Life Sciences 232 (2019) 116575

Contents lists available at ScienceDirect

Life Sciences

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

Cigarette smoke during lactation in rat female progeny: Late effects on endocannabinoid and dopaminergic systems

P.N. Soares[a,1], R.A. Miranda[a,1], T.C. Peixoto[a], F.A.H. Caramez[a], D.S. Guarda[a], A.C. Manhães[b], E. de Oliveira[a], E.G. de Moura[a], P.C. Lisboa[a,*]

[a] Endocrine Physiology Laboratory, Department of Physiological Sciences, State University of Rio de Janeiro, 20550-030 Rio de Janeiro, RJ, Brazil
[b] Neurophysiology Laboratory, Department of Physiological Sciences, State University of Rio de Janeiro, 20550-030 Rio de Janeiro, RJ, Brazil

ARTICLE INFO

Keywords:
Cigarette smoke
Endocannabinoids
Dopaminergic pathway
Metabolic programming
Breastfeeding

ABSTRACT

Aims: Maternal smoking is considered a risk factor for childhood obesity. In a rat model of tobacco exposure during breastfeeding, we previously reported hyperphagia, overweight, increased visceral fat and hyperleptinemia in adult female offspring. Obesity and eating disorders are associated with impairment in the endocannabinoid (EC) and dopaminergic (DA) systems. Considering that women are prone to eating disorders, we hypothesize that adult female Wistar rats that were exposed to cigarette smoke (CS) during the suckling period would develop EC and DA systems deregulation, possibly explaining the eating disorder in this model.

Material and methods: To mimic maternal smoking, from postnatal day 3 to 21, dams and offspring were exposed to a smoking machine, 4×/day/1 h (CS group). Control animals were exposed to ambient air. Offspring were evaluated at 26 weeks of age.

Key findings: Concerning the EC system, the CS group had increased expression of diacylglycerol lipase (DAGL) in the lateral hypothalamus (LH) and decreased in the liver. In the visceral adipose tissue, the EC receptor (CB1r) was decreased. Regarding the DA system, the CS group showed higher dopamine transporter (DAT) protein expression in the prefrontal cortex (PFC) and lower DA receptor (D2r) in the arcuate nucleus (ARC). We also assessed the hypothalamic leptin signaling, which was shown to be unchanged. CS offspring showed decreased plasma 17β-estradiol.

Significance: Neonatal CS exposure induces changes in some biomarkers of the EC and DA systems, which can partially explain the hyperphagia observed in female rats.

1. Introduction

Smoking is a risk factor for the onset of several diseases and is responsible for approximately 6 million annual deaths worldwide [1]. Tobacco is still the drug most commonly used by women during gestation and lactation, which are critical periods for fetus development, and it is known that cigarette components are transferred through placenta and breast milk, leading to negative consequences for the mother and possibly programming the baby's metabolism [2–4]. Metabolic programming can be caused by nutritional and/or environmental alterations during plasticity periods (pregnancy and breastfeeding) that cause epigenetic changes, increasing the risk of occurrence of diseases at adulthood, such as obesity [5,6]. In rodents, studies have already demonstrated that adult offspring from both sexes that were exposed to cigarette smoke exclusively during lactation were programmed for hyperphagia, overweight, increased visceral fat, hyperleptinemia and dyslipidemia [7,8]. In this experimental model, Pinheiro et al. (2015) observed an increase in the preference for a fat-rich palatable diet in males, which was attributed to changes in the dopaminergic system, such as lower dopamine production, consequently decreasing the effects of reward mechanisms [9].

Hyperphagia, obesity and metabolic programming have been associated with changes in the endocannabinoid (EC) system [10–12]. The EC system directly controls the central and peripheral mechanisms involved in energy homeostasis and food intake. EC action is mediated by the specific receptors, CB1r and CB2r, and their endogenous ligands, anandamide (AEA) and 2-aracdonoylglycerol (2-AG) [13–16]. The AEA is synthesized by *N*-acylphosphatidylethanolamide-phospholipase D

* Corresponding author at: Universidade do Estado do Rio de Janeiro, Instituto de Biologia, Departamento de Ciências Fisiológicas, 5° andar, Av. 28 de setembro, 87, Rio de Janeiro, RJ 20551-031, Brazil.
E-mail address: pclisboa@uerj.br (P.C. Lisboa).
[1] Equally contributed to this work.

https://doi.org/10.1016/j.lfs.2019.116575
Received 9 May 2019; Received in revised form 11 June 2019; Accepted 14 June 2019
Available online 15 June 2019
0024-3205/

(NAPE-PLD) and is metabolized to arachidonic acid (AA) and ethanolamine by the enzyme fatty acid amide hydrolase (FAAH). The 2-AG is mainly generated by diacylglycerol lipase (DAGL) and is metabolized to AA and glycerol through the enzyme monoacylglycerol lipase (MAGL) [14,16–21]. CB1r is expressed in the central nervous system (CNS), adipose tissues, liver and reproductive tract [15,22–25]. In the lateral hypothalamus (LH), the EC increases appetite through changes in neuron excitability [26–29]. This effect is suppressed by leptin [30,31]. In white adipose tissue (WAT), the EC stimulates lipogenesis and adipogenesis [32–34]. In the liver, EC is associated with the development of steatosis, inflammation and fibrosis [24,35–37]. The EC system also interacts with female reproductive tract inhibiting folliculogenesis, oocyte maturation and ovarian hormone secretion [16].

Substances that cause addiction, such as nicotine, activate the mesocorticolimbic dopaminergic (DA) system, which is composed of neurons with cellular bodies in the ventral tegmental area (VTA), releasing dopamine and activating its receptors (D1r and D2r) in the dorsal striatum (DS), nucleus accumbens (NAc) and prefrontal cortex (CPF) [38–40]. Adult male rats programmed by postnatal exposure to tobacco smoke or nicotine alone have complex alterations that decrease DA system actions [9,38]. In addition, these animals have higher serum leptin and this hormone has already been associated with a reduction in dopamine release from the VTA, and an increase in D2r levels in the arcuate nucleus (ARC) [41,42].

Considering that (i) women are more susceptible to eating disorders [43,44] and (ii) disturbances in appetite-regulating neural circuits involve the impairment of EC signaling [45] and high level of AEA [46], as well as deregulation of DA signaling, in different areas of the CNS [47,48], the current study was designed to evaluate whether adult female rat offspring that were programmed by cigarette smoke during breastfeeding (which show hyperphagia and abdominal fat accumulation) develops central and peripheral EC system dysfunction accompanied by a disruption of DA pathway. Therefore, using tissues from a previously published model [8], the EC system was evaluated in the LH, visceral adipose tissue and liver. Furthermore, the DA system was evaluated in the VTA, NAc, PFC, DS and ARC. Since leptin interacts with both EC and DA systems, the JAK/STAT pathway was evaluated in LH and ARC to provide some insight concerning leptin resistance in our model.

2. Materials and methods

2.1. Ethical and experimental model

This experimental protocol was conducted under the institutional approval from the Universidade do Estado do Rio de Janeiro (Number: 018/2014) and the experiment was carried out in accordance to the Brazilian Law no. 11.794/2008.

Wistar rats were appropriately allocated in a temperature-controlled room (23–24 °C) with a light/dark cycle (7 a.m.–7 p.m.) and received water and standard chow (Nuvilab, Sogorb, São Paulo, SP, Brazil) ad libitum. Three-month-old virgin female rats were mated with male rats in a 2:1 proportion for one week. After pregnancy confirmation, females were placed in individual cages during the gestation and breastfeeding periods. At birth, litters were adjusted to 6 pups (both sexes) per dam. Three days after birth, rat dams and their offspring were randomly assigned to each experimental group: Cigarette Smoke (CS) and Control (C). In the CS group ($n = 7$ litters), dams and offspring were placed into a smoking machine (TE-10, Teague Enterprises, CA, USA), 4 times per day (1 h for each exposure). In the C group ($n = 7$ litters), dams and offspring were exposed to ambient air in a chamber that was similar to the one used for tobacco smoke exposure. The tobacco smoke used was obtained from burning one cigarette at a time. The 3R4F reference cigarettes (0.73 mg nicotine) were obtained from the University of Kentucky. The number of experimental cigarettes used are similar to that used by moderate to heavy smokers. During the four weeks that preceded euthanasia, the estrous cycle of CS and C offspring was daily evaluated; the two groups showed regular estrous cycles (4 to 5 days). Only one female rat offspring per litter was randomly studied here; the remaining animals of the same litter were used for another study.

The food intake and body mass were assessed every 4 days from weaning (3 weeks of age) until 26 weeks of age. The amount of food intake was estimated from the difference between the mass of food left in the cage and the total mass initially placed in the cage 4 days before.

2.2. Euthanasia and tissue collection

At 26 weeks of age, the animals were weighed and euthanized with a lethal dose of ketamine hydrochloride/xylazine hydrochloride (2:1). Blood was obtained by cardiac puncture and collected in a heparin tube and centrifuged (1.260 $\times g$, 25 min, 4 °C). The plasma was collected and stored at −20 °C for posterior analyses. Brain, visceral adipose tissue and liver were collected and stored at −80 °C.

2.3. Hormone analyses

Plasma sex steroids were evaluated through specific radioimmunoassay kits in accordance with the manufacturer's instructions (MP Biomedicals, LLC, NY, EUA). Samples were analyzed in duplicate and in a single assay. The sensitivities of the assays were: 0.15 ng/mL for progesterone, 0.1 ng/mL for testosterone and 10 pg/mL for estradiol. The intra-assay coefficients of variation were: 1.8% for progesterone, 1.5% for testosterone and 1.6% for estradiol.

2.4. Punch technique

The whole brain was carefully collected and stored at −80 °C. Coronal brain sections were cut using a cryostat (Hyrax C25, Zeiss, Germany) and punches of the lateral hypothalamus (LH, bregma 2.04–3.60 mm), dorsal striatum (DS, bregma 2.16–0.96 mm), nucleus accumbens (NAc, bregma 2.16–0.96 mm), arcuate nucleus of hypothalamus (ARC, bregma 2.04–3.60 mm), prefrontal cortex (PFC, bregma 4.20–2.52 mm) and ventral tegmental area (VTA, bregma 5.64–6.36 mm) were extracted from the brains according to Paxinos and Watson (1998) stereotaxic coordinate atlas [49].

2.5. Western blotting

In the LH, adipose tissue and liver, we measured the following biomarkers of EC system: DAGL, NAPE-PLD, MAGL, FAAH and CB1r. In addition, the estrogen receptor alpha (ERα) was determined in these 3 tissues. Concerning the DA system, the following parameters were measured: 1) Tyrosine hydroxylase (TH) in the VTA; 2) TH, dopamine transporter (DAT) and dopamine receptors (D1r and D2r) in the NAc, DS and PFC; and 3) D2r in the ARC. The following leptin signaling parameters were in the ARC and LH: phosphorylated-janus kinase 2 (pJAK2), and phosphorylated-signal transducer and activator of transcription 3 (pSTAT3). Different extract buffers were used depending on each tissue. T-PER tissue protein extraction (Thermo Fisher Scientific CA, USA), RIPA buffer [50 mM Tris-HCl (pH 7.4), 1% NP-40, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM Na3VO4, 1 mM NaF], for LH, adipose tissue and liver, respectively. These buffers contained protease inhibitor cocktail (F. Hoffmann-La Roche Ltda., Basel, CH). The punches were sonicated twice in an ultrasonic processor for 10 s (15 s interval, 40% amplitude) with a RIPA buffer. The other samples were centrifuged at 18,506 $\times g$ for 25 min. The protein concentrations of all tissues were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, CA, USA). Homogenates were analyzed by SDS-PAGE using approximately 10–30 μg of total protein content. Proteins were then transferred from the gel to a polyvinylidene difluoride membrane (Hybond ECL; Amersham Pharmacia Biotech, London, UK) and incubated with Tris-buffered saline (TBS) containing

Table 1
Antibodies used in the western blotting.

Primary antibodies			Secondary antibodies		
Antibody	Distributed by	Dilution	Distributed by	Dilution	Specificity
DAGL-α	Cell Signaling Technology MA, USA	1:500	Sigma-Aldrich MO, USA	1:20000	Anti-goat
NAPE-PLD	Abcam, UK	1:1000	Sigma-Aldrich MO, USA	1:10000	Anti-mouse
MAGL	Santa Cruz Biotechnology MA, USA	1:400	Sigma-Aldrich MO, USA	1:10000	Anti-rabbit
FAAH	Sigma-Aldrich MO, USA	1:1000	Sigma-Aldrich MO, USA	1:10000	Anti-rabbit
CB1r	Abcam, UK	1:100	Sigma-Aldrich MO, USA	1:7000	Anti-rabbit
ERα	Millipore Corporation, Temecula, CA, SA	1:500	Sigma-Aldrich MO, USA	1:10000	Anti-mouse
TH	Sigma-Aldrich MO, USA	1:1000	Sigma-Aldrich MO, USA	1:10000	Anti-mouse
DAT	Millipore	1:500	Sigma-Aldrich MO, USA	1:10.000	Anti-rabbit
D1r	Abcam	1:500	Sigma-Aldrich MO, USA	1:10.000	Anti-rabbit
D2r	Millipore	1:500	Sigma-Aldrich MO, USA	1:7.000	Anti-rabbit
p-JAK2	Santa Cruz Biotechnology MA, USA	1:500	Sigma-Aldrich MO, USA	1:10.000	Anti-goat
p-STAT3	Santa Cruz Biotechnology MA, USA	1:500	Sigma-Aldrich MO, USA	1:10.000	Anti-rabbit
β-actin	Santa Cruz Biotechnology MA, USA	1:1000	Sigma-Aldrich MO, USA	1:10000	Anti-rabbit
GAPDH	Novus Biological Centennial, CO, USA	1:500	Sigma-Aldrich MO, USA	1:10000	Anti-mouse

DAGL-α: diacylglycerol lipase alpha; NAPE-PLD: *N*-arachidonoylphospatidylethanolamine phospholipase D; MAGL: monoacylglycerol lipase; FAAH: fatty acid amide hydrolase; CB1r: cannabinoid type-1 receptor; ERα: estrogen receptor alpha; TH: tyrosine hydroxylase; DAT: dopamine transporter; D1r and D2r: types 1 and 2 dopaminergic receptors; p-JAK2: phosphorylated-Janus kinase 2; pSTAT3: phosphorylated-signal transducer and activator of transcription 3; GAPDH: glyceraldehyde-3-phosphate 5 dehydrogenase.

5% albumin for 1 h. Membranes were then washed with tween-tris-buffered saline (0.1%) and incubated overnight at 4 °C with a specific primary antibody (Table 1). After this, membranes were washed three times with TweenTBS (0.1%) and incubated for 1 h with appropriate secondary antibody conjugated with biotin (Table 1) at room temperature. Then, membranes were again washed three times with Tween-TBS (0.1%) and incubated with streptavidin-conjugated horseradish peroxidase (Caltag Laboratories, Burlingame, CA, USA). The protein bands were visualized by chemiluminescence kit (ClarityTM Western ECL Substrate, Bio-Rad Laboratories Inc., USA) followed by exposure in Image Quant LAS (GE Healthcare, Buckinghamshire, UK). The area and density of the bands were quantified using the Image J software (Wayne Rasband National Institute of Health, MA, USA) and were normalized by β-actin or glyceraldehyde-3-phosphate 5 dehydrogenase (GAPDH). Results were expressed as percentages (%) of the control group.

2.6. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) and analyzed through the statistical program GraphPad Prism 6.0 (San Diego, CA, USA). Only one pup/litter was randomly used for analysis. Student's *t*-tests were used and statistical difference was considered significant when $p < 0.05$.

3. Results

As previously reported [8], adult CS female offspring had a significant increase food intake during life (~6%), were overweight (~20%) and had greater visceral fat mass (~27%).

3.1. EC system biomarkers

In the lateral hypothalamus (LH, Fig. 1A), an increase in DAGL protein levels (+54%, $p = 0.031$) in CS offspring was observed; no changes in NAPE-PLD, MAGL, FAAH and CB1r protein contents were found. In the visceral adipose tissue (VAT, Fig. 1B), a decrease in CB1r protein levels (−52%, $p = 0.033$) was observed in CS offspring; DAGL, NAPE-PLD, MAGL and FAAH protein contents were not significantly altered. In the liver (Fig. 1C), the CS group showed lower DAGL levels (−41%, $p = 0.026$); significant changes in NAPE-PLD, MAGL, FAAH and CB1r protein contents were not observed in these animals.

3.2. DA system biomarkers

Dopaminergic pathway is depicted in Figs. 2 and 3. In the PFC, the CS group showed an increase in DAT protein levels (+1.4 folds, $p = 0.033$, Fig. 2C); no changes in TH, D1r and D2r were observed. No alterations were found in the biomarkers of the DA system in the VTA (Fig. 2A), NAc (Fig. 2B) and DS (Fig. 3A) in CS offspring. However, in the ARC, D2r protein expression was decreased in the CS group (−35%, $p = 0.044$, Fig. 3B).

3.3. Leptin signaling

The protein contents of p-JAK2 and p-STAT3 were not altered in the ARC (Fig. 4A) or LH (Fig. 4B) of CS offspring.

3.4. Sex hormones and estrogen receptor (ERα)

A decrease in 17β-estradiol concentrations in CS group (−22%, $p < 0.05$, Fig. 5A) was observed; no changes in progesterone and testosterone levels (Fig. 5B and C respectively) were found. Also, no significant changes were detected in ERα in the LH, VAT or liver (Fig. 5D, E and F respectively).

4. Discussion

As previously described by Lisboa et al., (2017), adult females programmed by cigarette smoking during breastfeeding display hyperphagia, hyperleptinemia and increased fat accumulation [8]. Thus, in the present study, we focused on understanding the female's hyperphagia phenotype in this experimental model by evaluating central and peripheral EC and DA systems, as well as leptin signaling. Regarding the EC system, CS females showed that DAGL was increased in the LH but reduced in the liver, while reduced CB1r was observed in VAT. Concerning the DA system, we observed increased DAT in the PFC and reduced D2r in the ARC. Conversely, CS females had no differences in the leptin pathway in both the ARC and LH. Additionally, we demonstrated a reduction of plasma 17β–estradiol levels in the CS group, which may have a role in the aforementioned dysfunctions.

The LH expresses orexigenic neuropeptides directly involved in the increase of food intake via increased neurons excitability [26,50]. In the CS female group, the increase in DAGL expression in the LH suggests an augmented 2-AG synthesis, which could be related with their hyperphagic phenotype. Cristino et al., (2013) demonstrated that an increase

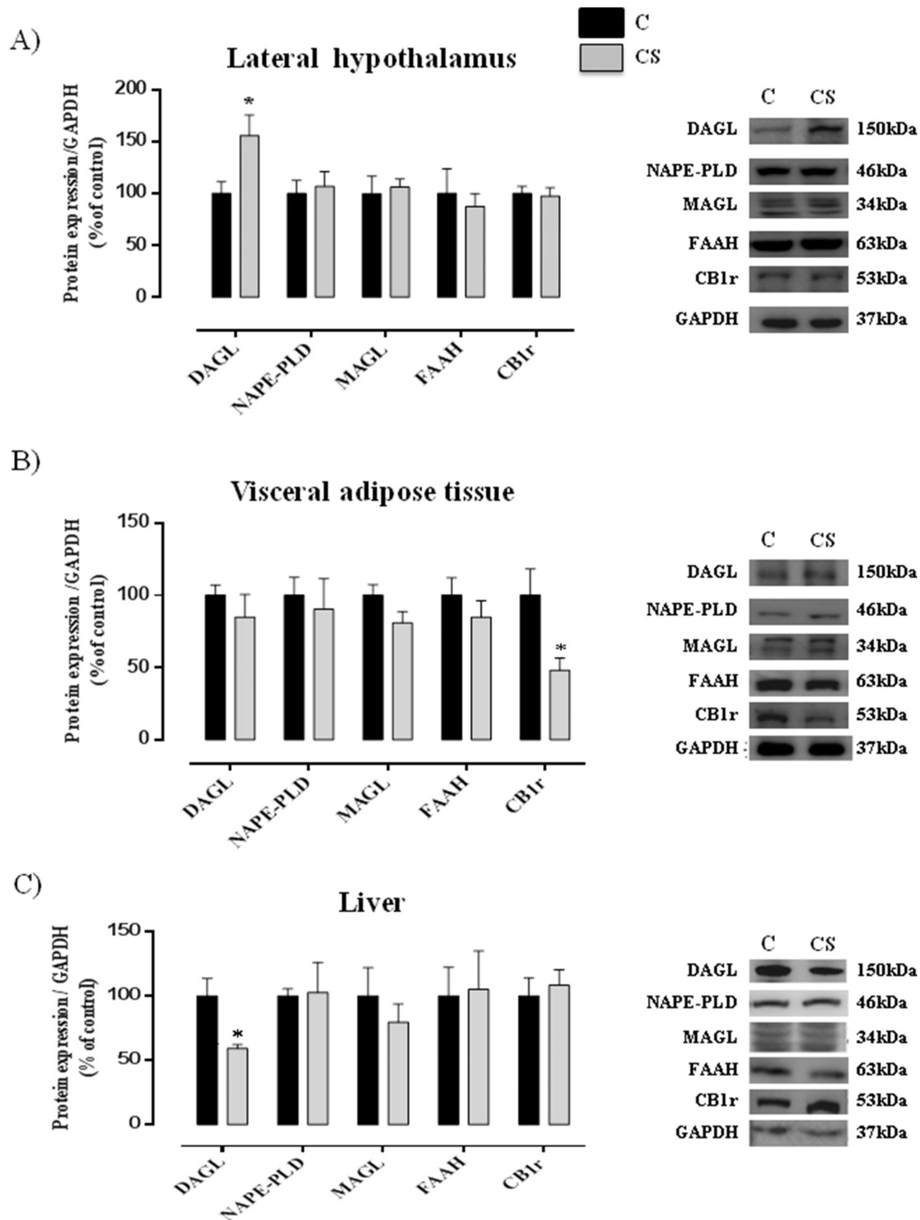

Fig. 1. Effects of cigarette smoke during breastfeeding on central and peripheral endocannabinoid system of female rat offspring at 26 weeks of age. Lateral hypothalamus (A), visceral adipose tissue (B) and liver (C). Diacylglycerol lipase (DAGL), N-acylphosphatidylethanolamine (NAPE-PLD), monoacylglycerol lipase (MAGL), fatty acid amide hydrolase (FAAH) and type 1 cannabinoid receptor (CB1r). Control group (C); Cigarette Smoking group (CS). Representative western blotting bands are shown. Student's unpaired *t*-tests were used and statistical difference was considered significant when $p < 0.05$; $n = 5–7$ rats/litter/group.

in 2-AG is intimately related with the hypothalamic orexigenic neurons differentiation in rodents during pre- and post-gestational periods [51]. Besides that, injections of 2-AG and AEA in the hypothalamus and NAc stimulate food ingestion in rats under satiety conditions [52,53].

It has been shown that the EC system acts on the adipose tissue metabolism throughout CB1r, inducing adipocyte hypertrophy and facilitating triglyceride accumulation [54–56,58]. In a model of mice lacking CB1r, Cota and colleagues (2003) observed a decrease in body mass and fat pad accumulation, accompanied by hypophagia [57]. In our study, the results regarding the EC system in the VAT were controversial. The CS female group (which had increased fat pad storage and hyperphagia) showed decreased CB1r in the VAT; the downregulation of this receptor possibly constitutes an attempt to reverse the fat accumulation. In the women's adipose tissue, decreased CB1r expression has been demonstrated and was related to increased levels of circulating EC as a negative feedback loop regulation [58]. The counterregulation mechanism in the adipose tissue of the CS group is speculative, mainly because we did not measure tissue and serum levels of EC. Thus, one limitation of the current investigation was to have failed to measure plasma and tissue levels of 2-AG and AEA in CS females. Notwithstanding, prolonged obesity cause altered CB1r expression, leading to hyperphagia [59].

Similarly, the EC system hyperactivation is related to liver disorders, such as non-alcoholic fat liver disease and steatosis [60,61]. Rats and mice with steatosis induced by a high-fat-diet and treated with a CB1r antagonist showed reversal of hepatic steatosis and dyslipidemia [62–64]. Here, CS offspring showed reduced DAGL in the liver, suggesting a decrease in 2-AG synthesis. Indeed, as already demonstrated by our group, CS females did not show changes in cholesterol and triglycerides content in the liver, as well as changes in hepatic morphophysiology [65]. Thus, the possible decrease in 2-AG levels caused by lower DAGL in the liver protects this tissue against steatosis

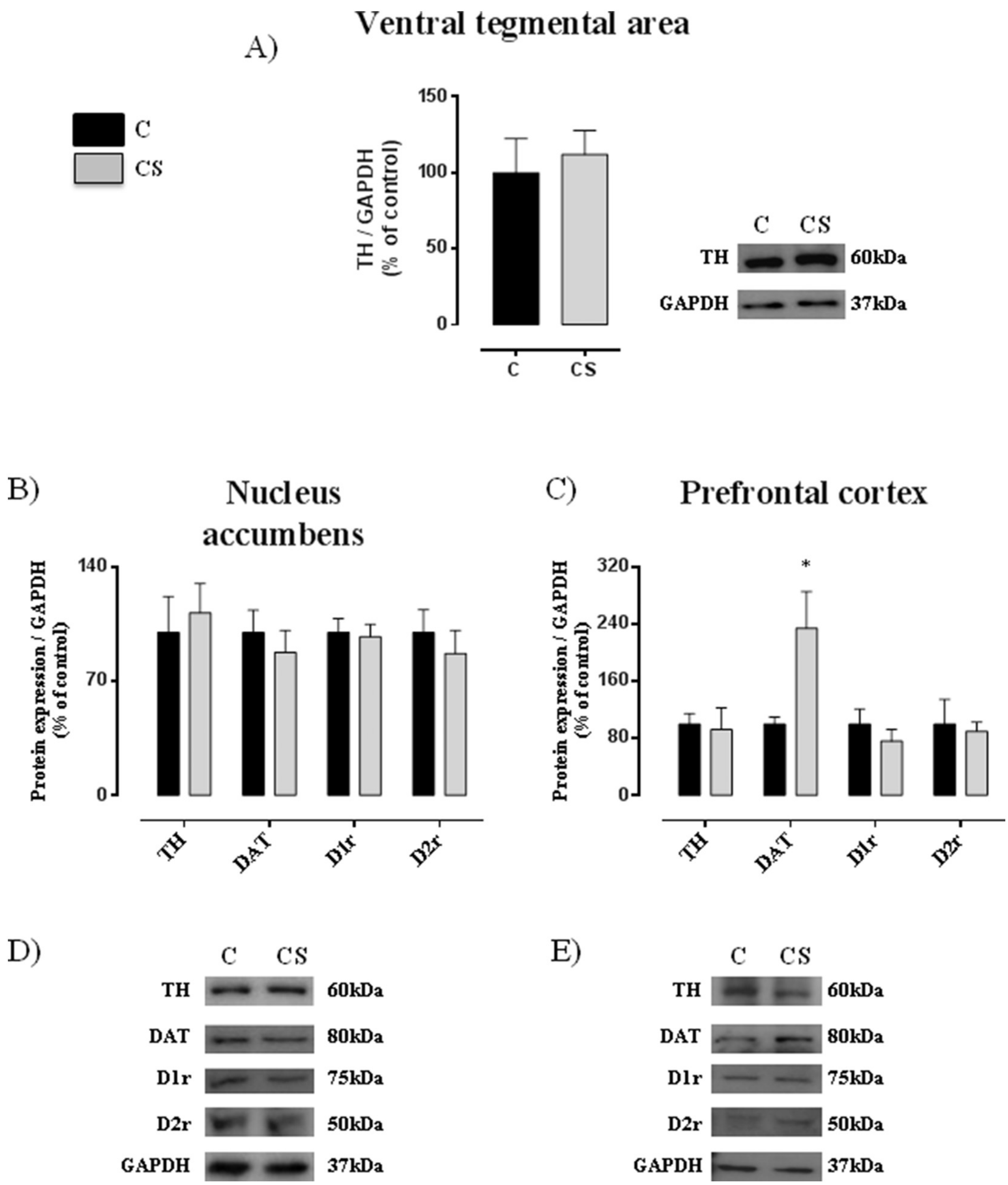

Fig. 2. Effects of cigarette smoke during breastfeeding on the dopaminergic system of female rat offspring at 26 weeks of age. Ventral tegmental area (A), nucleus accumbens (B) and prefrontal cortex (C). Tyrosine hydroxylase (TH), dopamine transporter (DAT) and dopamine receptors (D1r and D2r). Control group (C); Cigarette Smoking group (CS). Representative western blotting bands are shown (D and E). Student's unpaired *t*-tests were used and statistical difference was considered significant when $p < 0.05$; n = 5–7 rats/litter/group.

development in the CS group.

Alterations in the reproductive system were also observed in response to EC system dysfunction, affecting the hypothalamus-pituitary-ovarian axis and hormones synthesis as well as secretion [16,66]. According to Brents (2016), cannabinoids disrupt GnRH in the hypothalamus and consequently reduce estrogen levels [66]. Reduced estradiol levels accompanied by increased progesterone levels are associated with higher food intake [67–71]. It is well known that estrogens have anorexigenic effects [72] and that hyperphagia is observed in an ovariectomized rodent model of menopause [73]. Here, we observed that CS females had reduced 17β-estradiol, in spite of the fact that no variations in estrous cycle and/or progesterone and testosterone levels were identified. Therefore, this hypoestrogenism could be related to increased DAGL in the LH and possible higher 2-AG levels, helping to explain the hyperphagic phenotype of the CS group.

As the changes in the EC system were discrete and did not totally explain the mechanisms involved in hyperphagia in our model, we also evaluated the DA system and the leptin signaling in some brain regions that controls food intake. We demonstrated that DAT was increased in the PFC and that D2r was reduced in the ARC, suggesting a reduction of DA action in these nuclei. In fact, it was r previously reported, in adult males programmed by CS (same experimental model), that the DA system was also compromised, which was characterized by a reduced TH in the VTA and NAc, accompanied by lower D2r and higher D1r in the NAc [9]. Thus, postnatal tobacco smoke exposure reduces DA system actions in both sexes by different mechanisms.

Although the hyperphagia and hyperleptinemia evidenced in CS females is suggestive of the development of leptin resistance, these animals did not show any alteration in the JAK2/STAT3 pathway in both the ARC and LH. Despite the unchanged canonical leptin signaling,

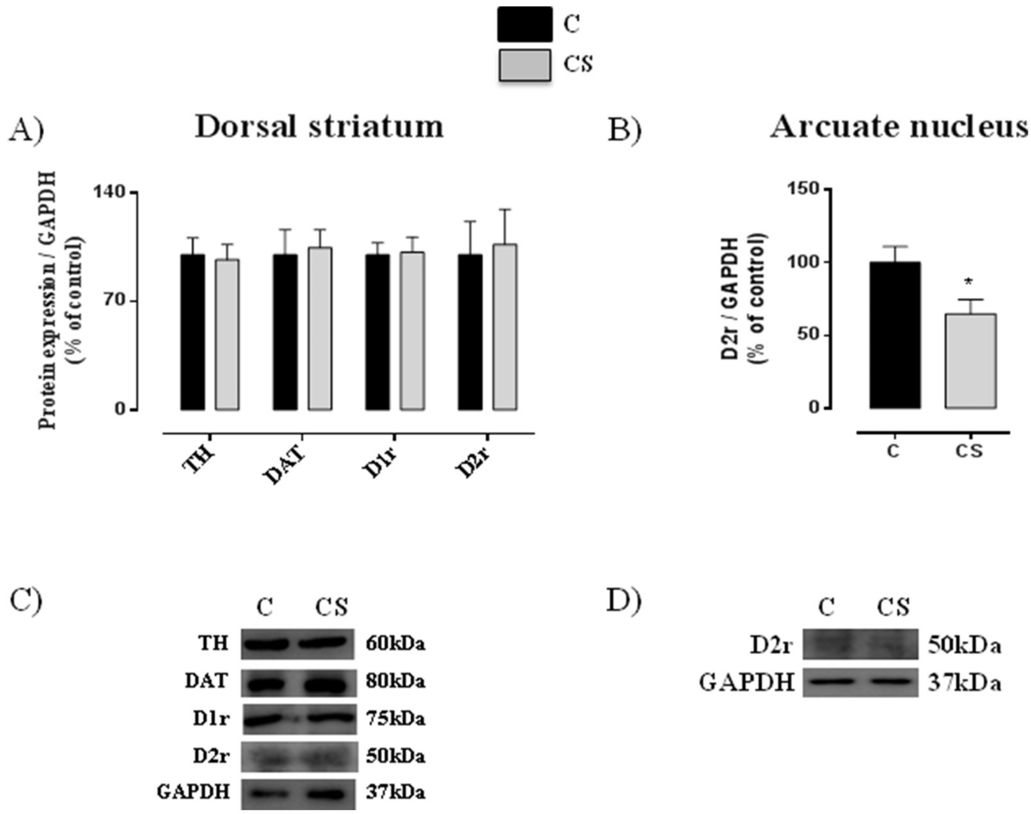

Fig. 3. Effects of cigarette smoke during breastfeeding on the dopaminergic system of female rat offspring at 26 weeks of age. Dorsal striatum (A) and arcuate nucleus (B). Tyrosine hydroxylase (TH), dopamine transporter (DAT) and dopamine receptors (D1r and D2r). Control group (C); Cigarette Smoking group (CS). Representative western blotting bands are shown (C and D). Student's unpaired *t*-tests were used and statistical difference was considered significant when $p < 0.05$; n = 5–7 rats/litter/group.

it is possible that other two events can be occurring in our model: 1) an impairment of leptin transport through the blood-brain barrier (BBB), and 2) a change in the alternative leptin pathway, involving the activation of PDE3B-PI3K and reduction of cAMP. A limitation of our study was not to have measured this signaling pathway. In addition, the integration between the EC system and the leptin signaling regulates the excitability of neurons in appetite-regulated circuits [30]. Our results indicated that leptin resistance in the LH blocks the integration of both

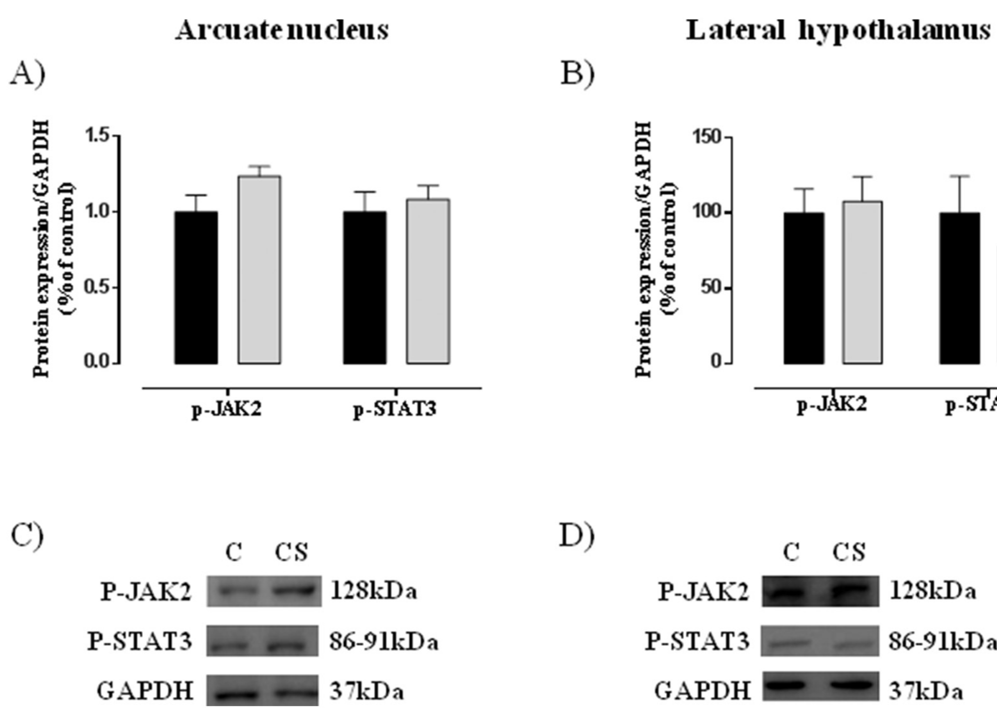

Fig. 4. Effects of cigarette smoke during breastfeeding on leptin signaling of female rat offspring at 26 weeks of age. Arcuate nucleus (A) and lateral hypothalamus (B). Phosphorylated-janus kinase 2 (p-JAK2), phosphorylated-signal transducer and activator of transcription 3 (pSTAT3). Control group (C); Cigarette Smoking group (CS). Representative western blotting bands are shown (C and D). Student's unpaired *t*-tests were used and statistical difference was considered significant when $p < 0.05$; n = 5–7 rats/litter/group.

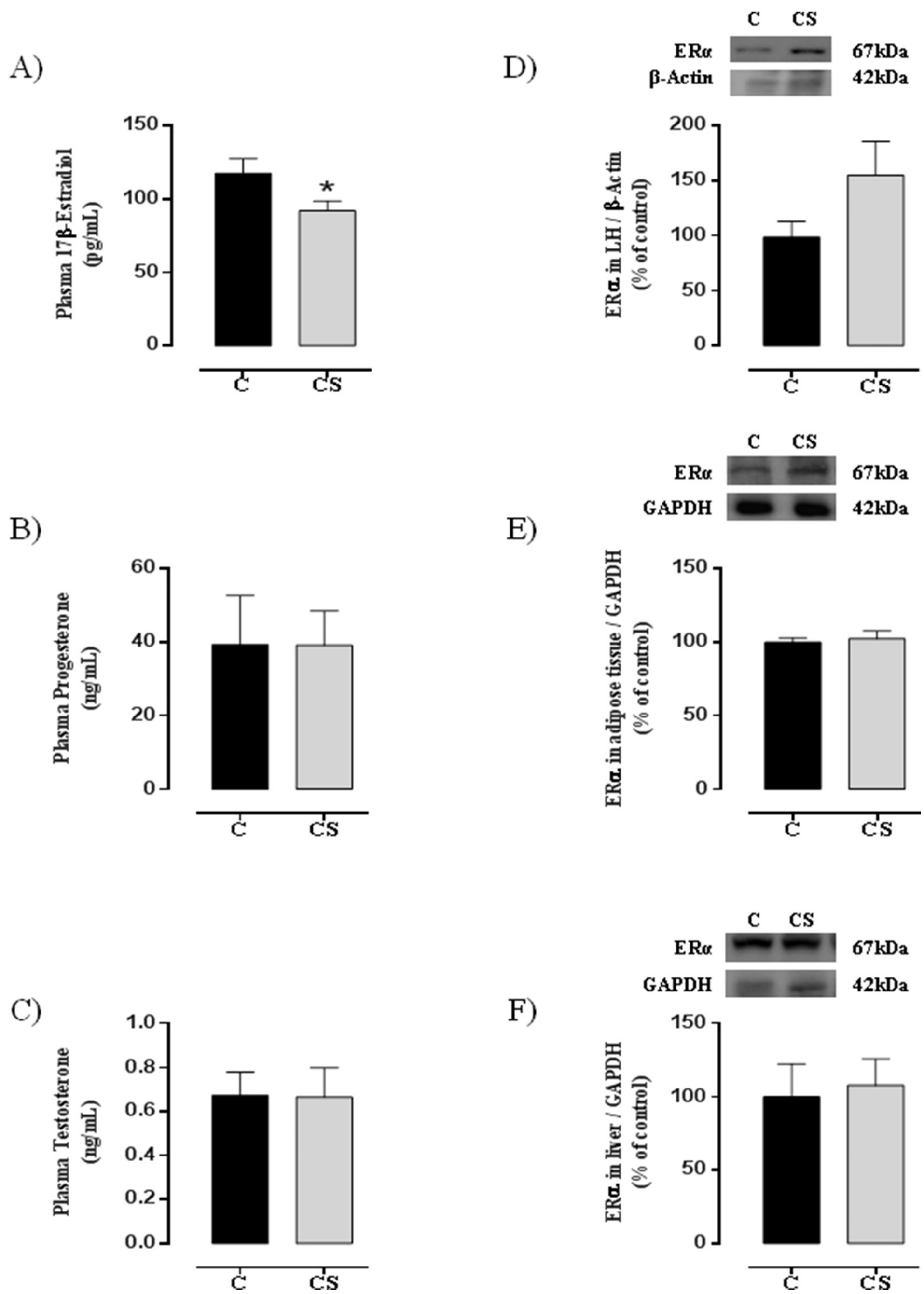

Fig. 5. Effects of cigarette smoke during breastfeeding on sex hormones and on central and peripheral estrogen receptor (ERα) expression of female rat offspring at 26 weeks of age. 17β-estradiol (A), progesterone (B), testosterone (C), ERα in lateral hypothalamus (D), ERα in visceral adipose tissue (E) and ERα in liver (F). Control group (C); Cigarette Smoking group (CS). Representative western blotting bands are shown. Student's unpaired *t*-tests were used and statistical difference was considered significant when $p < 0.05$; n = 5–7 rats/litter/group.

pathways, allowing an EC synthesis increase, as demonstrated by the higher DAGL expression in this nucleus. LH neurons in leptin deficient mice (*ob/ob*) have larger voltage-gated calcium channels, indicating upregulation of EC signaling, enhancing excitability, leading to hyperphagia [26].

5. Conclusion

In conclusion, early neonatal CS exposure induces changes in some biomarkers of the EC and DA systems, which could be related to a hyperleptinemic status and explain, at least in part, the hyperphagia of the females. Considering that women are more susceptible to eating disorders, smoking during critical stages of development could directly or indirectly influence the disturbances in appetite-regulating pathways. It is possible that nicotine, the main addictive component of cigarette, plays an important role in these long-term effects of tobacco smoke, which deserves further investigations.

Funding

The research was supported by the National Council for Scientific and Technological Development (CNPq), the Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (FAPERJ) and Coordination for the Enhancement of Higher Education

Personnel (CAPES).

Authors' contribution

EGM and PCL conceived and designed the study;

PNS, RAM, TCP, FAHC, DGS, ACM and EO performed the tobacco smoke treatment and collected/processed the biological samples;

PNS, RAM and ACM performed the statistical analyses:

PNS, RAM, EO, EGM and PCL interpreted the data:

PNS, RAM and PCL wrote the first draft of the manuscript:

PNS, RAM, ACM, EO, EGM and PCL revised subsequent drafts, consolidated the manuscript and contributed to its final version.

All authors have approved the final article.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors are grateful to Mr. Ulisses Risso, Mrs. Fabiana Gallaulckydio and Miss Monica Moura for technical assistance.

References

[1] H. Van Minh, K.B. Giang, N.B. Ngoc, P.T. Hai, D.T. Huyen, L.N. Khue, N.T. Lam, P.T. Nga, N.T. Quan, N.T. Xuyen, Prevalence of tobacco smoking in Vietnam: findings from the global adult tobacco survey 2015, Int. J. Public Health 62 (2017) 121–129.

[2] S.L. Buka, E.D. Shenassa, R. Niaura, Elevated risk of tobacco dependence among offspring of mothers who smoked during pregnancy: a 30-year prospective study, Am. J. Psychiatry 160 (11) (2003) 1978–1984.

[3] M. Rydell, C. Magnusson, S. Cnattingius, F. Granath, A.C. Svensson, M.R. Galanti, Exposure to maternal smoking during pregnancy as a risk factor for tobacco use in adult offspring, Am. J. Epidemiol. 179 (12) (2014) 1409–1417.

[4] C.A. Patten, R. Boyle, D. Tinkelman, T.A. Brockman, A.V. Lukowski, P.A. Decker, J. D'Silva, E. Lichtenstein, S.H. Zhu, Linking smokers to a quitline: randomized controlled effectiveness trial of a support person intervention that targets non-smokers, Health Educ. Res. 32 (2017) 318–331.

[5] D.J. Barker, The fetal and infant origins of adult disease, BMJ 301 (6761) (1990) 1111.

[6] P. Gonzalez-Muniesa, M.A. Mártinez-González, F.B. Hu, J.P. Després, Y. Matsuzawa, R.J.F. Loos, L.A. Moreno, G.A. Bray, J.A. Martinez, Obesity, Nat. Rev. Dis. Primers 3 (2017) 17034.

[7] A.P. Santos-Silva, E. Oliveira, C.R. Pinheiro, A.C. Santana, C.C. Nascimento-Saba, Y. Abreu-Villaça, E.G. Moura, P.C. Lisboa, Endocrine effects of tobacco smoke exposure during lactation in weaned and adult male offspring, J. Endocrinol. 218 (1) (2013) 13–24.

[8] P.C. Lisboa, P.N. Soares, T.C. Peixoto, J.C. Carvalho, C. Calvino, V.S.T. Rodrigues, D.N. Bernardino, V. Younes-Rapozo, A.C. Manhães, E. de Oliveira, E.G. de Moura, Effects of cigarette smoke exposure during suckling on food intake, fat mass, hormones, and biochemical profile of young and adult female rats, Endocrine 57 (1) (2017) 60–71.

[9] C.R. Pinheiro, E.G. Moura, A.C. Manhães, M.C. Fraga, S. Claudio-Neto, Y. Abreu-Villaça, E. Oliveira, P.C. Lisboa, Concurrent maternal and pup postnatal tobacco smoke exposure in Wistar rats changes food preference and dopaminergic reward system parameters in the adult male offspring, Neuroscience 301 (2015) 178–192.

[10] H. Schwarz, F.J. Blanco, M. Lotz, Anadamide, an endogenous cannabinoid receptor agonist inhibits lymphocyte proliferation and induces apoptosis, J. Neuroimmunol. 55 (1994) 107–115.

[11] R.A. Miranda, M.M. Almeida, C.P.D. Rocha, L.B. Fassarella, L.L. Souza, A.F.P. Souza, C.B.V. Andrade, R.S. Fortunato, C.C. Pazos-Moura, I.H. Trevenzoli, Maternal high-fat diet consumption induces sex-dependent alterations of the endocannabinoid system and redox homeostasis in liver of adult rat offspring, Sci. Rep. 8 (1) (2018) 14751.

[12] M.M. Almeida, C.P. Dias-Rocha, C.F. Reis-Gomes, H. Wang, Cordeiro A. Atella, C.C. Pazos-Moura, L. Joss-Moore, I.H. Trevenzoli, Maternal high-fat diet impairs leptin signaling and up-regulates type-1 cannabinoid receptor with sex-specific epigenetic changes in the hypothalamus of newborn rats, Psychoneuroendocrinology 103 (2019) 306–315.

[13] M.A. Ruby, D.K. Nomura, C.S.S. Hudak, A. Barber, J.E. Casida, R.M. Krauss, Acute overactive endocannabinoid signaling induces glucose intolerance, hepatic steatosis, and novel cannabinoid receptor 1 responsive genes, PLoS One 6 (11) (2011) e26415.

[14] C. D'Addario, M.V. Micioni Di Bonaventura, M. Pucci, A. Romano, S. Gaetani, R. Ciccocioppo, C. Cifani, M. Maccarrone, Endocannabinoid signaling and food addiction, Neurosci. Biobehav. Rev. 47 (2014) 203–224.

[15] F. Schreiner, M. Ackermann, M. Michalik, E. Hucklenbruch-Rother, A. Bilkei-Gorzo, I. Racz, L. Bindila, B. Lutz, J. Dötsch, A. Zimmer, J. Woelfle, Developmental programming of somatic growth, behavior and endocannabinoid metabolism by variation of early postnatal nutrition in a cross-fostering mouse model, PLoS One 12 (8) (2017) e0182754.

[16] O.S. Walker, A.C. Holloway, S. Raha, The role of the endocannabinoid system in female reproductive tissues, J. Ovarian. Res. 12 (1) (2019) 3.

[17] A.R. Alvheim, B.E. Torstensen, Y.H. Lin, H.H. Lillefosse, E.J. Lock, L. Madsen, J.R. Hibbeln, M.K. Malde, Dietary linoleic acid elevates endogenous 2-arachidonoylglycerol and anandamide in Atlantic salmon (Salmo salar L.) and mice, and induces weight gain and inflammation in mice, Br. J. Nutr. 109 (8) (2013) 1508–1517.

[18] A.R. Alvheim, B.E. Torstensen, Y.H. Lin, H.H. Lillefosse, E.J. Lock, L. Madsen, L. Froyland, J.R. Hibbeln, M.K. Malde, Dietary linoleic acid elevates the endocannabinoids 2-AG and anandamide and promotes weight gain in mice fed a low fat diet, Lipids 49 (1) (2014) 59–69.

[19] Y. Lee, J. Jo, H.Y. Chung, C. Pothoulakis, E. Im, Endocannabinoids in the gastrointestinal tract, Am. J. Physiol. Gastrointest. Liver Physiol. 311 (2016) G655–G666.

[20] L. Demizieux, F. Piscitelli, S. Troy-Fioramonti, F.A. Iannotti, S. Borrino, J. Gresti, T. Muller, J. Bellenger, C. Silvestri, V. Di Marzo, P. Degrace, Early low-fat diet enriched with linolenic acid reduces liver endocannabinoid tone and improves Late glycemic control after a high-fat diet challenge in mice, Diabetes 65 (7) (2016) 1824–1837.

[21] L.H. Torres, N.T. Balestrin, L.E.W. Spelta, S.O. Duro, M. Pistis, T. Marcourakis, Exposure to tobacco smoke during the early postnatal period modifies receptors and enzymes of the endocannabinoid system in the brainstem and striatum in mice, Toxicol. Lett. 302 (2019) 35–41.

[22] C.M. Williams, T.C. Kirkham, Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors, Psychopharmacology 143 (1999) 315–317.

[23] R. Roche, L. Hoareau, S. Bes-Houtmann, M.P. Gonthier, C. Laborde, J.F. Baron, Y. Haffaf, M. Cesari, F. Festy, Presence of the cannabinoid receptors, CB1 and CB2, in human omental and subcutaneous adipocytes, Histochem. Cell Biol. 126 (2006) 177–187.

[24] K.A. Alswat, The role of endocannabinoids system in fatty liver disease and therapeutic potentials, Saudi J. Gastroenterol. 19 (4) (2013) 144–151.

[25] M. Wang, N. Meng, Y. Chang, W. Tang, Endocannabinoids signaling: molecular mechanisms of liver regulation and diseases, Front. Biosci. 21 (2016) 1488–1501.

[26] Y.H. Jo, Y.J. Chen, S.C. Chua Jr., D.A. Talmage, L.W. Role, Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit, Neuron 48 (6) (2005) 1055–1066.

[27] A. Sánchez-Fuentes, B.A. Marichal-Cancino, M. Méndez-Díaz, A.L. Becerril-Meléndez, A.E. Ruiz-Contreras, O. Prospéro-Garcia, mGluR1/5 activation in the lateral hypothalamus increases food intake via the endocannabinoid system, Neurosci. Lett. 631 (2016) 104–108.

[28] Bermudez-Silva F.J., Cardinal P., D. 2012. The role of the endocannabinoid system in the neuroendocrine regulation of energy balance. J. Psychopharmacol.. 26(1) 114–124.

[29] W. Mazier, N. Saucisse, B. Gatta-Cherifi, D. Cota, The endocannabinoid system: pivotal orchestrator of obesity and metabolic disease, Trends Endocrinol. Metab. 26 (10) (2015) 524–537.

[30] V. Di Marzo, S.K. Goparaju, L. Wang, J. Liu, S. Bátkai, Z. Járai, F. Fezza, G.I. Miura, R.D. Palmiter, T. Sugiura, G. Kunos, Leptin-regulated endocannabinoids are involved in maintaining food intake, Nature 410 (6830) (2001) 822–825.

[31] L. Palomba, C. Silvestri, R. Imperatore, G. Morello, F. Piscitelli, A. Martella, L. Cristino, V. Di Marzo, Negative regulation of leptin-induced reactive oxygen species (ROS) formation by cannabinoid CB1 receptor activation in hypothalamic neurons, J. Biol. Chem. 290 (22) (2015) 13669–13677.

[32] M. Maccarrone, M. Guzmán, K. Mackie, P. Doherty, T. Harkany, Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies, Nat. Rev. Neurosci. 15 (2014) 786–801.

[33] L. Cristino, T. Becker, V. Di Marzo, Endocannabinoids and energy, Biofactors 40 (4) (2014) 389–397.

[34] R. Van Eenige, M. Van der Stelt, P.C.N. Rensen, S. Kooijman, Regulation of adipose tissue metabolism by the endocannabinoid system, Trends Endocrinol. Metab. 29 (5) (2018) 326–337.

[35] A. Parfieniuk, R. Flisiak, Role of cannabinoids in chronic liver diseases, World J. Gastroenterol. 14 (40) (2008) 6109–6114.

[36] P. Huang, Z. He, S. Ji, H. Sun, D. Xiang, C. Liu, Y. Hu, X. Wang, L. Hui, Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors, Nature 475 (7356) (2011) 386–389.

[37] E. Dai, L. Zhang, L. Ye, S. Wan, L. Feng, Q. Qi, F. Yao, Z. Li, Hepatic expression of cannabinoid receptors CB1 and CB2 correlate with fibrogenesis in patientswith chronic hepatitis B, Int. J. Infect. Dis. 59 (2017) 124–130.

[38] C.R. Pinheiro, E. Oliveira, A.C. Manhães, M.C. Fraga, S. Claudio-Neto, V. Younes-Rapozo, B.M. Lotufo, E.G. Moura, P.C. Lisboa, Exposure to nicotine increases dopamine receptor content in the mesocorticolimbic pathway of rat dams and offspring during lactation, Eur. J. Pharm. Sci. 57 (2015) 2–10.

[39] D. Quarta, I. Smolders, Rewarding, reinforcing and incentive salient events involve orexigenic hypothalamic neuropeptides regulating mesolimbic dopaminergic neurotransmission, Eur. J. Pharm. Sci. 57 (2014) 2–10.

[40] M.A. Bloomfield, A.H. Ashok, N.D. Volkow, O.D. Howes, The effects of Δ^9-tetrahydrocannabinol on the dopamine system, Nature 539 (7629) (2016) 369–377.

[41] J.D. Hommel, R. Trinko, R.M. Sears, D. Georgescu, Z.W. Liu, X.B. Gao, J.J. Thurmon, M. Marinelli, R.J. DiLeone, Leptin receptor signaling in midbrain dopamine neurons regulates feeding, Neuron 51 (6) (2006) 801–810.

[42] S.K. Billes, S.E. Simonds, M.A. Cowley, Leptin reduces food intake via a dopamine D2 receptor-dependent mechanism, Mol. Metab. 1 (1–2) (2012) 86–93.

[43] J.H. Baker, C.D. Runfola, Eating disorders in midlife women: a perimenopausal eating disorder? Maturitas 85 (2016) 112–116.

[44] C.L. Jones, J.L. Fowle, R. Ilyumzhinova, J. Berona, K. Mbayiwa, A.B. Goldschmidt, L.P. Bodell, S.D. Stepp, A.E. Hipwell, K.E. Keenan, The relationship between body mass index, body dissatisfaction, and eating pathology in sexual minority women, Int. J. Eat. Disord. (2019) 1–5.

[45] H. Frieling, H. Albrecht, S. Jedtberg, A. Gozner, B. Lenz, J. Wilhelm, T. Hillemacher, M. de Zwaan, J. Kornhuber, S. Bleich, Elevated cannabinoid 1 receptor mRNA is linked to eating disorder related behavior and attitudesin females with eating disorders, Psychoneuroendocrinology 34 (4) (2009) 620–624.

[46] P. Monteleone, M. Fabrazzo, V. Martiadis, C. Serritella, M. Pannuto, M. Maj, Circulating brain-derived neurotrophic factor is decreased in women with anorexia and bulimia nervosa but not in women with binge-eating disorder: relationships to co-morbid depression, psychopathology and hormonal variables, Psychol. Med. 35 (6) (2005) 897–905.

[47] D.J. Heal, M. Hallam, M. Prow, J. Gosden, S. Cheetham, Y.K. Choi, F. Tarazi, P. Hutson, Dopamine and μ-opioid receptor dysregulation in the brains of binge-eating female rats – possible relevance in the psychopathology and treatment of binge-eating disorder, J. Psychopharmacol. 31 (6) (2017) 770–783.

[48] J.L. Carlin, S.E. Mckee, t. Hill-Smith, N.M. Grissom, R. George, I. Lucki, T.M. Reyes, Removal of high-fat diet after chronic exposure drives binge behavior and dopaminergic dysregulation in female mice, Neuroscience 326 (2016) (2016) 170–179.

[49] G.M. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, San Diego, 1998.

[50] L. Bellocchio, P. Lafenêtre, A. Cannich, D. Cota, N. Puente, P. Grandes, F. Chaouloff, P.V. Piazza, G. Marsicano, Bimodal control of stimulated food intake by the endocannabinoid system, Nat. Neurosci. 13 (3) (2010) 281–283.

[51] L. Cristino, G. Busetto, R. Imperatore, I. Ferrandino, L. Palomba, S. Silvestri, S. Petrosino, P. Orlando, M. Bentivoglio, K. Mackie, V. Di Marzo, Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons, PNAS (2013) E2229–E2238.

[52] T.C. Kirkham, C.M. Williams, F. Fezza, M. Di, Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol, Br. J. Pharmacol. 136 (2002) 550–557.

[53] N. Jamshidi, D.A. Taylor, Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats, Br. J. Pharmacol. 134 (2001) 1151–1154.

[54] I. Matias, M.P. Gonthier, P. Orlando, V. Martiadis, L. De Petrocellis, C. Cervino, S. Petrosino, L. Hoareau, F. Festy, R. Pasquali, R. Roche, M. Maj, U. Pagotto, P. Monteleone, V. Di Marzo, Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia, J. Clin. Endocrinol. Metab. 91 (2006) 3171–3180.

[55] C. Pagano, C. Pilon, A. Calcagno, R. Urbanet, M. Rossato, G. Milan, K. Bianchi, R. Rizzuto, P. Bernante, G. Federspil, R. Vettor, The endogenous cannabinoid system stimulates glucose uptake in human fat cells via phosphatidylinositol 3-kinase and calcium-dependent mechanisms, J. Clin. Endocrinol. Metab. 92 (2007) 4810–4819.

[56] C. Silvestri, A. Ligresti, V. Di Marzo, Peripheral effects of the endocannabinoid system in energy homeostasis: adipose tissue, liver and skeletal muscle, Rev. Endocr. Metab. Disord. 12 (2011) 153–162.

[57] D. Cota, G. Marsicano, M. Tschöp, Y. Grübler, C. Flachskamm, M. Schubert, D. Auer, A. Yassouridis, C. Thöne-Reineke, S. Ortmann, F. Tomassoni, C. Cervino, E. Nisoli, A.C. Linthorst, R. Pasquali, B. Lutz, G.K. Stalla, U. Pagotto, The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis, J. Clin. Invest. 112 (3) (2003) 423–431.

[58] S. Engeli, J. Böhnke, M. Feldpausch, K. Gorzelniak, J. Janke, S. Bátkai, P. Pacher, J. Harvey-White, F.C. Luft, A.M. Sharma, J. Jordan, Activation of the peripheral endocannabinoid system in human obesity, Diabetes 54 (10) (2005) 2838–2843.

[59] K. Kempf, J. Hector, T. Strate, B. Schwarzloh, B. Rose, C. Herder, S. Martin, P. Algenstaedt, Immune-mediated activation of the endocannabinoid system in visceral adipose tissue in obesity, Horm. Metab. Res. 39 (8) (2007) 596–600.

[60] R.S. Ahima, Digging deeper into obesity, J. Clin. Invest. 121 (2011) 2076–2079.

[61] D. Osei-Hyiaman, M. DePetrillo, P. Pacher, J. Liu, S. Radaeva, S. Bátkai, J. Harvey-White, K. Mackie, L. Offertáler, L. Wang, G. Kunos, Endocannabinoid activation at hepatic CB1receptors stimulates fatty acid synthesis and contributes to dietinduced obesity, J. Clin. Invest. 115 (5) (2005) 1298–1305.

[62] M. Gary-Bobo, G. Elachouri, J.F. Gallas, P. Janiak, P. Marini, C. Ravinet-Trillou, M. Chabbert, N. Cruccioli, C. Pfersdorff, C. Roque, M. Arnone, T. Croci, P. Soubrié, F. Oury-Donat, J.P. Maffrand, B. Scatton, F. Lacheretz, G. Le Fur, J.M. Herbert, M. Bensaid, Rimonabant reduces obesity-associated hepatic steatosis and features of metabolic syndrome in obese Zucker fa/fa rats, Hepatology 46 (1) (2007) 122–129.

[63] T. Jourdan, L. Djaouti, L. Demizieux, J. Gresti, B. Verges, P. Degrace, CB1 antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice, Diabetes 59 (4) (2010) 926–934.

[64] J. Tam, V.K. Vemuri, J. Liu, S. Bátkai, B. Mukhopadhyay, G. Godlewski, D. Osei-Hyiaman, S. Ohnuma, S.V. Ambudkar, J. Pickel, A. Makriyannis, G. Kunos, Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity, J. Clin. Invest. 120 (8) (2010) 2953–2966.

[65] P.N. Soares, V.S.T. Rodrigues, T.C. Peixoto, C. Calvino, R.A. Miranda, B.P. Lopes, N. Peixoto-Silva, L.L. Costa, S. Claudio-Neto, A.C. Manhães, E. Oliveira, E.G. Moura, P.C. Lisboa, Cigarette smoke during breastfeeding in rats changes glucocorticoid and vitamin D status in obese adult offspring, Int. J. Mol. Sci. 19 (10) (2018) 3084.

[66] L.K. Brents, Marijuana, the endocannabinoid system and the female reproductive system, Yale J. Biol. Med. 27;89 (2) (2016) 175–191.

[67] L. Asarian, N. Geary, Modulation of appetite by gonadal steroid hormones, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 361 (1471) (2006) 1251–1263.

[68] L.A. Eckel, Estradiol: a rhythmic, inhibitory, indirect control of meal size, Physiol. Behav. 82 (1) (2004) 35–41.

[69] M. Varma, J.K. Chai, M.M. Meguid, A. Laviano, J.R. Gleason, Z.J. Yang, V. Blaha, Effect of estradiol and progesterone on daily rhythm in food intake and feeding patterns in Fischer rats, Physiol. Behav. 68 (1999) 99–107.

[70] G.N. Wade, Gonadal hormones and behavioral regulation of body weight, Physiol. Behav. 8 (1972) 523–534.

[71] G.N. Wade, Some effects of ovarian hormones on food intake and body weight in female rats, J. Comp. Physiol. Psychol. 88 (1) (1975) 183–193.

[72] C.J. Gruber, W. Tschugguel, C. Schneeberger, J.C. Huber, Production and actions of estrogens, N. Engl. J. Med. 346 (2002) 340–352.

[73] K. Shimomura, H. Shimizu, T. Tsuchiya, Y. Abe, Y. Uehara, M. Mori, Is leptin a key factor which develops obesity by ovariectomy? Endocr. J. 49 (4) (2002) 417–423.