Biomedicine & Pharmacotherapy 141 (2021) 111807

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Agomelatine reduces circulating triacylglycerides and hepatic steatosis in fructose-treated rats

Vanessa Barbosa Veronesi [a], Mariana Rodrigues Pioli [a], Dailson Nogueira de Souza [a], Caio Jordão Teixeira [b], Gilson Masahiro Murata [b,1], Junia Carolina Santos-Silva [a], Fernanda Ballerini Hecht [a], Julia Modesto Vicente [a], Silvana Bordin [b], Gabriel Forato Anhê [a,*]

[a] Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, 105 Alexander Flemming St., Zip Code: 13083-881, Campinas, SP, Brazil
[b] Department of Physiology and Biophysics, Institute of Biomedical Science, University of Sao Paulo, 1524 Prof. Lineu Prestes Ave., ICB 1, Zip Code: 05508-000, Sao Paulo, SP, Brazil

ARTICLE INFO

Keywords:
Agomelatine
Fructose
Lipid metabolism
Hepatic steatosis
Melatonin

ABSTRACT

Agomelatine (AGO) is an antidepressant drug with agonistic activity at melatonin receptor 1 (MT1) and MT2 and with neutral antagonistic activity at serotonin receptor 5-HT2$_C$. Although experimental studies show that melatonin reduces hypertriglyceridemia and hepatic steatosis induced by excessive fructose intake, no studies have tested if AGO exerts similar actions. To address this issue we have treated male Wistar rats with fructose (15% in the drinking water) and/or AGO (40 mg/kg/day) for two weeks. AGO reduced body weight gain, feeding efficiency and hepatic lipid levels without affecting caloric intake in fructose-treated rats. AGO has also decreased very low-density lipoprotein (VLDL) production and circulating TAG levels after an oral load with olive oil. Accordingly, treatment with AGO reduced the hepatic expression of fatty acid synthase (*Fasn*), a limiting step for hepatic *de novo lipogenesis* (DNLG). The expression of apolipoprotein B (*Apob*) and microsomal triglyceride transfer protein (*Mttp*) in the ileum, two crucial proteins for intestinal lipoprotein production, were also downregulated by treatment with AGO. Altogether, the present data show that AGO mimics the metabolic benefits of melatonin when used in fructose-treated rats. This study also suggests that it is relevant to evaluate the potential of AGO to treat metabolic disorders in future clinical trials.

1. Introduction

Circadian production of melatonin by the pineal gland is a key endocrine signal that translates environmental information (such as the length of the dark period) to living organisms and synchronizes the circadian clock in the hypothalamic suprachiasmatic nucleus (SCN) [1]. At the cellular level, melatonin acts by binding at the G protein-coupled receptors melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2) [2,3]. MT1 and MT2 receptors are also expressed in peripheral tissues where they play pivotal roles in energy metabolism [4].

Mice knockout for MT1 display glucose intolerance and impaired insulin signaling in the liver [5]. Mice knockout for MT1 also exhibit whole-body insulin resistance and exacerbated body weight gain when fed with a high fat diet (HFD) [4,6]. In rats, surgical ablation of the pineal gland leads to increased hepatic glucose production, glucose intolerance and insulin resistance in adipocytes [7–9].

On the other hand, the metabolic benefits of melatonin have been consistently described in rats exposed to fructose-enriched diets or beverages. Melatonin is able to improve glucose tolerance and insulin sensitivity and reduce hepatic gluconeogenesis in rats exposed to high fructose intake [10–12]. Melatonin is also able to reduce circulating triacylglycerides (TAG), free fatty acids (FFA) and total cholesterol levels and hepatic lipids in rats exposed to excessive fructose [11,12]. Noteworthy, chronic consumption of fructose-enriched diets or beverages was previously described to reduce melatonin production by rats [10,13].

Agomelatine (AGO) is a commercially available antidepressant with agonistic activity at MT1 and MT2 and neutral antagonistic activity at serotonin receptor 5-HT$_{2\ C}$ [14]. Recent studies demonstrate that AGO alleviates anxiety and depression and reduces the expression of TNF-α,

* Corresponding author.
E-mail address: anhegf@unicamp.br (G.F. Anhê).
[1] Present address: Department of Medical Clinic, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil, Zip Code 01246–403

https://doi.org/10.1016/j.biopha.2021.111807
Received 9 March 2021; Received in revised form 28 May 2021; Accepted 7 June 2021
Available online 11 June 2021

IL-6 and IL-1β in the hippocampus of rats fed with a HFD [15]. AGO was also described to reduce gonadal TNF-α expression, but not glucose levels, in rats rendered diabetic by streptozotocin injections [16]. On the other hand, data from clinical trials with small sample sizes suggest potential benefits of AGO for the glucose control of diabetic patients with depressive disorders [17,18]. In spite of these observations, no detailed experimental investigation has evaluated if AGO exerts metabolic actions similar to that of melatonin [19].

In order to address this issue, we presently evaluated the metabolic effects of AGO in rats exposed to water containing 15% fructose. We have performed *in vivo* tests to evaluate the production of very-low density lipoprotein (VLDL) as well as the kinetics of circulating TAG after *gavage* with olive oil. We have also assessed intestinal and hepatic TAG contents, hepatic neutral lipid staining by Oil Red O and the genes expression and enzymatic activity of key steps of lipid metabolism.

2. Materials and methods

2.1. Experimental design and treatments

The experimental design was approved by Committee for Ethics in Animal Experimentation of the State University of Campinas (Protocols 4545–1/2017 and 5006–1/2018). Four week-old male Wistar rats were purchased from the Animal Breeding Center of the State University of Campinas (CEMIB, Campinas, Sao Paulo, Brazil) and, unless otherwise stated, housed in collective cages with standard chow (Nuvilab CR1; Nuviltal, Colombo, PR, Brazil) and water *ad libitum*. The cages were kept in ventilated chambers under a 12 h light/12 h dark cycle with room temperature was set to 22 ± 2 °C.

By reaching 7 weeks of age, all rats were subjected to an adaptation period of seven days with one daily *gavage* of 0.5 ml of 0.9% NaCl solution. After the adaptation period, the rats were assigned to two groups. One group was kept with tap water available *ad libitum,* while the other group had the water replaced by 15% fructose solution. This treatment was carried out for two weeks because this interval of exposure to fructose was described to be sufficient to increase TAG in rats [20]. The concentration of fructose (15%) was based on a previous investigation reporting that this amount of sugar increases circulating TAG and VLDL and cause hepatic steatosis in rats [21].

Simultaneously to the treatment with fructose, half of the rats belonging to both groups received daily *gavages* containing AGO. Commercially available tablets containing 25 mg of AGO (Valdoxan; Servier Laboratories, Suresnes, France) were ground to a homogeneous powder using a mortar and a pestle. The powder was mixed with 0.9% NaCl solution (1 ml/tablet) to make a 25 mg/ml AGO suspension. The volume administered to the rats was calculated to yield a final AGO dose of 40 mg/kg of body weight. This dose was based on previous studies reporting that AGO reduces corticosterone levels and neuronal/behavioral parameters in rats exposed to acute stress or HFD [15,22,23]. When treated with AGO, rats drinking either tap water or 15% fructose were respectively designed as AGO or FRU+AGO rats.

The remaining rats drinking either tap water or 15% fructose were treated with daily *gavages* containing 0.5 ml of a vehicle solution with lactose (62 mg/ml). Lactose (62 mg/tablet) was described by the manufacturer as the main excipient of the tablet (https://www.medicines.org.uk/emc/medicine/21830#gref). When treated with the vehicle, rats drinking either tap water or 15% fructose were respectively designed as CTL or FRU rats.

A separate cohort of rats (10 week-old) was assigned to a pilot protocol to test if hypertriglyceridemia induced by fructose could be attenuated by a drug with known lipid lowering effect. The rats used in this protocol were assigned to 3 different groups, as follows: 6 rats were kept with standard chow *ad libitum* and water (CTL), 6 rats were kept with standard chow *ad libitum* and water with 15% fructose (FRU) and 6 rats received gemfibrozil-enriched chow *ad libitum* plus water with 15% fructose (FRU+GEM). This treatment was carried out during 2 weeks. Gemfibrozil-enriched chow was prepared by adding gemfibrozil solution (31.5 mg/ml in ethanol) to pellets of standard chow (0.1 ml/g of chow). The pellets were then incubated for 48 h at 45 °C to allow complete ethanol evaporation. Taking into account the approximate daily food intake of 20 g/day and the mean initial body weight of 350 g, rats assigned to the FRU+GEM group consumed an average of 180 mg/kg/day of gemfibrozil.

2.2. Feeding efficiency

The rats dedicated to the measurement of feeding efficiency were housed in isolated cages at the beginning of the adaptation protocol. All treatments were carried out as described above. Body mass was assessed before and after treatments. Chow and liquid intake were daily monitored. Feeding efficiency during the two-week protocol was expressed as the body mass gained (g) relative to the amount of calories consumed (kcal). The 15% fructose solution and the standard chow were assumed to contain 0.54 kcal/ml and 3.4 kcal/g, respectively.

2.3. Tissue sampling

Rats were overnight fasted before euthanasia performed with an i.p. injection with sodium thiopental (80 mg/kg). Body mass was acquired immediately before decapitation. Trunk blood was collected to extract the serum. The retroperitoneal and epididymal fat pads and fragments of liver were dissected, weighted and frozen at − 80 °C for further processing.

Serum samples were used for total cholesterol and TAG measurements with enzymatic/colorimetric kits (LABORLAB, Sao Paulo, SP, Brazil). Blood glucose was measured in blood samples obtained from tail punctures using a glucometer and appropriate reactive strips (On Call Plus; Acon Laboratories, San Diego, CA, USA).

Segments with 6 cm of the duodenum, jejunum and the ileum were dissected following anatomical references previously described [24]. The segments were gently washed in iced-cold 0.9% NaCl solution and transversally cut into three samples. One sample was frozen in liquid N_2 for subsequent lipid extraction. The other samples were longitudinally opened and the mucosa was scraped and frozen at − 80 °C for RNA extraction or enzymatic activity assays.

2.4. Lipid extraction and TAG determination

Samples of liver, duodenum, jejunum and ileum were subjected to lipid extraction as previously described [25]. Briefly, the samples were homogenized in $CHCl_3$:methanol solution and gently shook overnight at 4 °C. After the incubation, the samples were added with 0.6% NaCl solution and centrifuged for removal of the organic layer. Organic fraction was dried at room temperature, reconstituted in isopropanol and used for TAG quantification with enzymatic/colorimetric kits (LABORLAB, Sao Paulo, SP, Brazil).

2.5. Neutral lipids staining with Oil Red O (ORO)

Liver sections were obtained, processed and analyzed as previously described studies [25,26]. Briefly, liver fragments were embedded in Tissue-Tek O.C.T. compound (Sakura Finetek Europe, Netherlands) and immediately frozen in n-hexane with liquid nitrogen (N_2). Serial cryostat sections (15 μm-thick sections 200 μm apart) obtained from each block were mounted onto aminopropyltriethoxysilane-coated glass slides. Three sections from different parts of the samples were disposed per slide, and two slides per animal were used. Glass slides with the sections were incubated with ORO (3.75 μg/ml) for 10 min at room temperature and then rinsed with water. After rinsing, glass slides were coated with water-soluble mounting medium and coverslips. Three different fields of each section were captured using a light microscope (Olympus BX51TF) coupled to a digital camera (Olympus DP72) with

20 × objective magnification.

Analysis was performed using the free software ImageJ (http://imagej.nih.gov/ij). Colored images were converted to 8-bit and the ORO staining of each field were recorded. Analysis was performed assuming an individual background signal for each image. The data are presented as optical density of positive area for staining with ORO.

2.6. VLDL production rate

Overnight fasted rats received an i.p. injection with tyloxapol (Triton WR-1339; Sigma Aldrich, St Louis, USA). Tyloxapol solution was prepared as previously described [27] and injected at the dose of 500 mg/kg. All rats were kept with water during the test. Blood samples were collected from tail punctures before and 1.5, 3 and 6 h after tyloxapol injection. Serum was extracted from blood samples and subjected to TAG quantification. Linear regressions of the curves (TAG concentration *vs.* time) were presented as VLDL production rate (h^{-1}).

2.7. Olive oil gavage test

Rats received a *gavage* with extra virgin olive oil (5 ml/kg). All rats were kept without chow and with water during the test. Blood samples were collected from tail punctures before and 1.5 and 6 h after the *gavages*. Serum samples were used for TAG determination using the same kit described above.

2.8. RNA extraction and qPCR

Total RNA of liver and mucosal scrapings were extracted and processed for reverse transcription as previously described [28,29]. The sequences of the primers used in the study are shown in Table 1.

Values of mRNA expression were normalized with the reference genes *Rpl37a* and *Gapdh*. Fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1
Sequences of primers and GenBank numbers.

Gene / GenBank number	Forward and Reverse Sequences
fatty acid synthase (*Fasn*) / NM_017332	5′-TGGTGAAGCCCAGAGGGATC-3′ 5′-CACTTCCACACCCATGAGCG-3′
Aldolase, fructose-bisphosphate B (*Aldob*) / NM_012496	5′-CACACCTGAGCAAGTGGCTATG-3′ 5′-CTAGGTAGAGGGCAACGGTTG-3′
stearoyl-Coenzyme A desaturase 2 (*Scd2*) / NM_031841	5′-TGCGTCAGCACTTTCTTACGG-3′ 5′-GCGTGATGGTAGTTGTGGAAGC-3′
acetyl-CoA carboxylase alpha (*Acc*) / NM_022193)	5′-TGCTTATATTGTGGATGGCTTG-3′ 5′-TTCTACTGTCCCTTCTGGTTCC-3′
apolipoprotein b (*Apob*) / NM_019287	5′-CTGCGGTGGCAGAAATAACG-3′ 5′-CCTTGAGCAAACCTTAGGTAGGG-3′
acyl CoA:diacylglycerol acyltransferase 1 (*Dgat1*) / NM_053437	5′-AGGATGTTCCGCCTTTGGG-3′ 5′-CGTGGACATACATGAGCACAGC-3′
acyl CoA:diacylglycerol acyltransferase 2 (*Dgat2*) / NM_001012345	5′-AAGCCCATCACCACCGTTG-3′ 5′-TTCCTTCCAGGAGCTGGCAC-3′
microsomal triglyceride transfer protein (*Mttp*) / NM_001107727	5′-TATGACCGTTTCTCCAAGAGTGG-3′ 5′-TCAAGGTTCTCCTCTCCCTCATC-3′;
vesicle trafficking protein homolog B (*Sec22b*) / NM_001025686	5′-CGTGCTCGGAGAAATCTCGG-3′ 5′-AACACGGCTACTGCTGCAAGC-3′
glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) / M17701	5′-TACAGCAACAGGGTGGTGGACC-3′ 5′-TGGGATGGAATTGTGAGGGAGAG-3′
ribosomal protein L37a (*Rpl37a*) / X14069	5′-CAAGAAGGTCGGGATCGTCG-3′ 5′-ACCAGGCAAGTCTCAGGAGGTG-3′.

2.9. Enzymatic activity

The activities of L-3-hydroxyacyl-CoA dehydrogenase (β-HADH) and carnitine palmitoyltransferase-1 (CPT1) were measured in mucosal scrapings of ileum and liver fragments as described previously [27].

Fructokinase (FK) activity was determined using an enzyme-coupled assay. Tissue homogenate (10 μl) was added with a buffer (pH 8.2) containing 50 mM Tris-HCl, 2 mM $MgSO_4$, 5 mM KCl, 0.2 mM NADH, 1 mM ATP, 3 mM Fructose, 2 mM Phosphoenolpyruvate (PEP), 2 units of lactate dehydrogenase, 4 units of pyruvate kinase and 0.05% Triton X-100. The total assay volume was 165 μl.

ADP formed by the FK reaction, *i.e.*, phosphorylation of fructose to fructose-1-phosphate (F1P), was used by pyruvate kinase to convert phosphoenolpyruvate into pyruvate. Then, lactate dehydrogenase converted pyruvate into lactate using NADH as a cofactor. FK activity was measured by monitoring the decrease in absorbance at 340 nm, which corresponds to the oxidation of NADH into NAD^+.

2.10. Statistical analysis

All results were presented as the mean ± standard deviation (S.D.). Parametric distribution was checked using the Shapiro-Wilk test. Data analysis were performed with two-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparisons. We considered the following two factors: type of drinking solution (water or 15% fructose) and type of treatment *via gavage* (vehicle or AGO). For the concentration of TAG during the tests, the following two factors were considered: time after stimulus (either tyloxapol or olive oil) and experimental group (CTL, FRU, AGO or FRU+AGO). All analyses were performed with GraphPad Prism (Graph Pad Software, Inc., Version 8.4, San Diego, USA). P values < 0.05 indicated significant differences.

3. Results

3.1. AGO reduces body mass gain and feeding efficiency in fructose-treated rats

Before testing the metabolic effects of AGO, we performed a pilot experiment to validate if the protocol with fructose was able to increase circulating TAG. We also tested if the effect of fructose was attenuated by gemfibrozil, a drug with known lipid lowering effect. Our pilot data showed that rats treated with fructose had higher levels of circulating TAG (193.0 ± 12.4 mg/dl, n = 6) when compared to CTL rats (113.0 ± 9.4 mg/dl, n = 6) ($P < 0.0001$). The values of TAG in rats treated with fructose and gemfibrozil (135.3 ± 5.7 mg/dl, n = 6) were lower than in rats treated only with fructose ($P < 0.01$) but similar to CTL.

Having established that our protocol was efficient to induce hypertriglyceridemia, we next evaluated the potential metabolic effects of AGO. Our results showed that both AGO and fructose were able to influence body mass gain during the period of treatment ($P < 0.01$). However, the post-hoc analysis revealed that only FRU rats, but not FRU+AGO rats, exhibited increased body mass gain (40% higher than AGO and 25% higher than FRU+AGO; $P < 0.001$ and $P < 0.05$) (Fig. 1A). On the other hand, the daily intake of liquid calories was similar between FRU and FRU+AGO rats (Fig. 1B). The daily intake of solid calories was reduced by treatment with fructose ($P < 0.0001$), but the values of FRU and FRU+AGO rats were similar to each other (Fig. 1C). The daily caloric intakes were similar among the four groups of the study (Fig. 1D).

The body mass gain relative to the caloric intake, here presented as feeding efficiency, was influenced by AGO ($P < 0.001$). The post-hoc analysis showed that only FRU+AGO presented significant reductions in feeding efficiency (27% lower than CTL and 34% lower than FRU; $P < 0.05$ and $P < 0.01$) (Fig. 1E).

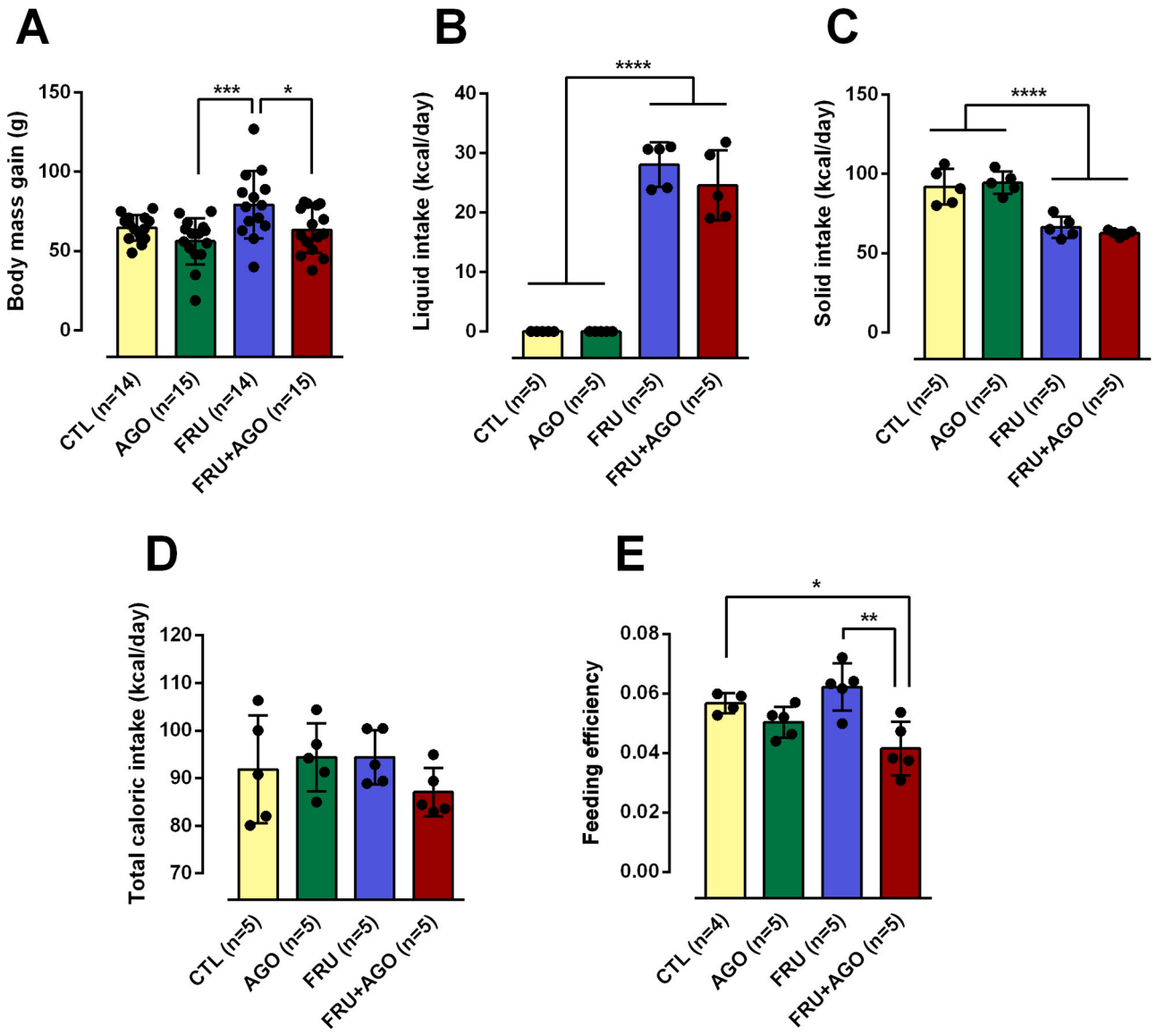


Fig. 1. Body mass gain, caloric intake and feeding efficiency in rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). Cumulative body mass gain was assessed over the 2 weeks of treatment (A). Intake of liquid calories (B), solid calories (C) and the sum of liquid and solid calories (D) were expressed on the daily basis. Feeding efficiency was calculated over the 2 weeks of treatment (E). Data are shown as mean ± S.D. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$.

3.2. AGO decreases fasting circulating triacylglycerides in fructose-treated rats

In order to clarify if the reduction in body mass induced by AGO in fructose-treated rats would be due to reduced adiposity, we next evaluated the relative mass of epididymal and retroperitoneal fat pads. However, no changes in the relative mass of these two fat pads were found when comparing the four experimental groups (Fig. 2A and B).

Although we could not detect differences in adiposity, treatment with fructose affected fasting TAG in serum samples obtained from trunk blood ($P < 0.001$). Interestingly, the post-hoc analyses revealed that increased fasting TAG detected in FRU rats (150% higher than CTL and 140% higher than AGO; $P < 0.05$ and $P < 0.01$) was not seen in FRU+AGO rats (Fig. 2C). Serum total cholesterol was not modulated by AGO, fructose or the combination of both (Fig. 2D). Blood glucose levels were affected by fructose treatment ($P < 0.0001$) and the post-hoc analyses indicated increased glycemia in both FRU and FRU+AGO rats (respectively 61% and 68% higher than CTL; $P < 0.001$ and $P < 0.0001$) (Fig. 2E).

3.3. AGO decreases VLDL production and hepatic lipids in fructose-treated rats

With the attempt to elucidate if changes in circulating TAG seen in rats treated with fructose and AGO were associated with the modulation of hepatic VLDL production, we performed a metabolic test based on whole-body LPL inhibition with tyloxapol. In this test, serum TAG was monitored in peripheral blood samples obtained by caudal punctures. The changes in circulating TAG found in peripheral blood samples before tyloxapol injection (basal) were similar to those seen in samples from serum of trunk blood. Fructose consumption affected peripheral TAG levels ($P < 0.01$), and the post-hoc analysis showed a specific increase in FRU (248% higher than CTL; $P < 0.01$), but not in FRU+AGO rats (Fig. 3A).

As expected, tyloxapol injection induced a progressive increase in TAG levels in CTL reaching significant differences at 6 h after injection (7.8-fold basal levels of CTL; $P < 0.0001$). FRU rats exhibited a kinetics hallmarked by an increase in TAG that was detected as early as 3 h after tyloxapol injection (2.4-fold basal levels of FRU; $P < 0.01$). This increase

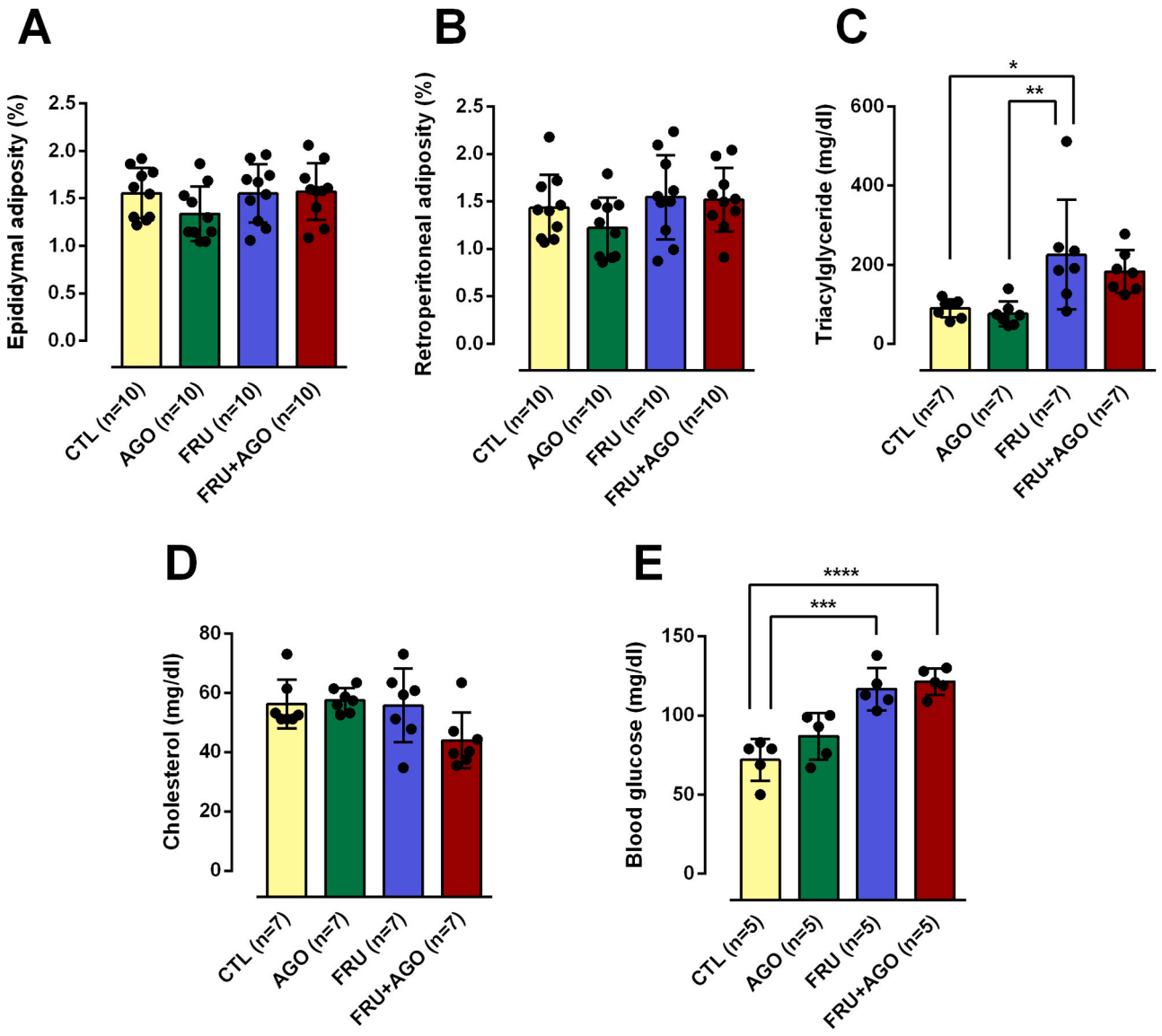


Fig. 2. Adiposity and fasting triacylglycerides, cholesterol and glucose levels in rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). After treatment, epididymal (A) and retroperitoneal (B) fat pads were removed from fasted animals to assess adiposity. Trunk blood was removed and serum was extracted for dosage of triacylglycerides (C) and total cholesterol (D). Fasting glycemia was assessed in whole blood obtained from tail (E). Data are shown as mean ± S.D. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

was sustained up to the 6th h post-injection (3.7-fold basal levels of FRU; $P < 0.0001$). The kinetics of circulating TAG detected in AGO and FRU+AGO rats were similar to CTL, and significant increases were only detected at 6 h after tyloxapol injection (5.2-fold basal levels in AGO group and 3.9-fold basal levels in FRU+AGO group; $P < 0.01$ and $P < 0.001$) (Fig. 3B).

The VLDL production rate was affected by both fructose and AGO treatments ($P < 0.01$), but the post-hoc analysis indicated that this parameter was increased exclusively in FRU rats (280% higher than CTL and 254% higher than FRU+AGO; $P < 0.01$ and $P < 0.05$) (Fig. 3C).

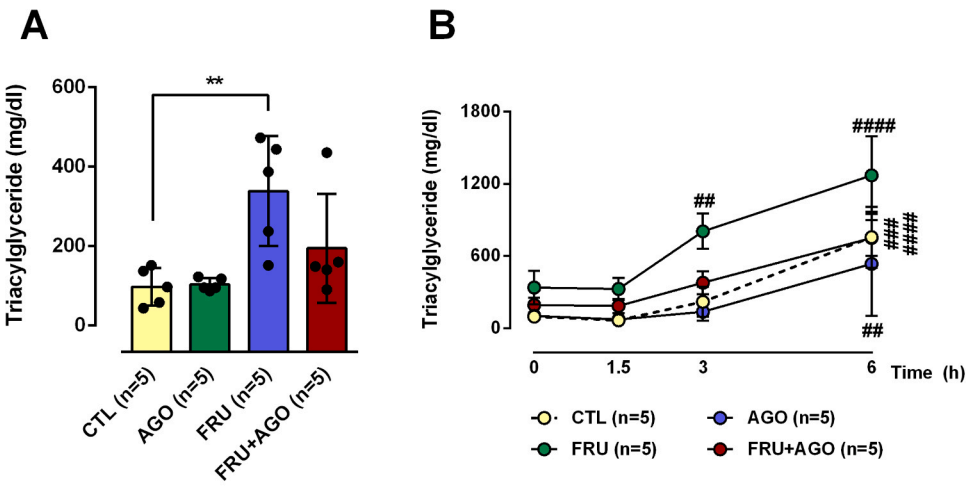
Hepatic TAG content was influenced by AGO treatment ($P < 0.01$) and the post-hoc analysis showed a significant reduction in FRU+AGO rats (59% lower than CTL rats; $P < 0.05$) (Fig. 3D).

We also evaluated the hepatic content of neutral lipids. Representative images of the ORO-stained sections show a more pronounced staining in the FRU rats than in the other groups (Fig. 4A). The quantitative analysis of the images revealed that the hepatic content of neutral lipids was affected by both fructose and AGO treatments ($P < 0.05$). The post-hoc analysis revealed increased hepatic content of neutral lipids only in FRU rats (146% higher than CTL and 131% higher than FRU+AGO; $P < 0.01$) (Fig. 4B).

3.4. AGO reduces the expression of the DNLG enzyme Fasn in the liver of fructose-treated rats

We next assessed whether AGO could modulate the expression or the activity of key enzymes related to the hepatic metabolism of fructose. The activity of FK was modulated by fructose consumption ($P < 0.0001$) and the post-hoc analysis revealed a significant increase in the liver of both FRU and FRU+AGO rats (respectively 29% and 44% higher than CTL; $P < 0.01$ and $P < 0.0001$) (Fig. 5A). Similarly, the expression of *Aldob* was also affected by the consumption of fructose ($P < 0.01$) and increased levels of its mRNA were found in the liver of both FRU and FRU+AGO rats (respectively 143% and 104% higher than CTL; $P < 0.01$ and $P < 0.05$) (Fig. 5B).

In addition to the enzymes involved in the first steps of fructose metabolism, we also evaluated the expression of key targets that mediate DNLG. The expression of *Fasn* was modulated by treatment with



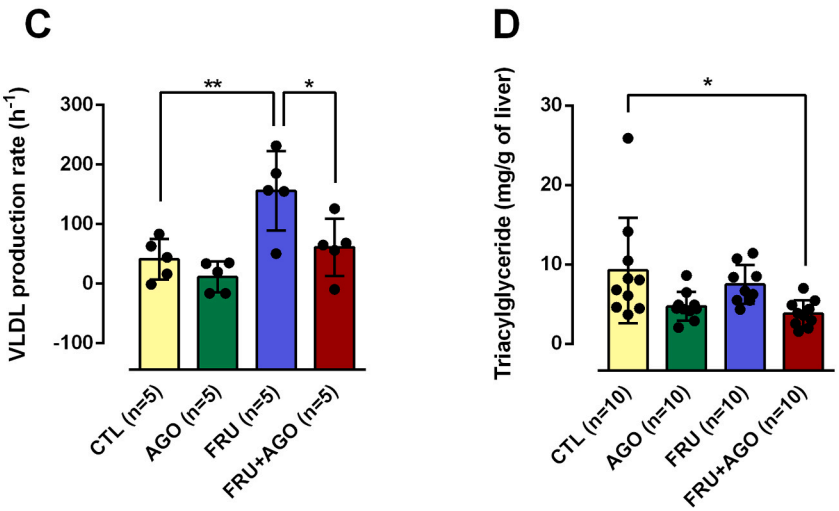


Fig. 3. VLDL production and hepatic triacylglycerides levels in rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). Fasting triacylglycerides levels were assessed in serum obtained from tail blood (A). An i.p. tyloxapol injection was performed and triacylglycerides levels were further assessed 1.5, 3 and 6 h after injection (B) in order to calculate VLDL production (C). Hepatic triacylglycerides levels were assessed in independent animals (D). Data are shown as mean ± S.D. $^{*}P < 0.05$ and $^{**}P < 0.01$; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ and $^{\#\#\#\#}P < 0.0001$ *vs.* in-group basal levels.

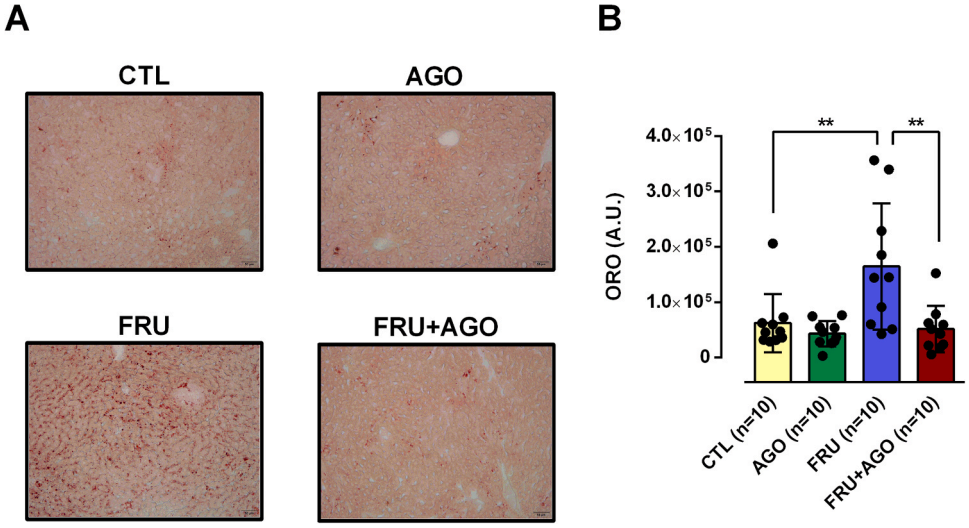


Fig. 4. Neutral lipid staining in liver of rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). Sections of liver were stained with oil red O (A) and the images were analyzed using the ImageJ software (B). Black bar represents a scale of 50 μm, objective 20 ×. Data are shown as mean ± S.D. $^{**}P < 0.01$.

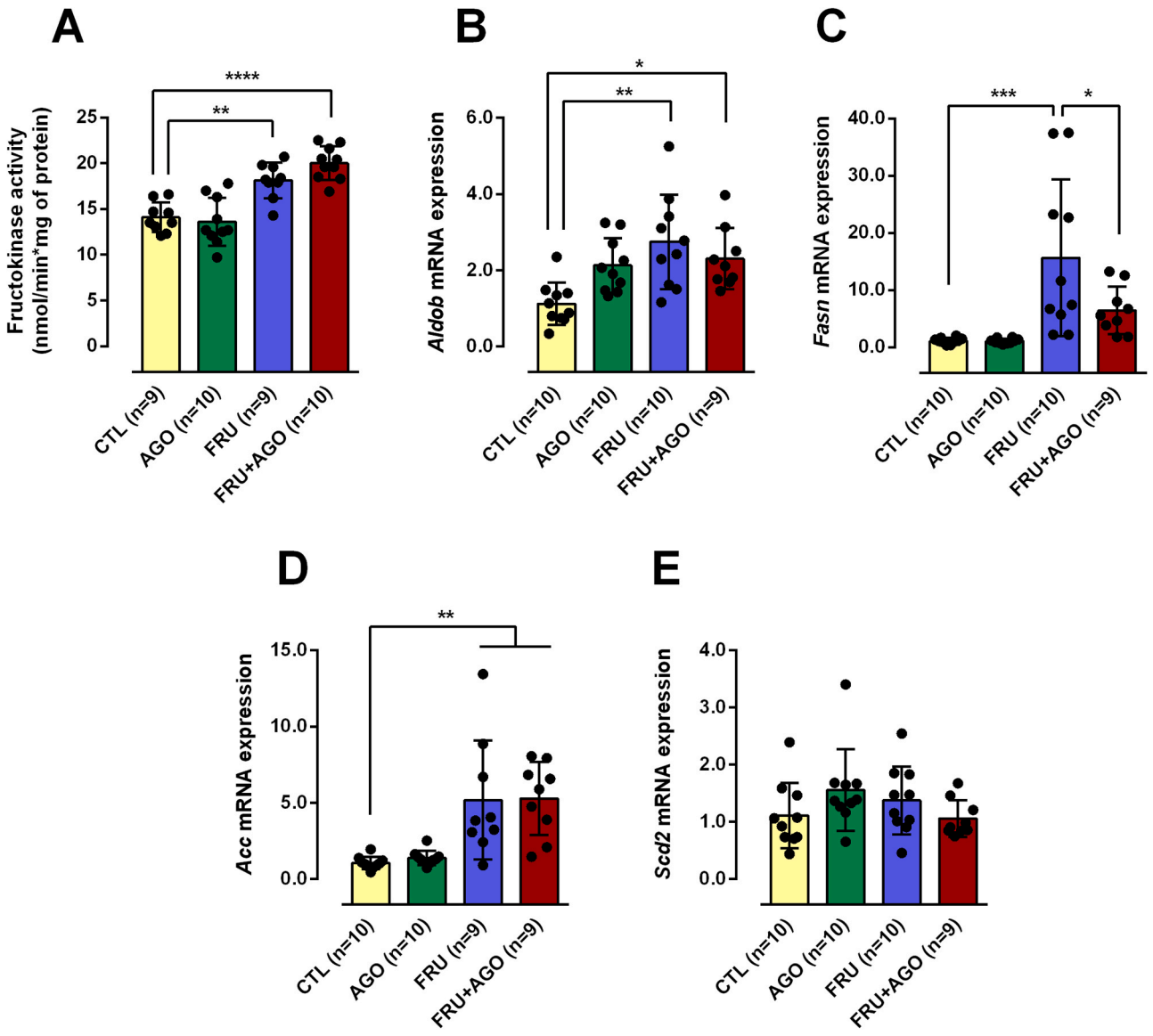


Fig. 5. Fructokinase activity and *Aldob*, *Fasn*, *Acc* and *Scd2* expression in the liver of rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). Fragments of liver were processed for measurement of fructokinase activity (A). Additional fragments were processed for RNA extraction and qPCR for *Aldob* (B), *Fasn* (C), *Acc* (D) and *Scd2* (E). Data are shown as mean ± S.D. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$.

fructose ($P < 0.001$) with a significant increase detected only in FRU rats (13.7-fold CTL levels and 2.45-fold FRU+AGO levels; $P < 0.001$ and $P < 0.05$) (Fig. 5C). The expression of *Acc* was also influenced by treatment with fructose ($P < 0.0001$) but significant increases were found in both FRU and FRU+AGO rats (approximately 5-fold CTL values; $P < 0.01$) (Fig. 5D). The hepatic expression of *Scd2* was not modulated in any of the four groups of the study (Fig. 5E).

The expression of the enzymes involved in fatty acid re-esterification *Dgat1* and *Dgat2* and the proteins that mediate hepatic VLDL assembly and secretion *Mttp* and *Sec22b* were not modulated in any of the groups of the study (Fig. 6A, B, C and D). The hepatic activity of CPT1 and β-HADH, two key enzymes involved in FFA oxidation, were also assessed. Although there was clear trend towards an increase in hepatic CPT1 activity induced by AGO treatment ($P = 0.0502$), a significant increase was only detected in AGO group (20% higher than the CTL group; $P < 0.05$) (Fig. 6E). No changes in hepatic β-HADH activity were detected in any of the four groups present in the study (Fig. 6F).

3.5. AGO reduces triglyceridemia induced by an oral load with olive oil in fructose-treated rats

Having established that AGO modulates VLDL production as well as hepatic *Fasn* expression, we next investigated if this drug could also affect lipid metabolism in fructose-treated rats challenged with of exogenous FFAs.

In contrast to the fasting TAG levels, TAG levels in fed rats before challenge with olive oil was affected by fructose treatment ($P < 0.001$) but equally increased in both FRU and FRU+AGO rats (respectively 80% and 62% higher than AGO rats; $P < 0.001$ and $P < 0.05$) (Fig. 7A). On the other hand, circulating TAG levels 1.5 h after the *gavage* with olive oil was higher in FRU rats when compared to AGO and FRU+AGO rats (respectively 84% and 48% higher than AGO and FRU+AGO 1.5 h after *gavage*; $P < 0.01$ and $P < 0.05$) (Fig. 7B). Accordingly, the AUC values were influenced by fructose treatment ($P = 0.0129$) and increased exclusively in FRU rats (44% higher than AGO rats; $P < 0.05$) (Fig. 7C).

In order to elucidate which segment of the small intestine could account for the lower TAG levels seen in FRU+AGO rats orally

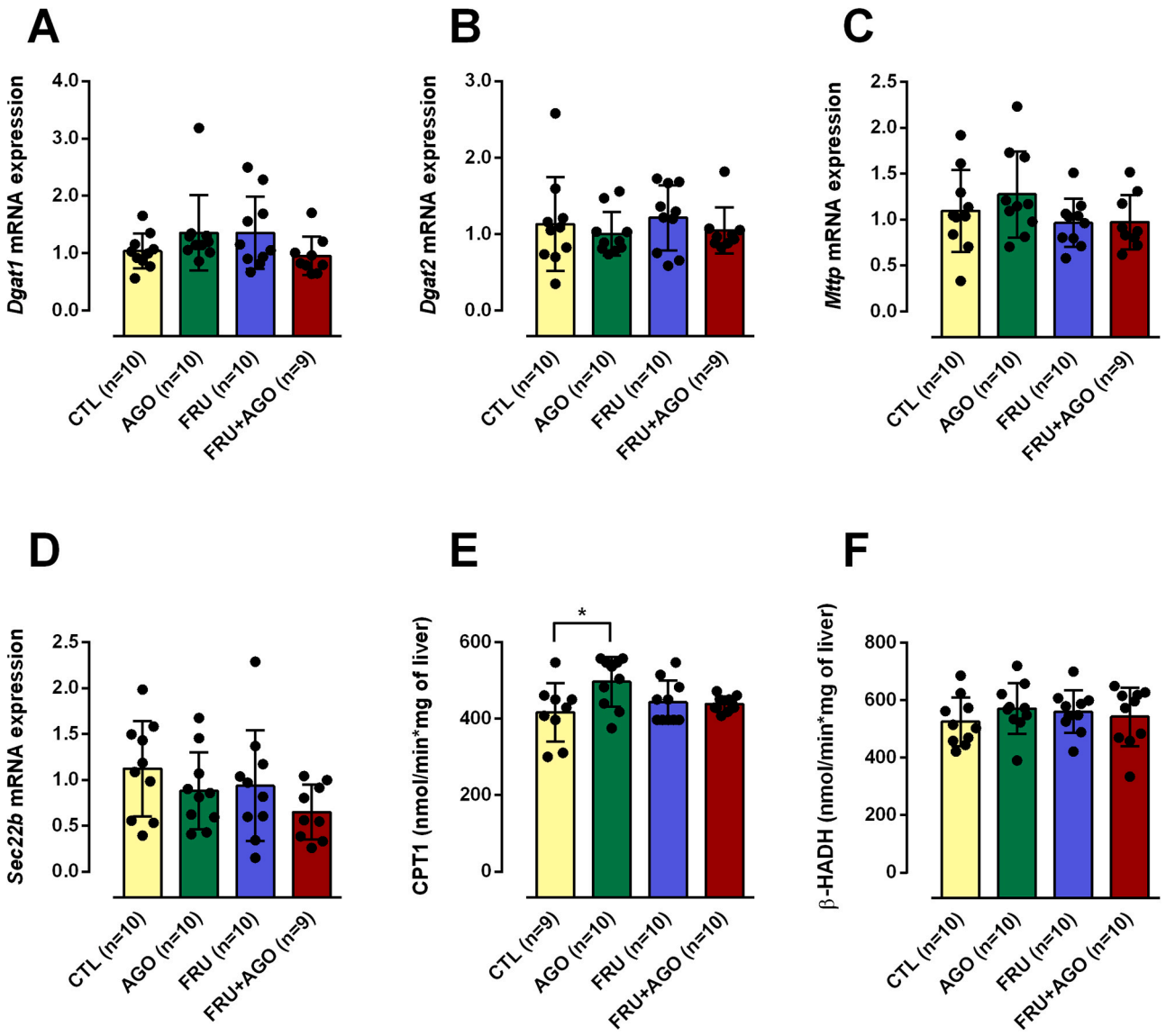


Fig. 6. *Dgat1, Dgat2, Mttp and Sec22b* expression and CPT1 and β-HADH activities in the liver of rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). Fragments of liver were processed for RNA extraction and qPCR for *Dgat1* (A), *Dgat2* (B), *Mttp* (C) and *Sec22b* (D). Fragments of liver were also used to assess CPT1 (E) and β-HADH (F). Data are shown as mean ± S. D. $^{*}P < 0.05$.

challenged with olive oil, we assessed the TAG content in samples of duodenum, jejunum and ileum. TAG contents in duodenum and jejunum samples were not different among the four groups of the study (Fig. 7D and E). TAG content in ileum samples instead, was modulated by AGO treatment ($P < 0.05$) and reduced in FRU+AGO rats (49% lower than CTL rats; $P < 0.05$) (Fig. 7F).

3.6. AGO reduces the expression of Mttp in the ileum of fructose-treated rats

We also evaluated the expression of genes involved in fatty acids re-esterification and chylomicron assembly and secretion in ileal mucosal scrapings. *Dgat1* and *Dgat2* expression were not modulated in the groups belonging to the study (Fig. 8A and B). Although no specific changes have been found in the group-by-group comparisons of the post-hoc analysis, the ileal expression of *Apob* was reduced by AGO treatment ($P = 0.0135$) (Fig. 8C). On the other hand, the ileal *Mttp* expression was influenced by AGO treatment ($P < 0.01$) and significant reductions were found in AGO and FRU+AGO rats (approximately 66% lower than CTL rats; $P < 0.05$) (Fig. 8D). The ileal activity of CPT1 and β-HADH were not modulated in the groups present in the study (Fig. 8E and F).

4. Discussion

The present study supports the previously unappreciated notion that the melatonin receptor agonist AGO reduces circulating TAG levels, hepatic lipid content and VLDL production in rats treated with fructose. These effects are in agreement with previous studies showing that melatonin is able to reduce TAG accumulation in liver of mice, hamsters and rats exposed to HFD [30–33]. Melatonin has also been reported to reduce hepatic steatosis in rats consuming fructose-enriched diet [11].

Our data also reveal that the reduction in hepatic lipid content induced by AGO was accompanied by a reduction in body mass with no changes in caloric intake. Consequently, AGO reduced feeding efficiency in fructose-treated rats. These effects a very similar to those already reported in aged-rats treated with melatonin [34]. On the other hand, the previously reported ability of melatonin in reduce adiposity of aged-rats [34,35] was not recapitulated by AGO in fructose-treated rats.

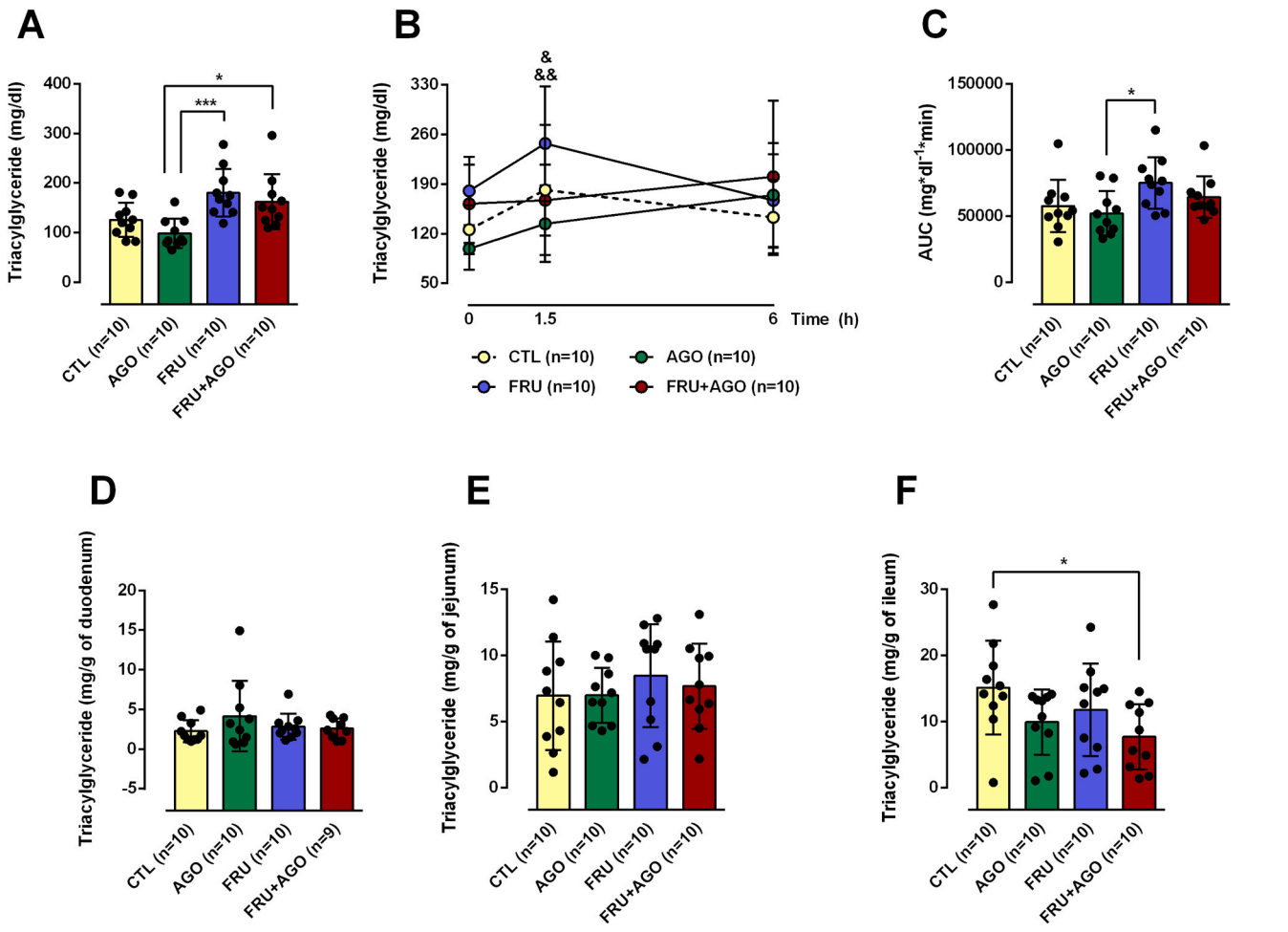


Fig. 7. Dynamics of triacylglycerides after a *gavage* with olive oil in rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). Basal triacylglycerides levels were assessed in serum obtained from tail blood (A). A *gavage* with olive oil was performed and triacylglycerides levels were assessed 1.5 and 6 h later (B). These values were used to calculate the area under the curve (AUC) (C). Triacylglycerides levels in segments of duodenum (D), jejunum (E) and ileum (F) were also assessed. The Data are shown as mean ± S.D. $^{*}P < 0.05$ and $^{***}P < 0.001$; $^{\&}P < 0.05$ *vs.* FRU+AGO 1.5 h after *gavage* and $^{\&\&}P < 0.01$ *vs.* AGO 1.5 h after *gavage*.

Absorbed fructose is rapidly metabolized into fructose-1-phosphate by hepatic fructokinase (FK). Fructose-1-phosphate is then converted into three carbon units by aldolase B (*Aldob*). Triose-P is then converted into pyruvate and lactate that serve as substrates for gluconeogenesis [36]. Based on our data on FK activity, *Aldob* expression and fasting glycemia, we concluded that AGO does not modulate the early steps of hepatic fructose metabolism and, by extension, is not able to alter the flux of fructose metabolites to *de novo* glucose synthesis.

Lactate derived of fructose metabolism also serves as a lipogenic substrate that contributes to increase hepatic lipids and circulating TAG [36]. In addition to provide intermediates for *de novo* lipogenesis (DNLG), fructose can also increase the expression and the activity of key DNLG enzymes in the liver, such as *Acc* and *Fasn* [37–40]. Treating rats with drinking water containing 10% fructose for 21 days was already reported to increase hepatic VLDL production rate [38].

It is relevant to point out that in the present experiments fructose by itself did not increase hepatic TAG but did increase hepatic neutral lipids staining with ORO. The reasonable explanation for such differences might rely on the fact that ORO stains a broad range of neutral lipids other than triacylglycerides such as diacylglycerides and esterified cholesterol [41,42]. Fructose feeding for only 11–12 days, in turns, was already reported to increase hepatic diacylglycerides and cholesterol levels [43,44].

Notably, we found that AGO reduced hepatic *Fasn* expression, VLDL production and hepatic lipids in fructose-treated rats. Accordingly, *Fasn* mRNA in the liver correlates with the rate of fatty acid synthesis [45]. Increased mRNA of *Fans* was also associated with increased hepatic steatosis in obese ob/ob mice [46]. Such finding suggests that DNLG might be the main hepatic biochemical route affected by AGO that would impact the hepatic lipid metabolism. Favoring this proposition, we found that AGO did not modulate the hepatic expression of *Sec22b* and *Mttp*, two genes that play a pivotal role in VLDL production [47,48]. AGO was not equally able to increase the hepatic activity of CPT1 and β-HADH in the liver of fructose-treated rats, two critical steps for fatty acid β-oxidation [49,50]. In consonance with the present data, a similar lipid-lowering effect was described for Piromelatine, a distinct MT1/MT2 agonist [51].

Previous studies have also shown that the ability of melatonin in reduce hepatic TAG in HFD rodents is associated to the downregulation of *Fasn* expression in the liver [31]. Melatonin has also been described to act *in vitro* in HepG2 cells to specifically inhibit *Fasn*, but not *Acc*, expression [52]. Although our experiments do not test the mechanism for such specificity, it is important to acknowledge that *Fasn* is under the control of transcription factors that do not modulate *Acc* transcription, such as signal transducer and activator of transcription 3 (STAT3) and Pre-B cell leukaemia transcription factor-regulating protein 1 (Prep1) [53,54]. Evidence regarding Prep1 activation is still lacking but studies show that melatonin is able to activate STAT3 in the liver [55,56].

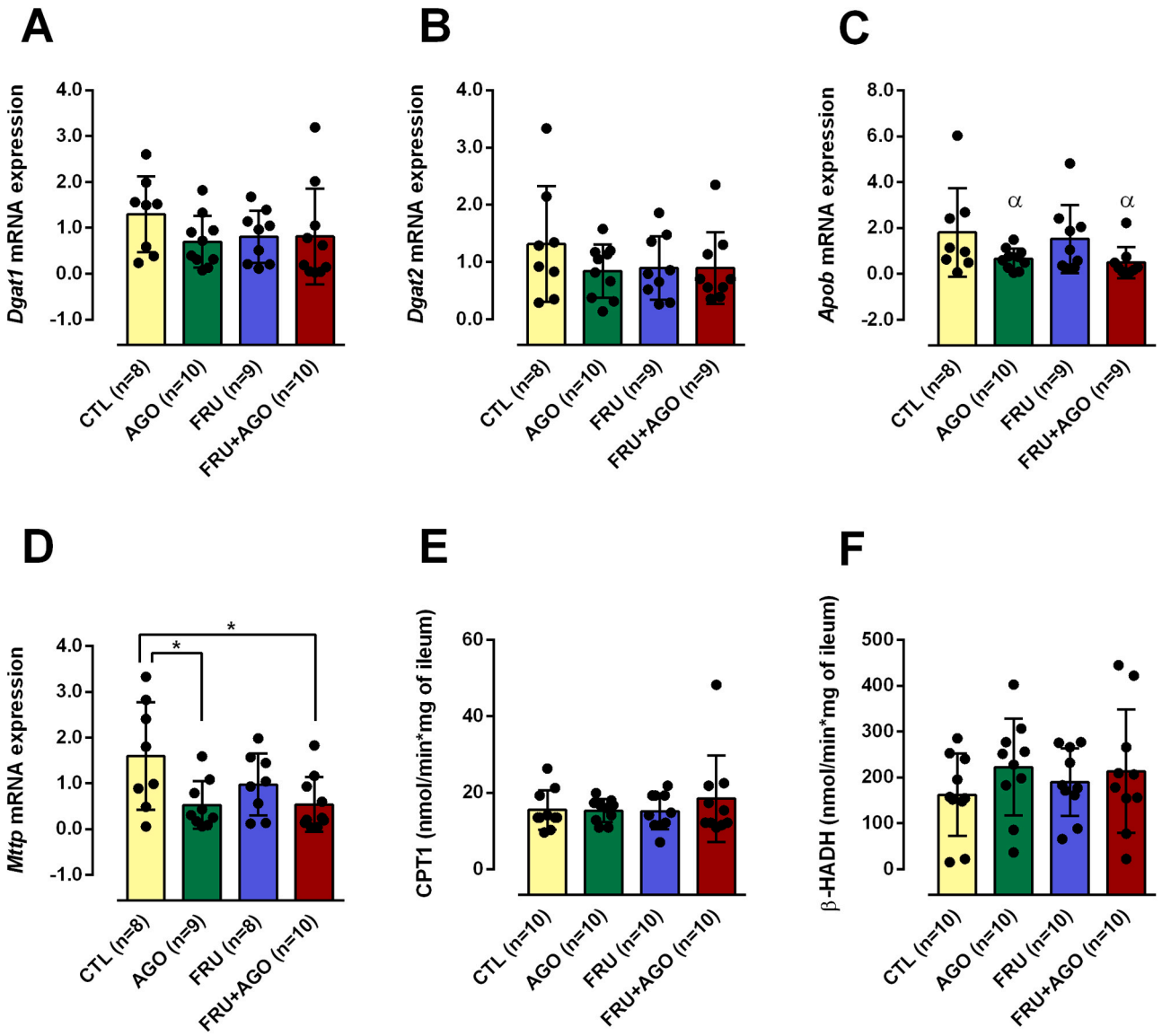


Fig. 8. *Dgat1, Dgat2, Apob* and *Mttp* expression and CPT1 and β-HADH activities in the ileum of rats treated with fructose and/or agomelatine. Rats were treated with agomelatine (AGO), fructose (FRU) or a combination of both (FRU+AGO). CTL rats received the vehicle (CTL). Fragments of ileum were processed for RNA extraction and qPCR for *Dgat1* (A), *Dgat2* (B), *Apob* (C) and *Mttp* (D). Fragments of ileum were also used to assess CPT1 (E) and β-HADH (F). Data are shown as mean ± S.D. * $P < 0.05$; $^{\alpha}P < 0.05$ for effect of the AGO factor with no difference in the post-hoc analysis.

The participation of melatonin receptors in the control of intracellular TAG content was already addressed by other studies in non-hepatic cells using ligands other than AGO. Melatonin was also described to reduce TAG accumulation in osteoblasts and this effect was reported to be blocked by the MT1/MT2 antagonists luzindole and S20928 [57]. Piromelatine was also described to reduce TAG levels in 3T3-L1 adipocytes through a mechanism that is sensitive to luzindole [58].

Although fructose was described to be a poor substrate for intestinal DNLG, high fructose intake increases postprandial TAG levels due an upregulation of intestinal *Mttp* and *Apob* expression that leads to increased intestinal lipoprotein secretion rate [59]. The simultaneous ingestion of fructose and fat was also reported to increase circulating chylomicrons in humans [60,61].

In agreement with these publications, we found that AGO reduced circulating TAG levels after a *gavage* with olive oil as well as the expression of *Apob* and *Mttp* in the mucosal scraping of ileal fragments. MTTP and ApoB are two key proteins that play an indispensable role in intestinal lipoprotein production [62,63]. It was already described that increased MTTP activity and protein content correlates with increased *Mttp* mRNA after postpandrial hypertriglyceridemia induced by short-term feeding with a HFD [64]. Similarly, changes in *Apob* mRNA correlate with changes in ApoB protein content [65,66]. Therefore, the current data suggest that AGO may also reduce TAG levels probably by decreasing intestinal chylomicrons secretion.

There are few studies addressing the effect of melatonin itself on chylomicrons production but the available data is in accordance with the properties presently attributed to AGO. MT1 and MT2 are expressed in rat small intestine and melatonin has been recently shown to reduce intestinal TAG absorption in mice fed a HFD [67,68].

In summary, the present study shows that AGO can mimic some metabolic benefits of melatonin when used in fructose-treated rats. AGO can reduce circulating TAG, hepatic lipids, VLDL production and triglyceridemia after a *gavage* with olive oil. The metabolic actions of AGO are likely to result of hepatic modulation of *Fasn* and intestinal modulation of *Apob* and *Mttp*. Altogether, the present results indicate that this antidepressant drug exerts beneficial metabolic effects in experimental conditions and its repurposing should be addressed in future clinical trials.

Funding

This study was funded by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2013/07607-8; 2015/23285-6; 2016/13138-9; 2017/20742-2; 2019/03196-0; 2019/19488-0; 2020/06397-3), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Finance Code 001).

CRediT authorship contribution statement

Vanessa Barbosa Veronesi: Validation, Investigation, Data curation, Formal analysis. **Mariana Rodrigues Pioli:** Investigation. **Dailson Nogueira de Souza:** Investigation. **Caio Jordão Teixeira:** Validation, Investigation, Writing - original draft, Writing - review & editing. **Gilson Murata:** Investigation, Formal analysis. **Junia Carolina Santos-Silva:** Investigation. **Fernanda Ballerini Hecht:** Investigation. **Julia Modesto Vicente:** Investigation. **Silvana Bordin:** Funding acquisition, Writing - original draft, Writing - review & editing. **Gabriel Forato Anhê:** Conceptualization, Formal analysis, Data curation, Supervision, Funding acquisition, Writing - original draft, Writing - review & editing.

Conflicts of interest statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgements

The authors are grateful to Edmyr Rosa dos Reis, Agnaldo Fernando de Azevedo, Marcos Donizete and Ivani Franco Correia dos Santos for their technical assistance.

References

[1] P.J. Morgan, P. Barrett, H.E. Howell, R. Helliwell, Melatonin receptors: localization, molecular pharmacology and physiological significance, Neurochem. Int. 24 (1994) 101–146, https://doi.org/10.1016/0197-0186(94)90100-7.

[2] M.L. Dubocovich, Melatonin receptors: are there multiple subtypes? Trends Pharmacol. Sci. 16 (1995) 50–56, https://doi.org/10.1016/s0165-6147(00)88978-6.

[3] V. Audinot, A. Bonnaud, L. Grandcolas, M. Rodriguez, N. Nagel, J.-P. Galizzi, A. Balik, S. Messager, D.G. Hazlerigg, P. Barrett, P. Delagrange, J.A. Boutin, Molecular cloning and pharmacological characterization of rat melatonin MT1 and MT2 receptors, Biochem. Pharmacol. 75 (2008) 2007–2019, https://doi.org/10.1016/j.bcp.2008.02.022.

[4] S. Owino, D.D.C. Buonfiglio, C. Tchio, G. Tosini, Melatonin signaling a key regulator of glucose homeostasis and energy metabolism, Front. Endocrinol. 10 (2019) 488, https://doi.org/10.3389/fendo.2019.00488.

[5] S. Owino, A. Sánchez-Bretaño, C. Tchio, E. Cecon, A. Karamitri, J. Dam, R. Jockers, G. Piccione, H.L. Noh, T. Kim, J.K. Kim, K. Baba, G. Tosini, Nocturnal activation of melatonin receptor type 1 signaling modulates diurnal insulin sensitivity via regulation of PI3K activity, J. Pineal Res. 64 (2018), https://doi.org/10.1111/jpi.12462.

[6] S. Contreras-Alcantara, K. Baba, G. Tosini, Removal of melatonin receptor type 1 induces insulin resistance in the mouse, Obesity 18 (2010) 1861–1863, https://doi.org/10.1038/oby.2010.24.

[7] T.C. Nogueira, C. Lellis-Santos, D.S. Jesus, M. Taneda, S.C. Rodrigues, F.G. Amaral, A.M.S. Lopes, J. Cipolla-Neto, S. Bordin, G.F. Anhê, Absence of melatonin induces night-time hepatic insulin resistance and increased gluconeogenesis due to stimulation of nocturnal unfolded protein response, Endocrinology 152 (2011) 1253–1263, https://doi.org/10.1210/en.2010-1088.

[8] M.I.C. Alonso-Vale, G.F. Anhê, C. das, N. Borges-Silva, S. Andreotti, S.B. Peres, J. Cipolla-Neto, F.B. Lima, Pinealectomy alters adipose tissue adaptability to fasting in rats, Metabolism 53 (2004) 500–506, https://doi.org/10.1016/j.metabol.2003.11.009.

[9] F.B. Lima, U.F. Machado, I. Bartol, P.M. Seraphim, D.H. Sumida, S.M. Moraes, N. S. Hell, M.M. Okamoto, M.J. Saad, C.R. Carvalho, J. Cipolla-Neto, Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats, Am. J. Physiol. 275 (1998) E934–E941, https://doi.org/10.1152/ajpendo.1998.275.6.E934.

[10] J. de, A. Faria, T.M.F. de Araújo, D.S. Razolli, L.M. Ignácio-Souza, D.N. Souza, S. Bordin, G.F. Anhê, Metabolic impact of light phase-restricted fructose consumption is linked to changes in hypothalamic ampk phosphorylation and melatonin production in rats, Nutrients 9 (2017), https://doi.org/10.3390/nu9040332.

[11] A. Kitagawa, Y. Ohta, K. Ohashi, Melatonin improves metabolic syndrome induced by high fructose intake in rats, J. Pineal Res. 52 (2012) 403–413, https://doi.org/10.1111/j.1600-079X.2011.00955.x.

[12] D.P. Cardinali, P.A.S. Bernasconi, R. Reynoso, C.F.R. Toso, P. Scacchi, Melatonin may curtail the metabolic syndrome: studies on initial and fully established fructose-induced metabolic syndrome in rats, Int. J. Mol. Sci. 14 (2013) 2502–2514, https://doi.org/10.3390/ijms14022502.

[13] A. Leibowitz, E. Peleg, Y. Sharabi, Z. Shabtai, A. Shamiss, E. Grossman, The role of melatonin in the pathogenesis of hypertension in rats with metabolic syndrome, Am. J. Hypertens. 21 (2008) 348–351, https://doi.org/10.1038/ajh.2007.60.

[14] B. Guardiola-Lemaitre, C. De Bodinat, P. Delagrange, M.J. Millan, C. Munoz, E. Mocaër, Agomelatine: mechanism of action and pharmacological profile in relation to antidepressant properties, Br. J. Pharmacol. 171 (2014) 3604–3619, https://doi.org/10.1111/bph.12720.

[15] R. Rebai, L. Jasmin, A. Boudah, Agomelatine effects on fat-enriched diet induced neuroinflammation and depression-like behavior in rats, Biomed. Pharmacother. 135 (2021), 111246, https://doi.org/10.1016/j.biopha.2021.111246.

[16] G. Yigitturk, A.C. Acara, O. Erbas, F. Oltulu, N.U.K. Yavasoglu, A. Uysal, A. Yavasoglu, The antioxidant role of agomelatine and gallic acid on oxidative stress in STZ induced type I diabetic rat testes, Biomed. Pharmacother. 87 (2017) 240–246, https://doi.org/10.1016/j.biopha.2016.12.102.

[17] T. Che, X. Teng, Q. Huang, Y. Mu, X. Tang, X. Mu, Y. Wei, Agomelatine versus fluoxetine in glycemic control and treating depressive and anxiety symptoms in type 2 diabetes mellitus subjects: a single-blind randomized controlled trial, Neuropsychiatr. Dis. Treat. 14 (2018) 1527–1533, https://doi.org/10.2147/NDT.S167608.

[18] R. Kang, Y. He, Y. Yan, Z. Li, Y. Wu, X. Guo, Z. Liang, J. Jiang, Comparison of paroxetine and agomelatine in depressed type 2 diabetes mellitus patients: a double-blind, randomized, clinical trial, Neuropsychiatr. Dis. Treat. 11 (2015) 1307–1311, https://doi.org/10.2147/NDT.S85711.

[19] D.P. Cardinali, R. Hardeland, Inflammaging, metabolic syndrome and melatonin: a call for treatment studies, Neuroendocrinology 104 (2017) 382–397, https://doi.org/10.1159/000446543.

[20] X. Huang, S. Zhao, L. Bai, M. Hu, W. Zhao, Q. Zhang, Atorvastatin and fenofibrate increase apolipoprotein AV and decrease triglycerides by up-regulating peroxisome proliferator-activated receptor-alpha, Br. J. Pharmacol. 158 (2009) 706–712, https://doi.org/10.1111/j.1476-5381.2009.00350.x.

[21] R. Mathur, S. Dutta, T. Velpandian, S.R. Mathur, Psidium guajava Linn. leaf extract affects hepatic glucose transporter-2 to attenuate early onset of insulin resistance consequent to high fructose intake: an experimental study, Pharmacogn. Res. 7 (2015) 166–175, https://doi.org/10.4103/0974-8490.151459.

[22] G. Dagyte, A. Trentani, F. Postema, P.G. Luiten, J.A. Den Boer, C. Gabriel, E. Mocaër, P. Meerlo, E.A. Van der Zee, The novel antidepressant agomelatine normalizes hippocampal neuronal activity and promotes neurogenesis in chronically stressed rats, CNS Neurosci. Ther. 16 (2010) 195–207, https://doi.org/10.1111/j.1755-5949.2009.00125.x.

[23] C.A. Grillo, M. Risher, V.A. Macht, A.L. Bumgardner, A. Hang, C. Gabriel, E. Mocaër, G.G. Piroli, J.R. Fadel, L.P. Reagan, Repeated restraint stress-induced atrophy of glutamatergic pyramidal neurons and decreases in glutamatergic efflux in the rat amygdala are prevented by the antidepressant agomelatine, Neuroscience 284 (2015) 430–443, https://doi.org/10.1016/j.neuroscience.2014.09.047.

[24] M. Chen, Y. Yang, E. Braunstein, K.E. Georgeson, C.M. Harmon, Gut expression and regulation of FAT/CD36: possible role in fatty acid transport in rat enterocytes, Am. J. Physiol. Endocrinol. Metab. 281 (2001) E916–E923, https://doi.org/10.1152/ajpendo.2001.281.5.E916.

[25] J.M. Vicente, C.J. Teixeira, J.C. Santos-Silva, D.N. de Souza, N. Tobar, F. S. Furtuoso, I.G. Adabo, F.S. Sodré, G. Murata, S. Bordin, G.F. Anhê, The absence of lactation after pregnancy induces long-term lipid accumulation in maternal liver of mice, Life Sci. 217 (2019) 261–270, https://doi.org/10.1016/j.lfs.2018.12.026.

[26] A. Mehlem, C.E. Hagberg, L. Muhl, U. Eriksson, A. Falkevall, Imaging of neutral lipids by oil red O for analyzing the metabolic status in health and disease, Nat. Protoc. 8 (2013) 1149–1154, https://doi.org/10.1038/nprot.2013.055.

[27] D.N. de Souza, C.J. Teixeira, V.B. Veronesi, G.M. Murata, J.C. Santos-Silva, F. B. Hecht, J.M. Vicente, S. Bordin, G.F. Anhê, Dexamethasone programs lower fatty acid absorption and reduced PPAR-γ and fat/CD36 expression in the jejunum of the adult rat offspring, Life Sci. 265 (2021), 118765, https://doi.org/10.1016/j.lfs.2020.118765.

[28] J.M. Vicente, J.C. Santos-Silva, C.J. Teixeira, D.N. De Souza, J.F. Vettorazzi, F. S. Furtuoso, I.G. Adabo, F.T. Sato, M.A.R. Vinolo, E.M. Carneiro, S. Bordin, G. F. Anhê, Long-term increase of insulin secretion in mice subjected to pregnancy and lactation, Endocr. Connect. 9 (2020) 299–308, https://doi.org/10.1530/EC-20-0020.

[29] C.J. Teixeira, J.C. Santos-Silva, D.N. de Souza, A. Rafacho, G.F. Anhe, S. Bordin, Dexamethasone during pregnancy impairs maternal pancreatic beta-cell renewal during lactation, Endocr. Connect. 8 (2019) 120–131, https://doi.org/10.1530/EC-18-0505.

[30] D.-J. Li, J. Tong, Y.-H. Li, H.-B. Meng, Q.-X. Ji, G.-Y. Zhang, J.-H. Zhu, W.-J. Zhang, F.-Y. Zeng, G. Huang, X. Hua, F.-M. Shen, P. Wang, Melatonin safeguards against fatty liver by antagonizing TRAFs-mediated ASK1 deubiquitination and stabilization in a β-arrestin-1 dependent manner, J. Pineal Res. 67 (2019) 12611, https://doi.org/10.1111/jpi.12611.

[31] T.-H. Ou, Y.-T. Tung, T.-H. Yang, Y.-W. Chien, Melatonin improves fatty liver syndrome by inhibiting the lipogenesis pathway in hamsters with high-fat diet-

induced hyperlipidemia, Nutrients 11 (2019), https://doi.org/10.3390/nu11040748.
[32] J.-I. Heo, D.W. Yoon, J.H. Yu, N.H. Kim, H.J. Yoo, J.A. Seo, S.G. Kim, K.M. Choi, S. H. Baik, D.S. Choi, N.H. Kim, Melatonin improves insulin resistance and hepatic steatosis through attenuation of alpha-2-HS-glycoprotein, J. Pineal Res. 65 (2018) 12493, https://doi.org/10.1111/jpi.12493.
[33] M. Pan, Y.-L. Song, J.-M. Xu, H.-Z. Gan, Melatonin ameliorates nonalcoholic fatty liver induced by high-fat diet in rats, J. Pineal Res. 41 (2006) 79–84, https://doi.org/10.1111/j.1600-079X.2006.00346.x.
[34] T. Wolden-Hanson, D.R. Mitton, R.L. McCants, S.M. Yellon, C.W. Wilkinson, A. M. Matsumoto, D.D. Rasmussen, Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat, Endocrinology 141 (2000) 487–497, https://doi.org/10.1210/endo.141.2.7311.
[35] D.D. Rasmussen, D.R. Mitton, S.A. Larsen, S.M. Yellon, Aging-dependent changes in the effect of daily melatonin supplementation on rat metabolic and behavioral responses, J. Pineal Res. 31 (2001) 89–94, https://doi.org/10.1034/j.1600-079x.2001.310113.x.
[36] L. Tappy, K.-A. Lê, Metabolic effects of fructose and the worldwide increase in obesity, Physiol. Rev. 90 (2010) 23–46, https://doi.org/10.1152/physrev.00019.2009.
[37] R. Fiebig, M.A. Griffiths, M.T. Gore, D.H. Baker, L. Oscai, D.M. Ney, L.L. Ji, Exercise training down-regulates hepatic lipogenic enzymes in meal-fed rats: fructose versus complex-carbohydrate diets, J. Nutr. 128 (1998) 810–817, https://doi.org/10.1093/jn/128.5.810.
[38] T. Kazumi, H. Odaka, T. Hozumi, Y. Ishida, N. Amano, G. Yoshino, Effects of dietary fructose or glucose on triglyceride production and lipogenic enzyme activities in the liver of Wistar fatty rats, an animal model of NIDDM, Endocr. J. 44 (1997) 239–245, https://doi.org/10.1507/endocrj.44.239.
[39] Y. Nagai, Y. Nishio, T. Nakamura, H. Maegawa, R. Kikkawa, A. Kashiwagi, Amelioration of high fructose-induced metabolic derangements by activation of PPARalpha, Am. J. Physiol. Endocrinol. Metab. 282 (2002) E1180–E1190, https://doi.org/10.1152/ajpendo.00471.2001.
[40] J.K. DiStefano, Fructose-mediated effects on gene expression and epigenetic mechanisms associated with NAFLD pathogenesis, Cell. Mol. Life Sci. 77 (2020) 2079–2090, https://doi.org/10.1007/s00018-019-03390-0.
[41] H.A. Talbott, M.R. Plewes, C. Krause, X. Hou, P. Zhang, W.B. Rizzo, J.R. Wood, A. S. Cupp, J.S. Davis, Formation and characterization of lipid droplets of the bovine corpus luteum, Sci. Rep. 10 (2020) 11287, https://doi.org/10.1038/s41598-020-68091-2.
[42] J.L. Ramírez-Zacarías, F. Castro-Muñozledo, W. Kuri-Harcuch, Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O, Histochemistry 97 (1992) 493–497, https://doi.org/10.1007/BF00316069.
[43] H.J. Fallon, J. Barwick, R.G. Lamb, H. van den Bosch, Studies of rat liver microsomal diglyceride acyltransferase and cholinephosphotransferase using microsomal-bound substrate: effects of high fructose intake, J. Lipid Res. 16 (1975) 107–115.
[44] Y. Ichigo, A. Takeshita, M. Hibino, T. Nakagawa, T. Hayakawa, D. Patel, C.J. Field, M. Shimada, High-fructose diet-induced hypertriglyceridemia is associated with enhanced hepatic expression of ACAT2 in rats, Physiol. Res. 68 (2019) 1021–1026, https://doi.org/10.33549/physiolres.934226.
[45] C.M. Nepokroeff, J.W. Porter, Translation and characterization of the fatty acid synthetase messenger RNA, J. Biol. Chem. 253 (1978) 2279–2283.
[46] S.E. Schadinger, N.L.R. Bucher, B.M. Schreiber, S.R. Farmer, PPARgamma2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes, Am. J. Physiol. Endocrinol. Metab. 288 (2005) E1195–E1205, https://doi.org/10.1152/ajpendo.00513.2004.
[47] S. Siddiqi, A.M. Mani, S.A. Siddiqi, The identification of the SNARE complex required for the fusion of VLDL-transport vesicle with hepatic cis-Golgi, Biochem. J. 429 (2010) 391–401, https://doi.org/10.1042/BJ20100336.
[48] K. Minehira, S.G. Young, C.J. Villanueva, L. Yetukuri, M. Oresic, M.K. Hellerstein, R.V.J. Farese, J.D. Horton, F. Preitner, B. Thorens, L. Tappy, Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice, J. Lipid Res. 49 (2008) 2038–2044, https://doi.org/10.1194/jlr.M800248-JLR200.
[49] O. Abo Alrob, G.D. Lopaschuk, Role of CoA and acetyl-CoA in regulating cardiac fatty acid and glucose oxidation, Biochem. Soc. Trans. 42 (2014) 1043–1051, https://doi.org/10.1042/BST20140094.
[50] B.B. Rasmussen, U.C. Holmbäck, E. Volpi, B. Morio-Liondore, D. Paddon-Jones, R. R. Wolfe, Malonyl coenzyme A and the regulation of functional carnitine palmitoyltransferase-1 activity and fat oxidation in human skeletal muscle, J. Clin. Invest. 110 (2002) 1687–1693, https://doi.org/10.1172/JCI15715.
[51] M. She, X. Hu, Z. Su, C. Zhang, S. Yang, L. Ding, M. Laudon, W. Yin, Piromelatine, a novel melatonin receptor agonist, stabilizes metabolic profiles and ameliorates insulin resistance in chronic sleep restricted rats, Eur. J. Pharmacol. 727 (2014) 60–65, https://doi.org/10.1016/j.ejphar.2014.01.037.
[52] Y. Mi, D. Tan, Y. He, X. Zhou, Q. Zhou, S. Ji, Melatonin modulates lipid metabolism in HepG2 cells cultured in high concentrations of oleic acid: AMPK pathway activation may play an important role, Cell Biochem. Biophys. 76 (2018) 463–470, https://doi.org/10.1007/s12013-018-0859-0.
[53] K. Wu, X.-Y. Tan, Y.-H. Xu, G.-H. Chen, M.-Q. Zhuo, Functional analysis of promoters of genes in lipid metabolism and their transcriptional response to STAT3 under leptin signals, Genes 9 (2018) 334, https://doi.org/10.3390/genes9070334.
[54] F. Oriente, S. Cabaro, A. Liotti, M. Longo, L. Parrillo, T.B. Pagano, G.A. Raciti, D. Penkov, O. Paciello, C. Miele, P. Formisano, F. Blasi, F. Beguinot, PREP1 deficiency downregulates hepatic lipogenesis and attenuates steatohepatitis in mice, Diabetologia 56 (2013) 2713–2722, https://doi.org/10.1007/s00125-013-3053-3.
[55] J. Chen, H. Xia, L. Zhang, H. Zhang, D. Wang, X. Tao, Protective effects of melatonin on sepsis-induced liver injury and dysregulation of gluconeogenesis in rats through activating SIRT1/STAT3 pathway, Biomed. Pharmacother. 117 (2019), 109150, https://doi.org/10.1016/j.biopha.2019.109150.
[56] Z. Song, B. Humar, A. Gupta, E. Maurizio, N. Borgeaud, R. Graf, P.-A. Clavien, Y. Tian, Exogenous melatonin protects small-for-size liver grafts by promoting monocyte infiltration and releases interleukin-6, J. Pineal Res. 65 (2018) 12486, https://doi.org/10.1111/jpi.12486.
[57] M. Sanchez-Hidalgo, Z. Lu, D.-X. Tan, M.D. Maldonado, R.J. Reiter, R. I. Gregerman, Melatonin inhibits fatty acid-induced triglyceride accumulation in ROS17/2.8 cells: implications for osteoblast differentiation and osteoporosis, Am. J. Physiol. Regul. Integr. Comp. Physiol. 292 (2007) R2208–R2215, https://doi.org/10.1152/ajpregu.00013.2007.
[58] P.-P. Wang, M.-H. She, P.-P. He, W.-J. Chen, M. Laudon, X.-X. Xu, W.-D. Yin, Piromelatine decreases triglyceride accumulation in insulin resistant 3T3-L1 adipocytes: role of ATGL and HSL, Biochimie 95 (2013) 1650–1654, https://doi.org/10.1016/j.biochi.2013.05.005.
[59] M. Haidari, N. Leung, F. Mahbub, K.D. Uffelman, R. Kohen-Avramoglu, G.F. Lewis, K. Adeli, Fasting and postprandial overproduction of intestinally derived lipoproteins in an animal model of insulin resistance. Evidence that chronic fructose feeding in the hamster is accompanied by enhanced intestinal de novo lipogenesis and ApoB48-containing l, J. Biol. Chem. 277 (2002) 31646–31655, https://doi.org/10.1074/jbc.M200544200.
[60] H. Saito, M. Kato, A. Yoshida, M. Naito, The ingestion of a fructose-containing beverage combined with fat cream exacerbates postprandial lipidemia in young healthy women, J. Atheroscler. Thromb. 22 (2015) 85–94, https://doi.org/10.5551/jat.22681.
[61] H. Saito, M. Kagaya, M. Suzuki, A. Yoshida, M. Naito, Simultaneous ingestion of fructose and fat exacerbates postprandial exogenous lipidemia in young healthy Japanese women, J. Atheroscler. Thromb. 20 (2013) 591–600, https://doi.org/10.5551/jat.17301.
[62] S.G. Young, C.M. Cham, R.E. Pitas, B.J. Burri, A. Connolly, L. Flynn, A.S. Pappu, J. S. Wong, R.L. Hamilton, R.V.J. Farese, A genetic model for absent chylomicron formation: mice producing apolipoprotein B in the liver, but not in the intestine, J. Clin. Investig. 96 (1995) 2932–2946, https://doi.org/10.1172/JCI118365.
[63] J. Iqbal, J.S. Parks, M.M. Hussain, Lipid absorption defects in intestine-specific microsomal triglyceride transfer protein and ATP-binding cassette transporter A1-deficient mice, J. Biol. Chem. 288 (2013) 30432–30444, https://doi.org/10.1074/jbc.M113.501247.
[64] S.J. Hernández Vallejo, M. Alqub, S. Luquet, C. Cruciani-Guglielmacci, P. Delerive, J.-M. Lobaccaro, A.-D. Kalopissis, J. Chambaz, M. Rousset, J.-M. Lacorte, Short-term adaptation of postprandial lipoprotein secretion and intestinal gene expression to a high-fat diet, Am. J. Physiol. Gastrointest. Liver Physiol. 296 (2009) G782–G792, https://doi.org/10.1152/ajpgi.90324.2008.
[65] Q. Cao, X. Cui, R. Wu, L. Zha, X. Wang, J.S. Parks, L. Yu, H. Shi, B. Xue, Myeloid deletion of α1AMPK exacerbates atherosclerosis in LDL receptor knockout (LDLRKO) mice, Diabetes 65 (2016) 1565–1576, https://doi.org/10.2337/db15-0917.
[66] Q. Su, C. Baker, P. Christian, M. Naples, X. Tong, K. Zhang, M. Santha, K. Adeli, Hepatic mitochondrial and ER stress induced by defective PPARα signaling in the pathogenesis of hepatic steatosis, Am. J. Physiol. Endocrinol. Metab. 306 (2014) E1264–E1273, https://doi.org/10.1152/ajpendo.00438.2013.
[67] P. Sallinen, S. Saarela, M. Ilves, O. Vakkuri, J. Leppäluoto, The expression of MT1 and MT2 melatonin receptor mRNA in several rat tissues, Life Sci. 76 (2005) 1123–1134, https://doi.org/10.1016/j.lfs.2004.08.016.
[68] F. Hong, S. Pan, P. Xu, T. Xue, J. Wang, Y. Guo, L. Jia, X. Qiao, L. Li, Y. Zhai, Melatonin orchestrates lipid homeostasis through the hepatointestinal circadian clock and microbiota during constant light exposure, Cells 9 (2020), https://doi.org/10.3390/cells9020489.