18:3 n-3 or 9,12,15-octadecatrienoic acid, ALA) and linoleic acid (C18:2 n-6 or cis,cis-9,12-octadecadienoic acid, LA) are essential fatty acids (FAs) for mammals including humans [1]. These two polyunsaturated FAs (PUFAs) cannot be biosynthesized from the novo, because the necessary enzymatic machinery is not available to incorporate a double bond at the n-3 position for ALA and at the n-6 position for LA [2]. ALA is the metabolic precursor of eicosapentaenoic acid (C20:5 n-3 or all-cis-5,8,11,14,17-icosapentaenoic acid, EPA) and docosahexaenoic acid (C22:6 n-3 or all-cis-4,7,10,13,16,19-docosahexaenoic acid, DHA), two long chain PUFAs (C20-22; LCPUFAs) that have several and important biochemical and physiological functions. EPA has regulatory role in the vascular homeostasis and in the protection of cardiovascular health [3]. DHA is specially concentrated at the membrane phospholipids of neuronal and visual cells having crucial roles in the development and function of brain and in visual acuity, particularly during the perinatal and infant life [4]. Consumption of oils rich in EPA and DHA (such as fish oil) has been associated with cardiovascular and cerebral protection [5]. LA is the metabolic precursor of arachidonic acid (C20:4 n-6 or 5,8,11,14-eicosatetraenoic acid, AA), a FA having a key role in inflammation and immunological responses [6]. AA is also incorporated to membrane phospholipids, particularly at neuronal and retina levels having, such as DHA, a significant function during pregnancy and the first years of life of the newborn [7].

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The transformation of ALA and LA to their respective metabolic products in mammals occurs through sequential desaturation, elongation, and partial degradation step [8]. Desaturation is accomplished by membrane-bound Δ-6 desaturase (FADS2) and Δ-5 desaturase (FADS1), which are non-heme iron containing enzymes that introduce double bonds at defined carbons of the acyl-CoA chain, a process that requires molecular oxygen, NAD(P)H, and an electron transport system [8–10]. Δ-6 desaturase, the rate-limiting step for the production of LCPUFA, is known to have desaturation activity on ALA and LA [10] and to participate in the peroxisomal production of DHA [9]. Microsomal PUFA elongation is catalyzed by elongases ELOVL5 and ELOVL2 using shorter (C18-C20) and longer (C20-C22) acyl-CoA substrates, respectively, which add two carbon units employing malonyl-CoA as the donor and NADPH as the reducing agent [8,9]. FA elongation is a four reactions cycle, in which the initial condensation step catalyzed by ELOVLs constitutes the rate-controlling process [8]. In mammalian cells, both desaturases Δ-6 and Δ-5 in conjunctions with elongases ELOVL5 and ELOVL2 play critical roles in regulating the degree of insaturation and length of PUFA derivatives and thereby their functions and metabolic fates, enzymes that are subjected to hormonal and/or end-product regulation [8,11,12] and whose activities may experience dietary influences. Accordingly, the objective of the present study was to evaluate the effect of a high dietary ingestion of fats (high fat diet, HFD) on the activity of liver desaturase Δ-6 and Δ-5 in association with the levels of n-3 and n-6 LCPUFAs in the liver and extrahepatic tissues. These parameters were correlated with HFD-induced changes in liver morphological characteristics and oxidative stress status.

## 2. Material and methods

### 2.1. Ethics statement

Experimental animal protocols and animal procedures complied with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, NIH Publication 6-23, revised 1985) and were approved by the Bioethics Committee for Research in Animals, Faculty of Medicine, University of Chile (protocols CBA#580 FMUCH and CBA#0630 FMUCH).

### 2.2. Animals and diet

Weaning male C57BL/6J mice weighing 12–14 g (Bioterio Central, ICBM, Faculty of Medicine, University of Chile) were randomly assigned to each experimental group and allowed free access to specially formulated control or high fat diets. The composition of the control diet (CD) (expressed as % total calories) was 10% fat, 20% protein, and 70% carbohydrate, with a caloric value of 3.85 kcal/g, free of EPA and DHA. The composition of the HFD was 60% fat, 20% protein, and 20% carbohydrate, with a caloric value of 5.24 kcal/g, free of EPA and DHA, and containing 0.7 g of ALA/100 g of diet (Research Diet INC, Rodent Diet, Product data D12450K and D12492, USA). Supplementary Table 1S depicts the fatty acid composition of CD and HFD used in this study. Animals received water ad libitum and were housed on a 12-h light/dark cycle from days 1 to 84 (12 weeks). Weekly controls of body weight and diet intake were performed through the whole period. At the end of the 12th week, animals were fasted (6–8 h), anesthetized with ketamine and xylazine (150 and 10 mg/kg, respectively), and blood samples were obtained by cardiac puncture for serum AST, ALT, glucose, insulin, triacylglycerols, total cholesterol, LDL-cholesterol, and HDL-cholesterol assessments. Blood, liver, heart, adipose tissue, and brain samples were frozen in liquid nitrogen for the determination of FA composition; in addition, liver samples were fixed in phosphate-buffered formalin, embedded in paraffin, stained with hematoxylin-eosin and analyze by optical microscopy in a blind fashion describing the presence of steatosis and inflammation, both graded as absent, mild, moderated and severe [13].

### 2.3. Measurements of serum glucose, insulin, cholesterol, triacylglycerol levels, transaminases (AST and ALT) activities, and interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels

Serum glucose (mM), cholesterol (mg/100 mL), LDL cholesterol (mg/100 mL), HDL cholesterol (mg/100 mL) and triacylglycerol levels (mg/dL) were measured using specific diagnostic kits (Wiener Lab, Argentina). A commercial immunoassay kit for mice serum insulin assessment (μU/mL) was used, according to the manufacturer's instructions (Mercodia, Uppsala, Sweden). Insulin resistance was estimated by the homeostasis model assessment method (HOMA) [fasting insulin (μU/mL) × fasting glucose (mM)/22.5] [14]. Serum aspartate transaminase (AST) and alanine transaminase (ALT) activities (units/L) were measured using specific diagnostic kits (Biomerieux SA, Marcy l,Etoile, France). ELISA kits were used for assessment of serum levels (pg/mL) of IL-6 and TNF-α (Thermo, Meridian, Rd, USA).

### 2.4. Assays for oxidative stress-related parameters in liver and plasma

In anesthetized animals, livers were perfused in situ with a cold solution containing 150 mM KCl and 5 mM Tris (pH 7.4) to remove blood for glutathione and protein carbonylation assessments. Reduced glutathione (GSH) and glutathione disulfide (GSSG) contents were assessed with an enzymatic recycling method [15]. Contents of protein carbonyls, F-2 isoprostanes, and thiobarbituric acid reactants (TBARs) in liver and the plasma levels of TBARs and the antioxidant capacity of plasma were measured using specific kits, according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, USA).

### 2.5. Determination of liver Δ-6 and Δ-5 desaturase activities

Liver samples frozen in liquid nitrogen were homogenized (500 mg) in a buffer solution pH 7.9 containing 10 mmol/l HEPES, 1 mmol/l EDTA, 0.6% Nonidet P-40, 150 mmol/l NaCl, and protease inhibitors (1 mmol/l phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 mmol/l orthovanadate). Liver homogenates were centrifuged three at 4 °C, first at 2000 rpm for 30 s, followed by centrifugation of the supernatants at 5000 rpm for 5 min, and finally at 100,000 rpm for 60 min, to obtain the extracts for desaturase activity measurements. The activity of Δ-6 desaturase was assayed by measuring the amount of 18:3, n-6 produced from 18:2, n-6, whereas that of Δ-5 desaturase was determined by the amount of 20:3, n-6 being converted into 20:4, n-6, using albumin-bound fatty acid precursors (18:2, n-6 and 20:3, n-6) [16]. Desaturase activity was assayed using 1 ml of incubation medium containing 4 μmol ATP, 0.1 μmol coenzyme-A, 1.28 μmol NADH, 2.42 μmol *N*-acetylcysteine, 0.5 μmol nicotinamide, 5 μmol $MgCl_2$, 62.5 μmol NaF, and 62.5 μmol phosphate buffer pH 7, supplemented with 100 nmol albumin-bound FA precursor and 1 mg protein of cytosolic extract in a total volume of 100 μl, incubated at 37 °C for 30 min in a shaking bath. Δ-6D and Δ-5D assays were conducted simultaneously. The reaction was stopped by adding 6 ml of a methanol/chloroform mixture (2/1 v/v). Freshly prepared heptadecanoic acid (17:0; 99%+ pure) was added (20 μg) as internal standard. To determine the changes in the levels of products or precursors achieved after incubation, lipids were extracted and derivatized to fatty acid methyl esters (FAME), which were analyzed by gas–liquid chromatographic analysis, as described previously [17]. The individual fatty acid methyl ester (FAME) peaks were identified by comparison of the retention times of the individual fatty acid from an authentic standard mixture and converted to concentration using the 17:0 internal

standard. Δ-6 desaturase (net increase in 18:3, n-6) and Δ-5 desaturase (net decrease in 20:3, n-6) activities were calculated from GC-FID results as differences between baseline values and those obtained after 30 min incubation, and results were expressed as nmol/mg protein/min.

### 2.6. Fatty acid profile

Quantitative extraction and separation of total lipids from erythrocytes, liver, heart, adipose tissue and brain was carried out according to Bligh and Dyer [18] containing butylated hydroxytoluene (BHT) as antioxidant. Erythrocytes and tissues samples were homogenized in ice-cold chloroform/methanol (2:1 v/v) containing 0.01% BHT in an Ultraturrax homogenized (Janke & Kunkel, Stufen, Germany). Total lipids from erythrocytes were extracted with chloroform/isopropanol (2:1 v/v). Phospholipids from liver, erythrocytes, heart, adipose tissue and brain were separated from total lipid extracts by thin layer chromatography (TLC) on silica gel plates (aluminum sheets $20 \times 20$ cm$^2$, silica gel 60F-254; Merck), using the solvent system hexane/diethyl ether/acetic acid (80:20:1 v/v). After the development of plates and solvent evaporation lipid spots were visualized by exposing the plates to a Camag UV (250 nm) lamp designed for TLC. The solvent system allows the separation of phospholipids, cholesterol, triacilglycerols and cholesterol ester according to their relative mobility. Phospholipid spots were removed from the plate with either diethyl ether or chloroform/methanol (2:1 v/v), according to Ruiz-Gutierrez et al. [19].

### 2.7. Preparation and gas chromatographic analysis of fatty acid methyl esters (FAME)

FAMEs from liver, erythrocytes, heart, adipose tissue, and brain phospholipids were prepared with boron trifluoride (12% methanolic solution) according to the Morrison and Smith [20], and followed by methanolic sodium hydroxide (0.5 N) solution. Phospholipids for FAME synthesis were extracted from the silica gel spots with 15 mL of chloroform/methanol/water (10:10:1) and evaporated under nitrogen stream. FAME samples were cooled and extracted with 0.5 mL of hexane.

FAME were separated and quantified by gas–liquid chromatography in an Agilent Hewlett-Packard equipment (model 7890 A, CA, USA) using a capillary column (Agilent HP-88, 100 m × 0.250 mm; I.D. 0.25 μm) and a flame ionization detector (FID). The injector temperature was set at 250 °C and the FID temperature at 300 °C. The oven temperature at injection was initially set at 140 °C and was programmed to increase to 220 °C at a rate of 5 °C per min. Hydrogen was utilized as the carrier gas (35 cm/s flow rate) in the column and the inlet split ratio was set at 20:1. The identification and quantification of FAME were achieved by comparing the retention times and the peak area values (%) of the unknown samples with those of a commercial lipid standard (Nu-Chek Prep Inc). C23:0 was used as internal standard (Nu-Chek Prep Inc, Elysian MN, USA) and a Hewlett-Packard Chemstation (Palo Alto, CA, USA) data system was used for peak analysis.

### 2.8. Statistical analysis

Statistical analysis was performed with GraphPad Prism 6.0 software (GraphPad Prism Software, Inc. San Diego, USA). Values shown represent the mean ± SEM for each experimental group. Evaluations of normality data distribution was performed using the Shapiro Wilk test. Assessment of the statistical significance of differences between mean values was performed by *t*-test for unpaired data. A *p*-value less than 0.05 was considered as significant. To analyze the association between different variables, the Pearson correlation coefficient was used.

## 3. Results

### 3.1. HFD-induced changes in parameters related to lipid metabolism, insulin resistance, pro-inflammatory cytokines, liver morphological characteristics, and oxidative stress status in liver and plasma

Mice subjected to HFD for 12 weeks exhibited significant increase in body weight and visceral adipose tissue, with concomitant enhancement in liver total fat and in the content of triacylglycerols and free fatty acids, changes that coincide with elevated levels of triacylglycerols, total cholesterol and LDL- and HDL-cholesterol in serum over animals given a CD (Table 1). Under these conditions, HFD induced higher plasma levels of glucose and insulin in fasted state, with a substantial increase in the HOMA index of insulin resistance, a parallel raise in IL-6 and TNF-α, and comparable values of AST and ALT compared to controls (Table 1). Mice subjected to control diet (Fig. 1A) exhibited normal histology, whereas those in the HFD group showed macrovesicular steatosis without indications of an inflammatory response (Fig. 1B).

The evaluation of oxidative stress-related parameters under the influence of HFD revealed major changes in liver, as evidenced by the significant diminution in GSH content without changes in that of GSSG, leading to reduction in total GSH equivalents and in the GSH/GSSG ratios ($p < 0.05$) (Table 2). Furthermore, the indicators of the pro-oxidant status of the liver, namely, protein carbonyls, F2-isoprostanes, and TBARs were enhanced over controls values ($p < 0.05$), with 140% to 200% increments in protein carbonyl/total GSH, F2-isoprostane/total GSH, and TBAR/total GSH ratios over control mice (Table 2). These data are in agreement with higher TBARs levels in plasma and decline in the antioxidant capacity of plasma ($p < 0.05$) (Table 2), TBARs levels in plasma being correlated with those in liver ($r = 0.86$; $p < 0.0001$) and with hepatic TBAR/total GSH ratios ($r = 0.88$; $p < 0.0001$). Besides, liver TBAR/total GSH ratios were associated with the HOMA index of insulin resistance ($r = 0.96$; $p < 0.0001$).

### 3.2. HFD induces significant changes in liver fatty acid composition with loss of phospholipid insaturation

The content of hepatic 18:3,n-3 (ALA), 18:4, n-3, 20:3, n-6, 20:4, n-6 (AA), 20:4, n-3, 20:5, n-3 (EPA), and 22:6, n-3 (DHA) was

**Table 1**
General and biochemical parameters in control mice and animals subjected to high fat diet (HFD).

| | Groups | | |
|---|---|---|---|
| | CD | HFD | % effect |
| General parameters | | | |
| Initial body weight (g) | 14.6 ± 2.4 | 14.2 ± 1.7 | – |
| Final body weight (g) | 32.1 ± 3.4 | 40.8 ± 3.4* | +27 |
| Liver weight (g) | 1.42 ± 0.06 | 1.81 ± 0.04 | – |
| Visceral adipose tissue (g) | 1.4 ± 0.03 | 5.4 ± 0.4* | +286 |
| Liver parameters | | | |
| Total fat (mg/g liver) | 38.5 ± 3.4 | 99.5 ± 4.7* | +158 |
| Triacylglycerols (mg/g liver) | 31.6 ± 1.1 | 84.4 ± 3.2* | +167 |
| Free fatty acid (μM/g liver) | 275.1 ± 19.3 | 705.3 ± 44.5* | +156 |
| Serum parameters | | | |
| Triacylglycerols (mg/dL) | 124.6 ± 7.5 | 161.4 ± 11.5* | +30 |
| Total cholesterol (mg/dL) | 70.5 ± 6.8 | 121.4 ± 10.2* | +72 |
| LDL-cholesterol (mg/dL) | 48.5 ± 4.1 | 82.7 ± 6.6* | +71 |
| HDL-cholesterol (mg/dL) | 18.4 ± 2.0 | 30.2 ± 2.7* | + 64 |
| Fasting glucose (mg/dL) | 125.3 ± 4.3 | 230.4 ± 7.8* | +84 |
| Fasting insulin (units/mL) | 5.61 ± 1.2 | 15.4 ± 2.8 | +175 |
| HOMA | 1.81 ± 0.06 | 8.8 ± 1.1* | +386 |
| IL-6 (pg/mL) | 34.6 ± 6.4 | 72.5 ± 9.1* | +110 |
| TNF-α (pg/mL) | 24.1 ± 2.2 | 44.6 ± 4.1* | +85 |
| AST (U/L) | 147.5 ± 10.5 | 158.4 ± 12.4 | – |
| ALT (U/L) | 74.2 ± 5.5 | 83.2 ± 3.7 | – |

Values represent means ± SEM for 12 mice per experimental group. The groups were compared by Student's *t*-test for unpaired data ($p < 0.05$), with significant differences being indicated by asterisks (*).

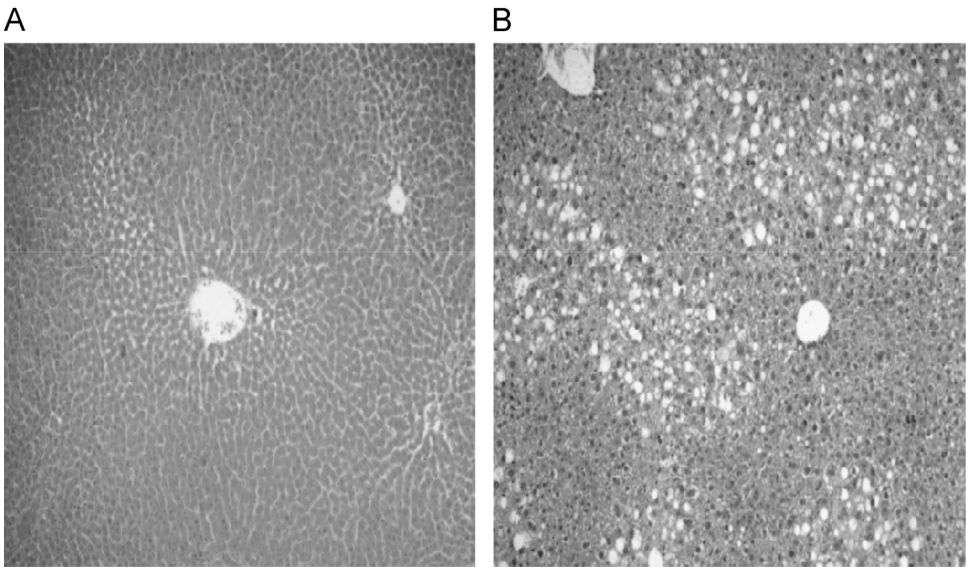


**Fig. 1.** Morphological characteristics in the liver of mice subjected to a control diet (CD) (A) and a high fat diet (HFD) (B). Representative liver sections from a total of 12 animals per experimental group (hematoxylin-eosin; original magnification × 10).

**Table 2**
Oxidative stress-related parameters in control mice and animals subjected to high fat diet (HFD).

| | Groups | | |
|---|---|---|---|
| | CD | HFD | % effect |
| Liver parameters | | | |
| GSH (μmol/g liver) | 5.80 ± 0.7 | 3.78 ± 0.4* | −35 |
| GSSG (μmol/g liver) | 0.44 ± 0.05 | 0.42 ± 0.04 | – |
| Total GSH equivalents (μmol/g liver) | 6.68 ± 0.8 | 4.62 ± 0.5* | −31 |
| GSH/GSSG ratio | 13.2 ± 1.4 | 8.64 ± 1.1* | −35 |
| Protein carbonyls (nmol/mg protein) | 2.40 ± 0.5 | 4.03 ± 0.7* | +68 |
| F2-isoprostanes (pg/mg protein) | 9.17 ± 1.4 | 15.66 ± 2.3* | +71 |
| TBARs (nmol/mg protein) | 0.07 ± 0.01 | 0.14 ± 0.01* | +100 |
| Protein carbonyl/total GSH ratio (arbitrary units) | 0.36 ± 0.004 | 0.87 ± 0.03* | +142 |
| F2-isoprostane/total GSH ratio (arbitrary units) | 1.37 ± 0.05 | 3.39 ± 0.15* | +147 |
| TBAR/total GSH ratio (arbitrary units) | 0.010 ± 0.0005 | 0.030 ± 0.0008* | +203 |
| Plasma parameters | | | |
| TBARs (nmol/mg protein) | 0.04 ± 0.01 | 0.12 ± 0.04* | +200 |
| Antioxidant capacity (mM Trolox equivalents) | 1.23 ± 0.04 | 0.86 ± 0.06* | −30 |

Values represent means ± SEM for 10 to 12 mice per experimental group. The groups were compared by Student's *t*-test for unpaired data ($p < 0.05$), with significant differences being indicated by asterisks (*). Total GSH equivalents = GSH + 2GSSG.

decreased by HFD over that observed with CD ($p < 0.05$), whereas total SFA levels exhibited 22% increase (mainly 16:0) and those of total MUFA remained unaltered (Table 3). Accordingly, total levels of liver PUFAs, LCPUFAs, n-6 LCPUFAs, and n-3 LCPUFAs were significantly diminished by 33%, 43%, 41%, and 50% by HFD over controls, respectively ($p < 0.05$) (Table 3). Similar changes were observed in total liver lipid fraction (data not shown). Total levels of LCPUFAs in liver exhibited an inversed correlation with oxidative stress status represented by the respective TBAR/total GSH ratios ($r = -0.93$; $p < 0.0001$) and with the index of insulin resistance HOMA ($r = -0.95$; $p < 0.0001$).

### 3.3. Liver Δ5- and Δ6-desaturase activities and the respective n-3 and n-6 product/precursor ratios are depressed by HFD

HFD led to 41.6% diminution in the hepatic activity of Δ-5 desaturase ($p < 0.05$; Fig. 2A) and 61% decreased in that of Δ-6 desaturase ($p < 0.05$; Fig. 2B) compared to control values. Calculation of the respective liver n-3 (EPA + DHA/ALA) (Fig. 2C) and n-6 (AA/LA) (Fig. 2D) product precursor ratios revealed 43% and 28% reduction by HFD ($p < 0.05$), respectively. The activities of Δ5-desaturase and Δ6-desaturase in the studied groups showed a significant correlation ($r = 0.81$, $p < 0.0001$), parameters that were also associated with the liver n-6 product/precursor ratios ($r = 0.82$ and $r = 0.84$; $p < 0.0001$, respectively), with the contents of total LCPUFAs ($r = 0.86$ and $r = 0.82$; $p < 0.0001$, respectively), and inversely correlated with HOMA ($r = -0.87$ and $r = -095$; $p < 0.0001$, respectively).

### 3.4. Fatty acid composition in erythrocytes, heart, adipose tissue, and brain is also altered by HFD

Mice subjected to HFD showed changes in fatty acid composition in erythrocytes, heart (Table 4), adipose tissue, and brain (Table 5) phospholipids that are similar to those found in the liver. These changes are represented by (i) increases in the content of SFA in all tissues ($p < 0.05$) and (ii) reductions in the levels of total LCPUFAs and total n-3 LCPUFA over control values ($p < 0.05$) (Tables 4 and 5).Similar effects induced by HFD were observed in total lipid fractions from the studied tissues (data not shown).Total levels of LCPUFAs in liver were correlated with those in erythrocytes ($r = 0.87$; $p < 0.0001$), heart ($r = 0.93$; $p < 0.0001$), adipose tissue ($r = 0.92$; $p < 0.0001$), and brain ($r = 0.66$; $p < 0.001$).

## 4. Discussion

Studies employing monogenic or pharmacologically induced obesity models in rodents have limited relevance in human obesity [21], with the exception of HFDs comprising ≤60% energy as fat in inbred strains of rodents prone to develop obesity such as AKR/J, DBA/2J, and C57BL/6J mice [22]. In agreement with these views, our studies in C57BL/6Y mice subjected to a HFD with 60% of energy as fat for 12 weeks showed enhancements in body weight and visceral adipose tissue, with hypertriglyceridemia, hypercholesterolemia, and elevation of fasting glucose and insulin levels in serum leading to insulin resistance development, thus resembling human obesity [22,23]. Under these conditions, liver macrovesicular steatosis is induced by HFD, with increases in the contents of total fat, triacylglycerols, and

**Table 3**
Fatty acid composition of liver phospholipids obtained from control mice and animals subjected to high fat diet (HFD).

| | Fatty acid composition (g/100 g FAME) | | |
|---|---|---|---|
| | Groups | | |
| Fatty acid | CD | HFD | % effect |
| 16:0 | 38.5 ± 3.7 | 46.8 ± 3.6* | +22 |
| 18:1, n-9 | 21.5 ± 2.3 | 23.6 ± 2.4 | – |
| 18:2, n-6 (LA) | 13.4 ± 1.2 | 11.2 ± 1.4 | – |
| 18:3, n-6 | 1.04 ± 0.1 | 0.86 ± 0.05 | – |
| 18:3, n-3 (ALA) | 1.12 ± 0.1 | 0.84 ± 0.1* | −25 |
| 18:4, n-3 | 0.23 ± 0.03 | 0.12 ± 0.02* | −48 |
| 20:3, n-6 | 0.19 ± 0.01 | 0.07 ± 0.03* | −63 |
| 20:4, n-6 (AA) | 12.4 ± 1.1 | 7.43 ± 1.10* | −40 |
| 20:4, n-3 | 0.14 ± 0.01 | 0.06 ± 0.01* | −57 |
| 20:5, n-3 (EPA) | 1.04 ± 0.2 | 0.44 ± 0.03* | −58 |
| 22:5, n-6 (DPAn-6) | 0.07 ± 0.01 | 0.03 ± 0.01* | −57 |
| 22:5, n-3 (DPAn-3) | 0.10 ± 0.02 | 0.06 ± 0.01* | −40 |
| 22:6, n-3 (DHA) | 4.23 ± 0.4 | 2.27 ± 0.2* | −46 |
| Total SFA | 38.5 ± 4.5 | 46.8 ± 3.1* | +22 |
| Total MUFA | 26.2 ± 2.2 | 29.4 ± 3.1 | – |
| Total PUFA | 35.3 ± 3.4 | 23.8 ± 2.7* | −33 |
| Total LCPUFA | 18.4 ± 2.1 | 10.4 ± 1.0* | −43 |
| Total n-6 LCPUFA | 12.7 ± 1.0 | 7.52 ± 0.1* | −41 |
| Total n-3 LCPUFA | 5.60 ± 0.6 | 2.79 ± 0.04* | −50 |

Values are expressed as g fatty acid per 100 g FAME and represent t the mean ± -SEM for $n=12$ mice per experimental group. The groups were compared by Student's $t$-test for unpaired data ($p<0.05$), with significant differences being indicated by asterisks (*). Saturated fatty acids (SFA) correspond to 14:0, 16:0 and 18:0. Monounsaturated fatty acids (MUFA) correspond to 14:1, n-7, 16:1, n-7 and 18:1, n-9. Polyunsaturated fatty acids (PUFA) correspond to 18:2, n-6, 18:3, n-3, 20:4, n-6, 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6 long-chain polyunsaturated fatty acids (LCPUFA) are 20:4, n-6; n-3 LCPUFA are 20:5, n-3, 22:5, n-3, and 22:6, n-3; n-6/n-3 ratio: 20:4, n-6/ (20:5, n-3+22:5, n-3+22:6, n-3).

**Table 4**
Fatty acid composition of erythrocytes and heart phospholipids obtained from control mice and animals subjected to high fat diet (HFD).

| | Fatty acid composition (g/100 g FAME) | | | |
|---|---|---|---|---|
| | Erythrocyte Groups | | Heart Groups | |
| Fatty acid | CD | HFD | CD | HDF |
| 16:0 | 21.6 ± 1.5 | 25.8 ± 1.0* | 21.2 ± 1.2 | 25.8 ± 1.0* |
| 18:1,n-9 | 22.3 ± 2.4 | 9.4 ± 2.1 | 21.0 ± 1.5 | 20.9 ± 2.0 |
| 18: 2, n-6 (LA) | 12.3 ± 1.4 | 10.5 ± 1.3 | 14.1 ± 1.8 | 11.3 ± 1.3 |
| 18: 3, n-3 (ALA) | 1.15 ± 0.3 | 0.98 ± 0.04 | 1.21 ± 0.08 | 0.91 ± 0.1* |
| 20: 4, n-6 (AA) | 14.5 ± 1.1 | 13.4 ± 0.5 | 13.1 ± 1.3 | 12.3 ± 1.3 |
| 20: 5, n-3 (EPA) | 2.07 ± 0.2 | 0.83 ± 0.02* | 2.73 ± 0.04 | 1.02 ± 0.2* |
| 22:5, n-6 (DPAn-6) | 0.13 ± 0.03 | 0.06 ± 0.02* | 0.71 ± 0.12 | 0.43 ± 0.03* |
| 22:5, n-3 (DPAn-3) | 0.82 ± 0.04 | 0.04 ± 0.01* | 0.65 ± 0.08 | 0.38 ± 0.07* |
| 22: 6, n-3 (DHA) | 4.05 ± 0.3 | 2.14 ± 0.04* | 5.01 ± 0.2 | 2.31 ± 0.4 |
| Total SFA | 39.8 ± 3.2 | 47.8 ± 2.4* | 38.4 ± 3.7 | 46.7 ± 2.4* |
| Total MUFA | 25.6 ± 2.8 | 23.1 ± 2.6 | 24.8 ± 2.0 | 24.4 ± 1.7 |
| Total PUFA | 34.6 ± 3.5 | 29.1 ± 3.1* | 36.8 ± 2.9 | 28.9 ± 1.3* |
| Total LCPUFA | 21.0 ± 2.2 | 16.6 ± 1.3* | 21.4 ± 1.2 | 16.3 ± 0.8* |
| Total n-6 LCPUFA | 14.8 ± 0.9 | 13.6 ± 1.1 | 13.4 ± 0.8 | 12.8 ± 0.9 |
| Total n-3 LCPUFA | 6.13 ± 0.4 | 2.98 ± 0.04* | 7.91 ± 0.4 | 3.38 ± 0.6* |

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for $n=10$–12 mice/experimental group. The groups were compared by Student's $t$-test for unpaired data ($p<0.05$), with significant differences being indicated by asterisks (*). Identification of saturated and unsaturated fatty acids and their relationships are shown in Table 3.

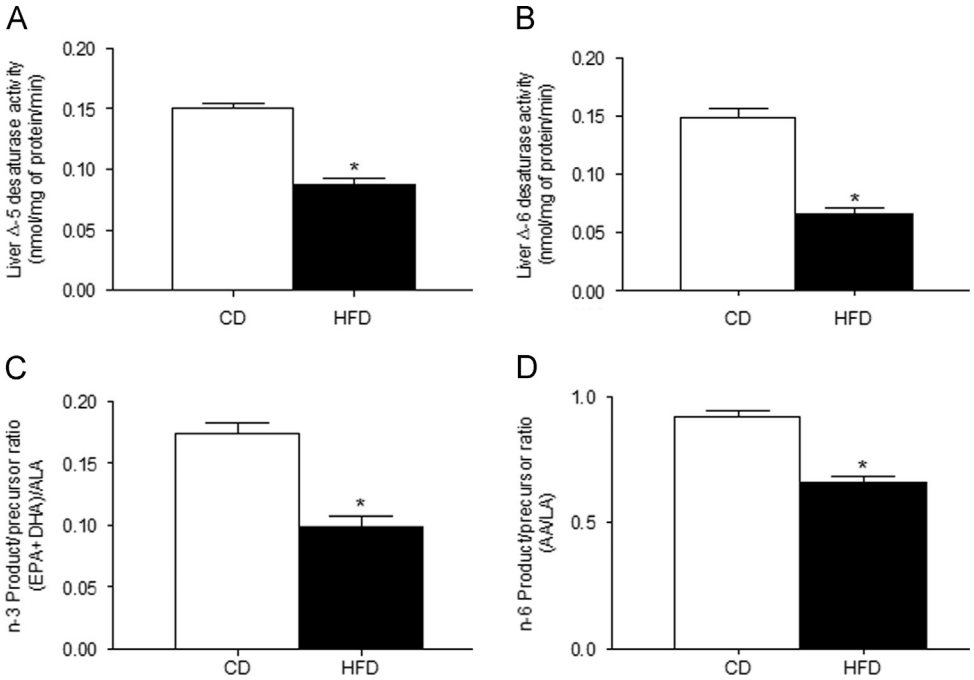


**Fig. 2.** Liver Δ-5 desaturase (A) and Δ-6 desaturase (B) activity and product/precursor ratio for n-3 (C) and n-6 (D) LCPUFA from control mice and animals subjected to high fat diet (HFD). Values represent means ± SEM for 8 to 9 mice per experimental group. The groups were compared by Student's $t$-test for unpaired data ($p<0.05$), with significant differences being indicated by asterisks (*).

**Table 5**
Fatty acid composition of adipose tissue and brain phospholipids obtained from control mice and animals subjected to high fat diet (HFD).

| | Fatty acid composition (g/100 g FAME) | | | |
|---|---|---|---|---|
| | Adipose tissue Groups | | Brain Groups | |
| Fatty acid | CD | HFD | CD | HDF |
| 16:0 | 25.8 ± 1.4 | 30.3 ± 1.1* | 20.6 ± 1.4 | 27.6 ± 1.7* |
| 18:1, n-9 | 22.3 ± 1.5 | 18.8 ± 1.0 | 21.1 ± 1.1 | 19.1 ± 1.4 |
| 18: 2, n-6 (LA) | 7.34 ± 0.5 | 8.20 ± 0.8 | 4.06 ± 0.5 | 3.97 ± 0.4 |
| 18: 3, n-3 (ALA) | 1. 84 ± 0.4 | 0.62 ± 0.04* | 1.24 ± 0.6 | 0.64 ± 0.1* |
| 20: 4, n-6 (AA) | 7.96 ± 0.3 | 5.44 ± 0.5 | 16.6 ± 1.7 | 12.3 ± 0.8* |
| 20: 5, n-3 (EPA) | 1.08 ± 0.06 | 0.34 ± 0.02* | 0.84 ± 0.06 | 0.55 ± 0.04* |
| 22:5, n-6 (DPAn-6) | 0.09 ± 0.01 | 0.05 ± 0.01* | 0.28 ± 0.02 | 1.27 ± 0.3* |
| 22:5, n-3 (DPAn-3) | 0.08 ± 0.01 | 0.03 ± 0.01* | 0.41 ± 0.08 | 0.29 ± 0.07* |
| 22: 6, n-3 (DHA) | 2.62 ± 0.2 | 0.98 ± 0.3* | 10.1 ± 1.4 | 7.27 ± 0.4* |
| Total SFA | 48.1 ± 3.7 | 57.3 ± 3.4* | 41.4 ± 2.6 | 50.9 ± 3.1* |
| Total MUFA | 30.6 ± 3.4 | 26.5 ± 3.0 | 24.6 ± 1.8 | 22.7 ± 1.6 |
| Total PUFA | 21.3 ± 1.9 | 16.2 ± 1.6* | 34.0 ± 2.3 | 26.4 ± 1.8* |
| Total LCPUFA | 11.9 ± 0.9 | 7.01 ± 0.6* | 28.0 ± 2.5 | 21.5 ± 1.6* |
| Total n-6 LCPUFA | 8.10 ± 0.5 | 5.68 ± 0.3* | 16.9 ± 1.2 | 13.6 ± 0.8* |
| Total n-3 LCPUFA | 3.84 ± 0.2 | 1.32 ± 0.1* | 11.0 ± 0.8 | 7.90 ± 0.4* |

Values are expressed as g fatty acid per 100 g FAME and represent the mean ± SEM for $n = 10–12$ mice per experimental group. The groups were compared by Student's *t*-test for unpaired data ($p < 0.05$), with significant differences being indicated by asterisks (*). Identification of saturated and unsaturated fatty acids and their relationships are shown in Table 3.

free FAs, but devoid of histological evidence of an inflammatory response, which agrees with the constancy of serum AST and ALT levels found after 12 weeks of HFD administration (Table 1) or following treatment for 10 to 19 weeks [23]. These data suggest that steatohepatitis is a late event after chronic exposure to HFDs, which is fully developed after 34 and 50 weeks [23]. Enhancements in the levels of TNF-α, IL-6 (Table 1), and IL-1β [24] in serum and in liver TNF-α and IL-1β mRNA expression [25] found in mice subjected to HFD for 12 weeks represent a pre-inflammatory state associated with its pro-steatotic effect, a response that might be due to activation of transcription factor NF-κB controlling pro-inflammatory cytokine expression [25]. HFD-induced liver steatosis was associated with two major alterations in hepatic physiology, namely, oxidative stress development and LCPUFA depletion, parameters that exhibited a significant inverse correlation.

Oxidative stress in the liver of HFD-fed mice is evidenced by depletion of total GSH equivalents, with a fall in the GSH/GSSG ratio, and enhancements in protein carbonyl, F2-isoprostane, and TBAR levels over control values. These data support the establishment of a higher free radical activity in the liver by HFD, a finding that may be associated with greater rates of mitochondrial FA oxidation [26] with a consequent ketogenic response [27,28], as result of the insulin resistance developed that favors FA oxidation, in addition to the free FA overload observed, thus representing a nutritional redox imbalance [29] also occurring in obese patients with liver steatosis [30]. In addition, upregulation of TNF-α by HFD may contribute to hepatic oxidative stress, a cytokine effectively inhibiting mitochondrial respiration with concomitant reactive oxygen species (ROS) generation [31,32]. Oxidative stress evidenced by significant 142% to 203% increments in hepatic protein carbonyl/total GSH, F2-isoprostane/total GSH, and TBAR/total GSH ratios is associated with related parameters measured in plasma, a feature that may be of importance in contributing to the induction of insulin resistance [33].

Depletion of PUFAs is an additional derangement in the liver of HFD-fed mice affecting both n-6 and n-3 LCPUFAs, as reported in human obesity [34]. Several mechanisms may trigger liver LCPUFA depletion after HFD feeding. These include (i) substantial peroxidation of LCPUFA due to their high susceptibility to free-radical reactions and the ROS activity achieved, a view that is supported by the inverse correlation between LCPUFA levels with the oxidative stress status attained; (ii) reduced availability of ALA in the case of n-3 LCPUFA; and (iii) defective desaturation and/or elongation of ALA and LA [35]. In this respect, HFD elicited significant reduction in the Δ5- and Δ6-desaturase activity of the liver, which is associated with the respective product/precursor ratios of the n-6 and n-3 series of LCPUFAs, suggesting a functional loss of desaturation capacity. In this respect, decreases in the desaturation indexes AA/LA and (EPA+DHA)/ALA were found in the liver of obese non-alcoholic fatty liver disease (NAFLD) patients with simple steatosis or steatohepatitis compared to controls [34], a feature also reported in chronic hepatitis C (CHC) patients with steatosis over those with CHC without steatosis [36] and in HIV-infected men with NAFLD compared to healthy controls [37]. HFD-induced diminution in liver Δ5- and Δ6-desaturase activity may be explained by several means including hormonal and dietary mediated mechanisms. (i) Insulin resistance induced by HFD at the hepatic level, as reflected in that inhibition of liver glucose production does not properly respond to insulin thus establishing hyperglycemia, may downregulate the protein content of Δ5- and Δ6-desaturase, considering that insulin upregulates the mRNA expression of both desaturases [38]. (ii) HFD-induced depletion of LCPUFAs in liver cell membrane phospholipids decreasing cell membrane fluidity may compromise the activity of both desaturases, which are membrane-bound enzymes localized in the endoplasmic reticulum (ER) [8,39], a factor proposed to predispose to insulin resistance development [39]. (iii) The enhanced free radical activity induced by HFD in the liver is likely to produce free radical-protein interactions, as evidenced by the high levels of protein carbonyls found, an oxidative process that may involve Δ5- and Δ6-desaturases leading to their inactivation due to protein unfolding followed by proteasomal degradation [40]. (iv) Defective liver desaturase activity can also be accomplished by a higher-than normal consumption of *trans*-fatty acids that are effective Δ6-desaturase inhibitors [41]. Although this factor is unlikely to play a role in our study due to the use of commercial diets devoid of *trans* isomers, greater levels of elaidic acid (18:1, n-9 *trans*) were detected in the adipose tissue from obese patients than in controls [34], a parameter considered a suitable biomarker of dietary FA intake [42]. In addition to HFD-induced liver LCPUFA depletion, hepatic steatosis was accompanied by a significant increment in the content of SFA, palmitic acid specifically, a pro-lipogenic condition that may be secondary to the development of endoplasmic reticulum stress, rather than an insulin-mediated response due to the high insulin resistant status achieved [43].

A part from the decreasing effect of HFD on liver Δ5- and Δ6-desaturase activity, a second major finding of our work is that the HFD-induced depletion of total LCPUFAs is not only observed in the liver, but in extrahepatic tissues as well, with hepatic total LCPUFA levels being significantly correlated with those in erythrocytes, heart, adipose tissue, and brain, besides their direct association with hepatic Δ5- and Δ6-desaturase activities. In this respect, previous studies have shown that liver is one of the most active organs in the synthesis and secretion of LCPUFAs from ALA and LA, for provision for less active tissues [44,45], particularly the brain [46,47]. In fact, using stable isotope tracer, techniques, it was demonstrated that liver DHA synthesis rates from ALA markedly exceed brain and heart DHA synthesis [48], and that only liver is able to upregulate both DHA synthesis and mRNA expression and activity of desaturases and elongases when dietary ALA is reduced [49]. It is also interesting to note that depletion of brain DHA levels is offset by an increase in C22: 5 n-6 content, a situation described as a way to maintain a sufficient level of polyunsaturated fatty acids at the neuronal level [50]. These data support the contention that the liver has a fundamental role in extrahepatic LCPUFA homeostasis, a function that may be accomplished by adequate hormonal and end-product regulation, with involvement of LCPUFA secretion in the form of

very low density lipoproteins [44,46], which is markedly deranged by HFD.

In conclusion, using a well-established HFD eliciting metabolic change leading to liver steatosis in mice, which resemble those in human obesity, we demonstrate that high dietary ingestion of fats (i) significantly reduces liver Δ5- and Δ6-desaturase activities with depletion of hepatic LCPUFA levels; and (ii) consequently deranges extrahepatic LCPUFA homeostasis, pointing to the importance of the liver as the main site for LCPUFA biosynthesis and secretion. These findings strengthen the importance of dietary supplementation with n-3 LCPUFA in the treatment of liver steatosis [51], either alone or in combination with other nutrients such as B vitamins, antioxidants (vitamins C and E, selenium) and uridine monophosphate, as recommended for the prevention of cognitive decline in ageing [52].

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.plefa.2015.04.002.

## References

[1] A. Hansen, H. Wiese, A. Boelsche, M. Haggard, D. Adam, H. Davis, Role of linolenic acid in infant nutrition: clinical and chemical study of 428 infants fed on milk mixtures varying in kind and amount of fat, Pediatrics 31 (1963) 171–192.
[2] M.A. Montanaro, O.J. Rimoldi, R.A. Igal, S. Montenegro, M.C. Tarrés S.M. Martínez, et al., Hepatic delta9, delta6, and delta5 desaturations in non-insulin-dependent diabetes mellitus eSS rats, Lipids 38 (2003) 827–832.
[3] M. Doi, K. Nosaka, T. Miyoshi, M. Iwamoto, M. Kajiya, K. Okawa, et al., Early eicosapentaenoic acid treatment after percutaneous coronary intervention reduces acute inflammatory responses and ventricular arrhythmias in patients with acute myocardial infarction: a randomized, controlled study, Int. J. Cardiol. 176 (2014) 577–582.
[4] R.K. McNamara, J. Able, R. Jandacek, T. Rider, P. Tso, J.C. Eliassen, et al., Docosahexaenoic acid supplementation increases prefrontal cortex activation during sustained attention in healthy boys: a placebo-controlled, dose-ranging, functional magnetic resonance imaging study, Am. J. Clin. Nutr. 91 (2010) 1060–1067.
[5] Y.B. Lombardo, A.G. Chicco, Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. a review, J. Nutr. Biochem. 17 (2006) 1–13.
[6] L.K. Lee, K.J. Bryant, R. Bouveret, P.W. Lei, A.P. Duff, S.J. Harrop, et al., Selective inhibition of human group IIA-secreted phospholipase A2 (hGIIA) signaling reveals arachidonic acid metabolism is associated with colocalization of hGIIA to vimentin in rheumatoid synoviocytes, J. Biol. Chem. 288 (2013) 15269–15279.
[7] V.H. Ryan, C.T. Primiani, J.S. Rao, K. Ahn, S.I. Rapoport, H. Blanchard, Coordination of gene expression of arachidonic and docosahexaenoic acid cascade enzymes during human brain development and aging, PLoS One 9 (2014) e100858.
[8] H. Guillou, D. Zadravec, G.P.G. Martin, A. Jacobsson, The key roles of elongases and desaturases in mammalian fatty acid metabolism: insights from transgenic mice, Prog. Lipid Res. 49 (2010) 186–199.
[9] H. Lee, W.J. Park, Unsaturated fatty acids, desaturases and human health, J. Med. Food 17 (2014) 189–197.
[10] M.T. Nakamura, T.Y. Nara, Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases, Annu. Rev. Nutr. 24 (2004) 345–376.
[11] P. Sjögren, J. Sierra-Johnson, K. Gertow, M. Rosell, B. Vessby, U. de Faire, et al., Fatty acid desaturases in human adipose tissue: relationships between gene expression, desaturation indexes and insulin resistance, Diabetologia 51 (2008) 328–335.
[12] R. Hofacer, R. Jandacek, T. Rider, P. Tso, I.J. Magrisso, S.C. Benoit, et al., Omega-3 fatty acid deficiency selectively up-regulates delta6-desaturase expression and activity indices in rat liver: prevention by normalization of omega-3 fatty acid status, Nutr. Res. 31 (2011) 715–722.
[13] S. Piro, L. Spadaro, M. Russello, D. Spampinato, C.E. Oliveri, E. Vasquez, et al., Molecular determinants of insulin resistance, cell apoptosis and lipid accumulation in non-alcoholic steatohepatitis, Nutr. Metab. Cardiovasc. Dis. 18 (2008) 545–552.
[14] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.
[15] I. Rahman, A. Kode, S.K. Biswas, Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method, Nat. Protoc. 1 (2006) 3159–3165.
[16] H.M. Su, J.T. Brenna, Simultaneous measurement of desaturase activities using stable isotope tracers or a nontracer method, Anal. Biochem. 261 (1998) 43–50.
[17] R. Valenzuela, C. Barrera, M. González-Astorga, J. Sanhueza, A. Valenzuela, Alpha linolenic acid (ALA) from Rosa canina, sacha inchi and chia oils may increase ALA accretion and its conversion into n-3 LCPUFA in diverse tissues of the rat, Food Funct. 5 (2014) 1564–1572.
[18] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[19] V. Ruiz-Gutierrez, A. Cert, J.J. Rios, Determination of phospholipid fatty acid and triacylglycerol composition of rat caecal mucosa, J. Chromatogr. 575 (1992) 1–6.
[20] W.R. Morrison, L.M. Smith, Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride – methanol, J. Lipid Res. 5 (1964) 600–608.
[21] Y. Takahashi, Y. Soejima, T. Fukusato, Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, World J. Hepatol. 18 (2012) 2300–2308.
[22] R. Buettner, J. Schölmerich, L.C. Bollheimer, High-fat diets: modeling the metabolic disorders of human obesity in rodents, Obesity 15 (2007) 798–808.
[23] M. Ito, J. Suzuki, S. Tsujioka, M. Sasaki, A. Gomori, T. Shirakura, et al., Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet, Hepatol. Res. 37 (2007) 50–57.
[24] R. Valenzuela, A. Espinosa, D. González-Mañán, A. D Espessailles, V. Fernández, L.A. Videla, et al., N-3 long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis, PLoS One 7 (10) (2012) e46400.
[25] G. Tapia, R. Valenzuela, A. Espinosa, P. Romanque, C. Dossi, D. González-Mañán, et al., N-3 long-chain PUFA supplementation prevents high fat diet induced mouse liver steatosis and inflammation in relation to PPAR-α upregulation and NF-κB DNA binding abrogation, Mol. Nutr. Food Res. 58 (2014) 1333–1341.
[26] A. Aronis, Z. Madar, O. Tirosh, Mechanism underlying oxidative stress-mediated lipotoxicity: exposure of J774.2 macrophages to triacylglycerols facilitates mitochondrial reactive oxygen species production and cellular necrosis, Free Radic. Biol. Med. 38 (2005) 1221–1230.
[27] S. Satapati, N.E. Sunny, B. Kucejova, X. Fu, T.T. He, A. Méndez-Lucas, et al., Elevated TCA cycle function in the pathology of diet-induced hepatic resistance and fatty liver, J. Lipid Res. 53 (2012) 1080–1092.
[28] H.C. Barrena, F.P.M. Schiavon, M.A. Carrara, Marques ACR, C.R. Schamber, R. Curi, et al., Effect of linseed oil and macadamia oil on metabolic changes induced by high-fat diet in mice, Cell Biochem. Funct. 32 (2014) 333–340.
[29] H. Sies, W. Stahl, A. Sevanian, Nutritional, dietary and postprandial oxidative stress, J. Nutr. 135 (2005) 969–972.
[30] L.A. Videla, R. Rodrigo, M. Orellana, V. Fernández, G. Tapia, L. Quiñones, et al., Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients, Clin. Sci. 106 (2004) 261–268.
[31] J. Stadler, B.G. Bentz, B.G. Harbrecht, M. Di Silvio, R.D. Curran, T.R. Billiar, et al., Tumor necrosis factor alpha inhibits hepatocyte mitochondrial respiration, Ann. Surg. 216 (1992) 539–546.
[32] K. Schulze-Osthoff, A.C. Bakker, B. Vanhaesebroeck, R. Beyaert, W.A. Jacob, W. Fiers, Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions, J. Biol. Chem. 267 (1992) 5317–5323.
[33] N. Houstis, E.D. Rosen, E.S. Lander, Reactive oxygen species have a causal role in multiple forms of insulin resistance, Nature 440 (2006) 944–948.
[34] J. Araya, R. Rodrigo, L.A. Videla, L. Thielemann, M. Orellana, P. Pettinelli, et al., Increase in long-chain polyunsaturated fatty acid n-6/n-3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease, Clin. Sci. 106 (2004) 635–643.
[35] R. Valenzuela, L.A. Videla, The importance of the long-chain polyunsaturated fatty acid n-6/n-3 ratio in development of non-alcoholic fatty liver associated with obesity, Food Funct. 2 (2011) 644–648.
[36] B.M. Arendt, S.S. Mohammed, E. Aghdassi, N.R. Prayitno, Ma. DWL, A. Nguyen, et al., Hepatic fatty acid composition differs between chronic hepatitis C patients with and without steatosis, J. Nutr. 139 (2009) 691–695.
[37] B.M. Arendt, S.S. Mohammed, Ma. DWL, E. Aghdassi, I.E. Salit, D.K. Wong, et al., Non-alcoholic fatty liver disease in HIV infection associated with altered hepatic fatty acid composition, Curr. HIV Res. 9 (2011) 128–135.
[38] R.R. Brenner, Hormonal modulation of Δ6 and Δ5 desaturases: case of diabetes, Prostaglandins Leukot Essent Fat. Acids 68 (2003) 151–162.
[39] U.N. Das, A defect in the activity of Δ6 and Δ5 desaturases may be a factor predisposing to the development of insulin resistance syndrome, Prostaglandins Leukot Essent Fat. Acids 72 (2005) 343–350.
[40] F. Shang, A. Taylor, Ubiquitin-proteasome pathway and cellular responses to oxidative stress, Free Radic. Biol. Med. 51 (2011) 5–16.
[41] E. Larqué, P.A. García-Ruiz, F. Perez-Llamas, S. Zamora, A. Gil, Dietary *trans* fatty acids alter the compositions of microsomes and mitochondria and the activities of microsome Δ6-fatty acid desaturase and glucose-6-phosphase in livers of pregnant rats, J. Nutr. 133 (2003) 2526–2531.

[42] A. Baylin, E.K. Kabagambe, X. Siles, H. Campos, Adipose tissue biomarkers of fatty acid intake, Am. J. Clin. Nutr. 76 (2002) 750–757.
[43] N. Anderson, J. Borlak, Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis, Pharmacol. Rev. 60 (2008) 311–357.
[44] J. Bézard, J.P. Blond, A. Bernard, P. Cloeut, The metabolism and availability of essential fatty acids in animal and human tissues, Reprod. Nutr. Dev. 34 (1994) 539–568.
[45] Y.H. Lin, N. Salem Jr., Whole distribution of deuterated linoleic and α-linolenic acids and their metabolites in the rat, J. Lipid. Res. 48 (2007) 2709–2724.
[46] B.L. Scott, N.G. Bazan, Membrane docosahexaenoate is supplied to the development brain and retina by the liver, Proc. Natl. Acad. Sci. USA 86 (1989) 2903–2907.
[47] S.I. Rapoport, J.S. Rao, M. Igarashi, Brain Metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver, Prostaglandins Leukot Essent Fat. Acids 77 (2007) 251–261.
[48] S.I. Rapoport, M. Igarashi, F. Gao, Quantitative contributions of diet and liver synthesis to docosahexaenoic acid homeostasis, Prostaglandins Leukot Essent Fat. Acids 82 (2010) 273–276.
[49] M. Igarashi, K. Ma, L. Chang, J.M. Bell, S.I. Rapoport, Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not in brain, J. Lipid Res. 48 (2007) 2463–2470.
[50] N. Gazzah, A. Gharib, M. Croset, P. Bobillier, M. Lagarde, N. Sarda, Decrease of brain phospholipid synthesis in free-moving n-3 fatty acid deficient rats, J Neurochem. 64 (1995) 908–918.
[51] L.A. Videla, P. Pettinelli, Misregulation of PPAR functioning and its pathogenic consequences associated with nonalcoholic fatty liver disease in human obesity, PPAR Res. 2012 (2012) 107434.
[52] P. Barberger-Gateau, Nutrition and brain aging: how can we move ahead? Eur. J. Clin. Nutr. 68 (2014) 1245–1249.