

Duodenal jejunal bypass attenuates non-alcoholic fatty liver disease in western diet-obese rats¹

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ABSTRACT

PURPOSE: To evaluate the effects of duodenal-jejunal bypass (DJB) on serum and hepatic profiles of obese rats fed on a western diet (WD).

METHODS: Twenty eight male Wistar rats were fed a standard rodent chow diet (CTL group) or WD *ad libitum*. After 10 weeks, WD rats were submitted to sham (WD SHAM) or duodenal-jejunal bypass (WD DJB). Body weight, fat pad depots, glycemia, insulinemia, HOMA-IR, TyG, lipids profile and hepatic analyses were evaluated two months after surgery.

RESULTS: The WD SHAM group presented greater obesity, hyperglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemia and hepatic steatosis than the CTL group. WD DJB rats presented decreased serum glucose and insulin resistance, when compared to WD SHAM animals, without changes in insulinemia. In addition, DJB surgery normalized serum TG and attenuated TG accumulation and steatosis in the liver of the WD DJB group. Hepatic ACC and FAS protein expressions were similar in all groups.

CONCLUSION: Duodenal-jejunal bypass attenuates hepatic parameters of non-alcoholic fatty liver disease in obese rats fed on a western diet.

Key words: Jejunoileal Bypass. Fatty Liver. Obesity. Rats.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease worldwide, affecting up to 30% of adults in Western countries and nearly 10% of individuals in developing nations¹. Since NAFLD is strongly associated with obesity², insulin resistance, type 2 diabetes mellitus (T2DM) and dyslipidemia, it has been recognized as the main hepatic manifestation of metabolic syndrome³. The first sign of NAFLD is hepatic triglyceride accumulation (steatosis), which may progressively evolve to non-alcoholic steatohepatitis (NASH), liver inflammation, fibrosis, cirrhosis and hepatocellular carcinoma (HCC)⁴.

While pathophysiologic mechanisms of NAFLD are still poorly elucidated, weight reduction has been considered the best treatment for NAFLD⁵. Life-style modifications and physical activity improvements associated, or not, with pharmacotherapy are options for weight loss. However, in cases where behavioral and pharmacological strategies are insufficient, bariatric surgery has offered a powerful alternative to treat both obesity and its comorbidities⁶. In humans, body weight loss after bariatric surgery improves histopathologic features of NAFLD and decreases the risk of progressive liver impairment^{5,7}. The mechanisms mediating improvements in NAFLD following bariatric surgery are complex and not fully understood, but a diminished insulin resistance and dyslipidemia, paralleled with alterations in gut hormones production, have emerged as the main reasons⁸.

The cafeteria diet- or western diet (WD) is an experimental rodent diet model that more accurately reflects the variety of highly palatable, energy dense foods that are prevalent in Western society and associated with the current obesity pandemic. In this model, animals are allowed free access to standard chow and water while concurrently offered highly palatable, energy dense, unhealthy human foods *ad libitum*⁹. WD promotes rapid weight gain, fat accumulation, insulin resistance and a fatty liver^{9,10}. On the other hand, the duodenal-jejunal bypass (DJB), a malabsorptive bariatric surgery described by Rubino¹¹, improves glucose homeostasis in diabetic rodents¹¹ and humans¹². In a recent report, we showed that DJB totally restored insulinemia and insulin sensitivity, and ameliorated beta-cell secretory capacity in WD-obese rats¹³. Nevertheless, few experimental studies have focused on the effects of DJB on NAFLD parameters. Thus, the current study was conducted to determine the effects of DJB on the plasma profile, liver histopathology and protein expression of enzymes involved in fatty acid synthesis in WD obese rats.

Methods

The experimental procedures are agreement with the Ethical Principles for Animal Research established by the National Council for Control of Animal Experimentation (CONCEA) and approved by the institutional Committee for Ethics in Animal Experimentation (CEUA/UNIOESTE protocol number 8709).

Twenty eight male Wistar rats (aged eight weeks) were divided into three groups: Control group (CTL, n=10), which received a standard rodent chow diet and water *ad libitum*; WD sham operation group (WD SHAM, n=09), which received a WD and soft drink *ad libitum* for all experimental procedures and was submitted to a sham operation after 10 weeks of diet; and WD duodenal-jejunal bypass group (WD DJB, n=09), which received the WD diet for the entire experimental period and was subjected to duodenal-jejunal bypass (DJB) 10 weeks after starting the WD diet. Seven days before and after the surgery, the WD groups received a high-energy liquid diet and the CTL group received standard liquid chow diet. The rats were housed in standard cages and maintained on a 12h light/dark cycle (lights on 06:00-18:00h) and controlled temperature ($22^{\circ} \pm 1^{\circ}\text{C}$).

Duodenal-jejunal bypass (DJB) and sham surgeries

DJB and SHAM surgeries were performed after 10 weeks of WD feeding. Preoperative procedures were performed as described by Araujo *et al.*¹³ and DJB was performed as described by Jurowich *et al.*¹⁴. Briefly, rats were deprived of food for 16-18h and were anesthetized with isoflurane (Isoforine®, Cristália, SP, Brazil). After laparotomy, DJB surgery was performed by postpyloric transection of the duodenum, closure of the duodenal stump, and reconstruction of the intestinal passage by terminal lateral duodenojejunosomy 5cm aboral of the flexura duodenojejunalis. The effectiveness of duodenal ligation was determined by injection of saline through the stomach and observing that no saline passed beyond the area of ligation. In the Sham group, a midline incision in the anterior abdominal wall was made; the stomach, duodenum and intestines were massaged, and the incision was closed. In the DJB group, three rats died due to postoperative complications.

Diet

The CTL group received a standard diet (Biobase, Brazil) comprised of 3.8 kcal/g (70% carbohydrate, 20% protein and 10% fat) and water *ad libitum*. WD SHAM and DJB groups

received the high palatable, hypercaloric WD diet, consisting of standard chow (Biobase, Brazil), Italian salami (Sadia, Brazil), mini bread (Nutrella, Brazil), corn snacks (Cheetos, Pepsico, Brazil), marshmallow (Fini, Brazil), mixed sausage (Sadia, Brazil), chocolate cake (Renata, Selmi, Brazil), cookie cornstarch (Zadimel, Brazil), mortadella (Frimesa, Brazil), bacon flavor crisps (Trophy, Helena, Brazil), wafer chocolate (Bauduco, Brazil) and 350 ml of degassed Coca-Cola daily (Coca-Cola, Brazil) or Guarana (Antarctica, AmBev, Brazil).

Obesity parameters

The animals' body weights were measured during the experimental period (from 8 to 18 weeks after the start of the diet). One week before euthanasia, five animals from each group were maintained in metabolic cages for three days to determine food ingestion and stool production during 12h. Two months after surgery, all animals were euthanized. Final body weight (BW) and nasoanal length were measured in all groups to obtain the Lee Index [from the ratio of body weight (g)^{1/3}/Nasoanal length (cm) x 1000], which was used as a predictor of obesity in rodents. Retroperitoneal and periepididymal fat pads were removed and weighed.

Plasma biochemical analysis

Triglycerides (TG) and total cholesterol (CHOL) were measured in the fasting (8h) state using standard commercial kits, according to the manufacturer's instructions (Laborclin®, Bioliquid, Pinhas, PR, BR). Glucose levels were measured using a glucose analyzer (Abbott®, Optium Xceed, Alameda, CA) and plasma insulin was measured by RIA, using rat insulin standard.

HOMA and TyG index

Tissue insulin sensitivity was also evaluated by the homeostasis model assessment (HOMA), using the HOMA index of insulin resistance [(HOMA-IR) = fasting insulin (U/mL) x fasting glucose (mM)/22.5]. The product of fasting glucose and triglycerides (TyG) was calculated as the Ln [fasting triglycerides (mg/dL) x fasting glucose (mg/dL)/2]. The TyG index is expressed in a logarithmic scale.

Histopathology

Liver samples were fixed in 10% formalin, embedded in Paraplast (Sigma Co, Saint Louis, MO), sectioned into slices of seven microns in thickness, and stained with hematoxylin and eosin (H&E). Liver histopathology was examined and graded according to the magnitude of steatosis, based on Brunt's classification with modifications for rodent models. Briefly, steatosis was graded (0-3), as follows: 0, none to 5% of hepatocytes affected; 1, >5% to 30% affected; 2, >30% to 60% affected; and 3, >60% affected.

TG and CHOL content of liver

The liver was weighed and a fragment was removed for determination of the TG and CHOL content. Liver lipids were extracted by the method of Folch. The extract was evaporated and then diluted in isopropanol. TG and CHOL contents were measured as described above.

Protein expression

For protein expression determination, a fragment of liver was solubilized in homogenization buffer at 4°C (containing: 100 mM tris pH 7.5, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride and 1% Triton-X 100) using a Polytron MA 102 generator (model MA 102/Mini; Piracicaba, SP, Brazil). Extracts were then centrifuged at 12.600 g at 4°C for 40 min to remove insoluble material. Protein concentration was measured by the Bradford dye method, using BSA to form a standard curve and Bradford reagent (Bio-Agency Lab., São Paulo, SP, Brazil). For SDS gel electrophoresis and Western blot analysis, the samples were homogenized with loading buffer containing beta-mercaptoethanol. After heating at 95°C for 5 min, the proteins were separated by electrophoresis (150 µg protein/lane, 6.5% gels) and afterwards transferred to nitrocellulose membranes that were subsequently blotted with polyclonal antibodies to Acetyl-CoA carboxylase (ACC, cat. sc-7985R) and Fatty Acid Synthase (FAS, cat. sc-20140, Santa Cruz, CA, USA). α -Tubulin was used as an internal control (1:1.000, cat. T5168, Sigma-Aldrich Chemicals, St Louis, MO, USA). Visualization of specific protein bands was performed by incubating the membranes with goat anti-rabbit secondary antibody (1:10.000; cat. 7074, Cell Signaling Technology, Boston, MA, USA), followed by exposure to X-ray film. The band intensities were quantified by optical densitometry using the free software.

Statistical analysis

Data are expressed as means \pm SEM accompanied by the indicated number of independent experiments. For statistical analyses, the groups were compared using one-way analysis of variance (ANOVA) followed by the Tukey post-test ($p < 0.05$). Graphs were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software®).

Results

Animal features

Results in Figure 1A clearly show that WD SHAM and WD DJB rats had a massive weight gain, which started as early on as two weeks after dietetic intervention, as compared to CTL rats. Following DJB or sham-operation, at week 10, the WD DJB rats presented a transient weight loss during the first post-operative week, which was not sustained. When body weight was analyzed during the whole period (Figure 1B), the WD SHAM animals demonstrated an increase in this parameter, compared to CTL, and no difference was found between the WD DJB rats and WD SHAM animals. The WD SHAM rats showed an increased food intake in 12 hours (24.50 ± 0.96 g/day, $p < 0.05$), as compared to the CTL group (19.0 ± 1.0 g/day), which was not reversed by DJB (26.80 ± 1.55 g/day, $p < 0.05$). Moreover, stool production for both the WD DJB and WD SHAM animals (0.67 ± 0.23 and 1.23 ± 0.38 g/day, respectively, $p < 0.01$) was lower than those fed a regular chow (3.57 ± 0.30 g/day), $p < 0.01$).

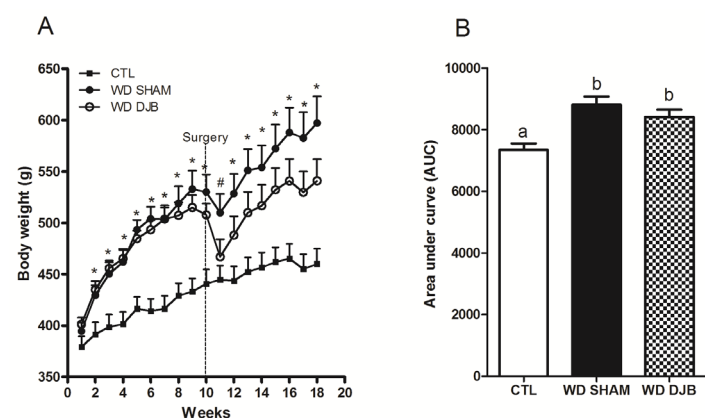


FIGURE 1 - Evolution of body weight during entire experimental period (A) and AUC of body weight in CTL, WD SHAM and WD DJB rats. *WD SHAM and WD DJB vs. CTL. # WD SHAM vs. CTL. One-way ANOVA with Tukey post hoc test. $p < 0.05$. $n = 6-10$.

Notably, DJB surgery attenuated white adipose tissue accumulation in WD DJB rats, since their retroperitoneal and perigonadal fat pads did not differ from those of either WD SHAM or CTL rats (Table 1). The combination of these alterations resulted in a decrease of 4% in the Lee Index of the WD DJB rats, as compared to the WD SHAM rats (Table 1), two months after surgery.

TABLE 1 - Obesity parameters in CTL, WD SHAM and WD DJB rats.

	CTL	WD SHAM	WD DJB
Final Body weight (g)	447 \pm 14 ^a	591 \pm 27 ^b	545 \pm 21 ^b
Lee index	320 \pm 2.3 ^a	340 \pm 2.4 ^b	326 \pm 4.6 ^a
Retroperitoneal fat pad (% BW)	2.4 \pm 0.23 ^a	3.72 \pm 0.14 ^b	3.0 \pm 0.15 ^{ab}
Perigonadal fat pad (% BW)	1.7 \pm 0.15 ^a	2.6 \pm 0.12 ^b	2.2 \pm 0.18 ^{ab}

Data are means \pm SEM ($n = 6-10$). Different letters indicate significant difference. One-way ANOVA with Tukey post hoc test ($p < 0.05$).

Biochemical parameters and insulin resistance

WD SHAM animals presented hypertriglyceridemia, when compared to CTL rats (Figure 2A, $p < 0.01$); furthermore, DJB surgery normalized fasting TG levels after two months. Total CHOL was similar in all groups (Figure 2B). The fasting glucose and insulin levels were higher in the WD SHAM than CTL animals (Figure 2C and D, respectively, $p < 0.001$). There was a significant improvement in fasting glucose in the WD DJB group ($p < 0.05$) despite insulinemia being similar to that of the WD SHAM animals. To reinforce these findings, we investigated insulin resistance using HOMA-IR and the TyG index. The WD SHAM rats demonstrated an increase in HOMA-IR and in the TyG index, compared to the CTL group (Figure 2E and F, respectively, $p < 0.001$); DJB surgery normalized these parameters in WD DJB animals, compared to the WD SHAM group ($p < 0.05$ and $p < 0.01$, respectively). Taken together, these results indicate that WD feeding induced metabolic changes compatible with the features of metabolic syndrome, which were partially restored two months after DJB surgery despite the maintenance of the cafeteria diet.

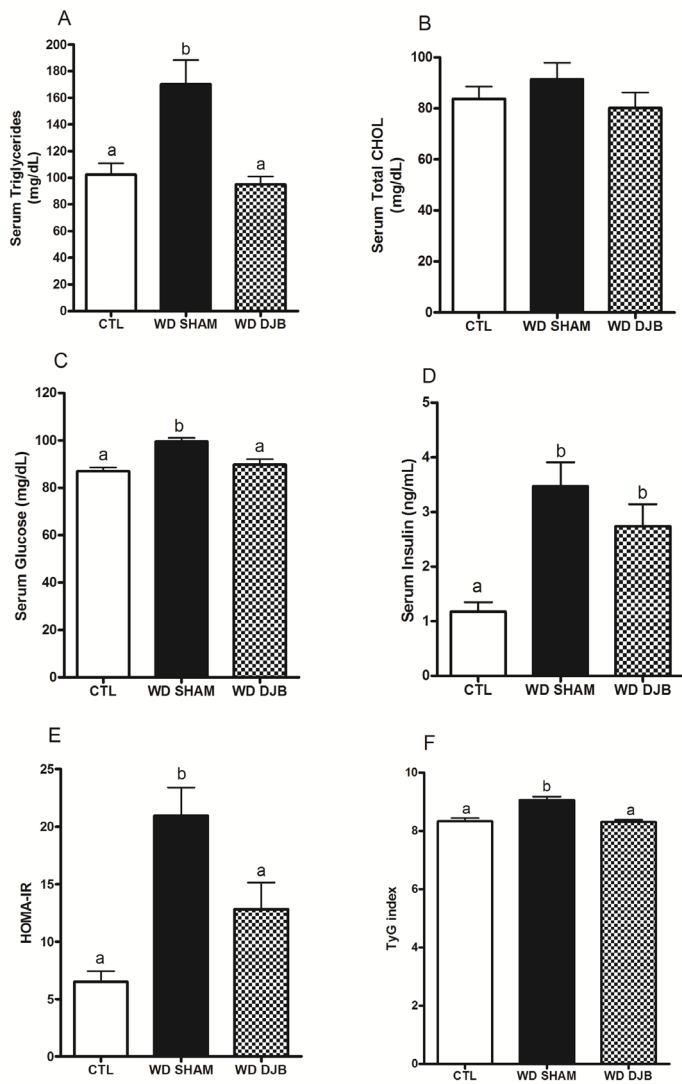


FIGURE 2 - Fasting serum level of triglycerides (A) total cholesterol (B), glucose (C) and insulin (D). HOMA-IR (E) and TyG index (F) in CTL, WD SHAM and WD DJB rats. Different letters indicate significant difference. One-way ANOVA with Tukey post hoc test. $p < 0.05$. $n = 6-10$.

Histological analyses of liver

We next evaluated the impact of DJB on liver histological patterns and the fat accumulation of WD obese rats. Histological analysis of the CTL and WD livers, according to the Brunt’s NAFLD score, revealed that all the WD SHAM obese rats had grade 1 or 2 fatty degeneration (Table 2), mainly characterized by microvacuolar steatosis (Figure 3C and D, respectively). No changes were found in the CTL rats (Figure 3A and B, respectively and Table 2). Importantly, approximately 30% of WD DJB rats had no steatosis (Table 2), demonstrating the effectiveness of DJB for ameliorating fat degeneration (Figure 3E and F, respectively). Other NAFLD/NASH features, such as ballooning or inflammation were not detected in any group. To reinforce histological analyses,

after 18 weeks of WD feeding, WD SHAM rats were found to present increased liver weight and TG content, without any effect on CHOL content (Figure 3G, H and I, respectively, $p < 0.001$ and $p < 0.01$). WD obese rats submitted to DJB had lower liver TG levels than those of the WD SHAM rats, although these levels were still significantly higher than those of CTL rats. No effects of DJB were observed on either liver weight or on CHOL content (Figure 3G and I, respectively).

TABLE 2 – Degree of steatosis in the liver of rats CTL, WD SHAM and WD DJB.

Groups	Degree 0	Degree 1	Degree 2
CTL	100%	0%	0%
WD SHAM	0%	50%	50%
WD DJB	28.6%	28.6%	42.8%

N=4-7

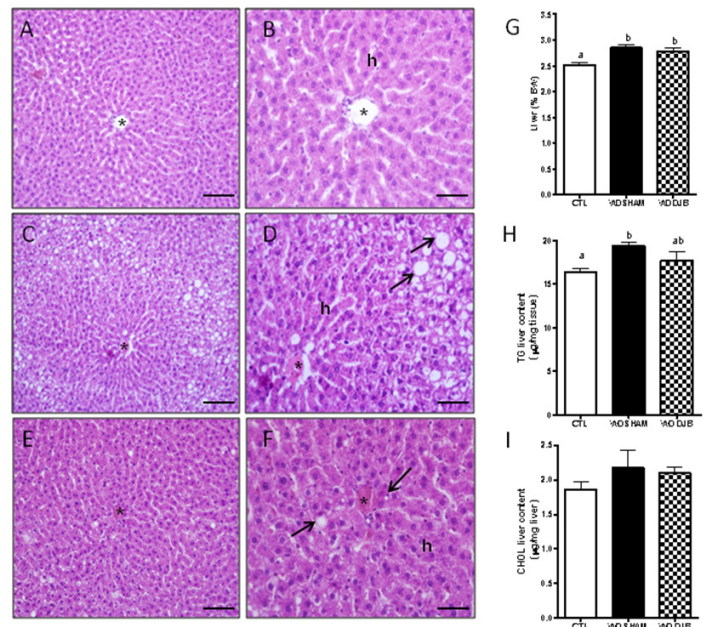


FIGURE 3 - Representative photomicrographs of hematoxylin and eosin-stained sections of livers from CTL (A,B), WD SHAM (C,D) and WDDJB (E,F) animals. Arrows indicate steatosis. *: central veins; h: hepatocytes. Figures A, C, E: Bars=100µm. Figures B, D, F Bars= 50µm. (G) Liver weight, (H) TG and (I) CHOL liver content in CTL, WD SHAM and WD DJB animals. Different letters indicate significant difference. One-way ANOVA with Tukey post hoc test. $p < 0.05$. $n = 6-10$.

Protein expression

In order to explain the improvement in steatosis in WD obese rats submitted to DJB surgery, we investigated the expressions of enzymes involved in fatty acid synthesis. We observed that hepatic ACC and FAS protein expressions were similar in all groups (Figure 4A and B, respectively).

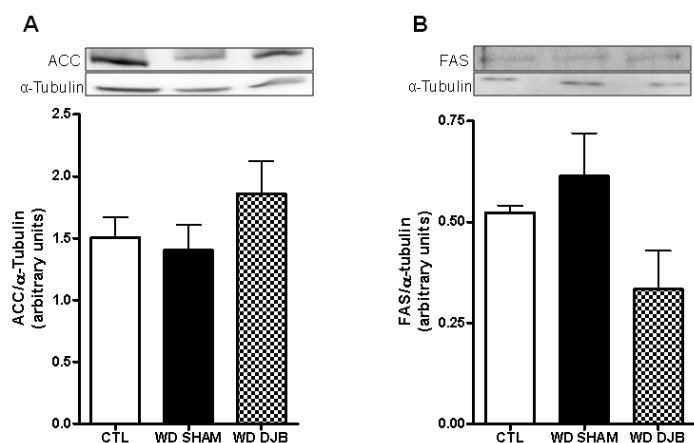


FIGURE 4 - Protein expression of ACC (A) and FAS (B) in the liver of CTL, WD SHAM and WD DJB animals. One-way ANOVA with Tukey post hoc test. $p < 0.05$. $n = 3-4$.

Discussion

Different techniques of bariatric surgery lead to improvements in steatosis, inflammation and fibrosis^{5,15}, however, there are no reports demonstrating the effect of DJB on NAFLD. Therefore, in the present study, we used WD obese rats to demonstrate the effects of the DJB on NAFLD.

WD is known to promote obesity and features of T2DM in rats^{9,13} and provides a highly relevant model for studies on diet-induced metabolic syndrome. WD was found to effectively induce obesity, hyperglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemic and hepatic steatosis. Currently, hepatic steatosis is considered the most common chronic liver disease and its incidence has grown in parallel with the pandemic rates of obesity and metabolic syndrome². In both humans¹⁵ and rodents⁹, TG accumulation in hepatocytes is the main factor in the development of hepatic steatosis. Many studies have consistently shown that TG accumulation is due to insulin resistance, which also causes increased VLDL secretion¹⁶. Data herein presented show that WD SHAM rats had a higher liver TG content and steatosis, which were associated with hyperinsulinemia and insulin resistance. Nevertheless, the assessment of liver insulin resistance, via TyG Index calculation, showed that WD SHAM animals were more resistant than CTL rats.

At two months after DJB surgery, partial reductions in retroperitoneal and perigonadal fat accumulation were observed, as well as improved glycemia and insulin sensitivity, without modifications in body weight and fasting insulin. Ours results are in accordance with others reporting that DJB surgery is characterized as a malabsorptive bariatric procedure that improves glucose homeostasis in genetically and diet-induced obesity^{11,13,14} without changing body weight.

Weight loss is effective for improving NASH^{5,7}; many options are available to induce weight loss, but bariatric surgery is the most effective therapy available, causing a reduction of about 20-30% in body weight. Approximately, 70% of patients referred for bariatric surgery have NAFLD and 25% present NASH; of these, half have advanced fibrosis and 1-2% has cirrhosis¹⁷. Different bariatric surgery techniques can be used to treat obesity and also lead to improvements in steatosis, inflammation and fibrosis⁵, but, the effect of the DJB on NAFLD has not been investigated to date. For the first time, we demonstrate that DJB attenuated TG liver content and steatosis and totally restored triglyceridemia in rats submitted to a cafeteria diet. Since no changes in body weight and serum insulin levels were observed in WD DJB rats, when compared to WD SHAM animals WD (Figure 1 and Table 2, respectively), we suggest that other factors may be associated with the improvement in hepatic steatosis in these animals. Patients undergoing biliopancreatic diversion, adjustable gastric banding or Roux-en-Y gastric bypass showed improvements in steatosis and ballooning degeneration, which were associated with changes in insulin sensitivity^{15,18}. Since DJB surgery improved insulin resistance and hyperglycemia in our rats, we suggest that these factors may contribute to the partial reduction in the total content of hepatic TG and the histological changes observed in WD DJB animals, independently of weight loss.

NAFLD can be caused by multiple factors, including lipolysis from fat cells, increased intake of dietary fat, and the impairment of fatty acid beta oxidation by mitochondrial dysfunction¹⁹. In addition, fatty acid synthesis by de novo lipogenesis in the liver contributes greatly to hepatic steatosis in obesity and diabetes²⁰. Hepatic protein expression of ACC, which converts acetyl-CoA to malonyl-CoA and FAS, which use malonyl-CoA to form palmitic acid, were not altered by WD-obesity or DJB surgery (Figure 4). Other enzymes involved in fatty acid synthesis, as well as beta oxidation should be investigated.

Conclusion

Duodenal-jejunal bypass attenuates hepatic parameters of non-alcoholic fatty liver disease in obese rats fed on a western diet.

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