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Supplementation with CO induces lipogenesis in adipose tissue, leptin and insulin resistance in healthy *Swiss* mice

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ABSTRACT

The consumption of saturated fatty acids (SFA), the main compound in coconut oil (CO), can promote insulin and leptin resistance and are associated with inflammation and obesity. We investigated the effects of CO supplementation on leptin signaling in healthy mice. *Swiss* male mice received oral supplementation for eight weeks with 300 μL of water for the control group (CV) or CO (100 or 300 μL). Sensitivity to leptin/insulin was evaluated after eight weeks of supplementation. The CO induced endoplasmic reticulum stress and leptin resistance in the hypothalamus, as demonstrated by reduced effect on the energy expenditure, hypothalamic pJAK2 and pSTAT3, and POMC expression. In the adipose tissue, lipogenesis was favored and STAT3 and JAK2 signaling was impaired after CO supplementation. Furthermore, the supplementation with CO reduced pAKT in the hypothalamus, liver and white adipose tissue. These results show that CO induces hypothalamic and peripheral resistance to leptin and insulin in healthy mice.

1. Introduction

Saturated fatty acid (SFA) is the main component of a fat diet (Krauss and Kris-Etherton, 2020; Harrison et al., 2020; Vandevijvere et al., 2015; German and Dillard, 2004) and rich diet in SFA can promote insulin and leptin resistance in peripheral and hypothalamic tissues (Lieu et al., 2021). Leptin resistance may be linked to a failure in leptin transport or deficits in intracellular signaling mechanisms downstream of leptin (Yazdi et al., 2015). Simultaneously to the development of leptin resistance, white adipose tissue also develops local resistance to leptin, since decreased STAT3 activation is observed in adipose tissue of diet-induced obese animals in basal conditions and stimulated by leptin (Wang et al., 2000). Besides, the peripheral and central signaling of the insulin also have an important role in the control of whole-body glucose and energy metabolism (Obici et al., 2002; Obici et al., 2002).

SFAs, especially medium chain saturated fatty acids (MCFAs), are the main compound in coconut oil (CO) (Rahim et al., 2017). Studies indicate that SFA from coconut oil can play a beneficial role (Nagao and Yanagita, 2010), this is because MCFAs are absorbed in the intestine and are transported through the portal system directly to the liver. Moreover, MCFAs do not depend on the presence of carnitine palmitoyltransferase-1 (CPT-1) to enter the mitochondrial (Friedman et al., 1990). In addition, studies have associated benefits of virgin CO with antioxidant properties of phenolics compounds and its beneficial role in both prevention and treatment of metabolic syndrome (Nagao and Yanagita, 2010).

On the other hand, SFA stimulates an inflammatory response via TLR2 and TLR4 signaling pathways (Huang et al., 2012; Hwang et al., 2016). Accordingly, in a previous study we showed that CO consumption for eight weeks led to weight gain, higher percentage of fat, reduced energy expenditure, activated inflammatory pathways, both centrally and peripherally, and triggered an anxious behavior in healthy *Swiss* mice, suggesting the deleterious effect on the body homeostasis (Veras et al., 2021). Thus, to evaluate if the CO supplementation also impaired hormonal signaling, we investigated the capacity of leptin to modulate the behavior and energy homeostasis in healthy *Swiss* mice.

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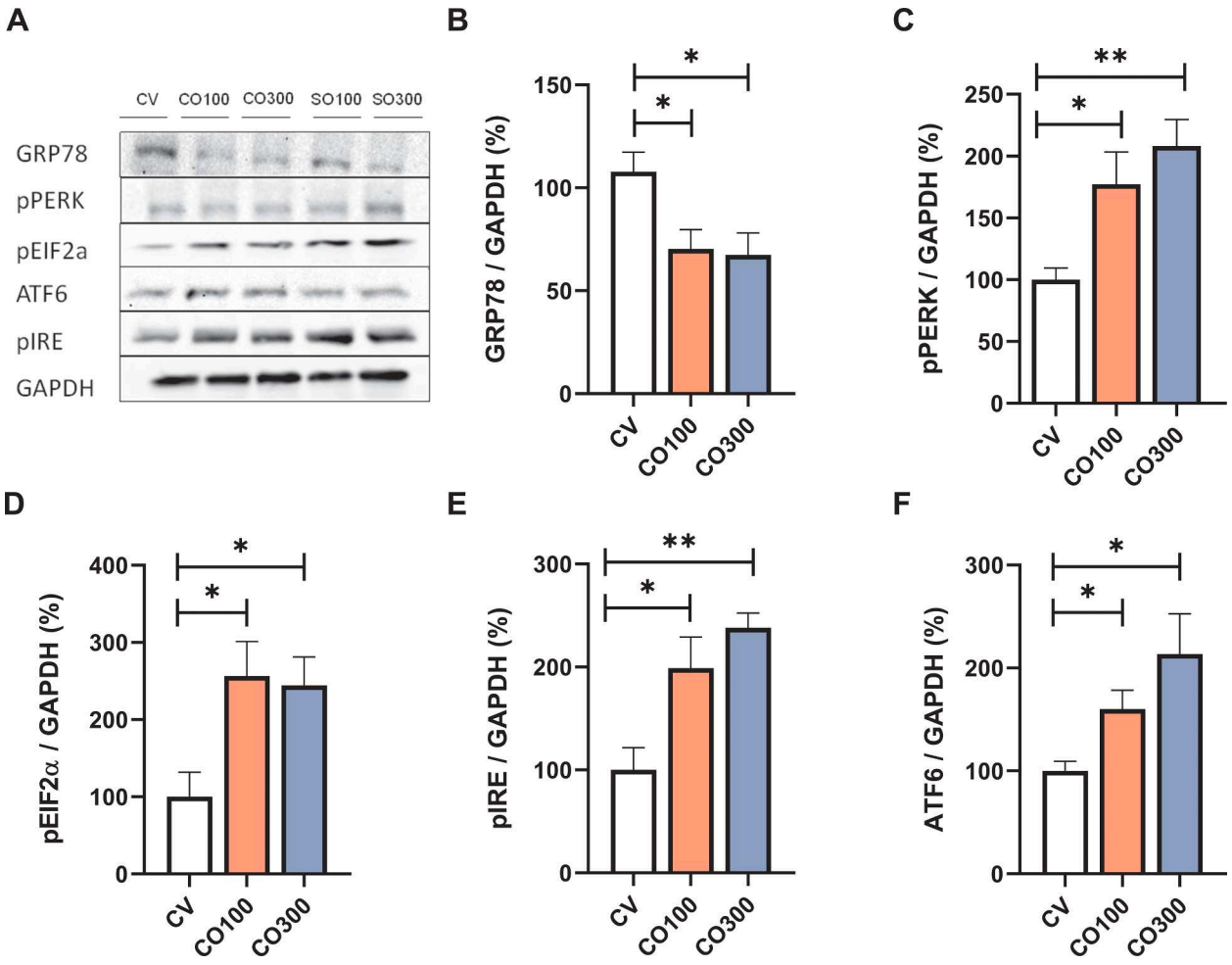


Fig. 1. Reticulum Stress parameters in the hypothalamus of mice supplemented, for 8 weeks, with coconut oil (CO100 or CO300) or water (CV). (A-F) Western-blotting analysis of Reticulum stress pathways markers, in the hypothalamus. Values are shown as mean ± SEM; * $p < 0,05$ versus CV; **$p < 0,01$ versus CV; $n = 3$ animals per group.

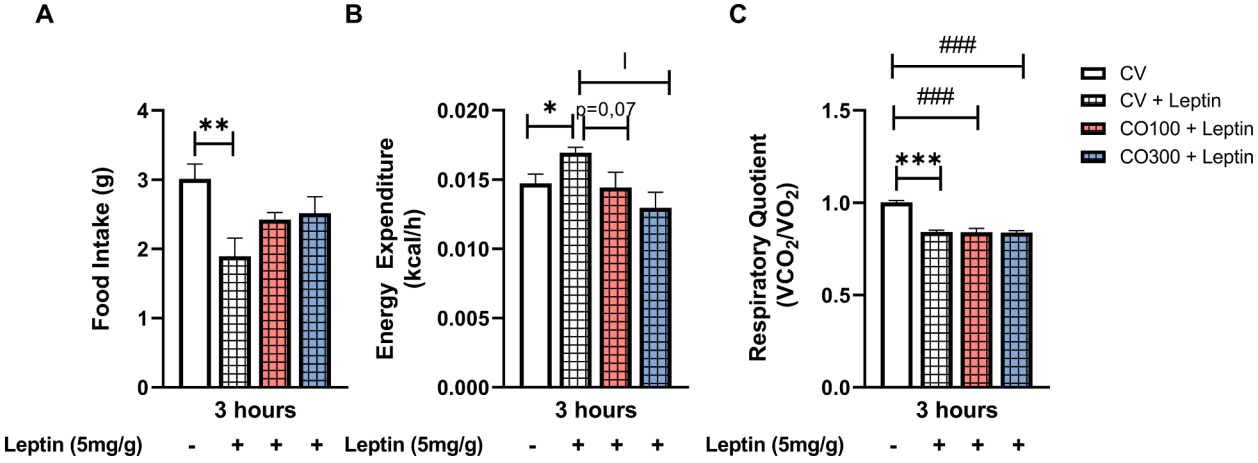


Fig. 2. Effects of leptin stimulation in mice supplemented, for 8 weeks, with coconut oil (CO100 or CO300) or water (CV). (A) Food intake after 3 h of leptin stimulation (5 mg/g, IP); (B) Energy expenditure after 3 h of leptin stimulation (5 mg/g, IP); (C) Respiratory Quotient (RER) after 3 h of leptin stimulation (5 mg/g, IP); Values are shown as mean ± SEM; * $p < 0,05$ versus respective basal group; **$p < 0,01$ versus respective basal group; ***$p < 0,001$ versus respective basal group; ### $p < 0,001$ versus CV; ł $p < 0,05$ versus CV + Leptin; $n = 4–6$ animals per group.

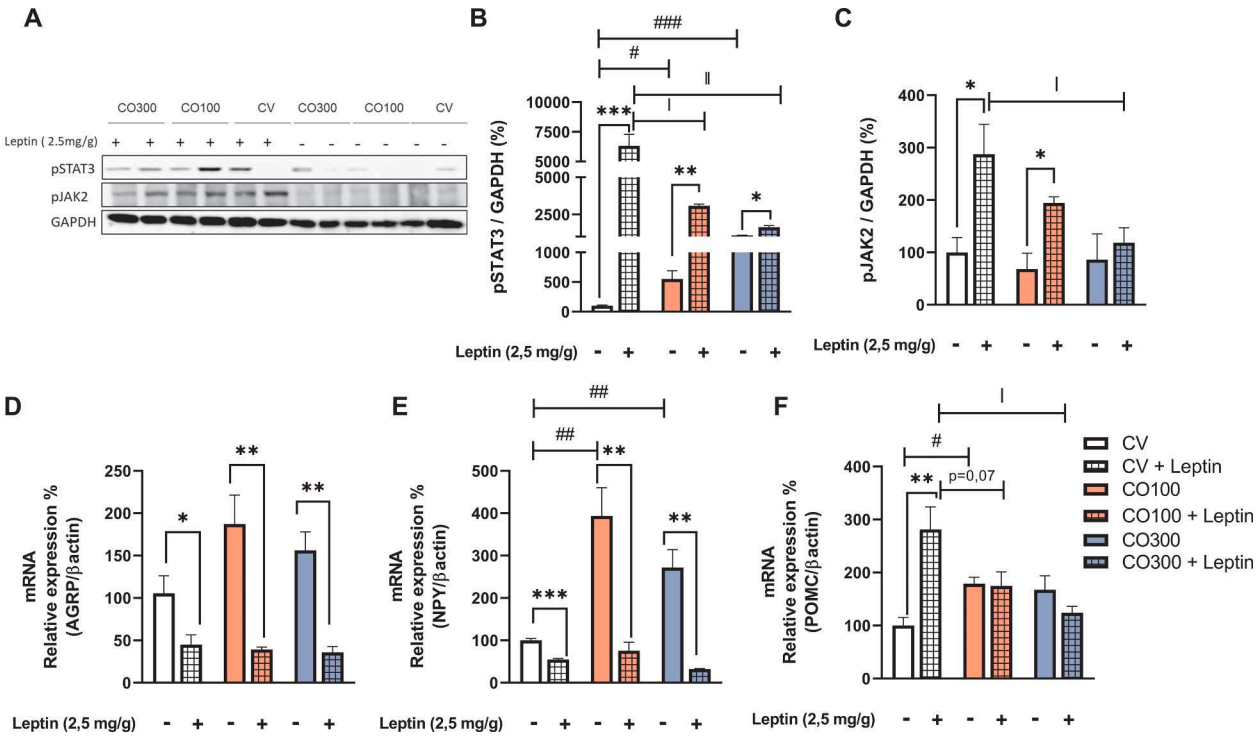


Fig. 3. Effects of leptin stimulation in the hypothalamus of mice supplemented, for 8 weeks, with coconut oil (CO100 or CO300) or water (CV). (A-C) Western-blotting analysis of Leptin pathways markers in the hypothalamus of mice supplemented with coconut oil or water after leptin stimulation (2.5 mg/g, IP); (D-F) mRNA expression of neuropeptides, in the hypothalamus of mice supplemented with coconut oil or water after leptin stimulation (2.5 mg/g, IP), AGRP, NPY and POMC, respectively. Values are shown as mean ± SEM; * $p < 0,05$ versus respective basal group; **$p < 0,01$ versus respective basal group; ***$p < 0,001$ versus respective basal group; # $p < 0,05$ versus CV; ## $p < 0,01$ versus CV; ### $p < 0,001$ versus CV; ł $p < 0,05$ versus CV + Leptin; ǂ $p < 0,01$ versus CV + Leptin; ǂǂ $p < 0,001$ versus CV + Leptin; n = 3–6 animals per group.

Additionally, insulin signaling in hypothalamus, white adipose tissue and liver was evaluated.

2. Materials and methods

2.1. Animals

This investigation was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the Brazilian Society of Science in Laboratory Animals. All procedures were approved by the Research Ethics Committee for Animal Use of the University of Campinas (Protocol number: 5124–1/2019). Five-week-old male mice of *Swiss* lineage, weighing approximately 20 g, were provided by the Animal Breeding Center of the University of Campinas. The animals were kept in individual cages, with *ad libitum* water and chow diet (NUVILAB® Cr-1-Nuvital, Brazil), in a room with controlled temperature (22–24 °C) and a light/dark cycle (12 h).

2.2. Experimental design

The mice (n = 60) were randomly distributed into three groups and received oral supplement with pipette tip, for eight weeks with 300 μL of water for the control group (CV, n = 20), 100 or 300 μL of commercial extra-virgin coconut oil (Copra brand) (CO100, n = 20 and CO300 n = 20, respectively). The supplementation was calculated according to the recommendation of saturated fat intake, corresponding to a maximum of 10% of the diet (Reeves et al., 1993), that is, 100 μL. Meanwhile, 300 μL is a volume recommended to mimic the additional consumption of CO. At the end of the experimental period, after 12 h overnight -fasting, the mice were anesthetized with a mixture containing (Ketamine 100 mg/kg; Xylazine 5 mg/kg, ip) and then decapitated. Samples of hypothalamus, liver and epididymal adipose tissue were weighted, frozen in liquid nitrogen and stored at − 80 °C until processing.

2.3. Leptin and insulin administration

The animals were fasted for 24 h and then stimulated with saline or leptin (5 mg/g) to evaluate sensitivity to leptin after eight weeks of CO supplementation, intraperitoneally, at the beginning of the waking period. During this same period, the animals were stimulated and re-fed to measure food intake, energy expenditure, and respiratory quotient three hours after the leptin injection (Garcia-Galiano et al., 2017). However, to assess leptin signaling at the end of the experimental period, saline or leptin (2.5 mg/g) was administered intraperitoneally in mice, which were fasting for 12 h, and 30 min after stimulation (Garcia-Galiano et al., 2017) the animals were anesthetized and subsequently decapitated.

To investigate insulin sensitivity in peripheral tissues, at the end of the experiment for 12 h, and 20 min after the stimulus, the animals were anesthetized and subsequently decapitated. At the end of the supplementation period the hypothalamus was collected after anesthesia and decapitation of mice in order to investigate insulin sensitivity in the central nervous system. Then, hypothalamic tissue was exposed to DMEM with or without insulin (50 nM) for 10 min.

2.4. Energy expenditure

At the end of the eighth week of supplementation and after leptin stimulation (5 mg/g), the animals were tested in the Comprehensive Lab Animal Monitoring System (CLAMS, Columbus Instruments, Columbus, OH), measuring energy expenditure (expressed in Heat – kcal/min /body weight) and respiratory quotient (RER - VO2/O2 ratio) by indirect calorimetry. CLAMS was installed under constant room temperature (22 °C) and light/dark cycle (12 h). For this assay, mice were previously

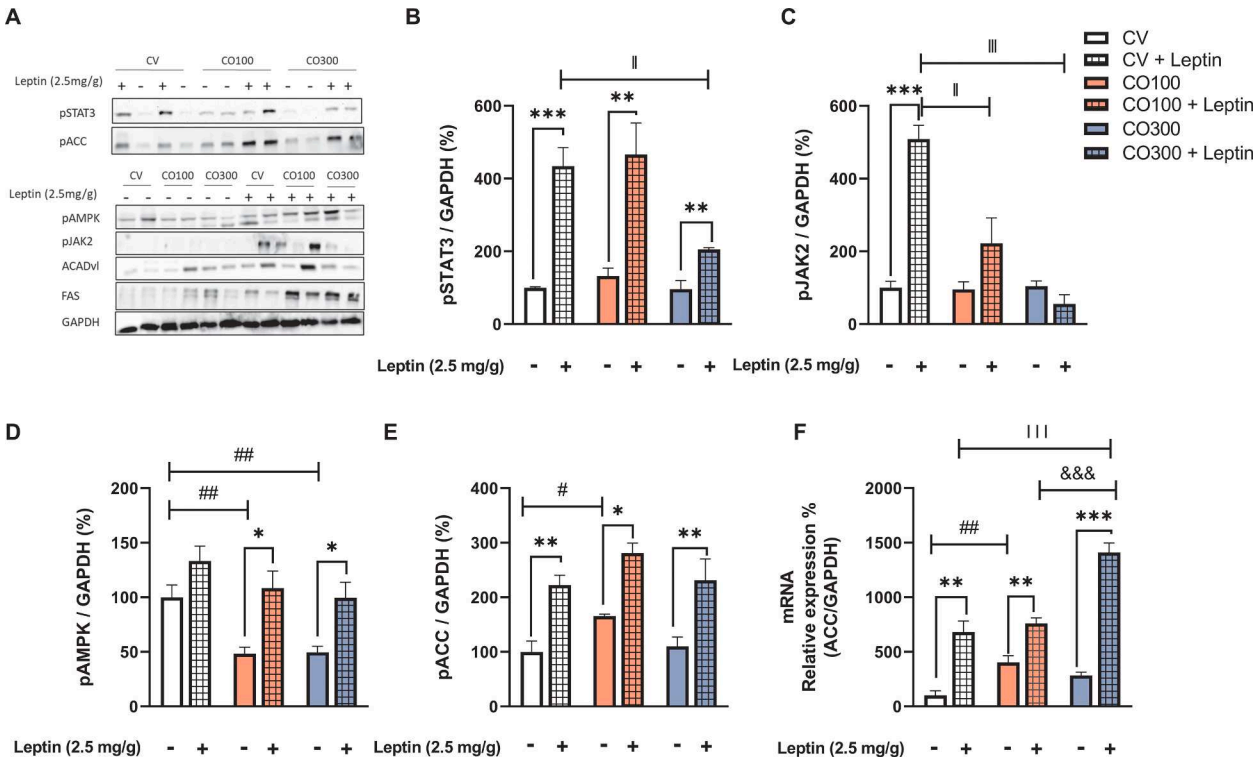


Fig. 4. Effects of leptin stimulation in the epididymal adipose tissue of mice supplemented, for 8 weeks, with coconut oil (CO100 or CO300) or water (CV). (A-E) Western-blotting analysis of Leptin pathways markers in the epididymal adipose tissue of mice supplemented with coconut oil or water after leptin stimulation (2.5 mg/g, IP); (F) ACC mRNA expression. * $p < 0,05$ versus respective basal group; **$p < 0,01$ versus respective basal group; ***$p < 0,001$ versus respective basal group; # $p < 0,05$ versus CV; ## $p < 0,01$ versus CV; ### $p < 0,001$ versus CV; ǂ $p < 0,05$ versus CV + Leptin; ǂǂ $p < 0,01$ versus CV + Leptin; ǂǂǂ $p < 0,001$ versus CV + Leptin; $$ $p < 0,01$ versus CO100; && $p < 0,01$ versus CO100 + Leptin; n = 3–6 animals per group.

adapted for 12 h in individual cages.

2.5. qPCR- real time analysis

Frozen epididymal adipose tissue (100 mg) and hypothalamus were homogenized using Trizol reagent (Invitrogen Corporation, CA, USA). Total RNA was extracted according to the manufacturer's guidelines and quantified on a Nanodrop ND-2000 spectrophotometer (Thermo Electron, WI, USA). Reverse transcription was performed with 3 μg of total RNA and a High-Capacity cDNA Reverse Transcription kit (Life Technologies Corporation, Carlsbad, CA, USA). Relative expression was determined using the TaqMan PCR Master Mix (Applied Biosystems) and all primers were obtained from the Applied Biosystems: NPY (Mm01410146_m1), POMC (Mm00435874_m1), AGRP (Mm00475829_g1), ACADvl (Mm0044293_m1), ACADm (Mm01323360_g1), AGPAT (Mm00479699_g1), ACACA (Mm01304277_m1), and beta-actin (4351315) or GAPDH (4351309) as endogenous control. Real-time PCR was performed in an ABI Prism 7700 sequence detection system (Applied Biosystems). Each PCR reaction contained 20 ng of cDNA. Data were analyzed using Sequence Detection System 2.0.5 (Life Technologies Corporation, Carlsbad, CA, USA) and expressed as relative values determined by the comparative threshold cycle (Ct) method (2DDCt) according to the manufacturer's guidelines.

2.6. Western blotting analysis

Frozen epididymal adipose tissue (100 mg), liver (100 mg) and hypothalamus were homogenized in freshly prepared ice-cold buffer [1% (v/v) Triton X-100, 0.1 M Tris, pH 7.4, 0.1 M sodium pyrophosphate, 0.1 M sodium fluoride, 0.01 M EDTA, 0.01 M sodium vanadate, 0.002 M PMSF and 0.01 M aprotinin] using a tissue homogenizer (Bead Ruptor 12 Homogenizer, Omni International, Kennesaw, GA, USA). Insoluble materials were removed by centrifugation (12,000 rpm) for 30 min at 4 °C. The protein concentration of the supernatant was determined using the Biuret dye-binding method for adipose tissue and liver, and Bradford assay (Bradford, 1976) for the hypothalamus. The supernatant was suspended in Laemmli sample buffer and boiled for 5 min before separation by SDS-PAGE using a miniature slab gel apparatus (BioRad, Richmond, CA, USA). Electrotransfer of proteins from the gel to a nitrocellulose membrane was performed for 120 min at 120 V (constant) in a transfer buffer with methanol. The membranes were subsequently blocked with a 5% skim milk solution in Tris-buffered saline (TBS)-Tween 20 (TTBS; 10 mmol Tris/L,150 mmol NaCl/L, 0.5% Tween 20) for 2 h at room temperature. After blocking, the membranes were washed three times with TBST for 5 min each and then incubated overnight at 4 °C with specific primary antibodies, from Cell Signaling, pSTAT3 (9145S), pJAK2 (3776S), pAMPK (2535S), pAKT (4060S), pEIF2a (9721), from Imgenex, ATF6 (IMG273), from Abcam, pIRE (ab48187) and from Santa Cruz, pACCα (sc-30447-R), ACADvl (sc-376239), FAS (sc-20140), GRP78 (sc-13968), pPERK (sc-32577), GAPDH (T5168) as endogenous control. Then, the membranes were washed three times with TBST for 5 min and incubated for 2 h at room temperature with secondary antibodies diluted in TTBS containing 3% dry skimmed milk). Proteins were detected by chemiluminescence (Super Signal West Pico Chemiluminescent Substrate, ThermoFisher Scientific, MA, USA). Band intensity was determined by the software application ImageJ and the results are expressed as the ratio between specific protein and endogenous protein (GAPDH).

2.7. Statistical analysis

All data were expressed as means ± SEM. Statistical analysis was initially conducted by applying the Kolmogorov-Smirnov to certify normality. Then, the following was applied: Student's *t*-test for

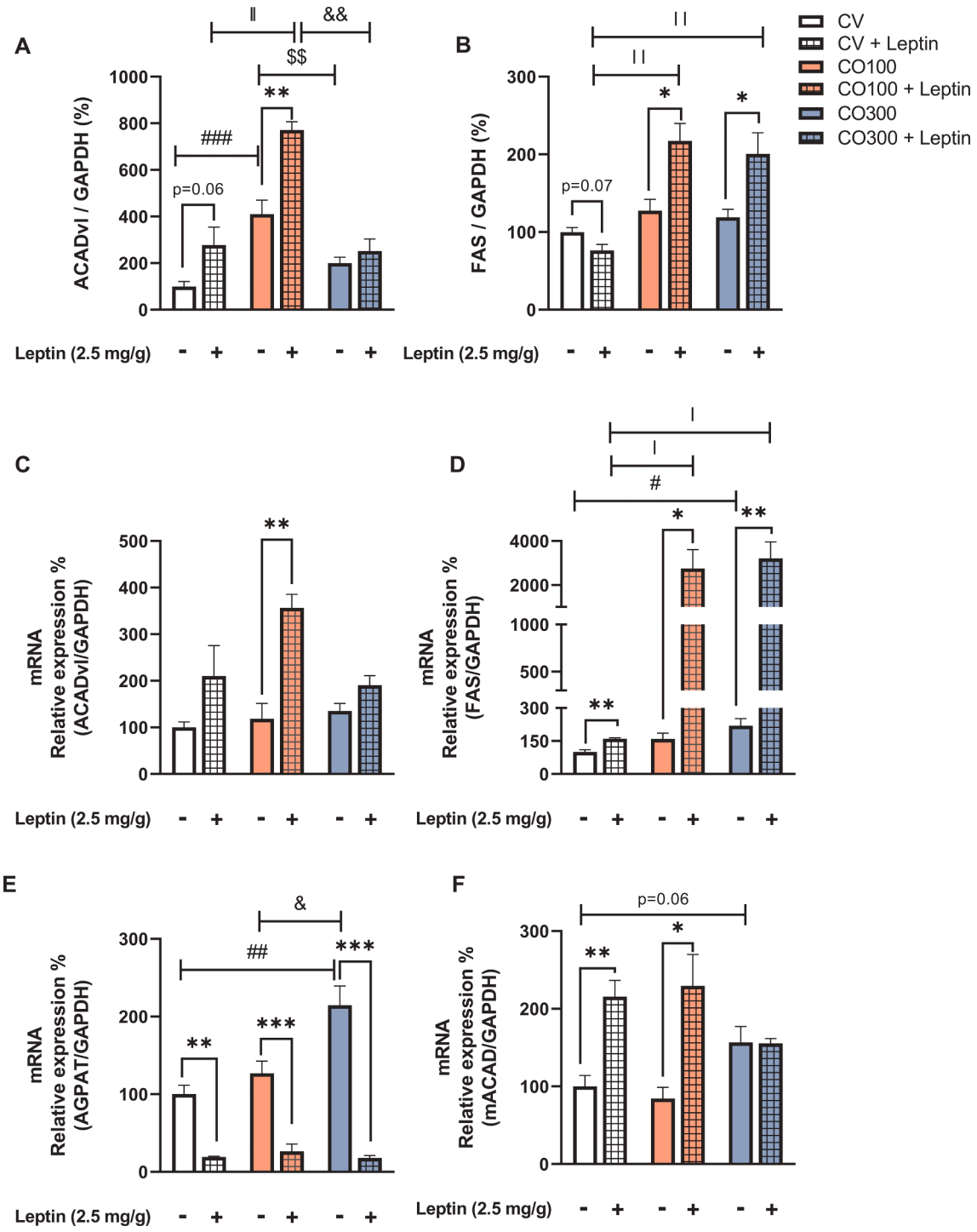


Fig. 5. Effects of leptin stimulation related to fatty acid metabolism in the epididymal adipose tissue of mice supplemented, for 8 weeks, with coconut oil (CO100 or CO300) or water (CV). (A-B) Western-blotting analysis of ACADvl and FAS, respectively. (C-D) mRNA expression of genes related to fatty acid metabolism in the epididymal adipose tissue of mice supplemented with coconut oil or water after leptin stimulation (2.5 mg/g, IP). Values are shown as mean ± SEM; * $p < 0,05$ versus respective basal group; **$p < 0,01$ versus respective basal group; ***$p < 0,001$ versus respective basal group; # $p < 0,05$ versus CV; ## $p < 0,01$ versus CV; ### $p < 0,001$ versus CV; ǂ $p < 0,05$ versus CV + Leptin; ǂǂ $p < 0,01$ versus CV + Leptin; ǂǂǂ $p < 0,001$ versus CV + Leptin; $$ $p < 0,01$ versus CO100; && $p < 0,01$ versus CO100 + Leptin; n = 3–6 animals per group.

comparisons between two independent groups and one-way analysis of variance (ANOVA) followed by Tukey's test for comparison between all experimental groups. GraphPad Prism 8 was used for all statistical analyses. Also, *p*-values < 0.05 were considered statically significant.

3. Results

3.1. Coconut oil consumption for 8 weeks induces reticulum stress in the hypothalamus

CO supplementation decreased GRP78 protein level (Fig. 1B) and increased expression of ATF6 (Fig. 1F) in the hypothalamus. Moreover,

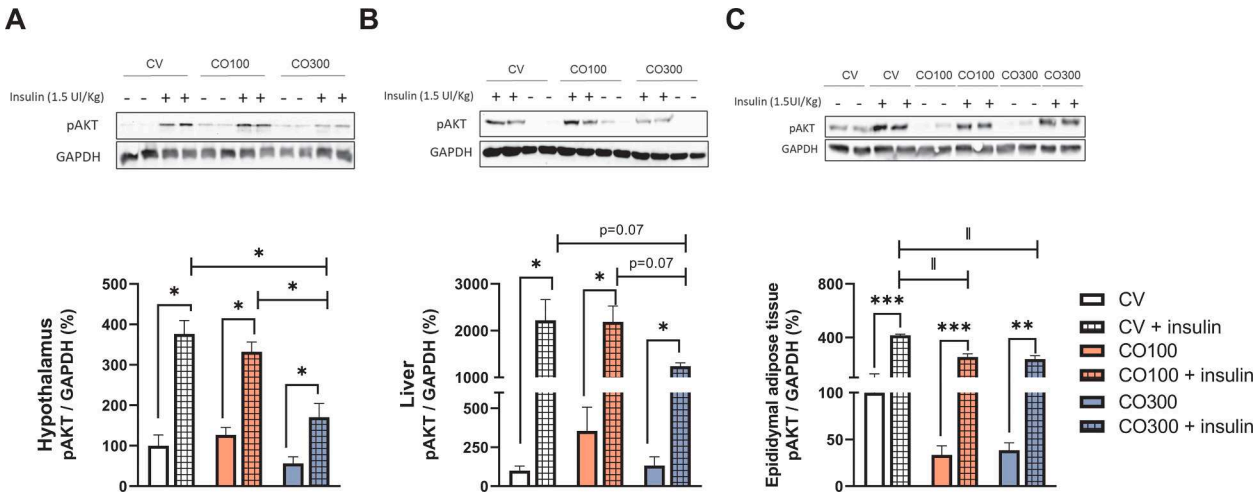


Fig. 6. Effects of insulin stimulation in mice supplemented, for 8 weeks, with coconut oil (CO100 or CO300) or water (CV). (A-D) pAKT expression in the hypothalamus, liver, and epididymal adipose tissue, respectively. Values are shown as mean ± SEM; **p < 0,01 versus respective basal group; ***p < 0,001 versus respective basal group; ǂ p < 0,01 versus CV + Insulin; n = 3 per group.

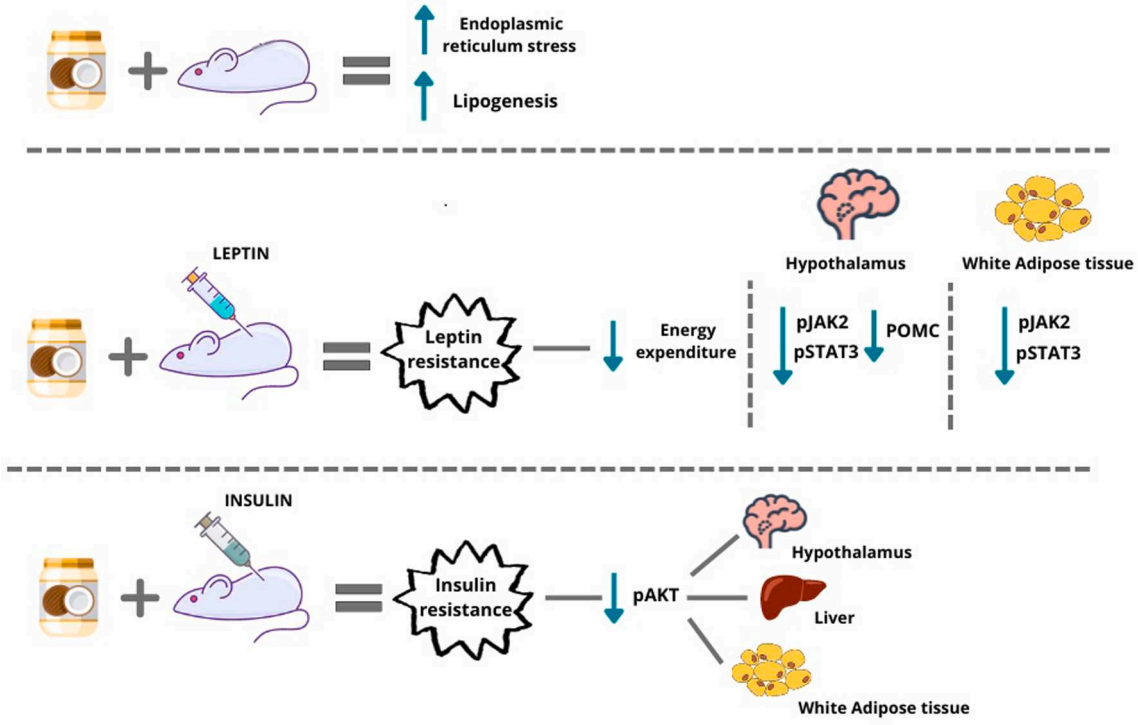


Fig. 7. Summary of the effects of coconut oil supplementation with or without leptin or insulin stimulation in healthy *Swiss* mice.

CO consumption increased phosphorylation of PERK, EIF2α, and IRE (Fig. 1B-1E, respectively). These results show that the CO consumption for eight weeks triggers endoplasmic reticulum stress in the hypothalamus of healthy *Swiss* mice.

3.2. Coconut oil supplementation alters leptin function and signaling pathway in the hypothalamus

Three hours after leptin stimulation, we identified a reduction in food intake and an increase in energy expenditure in the CV group. However, leptin could not change the food intake and energy expenditure of animals supplemented with CO (Fig. 2A and B). Furthermore, after leptin administration, the respiratory coefficient was lower in all experimental groups (Fig. 2C).

CO supplementation for eight weeks increased basal STAT3 phosphorylation when compared to the CV group (Fig. 3A). As expected, the presence of leptin increased hypothalamic STAT3 and JAK2 phosphorylation in animals from the CV group (Fig. 3B and 3C). However, in mice that consumed CO the hypothalamic STAT3 and JAK2 phosphorylation stimulated by leptin was lower than CV + Leptin mice (Fig. 3B and C).

Furthermore, we evaluated the capacity of leptin to modulate neuropeptides expression in the hypothalamus. The CO consumption increased NPY and AGRP mRNA expression (Fig. 3D and 3E). The leptin reduced NPY and AGRP transcripts in all experimental groups (Fig. 3D and 2E). However, the leptin was only able to increase POMC gene expression in the CV group (Fig. 3F).

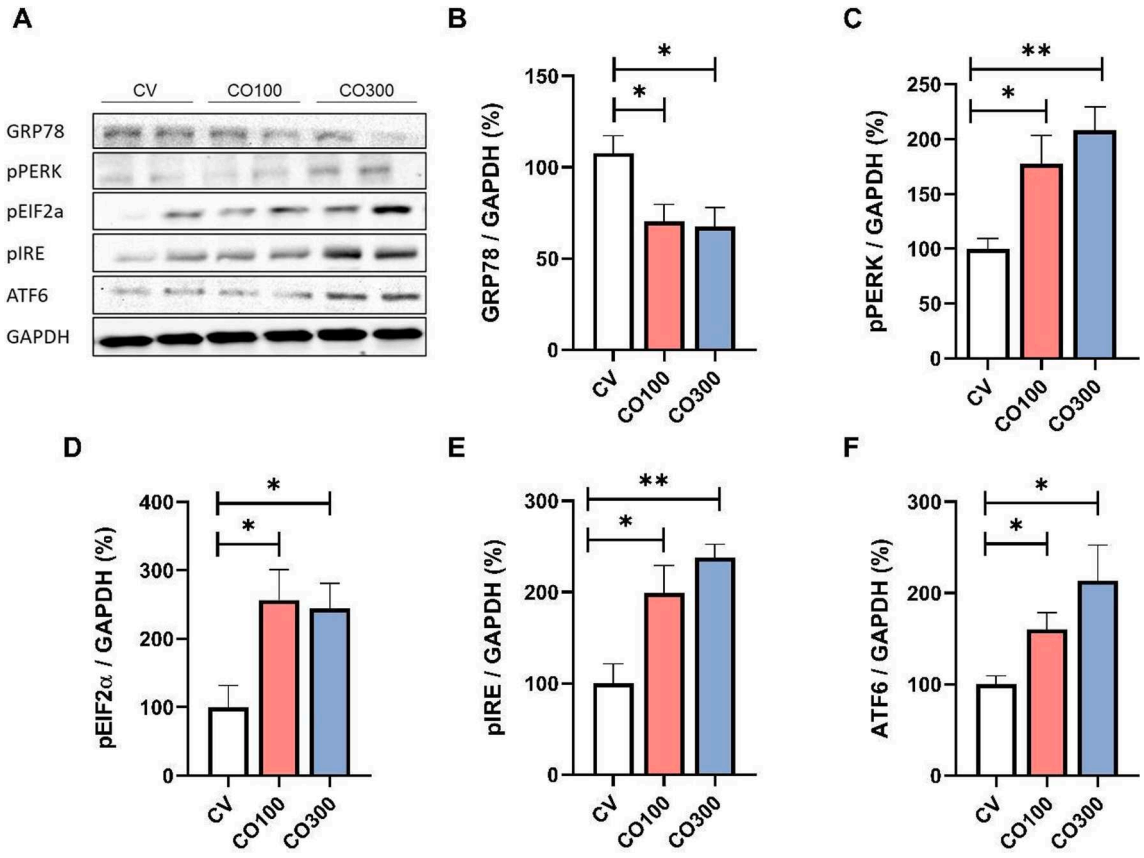


Fig. A1.

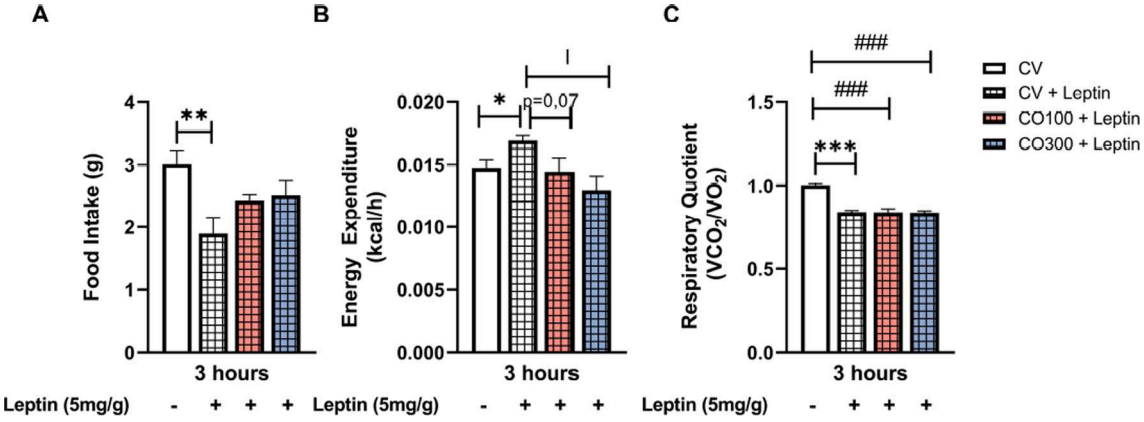


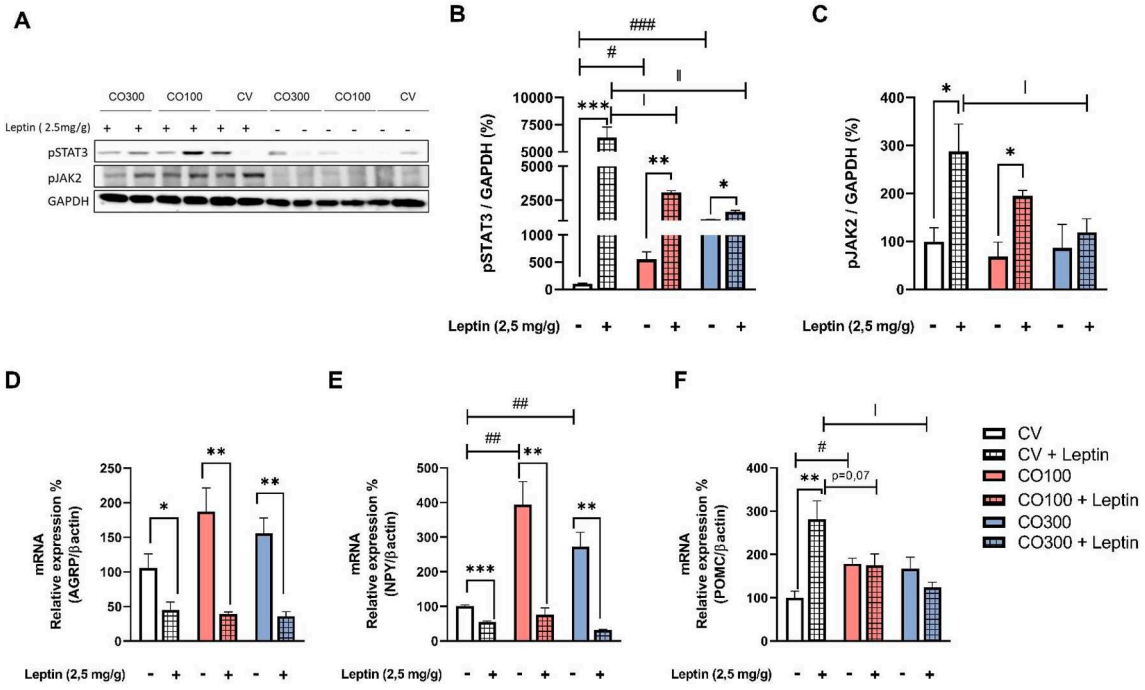
Fig. A2.

3.3. Coconut oil supplementation changes leptin signaling pathway and fatty acid metabolism in the epididymal adipose tissue

The supplementation with CO did not modify the basal phosphorylation of STAT3 and JAK2 (Fig. 4B and C) in the epididymal adipose tissue. JAK2 phosphorylation stimulated by leptin was significantly reduced in CO100 and CO300 compared to CV mice (Fig. 4C). However, STAT3 phosphorylation stimulated by leptin significantly increased in the CV, CO100, and CO300 mice, with a smaller increase in the CO300 group, as shown in Fig. 4B. Furthermore, the supplementation with CO reduced AMPK phosphorylation, but did not reduce the capacity of leptin to stimulate AMPK phosphorylation compared to the CV group (Fig. 4D). The level of total ACC and pACC, a target protein of AMPK, was not altered by CO supplementation and the stimulus with leptin induced ACC phosphorylation (Fig. 4E and F), similarly to that observed for AMPK.

Furthermore, we evaluated the proteins related to fatty acid metabolism in the epididymal adipose tissue. In CO100 mice the basal protein and transcript level of FAS and AGPAT mRNA were not different from control mice (CV). However, in CO300 mice FAS and AGPAT were increased compared to CV mice (Fig. 5A, B and C). Leptin stimulation reduced significantly AGPAT mRNA in all groups evaluated (CV and CO), while FAS expression was increased by leptin stimulation (Fig. 5A, B and C).

Regarding fatty acid oxidation, ACADvl and ACADm expression were similarly modulated by CO supplementation. While the ACADvl was induced in CO100 mice, ACADm was increased in CO300 mice (Fig. 5D, E and F). After leptin administration, the protein expression of ACADvl



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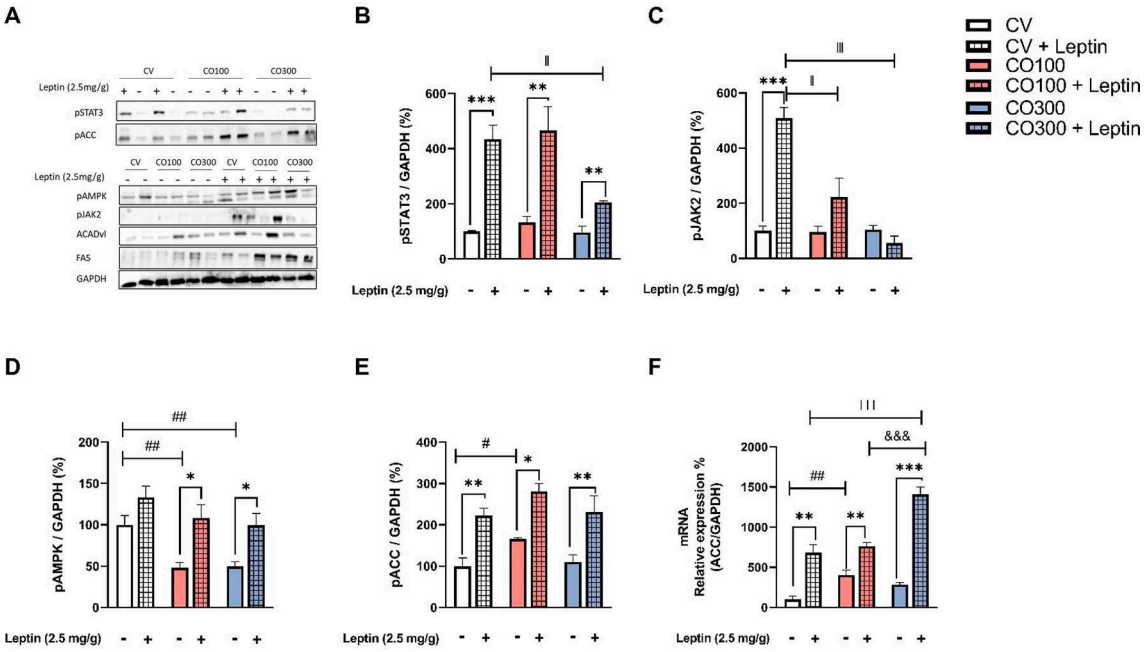

Fig. A4.

and ACADm increased in both CV and CO100 groups, but not in the CO300 group (Fig. 5D, E and F).

3.4. Coconut oil consumption does not induce insulin resistance

Basal AKT phosphorylation after supplementation with CO (CO100 and CO300) was similar to CV mice in all tissues investigated (hypothalamus, liver, and epididymal adipose tissue). The stimulus with insulin increased significantly AKT phosphorylation in the hypothalamus, liver, and epididymal adipose tissue of CO100 compared to CV mice (Fig. 6A, B and C). However, in CO300 mice the insulin-induced phosphorylation was lower than that observed in the CV and CO100 mice (Fig. 6A, B and C).

4. Discussion

In an initial study, we found that healthy *Swiss* mice supplemented with CO for eight weeks showed greater weight gain, increased body fat percentage and activation of inflammatory pathways, depending on TLR4, at the hypothalamic and peripheral tissues (Veras et al., 2021). In addition, food intake of control and the supplemented groups were not different, and we stress that the caloric gain at the end of the 8 weeks of

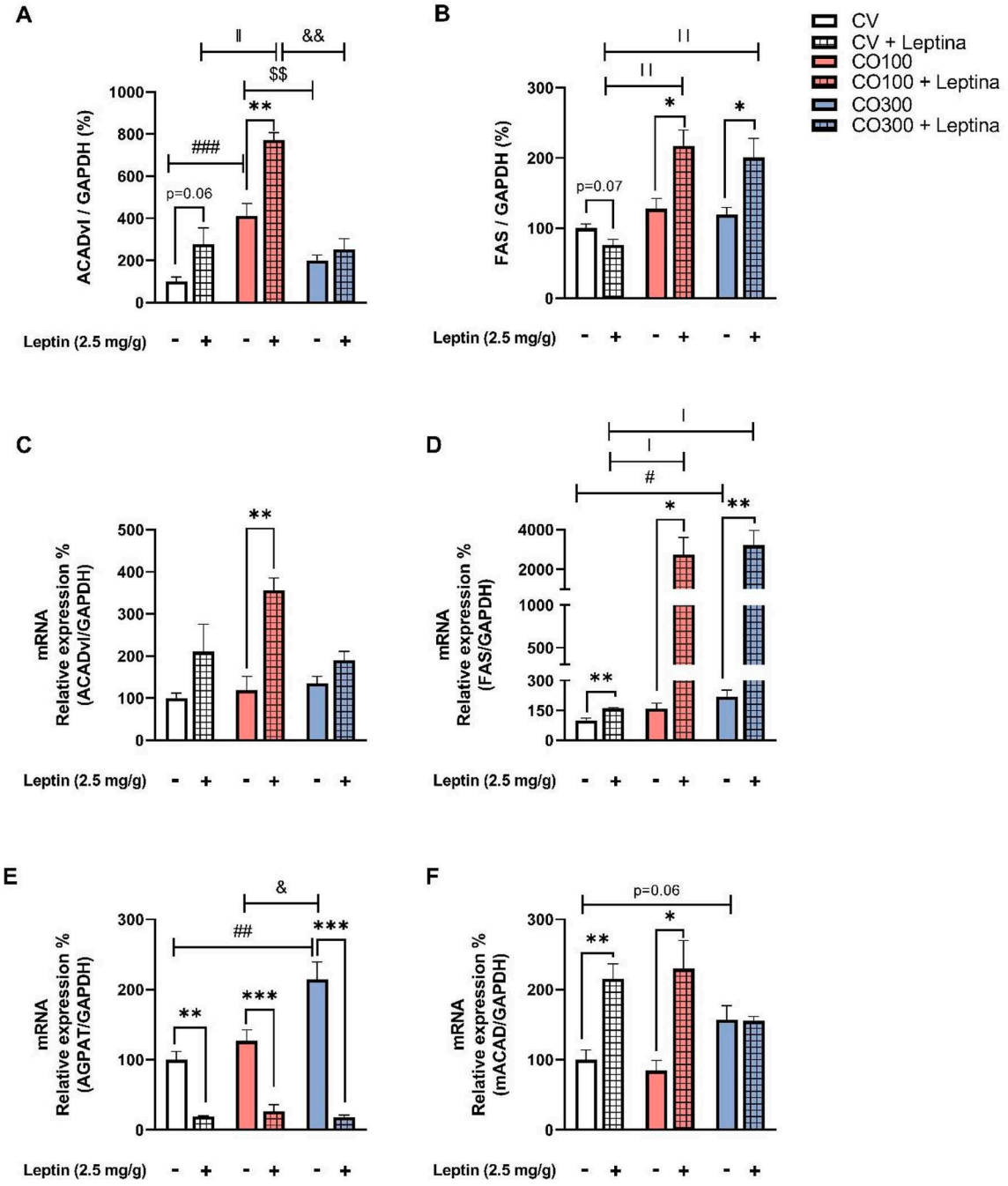

Fig. A5.

supplementation was only 50.4 and 151.2 kcal for the animals that consumed 100 uL and 300 uL of oil, respectively. Based on these previous findings and knowing that dietary fat consumption can trigger endoplasmic reticulum stress (ERS) (Milanski et al., 2009) we initially aimed to investigate whether CO supplementation for eight weeks would activate endoplasmic reticulum and oxidative stress in the hypothalamus of healthy mice. Thus, we have shown that consumption of CO induces hypothalamic ERS activation, by increasing protein expression of ATF6 and phosphorylation of IRE, PERK and EIF2α, but not oxidative stress, since there was no difference in the levels of MDA in the liver and NFR2 transcript levels in the hypothalamus of mice supplemented with CO (data not show). It is known that consumption diets high in animal fat (SFA rich) induces hypothalamic inflammation and subsequently ERS (De Souza et al., 2005; Carraro et al., 2018). However, here we showed that the consumption of coconut oil also activates inflammatory and ERS pathways in mice, probably through activation of TLR4 by SFAs, as demonstrated in previous study (Veras et al., 2021). One of the consequences of ERS activation is the detrimental effect on the ability of leptin and insulin to modulate glucose and energy homeostasis.

One of the mechanisms that triggers leptin resistance is hypothalamic inflammation (Cui et al., 2017; Velloso and Schwartz, 2011) and ERS emerged as a key factor in obesity-associated inflammation and leptin resistance (Cui et al., 2017). ERS is activated in several tissues of obese and leptin-resistant animals, including adipose tissue and the brain (Ozcan et al., 2004; Zhang et al., 2008; Ozcan et al., 2009). Hence, we evaluated sensitivity to leptin after eight weeks of CO supplementation and observed that leptin could not change food intake or increase the energy expenditure of animals that consumed coconut oil, suggesting hypothalamic leptin resistance. This hypothesis was confirmed by

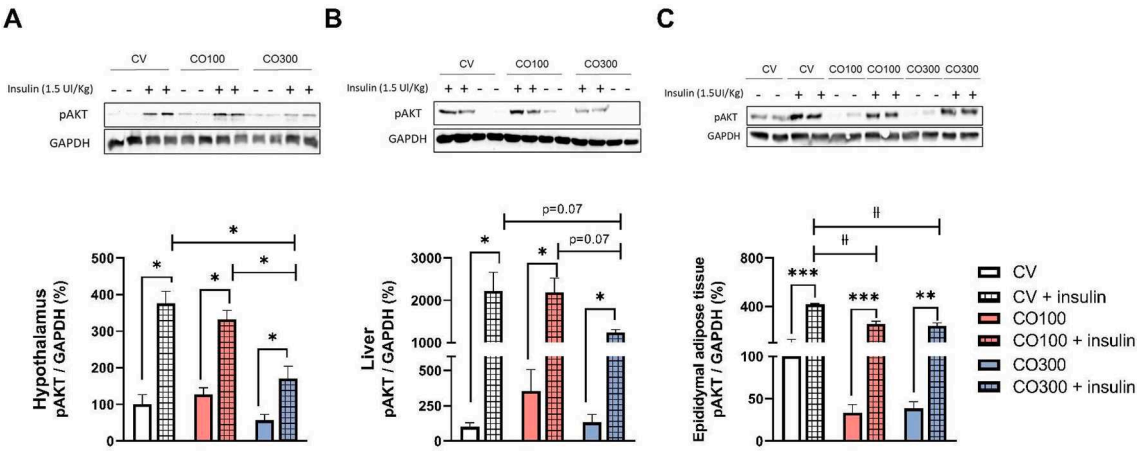

Fig. A6.

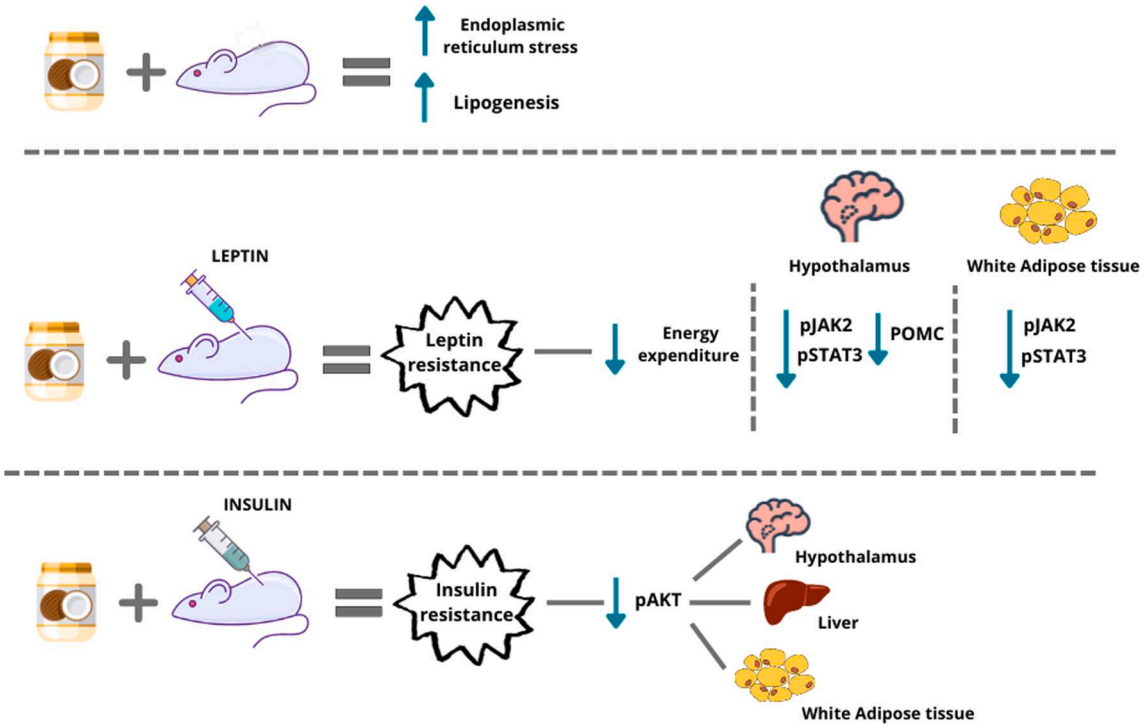

Fig. A7.

observing impairment in the leptin signaling, including the reduced effect on POMC expression, and phosphorylation of STAT3 and JAK2 in the hypothalamus and adipose tissue of mice that consumed coconut oil. Previous studies showed that pharmacological inhibition of ERS *in vitro* improves leptin signaling (Hosoi et al., 2008; Ozcan et al., 2008), as well as the inhibition of ERS either by genetic or pharmacological improves leptin sensitivity and decreases both food intake and body weight (Zhang et al., 2008; Ozcan et al., 2008). Recently, we showed that mice consuming coconut oil presented increased expression of pro-inflammatory cytokines (IL-6 and TNF-α) as well as activation of TLR4-dependent inflammatory pathways in the hypothalamus and adipose tissue (Veras et al., 2021). Thus, we believe that the leptin resistance triggered by coconut oil consumption is induced by inflammatory cytokines and the activation of ERS, given that the consumption of a high-fat diet (HFD) increases the expression of pro-inflammatory cytokines in the hypothalamus, such as IL-1, IL-6 and TNF-α, activating inflammatory pathways, such as the NFκB pathway (De Souza et al., 2005), resulting in ERS in the hypothalamus, which, in turn, can cause leptin resistance(Zhang et al., 2008).

Based on the previous outcome and the fact that leptin induces lipolysis (Stern et al., 2016), fatty acid oxidation (William et al., 2002), and decreases lipogenesis by ACC-1 phosphorylation (Huang et al., 2006), we investigated whether the administration of exogenous leptin would be able to promote changes in fatty acid metabolism in the adipose tissue of mice that consumed CO. Leptin increased the expression and the transcription of enzymes associated with fatty acid oxidation (ACADvl and ACADm) in the CO100 group alone. On the other hand, leptin stimulation, associated with coconut oil supplementation, led to an increase in ACC phosphorylation, probably inhibiting fatty acid synthesis. Although coconut oil consumption reduced AMPK phosphorylation favoring lipogenesis, leptin was able to reverse this parameter. Eventually, AMPK is the major mediator of leptin effects on fatty-acid metabolism (Minokoshi et al., 2002). The predominant effects of leptin on adipose tissue may be mediated by the peripheral nervous

system (Stern et al., 2016), regardless of the direct action of leptin in the adipose tissue. Leptin increases the sympathetic efferent signal to white adipose tissue to increase lipolysis (Geerling et al., 2014).

Finally, a correlation between insulin and leptin can be established. Insulin is capable of inducing leptin secretion (Vital et al., 2006; Inui et al., 2012), while leptin activates the main insulin signaling pathway, the phosphatidylinositol 3-kinase (PI3K) pathway (Niswender et al., 2003), via phosphorylation of insulin receptor substrates by JAK2 culminating in PI3K activation. Leptin or LepR deficiency produces a phenotype of hyperphagia, obesity and insulin resistance, and leptin infusion at low doses, not affecting body weight, can correct hyperglycemia and hyperinsulinemia in ob/ob mice (Pelleymounter et al., 1995). Furthermore, increased leptin sensitivity of LepR-expressing cells does not prevent diet-induced obesity, but protects mice from insulin resistance induced by obesity (Pedroso et al., 2014). We observed a scenario of insulin resistance in mice that consumed coconut oil (CO300), but not in CO100 mice, which may require a longer exposure to coconut oil to be triggered. Since adiposity plays an important role in insulin resistance, it is difficult to determine whether leptin signaling in a specific brain structure directly controls glucose homeostasis or whether the observed effects are secondary to changes in body weight and adiposity (Ramos-Lobo and Donato, 2017).

In summary, supplementation with coconut oil for eight weeks, in healthy *Swiss* mice, induces ERS in the hypothalamus and promotes leptin and insulin resistance, both centrally and peripherally, especially for high doses of CO, harming the control of energy expenditure, expression of neuropeptides and food intake. Additionally, the consumption of CO, by itself, promoted the reduction of AMPK phosphorylation and increased gene expression of ACC and AGPAT, possibly favoring lipogenesis (Fig. 7).

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CRediT authorship contribution statement

Alana Carolina Costa Veras: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Larissa da Silva Bruzasco:** Methodology, Investigation, Data curation, Formal analysis. **Ana Beatriz Profiro Lopes:** Methodology, Investigation, Data curation, Formal analysis. **Beatriz da Silva Franco:** Investigation, Validation. **Alessandro Spencer de Souza Holanda:** Investigation, Validation. **Andrea Maculano Esteves:** . **Marciane Milanski:** Resources. **Adriana Souza Torsoni:** Resources, Writing – review & editing. **Leticia Martins Ignacio-Souza:** Methodology, Resources. **Marcio Alberto Torsoni:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A

See Figs. A1-A7.

Appendix B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105600.

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