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ORIGINAL ARTICLE

# Maternal cinnamon intake during lactation led to visceral obesity and hepatic metabolic dysfunction in the adult male offspring

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## Abstract

**Purpose** Studies with foods, known to promote health benefits in addition to the nutritive value, show that their consumption by pregnant and/or lactating females could induce negative outcomes to the offspring. It is well characterized that cinnamon intake promotes benefits to energy homeostasis. The present study aimed to analyze the effects of the consumption of an aqueous extract of cinnamon by lactating female rats on the endocrine-metabolic outcomes in the adult offspring.

**Methods** Lactating dams (Wistar rats) were supplemented with cinnamon aqueous extract (400 mg/kg body weight/day) for the entire lactating period. The male adult offspring were evaluated at 180 days old (CinLac).

**Results** The offspring presented visceral obesity ($P = 0.001$), hyperleptinemia ($P = 0.002$), and hyperinsulinemia ($P = 0.016$). In the liver, CinLac exhibited reduced p-IRβ ($P = 0.018$) suggesting insulin resistance. However, phosphorylation of IRS1 ($P = 0.041$) and AKT ($P = 0.050$) were increased. JAK2 ($P = 0.030$) and p-STAT3 ($P = 0.015$) expressions were higher, suggesting that the activation of IRS1/AKT in the CinLac group could have resulted from the increased activation of leptin signaling. Although we observed no changes in the gluconeogenic pathway, the CinLac group exhibited lower hepatic glycogen content ($P = 0.005$) accompanied by increased p-GSK3β ($P = 0.011$). In addition, the CinLac group showed increased hepatic triacylglycerol content ($P = 0.049$) and a mild steatosis ($P = 0.001$), accompanied by reduced PPARα mRNA expression ($P = 0.005$).

**Conclusion** We conclude that maternal intake of aqueous extract of cinnamon induces long-term molecular, metabolic, and hormonal changes in the adult progeny, including visceral obesity, higher lipid accumulation, and lower glycogen content in the liver.

**Keywords** Lactation · Metabolic programming · Cinnamon · Liver · Insulin · Leptin

## Introduction

Epidemiological, clinical, and experimental studies have demonstrated associations between nutritional changes during critical periods of life, such as pregnancy and/or lactation, and development of metabolic disturbances in the adulthood [1–3]. This phenomenon is termed "early life programming", which is mediated by epigenetic modifications [4].

The quality of the diet during lactation is important for maternal health and milk quality [5, 6]. Diet modifications can generate metabolic endocrine disturbances in the mother, and also alter the composition of the milk (nutritional and hormonal) [7], factors with a potential programming effect on the offspring. In this context, the search for healthy and balanced nutrition and consumption of natural products during the lactation phase is of maternal interest [8].

Some nutritional components bring health benefits beyond their nutritional values [9], however, studies have also shown that consumption of some of these foods by pregnant and/or lactating mothers can lead to adverse outcomes in the offspring. Green tea supplementation during gestation and lactation of obese rats was beneficial for the

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adult offspring and associated with lower serum cholesterol and insulin levels, higher insulin sensitivity and diminished lipogenesis in the liver [10]. In contrast, flaxseed supplementation in lactating rats promoted negative metabolic outcomes in the offspring, leading to the development of insulin resistance, visceral adipose tissue accumulation and hyperleptinemia in male progeny [11]. These studies emphasize the importance of investigating the effects of functional foods and nutritional components by pregnant and/or lactating females.

Cinnamon (*Cinnamomum zeylanicum*) is one of the most popular spices used worldwide, consumed not only for culinary but also as complementary and alternative medicine [12, 13] and its consumption generates several benefits for metabolic homeostasis. The supplementation with cinnamon leads to reduced hepatic triacylglycerol, improvement in serum lipid profile, as well as an improvement of body composition in obese animal models and type 2 diabetes patients [14–18]. In addition, cinnamon consumption leads to reduced body weight and lipogenesis in liver and adipose tissue in a healthy animal model [19]. Notably, cinnamon also has an important anti-diabetic effect, mostly by enhancing insulin action. Aqueous cinnamon extract modulates many insulin signaling pathway steps including the activation of the insulin receptor (IR), the substrate of insulin receptor (IRS1 and 2) and protein kinase B (AKT) [20–22].

Although many studies had shown physiological benefits associated with cinnamon consumption, the effect of cinnamon supplementation during the lactation period is poorly investigated. Recently, it was demonstrated that the consumption of cinnamon aqueous extract by lactating rats promoted lower visceral fat mass in their male progeny, at 100 days old [23]. However, animal studies exploring the impact of nutritional changes during lactation have demonstrated that significant endocrine-metabolic dysfunctions become evident in later adulthood [11, 24]. Therefore, the aim of the present study was to investigate the 180 days old male progeny of cinnamon-treated lactating rats, concerning the adipose tissue mass, serum insulin and leptin, and hepatic metabolism.

## Methods

### Experimental model

Animal use and experimental procedures were approved by Ethics Committee on Animal Use of Fluminense Federal University under number 0120/11, and complied with the ethical guidelines of the Brazilian Society of Laboratory Animal Science. Wistar rats were kept in the institutional animal care facility in a temperature-controlled room (23 ± 1 °C) with artificial light–darkness cycles (12 h, lights on at 7 a.m.), receiving commercial chow (Nuvilab Cr-1®, Nuvital Nutrientes S/A, Paraná, Brazil) and water ad libitum.

Virgin female rats (weighting ~260 g) were mated, and each female was placed in an individual cage with free access to water and commercial chow until the birth of the offspring. To maximize the lactation performance, the litters were adjusted to six male pups per dam [25]. Twenty-four hours after birth, dams were randomly divided into two groups: Control: received water by gavage during the entire lactation ($n = 9$); Cinnamon: received an aqueous extract of cinnamon (400 mg/kg body weight/day; $n = 11$), by gavage during the entire lactation (20 days). The choice of dosage was based on previous studies [19, 23, 26] and represents a human dose of 64 mg/kg (~3–5 g per individual), that was calculated based on body surface area [27]. In humans, studies have shown that the use of 1–6 g of cinnamon powder is sufficient to observe the beneficial effects of cinnamon in glucose and lipid homeostasis [15, 28].

After weaning, the offspring of control and cinnamon-treated dams were randomly divided into two subgroups: offspring of the control group (Ctr; $n = 12$) and offspring of the cinnamon-treated group (CinLac; $n = 16$). At least one pup from each litter was used per group. The pups were kept in polyethylene cages, four animals per unit, receiving water and commercial chow ad libitum, and body weight and food intake (per cage) were monitored throughout the experiment weekly.

The male offspring at 180 days old were sacrificed by decapitation, at fed state, and the blood collected from the trunk was centrifuged to separate serum, which was stored at −80 °C. The liver was harvested and immediately frozen in liquid nitrogen, and then stored at −80 °C for future analysis. After the location of the testes and identification of the attached white adipose tissue, known as the epididymal adipose in males, the deposits were carefully dissected out from the testes and weighed. This compartment represents a visceral adipose depot [29].

### Supplementation with aqueous extract of cinnamon

Aqueous extract of cinnamon was prepared, as described previously [19, 23, 30]. Briefly, cinnamon bark (*C. zeylanicum*), obtained from Indonesia (Emporium Importadora e Distribuidora de Alimentos Ltda), was macerated and mixed with distilled water (1 g/10 mL), then heated in a water bath at 60 °C for 1 h. After centrifugation (1000×*g*, 5 min, 4 °C) the supernatant was aliquoted, stored at −20 °C and used throughout the treatment. As described previously [23], the extract obtained was analyzed by high-performance liquid chromatography (DAD-HPLC-UV) and (-)-ESI–MS. The analysis showed that the major peak obtained is characteristic of the chemical marker, cinnamaldehyde, a common phytochemical in the *Cinnamomum*

genus [31]. In addition to cinnamaldehyde, the extract contained other compounds, such as cinnamic acid, gluconic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid [23].

## Basal glycaemia

Seventy-two hours before sacrifice, the rats were fasted for 5 h for blood glucose measurements, based on the glucose–glucose oxidase reaction, using a glucometer (ACCU-Chek Advantage, Roche, Baden-Württemberg, Germany).

## Hormone measurements

Serum leptin and insulin concentrations were measured using specific rodent radioimmunoassay kits (Linco Research, Missouri, USA) in accordance with the manufacturer's instructions. The sensitivity was 0.5 ng/mL for leptin and 0.1 ng/mL for insulin. All samples were measured using the same assay, and the intra-assay variation was 7.09% for leptin, and 6.12% for insulin.

## Western blotting

Frozen liver (50 mg) was homogenized in 1 mL of lysis buffer (50 mM Hepes, 1 mM $MgCl_2$, 10 mM EDTA and 1% Triton X) containing protease and phosphatase inhibitors (Pierce Protease and Phosphatase Inhibitor mini-tablet (Thermo Scientific, Illinois, USA)). After centrifugation (15,000×*g*, 30 min at 4 °C), the supernatant was collected and stored at −20 °C. The total protein concentration was quantified using a commercial kit (Pierce BCA Protein Assay Kit, Thermo Scientific). For protein analysis, we used 9–16 samples per group, and each sample was obtained from a different rat from different litters. Proteins were separated by electrophoresis in a polyacrylamide gel (10–12% SDS–PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Hybond Amersham, GE, Buckinghamshire, Germany). The membranes were blocked in 3% bovine serum albumin (BSA) diluted in TBS-T (saline buffer containing 25 mM Tris, 190 mM glycine, 20% methanol with 0.1% Tween) at room temperature, for 1 h to avoid non-specific binding of the primary antibody. Sequentially, membranes were incubated overnight at 4 °C with one of the following primary antibodies: IRβ; phosphorylated IRβ in tyrosine 1162/1163 residues; IRS1; phosphorylated IRS1 in tyrosine 632 residue; PTP1B; GLUT2; PEPCK; Glucose 6 phosphatase; GSK3β; phosphorylated GSK3β in serine 9 residue; ObRb; JAK2; STAT3; phosphorylated STAT3 in tyrosine 705 residue; AKT; phosphorylated AKT in serine 473 residue. Additional information about antibodies is described in the Supplementary Material. Cyclophilin was used as a loading control to normalize the data obtained. Next, the membranes were incubated with the peroxidase-conjugated secondary antibody, specific for each of the primary antibodies, for 2 h at room temperature. Blots were washed and incubated in commercial enhanced chemiluminescence reagents (ECL, GE Healthcare) and the images of the bands were obtained with the ChemiDoc$^{MP}$ system (Bio-Rad, California, USA). Stripping buffer (200 mM glycine, 1% SDS, 1% Tween, and pH 2.2) was used to remove the primary and secondary antibodies from membrane and reprobing with another target. The chemiluminescent intensity of bands was determined using the ImageJ Software (version 1.5, Massachusetts, USA). The CinLac group was compared to the control group, which was considered to be 1.

## Hepatic glycogen content

For assessment of hepatic glycogen content, liver samples were homogenized in 10% trichloroacetic acid in the proportion of 1:2. The samples were centrifuged at 425×*g* for 10 min at room temperature. Absolute ethanol was added to the supernatant and glycogen was precipitated overnight at 4 °C. After centrifugation, the pellet was dissolved in 1 mL 1 N HCl for hydrolysis of the glycogen. The samples were then boiled for 30 min and after cooling, the samples were neutralized with 1 mL of 1 N NaOH. Quantification was performed using the Glucose Liquiform Kit (Lab Test, Minas Gerais, Brazil) [32].

## Hepatic triacylglycerol content

For quantification of the liver triacylglycerol content, 50 mg of liver was homogenized in 0.5 mL isopropyl alcohol and then sonicated for 1 min. After centrifugation at 2400×*g* for 10 min at 4 °C, the supernatant was used for triacylglycerol quantification, with a commercial kit in accordance with the manufacturer's instructions (Triacylglycerol Liquiform Kit, Lab Test, Brazil) [33].

## Liver histology

Liver samples, previously stored at −80 °C, were defreeze and stained in formalin solution (37% of formalin, 0.5 M phosphate-buffered saline). The samples were dehydrated in graded alcohol of increasing concentration (70% to absolute alcohol), cleared in xylene and embedded in paraffin. After histological sections (5 µm), the slices were stained with Harris hematoxylin and eosin (HE) and were scanned (Slide Scanner Aperio CS2, Leica Biosystems, Nussloch, Germany). The photomicrography of 50 images (Ctr, $n = 4$) or 30 images (CinLac, $n = 5$) per animal was analyzed to assess hepatic lipid steatosis. The volume density of hepatic

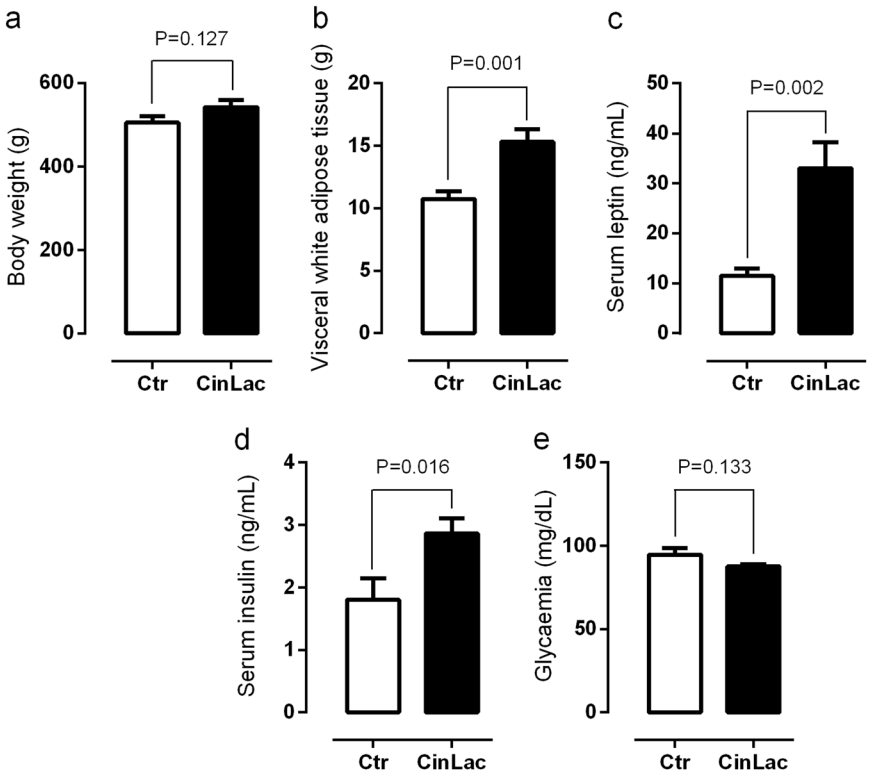


**Fig. 1** Body weight, visceral adipose tissue mass and serum parameters in offspring of cinnamon-treated mothers group (CinLac) and offspring of control mothers group (Ctr). **a** Body weight, **b** visceral white adipose tissue, **c** serum leptin, **d** serum insulin, and **e** blood glucose. Samples per group: 10–16. Values expressed as mean ± SEM. Statistically significant differences were determined by Student's unpaired *t*-test between Ctr and CinLac groups

steatosis (Vv[steatosis]) was estimated as the ratio of the points hitting the vesicles of fat (Pp) and the number of total points (PT, 36 points), in accordance with the formula: Vv [steatosis] = Pp/PT [33, 34].

## Real-time PCR

Total RNA was isolated from the liver using TRIzol® Reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. The integrity of total RNA was verified by electrophoresis on 0.8% agarose gel, and quality and purity were evaluated by measuring absorbance at 230, 260, and 280 nm. cDNA was synthesized using Superscript III Kit (Invitrogen) from 1 µg of total RNA. Genes of interest were amplified with a StepOne Real-Time PCR System (Applied BioSystems) using the GoTaq® qPCR Master Mix (Promega, WI, USA) following cycle parameters: initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Oligonucleotide primer sequences for SREBP1c (*Srebf1c* gene), PPARα (*Ppara* gene), and the reference gene 36β4 (*Rplp0* gene) were used, as described previously [19]. Relative mRNA expression levels were calculated by $2^{-\Delta\Delta Ct}$ method. The expression of the *Rplp0* gene was stable for the experimental groups and the efficiency of each reaction was calculated using a serial dilution and varied from 95% to 105%. The data are expressed relative to the control group, considered to be 1. The purity of the PCR products was assessed by melting curve analysis.

## Statistical analyses

The sample size was calculated using TIBCO Statistica software (version 13.3.0, CA, USA) and using visceral adipose tissue mass as primary outcome (data obtained from preliminary experiments) and assuming an average difference of 5% (effect size). The minimum of nine animals per group would provide the appropriate power (0.8) to identify significant differences ($\alpha = 0.05$).

Data normality was verified by the Shapiro–Wilk test and was analyzed by Student's *t*-test or Kolmogorov–Smirnov test using GraphPad Prism 5 software (version 5.03, CA, USA). Differences are expressed as the means ± standard error of the mean (SEM) and were considered significant at $P \leq 0.05$.

# Results

## Maternal consumption of cinnamon during lactation alters metabolism in CinLac offspring

Figure 1 shows that supplementation with EC during lactation altered the metabolic parameters of the 180 days old offspring. The results showed, in comparison to the controls, no changes in body weight (Fig. 1a), however, a significantly higher visceral adipose tissue mass ($P = 0.0001$; Fig.1b) as well as a marked hyperleptinemia ($P = 0.002$; Fig.1c). In addition, a significant hyperinsulinemia

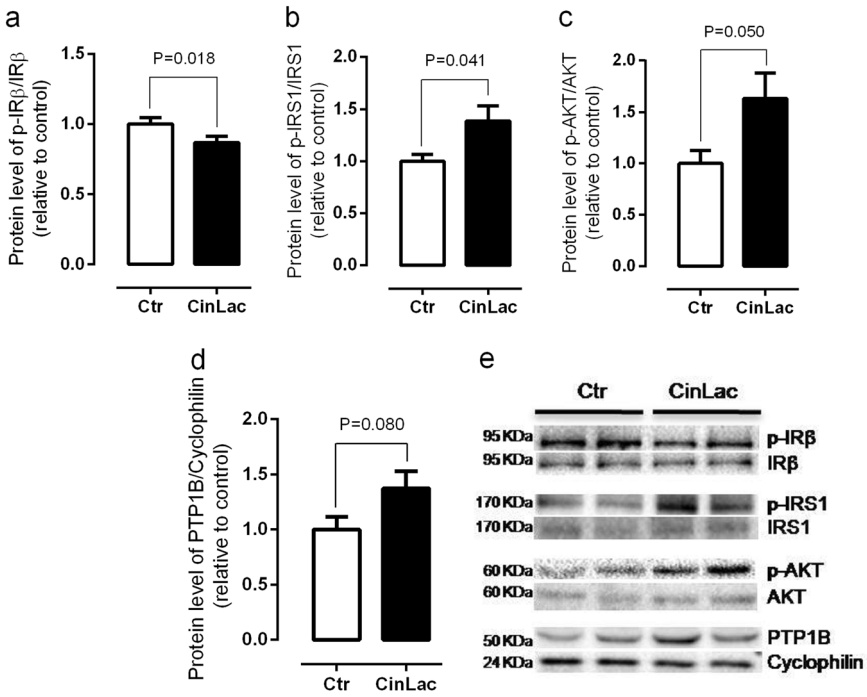


**Fig. 2** Protein expression of the insulin signaling pathway in the liver of the offspring of cinnamon-treated mothers group (CinLac) and offspring of control mothers group (Ctr). **a** Phosphorylated insulin receptor β (p-IRβ). **b** Phosphorylated insulin receptor substrate 1 (p-IRS1). **c** Phosphorylated protein kinase B (p-AKT). **d** Protein tyrosine phosphatase 1B (PTP1B). **e** Representative bands obtained by Western blot. The phospho-proteins were corrected to the correspondent total proteins after stripping. Samples per group: 9–16. Results are expressed relative to the values of the control group, which was set to 1. Values expressed as mean ± SEM. Statistically significant differences were determined by Student's unpaired *t*-test or Kolmogorov–Smirnov test between Ctr and CinLac group

occurred ($P = 0.016$; Fig.1d), but there were no changes in blood glucose in fasted (Fig. 1e) or fed state (Ctr: 188 ± 12.3 mg/dL; CinLac: 212 ± 8.8 mg/dL; $P = 0.911$) in the CinLac male offspring. The CinLac group did not exhibit changes in food intake throughout the experiments (data not shown). The serum lipid profiles also showed no differences in total cholesterol or triacylglycerol levels between the groups (data not shown). The cinnamon supplementation did not result in adverse events in the mothers or in the offspring during lactation.

## Maternal consumption of cinnamon during lactation alters insulin signaling in the liver of CinLac offspring

To evaluate possible changes at the molecular level in hepatic insulin signaling, some components of the insulin transduction pathway were studied. Phosphorylated IRβ was significantly lower in the CinLac group ($P = 0.018$; Fig.2a). In contrast, the level of phosphorylated insulin receptor substrate type 1 (IRS1) was higher in the CinLac group ($P = 0.041$; Fig. 2b). In accordance with this latter data, protein levels of phosphorylated AKT were higher in the CinLac group ($P = 0.050$; Fig.2c). Finally, the expression of PTP1B, an insulin pathway inhibitor, showed an increased trend and was 37% higher in the CinLac group (Fig. 2d).

## Maternal consumption of cinnamon during lactation alters leptin signaling in the liver of CinLac offspring

To evaluate the hepatic response to the serum hyperleptinemia, the expression of proteins involved in leptin signaling in this tissue were analyzed. There were no differences between groups in the protein expression of the leptin receptor (ObRb) (Fig. 3a). However, the expression of janus kinase 2 (JAK2) ($P = 0.030$; Fig. 3b) and signal transducer and activator of transcription 3 (STAT3) phosphorylated ($P = 0.015$; Fig. 3c) was significantly higher in CinLac group.

## Maternal consumption of cinnamon during lactation alters glucose metabolism in the liver of CinLac offspring

In relation to liver glucose metabolism, total liver glycogen content in the CinLac group was significantly lower compared with the control group ($P = 0.005$; Fig. 4a), accordance with this data, the levels of phosphorylated glycogen synthase kinase type 3 beta (GSK3β) were significantly

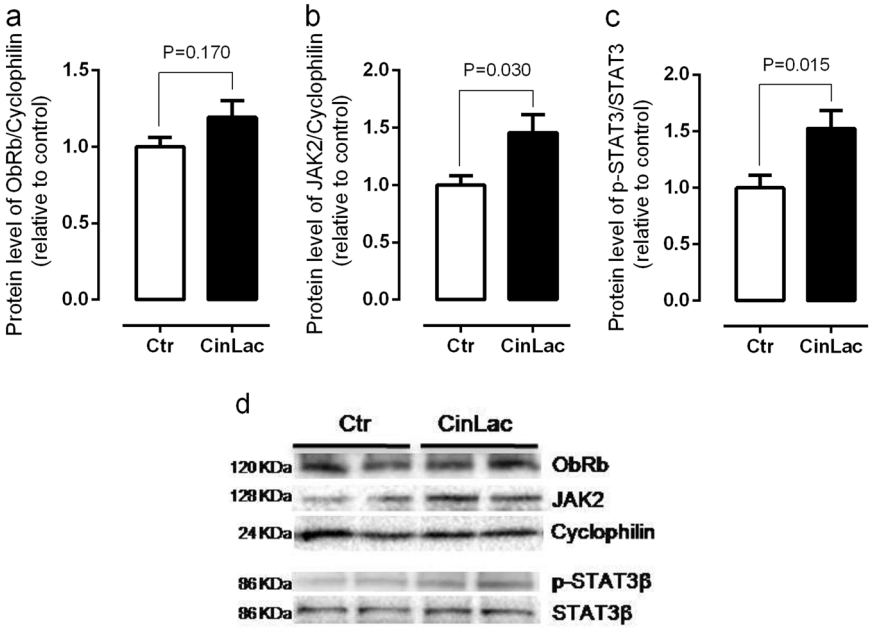


**Fig. 3** Protein expression of the leptin signaling pathway in the liver of the offspring of cinnamon-treated mothers group (CinLac) and offspring of control mothers group (Ctr). **a** Leptin receptor isoform b (ObRb). **b** Janus kinase 2 (JAK2). **c** Phosphorylated signal transducer and activator of transcription 3 (p-STAT3). **d** Representative bands obtained by Western blot. The p-STAT3 expression was corrected to the correspondent total STAT3 after stripping. The JAK2 and SOCS3 expression were analyzed after stripping of ObRb and Cyclophilin membranes, respectively. Samples per group: 9–16. Results are expressed relative to the values of the control group, which was set to 1. Values expressed as mean ± SEM. Statistically significant differences were determined by Student's unpaired *t*-test between Ctr and CinLac groups

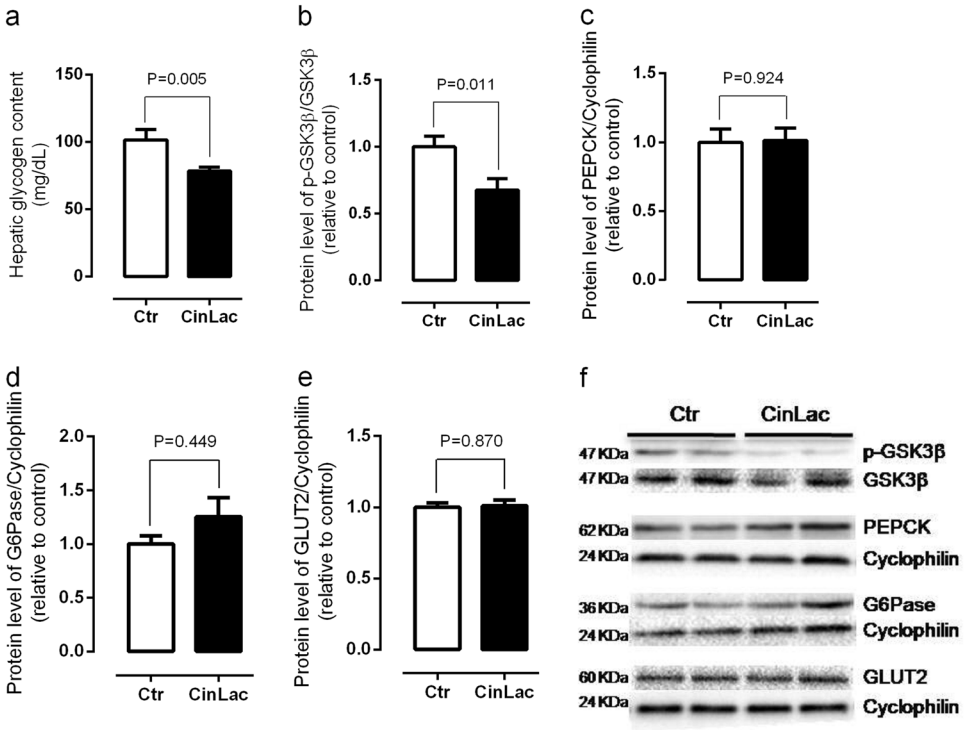


**Fig. 4** Glucose metabolism in the liver of the offspring of cinnamon-treated mothers group (CinLac) and offspring of control mothers group (Ctr). **a** Glycogen content, **b** phosphorylated glycogen synthase kinase 3 β (p-GSK3β), **c** phosphoenolpyruvate carboxykinase (PEPCK), **d** glucose-6 phosphatase (G6Pase), **e** glucose transporter 2 (GLUT2), and **f** representative bands obtained by Western blot. The p-GSK3β expression was corrected to the correspondent total GSK3β after stripping. Samples per group: 12–16. Results are expressed relative to the values of the control group, which was set to 1. Values expressed as mean ± SEM. Statistically significant differences were determined by Student's unpaired *t*-test or Kolmogorov–Smirnov test between Ctr and CinLac groups

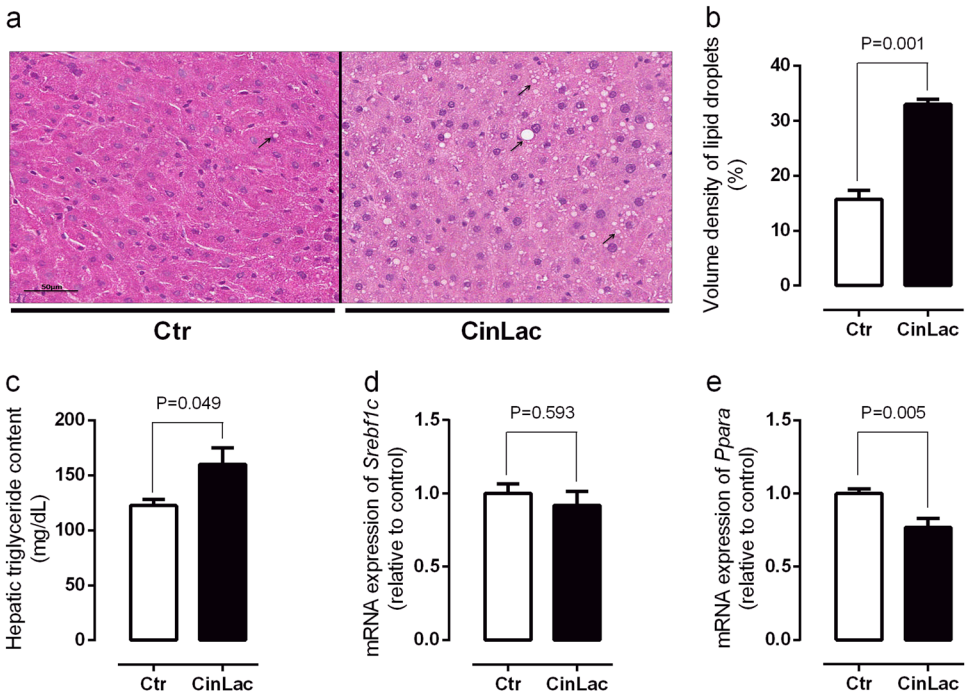


**Fig. 5** Lipid metabolism in the liver of the offspring of cinnamon-treated mothers group (CinLac) and offspring of control mothers group (Ctr). **a** Representative photomicrographs of liver sections (HE stain) of Ctr group with normal hepatocytes and CinLac group with an accumulation of fat (arrows) in the hepatocytes. **b** Volume density of lipid droplets. **c** Triacylglycerol content. **d** mRNA expression of sterol regulatory element-binding protein 1c (Srebf1c). **e** mRNA expression of peroxisome proliferator-activated receptor alpha (Ppara). Samples per group: 12–16. Results of real-time PCR were expressed relative to the values of the control group, which was set to 1. Values expressed as mean ± SEM. Statistically significant differences were determined by Student's unpaired *t*-test or Kolmogorov–Smirnov test between Ctr and CinLac group

lower in the CinLac group ($P = 0.011$; Fig. 4b). We also analyzed the gluconeogenesis enzymes and there was no difference in protein levels of the phosphoenolpyruvate carboxykinase (PEPCK) (Fig. 4c) or the glucose 6-phosphatase (G6Pase) (Fig. 4d) between the groups. In addition, the protein expression of the glucose transporter type 2 (GLUT2) (Fig. 4e) was the same in both groups.

### Maternal consumption of cinnamon during lactation leads to triacylglycerol accumulation in the liver of CinLac offspring

The histological analyses of the CinLac group showed lipid droplets accumulation in the hepatocytes, characteristics of mild to moderate steatosis, while control group exhibit a morphology characteristic of a healthy tissue (Fig. 5a), as represented in Fig.5b ($P = 0.0001$). The hepatic lipid accumulation in the CinLac group was confirmed by higher triacylglycerol content compared with the controls ($P = 0.049$; Fig. 5c). To understand possible alterations in the lipid metabolism, the gene expression involved in lipogenesis and lipid oxidation were evaluated. The mRNA expression of the lipogenic transcription factor sterol regulatory element-binding protein (*Srebf1c*) was similar to the controls (Fig. 5d). Furthermore, the expression of the peroxisome proliferator-activated receptor alpha (*Ppara*), a transcription factor involved in fatty acid oxidation, showed a significant reduction in the CinLac group ($P = 0.005$; Fig.5e).

## Discussion

The cinnamon treatment during 20 days of lactation in dams led to the reduction of visceral white adipose tissue mass, with no changes in serum insulin and leptin. Despite the beneficial effects for the mothers, previously described [23], the present study showed that their offspring at 180 days old presented metabolic disturbances, such as higher insulinemia, visceral obesity, and hepatic lipid accumulation.

The offspring of cinnamon-treated mothers group showed a higher visceral fat mass, with no changes in body weight. An excess of adipose tissue mass, mainly in the visceral compartment, is associated with important metabolic dysfunctions [35]. This phenotype could not be related to a catch-up growth, since in this model of cinnamon supplementation during lactation there are no changes in the weaning body weights [23]. The CinLac group also

exhibited higher levels of serum leptin, in accordance with the increased adiposity observed in this group. The classic effect of leptin occurs in the hypothalamus, decreasing food intake and increasing energy expenditure [36]. However, no changes in food intake behavior occurred, which suggests some level of hypothalamic resistance to leptin induction of satiety [37].

Insulin resistance and development of type 2 diabetes, characterized by hyperinsulinemia and hyperglycemia or normoglycemia in a pre-diabetic state, is a common disorder associated with visceral obesity [38, 39]. Our data showed that the CinLac group exhibited hyperinsulinemia accompanied by normoglycemia, suggesting a pre-diabetic condition with a modest impairment in insulin sensitivity. These data confirm our initial hypothesis that the progeny phenotype can be different in late adulthood, since our previous data in the male progeny at 100 days old was associated to lower visceral fat mass without changes in insulin homeostasis [23].

Despite the higher insulinemia of the CinLac group, a lower IR phosphorylation level was observed in the liver, suggesting a resistance to the hormone action in this tissue. The dephosphorylation of the tyrosine residue and deactivation of IRβ is mediated largely by PTP1B [40] that showed no statistically significant difference in the CinLac group. The insulin-signaling pathway in the liver includes the activation of IRS1 and AKT [41]. However, regardless the lower IRβ phosphorylation identified in the CinLac group, the phosphorylation level of IRS1 and AKT was higher in these animals. Although there is a dissociation between the activation of the IRβ and IRS1 in this model, activation of AKT is consistent with IRS1 phenotype, since increased phosphorylation of AKT, at the serine 473 residue, has been shown to be stimulated by IRS1 activation [42].

Beyond insulin, the isoforms of AKT can be regulated by other factors, such as growth factors and cytokines, including PDGF, IGF-1, TNFα, IL-2, and studies have demonstrated that leptin is able to modulate the AKT activation as well, including by a direct action of this hormone in the liver [43, 44]. In the present study, no differences were observed in the protein expression of the leptin receptor ObRb. However, a significant increase occurred in the expression of JAK2 and phosphorylation of STAT3 in the CinLac group. The actions of leptin in the liver, via the activation of the JAK2/STAT3 pathway, can stimulate the insulin signaling cascade [36, 45]. The administration of leptin can lead to the activation of IRS1 and IRS2 [46]. In addition, in animal models of type 1 diabetes, leptin treatment not only restore the insulin signaling by activating the IRS but it is also able to increase the phosphorylation of AKT [47]. Therefore, the increased phosphorylation of IRS1 and AKT observed in the CinLac group was induced, at least in part, by leptin signaling in the liver, which reflects the hyperleptinemia observed in these animals.

To further characterize how cinnamon exposure during lactation affects the insulin and/or leptin modulation of liver metabolic function, the gluconeogenesis pathway, the glycogen and the triacylglycerol content were investigated.

Insulin and leptin inhibit the expression of the hepatic gluconeogenic enzymes, such as PEPCK and G6Pase, which result in a decreased glucose production, via AKT pathway [48]. In the present model, no changes in the protein expression of liver PEPCK, G6Pase, and GLUT2 in the CinLac group were observed, despite the higher activation of AKT. Therefore, these data suggest that there are no major changes in hepatic glucose output, which should contribute to the normoglycemia observed in CinLac animals.

In the present study, decreased glycogen content was observed in the CinLac group accompanied by lower levels of phosphorylated GSK3β, indicating a reduction of glycogen synthesis in this group. The GSK3β activity is modulated by AKT, which phosphorylates and inhibits the enzyme, allowing the increase of glycogen synthesis and storage [49]. However, the increased activation of IRS1 and AKT in the CinLac animals do not correlate with the lower glycogen content, suggesting that the regulation of hepatic glycogen stores is, at least in part, independent of the IRS1/AKT pathway in this programming model. The role of leptin in glycogen metabolism is conflicting, both promoting [50, 51] or decreasing [52] hepatic glycogen storage, depending on the dosage, time, and nutritional status [53]. The reduction of hepatic glycogen content may have a negative consequence to the animal glycemic homeostasis, with impairment in the ability to maintain blood glucose in times of need [32, 54].

The animals of CinLac group presented high triacylglycerol content and moderate steatosis in the liver. Hepatic steatosis is a common metabolic complication associated with obesity in which an accumulation of triacylglycerol is mainly caused by an imbalance between lipogenesis and lipid oxidation [55]. A major contributor to the increased lipogenesis stimulated by insulin, via AKT, is the SREBP1c, a transcription factor that activates the lipogenic program [56]. In the present study, the mRNA expression of SREBP1c was evaluated and showed no differences between groups. Leptin, via STAT3, has been shown to play a role in protecting the liver against steatosis, at least in the initial stages of the disease [57], by reducing the SREBP1c expression in vitro [58] and in vivo [59]. It is possible that the protective effect of leptin on the liver contributes to the fat accumulation observed in the CinLac animals be presented as a mild phenotype. However, in the long-term obesity, the chronic hyperleptinemia may have a harmful effect in this tissue, by acting as a proinflammatory

and a profibrogenic adipokine contributing to the development of non-alcoholic fatty liver disease (NAFLD) [36].

To investigate if the hepatic triacylglycerol accumulation could be due to changes in lipid oxidation, the expression of PPARα was evaluated, a transcription factor that stimulates the expression of genes involved in lipid β-oxidation. This factor induces the expression of many enzymes involved in mitochondrial and peroxisomal β-oxidation; furthermore, it also stimulates the uptake and transport of fatty acids [60]. In the present study, the mRNA expression of PPARα had a significant reduction in the CinLac group compared to the controls. Thus, this reduction may induce a lower lipid oxidation rate, which possibly contributes to the accumulation of hepatic fat.

The intriguing phenotype observed in the CinLac group is most likely a consequence of biochemical changes in the milk composition, and the specific cinnamon-extract component responsible for triggering this phenotype should be further characterized. Besides that, it is possible that the outcome observed in the male offspring at 180 days old would be different at the female progeny. The sexual dimorphism is an important question that has been addressed in several programming models [23, 61, 62]. A previous study of our group showed that maternal cinnamon intake during lactation can trigger sex-specific metabolic phenotypes in pups at 21 and 100 days old [23].

The hepatic phenotype observed in this programming model is complex, and how the insulin and leptin pathways modulate the metabolic outcome needs to be better investigated. In summary, the present study highlights the negative impact of maternal cinnamon supplementation during lactation in the male adult offspring at 180 days old. The CinLac group develops visceral obesity, hyperinsulinemia and hyperleptinemia, associated with changes in liver metabolism, such as lower glycogen storage and increased lipid accumulation. In addition, evidence is provided for a possible increased crosstalk between leptin and insulin signaling in the liver that could contribute to the hepatic profile observed in the CinLac programming model.

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## Compliance with ethical standards

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

**Ethical approval** Animal use and experimental procedures were approved by Ethics Committee on Animal Use of Fluminense Federal University under number 0120/11, and complied with the ethical guidelines of the Brazilian Society of Laboratory Animal Science.

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