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Maternal coconut oil intake on lactation programs for endocannabinoid system dysfunction in adult offspring

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A B S T R A C T

Maternal exposure to coconut oil metabolically programs adult offspring for overweight, hyperphagia and hyperleptinemia. We studied the neuroendocrine mechanisms by which coconut oil supplementation during breastfeeding as well as continued exposure of this oil throughout life affect the feeding behavior of the progeny. At birth, pups were divided into two groups: Soybean oil (SO) and Coconut oil (CO). Dams received these oils by gavage (0.5 g/kg body mass/day) during lactation. Half of the CO group continued to receive CO in chow throughout life (CO + C). Adult CO and CO + C groups had overweight; the CO group had hyperphagia, higher visceral adiposity, and hyperleptinemia, while the CO + C group had hypophagia only. The CO group showed higher DAGLα (endocannabinoid synthesis) but no alteration of FAAH (endocannabinoid degradation) or CB1R. Leptin signaling and GLP1R were unchanged in the CO group, which did not explain its phenotype. Hyperphagia in these animals can be due to higher DAGLα, increasing the production of 2-AG, an orexigenic mediator. The CO + C group had higher preference for fat and lower hypothalamic GLP1R content. Continuous exposure to coconut oil prevented an increase in DAGLα. The CO + C group, although hypophagic, showed greater voracity when exposed to a hyperlipidemic diet, maybe due to lower GLP1R, since GLP1 inhibits short-term food intake.

1. Introduction

Coconut oil is considered an edible oil with numerous therapeutic benefits. It is obtained from the pulp of the ripe and dried fruit, or coconut milk, resulting in the virgin form of the product (Kumar, 2011; Marina et al., 2009). This oil is mainly composed of medium-chain saturated fatty acids (MCSFAs), with lauric acid being the most predominant, approximately 48% (Lockyer, Stanner, 2016). The intake of medium-chain triglycerides (MCTs) is associated with lower food intake and lower body mass (Bach, Babayan, 1982; Ferreira et al., 2014; St-Onge, Jones, 2002). As coconut oil is rich in MCSFAs, it has been used indiscriminately as a substitute for traditional sources of fat in the Western diet, including for women during pregnancy and breastfeeding. However, it is known that transient changes in maternal nutrition during these critical periods may predispose to obesity and other diseases in the adult progeny (Bispo et al., 2015; Guarda et al., 2016; Moura, Passos, 2005; Oben et al., 2010; Qasem et al., 2016), a phenomenon known as metabolic programming.

Abbreviations: 2-AG, 2-arachidonoylglycerol; ARC, Arcuate nucleus; CB1R, Cannabinoid receptor type 1; CNS, Central nervous system; CO, Coconut Oil group; CO + C, Coconut oil + chow supplemented with coconut oil; D1-R, Dopaminergic receptor 1; D2-R, Dopaminergic receptor 2; DAGLα, Diacylglycerol lípase α; DAT, Dopamine transporter; DDc, DOPA decaborxylase; DS, Dorsal striatum; EC, Endocannabinoids; ECS, Endocannabinoid system; FAAH, Fatty acid amide hydrolase; GHSR-1a, ghrelin receptor; GI, gastrointestinal; GLP-1, Glucagon like peptide −1; GLP1R, Glucagon like peptide −1 receptor; HFD, High-fat diet; HSD, High-sugar diet; JAK2, Janus Kinase 2; LH, Lateral hypothalamus; MAGL, Monoacylglycerol lípase; MCH, Melanin-concentrating hormone; MCT, Medium chain triglycerides; NAc, Nucleus accumbens; Ob-R, Leptin receptor; PFC, Prefrontal córtex; PN, Post natal; SN, Substantia nigra; SO, Soybean Oil group; SOCS3, Supressor of cytokine signaling 3; STAT3, Signal transducer and activator of transcription 3; TH, Tyrosine hydroxylase; VFM, Visceral fat mass; VTA, Ventral tegmental área

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The main lipid source of the Western diet of humans is soybean oil, which is also used in standard chow for animals. Our group has shown that partial or total replacement of soybean oil in the maternal diet during lactation by an unusual source, such as coconut oil (Quitete et al., 2018) or flaxseed oil (Guarda et al., 2016), lead adult offspring to develop endocrine-metabolic disorders compared to soybean oil.

In previous studies of metabolic programming, obesity, hyperleptinemia and changes in food intake that may be associated with changes in neuronal circuitry involved in energy balance regulation were observed in adult animals who were submitted to different imprinting factors as pups during lactation (Bonomo et al., 2007; Conceição et al., 2016; Kirk et al., 2009; Lima et al., 2011; de Oliveira et al., 2010; Pinheiro et al., 2015; Rodrigues et al., 2011; Santos-Silva et al., 2013; Younes-Rapozo et al., 2012). The control of food intake in the central nervous system (CNS) occurs through the integration of homeostatic and hedonic signals (Berthoud, 2006).

Nutrients, intestinal peptides and hormones compose homeostatic signals that act, mainly, in the arcuate nucleus of the hypothalamus (ARC) to inform the CNS of the body's energy status (Morton et al., 2014; Minor et al., 2009). Leptin, a hormone secreted mainly by white adipose tissue, is one of these signals. Leptin binds to its receptor (Ob-Rb) in the ARC, activating the JAK2-STAT3 (Janus Kinase 2-signal transducer and activator of transcription 3) pathway that regulates the expression of orexigenic and anorexigenic neuropeptides in order to reduce long-term food intake and also to control energy expenditure (Kwon et al., 2016; Münzberg, Morrison, 2015).

Gastrointestinal (GI) peptides, another set of homeostatic signals, play an important role in the control of short-term food intake in response to changes in the gut intraluminal food content (Murphy, Bloom, 2004; Perry, Wang, 2012). Mechanical and chemical stimuli promote the secretion of GI peptides (Adrian et al., 1985; Sam et al., 2012), such as ghrelin and glucagon-like peptide-1 (GLP-1). They act through specific receptors, GHSR-1a and GLP1-R, respectively, in specific neuronal populations (POMC/CART or NPY/AgRP) in the ARC or, at the peripheral level, through paracrine pathways via the vagus nerve, promoting alterations in satiety through feedback mechanisms on intestinal transit that increase or slow gastric emptying and acting to regulate energy expenditure (Sam et al., 2012).

Hedonic signals are a product of the interaction between external stimuli sent to the CNS through sensory systems and previous food experiences. The most well-characterized messenger of this system is dopamine, a neurotransmitter mainly synthesized in neurons of the ventral tegmental area (VTA) and substantia nigra (SN) (Arias-Carrión et al., 2014). These neurons send projections to other regions, such as the nucleus accumbens (NAc), dorsal striatum (DS) and prefrontal cortex (PFC), which express dopaminergic receptors 1 (D1-R) and 2 (D2-R) (Arias-Carrión et al., 2014). Thus, dopamine stimulates learning, motivation, pursuit and reward value (Arias-Carrión et al., 2014), such as the intake of palatable foods. In addition, other neurotransmitters have an influence on this system, such as μ-opioid receptor agonists, which increase the excitability of dopaminergic neurons (Johnson, North, 1992).

The integration of all these circuits occurs in the lateral hypothalamus (LH), in which there are neurons producing orexigenic neuropeptides: melanin-concentrating hormone (MCH) and orexin (Simpson et al., 2009). Additionally, LH is an important region for the synthesis and action of endocannabinoids (EC) (Di Marzo et al., 2009), which are lipid mediators that stimulate food intake through binding to cannabinoid receptor type 1 (CB1-R) (Cardinal et al., 2012; Di Marzo et al., 2009; Watkins, Kim, 2015). The EC synthesis in the LH is under direct or indirect influence of leptin, which inhibits EC synthesis (Cristino et al., 2013; Jo et al., 2005). In this context, the enzyme diacylglycerol lipase α (DAGLα) catalyzes the synthesis of the most abundant EC, 2-arachidonoylglycerol (2-AG), which is degraded by monoacylglycerol lipase (MAGL). Anandamide, another important EC, is mainly synthesized by N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) and is degraded by fatty acid amide hydrolase (FAAH) (Chanda et al., 2019; Lau et al., 2017).

The metabolic programming model of maternal supplementation with coconut oil during lactation seems to share similar characteristics to other models. We previously reported that adult offspring of dams supplemented with coconut oil during lactation show hyperphagia and hyperleptinemia as well as higher visceral adiposity compared with offspring of dams supplemented with soybean oil (Quitete et al., 2018). These alterations were prevented when the coconut oil supplementation occurred throughout life (Quitete et al., 2019). However, Gunasekaran et al. (2017) showed that the maternal supplementation with coconut oil from the periconceptional period to the end of lactation and during the whole life of the offspring caused a lower weight gain in offspring with no changes in food intake.

We hypothesized that alterations in the hypothalamic leptin signaling pathway or other central pathways involved in eating disorders common in obesity could explain the dysfunctions observed in hyperphagic overweight rats. Additionally, continued exposure of the imprinting factor (coconut oil), throughout life, may alter the imprinting mechanism of these central pathways, explaining why the animals do not show changes in food consumption (Quitete et al., 2019). Here, we investigated the central mechanisms, such as GLP-1 receptors and endocannabinoid and dopaminergic reward systems, involved in the control of food intake to better understand the mechanisms of hyperphagia observed in animals programmed by maternal supplementation with coconut oil as well as the consequences of continuous exposure to coconut oil throughout life.

2. Materials and methods

The experimental design was approved by the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro (CEUA/001/2014). Wistar rats were maintained in a temperature-controlled room (25 ± 1 °C) with artificial light-darkness cycles (lights on 0700 h, lights off 1900 h). Virgin female rats (n = 30) that were 3 months old were caged with male rats (2 females:1 male). After mating, each female rat was placed in individual cage with free access to water and food until delivery.

2.1. Experimental model of maternal supplementation with coconut oil

At birth, litters were adjusted to 6 male pups per dam to maximize the lactation performance (Passos et al., 2000). Dams and offspring were randomly assigned to one of two experimental groups: **SO** (Soybean Oil group, n = 10): dams received soybean oil (Santa Cruz Biotechnology, Inc., TX, USA; sc-215898, Lot #G3014) (0.5 g of oil/kg of body weight) via intragastric gavage throughout the lactation period - from postnatal day 1–21 (PN1 to PN21); and **CO** (Coconut Oil group, n = 20): dams received extra virgin coconut oil (Santa Cruz Biotechnology, Inc., TX, USA; sc-214752A, Lot #K0614) (0.5 g of oil/kg of body weight) via intragastric gavage throughout the lactation period.

At weaning (PN21), the animals of the CO group were subdivided into two groups according to the type of chow consumed until PN180. From weaning, the study was composed of the following experimental groups: **SO group** (n = 10): offspring of dams that received soybean oil during lactation and, after weaning, received a commercial standard chow for rodents (Nuvilab-NUVITAL Nutrients LTDA, Paraná, Brazil); **CO group** (n = 10): offspring of dams that received extra virgin coconut oil during lactation and, after weaning, received a commercial standard chow for rodents; and **CO + C group** (n = 10): offspring of dams that received extra virgin coconut oil during lactation and, after weaning, received chow supplemented with extra virgin coconut oil. The diets are described in Table 1. The three groups had free access to water until PN180.

Table 1
Macronutrients and micronutrients composition of standard chow for rats and coconut oil supplemented chow.

	Standard chow[a]	Coconut oil chow
Carbohydrate (g/kg)	660.0	570.0
Corn starch (g/kg)	–	363.3
% kJ	68.0	63.6
Protein (g/kg)	220.0	224.0
Textured soy (g/kg)	–	303.0
% kJ	22.7	25.0
Fat (g/kg)	40.0	45.0
Coconut oil (ml/kg)	–	30.3
% kJ	9.2	11.4
Vitamins and Minerals		
Mineral mix[b] (g/kg)	–	9.6
Vitamin mix[b] (g/kg)	–	1.6
Ca **(g/kg)**	10.0–14.0	–
P **(mg/kg)**	8000.0	–
Na **(mg/kg)**	2700.0	–
Fe **(mg/kg)**	50.0	–
Mn **(mg/kg)**	60.0	–
Zn **(mg/kg)**	60.0	–
Cu **(mg/kg)**	10.0	–
I **(mg/kg)**	2.0	–
Se **(mg/kg)**	0.05	–
Co **(mg/kg)**	1.5	–
F **(mg/kg)**	80.0	–
Vit A **(UI/kg)**	13000.0	–
Vit D3 **(UI/kg)**	2000.0	–
Vit E **(UI/kg)**	34.0	–
Vit K3 **(mg/kg)**	3.0	–
Vit B1 **(mg/kg)**	5.0	–
Vit B2 **(mg/kg)**	6.0	–
Vit B3 **(mg/kg)**	60.0	–
Vit B5 **(mg/kg)**	20.0	–
Vit B6 **(mg/kg)**	7.0	–
Vit B7 **mg/kg)**	0.05	–
Vit B9 **(mg/kg)**	1.0	–
Vit B12 **(mcg/kg)**	22.0	–
Choline **(mg/kg)**	1900.0	–
Aminoacids		
Lysine (mg/kg)	12000.0	50.0
L-cystine (mg/kg)	–	875.0
Methionine (mg/kg)	4000.0	150.0
BHT (mg/kg)	100.0	300.0

[a] Standard chow to rats (Nuvilab-NUVITAL Nutrientes LTDA, Paraná, Brazil). Composition of diet: Whole corn, soybean bran, wheat bran, calcium carbonate, dicalcium phosphate, sodium chloride, Vitamin A, Vitamin D3, Vitamin E, Vitamin K3, Vitamin B1, Vitamin B2, Vitamin B6, Vitamin B12, niacin, calcium pantothenate, folic acid, biotin, choline chloride, iron sulfate, manganese sulfate, zinc sulfate, copper sulfate, calcium iodate, sodium selenite, cobalt sulfate, lysine, methionine, BHT - butylatedhydroxytoluene.

[b] Vitamins and minerals mixture formulated as recommended by the American Institute of Nutrition AIN93G of rodents diet; contains the recommended amount of iodide (Reeves et al., 1993).

2.2. Chow supplemented with coconut oil

The standard chow was crushed, and textured soy protein, corn starch and coconut oil (Coconut oil from *Cocos nucifera*, Santa Cruz Biotechnology, Inc., TX, USA; sc-214752A, Lot #K0614) were added according to Table 1, in order to balance the macronutrients without changing sources of protein and carbohydrates, maintaining the chow with normocaloric, normoproteic, normoglycemic and normolipidemic profiles. Vitamins and mineral mix, amino acids and antioxidants were added according to the American Institute of Nutrition (AIN-93M) guidelines for rodents. Water was added to homogenize and prepare new pellets that were dried in a ventilated oven for 24 h and were subsequently stored in a refrigerator. This supplemented diet was offered for the CO + C group from PN21 to PN180 and was isocaloric in relation to standard chow.

2.3. Nutritional status evaluation

- **Body mass**: during 21 days of lactation, the body masses of dams and pups were assessed daily, and, after weaning, they were monitored every 4 days until PN180.
- **Food intake:** the food intake of offspring from weaning until PN180 was assessed every 4 days. The food intake was estimated from the difference between the weight of the food left in the cage and the total quantity put in the cage 4 days before.
- **Total adiposity:** At PN180, rats were anesthetized with a nonlethal dose of 2,2,2 tribromoethanol (Avertin®) and were carried to the Lunar DXA 200368 GE equipment (Lunar, Wisconsin, EUA) that had specific software (Encore 2008. Version 12,20 GE Healthcare, Wisconsin, EUA). Total body fat was expressed as a percentage.

2.4. Preference test for palatable food

Rats of SO, CO and CO + C groups were submitted to the preference test for palatable food at PN175 under fasting conditions for 12 h (from 9:00 to 21:00 – lights-on period). The diets were offered for one continuous period of 12 h, and the animals could freely select between the two palatable diets: high-fat diet (HFD) or high-sugar diet (HSD). To avoid stressing the animals, the tests were conducted in the vivarium, and two rats were kept per cage (n = 20 animals/group) during the entire testing period. In each cage, the food compartment was divided in two by a barrier. On one side, HFD was placed, and on the other side, HSD was placed. Both sides were equally accessible by the animals and were the same size. As the test was carried out during the active period, known amounts of HFD and HSD (measured in grams) were placed on the two sides of the food compartment at 21:00 (beginning of the lights-off period). At 21:30, the amount of chow left on each side of the food compartment was measured (30 min of consumption). The second measurement of the remaining chow on each side occurred at 9:00 the following day (12 h of consumption). The 30-min and the 12-h consumption values were determined by subtracting the original amount placed in the food compartments by the amount observed, respectively, after 30 min and 12 h, and the results were expressed as consumption (g)/animal (Pinheiro et al., 2015).

2.5. Composition of the palatable diets

The palatable diets were prepared according to the American Institute of Nutrition (AIN 93M) recommendations for rodent diets and are shown in Table 2. The standard chow was ground, and the butter or the sugar was mixed with the resulting bran using an industrial food mixer. To prepare 100 g of a given diet, we added: 38 g of sucrose in 62 g of standard chow for the preparation of the HSD and 20 g of butter in 62 g of standard chow for the preparation of the HFD. We also added 18 g of sucrose in the HFD. Then, the food pellet was submitted to an industrial heater for drying (60 °C, 12 h). The texture and shape of these food pellets were similar to those of the standard chow (Pinheiro et al., 2015).

Table 2
Composition of the experimental palatable diets.

	Sugar (g)	Fat (g)	Protein (g)	Energy value (kcal)
Standard chow	66.0	11.0	23.0	455
High-sugar diet	78.9	6.8	14.3	461
High-fat diet	58.9	26.8	14.3	561

The values are relative to 100 g of each chow.

2.6. Euthanasia

At PN180, all rats were submitted to 12 h of fasting, anesthetized with a nonlethal dose of ketamine/xylazine mixture (90 mL/kg of body weight (BW) of ketamine hydrochloride and 15 mL/kg of BW of xylazine hydrochloride, ip.) and were killed by cardiac puncture. The blood was collected in tubes containing EDTA and was centrifuged (4 °C, 20 min, 1.260 × *g*) to obtain plasma and were stored at −20 °C until analysis. After exsanguination of the rats, the brains were quickly excised and stored at −20 °C for 24 h and then stored at −80 °C. The visceral fat compartments were also excised, immediately weighed to obtain the visceral fat mass (VFM), and stored at −80 °C before subsequent processing according to the methods described below.

2.7. Plasma leptin levels

Leptin was determined by an immunoenzymatic assay specific to rats (ELISA) (EMD Millipore Corporation, Billerica, MA, USA) in a single test, dispensing the evaluation of the interassay coefficient of variation. The intra-assay coefficient of variation was 2.13%, and the assay sensitivity was 0.08 ng/mL.

2.8. Microdissection of brain nuclei

Frozen brains were cut in coronal sections using a cryostat (Hyrax C25, Zeiss, Stuttgart, Germany) at −12 °C. The regions of interest were located using bregma as a reference (Paxinos, Watson, 1998). Thus, the punch technique was applied to isolate the nucleus accumbens (NAc), arcuate nucleus (ARC), lateral hypothalamus (LH), and the ventral tegmental area (VTA) in the coronal sections located between bregma 1.70 and 0.48 mm, −1.6 and −2.6 mm, and −5.60 and −6.30 mm, respectively. The nuclei were stored at −80 °C until the analyses were performed.

2.9. Western blotting

The proteins of the leptin signaling pathway, endocannabinoid system (ECS), dopaminergic system and GLP1-R content were analyzed in specific brain regions by western blotting. For protein extraction, seven samples of each brain region, ARC, NAc, VTA and LH, were homogenized with RIPA buffer (50 mM TRIS, 150 mM NaCl, 0,1% SDS, 50 mM NaF, 1 mM sodium orthovanadate, 30 mM sodium pyrophosphate, 5 mM-EDTA, Triton X-100 1%) that included a protease inhibitor cocktail (cOmplete EDTA-free - Roche Applied Science, Mannheim, Germany). The protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). Then, the reading was made in a microplate reader (TU-1800 UV-VIS, USA) at a wavelength of 545–590 nm. Samples were stored at −20 °C until western blotting analysis. Before they were analyzed, all samples were denatured in the sample buffer (50 mM Tris-HCl, pH 6.8, 1% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.001% bromophenol blue) and were heated at 95 °C for 5 min.

Samples were separated by SDS-PAGE, with a polyacrylamide gel (10 or 12%) and were transferred onto nitrocellulose membranes (Hybond ECL; Amersham Pharmacia Biotech, Little Chalfont, Bucks, UK). Rainbow standard markers (Amersham Biosciences, Uppsala, Sweden) were run in parallel to estimate the molecular weights. To inhibit antibody nonspecific binding, the membranes were incubated with 25 ml Tween-Tris-buffered saline (T-TBS) (20 mM-Tris–HCl, pH 7.5, 500 mM NaCl, 0.1% Tween-20) with 1.25 g of bovine serum albumin (BSA) (Sigma-Aldrich Co, St. Louis, MO, USA) for 45 min at 25 °C. Then, the membranes of each nuclear sample were incubated overnight with specific primary antibodies: ARC- Ob-R (1:1000, Santa Cruz Biotechnology Inc., Dallas, TX, USA), phosphoJAK2 (pJAK2), phosphoSTAT3 (pSTAT3), total JAK2, total STAT3, suppressor of cytokine signaling 3 (SOCS3) (1:500, Santa Cruz Biotechnology Inc., Dallas, TX, USA) and GLP1-R (1:200, Abcam Inc., Cambridge, MA, USA); LH - CB1-R (1:100, Abcam Inc., Cambridge, MA, EUA), diacylglycerol lipase α (DAGLα) and fatty acid amide hydrolase (FAAH) (1:500, Abcam Inc., Cambridge, MA, EUA); VTA-tyrosine hydroxylase (TH) (1:500, Sigma-Aldrich Co, St. Louis, MO, USA); NAc – TH (1:1000, Sigma-Aldrich Co, St. Louis, MO, USA), DOPA decarboxylase (DDc) (1:1000, Abcam Inc., Cambridge, MA, EUA), dopamine transporter (DAT) (1:500, EMD Millipore Corporation, Billerica, MA, USA), D1-R (1:10000, Abcam Inc., Cambridge, MA, EUA) and μ-opioid receptor (1:2000, Abcam Inc., Cambridge, MA, EUA); DS - TH (1:1000, Sigma-Aldrich Co, St. Louis, MO, USA), DAT (1:500, EMD Millipore Corporation, Billerica, MA, USA), D1-R (1:10000, Abcam Inc., Cambridge, MA, EUA), D2-R (1:500, EMD Millipore Corporation, Billerica, MA, USA) and μ-opioid receptor (1:2000, Abcam Inc., Cambridge, MA, EUA). Then, the membranes were washed with T-TBS and were incubated with appropriate secondary antibodies for 1 h at 25 °C under constant agitation. After another series of washes, the membranes were incubated with streptavidin at the same concentration of secondary antibody for 1 h at 25 °C. After another series of washes, the targeted proteins were detected by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech, Piscataway, NJ, USA). The band images were quantified by densitometry using *ImageJ* software (Media Cybernetics). All results were normalized for actin (Sigma-Aldrich Co, St. Louis, MO, USA, mouse, 1:500).

2.10. Statistical analysis

Results are reported as the mean ± standard error of the mean (SEM). GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analyses and graphics. All data, except for the feeding test, were analyzed by one-way ANOVA, using Newman Keuls as a posttest, and differences were considered significant when $p < 0.05$. For the food preference test, Two-way ANOVA was used considering Groups and Diets as the between-subject factors. We tested whether the animals would display a clear preference toward one of the diets (HSD or HFD) and if the groups showed differences in the consumption of the two offered diets. Bonferroni posttest was used when differences were significant.

3. Results

At PN180, the CO and CO + C groups had higher body mass than the SO group (SO: 415 ± 6.9 g; CO: 455 ± 7.6 g; CO + C: 456 ± 8.9 g; +10% for both groups, $p < 0.05$). Concerning food intake, the CO group showed hyperphagia (CO: 23 ± 0.3 g; +8%, $p < 0.05$), and the CO + C group showed hypophagia (CO + C: 20 ± 0.8 g; −15%, $p < 0.05$) compared with the SO group (SO: 22 ± 0.3 g). This information is described because the tissues used in the present study belong to the animals of our previous publication (Quitete et al., 2019).

Although the CO and CO + C groups had higher body mass, only the CO group showed higher total adiposity compared to the SO group (+51%, $p < 0.05$, Fig. 1A), and the CO + C group demonstrated similar adiposity to the SO group.

Concerning the food preference test, after 30 min of challenge, all groups had a higher intake of HFD compared to HSD (5.2-, 9.2- and 10.3-fold increase in SO, CO and CO + C groups, respectively; diet: $F_{1,92} = 268.99$, $p < 0.001$; Fig. 1B). The preference for HFD was similar in both the CO and SO groups, but it was higher in the CO + C group, indicating an interaction between diet and treatment (+46% CO + C *Vs* SO; +20% CO + C *Vs* CO; diet X treatment: $F_{2,92} = 5.00$, $p = 0.0087$; Fig. 1B). After 12 h of challenge, HFD intake remained higher than HSD intake in all groups (5.9-, 13.0- and 8.8-fold increase in the SO, CO and CO + C groups, respectively; diet: $F_{1,106} = 636.80$, $p < 0.0001$; Fig. 1C), However, HFD intake was similar among the groups.

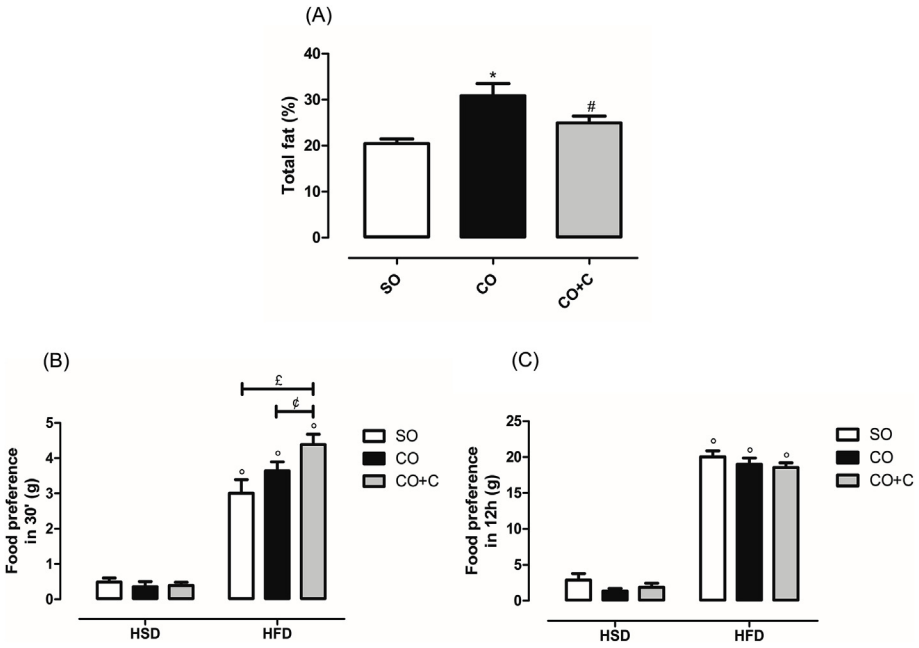

Fig. 1. Body adiposity and food preference test. (A) - Total fat content at PN180; (B) food preference after 30 min of ingestion of palatable diets and (C) food preference after 12 h of palatable diets of ingestion at PN175 of soybean oil group (SO), coconut oil group (CO) and coconut oil + chow supplemented with coconut oil group (CO + C). Data expressed as mean ± SEM; n = 10 to total fat content and n = 15 to food preference test. * *Vs* SO; # *Vs* CO; ° *Vs* HSD of each respective group; £ HFD CO + C *Vs* HFD SO; ¢ HFD CO + C *Vs* HFD CO; $p < 0.05$.

Plasma leptin was 2.2-fold higher in the CO group than in the SO and 1.3-fold higher than the CO + C group (SO: 0.98 ± 0.19; CO: 3.12 ± 0.74; CO + C: 1.39 ± 0.24, $p < 0.05$). No significant differences were observed between the SO and CO + C groups.

Fig. 2 shows the leptin signaling pathway proteins in the ARC. No differences were observed among groups regarding Ob-R (Fig. 2A), pJAK2/total JAK2 (Fig. 2B) and pSTAT3/total STAT3 ratios (Fig. 2C). However, SOCS3 protein content was lower in the CO and CO + C groups compared to the SO group (−23% and −25%, respectively, $p < 0.05$; Fig. 2D).

The GLP-1 receptor content was also analyzed in the ARC, and we observed a lower content only in the CO + C group compared to the SO and CO groups (−49% and −48%, respectively, $p < 0.05$; Fig. 3).

Concerning the proteins involved with the endocannabinoid system

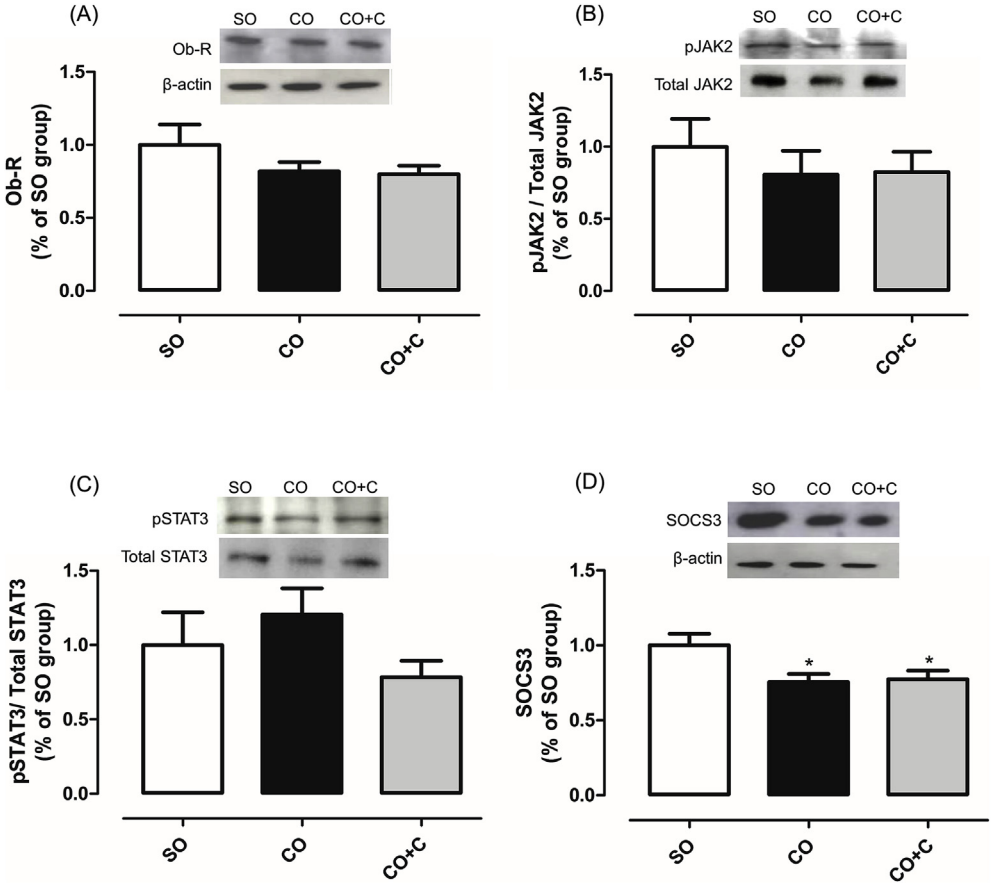

Fig. 2. Content of proteins of leptin signaling pathway in arcuate nuclei of hypothalamus at PN180 of soybean oil group (SO), coconut oil group (CO) and coconut oil + chow supplemented with coconut oil group (CO + C). (A) - OB-Rb content; (B) – pJAK2/Total JAK2 ratio; (C) - pSTAT3/Total STAT3 ratio; (D) – SOCS3 content. Data expressed as mean ± SEM; n = 07; *Vs SO; $p < 0.05$.

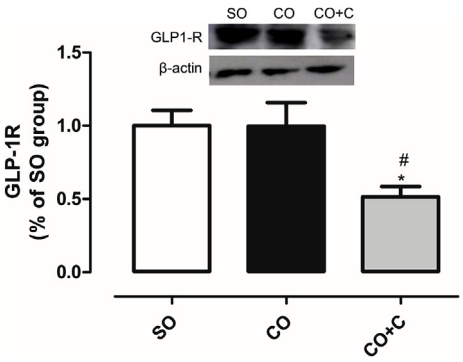

Fig. 3. GLP1-R content in arcuate nuclei of hypothalamus at PN180 of soybean oil group (SO), coconut oil group (CO) and coconut oil + chow supplemented with coconut oil group (CO + C). Data expressed as mean ± SEM; n = 07; *Vs SO; #Vs CO; $p < 0.05$.

in the LH, we detected no differences in the contents of CB1-R and FAAH (Fig. 4A and C, respectively), and a higher content of DAGLα, an enzyme of endocannabinoid synthesis, was detected in the CO group compared to the SO group (+58%, $p < 0.05$, Fig. 4B).

The dopaminergic system markers were evaluated in the regions of the mesolimbic system (VTA, Nac and DS). No differences were observed in TH the in VTA (Fig. 5A), Nac (Fig. 5B) or DS (Fig. 6A). Additionally, no differences were detected in the DDc (Fig. 5C), D1-R (Fig. 5D), DAT (Fig. 5E) or μ-opioid receptor (Fig. 5F) in the NAc. Additionally, no differences were found in D1-R (Fig. 6B), D2-R (Fig. 6C), DAT (Fig. 6D) or μ-opioid receptor (Fig. 6E) in the DS.

4. Discussion

Previous studies showed that the profile of fatty acids ingested by pregnant and lactating women (Mazurier et al., 2017) and rodents (Fernandes et al., 2012; Priego et al., 2013) can alter the quality of fatty acids in the milk. Thus, we hypothesize that maternal supplementation with coconut oil during lactation could alter the fatty acid profile of the milk, and this change in milk lipid content could be an imprinting factor for the changes observed in adult offspring. It is important to mention that the maternal supplementation with oils occurs through intragastric gavage, and, although baseline corticosterone levels are not a measure of stress responsivity, we have previously compared the baseline corticosterone levels of these groups (Quitete et al., 2018). The lack of differences between the groups suggests that changes in the mechanisms associated with the stress response are not playing a major role in our findings. In a previous study, we observed that maternal supplementation with coconut oil led the offspring fed with standard chow (CO group) after weaning to show higher total weight gain and hyperphagia (Quitete et al., 2019). This hyperphagia certainly contributed to the greater total adiposity of these animals in adulthood. Additionally, we demonstrated that when the offspring of these dams received a chow supplemented with coconut oil after weaning (CO + C group), they show higher body mass and unchanged visceral fat in adulthood, despite hypophagia, compared to control animals, which were fed with standard chow supplemented with soybean oil (Quitete et al., 2019). Here, we also showed that the total fat content was increased in adult offspring in the CO group and was unchanged in the CO + C group.

Coconut oil is rich in MCTs, which reduces the fatty acid deposition in adipose tissue and allows rapid hepatic β-oxidation, increasing energy expenditure and the production of ketone bodies, which are responsible for rapid satiety (St-Onge, Jones, 2002). Thus, replacing a diet rich in polyunsaturated fatty acids with a diet higher in MCTs may be responsible for the phenotype observed in the CO group. On the other hand, as the CO + C group was exposed to coconut oil throughout life, it is possible that the effects of MCT have contributed to the observed hypophagia and normal adiposity.

The leptinemia was proportional to the adiposity found in each experimental group, as expected (Münzberg, Morrison, 2015). The lifetime coconut oil intake not only prevented the increased adiposity in the CO group but also increased leptinemia in programmed rats.

The CO group showed hyperphagia; however, its plasma leptin was

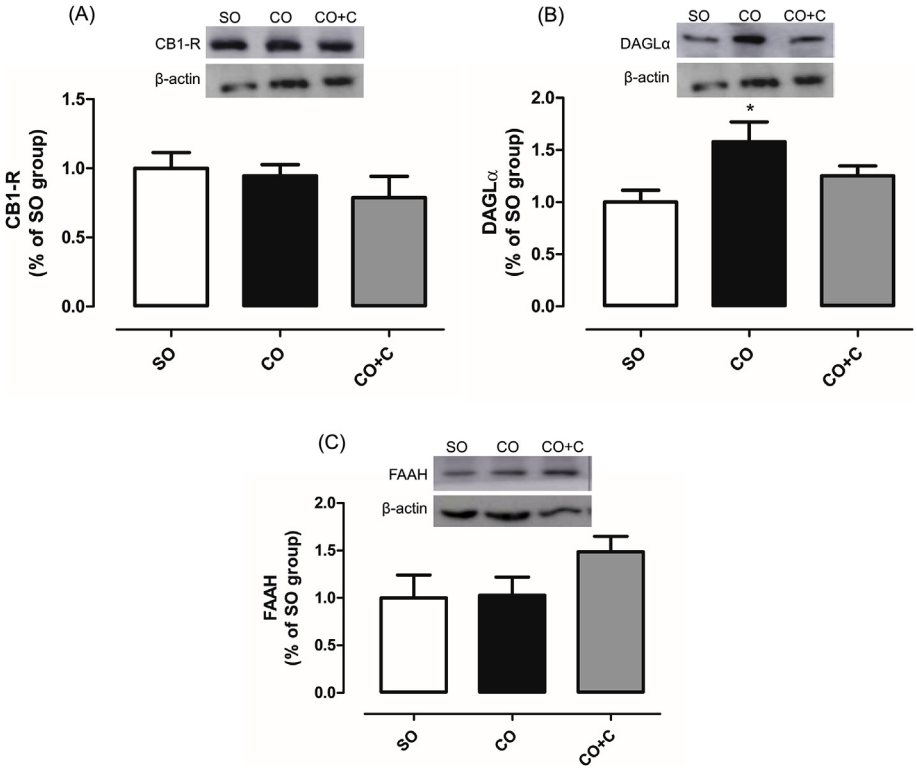

Fig. 4. Content of markers of endocannabinoid system in lateral hypothalamus at PN180 of soybean oil group (SO), coconut oil group (CO) and coconut oil + chow supplemented with coconut oil group (CO + C). (A) – CB1-Receptor content; (B) – DAGL-α content; (C) FAAH content. Data expressed as mean ± SEM; n = 07; *Vs SO; $p < 0.05$.

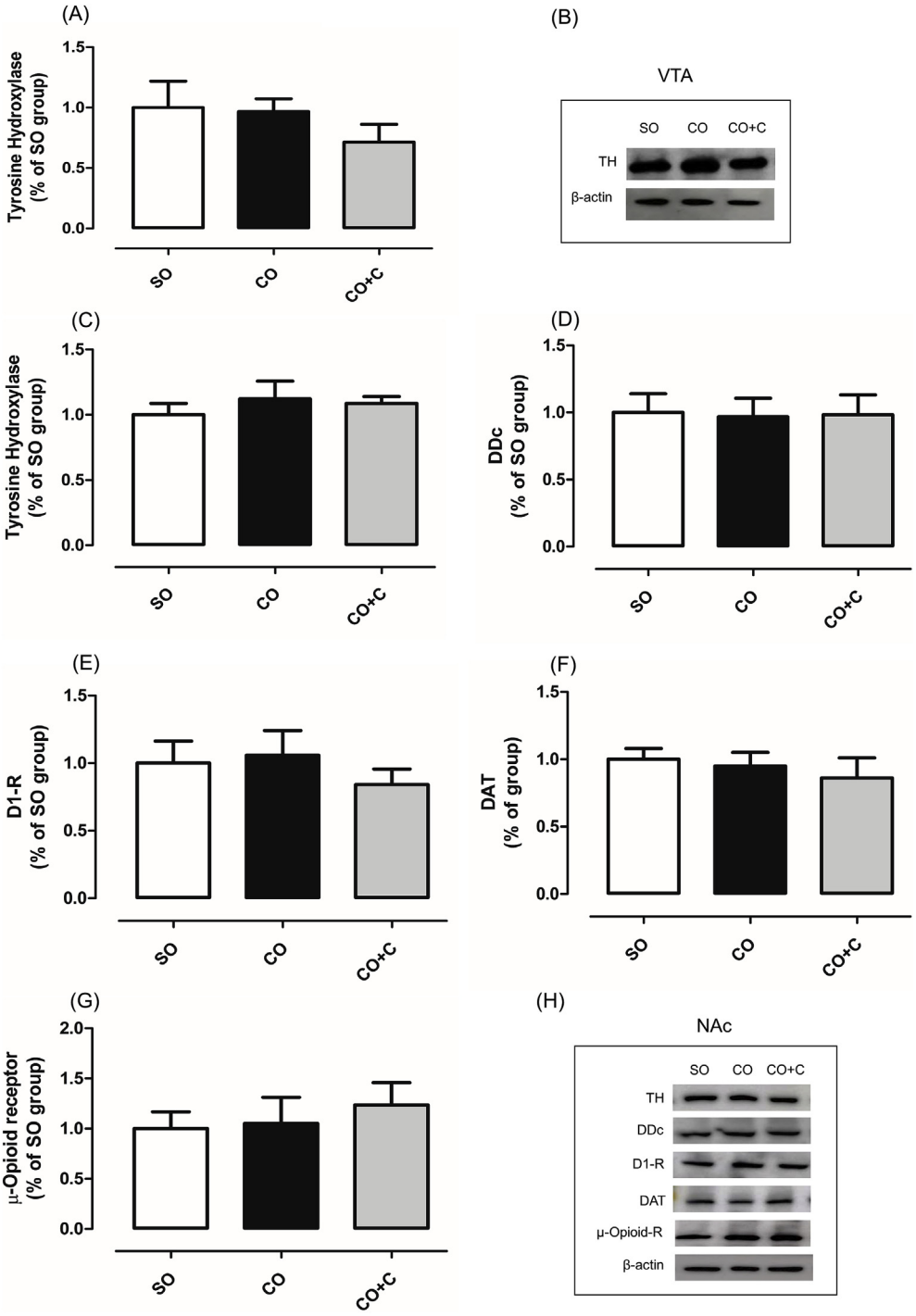

Fig. 5. Content of markers of dopaminergic system at PN180 of soybean oil group (SO), coconut oil group (CO) and coconut oil + chow supplemented with coconut oil group (CO + C). (A) – tyrosine hydroxylase content in VTA; (B) – tyrosine hydroxylase content in NAc; (C) –DOPA decarboxylase content in NAc; (D) – Dopamine receptor D1 content in NAc; (E) – DAT content in NAc; (F) - μ-Opioid receptor content in NAc; (G) – representative bands of TH and β-actin in VTA; (H) - representative bands of TH, DDc, D1-R, DAT, μ-opioid-receptor and β-actin in NAc. Data expressed as mean ± SEM; n = 07; $p < 0.05$.

higher than that of the SO group, suggesting a central resistance to the anorectic effects of this hormone, as already reported in other experimental models of obesity (Bonomo et al., 2007; Lima et al., 2011; Rodrigues et al., 2011). However, here no differences were observed in Ob-R, pJAK2/total JAK2 and pSTAT3/total STAT3 ratios in either the CO or CO + C groups. The only alteration was a lower SOCS3 expression in these groups. Although the proteins are similarly expressed in both groups, the end effect suggests that the underlying cause is different. Usually, SOCS3, which is stimulated by the JAK/STAT pathway, is responsible for the negative feedback of this pathway, so high levels of SOCS3 are associated with leptin resistance, as commonly observed in obese individuals (Bjørbaek et al., 1999). However, in the CO group, SOCS3 in reduced, showing that this is not a cause of leptin resistance. Therefore, the leptin resistance of the CO group is possibly due to a difficulty in accessing the CNS or to an alteration of other leptin signaling pathways, such as the PI3K pathway (Gomes et al., 2018; Kwon et al., 2016; Münzberg, Morrison, 2015).

Another possibility to explain the hyperphagia of the CO group is

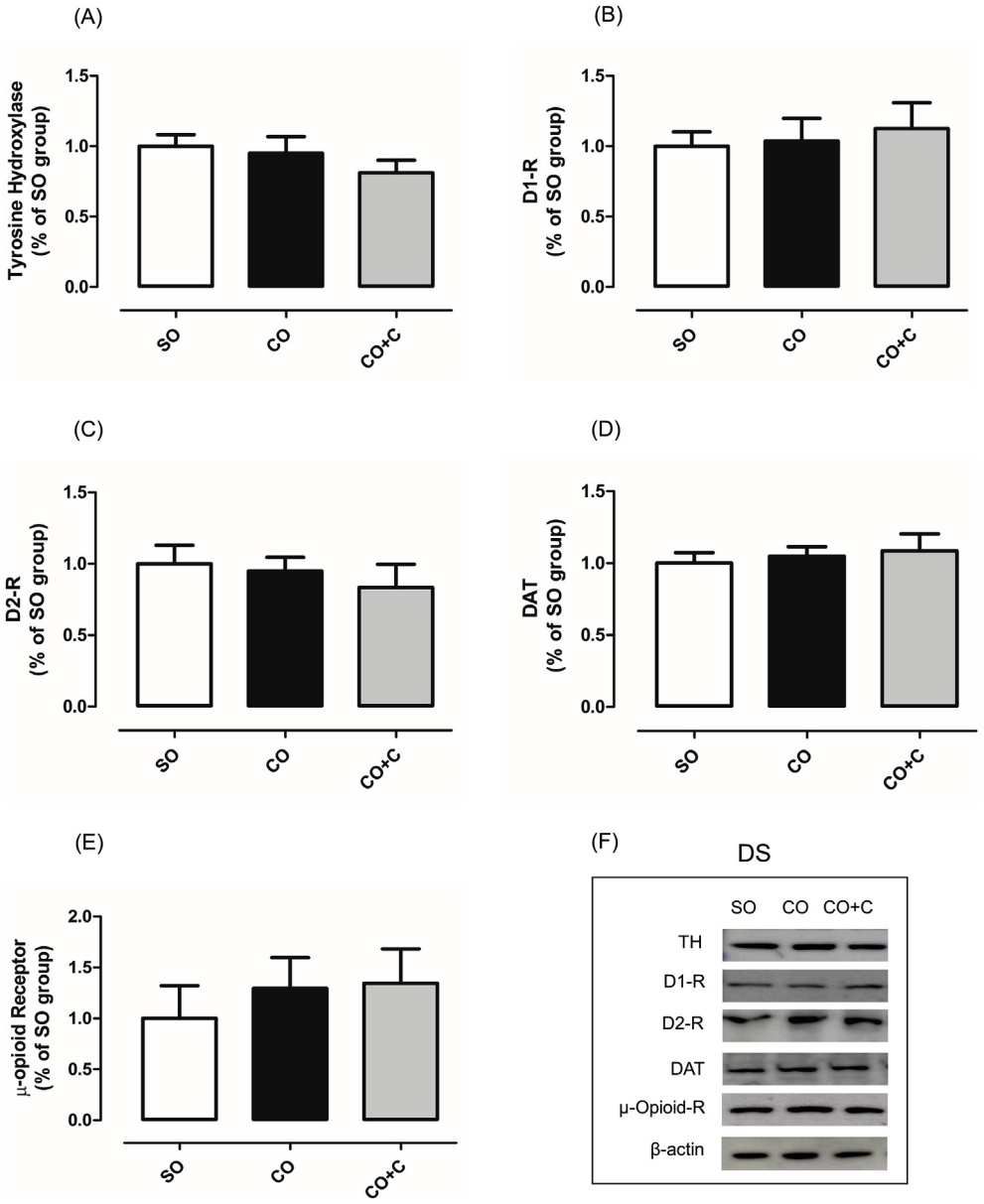

Fig. 6. Content of markers of dopaminergic system in dorsal striatum nuclei at PN180 of soybean oil group (SO), coconut oil group (CO) and coconut oil + chow supplemented with coconut oil group (CO + C). (A) – tyrosine hydroxylase content; (B) –Dopamine receptor 1 content (D1-R); (C) –Dopamine receptor 2 content (D2-R); (D) –DAT content; (E) –μ-Opioid receptor content; (F) - representative bands of TH, D1-R, D2-R, DAT, μ-opioid-receptor and β-actin in DS. Data expressed as mean ± SEM; n = 07; $p < 0.05$.

the higher expression of DAGLα in the LH. This enzyme catalyzes the synthesis of the most abundant endocannabinoid, 2-arachidonoylglycerol (2-AG), suggesting that its biosynthesis may be increased. Since we did not measure the degradation enzyme (MAGL), we do not know if 2-AG degradation is altered in the CO group. On the other hand, the content of FAAH, which degrades anandamide, was not altered. Despite the technical limitations, the increase in DAGLα in the LH is an important finding, helping us to understand the higher food intake in these programmed animals. In a model of obese mice with leptin resistance, Cristino et al. (2013) observed a higher DAGLα in LH with no changes in MAGL. Additionally, increased DAGLα in the LH is more evidence of central leptin resistance in the CO group because this hormone inhibits endocannabinoid production (Cristino et al., 2013; Jo et al., 2005; Maccarrone et al., 2005). Conversely, the CO + C group had no changes in the proteins of ECS, which can be attributed to normal sensitivity to leptin.

The orexin + neurons of LH send projections to dopaminergic neurons of the VTA, stimulating the release of dopamine in target regions, such as the nucleus accumbens and the prefrontal cortex (Flores et al., 2013; Hirasawa et al., 2007). Therefore, we investigated the expression of some proteins of the mesolimbic dopaminergic system, composed of VTA, NAc and DS, an important system that controls food behavior (Boekhoudt et al., 2017; Leigh, Morris, 2016). Alterations in this system are related to higher food intake, especially of palatable foods, and to obesity (Leigh, Morris, 2016). However, no changes were detected in the proteins related to dopamine signaling in the VTA, NAc and DS, as well as in the μ-opioid receptor in the NAc and DS, indicating that our animals did not have alterations in the reward system related to food behavior. Dopamine synthesis can be modulated by the ECS, which acts to disinhibit the dopaminergic neurons of the VTA, resulting in excitation of these neurons (Lau et al., 2017). Thus, we suppose that the ECS effect in the mesolimbic system is not altered in the CO group.

Surprisingly, during the feeding test, the CO + C group that shows hypophagia, demonstrated higher voracity for HFD after 30 min of the

challenge compared to the other groups. Since the dopaminergic pathway is normal and the higher HFD intake occurred only acutely, we think that the central action of GLP-1, a gastrointestinal peptide that inhibits short-term food intake, could be altered. We observed lower GLP1-R in ARC of the CO + C group, indicating a lower response to GLP-1, which can be the mechanism by which the highest voracity occurs in this group. However, the smallest GLP-1 response seems to be compensated over the period of the test, since the long-term regulators, such as leptin, apparently, have its action preserved. Despite the CO + C group being better adapted (hypophagia, lower adiposity and normoleptinemia), increased voracity for palatable food suggests that they may become obese if exposed to HFD long term.

Maternal supplementation with coconut oil followed by feeding with standard chow after weaning leads to hyperphagia, higher body mass and adiposity in adulthood. However, offspring from dams that received coconut oil only during lactation and that were fed with chow supplemented with coconut oil after weaning, showed hypophagia and unaltered fat mass. Thus, maybe the findings in the CO group are due to the absence of coconut oil in the diet after weaning. Throughout development, adiposity increased, leading to hyperleptinemia and resistance to leptin, reflected in changes in others regulators of food consumption, such as 2-AG biosynthesis in the LH. However, the CO group has the dopaminergic reward system preserved, whereas in the CO + C group, the permanent presence of coconut oil (imprinting factor) allowed these animals to become better adapted, as the programming concept states.

Based on current data together with the increasing use of functional foods and dietary supplements by women of a fertile age, we recommend caution when such modifications are made during critical windows of development, such as lactation, because that may result in future consequences to offspring, even after transitory interventions. More studies are needed to understand the effects of coconut oil supplementation during critical periods, especially its consequences on food intake, for which information is still scarce in the literature.

Author contributions

Conception and design: EO, FTQ, DNB, EGM, PCL.

Collection, analysis and interpretation of data: FTQ, DNB, DSG, FAHC, PNS, TCP, VSTR, IHT.

Drafting and/or revising the article critically for important intellectual content: EO, FTQ, IHT, EGM, PCL.

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Conflict of interest

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

Transparency document

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