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Basic nutritional investigation

Modulation of Δ5- and Δ6-desaturases in the brain-liver axis

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ABSTRACT

Objective: Obesity is associated with liver depletion of ω-3 polyunsaturated fatty acids (ω-3 PUFAS) promoting steatosis and inflammation, whose levels are maintained by diet or biosynthesis involving Δ-5D, Δ-6D desaturases and elongases.
Method: We aimed to assess Δ-5D and Δ-6D activities in liver and brain from mice fed a control diet (CD) or high-fat diet (HFD) for four to sixteen weeks.
Results: HFD led to (1) an early (4 weeks) enhancement in liver Δ-5D, Δ-6D, and PPAR-α activities, without changes in oxidative stress, liver damage or fat accumulation; (2) a latter progressive loss in hepatic desaturation with insufficient compensatory increases in mRNA and protein expression, leading to ω-3 PUFA depletion, PPAR-α down-regulation reducing FA oxidation, and liver steatosis with enhancement in lipogenesis; and (3) brain ω-3 PUFA depletion after 12 to 16 weeks of HFD feeding.
Conclusion: In conclusion, the brain-liver axis is drastically affected by obesity in a time dependent fashion.
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Introduction

Obesity and related comorbidities are a global public health problem in both developing and developed countries, due to the prevalence of a sedentary lifestyle and fast food containing high levels of fat and carbohydrates [1], which causes several cellular alterations, such as inflammation, mitochondrial dysfunction, and oxidative stress [2,3]. Moreover, obesity is a risk factor for several other diseases such as cardiovascular sicknesses, diabetes [2] and liver metabolic disorders [4]. In this context, obesity plays a crucial role in the development of metabolic dysfunction-associated fatty liver disease (MAFLD) [5,6], where the obesity related-MAFLD may be the result of a dysregulating relation among multiple extrahepatic organs and the liver. The above alterations could be mediated by metabolites such as lipids or glucose, apart from endocrine signals (e.g., adipokines), gut cytokines and macrophage polarization [7]. Considering that the liver has a role in the synthesis,

Abbreviations: ACC, Acetyl-CoA carboxylase; ACOX, acyl-CoA oxidase; ALA, α-linolenic acid (C18:3n-3); ALT, alanine aminotransferase; AP1, activator protein 1; ARA, arachidonic acid; AST, aspartate aminotransferase; CPT-1, carnitine-palmitoyl transferase-1; CD, control diet; CAT, catalase; Δ-5D, Δ5 desaturase; Δ-6D, Δ6 desaturase; DHA, docosahexaenoic acid (C22:6n-3, DHA); DGLA, dihomo-gamma-linolenic acid; EPA, eicosapentaenoic acid (C20:5n-3); FA, fatty acid; FAME, fatty acid methyl ester; FADS, fatty acid desaturase gene cluster; FAS, fatty acid synthase; GPX, glutathione peroxidase; GR, glutathione reductase; GSSG, glutathione disulfide; GSH, reduced glutathione; HDL, high density lipoprotein; HFD, high-fat diet; IL-6, interleukin-6; IR, insulin resistance; LA, linoleic acid (C18:2n-6); LDL, low density lipoprotein; LTs, leukotrienes; MAFLD, metabolic dysfunction-associated fatty liver disease; MASH, metabolic dysfunction-associated steatohepatitis; NF-κB, nuclear factor-κB; Nrf2, nuclear factor-E2-related factor-2; PPAR-α, peroxisome proliferator-activated receptor-α; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SDA, stearidonic acid; SOD, superoxide dismutase; SREBP-1c, sterol receptor element-binding protein-1c; TBARS, thiobarbituric acid reactive substances; STAT3, signal transducer and activator of transcription-3; TNF-α, tumor necrosis factor-α

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oxidation, accretion and export of lipids, while lipids are essential components of brain structure and function, the unrestrained hepatic lipid metabolism may lead to changes and alterations in brain functionality [7].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), are products of normal cellular metabolism, characterized by having unpaired electron(s) assigning a great reactivity to them, which need to be kept at low levels by antioxidant mechanisms [8]. In this context, oxidative stress is a redox imbalance between ROS production and neutralization in favor of the former, a cellular metabolic phenomenon that at low/moderate levels achieves beneficial effects (oxidative eutress) such as redox-regulatory mechanisms for redox homeostasis maintenance [8,9]. However, an overproduction of ROS and/or a deficiency of enzymatic and non-enzymatic antioxidants determine harmful consequences (oxidative distress), mediating damage to cell structures, nucleic acids, lipids and proteins, and, consequently, promoting various disease states including MAFLD [8,9]. These dual responses are carried out through changes in the activity of redox-sensitive transcription factors such as nuclear factor-κB (NF-κB), signal transducer and activator of transcription-3 (STAT3), activator protein-1 (AP1), and nuclear factor-E2-related factor-2 (Nrf2), which are involved in the development of low- and high-grade systemic inflammatory phenotypes [10].

There is sufficient evidence for the role of brain in lipid accumulation and the consequent excess weight [11,12]. Obese individuals have brain structure alterations, such as reduced gray matter volume in the postcentral gyrus (an area related with somatosensory input−output gain of a detector system for foods and drinks). It is believed that structural alterations in the postcentral gyrus promote obesogenic influences [13,14]. Interestingly, Cheng et al. showed that the excitatory neurons in anterior paraventricular thalamus play a crucial role in the high fat diet (HFD)-induced compulsive eating behavior [15]. The metabolic inflammation provoked by HFD augments neural activity in the anterior paraventricular thalamus, and the microglias in this brain area act as lipid sensing reward area, promoting obesity [15]. Additionally, HFD induces hippocampal alterations and hypothalamic injury, generating neurobehavioral changes, probably related to cellular lipid accumulation in microglia, inflammation and gliosis [16,17].

Inflammation is a process enabling survival in conditions of infection or injury, by eliminating harmful stimuli and initiating the recovery process, with immunonutrition playing an important role, mainly kept by arginine, nucleotides and ω-3 polyunsaturated fatty acids (ω-3 PUFAs) [18]. In the context of PUFAs biosynthesis, desaturases (a group of enzymes that insert appropriate number of double bonds in the acyl chains of fatty acids (FAs) molecules) are important in cell adaptation, membrane fluidity and membrane-bound protein function [19]. The PUFA synthesis occurring in the liver, but also in the brain in development, requires desaturation reactions catalyzed by Δ5 desaturase (Δ-5D) and Δ-6D, as well as elongation processes [19,20]. Importantly, the dietary essential FAs α-linolenic acid (C18:3ω-3, ALA) and linoleic acid (C18:2ω-6, LA) can be desaturated by Δ-5D and Δ-6D and elongated in the liver or other organs such as brain, kidney and testicles, being the liver who exhibits the highest enzymatic activities compared to the other organs [20]. It is well known that Δ-6D is affected by nutritional influences and dietary essential FAs, and deficiency of Δ-5D and Δ-6D are related to human disease [21]. Also, desaturase activity plays a role in the progress of hepatic steatosis, and the dietary intakes of EPA and DHA are beneficial in liver fat accumulation [22]. Changes in ω-3 PUFA biosynthesis by nutritional and hormonal factors, and oxidative stress, may increase inflammation, triggering MAFLD development, with potential progression to MASH and fibrosis [23,24]. Considering the previous antecedents related to food intake, lipid synthesis and desaturase behaviors, we analyzed the modification of Δ-5D and Δ-6D activity among liver and brain from mice fed a control diet (CD) or HFD for four to sixteen weeks.

Material and methods

Animals and diets

Twenty-one days after weaning, male C57BL/6J mice weighing 12−14 g (Animal Room, Faculty of Medicine, University of Chile) was randomly assigned to each experimental group and allowed free access to specially formulated control CD or HFD. The composition of the CD (expressed as % total calories) was 10% fat, 20% protein, and 70% carbohydrates, with caloric value of 3.85 kcal/g, and that of HFD was 60% fat, 20% protein and 20% carbohydrates, with a caloric value of 5.24 kcal/g, both diets being free of EPA and DHA, and contained 0.7 g of ALA/100 g of diet (Research diet INC, Rodent diet, Product data D12450K [CD] and D12492 [HFD], USA). Animals received water *ad libitum* and were housed on a 12-h light/dark periods and were assembled in four experimental groups during 4, 8, 12, and 16 weeks, respectively. Weekly controls of body weight and diet intake were performed throughout the whole period. Following 4, 8, 12, and 16 weeks of CD and HFD feeding, animals were fasted (6−8 h), anesthetized with isofluorane and blood samples were obtained by cardiac puncture for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, insulin, triacylglycerides, total cholesterol, LDH-cholesterol, HFD-cholesterol, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) levels. Then, animals were perfused *in situ* with a cold solution of phosphate-buffer saline (PBS) to remove blood for enzymatic activity measurements in liver and brain, and tissue 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 analyzed by optical microscopy in a blind fashion to describe the presence of steatosis, both graded as absent, mild, moderated, and severe [25].

Ethics statement

Experimental animal protocols and animal procedures complied with the Guide for the Care and Use of Laboratory Animals (National Academy of Science NHI Publication 6-23, revised 1985), and were approved by the Bioethics Committee for Research in Animals, Faculty of Medicine, University of Chile (Protocol # 24749−MED−UCH).

Measurements of liver and serum parameters

Hepatic total fat content (mg/g liver) was evaluated according to a previously described method [26], and hepatic triacylglycerols content (mg/g liver) (Wiener Lab, Argentina) and hepatic free FAs (μM/g liver) (Cayman Chemical Company, Ann Arbor, MI, USA) were measured using specific kits according to the manufacturer's instructions. Serum AST and ALT activities (Units/L) were determined using specific diagnostic kits (Biomerieux SA, Marcy l'Etoile, France). Cholesterol (mg/dL) and glucose (mg/dL) levels were evaluated using specific diagnostic kits (Wiener Lab, Argentina). An immunoassay kit for mice serum insulin assessment (units/mL) was used, according to the manufacturer's instruction (Wiener Lab, Argentina). Insulin resistance was estimated by the homeostasis model assessment method (fasting insulin (U/mL) x fasting glucose (mM)/22.5) [27]. ELISA kits were used for assessment of serum levels (pg/mL) of TNF-α and IL-6 (R&D Systems, USA).

Assays for oxidative stress-related parameters in liver

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 assessment. Reduced glutathione (GSH) and glutathione disulfide (GSSG) contents were assessed with an enzymatic recycling method [28]. Content of protein carbonyls, F-2 isoprostanes, and thiobarbituric acid reactants (TBARS) in liver were measured using specific kits, according to the manufacturer's instructions (R&D Systems, USA).

Quantification of the protein concentration of Δ-6D and Δ-5D in liver and brain

Assessment of Δ-6D and Δ-5D protein concentration (ng/g tissue) of each enzyme in liver and brain were carried out using commercially available enzyme-linked immunosorbent assay kits (MyBioSource, Inc., San Diego, CA, USA), according to the manufacturer's instructions. Briefly, samples were prepared as indicated by the manufacturer's instructions, plates were read at 450 nm and the protein concentration was quantified in each tissue (ng/g tissue) using a standard curve.

Determinations of liver and brain Δ-6D and Δ-5D activities

Liver and brain samples (500 mg) previously stored at -80°C were homogenized on ice in a buffer solution of pH 7.9 containing 10 mmol/L HEPES, 1.0 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). Tissue homogenates were centrifuged at 4°C, first at 2000 g for 30 s, followed by centrifugation of the supernatants at 5000 g for 5 min, and finally at 100,000 g for 60 min, to obtain the microsomal isolations for the assessment of desaturase activities. These were assayed using 1 mL of incubation medium containing 4 μmol ATP, 0.1 μmol coenzyme-A, 1.28 μmol NADPH, 2.42 μmol N-acetylcysteine, 0.5 μmol nicotinamide, 5 μmol MgCl2, 62.5 μmol NaF, and 62.5 μmol phosphate buffer pH 7, added with 100 nmol albumin-bound FA precursor and 1 mg protein of cytosolic extract in a total volume of 100 μL. The incubation reaction was carried out at 37°C for 30 min with shaking.

The activity of Δ-6D was determined by the amount of ALA converted to stearidonic acid (SDA, 18:4n-3), and the activity of Δ-5D was assessed by the amount of dihomo-gamma-linolenic acid (DGLA, 20:3n-6) transformed into AA. The respective PUFA products were measured as FA methyl esters (FAMES) by gas chromatographic analysis. The protein content in enzyme extracts was determined by the Bradford assay (Bio-Rad reagent, California, USA). This methodology allows the assessment of actual enzyme activities, expressed as nmol/mg protein/min. The specific identifications of products from precursors were previously developed and reported [20,29].

FA profile from liver and brain samples

Quantitative extraction of total lipids from liver and brain was carried out according to Bligh and Dyer [30]. Liver and brain samples were homogenized in ice-cold chloroform/methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (BHT) in an UltraTurrax homogenizer (Janke & Kunkel, Stufen, Germany). Total lipids were extracted with chloroform/methanol (2:1 v/v). FAMES from liver and brain were prepared according to Morrison and Smith [31] and analyzed as previously reported [32].

SREBP-1c, PPAR-α, NF-KB, and Nrf2 DNA-binding activity

Nuclear extracts from liver were obtained using a commercial extraction kit (Cayman Chemical Company, Ann Arbor, MI, USA). Sterol receptor element-binding protein-1c (SREBP-1c), peroxisome proliferator-activated receptor-α (PPAR-α), NF-κB-p65 subunit and Nrf2 DNA-binding activities were assessed with a commercial ELISA kit (Cayman Chemical Company, Ann Arbor, MI, USA) and according to the manufacturer's instructions. Values were expressed as percentage of SREBP-1c, PPAR-α, NF-κB (p65 subunit) and Nrf2 DNA-binding with respect to a positive control provided by the respective ELISA kit.

Activities of antioxidant and lipogenic enzymes

Liver samples were homogenized in three volumes of 30 mM phosphate buffer, pH 7.4, containing EDTA (1 mM) and sucrose (250 mmol). After centrifugation for 10 min at 1200 g and 4°C, one aliquot of the supernatant was used for the determination of both catalase (CAT) and superoxide dismutase (SOD) activities. Another aliquot was centrifuged at 100,000 g for 60 min at 4°C to carry out glutathione peroxidase (GPX) and glutathione reductase (GR) assays according to Chow [33]. CAT activity was measured according to Lück [34]. Enzyme unit is defined as the amount of oxygen liberated from a hydrogen peroxide solution in 100 s at 25°C. Assessment of SOD activity was carried out with a commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. GPX activity was determined using hydrogen peroxide as substrate according to the method of Paglia and Valentine [35]. Enzyme activity was evaluated at 340 nm by measuring the decrease in the absorbance of NADPH. Enzyme unit is defined as the number of μmoles of NADPH oxidized per min at 20°C. GR activity was determined by the method of Horn [36]. Enzyme unit is defined as the amount of enzyme that reduces 1 μmol of GSSG per min at pH 6.6 and 25°C. The fat of the upper layers was discarded, the supernatants were again centrifuged at 100,000 g for 60 min at 4°C and the aqueous fractions were used to study Acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) activities as previously described [37]. Activity of carnitine-palmitoyl transferase-1 (CPT-1) was determined spectrophotometrically according to the method described by Karlic et al. [38]. The protein content in enzyme extracts was determined by the Bradford assay (Bio-Rad reagent, California, USA).

Gene expression assays

Total RNA was isolated from liver samples using Trizol (Ambion, Carlsbad, CA, USA), according to the supplier's protocols. Purified RNA was treated with DNAase I (DNA-freeTM Kit; Invitrogen, Waltham, Ma, USA). Then 1 μg of total RNA was used to generate single-stranded cDNA, using High-Capacity cDNA Reverse Transcription Kit (Appliedbiosystems, Vilnus, Lithuania). The cDNA was amplified using the TaqMan® Gene Expression Assays (Appliedbiosystems) in a total volume of 20 μL. Real time PCR was performed in an AriaMx qPCR System (Agilent Technologies, Penang, Malaysia) following the manufacturer's recommendations (Appliedbiosystems). Expression levels of target genes studied were normalized by the expression of cyclophilin A (Ppia) as internal control. Fold change between groups was calculated by the $2^{-(\Delta\Delta Ct)}$ method, as established by Pfaffl [39].

Statistical analysis

Statistical analysis was performed with GraphPad Prism 9.0 software (GraphPad Prism Software, Inc, San Diego, CA, USA). Reported values represent the mean ± SEM for each experimental group. Evaluation of normality of data distribution was performed using the Shapiro Wilk test. Assessment of the statistical significance of differences between mean values was performed by two-way analysis of variance and Bonferroni post-test. $P < 0.05$ was considered significant.

Results

General and biochemical parameters

The HFD group showed a progressive weight increase over the study weeks (Table 1A). The animals gained 7.9, 15.7, 30.9, and 36 g every 4 weeks, respectively for HFD-fed animals ($P < 0.01$), being the total weight gain of 353.5% in HFD compared to 248.9% in CD animals. The liver weight in HFD animals increased by 160% from week 4 to 16, compared to 143% increase in control animals ($P < 0.001$). Thus, our model reflects the normal development and growth in the control group compared to a pathological model of obesity in HFD mice.

The hepatic index (liver weight (g) / body weight (g) x 100) at the end of the study was 2.49 for HFD versus 3.26 for CD, values that were not significantly different. In assessing the visceral adipose tissue, HFD animals displayed a significant increase at weeks 12 and 16, with a rise of 243% and 481% from week 4 to 12 and 4 to 16, respectively ($P < 0.01$). As expected, animals with CD did not show any change in visceral adipose tissue. Respect to the fat liver parameters (Table 1B), at week 12 all HFD animals experienced an elevation of 2.9, 3.0, and 2.9 times for total fat, triacylglycerols and free FAs ($P < 0.001$), respectively, and they continue increasing in an average of 1.27 times from week 12 to 16. The animals fed with CD exhibited a normal liver histology (4 to 16 weeks) (Fig. 1A–D). However, HFD-fed mice showed a significant increment in steatosis (micro- and macro-vascular focus) between 4 and 16 weeks compared to respective CD groups (Fig. 1E–H). Also, this increment was qualitatively significant from 4, 8, and 12 weeks compared with all CD groups (Fig. 1I). In the context of serum transaminases AST and ALT (Table 1C), their levels remained throughout the experiment except at week 16, where AST increased by over 68 U/L in the four last weeks and ALT increased by an average of 78.4 U/L in the first 12 weeks to 124.6 U/L at week 16 ($P < 0.01$). The lipid profile (Table 1D) revealed anticipated increases in triacylglycerols, total cholesterol, and LDL-cholesterol in HFD animals, with respective total increases of 159%, 205%, and 235% compared to CD at 16 weeks ($P < 0.01$). HDL-cholesterol increased significantly by 179% when comparing HFD with CD at 16 weeks ($P < 0.01$). Insulin resistance analysis (see Table 1E), corroborate HFD-induced fasting glucose increase (48 mg/dL every 4 weeks) and a substantial increase from weeks 4 to 8 (78.8 mg/dL, $P < 0.01$), reaching 268 mg/dL at the end of the study. Insulin increased by 4.1, 6.9, and 7.1 g/dL every 4 weeks, with a final value of 23.4 mg/dL (4.5 times more than control, $P < 0.01$). HOMA values related to insulin resistance emerged from week 8 (4.4 ± 0.7, $P < 0.01$), culminating in a final HOMA of 15.5 at week 16 ($P < 0.01$), while control HOMA remained at an average value of 1.51. In the case of serum cytokines (Table 1F), TNF-α and IL-6 were

Table 1
General and biochemical parameters in control diet (CD) and high-fat diet (HFD) fed mice after different feeding periods (wks, weeks)

Groups	CD 4 wks (a)	HFD 4 wks (b)	CD 8 wks (c)	HFD 8 wks (d)	CD 12 wks (e)	HFD 12 wks (f)	CD 16 wks (g)	HFD 16 wks (h)
A. General parameters								
Initial body weight (g)	14.2 ± 1.8	14.3 ± 1.6	14.1 ± 1.9	13.9 ± 1.7	14.4 ± 1.5	14.0 ± 1.3	14.3 ± 1.5	14.2 ± 1.9
Final body weight (g)	20.8 ± 2.2^{d,e,f,g,h}	22.2 ± 2.5^{d,e,f,g,h}	25.9 ± 2.1^{f,g,h}	29.6 ± 2.5^{a,b,f,g,h}	33.8 ± 2.8^{a,b,c,f,h}	44.9 ± 4.1^{a,b,c,d,e,g}	35.6 ± 3.1^{a,b,c,f,h}	50.2 ± 4.4^{a,b,c,d,e,f,g}
Liver weight (g)	0.81 ± 0.04^{e,f,g,h}	0.78 ± 0.03^{e,f,g,h}	0.91 ± 0.05	0.95 ± 0.04	1.04 ± 0.1^{a,b}	1.17 ± 0.2^{a,b}	1.16 ± 0.3 ^{a,b}	1.25 ± 0.3^{a,b}
Visceral adipose tissue (g)	1.2 ± 0.2^{d,f,h}	1.6 ± 0.5^{d,f,h}	1.3 ± 0.2^{d,f,h}	3.2 ± 0.5^{a,b,c,e,f,g,h}	1.5 ± 0.03^{d,f,h}	5.5 ± 0.4^{a,b,c,d,e}	1.6 ± 0.4^{d,f,h}	7.7 ± 0.9^{a,b,c,d,e,f,g}
B. Liver parameters								
Total fat (mg/g liver)	29.6 ± 3.1^{d,f,h}	34.6 ± 3.3^{d,f,h}	32.8 ± 3.5^{d,f,h}	68.2 ± 4.6^{a,b,c,e,f,g,h}	35.1 ± 3.3^{d,f,h}	98.2 ± 6.1^{a,b,c,d,e,g,h}	38.6 ± 4.7^{d,f,h}	125.4 ± 15.4^{a,b,c,d,e,f,g}
Triacylglycerols (mg/g liver)	27.8 ± 2.7^{d,f,h}	28.5 ± 2.9^{d,f,h}	26.6 ± 2.1^{d,f,h}	56.8 ± 4.1^{a,b,c,e,f,g,h}	31.7 ± 3.2^{d,f,h}	85.7 ± 3.5^{a,b,c,d,e,g,h}	33.4 ± 4.6^{d,f,h}	108.3 ± 9.4^{a,b,c,d,e,f,g}
Free fatty acid (μM/g liver)	212.1 ± 21.5^{d,f,h}	245.3 ± 35.5^{d,f,h}	218.3 ± 26.8^{d,f,h}	495.3 ± 36.7^{a,b,c,e,f,g,h}	233.1 ± 18.5^{d,f,h}	701.2 ± 45.9^{a,b,c,d,e,g,h}	241.4 ± 38.9^{d,f,h}	905.7 ± 87.2^{a,b,c,d,e,f,g}
C. Serum transaminases								
AST (U/L)	129.7 ± 11.2^{h}	133.5 ± 19.3^{h}	147.1 ± 24.6^{h}	152.9 ± 30.1^{h}	143.4 ± 21.7^{h}	161.7 ± 34.6^{h}	140.9 ± 30.7^{h}	218.2 ± 33.9^{a,b,c,d,e,f,g}
ALT (U/L)	75.1 ± 7.9^{h}	71.0 ± 9.5^{h}	77.3 ± 8.4^{h}	81.3 ± 10.2^{h}	72.5 ± 5.1^{h}	82.9 ± 5.5^{h}	75.5 ± 10.3^{h}	124.6 ± 15.2^{a,b,c,d,e,f,g}
D. Serum parameters								
Triacylglycerols (mg/dL)	119.3 ± 6.9^{d,f,h}	129.3 ± 7.4^{d,f,h}	122.7 ± 6.1^{d,f,h}	144.3 ± 12.9^{a,b,c,e,f,g,h}	128.9 ± 7.1^{d,f,h}	172.5 ± 12.9^{a,b,c,d,e,g}	125.1 ± 10.6^{d,f,h}	199.4 ± 15.5^{a,b,c,d,e,g}
Total cholesterol (mg/dL)	68.5 ± 7.3^{d,f,h}	70.1 ± 8.9^{d,f,h}	67.3 ± 7.9^{d,f,h}	88.9 ± 11.1^{a,b,c,f,g,h}	70.3 ± 6.2^{f,h}	123.5 ± 15.3^{a,b,c,d,e,g}	75.9 ± 8.0^{f,h}	156.1 ± 19.9^{a,b,c,d,e,g}
LDL-cholesterol (mg/dL)	41.9 ± 5.3 ^{d,f,h}	44.2 ± 5.7 ^{d,f,h}	40.6 ± 6.3 ^{d,f,h}	63.9 ± 8.8 ^{a,b,c,e,f,g,h}	46.6 ± 3.9 ^{d,f,h}	83.4 ± 9.9 ^{a,b,c,d,e,g,h}	45.2 ± 4.1 ^{d,f,h}	106.3 ± 14.5 ^{a,b,c,d,e,f,g}
HDL-cholesterol (mg/dL)	25.5 ± 2.7 ^{f,h}	25.8 ± 2.3 ^{f,h}	26.6 ± 2.0 ^{f,h}	24.5 ± 2.5 ^{f,h}	23.5 ± 2.9 ^{f,h}	40.1 ± 4.5 ^{a,b,c,d,e,g,h}	27.7 ± 3.7^{f,h}	49.7 ± 5.0 ^{a,b,c,d,e,g}
E. Insulin resistance								
Fasting glucose (mg/dL)	112.9 ± 12.4^{d,f,h}	124.3 ± 17.7^{d,f,g}	108.3 ± 18.0^{d,f,h}	173.5 ± 29.1 ^{a,b,c,e,f,g,h}	117.6 ± 16.8^{d,f,h}	252.3 ± 31.6^{a,b,c,d,e,g}	126.5 ± 24.8^{d,f,h}	268.7± 33.2^{a,b,c,d,e,g}
Fasting insulin (mg/dL)	4.92 ± 1.6 ^{d,f,g}	5.3 ± 2.7 ^{d,f,g}	5.01 ± 1.3 ^{d,f,g}	9.4 ± 2.4 ^{a,b,c,e,f,g,h}	5.44 ± 1.1 ^{d,f,h}	16.3 ± 3.9 ^{a,b,c,d,e,g}	5.17 ± 1.9 ^{d,f,h}	23.4 ± 3.5 ^{a,b,c,d,e,f,g}
HOMA	1.42 ± 0.3 ^{d,f,h}	1.65 ± 0.4 ^{d,f,h}	1.47± 0.3 ^{d,f,h}	4.40 ± 0.7 ^{a,b,c,e,f,g,h}	1.53 ± 0.05 ^{d,f,h}	9.94 ± 1.2 ^{a,b,c,d,e,g,h}	1.62 ± 0.5 ^{d,f,h}	15.5 ± 3.1 ^{a,b,c,d,e,f,g}
F. Inflammatory cytokines								
TNF-α (pg/mL)	17.9 ± 3.1 ^{d,f,h}	21.0 ± 2.8^{d,f,h}	19.5 ± 2.4 ^{d,f,h}	34.6 ± 3.9 ^{a,b,c,e,f,g,h}	24.3 ± 2.8 ^{d,f,h}	49.3 ± 5.7 ^{a,b,c,d,e,g,h}	22.5 ± 3.0 ^{d,f,h}	68.9 ± 7.2 ^{a,b,c,d,e,f,g}
IL-6 (pg/mL)	35.2 ± 2.9 ^{d,f,h}	38.0 ± 3.3^{d,f,h}	36.8 ± 3.0 ^{d,f,h}	50.1 ± 4.4 ^{a,b,c,e,f,g,h}	38.9 ± 6.1 ^{d,f,h}	75.1 ± 8.4 ^{a,b,c,d,e,g,h}	38.5 ± 3.6 ^{d,f,h}	94.2 ± 8.9 ^{a,b,c,d,e,f,g}

Values represent means ± SEM for 8 mice per experimental group. Significant differences between the groups are indicated by the superscript letter identifying each group (two-way analysis of variance and Bonferroni post-test; $P < 0.05$).

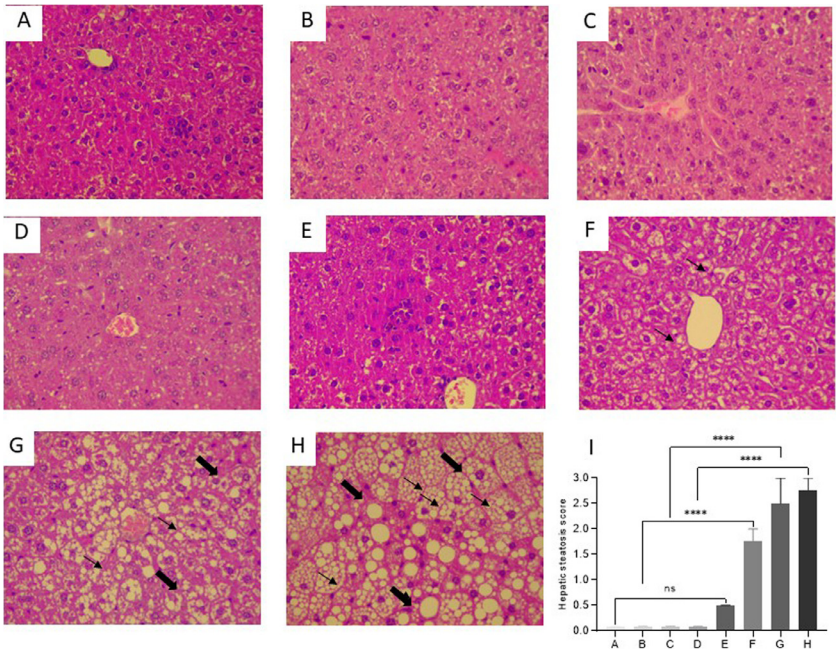


Fig. 1. Microscopy of liver sections from mice subjected to control diet (CD) or high fat diet (HFD) from week 4 (A), CD week 8 (B), CD week 12 (C), CD week 16 (D), HFD week 4 (E), HFD week 8 (F), HFD week 12 (G), HFD week 16 (H); figures are representative of liver sections from 8 animals per group. Hepatic steatosis score (I). Thin arrows indicate micro-vesicular steatosis and thick arrows show macro-vesicular steatosis. Data presented as mean and SD. Statistical analysis: (I) T-test; **** indicates significant difference ($P < 0.001$) between pairs (hematoxylin-eosin liver sections; original magnification × 40). →, microvesicular steatosis; ➡, macrovesicular steatosis.

significantly elevated from week 8 until the study's end for HFD animals. TNF-α increased by an average of 16 pg/mL every 4 weeks, resulting in a final increase of 300% compared to CD at week 16 ($P < 0.01$). For IL-6, the increase was 18.7 pg/dL every 4 weeks, leading to a final elevation of 244% at week 16 ($P < 0.01$). On the basis of the aforementioned, our results indicate an accumulative hepatic damage through the weeks in a HFD model that finally leads to a metabolic syndrome phenotype.

Activity of Δ-6D and Δ-5D desaturases

Content, expression and activity of desaturases were evaluated in HFD animals. In the liver (Fig. 2), Δ-6D activity increased by 143% at 4 weeks ($P < 0.0001$) but decreased by 45.6% at week 12 ($P < 0.0001$) and 77.2% at week 16 compared to CD. This decline correlates with a 128.5% increase in Δ-6D mRNA levels at week 8 ($P < 0.0001$), 163.1% at week 12 ($P < 0.0001$), and 189.7% at week 16 in HFD versus CD animals. Protein expression, significantly rises from week 8 (163.9%, $P < 0.0001$) to 12 weeks (209.4%, $P < 0.0001$), maintained at high levels at week 16 (212.4%, $P < 0.0001$) (Fig. 2A–C). Δ-5D in liver tissue (Fig. 2D–G) also exhibited increased activity at week 4 (143.5%, $P < 0.0001$), followed by a significant decline at weeks 12 (36.5%, $P < 0.0001$) and 16 (35.4%, $P < 0.0001$). This decline was observed together with increased mRNA expression at week 8 (123.2%, $P < 0.0001$) and 12 (152.8%, $P < 0.0001$), maintaining high levels at week 16 (152.8% versus 155.8%). Protein expression mirrored mRNA levels, being elevated at 8, 12, and 16 weeks (by 136.8%, 206.6%, and 215.9%, $P < 0.001$), with the highest increase observed at week 12, maintaining high levels at week 16. In the brain (Fig. 3A–C), Δ-6D activity decreases by 41.4% at week 16 respect to the CD ($P < 0.0001$). In opposition, lower Δ-6D activity stimulated gene expression, resulting in a 171.5% enhancement at week 16 ($P < 0.0001$) and a 138.7% increase in Δ-6D brain protein content ($P < 0.0001$). Similarly, in Δ-5D (Fig. 3D–G), activity decays by 37.9% ($P < 0.0001$), accompanied by a 149% increase in mRNA expression ($P < 0.0001$) and a 145.5% in Δ-5D brain protein content ($P < 0.0001$), all observed at 16 weeks.

Liver oxidative stress-related parameters

As Nrf2 and related genes present a characteristic pattern of enhanced activity at week 8, to decay at the end of the study in the HFD group, the oxidative stress parameters were assayed to verify if this affects the liver redox capability through the measure of GSH, GSSG, protein carbonyls, nonenzymatic F2-isoprostanes release, TBARS, and their redox relationships (see Table 2). As expected, GSH and total GSH equivalents were worn out as the weeks went by with a loss of 31.5% ($P < 0.01$) GSH equivalents and a decay of 44.3% ($P < 0.01$) in the GSH/GSSG ratio from week 4 to 16 in HFD animals. Also, the HFD demonstrated to enhance the oxidative damage at week 12 for protein carbonyls, nonenzymatic F2-isoprostanes release, TBARS in 1.7, 1.5, and 2.5 times respectively ($P < 0.01$) and an elevation of 2.6, 2.1, and 3.8 times respectively at the end of the study ($P < 0.01$).

DNA binding activity of nuclear receptors and regulated enzymes in liver tissue

The changes observed in liver desaturases suggest modifications in FA signaling, particularly those related to nuclear receptors. In this study, modifications in SREBP1c, PPAR-α, Nrf2, and NF-κB were analyzed in liver tissue, representing master receptors in lipid metabolism, inflammation, and oxidative stress (see Fig. 4). SREBP1c activity increased progressively in the HFD group, reaching increments of 130.7% ($P < 0.01$), 169.9% ($P < 0.0001$), and 175.7% ($P < 0.0001$) every 4 weeks from weeks 8 compared to CD. This increase was observed together with elevated SREBP1c mRNA levels at weeks 8 (124.7%, $P < 0.0001$) and 12 (144.6%, $P < 0.0001$), with the levels being sustained until the end of the study (Fig. 4A).

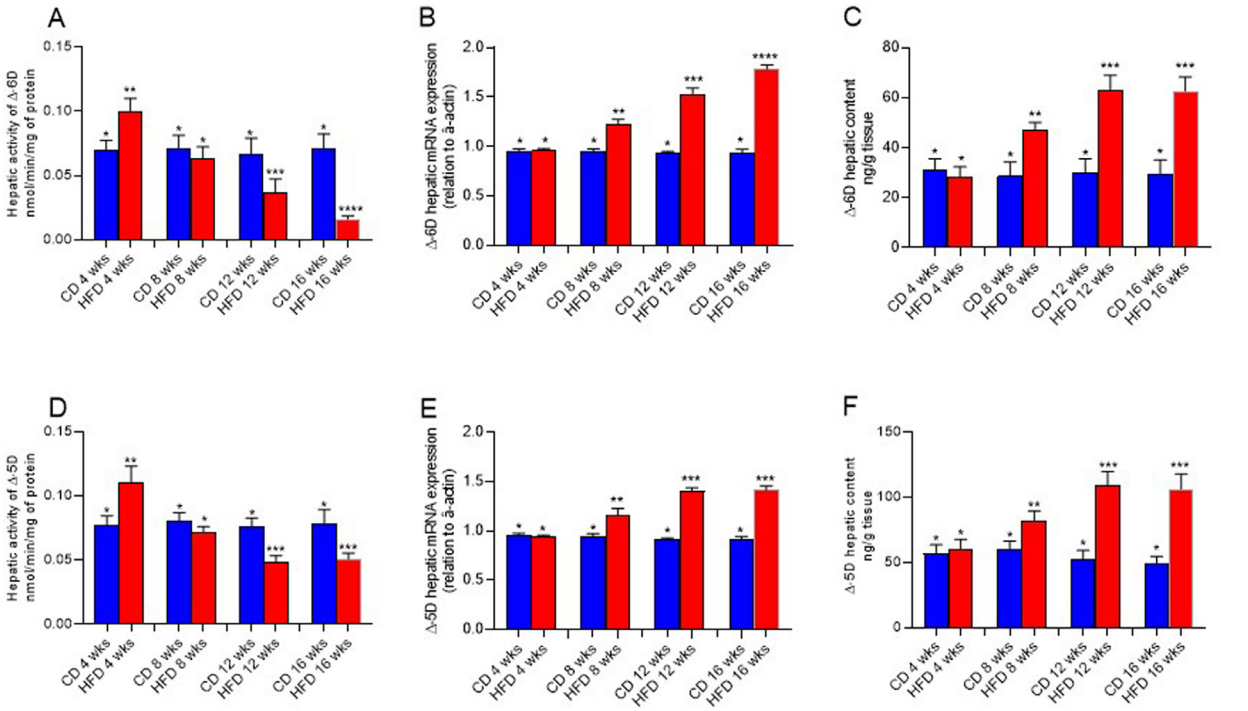


Fig. 2. Hepatic activity of Δ-6D (A); Hepatic Δ-6D mRNA expression (B); Δ-6D hepatic content (C); Hepatic activity of Δ-5D (D); Δ-5D mRNA expression (E); Δ-5D hepatic content (F) in mice subjected to control diet (CD) or high fat diet (HFD) from 4 to 16 weeks. Data are expressed as means ± SEM ($n = 8$), with significant differences being indicated by different asterisk (*) numbers (two-way ANOVA and the Bonferroni's post-test; $P < 0.05$).

SREBP1c genes associated with *de novo* lipogenesis and triglyceride synthesis, including ACC and FAS, exhibited increased mRNA expression levels from weeks 8 to 16 (1.4 and 1.6 times, respectively, $P < 0.0001$) (Fig. 5A, B). This mRNA elevation resulted in a sustained increase in ACC and FAS enzyme activity from weeks 4 to 16, with a total enhancement at week 16 of 2.23 and 1.95 times for ACC and FAS, respectively, in the HFD group ($P < 0.0001$) (Fig. 6E, F). NF-κB binding activity in liver tissue increased

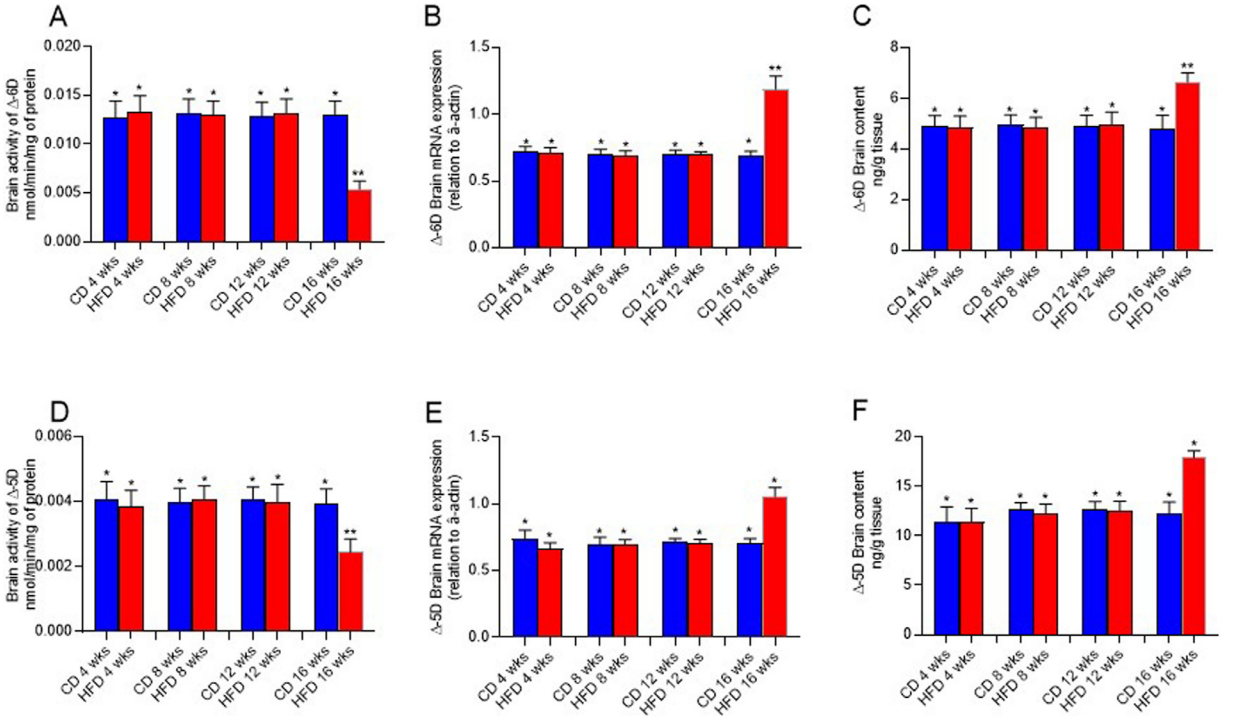


Fig. 3. Brain activity of Δ-6D (A); Δ-6D mRNA expression (B); Δ-6D brain content (C); Brain activity of Δ-5D (D); Brain Δ-5D mRNA expression (E); Δ-5D brain content (F) in mice subjected to control diet (CD) or high fat diet (HFD) from 4 to 16 weeks. Data are expressed as means ± SEM ($n = 8$), with significant differences being indicated by different asterisk (*) numbers (two-way ANOVA and the Bonferroni's post-test; $P < 0.05$).

Table 2
Oxidative stress-related parameters in control diet (CD) and high-fat diet (HFD) fed mice after different feeding periods (wks, weeks)

Groups	CD 4 wks (a)	HFD 4 wks (b)	CD 8 wks (c)	HFD 8 wks (d)	CD 12 wks (e)	HFD 12 wks (f)	CD 16 wks (g)	HFD 16 wks (h)
Liver parameters								
GSH (μmol/g liver)	5.05 ± 0.4 f,h	5.43 ± 0.5 f,h	5.19 ± 0.6 f,h	5.97 ± 0.5 f,h	5.62 ± 0.5 f,h	3.75 ± 0.2 a,b,c,d,e,g	5.51 ± 0.4 f,h	3.52 ± 0.6 a,b,c,d,e,g
GSSG (μmol/g liver)	0.36 ± 0.3	0.42 ± 0.6	0.47 ± 0.5	0.58 ± 0.6	0.40 ± 0.04	0.45 ± 0.06	0.43 ± 0.05	0.49 ± 0.08
Total GSH equivalents (μmol/g liver)	5.43 ± 0.6 f,h	5.87 ± 0.8 f,h	5.68 ± 0.5 f,h	6.56 ± 0.7 f,h	6.32 ± 0.6 f,h	4.35 ± 0.4 a,b,c,d,e,g	5.97 ± 0.5 f,h	4.02 ± 0.3 a,b,c,d,e,g
GSH/GSSG ratio	14.0 ± 0.9 f,h	12.9 ± 0.7 f,h	11.5 ± 0.8 f,h	10.4 ± 1.0 f,h	14.6 ± 1.6 f,h	8.39 ± 0.7 a,b,c,d,e,g	12.9 ± 1.2	7.19 ± 0.5 a,b,c,d,e,g
Protein carbonyls (nmol/mg protein)	2.05 ± 0.4 f,h	2.47 ± 0.4 f,h	2.21 ± 0.3 f,h	2.45 ± 0.4 f,h	2.28 ± 0.3 f,h	4.17 ± 0.5 a,b,c,d,e,g,h	2.41 ± 0.3 f,h	6.47 ± 0.8 a,b,c,d,e,f,g
F2-isoprostanes (pg/mg protein)	8.15 ± 1.0 d,f,h	10.9 ± 1.8 d,f,h	8.77 ± 1.1 d,f,h	13.5 ± 1.7 a,b,c,e,g,h	8.95 ± 1.5 d,f,h	16.3 ± 2.1 a,b,c,d,e,g,h	8.62 ± 1.2 d,f,h	22.3 ± 2.7 a,b,c,d,e,f,g
TBARS (nmol/mg protein)	0.07 ± 0.02 f,h	0.06 ± 0.03 f,h	0.05 ± 0.01 f,h	0.10 ± 0.04 a,b,c,e,f,gh	0.06 ± 0.01 d,f,h	0.15 ± 0.02 a,b,c,d,e,g,h	0.07 ± 0.03 d,f,h	0.23 ± 0.04 a,b,c,d,e,f,g
Protein carbonyls /total GSH ratio (arbitrary units)	0.33 ± 0.03 f,h	0.35 ± 0.04 f,h	0.36 ± 0.05 f,h	0.42 ± 0.06 f,h	0.37 ± 0.02 f,h	0.96 ± 0.03 a,b,c,d,e,g,h	0.40 ± 0.04 f,h	1.17 ± 0.09 a,b,c,d,e,f,g
F2-isoprostanes /total GSH ratio (arbitrary units)	1.50 ± 0.05^{b,d,f,h}	1.85 ± 0.1^{d,e,f,g,h}	1.54 ± 0.04^{b,d,f,h}	2.06 ± 0.2^{a,c,e,f,g,h}	1.42 ± 0.1^{b,d,f,h}	3.75 ± 0.2^{a,b,c,d,e,g,h}	1.44 ± 0.04^{b,d,f,h}	5.54 ± 0.5^{a,b,c,d,e,f,g}
TBARS /total GSH ratio (arbitrary units)	0.01 ± 0.005^{f,h}	0.01 ± 0.004 f,h	0.01 ± 0.005^{f,h}	0.02 ± 0.005 f,h	0.010 ± 0.002^{f,h}	0.034 ± 0.008 a,b,c,d,e,g,h	0.01 ± 0.004 f,h	0.06 ± 0.01 a,b,c,d,e,f,g

Values represent means ± SEM for 8 mice per experimental group. Significant differences between the groups are indicated by the superscript letter identifying each group (two-way analysis of variance and Bonferroni post-test; $P < 0.05$). Total GSH equivalents = GSH + 2GSSG.

significantly at weeks 12 (181.8%, $P < 0.0001$) and 16 (300.8%, $P < 0.0001$), correlating with NF-κB mRNA expression, with increases of 161.6% and 196.1% at weeks 12 and 16, respectively ($P < 0.0001$) (Fig. 4F, G). Consistent with these results, TNF-α and IL-6 liver mRNA expression increased at week 12 compared to previous weeks (2.4 and 1.8 times for TNF-α and IL-6, respectively, $P < 0.0001$), with a further increase of 1.6 times for each gene at week 16 compared to week 12 ($P < 0.0001$) (Fig. 5G, H). In opposition, PPAR-α levels (Fig. 4B) increased only at week 4 (161.2%, $P < 0.0001$), followed by a significant decline in DNA binding activity at week 12 (45%, $P < 0.01$) and the highest decrease at week 16 (91.9%, $P < 0.0001$). PPAR-α DNA binding activity modulation was related to mRNA expression, with an elevation at week 4 (146%, $P < 0.0001$), followed by significant decreases at weeks 12 (50.1%, $P < 0.0001$) and 16 (60%, $P < 0.0001$) (Fig. 4C, D). As PPAR-α induces CPT1, a similar pattern of activity was observed in this enzyme (Fig. 6G) in the HFD group, indicating an initial enhancement of 1.7 times ($P < 0.0001$) compared to CD and a subsequent decline from weeks 4 to 16 of 5.6 times ($P < 0.0001$). Similar patterns were observed for CPT1 mRNA and ACOX expression, both regulated by PPAR-α activity, with enhancements of 1.7 and 1.6 times, respectively, at week 4, followed by a decay at week 16 of 3.3 and 3.6 times, respectively, compared to week 4 in HFD animals ($P < 0.0001$) (Fig. 5C, D). Regarding Nrf2 DNA binding activity, HFD increased levels in the first 8 weeks (211.3%, $P < 0.0001$) but decreased at weeks 12 (44.9%, $P < 0.0001$) and 16 (60.5%, $P < 0.0001$). Nrf2 mRNA gene expression exhibited a similar pattern, with enhancements at weeks 4 (118.6%, $P < 0.05$) and 8 (157.4%, $P < 0.0001$), followed by declines at weeks 12 (39.3%, $P < 0.0001$) and 16 (72.2%, $P < 0.0001$) compared to CD (Fig. 4C). The activity of enzymes regulated by oxidative stress and Nrf2, including SOD, CAT, GPx, and GR, displayed a similar outline in HFD, with an increase from weeks 8 to 12 (1.26 times on average, $P < 0.0001$) and a total decay at week 16 (3.6 times on average from weeks 4 to 16, $P < 0.0001$) (Fig. 6A–D). Liver mRNA expression of GST and GCL, genes regulating oxidative stress, followed the same pattern as Nrf2 in HFD, with enhancements at week 8 (1.7 and 2.1 times for GST and GCL, respectively, $P < 0.0001$), and declines from weeks 8 to 16 (3.0 and 3.5 for GST and GCL, $P < 0.0001$) (Fig. 5E, F).

FAs' contents in liver and brain tissues

The FA profiles of liver and brain tissues were analyzed from normal and HFD animals using up-gas chromatography (Tables 3 and 4). In liver samples (Table 3), HFD administration increased C16:0 levels by 38% from week 4 to 12 ($P < 0.01$) and 25% compared to CD. This increment continued at week 16 (7% increase from week 12, $P < 0.01$). The highest increase (15%) was observed from week 4 to 8. Total SFA showed a significant increase from week 8, supporting the above findings. Total n-3 PUFA and DHA levels in liver tissue present a slightly decrease from week 4 to 12, but at week 12 to the end of the study, the decrease is statistically significantly. DHA levels decreased to an average of 11.3 mol % FAs every 4 weeks, resulting in a total decrease of 46 % at week 16 ($P < 0.01$). Total n-3 PUFA levels decreased by 49.4% from week 4 to 16 ($P < 0.01$). In brain tissue analysis (Table 4), total SFA increased by 22% and 25% from week 4 to 12 and 4 to 16, respectively ($P < 0.01$). PUFA levels in brain tissue showed significant changes, with DPAn-6 increasing by 61% from week 4 to 12, and 20% from 12 to 16 ($P < 0.01$) (or 94% from 4 to 16 week), whereas AA did not show statistical changes in animals on HFD. Conversely, DHA levels decreased slightly by 17.3% from week 4 to 8, and 37.3% from week 8 to 12 ($P < 0.01$), and 1.4% from week 12 to 16. The total decrease

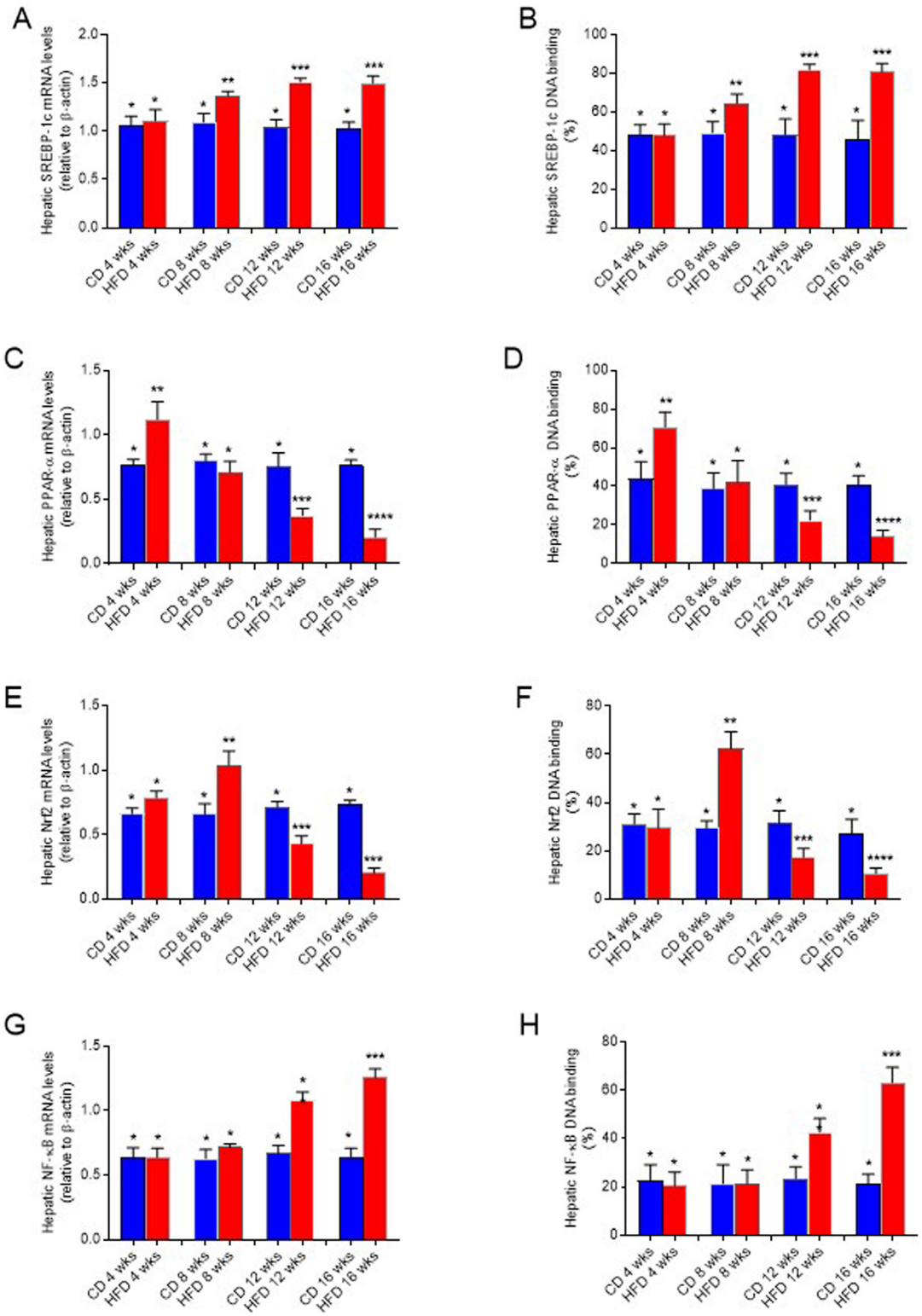


Fig. 4. Hepatic expression and DNA-binding activity of transcription factors involved in lipogénesis, oxidative stress and inflammation: SREBP-1c mRNA expression (A); SREBP-1c DNA-binding activity (B); PPAR-α mRNA expression (C); PPAR-α DNA-binding activity and (D); Nrf2 mRNA expression (E); Nrf2 DNA-binding activity (F); NF-κβ mRNA expression (G); NF-κβ DNA-binding activity (H) in mice subjected to control diet (CD) or high fat diet (HFD) from 4 to 16 weeks. Data are expressed as means ± SEM ($n = 8$), with significant differences being indicated by different asterisk (*) numbers (two-way ANOVA and the Bonferroni's post-test; $P < 0.05$).

in DHA levels in brain tissue from HFD-fed animals was 49% from week 4 to 16 ($P < 0.01$). As expected, total n-3 PUFA also decreased at week 12 and 16 ($P < 0.01$), emphasizing the loss of n-3 PUFAs induced by HFD over the weeks of survey.

Discussion

FAs are essential molecules, not only as fuel for different cell types but also as signaling molecules that modulate metabolism.

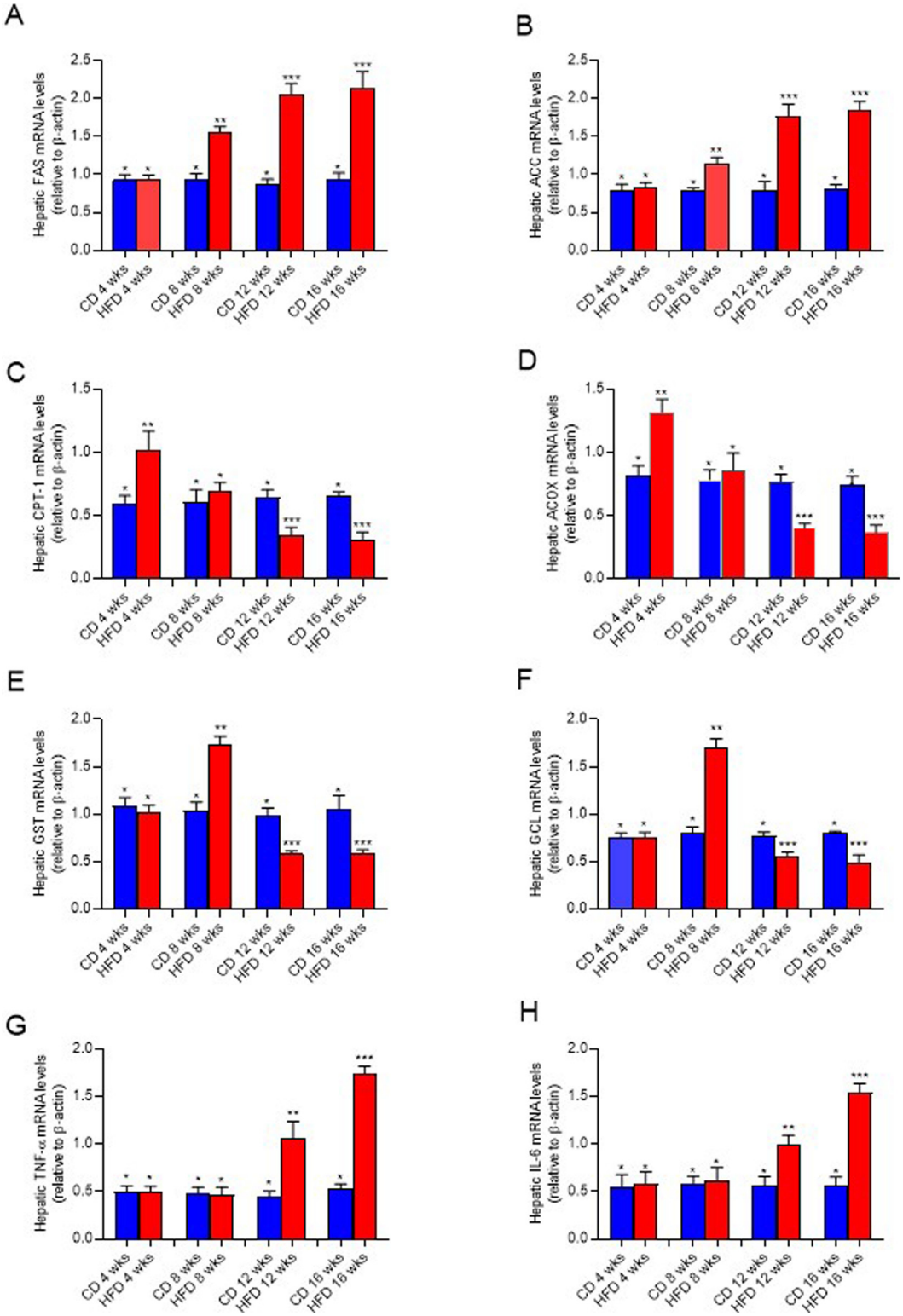


Fig. 5. Hepatic expression of enzymes involved in lipogenesis, β-oxidation and antioxidant defenses and pro-inflammatory cytokines: FAS mRNA expression (A); ACC mRNA expression (B); CPT-I mRNA expression (C); ACOX mRNA expression (D); GST mRNA expression (E); GLC mRNA expression (F); TNF-α mRNA expression (G); IL-6 mRNA expression (H) in mice subjected to control diet (CD) or high fat diet (HFD) from 4 to 16 weeks. Data are expressed as means ± SEM ($n = 8$), with significant differences being indicated by different asterisk (*) numbers (two-way ANOVA and the Bonferroni's post-test; $P < 0.05$).

Different types of FAs that can be obtained from diet and nutritional modifications, may then affect metabolic signaling in the organism. Obesity is one of the most prevalent illnesses worldwide, and due to the actual society behavior, where in 2022, 1 in 8 people in the world were living with obesity [40]. Obesity is caused by excessive fat accumulation deriving in pathophysiological conditions in different organs, being a risk factor for numerous diseases. These include type 2 diabetes mellitus, MAFLD, MASH,

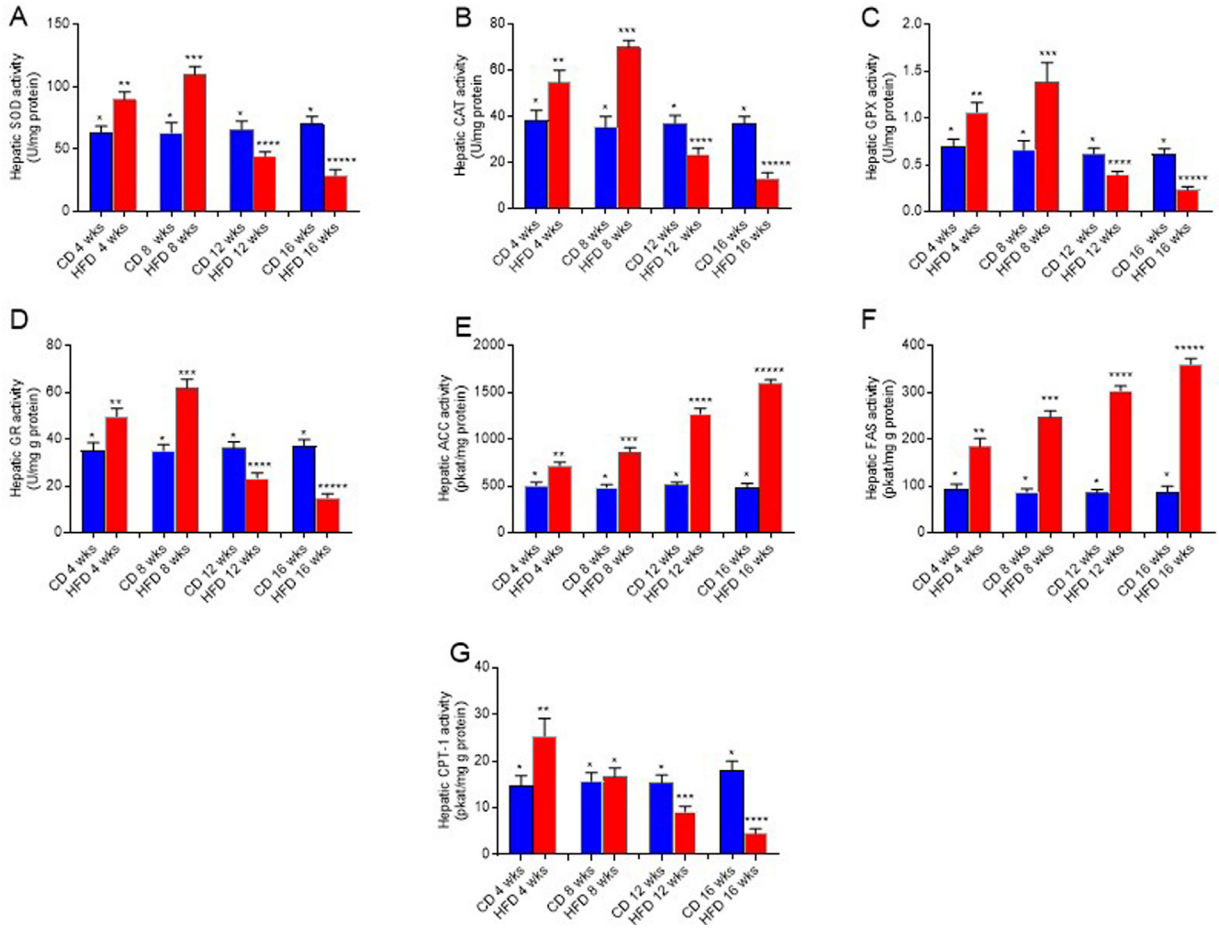


Fig. 6. Hepatic activities of enzymes involved in antioxidant defenses, lipogenesis, and β-oxidation: SOD specific activity (A); CAT specific activity (B); GPX specific activity (C); GR specific activity (D); ACC activity (E); FAS specific activity(F); CPT-I specific activity (G) in mice subjected to control diet (CD) or high fat diet (HFD) from 4 to 16 weeks. Data are expressed as means ± SEM (n = 8), with significant differences being indicated by different asterisk (*) numbers (two-way ANOVA and the Bonferroni's post-test; $P < 0.05$).

cardiovascular disease such as hypertension and stroke, and some kinds of cancer [41,42].

The accumulation of lipids and FAs leads to an imbalance in redox metabolism, a resulting increase in ROS production and an augmented immune response derived in chronic inflammation in different organs. Oxidative stress could be the consequence but also a trigger for obesity. For example, the intake of saturated FAs and *trans*-FAs increase ROS production, and ROS contribute to stimulate adipose tissue deposition, proliferation, growth, and adipocyte differentiation [43]. Moreover, different FAs can generate distinctive responses, for instance saturated and unsaturated FAs have almost contrasting effects over cellular responses, whereas PUFAs, mainly found in oily fish and fish oil supplements, promote beneficial effects [44–46]. The PUFAs ω-3 and ω-6 constitute a family of nutrients of great importance for the human being, which are directly involved in the generation of the inflammatory response and its termination. Metabolically speaking, ω-3 generally participates in metabolic pathways that have as final product anti-inflammatory agents, whereas the ω-6 usually participates in the synthesis of pro-inflammatory mediators [46]. The ω-6 precursor LA is transformed into arachidonic acid (ARA), which in turn occasionally originates a family of pro-inflammatory intermediates, such as prostaglandin E-2 (PGE2), PGD2, leukotrienes (LTs) like LTB4, and lipoxines (LXA4, LXB4), which finally exert the net pro-inflammatory effect of ω-6 [47]. These antecedents point to the importance of the balance between ω-3 and ω-6 PUFAs in the management of inflammation, which mainly relies on enzymes, where PUFA desaturation is mediated by Δ-6D and Δ-5D [19,22,47]. Data shows that after a HFD regimen the activity of Δ-6D and Δ-5D increases at first, in correlation with the maintenance of the mRNA and protein expression of both enzymes and with the greater incorporation of fat in the diet. Then, after twelve weeks of HFD, a decrease in their activity is observed, accompanied by an enhancement in their mRNA and protein levels. This implies that a probable adaptive compensatory mechanism of the liver is not capable of maintaining itself for long periods of time. Accordingly, Hucik et al. reported in a HFD diet model that the reduction of Δ-6D through Fads2+/- (a heterozygous model of Δ-6D deficiency) generates at 3 weeks a protection in whole-body glucose tolerance but a reduction of GLUT4 in skeletal muscle, where no changes in n-6/n-3 ratio were observed [48]. Besides, in a mouse model assay Valenzuela et al. showed that extended periods of HFD administration (12 weeks) generate insulin resistance (with increased HOMA) and, as expected, a MAFLD with SREBP1c DNA binding increase, all of these associated with a strong reduction of Δ-5D and Δ-6D, and a correlation with an impairment in the antioxidant capacity [49]. Complementarily, results reported show that the activity of desaturases at the brain level does not decrease until week 16. This could indicate that these enzymes in the brain have a different capacity than those found in the liver, and their activities are only affected when the damage of the HFD is declared and the liver is no longer capable of desaturating all the input of

Table 3
Most relevant fatty acids present in liver samples obtained from control diet (CD) fed mice and animals subjected to high fat diet (HFD) after different feeding periods (wks, weeks)

Groups Fatty acid	CD 4 wks (a)	HFD 4 wks (b)	CD 8 wks (c)	HFD 8 wks (d)	CD 12 wks (e)	HFD 12 wks (f)	CD 16 wks (g)	HFD 16 wks (h)
C16:0	35.9 ± 4.4	36.8 ± 3.9	33.9 ± 4.2	42.5 ± 4.8	37.4 ± 4.6	46.9 ± 5.1 a,b,c,g	33.6 ± 5.1	50.2 ± 6.3 a,b,c,g
C18:0	4.7 ± 0.5	4.2 ± 0.6	3.7 ± 0.4	3.8 ± 0.4	3.4 ± 0.7	4.5 ± 0.6	4.1 ± 0.5	4.3 ± 0.8
C18:1n-9	25.2 ± 3.8	23.8 ± 3.5	23.9 ± 2.5	21.4 ± 4.1	22.3 ± 3.7	19.9 ± 2.7	23.8 ± 4.6	20.4 ± 2.6
C18:2n-6 (LA)	10.5 ± 2.4	13.4 ± 2.6	11.7 ± 1.1	11.3 ±2.8	12.8 ± 1.9	10.6 ± 1.5	12.5 ± 2.7	10.1 ± 1.8
C18:3n-3 (ALA)	1.35 ± 0.2 f,h	1.17 ± 0.3 h	1.44 ± 0.5 f,h	0.95 ± 0.2	1.29 ± 0.2 h	0.79 ± 0.3 a,c	1.02 ± 0.4	0.71 ± 0.2 a,b,c,e
C20:4n-6 (AA)	11.6 ± 1.4	10.6 ± 1.2	12.1 ± 1.7	9.92 ± 1.1	12.7 ± 2.0	9.73 ± 0.9	13.5 ± 2.3	8.96 ± 1.4
C20:5n-3 (EPA)	2.37 ± 0.3 f,h	2.12 ± 0.4 f,h	2.31 ± 0.4 f,h	1.89 ± 0.3 f,h	2.25 ± 0.5 f,h	1.05 ± 0.2 a,b,c,d,e,g	2.22 ± 0.4 f,h	0.86 ± 0.4 a,b,c,d,e,f,g
C22:5n-6 (DPAn-6)	0.06 ± 0.03	0.04 ± 0.02	0.08 ± 0.04	0.05 ± 0.03	0.08 ± 0.02	0.04 ± 0.01	0.7 ± 0.04	0.03 ± 0.01
C22:5n-3 (DPAn-3)	0.18 ± 0.05 h	0.20 ± 0.04 h	0.015 ± 0.05	0.23 ± 0.05 h	0.17 ± 0.04 h	0.15 ± 0.03	0.18 ± 0.02 h	0.10 ± 0.02 a,b,d,e,g
C22:6n-3 (DHA)	4.57 ± 0.6 f,h	4.78 ± 0.5 f,h	4.49 ± 0.6 f,h	4.19 ± 0.6 f,h	4.36 ± 0.7 f,h	2.94 ± 0.3 a,b,c,d,e,g,h	4.31 ± 0.8 f,h	2.56 ± 0.4 a,b,c,d,e,g
Total SFA	41.1 ± 4.7 f,h	41.5 ± 4.4 f,h	38.0 ± 4.3 f.h	46.9 ± 5.1 a,b,c,e,g	41.3 ± 4.7 f,h	51.9 ± 5.2 a,b,c,d,e,g	41.0 ± 4.5 f,h	54.9 ± 5.7 a,b,c,d,e,g
Total MUFA	26.3 ± 2.9	24.6 ± 2.6	24.4 ± 2.7	22.3 ± 2.3	24.6 ± 2.8	20.3 ± 2.1	24.4 ± 3.2	20.8 ± 1.9
Total PUFA	32.6 ± 3.5 h	33.9 ± 3.9 h	37.6 ± 3.5 h	30.8 ± 3.4	34.1 ± 3.6 h	27.8 ± 3.4	34.6 ± 2.7 g	24.3 ± 2.8 a,b,c,e,g
Total n-6 PUFA	24.0 ± 2.9	25.2 ± 2.8	28.9 ± 3.6	23.1 ± 2.4	25.9 ± 2.5	22.6 ± 2.7	26.5 ± 2.8	19.5 ± 2.3
Total n-3 PUFA	8.52 ± 1.1 f,h	8.62 ± 1.3 f,h	8.65 ± 1.4 f,h	7.61 ± 1.1 f,h	8.20 ± 0.8 f,h	5.14 ± 0.6 a,b,c,d,e	8.74 ± 0.9 f,h	4.35± 0.5 a,b,c,d,e,g

Values are expressed as mol % fatty acid and represent the mean ± SEM for 8 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni post-test; $P < 0.05$). SFAs correspond to C14:0, C16:0, C18:0, and C20:0. MUFAs correspond to C14:1n-7, C16:1n-7, and C18:1n-9. PUFAs correspond to C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3, C22:5n-6, C22:5n-3, and C22:6n-6.

FAs, since at this point the levels of hepatic desaturases are maximally decreased. The supply of DHA to the brain is thought to be achieved from the blood, either from (1) the diet via the blood-brain barrier; and (2) by synthesis in the liver from dietary ALA; or by intrabrain (3) release from membrane phospholipids via phospholipases-A_2; and (4) by synthesis in the astrocytes from dietary ALA, which represents the lowest efficient process [50]. The importance of the levels of ω-3 PUFAs in brain is substantiated by the observation that the human orbitofrontal cortex (OFC), that plays a critical role in both cognitive and affective regulation, exhibits neuronal shrinkage and reductions in gray matter volume during aging and in major depressive disorders, which were associated with decreases in DHA content [51]. Furthermore, increasing age was related to transient augmentations in Δ-5D and Δ-6D and

Table 4
Most relevant fatty acids present in brain samples obtained from control diet (CD) fed mice and animals subjected to high fat diet (HFD) after different feeding periods (wks, weeks)

Groups Fatty acid	CD 4 wks (a)	HFD 4 wks (b)	CD 8 wks (c)	HFD 8 wks (d)	CD 12 wks (e)	HFD 12 wks (f)	CD 16 wks (g)	HFD 16 wks (h)
C16:0	34.8 ± 4.2	38.1 ± 4.4	34.1 ± 3.7	40.1 ± 4.4	36.7 ± 3.9	45.9 ± 5.2	37.9 ± 4.2	46.8 ± 5.5
C18:0	3.92 ± 0.5	3.77 ± 0.6	3.14 ± 0.4	3.62 ± 0.7	3.81 ± 0.7	4.20 ± 0.9	3.26 ± 0.5	4.31 ± 0.7
C18:1n-9	23.5 ± 2.9	20.6 ± 2.1	22.5 ± 2.5	21.5 ± 1.9	21.8 ± 2.0	19.8 ± 1.8	22.5 ± 2.7	19.4 ± 1.4
C18:2n-6 (LA)	3.15 ± 0.5	3.66 ± 0.4	3.45 ± 0.7	3.06 ± 0.6	3.54 ± 0.6	2.91 ± 0.5	3.11 ± 0.8	2.55 ± 0.7
C18:3n-3 (ALA)	1.37 ± 0.3 h	1.21 ± 0.2	1.24 ± 0.8	1.01 ± 0.5	1.21 ± 0.3	0.85 ± 0.3	0.99 ± 0.4	0.80 ± 0.2 a
C20:4n-6 (AA)	14.7 ± 2.5	14.1 ± 2.7	15.9 ± 2.7	13.5 ± 2.4	16.3 ± 2.2	13.3 ± 1.5	15.8 ± 2.8	13.5 ± 1.9
C20:5n-3 (EPA)	0.84 ± 0.2 f,h	0.81 ± 0.3	0. 89 ± 0.3 f,h	0.51 ± 0.1	0.81 ± 0.3 f,h	0.42 ± 0.1 a,c,e,g	0.87 ± 0.4 f,h	0.36 ± 0.1 a,c,e,g
C22:5n-6 (DPAn-6)	0.95 ± 0.2 f,h	0.97 ± 0.2 f,h	0.93 ± 0.3 f,h	1.14 ± 0.4	0.99 ± 0.2 h	1.57 ± 0.3 a,b,c	1.05 ± 0.3 h	1.89 ± 0.5 a,b,c,e,g
C22:5n-3 (DPAn-3)	0.43 ± 0.1 f,h	0.48 ± 0.2 f,h	0. 51 ± 0.3 f,h	0.38 ± 0.1	0.42 ± 0.1 f,h	0.25 ± 0.1 a,b,c,d,e,g	0.52 ± 0.2 f,h	0.21 ± 0.1 a,b,c,d,e,g
C22:6n-3 (DHA)	12.6 ± 2.5 f,h	13.9 ± 1.9 f,h	13.4 ± 1.5 f,h	11.5 ± 2.2 f,h	11.7 ± 1.8 f,h	7.21 ± 0.6 a,b,c,d,e,g	12.3 ± 0.7 f,h	7.11 ± 0.9 a,b,c,d,e,g
Total SFA	40.2 ± 3.9 f,h	42.2 ± 4.3 f,h	39.9 ± 4.2 f,h	44.5 ± 4.9	41.2 ± 4.4 f,h	51.5 ± 5.3 a,b,c,e,g	41.6 ± 4.4 f,h	52.8 ± 5.3 a,b,c,e,g
Total MUFA	24.3 ± 2.7	22.3 ± 2.1	23.7 ± 2.9	21.9 ± 2.3	22.2 ± 2.5	20.2 ± 2.8	22.8 ± 2.9	20.0 ± 1.7
Total PUFA	35.5 ± 3.3 f,h	35.5 ± 3.9 f,h	36.4 ± 4.1 f,h	33.6 ± 3.7 h	36.6 ± 3.7 f,h	28.3 ± 2.4 a,b,c,e,g	35.6 ± 3.8 f,h	27.2 ± 2.2 a,b,c,e,g
Total n-6 PUFA	20.1 ± 2.7	19.1 ± 1.9	20.3 ± 2.2	19.5 ± 2.3	21.4 ± 2.5	19.3 ± 1.5	20.8 ± 2.0	18.5 ± 1.7
Total n-3 PUFA	15.4 ± 1.4 f,h	16.9 ± 1.2 f,h	16.1 ± 1.7 f,h	14.1 ± 1.8 f,h	15.2 ± 1.6 f,h	8.96 ± 0.9 a,b,c,d,e,g	14.8 ± 1.1 f,h	8.70 ± 0.9 a,b,c,d,e,g

Values are expressed as mol % fatty acid and represent the mean ± SEM for 8 mice per experimental group. Significant differences between the groups are indicated by the letter identifying each group (two-way ANOVA and Bonferroni post-test; $P < 0.05$). Identification of saturated and unsaturated fatty acids and their relationships are shown in Table 3.

elongase mRNA expression during declining DHA levels, suggesting a compensatory up-regulating mechanism secondary to endogenous dietary and biosynthetic deficiencies [52]. In agreement with these views, an enhancement in mRNA expression and protein content of Δ-5D and Δ-6D in brain (Fig. 3) and liver (Fig. 2) was observed in HFD-fed mice, coinciding with DHA and EPA decline (Tables 3 and 4), parameters that were normalized in the liver by the supplementation with the antioxidant hydroxytyrosol [49]. Considering that (1) the content of Δ-6D was 6.1-fold higher in liver than in brain, as well as of the other enzymes involved in PUFA biosynthesis [20] and (2) that liver but not brain synthesis is upregulated when dietary n-3 PUFA content is diminished [53], reaffirm that hepatic n-3 PUFA depletion by HFD at 16 weeks feeding is a main contributor to the reduction observed in brain (Tables 3 and 4).

In this regard, Reynols et al. showed that the FA desaturase gene cluster (*FADS)* impacts the transcription of Fads1 (Δ-5D) and Fads2 (Δ-6D) in numerous tissues, establishing that tissues have their own endogenous capacity to synthesize PUFAs [54]. These authors found that at liver and brain level, the minor allele of rs174548 (G) was associated with lower expression of *Fads1 (*Δ-5D gene command), but was not associated with *Fads2,* what would explain the differential Δ-5D/Δ-6D pattern between these organs [54]. According to our knowledge, our work would be the first study that demonstrates changes in desaturases′ functions in the brain-liver axis in HFD over long periods of time. Of interest, humans with genetic variants of Fads2 gene show a reduction in Δ-6D activity with the consequent reduction of EPA and DHA levels [55]. As previously reported, there is a relation among Δ-5D and Δ-6D activity, FA composition, insulin resistance and metabolic syndrome, e.g., low estimated Δ-5D activity significantly predicted the development of metabolic syndrome [56,57]. Also, obese patients with NASH present an increase in Δ-6D/Fads2 mRNA expression and a decay in Δ-5D activity compared to normal liver function, where the Δ-5D and Δ-6D activities reflect liver metabolic functions [58]. Together with the aforementioned, Δ-6D deficiency (Fads2 knock-out) alters triacylglycerol/FA cycling in mouse white adipose tissue by promoting lipolysis and reducing FA re-esterification, due to a disruption in EPA and DHA production, indicating an early impairment in insulin sensitivity with Δ-6D deficiency [59]. Specifically, the drop in the function of the desaturases studied could be an important factor in the development of insulin resistance, but also in the deterioration of liver function; this hypothesis is coherent with our results in HOMA index and transaminases′ measurements, that are significantly altered after 16 weeks of HFD. These results suggest that obesity may be mimicking what can be seen during aging in the brain, where Δ-6D is significantly decreased [60], indicating an impairment in the organism with decrease chance of a metabolic adaptation.

In terms of lipid metabolism, a progressive increase in SREBP-1c indicates the activation of the lipogenic system, since the first 4 weeks of HFD this metabolic pathway is in constant activity and increases significantly until week 16, turning into a constant synthesis of lipid metabolites and contributing to metabolic stress. In parallel to this continuous activation, at first the liver begins to enhance its energy systems through the activation, for example, of biogenesis, with an increase in PPAR-α, which fails to compensate the continuity of the diet and begins to be affected until the end of treatment where it is significantly reduced. FAs also have differential effects on the activity of specific enzymes. Thus, α-linolenic acid, compared to linoleic and saturated FAs, has the greatest inducing effect on CPT I (limiting enzyme for the entry of FAs into the mitochondria), and the mitochondrial FA oxidation rate [61]. Therefore, the FA content and composition of the diet can regulate the activity and metabolic control of different enzymes. One of the main mechanisms participating in the development of MAFLD is the impairment of redox capacity in hepatocytes [9,24,43,62]. Our results show that before the injury in the liver is tangible (increase in AST and ALT), the first weeks of HFD (between 4 and 8 weeks) trigger an adaptative mechanism, which is also reflected in the antioxidant capacity through an increase in Nrf2 at the eighth week, but again the maintenance of the HFD, that ultimately causes the injury, leads to a diminution in this antioxidant defense mechanism and an enhancement in inflammatory processes, which is reflected in the transaminase's levels and lipid profile.

The influence of various dietary FAs on health has implications for lipid metabolism. The liver and adipose tissue are essential in sustaining energy and lipid equilibrium. An examination on gene expression linked to lipid metabolism and its relationship with FA composition revealed notable changes in crucial genes responsible for lipid balance. Particularly, SREBP-1c, PPARα, and FAS displayed noteworthy connections with different FA profiles [63,64]. Also, it is well documented that excessive caloric intake, not only causes metabolic dysfunction, but also triggers chronic low-grade inflammation in peripheral organs including adipose tissue, liver, skeletal muscle, and also central nervous system, where it is referred as neuroinflammation [65–67]. Sanchez et al. [65], described that dietary n-6/n-3 ratio influence microglial activation in hypothalamus and that high dietary ω-6/ω-3 ratio leads to a proinflammatory profile without microglial activation in hippocampus. The author's data strongly support a role for PUFAs on peripheral inflammation (systemic) and neuroinflammation. Added to this, Xu et al. [68], showed that the administration of DHA to a HFD aged-Wistar rats enhanced hippocampal insulin sensitivity with a reduction in systemic glucose homeostasis dysregulation, neuroinflammation, and oxidative stress. Reinforcing the above, Gonzalo–Gobernado et al. [69] demonstrated that DHA/EPA-supplementation reduces the damage and protect the brain when a stroke occurs in C57BL/6J model of cerebral ischemia, by reducing the infarct area. Moreover, daily administration of hydroxytyrosol, a polyphenol with powerful antioxidant activity in mice fed a HFD, prevented the decrease in the activity of hepatic Δ-5D and Δ-6D and the depletion of ω-3 LCPUFA. These results are associated with a reduction in oxidative stress and down-regulation of the lipogenic factor SREBP-1c, allowing the maintenance of ω-3 LCPUFA levels in extrahepatic tissues, including the brain [49]. Therefore, different supplementation strategies with FAs and bioactive compounds have proven to be an excellent alternative to preserve the hepatic and brain PUFA content, as well as the redox and inflammatory status at levels compatible with an optimal state of health.

Conclusions

Apart from being a resourceful metabolic center able to impact behavior, the liver interacts closely with the central nervous system through the autonomic nervous system, including the vagus nerve that modulates metabolic functions in the liver, therefore, establishing the dual interconnection defined as the brain-liver axis [70]. MAFLD is associated with several extrahepatic manifestations such as cognitive impairment, which is situated between normal aging and the inception of dementia, underlying a variety of factors such as insulin resistance, oxidative stress-induced lipotoxicity and inflammation [71]. Data presented indicate that the brain-liver axis is drastically affected by HFD consumption, in a time dependent fashion. This is characterized by (1) an early (4 weeks) increase in liver desaturation and PPAR-α activities, with no changes in oxidative stress, liver damage or fat

accumulation; (2) a latter progressive loss in hepatic desaturation activity with insufficient compensatory increases in mRNA and protein expression leading to ω-3 PUFA depletion, PPAR-α down-regulation reducing FA oxidation, and liver steatosis with enhancement in hepatic lipogenesis; and (3) brain ω-3 PUFA depletion also occurring after 12 to 16 weeks of HFD feeding similarly to liver, thus corroborating the metabolic interdependency between both organs. It is important to point that future research should include the gut, as it produces several endotoxins and signaling molecules (e.g., short-chain FAs) that act on the brain and liver conforming the brain-liver-gut axis, whose derangement can directly establish MAFLD [72].

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.

CRediT authorship contribution statement

Jessica Zúñiga-Hernández: Methodology, Formal analysis, Conceptualization. **Camila Farias:** Methodology, Investigation. **Alejandra Espinosa:** Methodology, Formal analysis. **Lorena Mercado:** Software, Methodology, Investigation. **Alexies Dagnino-Subiabre:** Writing – original draft, Visualization. **Andrea del Campo:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Paola Illesca:** Writing – original draft, Conceptualization. **Luis A. Videla:** Writing – review & editing, Writing – original draft, Conceptualization. **Rodrigo Valenzuela:** Conceptualization, Funding acquisition, Methodology, Formal analysis, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

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