Cellular and Molecular Neurobiology (2019) 39:963–974
https://doi.org/10.1007/s10571-019-00692-6

ORIGINAL RESEARCH

High-Fat Diets and LXRs Expression in Rat Liver and Hypothalamus

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Received: 26 February 2019 / Accepted: 25 May 2019 / Published online: 3 June 2019

Abstract

Disturbances on lipid metabolism are associated with health disorders. High-fat diets (HFDs) consumption promotes cardiovascular and neurodegenerative diseases where cholesterol plays an important role. Among regulators of this steroid homeostasis, the liver X receptors (LXRs) induce genes that protect cells from cholesterol overload. We previously described how both hypothalamic LXRα and LXRβ are sensitive to a high-fructose diet, suggesting that these receptors trigger responses related to control of energy and food intake. The present work's main objective was to study the effect of different HFDs on LXRs expression (in hypothalamus and liver), and lipid profile. Male rats received control diet (CD), HFD_1 (CD + bovine fat (BF)), HFD_2 (CD + BF + cholic acid (CA)), HFD_3 (CD + BF + cholesterol), or HFD_4 (CD + BF + CA + cholesterol) for different time periods. Hypothalamic LXRβ, both hepatic LXRs subtypes, and total cholesterol (TC) raised after 2 weeks of HFDs. Four and 8 weeks of HFD_3 and HFD_4 increased the LXRs subtypes in both tissues and TC levels. Only HFD_4 reduced triglycerides (TG) levels after 2 and 8 weeks. The TC and TG values correlated significantly with LXRs expression only in rats fed with HFD_4. These data add relevant information about how diet composition can produce different scales of hypercholesterolemia states accompanied with central and peripheral changes in the LXRs expression.

Keywords High-fat diets · Oxysterols · Cholic acid · Cholesterol · Triglycerides

Introduction

Cholesterol homeostasis disturbances are associated with serious health disorders. In fact, it is well known that regular high-fat diets (HFDs) consumption constitutes a risk factor for metabolic, cardiovascular, and neurodegenerative diseases (Cullen 2000; Ghibaudi et al. 2002; Riccioni and Sblendorio 2012; Shanmugasundaram et al. 1986; Sparks et al. 1993). One of the major sites of endogenous cholesterol synthesis is the liver. After a HFDs intake, this organ activates mechanisms to handle the elevated loads of cholesterol, suppressing novo cholesterol synthesis and promoting cholesterol elimination (Carey and Mazer 1984; Russell 2003).

The liver X receptors (LXRs) are key factors in cholesterol metabolism and their transcriptional function is activated by oxysterols (oxidized cholesterol derivatives; Baranowski 2008; Peet et al. 1998; Zelcer and Tontonoz 2006). The LXRs maintain normal cholesterol balance promoting sterol efflux from peripheral cells and hepatic sterol catabolism and excretion, increasing circulating high density lipoprotein (HDL-c), and inhibiting further sterol absorption. Also, they participate in the metabolism of bile acids (BAs), fatty acids, triglycerides (TG), glucose, processes related to steroidogenesis, the immune system, and inflammation (Cao et al. 2004; Cha and Repa 2007; Laurencikiene and Rydén 2012; Zelcer and Tontonoz 2006). There are two LXRs subtypes, LXRα (NR1H3) and LXRβ (NR1H2) that share ~78% identity of their amino acid sequences in both

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DNA and ligand-binding domains (Ulven et al. 2005). LXRα predominates in liver, small intestine, kidney, macrophages, and adipose tissue, whereas LXRβ is ubiquitously expressed. Although both subtypes are equally able to induce hepatic lipogenesis, studies in LXRs knockout mice suggest that LXRα is the dominant subtype in this pathway (Repa et al. 2000; Repa and Mangelsdorf 2000; Steffensen et al. 2003).

In the central nervous system (CNS), the cholesterol pool is separated from the peripheral one (Koch et al. 2001). The brain produces and regulates its own cholesterol requirements related to vital functions as signal transmissions. However, chronic HFDs consumption produces detrimental changes in the brain (Franciosi et al. 2009; Hwang et al. 2008; Morrison et al. 2010), there also is an association between cholesterol metabolism dysregulation and neurological, neurodegenerative and neurodevelopmental occurrence (Blain and Poirier 2004; Treiber-Held et al. 2003).

Both LXRs are present in the brain, but LXRβ is the main subtype (Whitney et al. 2002). To date, it is not known if they have different functions within the CNS. However, LXRβ alterations are related to several neurodegenerative diseases (Dai et al. 2012; Fan et al. 2008; Kim et al. 2008). Studies from our group suggest that hypothalamic LXRs play a critical role in lipid and glucose homeostasis. Six weeks of high-fructose beverage changes the LXRs expression in rat hypothalamus but not in neocortex, hippocampus, or cerebellum. In addition, the TG levels negatively correlate with both receptor subtypes (Kruse et al. 2012). Moreover, in hypothalamic explants from untreated rats, the LXRs expression is sensitive to cholesterol or glucose addition (Kruse et al. 2017). It is important to note that the hypothalamus receives metabolic signals from periphery through complex circuits of nutrient-sensing cells (Karnani and Burdakov 2011; Lam et al. 2005) and regulates the body energy homeostasis and body weight (BW; Bantubungi et al. 2012; De Souza et al. 2005; Gao and Horvath 2008; Morton et al. 2006). Several reports describe that sustained HFDs consumptions cause a localized inflammatory state on hypothalamus that contributes to the obesity development (Cai 2012; Dalvi et al. 2017; De Souza et al. 2005; Horvath et al. 2010; Thaler et al. 2012; Valdearcos et al. 2017).

The prevalence of health problems related to hypercholesterolemia is progressively increasing; thus, the understanding of how hypothalamic control of homeostasis and body energy metabolism are affected by changes in the peripheral system remains to be answered. Experimental models based on animals fed with HFDs are extensively used for these purposes. These diets include variations in fat type, content, and supplements like cholesterol and/or cholic acid (CA) that produce different scales of hypercholesterolemia (Charlton et al. 2011; Matsuzawa et al. 2007; Van Herck et al. 2017). Also, several works describe the capacity of HFDs to induce changes in LXRs expression but no one has explored such effects on the CNS (Côté et al. 2013; Mohammadi and Oshaghi 2014; Thornton et al. 2008; Zhu et al. 2017).

Taking into account the association between HFDs consumption and detrimental changes in the brain, the LXRs role on lipid metabolism, and our previous findings, the aim of the present work was to evaluate the effects of different HFDs consumption on LXRs expression (in hypothalamus and liver) and lipid metabolism parameters (total cholesterol (TC) and TG). The liver organ was selected for functional comparisons, as a metabolic reference tissue. The HFDs compositions were based on nutritional models described in the bibliography (Van Herck et al. 2017), including the addition of bovine fat (BF), CA, and cholesterol to normal chow diet (CD). The treatments were administered by different time lapses (2, 4, or 8 weeks), chosen according to the available literature. We hypothesized that the selected HFDs composition would produce changes in central and peripheral LXRs expression associated with different scales of hypercholesterolemia. In this manner, the obtained results will add more information on how the diet components influence lipid metabolism and LXRs expression.

Materials and Methods

Experimental Animals

Two-month-old adult male Sprague–Dawley rats (300–400 g; $n = 60–65$) were housed under standard laboratory conditions in a temperature- and humidity-controlled vivarium with a 12-h light–dark cycle, and ad libitum access to food and water. All procedures concerning animal care and use were carried out according to the European Community Council Directive (86/609/EEC), the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Instituto de Biologia y Medicina Experimental's ethical committee (CE050/2015) in the City of Buenos Aires, Argentine.

Experimental Procedure and Diets

After weaning and up to 2 months of age, animals were fed with a normal chow diet (CD; protein (18.2%), carbohydrates (56.9%), lipids (3.9%), vitamins and minerals (3%), fiber (5%), and humidity (13%); Gepsa feeds, Grupo Pilar SA, Argentine). Then, rats were maintained with CD (3.3 kcal/g) or with different HFDs (4.8 kcal/g). Experimental HFDs were prepared adding BF (38%; HFD_1), BF (38%) + CA (0.2%; HFD_2), BF (38%) + cholesterol (2%; HFD_3), or BF (38%) + CA (0.2%) + cholesterol (2%; HFD_4) to CD. Diet composition was based on nutritional models previously described (Van Herck et al. 2017). HFDs without CA are more closely related to human diets, but rats

are resistant to increase TC by fat consumption (Stark et al. 2000); therefore CA was added to promote cholesterol absorption (Matsuzawa et al. 2007; Nishina et al. 1990).

Animals were separated into 5 groups according to diets (CD, HFD_1, HFD_2, HFD_3, and HFD_4), each group comprised of 5 rats ($n=25$). At 2 months of age, HFDs feeding were administered by 2 weeks for an initial evaluation. Although in the experimental practice the most used HFDs resemble HFD_3 and HFD_4, we decided to evaluate HFD_1 and HFD_2 with the purpose to study their action on LXRs expression in hypothalamus and liver. Moreover, given the extensive bibliographic data that describe the hypercholesterolemic effects of HFDs containing 2% of cholesterol, these were also administered by 4 and 8 weeks ($n=5$ rats/group; Van Herck et al. 2017). In each case, the results were compared to CD group.

The fasted animals' body weight (BW) was registered weekly between 09:00 and 10:00 a.m. The BW gain was calculated as final BW (at 2, 4, or 8 weeks) minus the BW before the initiation of the treatments. The food consumption was quantified daily only in rats that received HFD_3 and/or HFD_4 by a 7-day period between the second and fourth week. The food intake (grams/calories) was relativized to animal BW. On the day of sacrifice, the rats were fasted by 8 h prior to taking blood samples from the tail vein before they were rendered unconscious by CO_2 and killed by decapitation. Liver and hypothalamus were dissected, frozen, and stored at − 80 °C (Coirini et al. 1983).

Lipid Profile

Blood samples were centrifuged at 3500×*g* for 15 min to obtain serum. TC and TG levels were measured by spectrophotometry using commercially available kits (Wiener Labs S.A.I.C., Argentine), and according to the manufacturers' instructions, with equal volumes of serum from each animal. Results were analyzed together according to each group.

Protein Expression Determination: Western and Immunoblot

Frozen tissue fragments of liver or hypothalamus were processed as previously described (Rey et al. 2013). The total homogenate protein concentration was determined by Bradford's method (Bradford 1976), using BSA (Sigma-Aldrich, USA) as standard. Aliquots from each tissue sample containing equal amounts of protein were suspended in SDS-containing sample buffer and heated at 100 °C for 2 min. The proteins (20 µg) were loaded onto a 10% SDS-PAGE gel in Tris–Glycine-SDS electrophoresis buffer and separated at 120 V for 90 min. Proteins were transferred from gels onto PVDF membranes (Bio-Rad, USA) in Tris-MetOH-Glycine transfer buffer at 30 V overnight. Membranes were blocked with TBS-T 0.1% (20 mmol/l Tris, 150 mmol/l NaCl, and 0.1% Tween-20, pH 7.6) containing 5% of fat-free milk at room temperature for 1 h. Blocked membranes were incubated with the primary antibody in TBS-T 0.1% containing 1% fat-free milk at 4 °C overnight. The primary antibodies used were LXRα (rabbit, 1:1000, Santa Cruz Biotechnology, USA), LXRβ (goat, 1:1000, Santa Cruz Biotechnology, USA), and β-Actin (goat, 1:3000, Santa Cruz Biotechnology, USA) as protein loading control. A pre-stained molecular ladder (Thermo Fisher Scientific, USA) was used to determine the molecular weights. Immunoblots were washed with TBS-T 0.1% 3 times and incubated at room temperature for 1 h with the respective HRP-conjugated secondary antibodies (rabbit, 1:3000, Bio-Rad, USA and goat, 1:5000, Santa Cruz Biotechnology, USA). Chemiluminescence was detected with the ECL system and exposed to hyperfilm (GE Healthcare Life Sciences, USA). Signals in the immunoblots were scanned and analyzed by Scion Image Software (National Institutes of Health, USA). The protein level was corrected for the loading control (β-actin). For each tissue and marker used, the amount of protein was referred as percentage of the CD. The time-line studies for HFD_3 and HFD_4 are presented as percentage of increase with respect to CD at 2, 4, and 8 weeks.

Statistical Analysis

Data were expressed as mean ± SEM (standard error of the mean). Differences among diets (CD, HFD_1, HFD_2, HFD_3, and HFD_4) at the second week and food/energy consumption for CD, HFD_3, and HFD_4 were determined by one-way analysis of variance (ANOVA). Significance among diets (CD, HFD_3, and HFD_4) and weeks was determined by two-way ANOVA followed by Newman–Keuls post hoc test. The statistical analysis was performed with commercial softwares GraphPad Prism (GraphPad Software Inc., v.4) and/or StatView (SAS Institute Inc. v5.0.1). Differences were considered significant at $p<0.05$.

Results

HFDs Feeding Effects After 2 Weeks

BW Gain and Lipid Metabolism Parameters

The HFDs did not modify the BW gain (data not shown). However, rats fed with HFDs presented changes in the lipid parameters (see Table 1 legend for statistical data). Compared to CD, HFD_2, HFD_3, and HFD_4 increased the TC levels (61.69, 49.81, and 82.00%, respectively; $p<0.05$). Only HFD_4 reduced TG levels (35.76% of decrease; $p<0.05$; Table 1).

Table 1 Lipid parameters (TC and TG) after 2 weeks of CD or HFDs feeding

	CD	HFD_1	HFD_2	HFD_3	HFD_4
TC (mg/dL)	52.20 ± 0.20	59.80 ± 2.70	84.40 ± 7.20	78.20 ± 1.40*	95.00 ± 5.80*
TC (mg/dL)	198.00 ± 25.20	234.80 ± 11.90	175.20 ± 3.90	168.40 ± 6.10	127.20 ± 2.20*

Results are expressed as mean ± SEM from two independent assays ($n = 5$ animals/group). Significant differences were determined by one-way ANOVA for TC ($F(4,20) = 15.79$; $p < 0.0001$) and TG ($F(4,20) = 9.39$; $p = 0.0002$)

*$p < 0.05$ Newman–Keuls post hoc test

LXRs Expression

The consumption of HFDs produced significant changes in the LXRs expression (see Fig. 1 legend for statistical data). Compared to CD, all HFDs raised the hepatic levels of LXRα (HFD_1:50.07%, HFD_2:49.07%, HFD_3:54.97%, and HFD_4:11.69%; $p < 0.05$). Similarly, the increases in the LXRβ expression were 90.03, 41.51, and 100.61% for HFD_1, HFD_3, and HFD_4, respectively ($p < 0.05$; Fig. 1a). In the hypothalamus, LXRα expression remained unchanged, but LXRβ increased with all HFDs

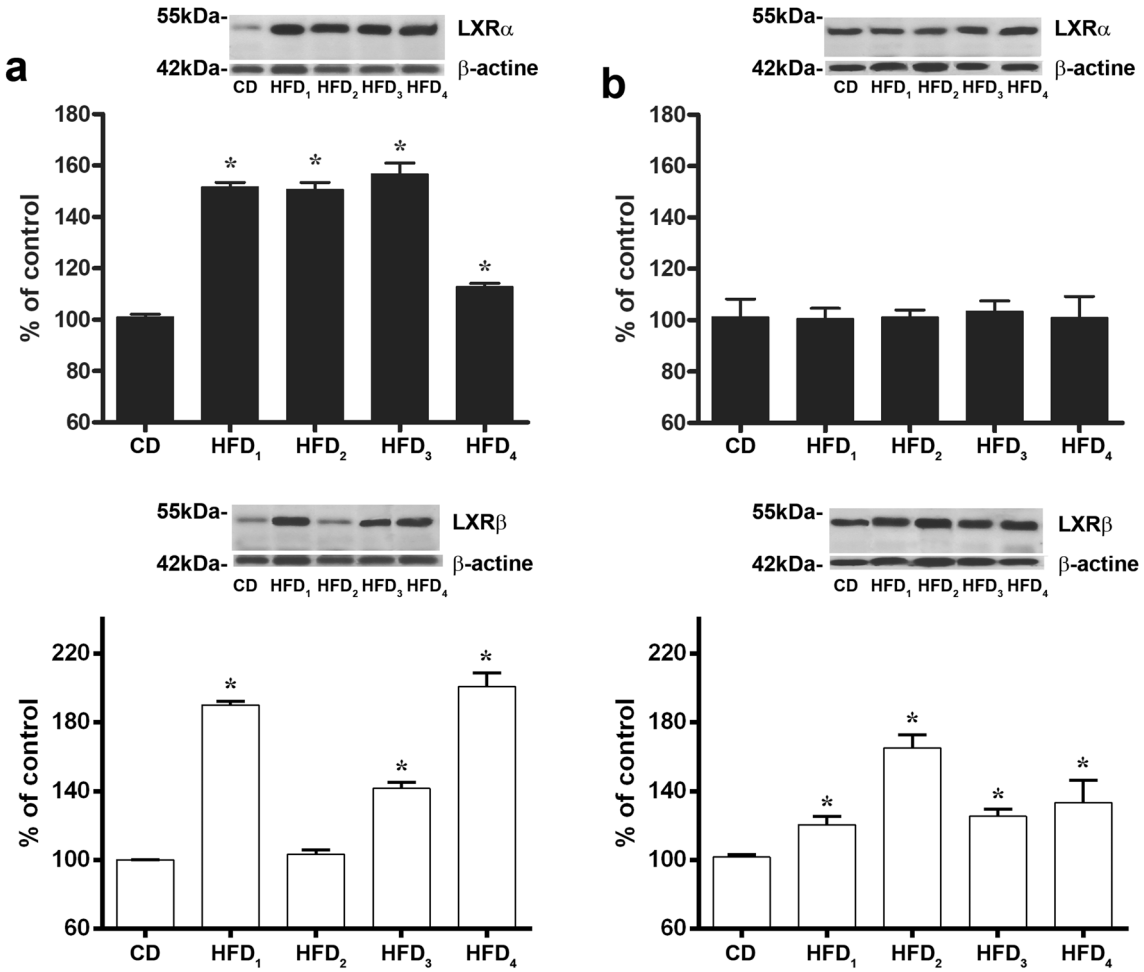

Fig. 1 LXRα (black bars) and LXRβ (white bars) expression in liver (**a**) and hypothalamus (**b**) after 2 weeks of CD or HFDs feeding. LXRs levels were quantified by Western blot and represented as percentage of CD. Representative Western blots are showed on the top of each graph. Results are expressed as mean ± SEM from two independent assays ($n = 5$ animals/group). Significant differences were determined by one-way ANOVA for liver ($F_{LXR\alpha}(4,20) = 101.24$; $p < 0.0001$ and $F_{LXR\beta}(4,20) = 127.39$; $p < 0.0001$) and hypothalamus ($F_{LXR\alpha}(4,20) = 0.207$; $p = 0.931$ and $F_{LXR\beta}(4,20) = 48.51$; $p < 0.0001$). Sub-index in each F indicates the LXRs subtype. *$p < 0.05$ Newman–Keuls post hoc test

feedings (HFD_1:18.47%, HFD_2: 62.16%, HFD_3:23.31%, and HFD_4:30.87%; $p < 0.05$; Fig. 1b).

HFDs Feeding Effects After 4 and/or 8 Weeks

These studies were done with HFD_3 and HFD_4. The selection was based on the 2-week results since these two HFDs were the only ones capable of increasing TC levels and LXRs expression. In this section we also include correlational studies between LXRs expression (in liver and hypothalamus) and TC and TG levels, like the comparison performed in our previous work (Kruse et al. 2012).

BW Gain and Food Consumption

HFD_3 and HFD_4 feeding did not modify the BW gain in any of the time evaluated (data not shown). However, a macroscopical observation of the dissected livers showed that animals fed with these HFDs presented an augmentation in the amount of fat storage (observed as white dots). It also seems that these inclusions were proportional to HFDs treatment duration (Fig. 2a).

One-way ANOVA revealed significant differences in food intake ($F_{Grams}(2,21) = 10.532$; $p < 0.0001$ and $F_{Calories}(2,21) = 11.394$; $p = 0.0004$). The animals fed with HFD_3 and HFD_4 ate less grams of food (16.31% and 12.92%, respectively; $p < 0.05$) but more calories (20.79% and 26.67%, respectively; $p < 0.05$) than CD. No differences were found between these HFDs.

Lipid Metabolism Parameters

The lipid parameters values were compared by two-way ANOVA using diet (CD, HFD_3, and HFD_4) and week (2, 4, and 8) as factors. Significant differences were found in both factors and their interaction for TC levels ($F_{Diet}(2,36) = 96.17$; $p < 0.0001$, $F_{Week}(2,36) = 3.91$; $p = 0.029$; $F_{Interaction}(4,36) = 5.61$; $p = 0.001$). Compared to CD, HFD_3 and HFD_4 raised the TC levels after weeks 2 (49.81% and 82.00%; $p < 0.05$), 4 (64.29% and 93.69%; $p < 0.05$), and 8 (62.40% and 42.80%; $p < 0.05$; Fig. 2b). Also, 2 and 4 weeks of HFD_4 produced higher TC increases than HFD_3, and this difference disappeared toward the eighth week. One-way ANOVA for time-line comparison revealed significant differences in each HFD (see Fig. 2 for statistical

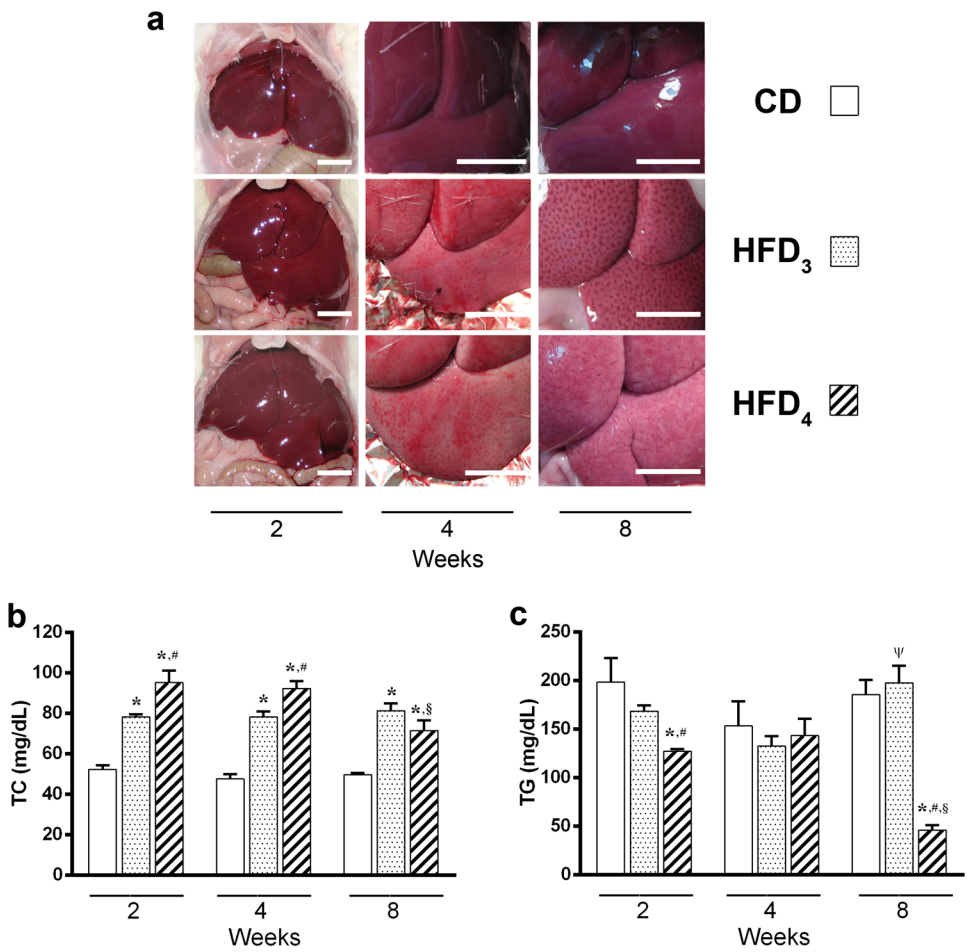

Fig. 2 Representative pictures of livers (scale bars: 5 mm) (**a**), levels of TC (**b**), and TG (**c**) from animals fed with CD, HFD_3, and HFD_4 after 2, 4, or 8 weeks. Results are expressed as mean ± SEM from six independent assays ($n = 5$ animals/group). The two-way ANOVA was followed by Newman–Keuls post hoc test ($p < 0.05$). One-way ANOVA for the levels of TC ($F_{CD}(2,12) = 1.54$; $p = 0.25$ $F_{HFD3}(2,12) = 0.43$; $p = 0.66$; $F_{HFD4}(2,12) = 6.88$; $p = 0.0102$) and TG ($F_{CD}(2,12) = 1.07$; $p = 0.37$; $F_{HFD3}(2,12) = 7.17$; $p = 0.009$; $F_{HFD4}(2,12) = 24.87$; $p < 0.0001$). Sub-index in each F indicates the used diet. *,# refer to CD and HFD_3, respectively, for each week, § refers to 4 week of the same diet

data). Through the weeks, rats fed with HFD_3 maintained TC levels elevated, whereas with HFD_4 these values decreased toward the last week (24.84%; $p<0.05$, Fig. 2b).

The two-way ANOVA for TG levels revealed significant differences by diet and interaction ($F_{Diet}(2,36)=18.12$; $p<0.0001$, $F_{Week}(2,36)=1.84$; $p=0.17$; $F_{Interaction}(4,36)=7.60$; $p=0.0002$). After 2 and 8 weeks, HFD_4 produced lower TG levels than CD (35.75% and 75.30%, respectively; $p<0.05$; Fig. 2c). One-way ANOVA for time-line comparison revealed significant differences for each HFD (see Fig. 2 for statistical data). Throughout time, HFD_3 produced a TG increase whereas HFD_4 did the opposite ($p<0.05$; Fig. 2c).

LXRs Expression and Their Correlation with Lipid Profile Parameters

Hepatic Results LXRs expression levels were analyzed by two-way ANOVA using diet (CD, HFD_3, and HFD_4) and week (4 and 8) as factors. Significant differences were found in the factor diet and interaction for LXRα ($F_{Diet}(2,24)=36.05$; $p<0.0001$, $F_{Week}(1,24)=1.24$; $p=0.2772$; $F_{Interaction}(2,24)=15.42$; $p<0.0001$), and in both factors and their interaction for LXRβ ($F_{Diet}(2,24)=111.91$; $p<0.0001$; $F_{Week}(1,24)=12.60$; $p=0.0016$; $F_{Interaction}(2,24)=9.33$; $p=0.001$). Feeding with HFD_3 and HFD_4 significantly raised the LXRs expression after weeks 4 (LXRα: 24.15% and 17.84% and LXRβ: 23.24% and 47.62%; $p<0.05$) and 8 (LXRα: 9.56% and 42.24% and LXRβ: 24.00% and 29.00%; $p<0.05$; Fig. 3a). One-way ANOVA for time-line comparison revealed significant differences in each HFDs treatment (see Figs. 4, 5 for their respective statistical data). Through the weeks, animals fed with HFD_3 presented a decrease in both LXRs expression ($p<0.05$; Fig. 4a). However, with HFD_4 the individuals showed a raise in LXRα and a decrease in LXRβ levels ($p<0.05$; Fig. 5a).

Correlational studies between LXRs expression and lipid profile parameters were made for each

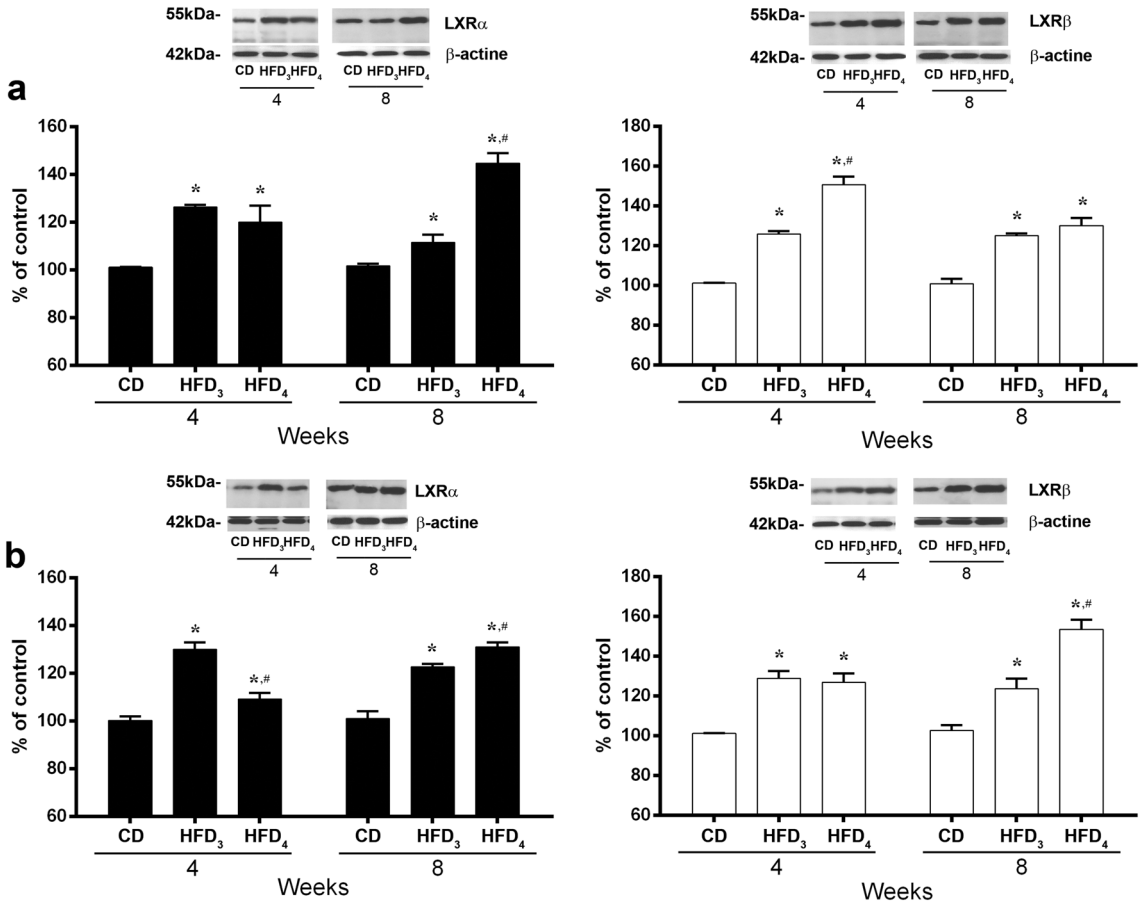

Fig. 3 LXRα (black bars) and LXRβ (white bars) expression in liver (**a**) and hypothalamus (**b**) after 4 and 8 weeks of CD, HFD_3, or HFD_4 feeding. In each tissue, the LXRs levels were quantified by Western blot and represented as percentage of CD. Representative Western blots are showed on the top of each graph. Results are expressed as mean ± SEM from four independent assays ($n=5$ animals/group). Significant differences were determined in each tissue, respectively, by two-way ANOVA followed by Newman–Keuls post hoc test ($p<0.05$). *,# refer to CD and HFD_3, respectively

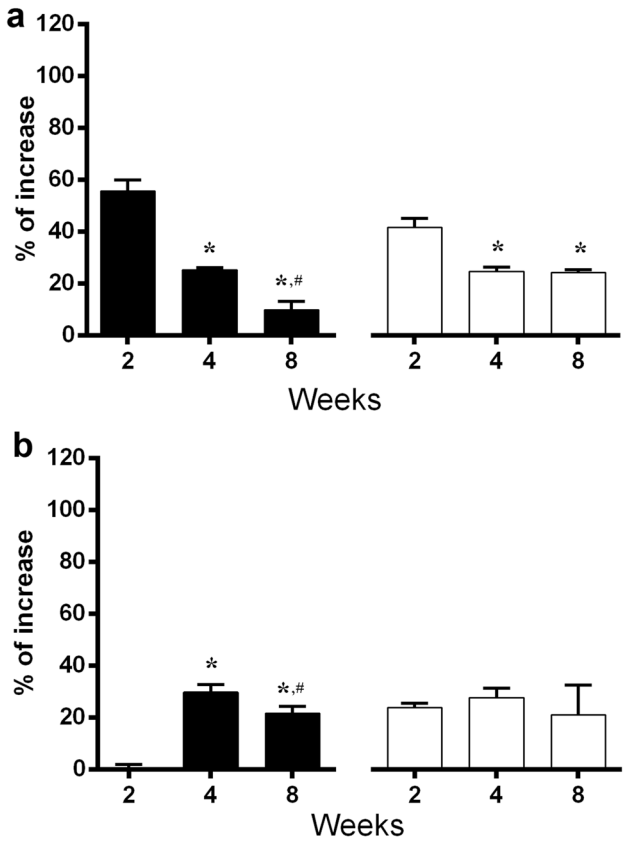

Fig. 4 LXRα (black bars) and LXRβ (white bars) expression in liver (**a**) and hypothalamus (**b**) after 2, 4, and 8 weeks of HFD_3 administration. In each tissue, the LXRs levels were quantified by Western blot and represented as percentage of increase with respect to CD at 2, 4, and 8 weeks. Results are expressed as mean ± SEM from six independent assays ($n = 5$ animals/group). Significant differences were determined in each tissue, respectively, by one-way ANOVA for liver ($F_{LXR\alpha}(2,12) = 52.39$; $p < 0.0001$ and $F_{LXR\beta}(2,12) = 16.39$; $p = 0.0004$) and for hypothalamus ($F_{LXR\alpha}(2,12) = 42.98$; $p < 0.0001$ and $F_{LXR\beta}(2,12) = 0.47$; $p = 0.64$; $p < 0.0001$). Sub-index in each F indicates the LXRs subtype. Newman–Keuls post hoc test ($p < 0.05$). *,# refer to 2 and 4 weeks values, respectively

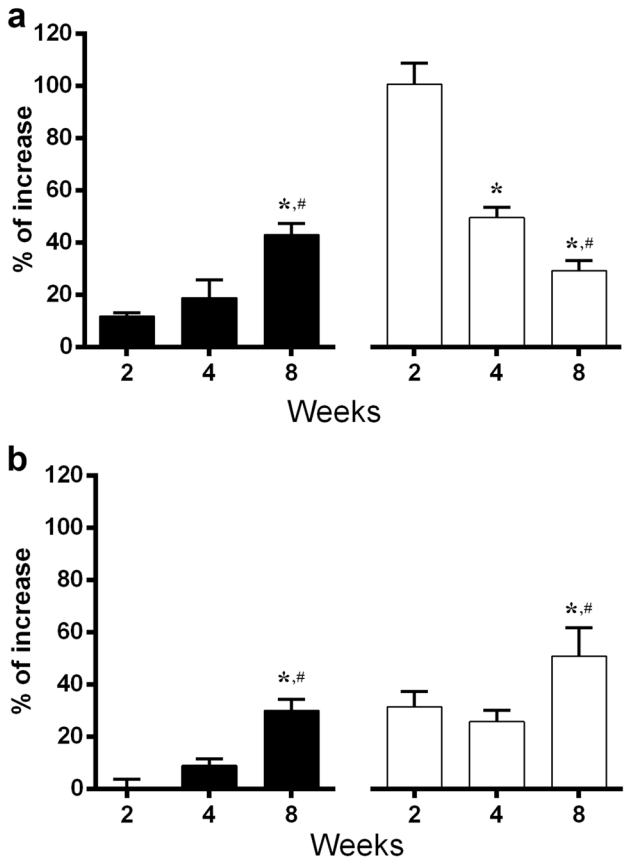

Fig. 5 LXRα (black bars) and LXRβ (white bars) expression in liver (**a**) and hypothalamus (**b**) after 2, 4, and 8 weeks of HFD_4 administration. In each tissue, the LXRs levels were quantified by Western blot and represented as percentage of increase with respect to CD at 2, 4, and 8 weeks. Results are expressed as mean ± SEM from six independent assays ($n = 5$ animals/group). Significant differences were determined in each tissue, respectively, by one-way ANOVA in liver ($F_{LXR\alpha}(2,12) = 12.50$; $p = 0.001$ and $F_{LXR\beta}(2,12) = 42.14$; $p < 0.0001$) and hypothalamus ($F_{LXR\alpha}(2,12) = 26.82$; $p < 0.0001$ and $F_{LXR\beta}(2,12) = 7.48$; $p = 0.008$). Sub-index in each F indicates the LXRs subtype. Newman–Keuls post hoc test ($p < 0.05$). *,# refer to 2 and 4 weeks values, respectively

individual considering animals from 2, 4, and 8 weeks. The statistical analysis showed significant correlations only for HFD_4. These correlations were negative for LXRα ($F_{TC}(1,14) = 8.93$; $p = 0.0105$; $r^2 = 0.41$ and $F_{TG}(1,14) = 11.85$; $p = 0.0044$; $r^2 = 0.48$) and positive for LXRβ expression ($F_{TC}(1,14) = 7.26$; $p = 0.0184$; $r^2 = 0.36$, $F_{TG}(1,14) = 5.76$; $p = 0.0320$; $r^2 = 0.31$; Fig. 6a).

Hypothalamic Results The two-way ANOVA using diet (CD, HFD_3, and HFD_4) and week (4 and 8) as factors indicated significant differences in both factors and their interaction for LXRα ($F_{Diet}(2,24) = 66.93$; $p < 0.0001$, $F_{Week}(1,24) = 7.33$; $p = 0.0123$; $F_{Interaction}(2,24) = 21.21$; $p < 0.0001$) and LXRβ ($F_{Diet}(2,24) = 50.53$; $p < 0.0001$, $F_{Week}(1,24) = 5.89$; $p = 0.0231$; $F_{Interaction}(2,24) = 9.4$; $p = 0.001$). HFD_3 and HFD_4 feeding raised LXRα expression after weeks 4 (29.71% and 8.92%, respectively; $p < 0.05$) and 8 (21.35% and 29.67%, respectively; $p < 0.05$). Similarly, the LXRβ levels increased after weeks 4 (HFD_3:27.36% and HFD_4:25.50%; $p < 0.05$) and 8 (HFD_3:20.45% and HFD_4:49.52%; $p < 0.05$; Fig. 3b).

The one-way ANOVA for time-line comparison showed significant changes in LXRs expression in rats fed with HFD_3 and HFD_4 (see Figs. 4, 5 legend for statistical data). In the animals fed with HFD_3, the LXRα expression fluctuated and LXRβ remained elevated (Fig. 4b). HFD_4 feeding raised both LXRs levels toward week 8 ($p < 0.05$; Fig. 5b).

The LXRs expression and lipid parameters correlational study were made for each individual data, considering all

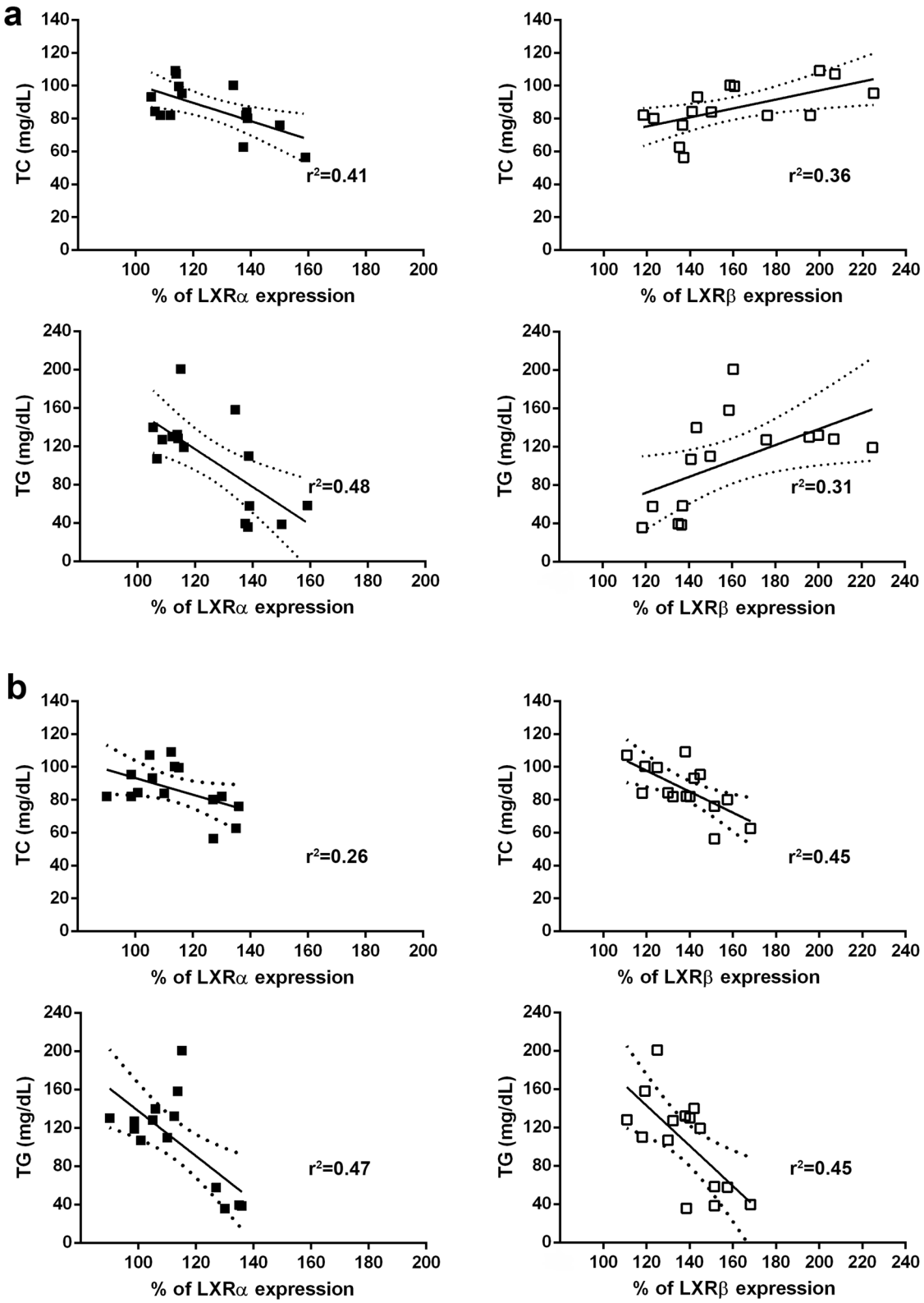

Fig. 6 Correlations between the lipid parameters TC (mg/dL) or TG (mg/dL) and the LXRα (filled square) or LXRβ (open square) expression in liver (**a**) and hypothalamus (**b**) from animals fed with HFD_4 throughout the evaluated weeks. Each point represents a value corresponding to an individual animal. The confident interval is represented by dotted curves. Correlation equations obtained for LXRα in liver ($y_{TC} = 1.558 - 0.006x$ and $y_{TG} = 3.539 - 0.02x$) and hypothalamus ($y_{TC} = 1.431 - 0.005x$ and $y_{TG} = 3.727 - 0.024x$), and for LXRβ in liver ($y_{TC} = 0.426 + 0.003x$ and $y_{TG} = -0.278 + 0.008x$) and hypothalamus ($y_{TC} = 1.739 - 0.006x$ and $y_{TG} = 3.955 - 0.021x$). Sub-index in each equation indicates the lipid parameter

weeks together. The statistical analysis indicated significant correlations only for HFD_4. These correlations were negative for LXRα ($F_{TC}(1,14) = 4.675$; $p = 0.0498$; $r^2 = 0.23$ and $F_{TG}(1,14) = 11.70$; $p = 0.0046$; $r^2 = 0.47$) and LXRβ expression ($F_{TC}(1,14) = 10.69$; $p = 0.0061$; $r^2 = 0.45$ and $F_{TG}(1,14) = 10.79$; $p = 0.0059$; $r^2 = 0.45$; Fig. 6b).

Discussion

Regular HFDs consumption is associated with metabolic, cardiovascular, and neurodegenerative diseases (Cullen 2000; Ghibaudi et al. 2002; Riccioni and Sblendorio 2012; Shanmugasundaram et al. 1986; Sparks et al. 1993). The prevalence of these health disorders is increasing; thus, the understanding of how cholesterol homeostasis is maintained is an important concern that remains in force. Based on the available bibliography, we developed different HFDs combining BF, CA, and/or cholesterol, to study the hypothalamic and hepatic LXRs expression. Initially, all HFDs feedings were studied after 2 weeks, and these results allowed us to select two HFDs for longer treatments (4 and 8 weeks).

HFDs feeding did not modify the BW gain in any of the evaluated times. However, rats fed with HFD_3 or HFD_4 ate less grams of food and more calories than CD. The BW gain results are in agreement with reports of murine fed with HFDs, even though the food intake levels are different (Côté et al. 2013; Nishina et al. 1990; Wang et al. 2010).

Regarding to lipid metabolism, after 2 weeks, all HFDs feeding increased TC levels, and HFD_4 produced the highest value. Also, this diet diminished TG levels (Table 1). Four weeks of HFD_3 and HFD_4 raised TC levels, and the highest value was found with the last one (Fig. 2b), but the TG levels remained unchanged (Fig. 2c). The HFD_3 effect on TC was similar to other works employing analogous diets, although the action on TG levels was different (Fidèle et al. 2017; Wang et al. 2010). Otherwise, HFD_4 increased TC values like Mohammadi and Oshaghi's report, who also describe an increase in TG levels after feeding with a similar HFD (Mohammadi and Oshaghi 2014). Eight weeks of HFD_3 or HFD_4 elevated TC levels, but the second diet reduced the TG levels (Fig. 2b, c). The effect of HFD_4 on TC was similar to those described by other authors, but the action on TG levels was different (Côté et al. 2013; Zhu et al. 2017). Although both HFDs significantly changed the lipid profile with respect to CD, the time-line comparison of HFD_3 and HFD_4 feeding revealed opposite results. HFD_3 kept the raise in TC levels and produced a non-significant increase in TG levels. The TC discrepancies between both HFDs may be due to the presence of CA in HFD_4, which elevates the TC levels increasing the absorption of cholesterol and suppressing its conversion to BAs (Nishina et al. 1990; Srivastava et al. 2000; Van Herck et al. 2017). Otherwise, the decrease in TG levels caused by HFDs may be due since during periods of high lipid influx the liver acts as a "lipid buffer" increasing its TG storage (Frayn 2002), which is macroscopically observed in the liver of animals fed with HFDs (Fig. 2a). HFD_4 results are in agreement with other reports showing that HFDs containing CA induce hepatic steatosis (Côté et al. 2013; Panchal and Brown 2011). However, the mechanism underlying the reciprocal relationship between BAs biosynthesis and TG production remains elusive. One possible explanation is the fact that BAs activate the farnesoid X receptor (FXR) which modulates the TG levels through transcriptional gene regulation (Kast et al. 2001; Lu et al. 2000; Pineda Torra et al. 2003). FXR suppresses BAs synthesis and lipogenesis (Côté et al. 2013), promotes nutrient absorption, and stimulates cholesterol and BAs efflux from the liver (Cave et al. 2016). Alternatively, at the metabolite level, a reduction in BAs biosynthesis could increase hepatic cholesterol and oxysterol levels which will reduce the function of the sterol regulatory element binding protein-1c (SREBP-1c), leading to decrease the TG production (Beigneux et al. 2002; Pullinger et al. 2002).

Several reports describe the HFDs capacity to induce changes in the hepatic LXRs expression (Côté et al. 2013; Mohammadi and Oshaghi 2014; Thornton et al. 2008; Zhu et al. 2017). In this work, 2 weeks of HFDs feeding raised the hepatic LXRα expression (Fig. 1a). Except for HFD_2, all HFDs elevated the LXRβ levels (Fig. 1a). The extension of HFD_3 and HFD_4 to 4 weeks increased both LXRs expression (Fig. 3). The HFD_4 results are in agreement with the report of Mohammadi and Oshaghi, where a similar HFD raises the hepatic LXRα mRNA in a murine model (Mohammadi and Oshaghi 2014). After 8 weeks, HFD_3 and HFD_4 elevated LXRα expression, but the second diet produced the highest increase (Fig. 3). This last diet results are in accordance with Zhu et al., who described an increase in LXRα levels after 6 weeks of treatment with a similar HFD (Zhu et al. 2017). However, 7 weeks of treatment with another diet like HFD_4 did not alter the hepatic LXR mRNA levels (Côté et al. 2013). The time-line comparison of LXRα expression revealed that HFD_3 and HFD_4 produced opposite effects on this subtype, but the LXRβ levels decreased with both HFDs (Figs. 4a, 5a). The correlational studies performed with HFD_4 indicated that TC and/or TG correlated with both LXRs expression (negatively to LXRα and positively to LXRβ; Fig. 6a), adding relevant information about the role of these receptors on lipid homeostasis.

The discrepancies of the HFDs action on LXRs expression could be explained by the fact that diet impact depends on the balance between LXR and FXR actions. Both receptors' functions are subjected to the heterodimerization with retinoid X receptor (RXR; Peet et al. 1998; Wang et al. 1999; Yoshikawa et al. 2003). Thus, FXR can inhibit LXR signaling by a competition for the limited

pool of their common dimeric partner (Wang et al. 1999; Yoshikawa et al. 2003). However, HFDs' effects on transcriptional level remain controversial. Some researchers found that high-cholesterol and CA diet actions on LXRα overrode the effects of FXR (Gupta et al. 2002). But others described that FXR is not activated by a high-cholesterol diet because the hepatic BAs pool does not increase; although its synthesis is stimulated, this is balanced by an increased excretion. However, the addition of CA activates FXR, which results in an impaired cholesterol metabolism and hypercholesterolemic state (Xu et al. 2004).

Although brain cholesterol levels remain separated from the peripheral pool (Koch et al. 2001), HFDs consumption can cause detrimental changes (Franciosi et al. 2009; Hwang et al. 2008; Morrison et al. 2010; Waise et al. 2015). Specifically, Waise et al. demonstrated that HFDs feeding produces inflammation in rodent hypothalamus (Waise et al. 2015). In the present work, 2 weeks of HFDs did not modify the hypothalamic LXRα expression, but increased LXRβ levels (Fig. 1b). Four weeks of HFD_3 and HFD_4 raised the LXRα and LXRβ, but the first diet produced the highest LXRα values. This pattern was reversed after 8 weeks, where HFD_4 feeding generated the highest LXRs increases (Fig. 3). The time-line comparison revealed that rats fed with HFD_3 presented a fluctuation in LXRα expression, whereas the elevated LXRβ level remained unchanged (Fig. 4b). Similar evaluation with HFD_4 showed that both LXRs subtypes increased toward the eighth week (Fig. 5b). LXRs levels correlated negatively with TC and/or TG levels (Fig. 6b), revealing that hypothalamic LXRs are sensitive to changes in these parameters. This reinforces our previous observation suggesting a possible role of these hypothalamic receptors on lipid homeostasis (Kruse et al. 2012). Although more studies will be necessary to understand the mechanisms involved on this action, the present work points out hypothalamic LXRs as interesting targets to explore possible therapeutic interventions for pathologies related to cholesterol disturbances.

Summarizing, distinct HFDs feedings were evaluated at different times: exploring changes on BW gain, lipid profile, and LXRs expression in hypothalamus and liver. Our results add relevant information about how diet composition can produce different scales of hypercholesterolemia states accompanied with peripheral and central changes in LXRs expression. Also to our knowledge, this is the first study that correlates changes in lipid profile with LXRs expression after HFDs feeding treatment.

Acknowledgements This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET; Grants Nos. PIP-860 and PIP-243). MR and RNM-H were, respectively, supported by postdoctoral and doctoral fellowships from CONICET.

Authors contribution The authors' responsibilities were as follow: MR and HC designed and conducted the research, had primary responsibility for the final content, and wrote the paper with contribution by coauthors; MR, MSK, and RNM-H performed the TC and TG analysis and the Western blot studies; MR statistically analyzed the data; and all authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Bantubungi K, Prawitt J, Staels B (2012) Control of metabolism by nutrient-regulated nuclear receptors acting in the brain. J Steroid Biochem Mol Biol 130:126–137

Baranowski M (2008) Biological role of liver X receptors. J Physiol Pharmacol 59:31–55

Beigneux A, Hofmann AF, Young SG (2002) Human CYP7A1 deficiency: progress and enigmas. J Clin Invest 110:29–31

Blain JF, Poirier J (2004) Cholesterol homeostasis and the pathophysiology of Alzheimer's disease. Expert Rev Neurother 4:823–829

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cai D (2012) One step from prediabetes to diabetes: hypothalamic inflammation? Endocrinology 153:1010–1013

Cao G, Liang Y, Jiang XC, Eacho PI (2004) Liver X receptors as potential therapeutic targets for multiple diseases. Drug News Perspect 17:35–41

Carey MC, Mazer NA (1984) Biliary lipid secretion in health and in cholesterol gallstone disease. Hepatology 4(5):31S–37S

Cave MC, Clair HB, Hardesty JE, Falkner KC, Feng W, Clark BJ, Sidey J, Shi H, Aqel BA, McClain CJ, Prough RA (2016) Nuclear receptors and nonalcoholic fatty liver disease. Biochim Biophys Acta 1859:1083–1099

Cha JY, Repa JJ (2007) The liver X receptor (LXR) and hepatic lipogenesis—the carbohydrate-response element-binding protein is a target gene of LXR. J Biol Chem 282(1):743–751

Charlton M, Krishnan A, Viker K, Sanderson S, Cazanave S, McConico A, Masuoko H, Gores G (2011) Fast food diet mouse: novel small animal model of nash with ballooning, progressive fibrosis, and high physiological fidelity to the human condition. Am J Physiol Gastrointest Liver Physiol 301:G825–G834

Coirini H, Marusic ET, De Nicola AF, Rainbow TC, McEwen BS (1983) Identification of mineralocorticoid binding sites in rat brain by competition studies and density gradient centrifugation. Neuroendocrinology 37:354–360

Côté I, Ngo Sock ET, Lévy É, Lavoie JM (2013) An atherogenic diet decreases liver FXR gene expression and causes severe hepatic steatosis and hepatic cholesterol accumulation: effect of endurance training. Eur J Nutr 52(5):1523–1532

Cullen P (2000) Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol 86:943–949

Dai YB, Tan XJ, Wu WF, Warner M, Gustafsson JÅ (2012) Liver X receptor β protects dopaminergic neurons in a mouse model of Parkinson disease. Proc Natl Acad Sci USA 109:13112–13117

Dalvi PS, Chalmers JA, Luo V, Han DY, Wellhauser L, Liu Y, Tran DQ, Castel J, Luquet S, Wheeler MB, Belsham DD (2017) High fat induces acute and chronic inflammation in thehypothalamus:

effect of high-fat diet, palmitate and TNF-alpha on appetite-regulating NPY neurons. Int J Obes 41:149–158
De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA (2005) Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146:4192–4199
Fan X, Kim HJ, Bouton D, Warner M, Gustafsson JA (2008) Expression of liver X receptor beta is essential for formation of superficial cortical layers and migration of later-born neurons. Proc Natl Acad Sci USA 105:13445–13450
Fidèle N, Joseph B, Emmanuel T, Théophile D (2017) Hypolipidemic, antioxidant and anti-atherosclerogenic effect of aqueous extract leaves of *Cassia occidentalis* Linn (*Caesalpiniaceae*) in diet-induced hypercholesterolemic rats. BMC Complement Altern Med 17(1):76. https://doi.org/10.1186/s12906-017-1566-x
Franciosi S, Gama Sosa MA, English DF, Oler E, Oung T, Janssen WG, De Gasperi R, Schmeidler J, Dickstein DL, Schmitz C, Gandy S, Hof PR, Buxbaum JD, Elder GA (2009) Novel cerebrovascular pathology in mice fed a high cholesterol diet. Mol Neurodegener 4:42
Frayn KN (2002) Adipose tissue as a buffer for daily lipid flux. Diabetologia 45(9):1201–1210
Gao Q, Horvath TL (2008) Neuronal control of energy homeostasis. FEBS Lett 582:132–141
Ghibaudi L, Cook J, Farley C, van Heek M, Hwa JJ (2002) Fat intake affects adiposity, comorbidity factors, and energy metabolism of Sprague-Dawley rats. Obes Res 10:956–963
Gupta S, Pandak WM, Hylemon PB (2002) LXR alpha is the dominant regulator of CYP7A1 transcription. Biochem Biophys Res Commun 293:338–343
Horvath TL, Sarman B, Garcia-Caceres C, Enriori PJ, Sotonyi P, Shanabrough M, Borok E, Argente J, Chowen JA, Perez-Tilve D, Pfluger PT, Brönneke HS, Levin BE, Diano S, Cowley MA, Tschöp MH (2010) Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. Proc Natl Acad Sci USA 107:14875–14880
Hwang IK, Kim IY, Kim DW, Yoo KY, Kim YN, Yi SS, Won MH, Lee IS, Yoon YS, Seong JK (2008) Strain-specific differences in cell proliferation and differentiation in the dentate gyrus of C57BL/6N and C3H/HeN mice fed a high fat diet. Brain Res 1241:1–6
Karnani M, Burdakov D (2011) Multiple hypothalamic circuits sense and regulate glucose levels. Am J Physiol Regul Integr Comp Physiol 300:R47–R55
Kast HR, Nguyen CM, Sinal CJ, Jones SA, Laffitte BA, Reue K, Gonzalez FJ, Willson TM, Edwards PA (2001) Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. Mol Endocrinol 15:1720–1728
Kim HJ, Fan X, Gabbi C, Yakimchuk K, Parini P, Warner M, Gustafsson JA (2008) Liver X receptor beta (LXRbeta): a link between beta-sitosterol and amyotrophic lateral sclerosis-Parkinson's dementia. Proc Natl Acad Sci USA 105:2094–2099
Koch S, Donarski N, Goetze K, Kreckel M, Stuerenburg HJ, Buhmann C, Beisiegel U (2001) Characterization of four lipoprotein classes in human cerebrospinal fluid. J Lipid Res 42:1143–1151
Kruse MS, Rey M, Vega MC, Coirini H (2012) Alterations of LXRα and LXRβ in the hypothalamus of glucose-intolerant rats. J Endocrinol 215:51–58
Kruse MS, Suarez LG, Coirini H (2017) Regulation of the expression of LXR in rat hypothalamic and hippocampal explants. Neurosci Lett 639:53–58
Lam TK, Pocai A, Gutierrez-Juarez R, Obici S, Bryan J, Aguilar-Bryan L, Schwartz GJ, Rossetti L (2005) Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis. Nat Med 11:320–327
Laurencikiene J, Rydén M (2012) Liver X receptors and fat cell metabolism. Int J Obes (Lond) 36:1494–1502
Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ (2000) Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol Cell 6:507–515
Matsuzawa N, Takamura T, Kurita S, Misu H, Ota T, Ando H, Yokoyama M, Honda M, Zen Y, Nakanuma Y, Miyamoto K, Kaneko S (2007) Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. Hepatology 46(5):1392–1403
Mohammadi A, Oshaghi EA (2014) Effect of garlic on lipid profile and expression of LXR alpha in intestine and liver of hypercholesterolemic mice. J Diabetes Metab Disord 13(1):20
Morrison CD, Pistell PJ, Ingram DK, Johnson WD, Liu Y, Fernandez-Kim SO, White CL, Purpera MN, Uranga RM, Bruce-Keller AJ, Keller JN (2010) High fat diet increases hippocampal oxidative stress and cognitive impairment in aged mice: implications for decreased nrf2 signaling. J Neurochem 114:1581–1589
Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW (2006) Central nervous system control of food intake and body weight. Nature 443:289–295
Nishina PM, Verstuyft J, Paigen B (1990) Synthetic low and high fat diets for the study of atherosclerosis in the mouse. J Lipid Res 31:859–869
Panchal SK, Brown L (2011) Rodent models for metabolic syndrome research. J Biomed Biotechnol 2011:351982. https://doi.org/10.1155/2011/351982
Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, Mangelsdorf DJ (1998) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. Cell 93:693–704
Pineda Torra I, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B (2003) Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. Mol Endocrinol 17:259–272
Pullinger CR, Eng C, Salen G, Shefer S, Batta AK, Erickson SK, Verhagen A, Rivera CR, Mulvihill SJ, Malloy MJ, Kane JP (2002) Human cholesterol 7alpha-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. J Clin Invest 110:109–117
Repa JJ, Mangelsdorf DJ (2000) The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. Annu Rev Cell Dev Biol 16:459–481
Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev 14:2819–2830
Rey M, Kruse MS, Alvarez LD, Ghini AA, Veleiro AS, Burton G, Coirini H (2013) Neuroprotective action of synthetic steroids with oxygen bridge. Activity on GABAA receptor. Exp Neurol 249:49–58
Riccioni G, Sblendorio V (2012) Atherosclerosis: from biology to pharmacological treatment. J. Geriatr Cardiol 9:305–317
Russell DW (2003) The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 72:137–174
Shanmugasundaram KR, Visvanathan A, Dhandapani K, Srinivasan N, Rasappan P, Gilbert R, Alladi S, Kancharla S, Vasanthi N (1986) Effect of high-fat diet on cholesterol distribution in plasma lipoproteins, cholesterol esterifying activity in leucocytes, and erythrocyte membrane components studied: importance of body weight. Am J Clin Nutr 44:805–815
Sparks DL, Liu H, Scheff SW, Coyne CM, Hunsaker JC (1993) Temporal sequence of plaque formation in the cerebral cortex of nondemented individuals. J Neuropathol Exp Neurol 52:135–142
Srivastava RA, Srivastava N, Averna M (2000) Dietary cholic acid lowers plasma levels of mouse and human apolipoprotein A-I primarily via a transcriptional mechanism. Eur J Biochem 267:4272–4280

Stark AH, Timar B, Madar Z (2000) Adaptation of Sprague Dawley rats to long-term feeding of high fat or high fructose diets. Eur J Nutr 39:229–234

Steffensen KR, Nilsson M, Schuster GU, Stulnig TM, Dahlman-Wright K, Gustafsson JA (2003) Gene expression profiling in adipose tissue indicates different transcriptional mechanisms of liver X receptors alpha and beta, respectively. Biochem Biophys Res Commun 310:589–593

Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschöp MH, Schwartz MW (2012) Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 122:153–162

Thornton SJ, Wong E, Lee SD, Wasan KM (2008) Effect of dietary fat on hepatic liver X receptor expression in P-glycoprotein deficient mice: implications for cholesterol metabolism. Lipids Health Dis 7:21

Treiber-Held S, Distl R, Meske V, Albert F, Ohm TG (2003) Spatial and temporal distribution of intracellular free cholesterol in brains of a Niemann-Pick type C mouse model showing hyperphosphorylated tau protein. Implications for Alzheimer's disease. J Pathol 200:95–103

Ulven SM, Dalen KT, Gustafsson JA, Nebb HI (2005) LXR is crucial in lipid metabolism. Prostaglandins Leukot Essent Fatty Acids 73:59–63

Valdearcos M, Douglass JD, Robblee MM, Dorfman MD, Stifler DR, Bennett ML, Gerritse I, Fasnacht R, Barres BA, Thaler JP, Koliwad SK (2017) Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility. Cell Metab 26:185–197.e3

Van Herck MA, Vonghia L, Francque SM (2017) Animal models of nonalcoholic fatty liver disease—a Starter's guide. Nutrients 9(10):E1072. https://doi.org/10.3390/nu9101072

Waise TMZ, Toshinai K, Naznin F, NamKoong C, Md Moin AS, Sakoda H, Nakazato M (2015) One-day high-fat diet induces inflammation in the nodose ganglion and hypothalamus of mice. Biochem Biophys Res Commun 464:1157–1162

Wang H, Chen J, Hollister K, Sowers LC, Forman BM (1999) Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 3(5):543–553

Wang YM, Zhang B, Xue Y, Li ZJ, Wang JF, Xue CH, Yanagita T (2010) The mechanism of dietary cholesterol effects on lipids metabolism in rats. Lipids Health Dis 9:4

Whitney KD, Watson MA, Collins JL, Benson WG, Stone TM, Numerick MJ, Tippin TK, Wilson JG, Winegar DA, Kliewer SA (2002) Regulation of cholesterol homeostasis by the liver X receptors in the central nervous system. Mol Endocrinol 16:1378–1385

Xu G, Pan LX, Li H, Shang Q, Honda A, Shefer S, Bollineni J, Matsuzaki Y, Tint GS, Salen G (2004) Dietary cholesterol stimulates CYP7A1 in rats because farnesoid X receptor is not activated. Am J Physiol Gastrointest Liver Physiol 286:G730–G735

Yoshikawa T, Shimano H, Yoshikawa T, Yahagi N, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Yatoh S, Iizuka Y, Tomita S, Ohashi K, Takahashi A, Sone H, Gotoda T, Osuga J, Ishibashi S, Yamada N (2003) Cross-talk between peroxisome proliferator-activated receptor (PPAR) a and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signaling. Mol Endocrinol 17:1240–1254

Zelcer N, Tontonoz P (2006) Liver X receptors as integrators of metabolic and inflammatory signaling. J Clin Invest 116(3):607–614

Zhu J, Zhang X, Chen X, Sun Y, Dai Y, Chen C, Zhang T, Yan Z (2017) Studies on the regulation of lipid metabolism and the mechanism of the aqueous and ethanol extracts of Usnea. Biomed Pharmacother 94:930–936