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Liver steatosis in hypothalamic obese rats improves after duodeno-jejunal bypass by reduction in *de novo* lipogenesis pathway

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ABSTRACT

Aims: Hypothalamic obesity is a severe condition without any effective therapy. Bariatric operations appear as an alternative treatment, but the effects of this procedure are controversial. We, herein, investigated the effects of duodeno-jejunal bypass (DJB) surgery upon the lipid profile and expression of genes and proteins, involved in the regulation of hepatic lipid metabolism, in hypothalamic obese (HyO) rats.
Methods: During the first 5 days of life, male newborn *Wistar* rats received subcutaneous injections of monosodium glutamate [4 g/kg body weight, HyO group] or saline (control, CTL group). At 90 days of life, HyO rats were randomly submitted to DJB (HyO DJB) or Sham-operations (HyO Sham group). Six months after DJB, adiposity, hepatic steatosis and lipid metabolism were verified.
Key findings: HyO Sham rats were obese, hyperinsulinemic, insulin resistant and dyslipidemic. These rats had higher liver contents of trygliceride (TG) and presented disorganization of the hepatocyte structures, in association with higher hepatic contents of acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase-1 mRNAs and protein. DJB surgery normalized insulinemia, insulin resistance, and dyslipidemia in HyO rats. TG content in the liver and the hepatic microscopic structures were also normalized in HyO DJB rats, while the expressions of ACC and FASN proteins were decreased in the liver of these rodents.
Significance: The DJB-induced amelioration in hepatic steatosis manifested as a late effect in HyO rats, and was partly associated with a downregulation in hepatic *de novo* lipogenesis processes, indicating that DJB protects against liver steatosis in hypothalamic obesity.

1. Introduction

The hypothalamus plays an important role in the control of energy expenditure and body adiposity [1]. Lesions in the hypothalamus may cause neuroendocrine and metabolic alterations, provoking hypothalamic obesity [2]. Hypothalamic obese (HyO) patients present deficiency in the secretion of pituitary hormones [3], disruption in the orexigenic and anorexigenic signals in the hypothalamus, excessive adiposity, hyperleptinemia, reduction in the sympathetic tonus and increased parasympathetic tonus [2], hyperinsulinemia, insulin resistance, dyslipidemias and nonalcoholic fatty liver disease (NAFLD) [4].

NAFLD is characterized by increased hepatic lipid deposition that can gradually progress to nonalcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma [5]. The accumulation of TG in hepatocytes, leading to hepatic steatosis, occurs due to increased fatty acid (FA) absorption from the circulation, due to a high caloric diet and/or from adipose tissue lipolysis. Additionally, increases in the activation of the transcription factors and enzymes involved in *de novo* (DN) lipogenesis, or downregulation in the FA β-oxidation, together or not, with reduced hepatic secretion of very low-density lipoproteins (VLDLs) are key mechanisms involved in NAFLD pathology [6].

Weight loss has been suggested to represent the best treatment for NAFLD and obesity. However, changes in feeding behavior, physical

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exercise or the use of traditional anti-obesity pharmacotherapies are not effective for hypothalamic obesity [2]. Bariatric operations can be an alternative approach for this syndrome, but there are fewer studies with HyO patients that have undergone this surgical procedure, and these report contrasting data regarding its benefits [7–9].

Of the bariatric surgeries employed in HyO patients for weight loss, restrictive procedures such as sleeve gastrectomy, gastric bypass, and gastric banding are often used [8,9]. However, the duodeno-jejunal bypass (DJB), a procedure that maintains the volume of the stomach, but avoids the passage of food from the duodenum and part of the jejunum, has been poorly investigated in this syndrome. DJB has been shown to ameliorate glucose homeostasis and hepatic steatosis, independently of reductions in body weight in genetic and diet-induced diabetic obese rodents [10,11]. However, these DJB benefits may be influenced by the type of obesity and the time after the surgery [12–14]. Recently, using neonatal monosodium glutamate (MSG) treatment in rats to induce lesions in the arcuate nuclei and median eminence of the hypothalamus [15], we demonstrated that HyO rats exhibited better peripheral actions of insulin [16], normalization of pancreatic islet morphofunction, but display persistent hepatic steatosis [17], at 2-months after DJB. We herein sought to determine whether the benefits of DJB, against hepatic lipid accumulation and lipogenesis regulation, manifest at a later stage in HyO rats.

2. Material and methods

2.1. Hypothalamic obesity induction

Hypothalamic obesity was obtained by daily subcutaneous injection of MSG [4 mg/g body weight (BW), HyO group, n = 34], for 5 consecutive days, in male newborn Wistar rats, while the control group (CTL, n = 17) received saline (1.25 mg/g BW). From 21 days to 90 days of age, all rats were maintained under controlled lighting (lights on 8:00–20:00 h) and temperature (22 ± 1 °C), and had free access to standard diet and water. All experiments were approved by the UNIOESTE's Committee on Ethics in Animal Experimentation.

2.2. Duodeno-jejunal bypass and sham operations

At 90 days of life, HyO rats were randomly submitted to DJB (HyO DJB group, n = 17) or sham operations (HyO Sham, n = 17). Preoperative procedures were performed as reported by Meguid et al. [18]. The DJB surgery was executed as described by Rubino and Marescaux [11]. For the sham surgery, a laparotomy was performed and the intestine was massaged to mimic the surgical movements.

2.3. Food intake and feces production

At 6 months after the DJB or sham operations, five rats from each experimental group were maintained, individually, in metabolic cages during 3 days for measurements of food consumption and excreted feces in 12 h.

2.4. Obesity parameters and serum biochemical analysis

Six months after the operation, glycemia was measured after 6 h of fasting in all rats using a glucometer. Subsequently, all rodents were weighed and the nasoanal length was measured in all groups to obtain the Lee index [BW $(g)^{1/3}$ / nasoanal length (cm) × 1000]. The rats were euthanized by decapitation and total blood was collected to obtain the serum, which was used to measure total cholesterol (CHOL), TG and non-esterified free FA (NEFA) using commercial kits (LaborClin®, Bioliquid, BRA and Wako®, Germany, respectively). Insulinemia was measured by radioimmunoassay. In addition, the retroperitoneal and perigonadal fat pads were removed and weighed.

2.5. TyG index and HOMA-IR

Insulin sensitivity was evaluated by the TyG index [19]. Tissue insulin resistance was also evaluated by HOMA of insulin resistance [20].

2.6. Lipids and glycogen content in the liver

Fragments of liver were collected and the lipids were extracted by Folch's method [21]. The extract was evaporated and diluted in isopropanol for the determination of TG and CHOL contents in the liver, as described above. The glycogen content in the liver was measured as reported by Ropelle et al. [22].

2.7. Liver histology

Liver samples were fixed in 10% formalin for 24 h, dehydrated in alcohol, permeabilized with xylene and then embedded in Paraplast® (Sigma-Aldrich, MO, USA). Sections of 7 μm in thickness were stained with hematoxylin and eosin. To identify liver collagen fibers, Mallory's trichrome staining was performed. For the descriptive analyses, 3 sections from each liver were analyzed by a blind researcher, using a light microscope (Olympus DP71; Tokyo, Japan) with a 400 × magnification lens.

2.8. Isolation of RNA and qPCR

Liver samples were separated for RNA extraction, which was performed using RNA minikit PuriLink® (Life Technologies, CA, USA). mRNA was quantified using the Fast System 7500 & 7500 Real-Time PCR System (Applied Biosystems, CA, USA) and the expression of each gene was normalized by the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA content. The absolute amount of gene expression was calculated by the use of standard curves (10^8–10^3 copies/DNA molecules in 2 μL), produced from the gene amplification products on 2% agarose gels. The primers sequences, showed in Table 1, were designed and purchased from Sigma-Aldrich®.

2.9. Protein expression

A fragment of the liver was solubilized in extraction buffer [16] using a mechanical homogenizer (Marconi®, SP, BRA). The extracts were then centrifuged at 12,600*g* at 4 °C for 30 min and the protein content was measured in the supernatant using the Bradford method. The samples (100 μg) were incubated at 100 °C for 5 min with Laemmli buffer and separated by electrophoresis in 6.5 or 10% biphasic polyacrylamide gel. Subsequently, samples were transferred to a nitrocellulose membrane and incubated with primary antibody for: adenosine monophosphate activated protein kinase (AMPK-α, 1:1000; #2532S, Cell Signaling), phosphorylated AMPK (pAMPK-α^{Thr172}, 1:1000; Cell Signaling #2531S), acetyl-CoA carboxylase (ACC, 1:1000; Cell Signaling #3662S), phosphorylated ACC (pACC^{Ser79}, 1:1000; Cell Signaling #3661), FA synthase (FASN, 1:500; Santa Cruz sc-20140), stearoyl-CoA desaturase-1 (SCD-1, 1:1000; Abcam ab19862), carnitine palmitoyl transferase-1a (CPT-1a, 1:500; Santa Cruz sc-20669) or microsomal triglyceride transfer protein (MTTP, 1:1000; Sigma-Aldrich AV43618). α-Tubulin was used as an internal control (1:1000; Sigma-Aldrich T5168). Specific bands were visualized by incubating the membranes with secondary antibodies (1:10,000; Cell Signaling), followed by the incubation with chemiluminescent reagents. The image was captured with the Chemi L-Pix Express photodocumentation system (Loccus Biotecnologia®, SP, BRA) and band densitometry was measured using the LabImage analysis 1D software (Loccus Biotecnologia®, SP, BRA).

Table 1
Primer sequences for real-time qPCR assays.

Gene	Forward (5′–3′)	Reverse (5′–3′)
ACC-1	AGGAAGATGGTGTCCCGCTCTG	GGGGAGATGTGCTGGGTCAT
ACO	CCCAAGACCCAAGAGTTCATTC	TCACGGATAGGGACAACAAAGG
ChREBP	GAAGACCCAAAGACCAAGATGC	TCTGACAACAAAGCAGGAGGTG
CPT-1a	CTCCTGAGCAGTTACCAATGC	GAACCTTGGCTGCGGTAAGAC
FASN	AGGTGCTAGAGGCCCTGCTA	GTGCACAGACACCTTCCCAT
FXR	GCAACTGCGTGATGGATATG	TTCGCTGTCCTCATTCACTG
LPK	GACCCGAAGTTCCAGACAAGG	ATGAGCCCGTCGTCAATGTAG
MTTP	CTTCTGCCTACACTGGCTACG	GTTCTCCTCTCCCTCATCTGG
PPAR-α	GTACGGTGTGTATGAAGCCATCTT	GCCGTACGCGATCAGCAT
PPAR-γ	GCCCTTTGGTGACTTTATGGAG	GCAGCAGGTTGTCTTGGATGT
SCD-1	CAGTTCCTACACGACCACCACTA	GGACGGATGTCTTCTTCCAGAT
SREBP-1c	GGAGCCATGGATTGCACATT	AGGAAGGCTTCCAGAGAG
GAPDH	GGAGAAACCTGCCAAGTATGATG	AACCTGGTCCTCAGTGTAGCCCC

ACC-1 – acetyl-CoA carboxylase, ACO – acyl-CoA oxidase, ChREBP – carbohydrate response element-binding protein, CPT-1a – carnitine palmitoyl transferase-1a, FASN – fatty acid synthase, FXR – farnesoid X receptor, LPK – liver pyruvate kinase, MTTP - microsomal triglyceride transfer protein, PPAR-α - peroxisome proliferator-activated receptor-α, PPAR-γ - peroxisome proliferator-activated receptor-γ, SCD-1 – stearoyl-CoA desaturase-1, SREBP-1c – sterol regulatory element binding protein-1c, GAPDH – glyceraldehyde 3-phosphate dehydrogenase.

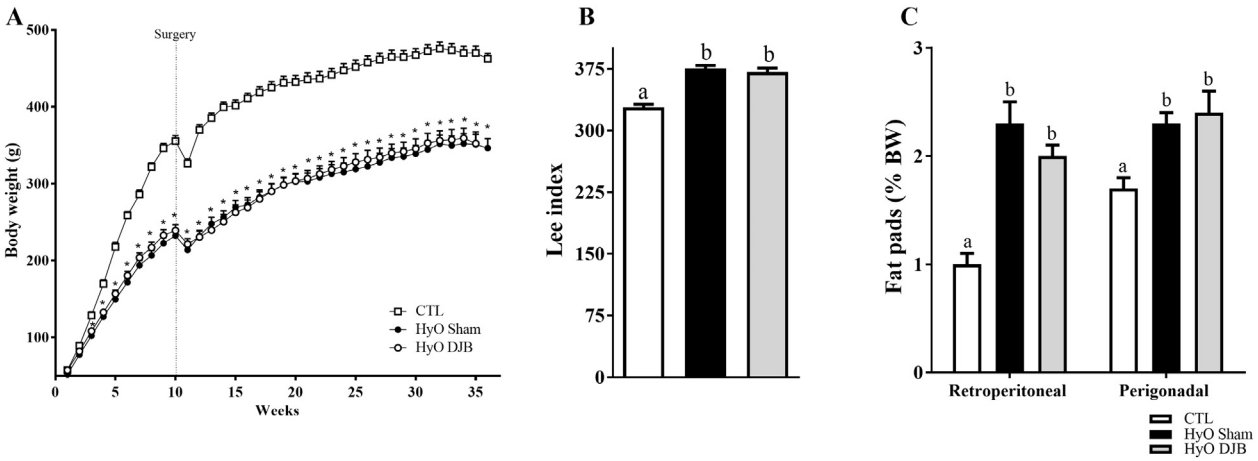

Fig. 1. HyO rats do not present alterations in BW or adiposity at 6 months after DJB surgery. (A) Means ± SEM (n = 17 rats) of BW measured weekly. *HyO DJB and HyO Sham groups are different from CTL ($P < 0.05$). (B) Lee index and (C) retroperitoneal and perigonadal fat pad weights in CTL, HyO Sham and HyO DJB rats (n = 17). Different letters over the bars indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Table 2
Final body weight, food intake and excreted feces during 12 h in CTL, HyO Sham and HyO DJB rats.

	CTL	HyO Sham	HyO DJB
Body weight (g)	440 ± 7.8[a]	337 ± 11.7[b]	337 ± 10.5[b]
Food intake during 12 h (g)	15.5 ± 0.7[a]	9.8 ± 0.7[b]	10.8 ± 0.5[b]
Food intake/BW (mg/g)	32 ± 0.1	28 ± 0.2	31 ± 0.2
Feces excreted in 12 h (g)	6.3 ± 0.3[a]	4.2 ± 0.3[b]	4.1 ± 0.3[b]

Data are mean ± SEM (n = 14–19 rats). Different letters indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Table 3
Fasting serum biochemical parameters, TyG index, HOMA-IR and liver glycogen content in CTL, HyO Sham and HyO DJB rats.

	CTL	HyO Sham	HyO DJB
Glucose (mg/dL)	65 ± 1.7	66 ± 2.5	66 ± 2.8
Insulin (ng/mL)	0.4 ± 0.1[a]	1.0 ± 0.2[b]	0.3 ± 0.1[a]
Triglycerides (mg/dL)	155 ± 7.9[a]	237 ± 16.7[b]	140 ± 16.5[a]
Cholesterol (mg/dL)	110 ± 4.3	122 ± 4.3	102 ± 5
NEFA (mol/L)	0.6 ± 0.02[a]	0.9 ± 0.1[b]	0.6 ± 0.1[a]
TyG index	9.2 ± 0.1[a]	9.6 ± 0.1[b]	9 ± 0.1[a]
HOMA-IR	1.5 ± 0.2[a]	2.8 ± 0.5[b]	1.6 ± 0.2[a]
Liver glycogen content (mg/100 mg liver)	25 ± 1.6[a]	38 ± 1.8[b]	31 ± 1.3[a]

Data are mean ± SEM (n = 8–14 rats). Different letters indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

2.10. Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey post-test ($P < 0.05$) and were performed using GraphPad Prism version 5.00 software (GraphPad Inc., CA, USA).

3. Results

3.1. Nutritional and obesity parameters

HyO displayed lower BW than the CTL rats, from the third week until the end of the experimental period ($P < 0.05$; Fig. 1A). This effect is also confirmed by a reduction in total BW calculated by area under curve of BW in the HyO group, in comparison with the CTL group, before (1165 ± 36 and 1674 ± 38, respectively, $P < 0.0001$) and after (7919 ± 272; 11,301 ± 189, respectively, $P < 0.0001$) the Sham surgery. The DJB operation did not alter the BW in HyO DJB rats, when compared to HyO Sham (Fig. 1A).

At the end of the experimental period, the HyO Sham rats presented lower food intake, excreted feces, and final BW than CTL rats ($P < 0.001$; Table 2). However, the feed efficiency did not differ between the HyO and CTL groups (Table 2). But, HyO rats presented

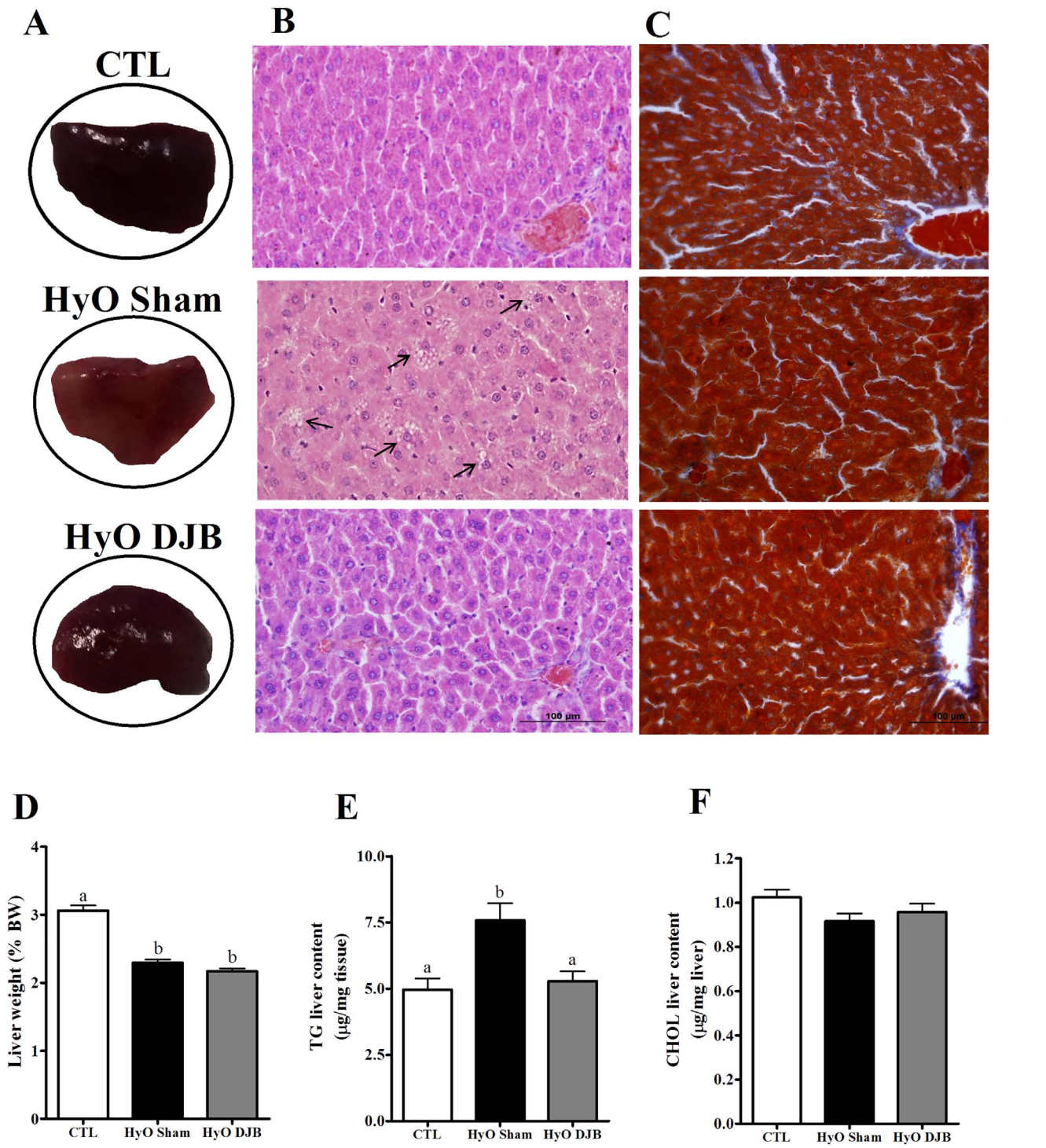

Fig. 2. DJB surgery ameliorates liver steatosis after six months in HyO rats. Macroscopic aspect of the liver (A), light microscopy of (B) hematoxylin and eosin and (C) Mallory's trichrome-stained liver sections from CTL, HyO Sham and HyO DJB rats. Means ± SEM (n = 11–17 rats) of the liver weight (D), and hepatic TG (E) and CHOL (F) contents in CTL, HyO Sham and HyO DJB rats. Arrows indicate microvesicular steatosis. Different letters over the bars indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

obesity, demonstrating increases of 13% in the Lee index and 130% and 36% in the perigonadal and the retroperitoneal fat stores, when compared to CTL ($P < 0.001$, $P < 0.001$, $P < 0.05$, respectively; Fig. 1B and C). At 6 months after the DJB operation, food consumption, excreted feces, and obesity, were similar in the HyO DJB and HyO Sham groups (Table 2 and Fig. 1B and C).

HyO rats displayed normoglycemia, but hyperinsulinemia, hypertrygliceridemia and higher NEFAs serum levels, when compared with CTL ($P < 0.05$; Table 3). The TyG and HOMA-IR indexes were higher in the HyO Sham group than in the CTL ($P < 0.01$; Table 3). At 6 months after the DJB operation, HyO DJB rats presented normalized serum insulin, TG, and NEFA concentrations (Table 3). The DJB operation also improved the action of insulin, since the TyG and HOMA-IR indexes were similar in the HyO DJB rats and the CTL (Table 3).

3.2. Liver morphology and lipid and glycogen contents

Fig. 2A shows that the macroscopic appearance of the liver of the

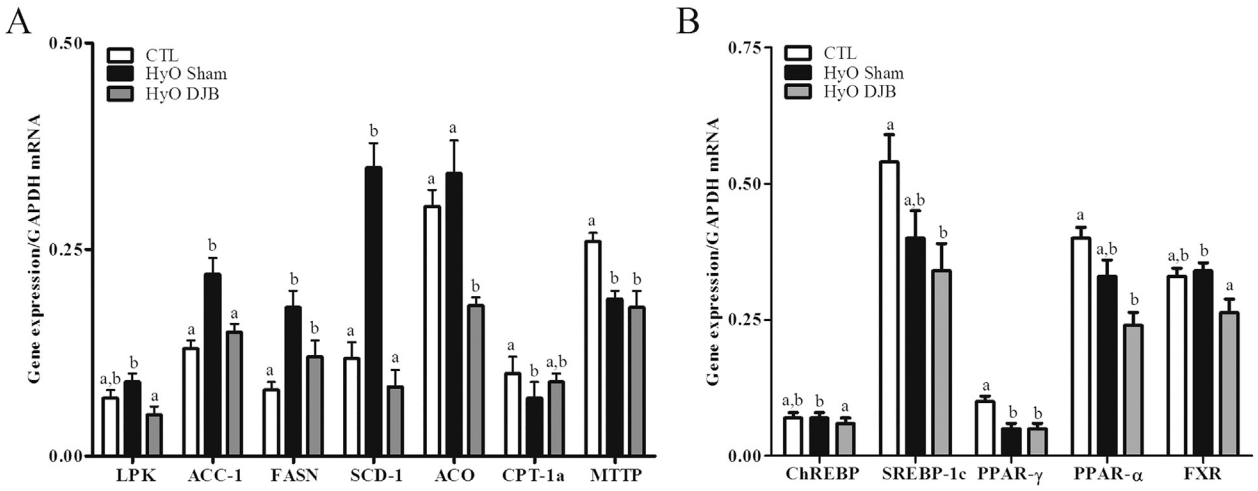

Fig. 3. DJB surgery improves the expression of hepatic genes involved in DN lipogenesis. Means ± SEM (n = 4–7 rats) of the mRNA expressions for (A) LPK, ACC-1, FASN, SCD-1, ACO, CPT-1a, MTTP; and (B) ChREBP, SREBP-1c, PPAR-γ, PPAR-α and FXR in the liver of CTL, HyO Sham and HyO DJB rats. Different letters over the bars indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

HyO Sham rats was uniformly pale, when compared to CTL. However, at 6 months after the DJB operation, the HyO DJB livers presented a partial reestablishment of the macroscopic appearance, which approached that observed in CTL rats (Fig. 2A). In addition, the liver weight was lower in the HyO Sham group, when compared to CTL ($P < 0.0001$; Fig. 2D). The DJB procedure did not alter liver weight in the HyO DJB rats.

Fig. 2B demonstrates that the liver of HyO rats presented hepatocytes with several cytoplasmic lipid vacuoles, associated with a displacement of the nuclei towards the cell periphery. These histological characteristics indicate that the liver of the HyO Sham rats presented steatosis and a profile of "microvesicular fat disease". These microscopic features differ from the liver histology of the CTL, in which the hepatocytes were arranged in rows, delimited by connective tissue containing sinusoidal capillaries (Fig. 2B). As expected, the CTL hepatocytes displayed abundant and homogeneous cytoplasm, central nuclei and absence of steatosis. DJB surgery normalized the hepatic steatosis, restoring the aspect of the hepatocyte cytoplasm and nuclear localization in the HyO DJB similar to CTL group (Fig. 2B). Liver fibrosis, which occurs in most types of chronic liver diseases, was not observed in the HyO rats (Fig. 2C).

Hepatic steatosis was also confirmed in the HyO rats by the higher hepatic TG content, when compared to CTL ($P < 0.002$; Fig. 2E). Hepatic glycogen content was also higher in the HyO Sham than in the CTL (Table 3). Six months after the DJB operation, hepatic TG and glycogen content in the HyO DJB rats was normalized (Fig. 2E and Table 3).

3.3. Expression of genes and proteins involved in DN lipogeneses and FA oxidation

The hepatic expression of the lipogenic genes, ACC-1, SCD-1 and FASN, was higher in the HyO Sham, when compared to CTL group ($P < 0.01$; Fig. 3A). However, the gene expressions of CPT-1a, involved in the β-oxidation process, and MTTP, involved in apolipoprotein B assembly, were reduced by approximately 26% in the HyO Sham liver, when compared with the CTL ($P < 0.05$; Fig. 3A). Accordingly, the hepatic ACC, SCD-1 and FASN protein contents were 73%, 34%, and 96% higher in the HyO Sham than in the CTL rats ($P < 0.05$; Fig. 4A, C and D). No changes in the expressions of the pACC/ACC, CPT-1a and MTTP proteins were observed (Fig. 4B, E and F).

Furthermore, the mRNA levels of the transcription factor, peroxisome proliferator-activated receptor (PPAR)-γ, was 50% lower in the HyO Sham liver than in the CTL ($P < 0.001$; Fig. 3B). However, no modifications in liver pyruvate kinase (LPK), acyl-CoA oxidase (ACO; Fig. 3A), carbohydrate-responsive element-binding protein (ChREBP), sterol regulatory element-binding protein (SREBP)-1c, farnesoid X receptor (FXR), and PPAR-α (Fig. 3B) mRNA expressions, were evidenced in the livers of the HyO Sham and CTL groups (Fig. 3A and B). At 6 months after the DJB operation, the LPK, ACC-1, SCD-1and ChREBP gene expressions were decreased in the HyO DJB liver to levels similar to those observed for CTL (Fig. 3). Accordingly, the protein expressions of the ACC and FASN enzymes were reduced in the livers of the HyO DJB rats, in comparison with the HyO Sham ($P < 0.02$; Fig. 4A and C). Remarkably, hepatic ACO and FXR mRNAs were lower in the HyO DJB, than in the HyO Sham group ($P < 0.05$; Fig. 3B). Finally, the pAMPK/AMPK protein expressions were similar between the groups (Fig. 4G).

4. Discussion

Hypothalamic obesity is a devastating condition that has no effective treatment at the present time. Bariatric surgery, frequently used to treat diet or genetic-induced obesity, shows no consistent benefits for hypothalamic obesity [2,7–9]. For the first time, we demonstrate that at 6 months after DJB surgery, HyO rats presented a normalized lipid profile in the plasma and liver, probably due to a reduction in the expression of enzymes involved in DN lipogenesis. Interestingly, the effect of DJB on liver steatosis in the HyO rats was not due to reductions in adiposity or BW.

Neonatal treatment with MSG can efficiently mimic the metabolic comorbidities that occur in human hypothalamic obesity [23]. As previously reported [24–30], HyO rats displayed lower BW, and presented an enhanced Lee index and fat stores. In addition, HyO rats were normoglycemic, hyperinsulinemic and insulin resistance. Also, they were hypertriglyceridemic, with increased NEFA serum levels, higher hepatic TG content and histological alterations in hepatocytes, consistent with hepatic steatosis. These alterations in lipids metabolism were associated with an enhanced hepatic mRNA and protein levels of enzymes involved in DN lipogenesis (ACC, FASN and SCD-1).

The majority of obese patients submitted to bariatric surgeries present NAFLD, which is linked to insulin resistance, type 2 diabetes and dyslipidemias [31–33]. The best treatment for NAFLD is weight loss, through lifestyle modifications. However, when it is unsuccessful, bariatric surgery may represent an alternative method for achieving substantial and sustained weight loss [34]. Accordingly, at 8 weeks following the DJB operation, genetic obese Otsuka Long-Evans Tokushima Fatty rats are reported to present improvements in hepatic steatosis, associated with weight loss, and increased glucagon-like

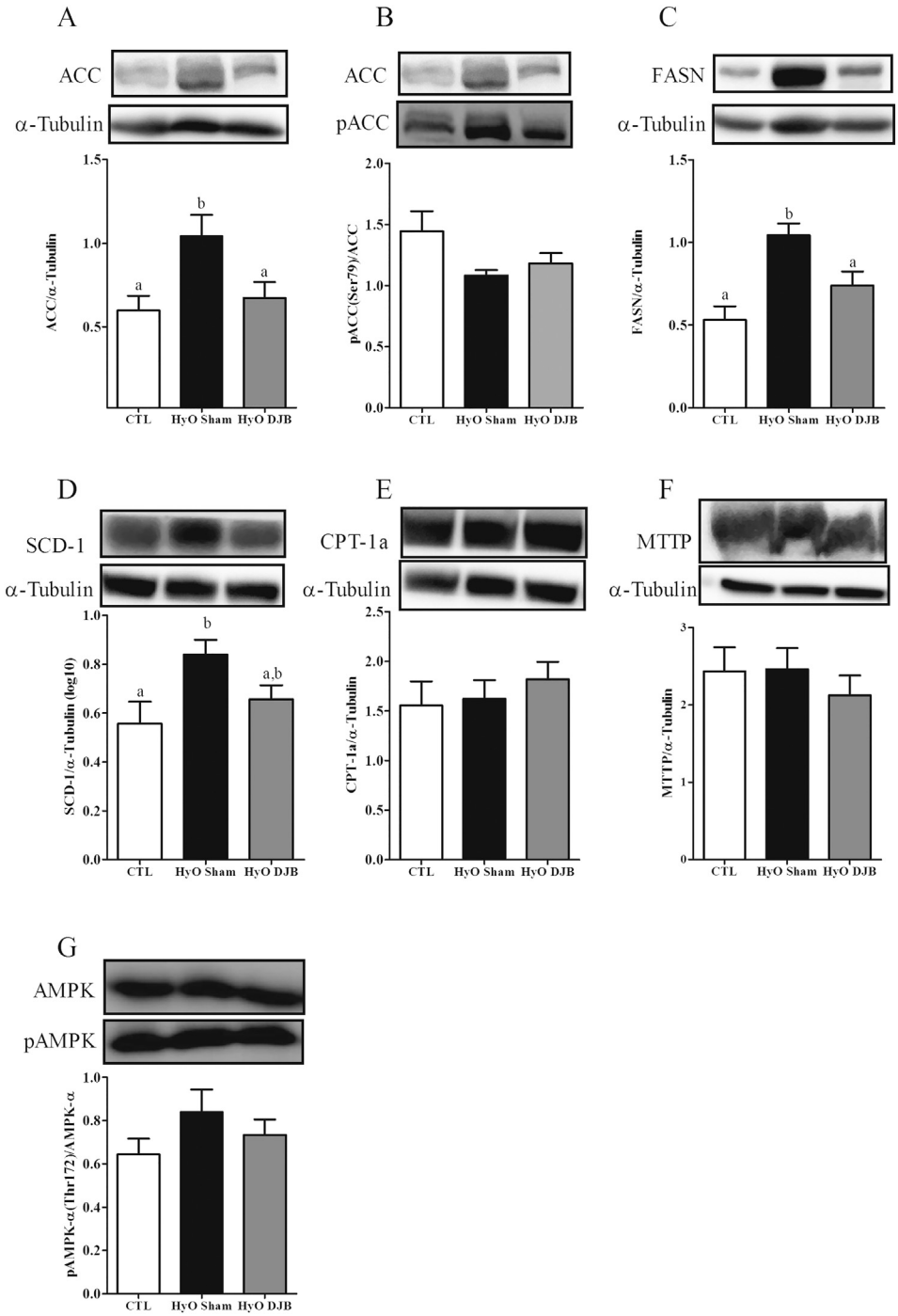

Fig. 4. DJB operation normalizes hepatic protein expression of DN lipogenesis enzymes in HyO rats. Means ± SEM (n = 6–10 rats) of the ACC (A), pACC/ACC (B), FASN (C), SCD-1 (D), CPT-1a (E), MTTP (F) and pAMPK/AMPK (G) protein expressions in the liver of CTL, HyO Sham and HyO DJB rats. Different letters over the bars indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

peptide 1 (GLP-1) and bile acids (BA) in the serum [35]. However, western-diet rats submitted to DJB demonstrated decreased hepatic TG content, without any alteration in body adiposity, at 1 or 2 months after surgery [10,12]. Similar effects of DJB were observed in high-fat diet type 2 diabetic rats, at 8 weeks after surgery [13]. Despite these reported improvements in hepatic steatosis in genetic and diet-induced obese rodents after DJB, there are few studies about the effect of this malabsorption procedure on hypothalamic obesity [17]. We, recently, evidenced that HyO DJB rats, display hepatic steatosis despite a reduction in serum TG levels [17] and an improvement in liver insulin resistance [16] at 2 months after surgery. These data demonstrate that the improvement in liver steatosis manifests later on after the DJB operation, in hypothalamic obesity and independent of the improvement in hepatic insulin resistance. Hepatic FA metabolism is normalized only at 6 months after the DJB operation, indicating that the benefits of bariatric surgeries may represent a slow phenomenon with a factor unknown yet.

NAFLD is characterized by an upregulation of hepatic lipogenic enzymes [36,37] or may manifest due to reductions in the expression/activity of enzymes involved in β-oxidation or VLDL assembling [6]. DJB surgery led to a reduction in the expressions of the LPK, ACC, and SCD-1 genes, and normalized the ACC and FASN protein contents in the HyO DJB liver. Additionally, these rodents displayed a lower hepatic mRNA content of ChREBP, a well-recognized transcription factor that mediates the transcriptional effect of glucose upon LPK and lipogenic genes [38]. Therefore, the downregulation in LPK, ACC, and SCD-1 mRNAs, induced by the DJB operation in HyO rats, may be linked to a reduction in ChREBP mRNA.

FXR is a ligand-activated transcription factor, whose activation can inhibit the expression of SREBP-1c, and its target lipogenic genes, preventing excessive FA synthesis [39]. In addition, FXR activates PPAR-α, which regulates the transcription of the FA oxidation genes; CPT-1 and ACO [39,40]. We observed that the DJB surgery reduced hepatic FXR and ACO mRNAs in the HyO DJB group. The mRNA for PPAR-α in the liver of the HyO DJB rats was lower than that of the CTL rats; it is possible that the downregulation of FXR/PPAR-α may decrease ACO transcription, although such an effect would reduce peroxisome β-oxidation in the HyO DJB rats. In addition, the FXR is a target for BA and regulates BA metabolism, *via* an autocrine mechanism [39]. Increased serum levels of BA were evidenced in diet and genetic-obese diabetic rats, submitted to DJB surgery [13,35]. An improvement in glucose homeostasis and hepatic steatosis, after DJB, in diabetic rats has been found to be associated with enhanced gut secretion of GLP-1by BA stimulation [35]. As such, the reduction in the gene expression of hepatic FXR, in HyO DJB rats, may suggest that the mechanism of action, by which the DJB operation exerts benefits in hypothalamic obesity, may differ from that observed in genetic and diet-induced obesity.

Another important finding of this study was that HyO rats exhibiting an increased content of hepatic glycogen. Enhanced glycogen content has also been seen in the livers of high-fat and western-diet obese rodents [10,41]. This effect may increase hepatic lipogenesis and the development of NAFLD. Lu et al. [41] demonstrated that, in insulin resistant high-fat diet mice, increased hepatic glycogen was linked to an enhanced gene and protein expression of the glycogenic scaffolding protein (PTG/R5). This protein regulates the mobilization and storage of glycogen and also directs the glucose excess to FA synthesis *via* a mechanism involving the activation of the target of rapamycin complex-1 (mTORC1), which regulates downstream lipogenic genes. Accordingly, the deletion of PTG/R5 prevents hepatic glycogen accumulation and steatosis in high-fat diet mice [41].

We, herein, investigated whether decreased lipogenesis that was achieved by DJB in HyO rats may be associated with AMP-activated protein kinase (AMPK) activation. AMPK is known to inhibit lipogenesis *via* phosphorylation of ACC, decreasing malonyl-CoA levels and enhancing the action of CPT-1a and FA oxidation [42]. Furthermore, AMPK may improve hepatic insulin sensitivity inhibiting lipogenesis by downregulation of the mTORC1 pathway [43]. However, no modifications in pAMPK/AMPK protein expressions were observed in the HyO DJB and HyO Sham rats. Therefore, new studies are necessary to determine how lipogenesis is downregulated in hypothalamic obesity by DJB surgery.

5. Conclusion

In summary, we report for the first time that DJB surgery has a delayed effect upon NAFLD in hypothalamic obesity, and that the improvement in hepatic steatosis in HyO rats is not associated with weight loss or reductions in adipose tissue stores. The benefits of DJB on steatosis in hypothalamic obesity were associated in part with a reduction in the expression of hepatic enzymes involved in DN lipogenesis. However, additional studies are necessary to appropriately dissect this mechanism.

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