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Leptin administration during lactation leads to different nutritional, biometric, hemodynamic, and cardiac outcomes in prepubertal and adult female Wistar rats

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Original Article

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Abstract

Literature reports that insults, such as hormonal disturbances, during critical periods of development may modulate organism physiology and metabolism favoring cardiovascular diseases (CVDs) later in life. Studies show that leptin administration during lactation leads to cardiovascular dysfunction in young and adult male Wistar rats. However, there are sex differences regarding CVD. Thus, the present work aimed to investigate neonatal leptin administration's consequences on different outcomes in female rats at prepubertal and adult age. Newborn Wistar female rats were divided into two groups, Leptin and Control, receiving daily subcutaneous injections of this adipokine (8 μg/100 g) or saline for the first 10 of 21 d of lactation. Nutritional, biometric, hemodynamic, and echocardiographic parameters, as well as maximal effort ergometer performance, were determined at postnatal days (PND) 30 and 150. Leptin group presented lower food intake ($p = 0.0003$) and higher feed efficiency ($p = 0.0058$) between PND 21 and 30. Differences concerning echocardiographic parameters revealed higher left ventricle internal diameter (LVID) in systole ($p = 0.0051$), as well as lower left ventricle ejection fraction (LVEF) ($p = 0.0111$) and fractional shortening (FS) ($p = 0.0405$) for this group at PND 30. Older rats treated with leptin during lactation presented only higher LVID in systole ($p = 0.0270$). Systolic blood pressure and maximum effort ergometer test performance was similar between groups at both ages. These data suggest that nutritional, biometric, and cardiac outcomes due to neonatal leptin administration in female rats are age-dependent.

Introduction

According to David Baker, insults, such as malnutrition or hormonal disturbances, during critical periods of development may modulate organism physiology and metabolism, favoring noncommunicable diseases later in life, including cardiovascular diseases (CVDs)[1]. Epigenetic mechanisms underlying cell plasticity and adaptative responses are discussed to guarantee survival upon adverse conditions[2–4]. As mammalian cardiomyocytes continue to differentiate and proliferate at the neonatal period, their hearts remain vulnerable after birth[5–7]. Thus lactation, as well as pregnancy, encompasses a critical period regarding cardiovascular development[4].

Literature reports higher levels of leptin in serum and breast milk of mothers with overweight and obesity. These leptin levels were also positively correlated to each other and maternal body mass index[8,9]. Breastfeeding allows this adipokine transference to the offspring, influencing body composition and health[9–11]. Leptin, a hormone secreted by adipocytes, participates in several physiological processes, including energy balance[12].

Leptin serum concentration in early life seems to be relevant to developmental plasticity thus, the increase in leptin serum lead hyperleptinemia associated with chronic diseases in adulthood in rodents[13,14]. Nevertheless, as important as the level itself is the exact moment in which leptin concentration changes in the suckling period[14]. Toste et al.[15] have demonstrated that leptin administration in newborn male Wistar rats during the first 10 of 21 d of lactation leads to several alterations: hyperleptinemia, hyperphagia, overweight, hyperinsulinemia, and hypertriglyceridemia in adulthood, explained by hypothalamic leptin resistance. Besides, Marques et al.[16,17] have also characterized diastolic dysfunction in this experimental model in youth that evolved to systolic dysfunction in adulthood along with changes in the spontaneous and sympathetic response of isolated heart preparations compatible to heart failure.

CVDs constitute the leading cause of death worldwide[18] and sex differences regarding these diseases have already been described by literature, which may affect clinical practice[19]. Although the cardioprotection addressed to estrogen in females during the reproductive period in animal models[20], there is a prospective study that reports a decrease of mortality rate due to CVD among men in the past years highlighting the relevance of their outcomes in women.[21]

There are studies that also point to sex dimorphism related to developmental plasticity[22,23]. Thus, this work aimed to describe the consequences of leptin administration during the first 10 d of lactation on biometric, nutritional, hemodynamic, and cardiac outcomes in female Wistar rats highlighting the hypothesis that leptin programming could also susceptible to sex dimorphism.

Methods

The Ethics Committee for the Use of Animals of Federal Fluminense University (Niterói, Brazil) had previously approved this research before its beginning. The study conduction was also accorded to the Brazilian Society of Animal Science Experimentation (Sociedade Brasileira de Ciência em Animais de Laboratório, SBCAL) guidelines[24]. The ARRIVE guidelines for reporting animal research have oriented all the steps of the work[25].

Animals and experimental model

All animals had free access to standard chow (Nuvilab®) and tap water at controlled conditions (22°C, 55–65% humidity, 12/12 h light/dark cycle). The breeding laboratory of the university has provided male ($n = 5$) and primiparous female ($n = 10$) Wistar rats about 3 months of age used for mating (F0 generation). They had no kinship and after 7 d of mating (two females for each male), the pregnant rats were placed in individual cages. Parameters observed to confirm pregnancy encompass increased abdominal circumference and behavioral changes[26].

A total of 10–12 puppies were born per dam after 21 d of gestation (postnatal day [PND] 0). Aiming to avoid genetic bias, litters adjustment (six pups per mother) was performed cross-fostering the offspring at PND day 1[27]. The offspring were divided into Leptin and Control groups by simple randomization (F1 generation)[28].

Control – six pups/mother (three males and three females)
Leptin – six pups/mother (three males and three females)

The litter adjustment comprised the proportion of one female for each male to ensure the dam's normal nursing behavior[29]. One dam died during lactation and its offspring were euthanized. Three female offspring have died throughout the experimental period. Male rats were evaluated on a different work. Thus, a total of 24 female rats from the F1 generation were included in this study:

Control – 9 female rats – 4 litters,
Leptin – 15 female rats – 5 litters.

Leptin group received daily subcutaneous injections of mouse leptin (PeproTech Inc., London, UK) diluted in saline within the first 10 of 21 d of lactation (8 µg/100 g of body mass). Control group have received vehicle (0.9% NaCl) throughout the same period instead[16]. Prepubertal and adult female offspring assessment happened at PND 30 and 150[30]. Body mass and food intake monitoring comprised all the experimental period (three animals/cage). The conduction of all the assays occurred as described by Marques et al.[16] and Araújo et al.[31].

Nutritional and biometric analysis

Body mass and food intake monitoring began upon weaning at PND 21. The determination of body weight gain occurred between PND 21–30 and 30–150 (Final body mass−Inicial body mass). The sum of food intake in the same periods allowed the calculation of feed efficiency (Weight gain/Σfood intake).

Nose-to-anus length was collected from anesthetized rats before echocardiography using a tape measure.

Systolic blood pressure recording

The rats were previously acclimated to restraint and tail-cuff inflation throughout 3 d for 10 min in the morning. The determination of systolic blood pressure occurred on the fourth day using awake rats and a noninvasively computerized tail-cuff system (NIBP controller, ML125; ADInstruments) connected to the ADInstruments PowerLab 8/30, ML870 digital–analog converter. Data were analyzed using LabChart 6 Pro software (ADInstruments, Bella Vista, New South Wales, Australia). The final values of systolic blood pressure used were the average of six successful recordings of each animal achieved in the absence of spontaneous tail movement.

Echocardiographic evaluation

The animals were previously anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg) intraperitoneally, and then submitted to the noninvasive transthoracic echocardiography using a portable ultrasound system (Acuson Cypress Plus, Siemens, DEU, Mountain View, CA, USA) and a 10-MHz transducer. Parameters assessed to allow cardiac structure and function evaluation: left ventricle internal diameter (LVID), interventricular septum thickness (IVS), and left ventricle posterior wall thickness (LVPW) in systole and diastole, as well as relative wall thickness (RWT), left ventricle mass (LVM), left atrium-to-aorta ratio (LA/Ao), systolic volume (SV), left ventricle ejection fraction (LVEF), fractional shortening (FS), and mitral deceleration time (DT). All parameters were measured at least three times per animal by a unique researcher. The assay conduction also followed the American Society of Echocardiography[32].

Maximal effort ergometer test

The experiment occurred after 3 d of acclimation (daily exercise sessions of 10 min at 0.7–0.9 km/h). Some animals were nonresponsive and has been categorized as sedentary rats. Because of them, it was not possible to evaluate all animals submitted to previous assays.

The test comprised an adapted treadmill for rats (Imbrasport®, Brasília) and the protocol does not include inclination. The initial speed of 0.9 km/h was followed by progressive increments of 0.3 km/h every 3 min until exhaustion (rats remaining still for at least 10 s despite stimuli). The parameters recorded were distance traveled, time spent, and maximum speed developed in the test.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 7.0 software (USA). Shapiro–Wilk test allowed the evaluation of data normality and homoscedasticity and guided the test used to compare Control and Leptin groups within the same age (unpaired Student's t-test – parametric data – or Mann–Whitney test – nonparametric data). Values are expressed as mean ± standard deviation. Significance accepted comprised a $p < 0.05$.

Results

Nutritional parameters

Data points differences regarding nutritional parameters only between PND 21 and 30. Table 1 shows that female rats from

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Table 1. Nutritional parameters

	Postnatal days 21–30			Postnatal days 30–150		
Groups						
Parameters	Control $n = 9$	Leptin $n = 15$	p	Control $n = 9$	Leptin $n = 15$	p
Body weight gain (g)	39.44 ± 4.81	42.48 ± 4.69	*0.1415*	162.20 ± 20.21	166.30 ± 12.66	*0.5422*
∑Food intake (g)	158.40 ± 11.60	136.40 ± 12.86*	*0.0003*	2002 ± 96.84	2144. ± 167.10	*0.0607*
Feed efficiency	0.25 ± 0.03	0.32 ± 0.06*	*0.0058*	0.08 ± 0.01	0.08 ± 0.01	*0.5020*

Values are expressed as mean ± standard deviation. Data were analyzed using unpaired *t*-test or Mann–Whitney test.
*$P < 0.05$ Leptin versus Control group.

Table 2. Biometric parameters

	Postnatal day 30			Postnatal day 150		
Groups						
Parameters	Control $n = 9$	Leptin $n = 15$	p	Control $n = 9$	Leptin $n = 15$	p
Body mass (g)	88.31 ± 6.60	90.42 ± 7.11	*0.1191*	250.50 ± 16.90	258.20 ± 10.90	*0.1861*
Nose-to-anus length (cm)	16.77 ± 0.58	17.18 ± 1.08	*0.3022*	21.88 ± 0.89	21.60 ± 0.53	*0.3455*

Values are expressed as mean ± standard deviation. Data were analyzed using unpaired *t*-test or Mann–Whitney test. Significance was accepted if $P < 0.05$ (Leptin versus respective Control group). No statistical difference were observed between groups at postnatal day 30 nor 150.

Table 3. Echocardiographic and hemodynamic parameters

	Postnatal day 30			Postnatal day 150		
Groups						
Parameters	Control $n = 9$	Leptin $n = 15$	p	Control $n = 9$	Leptin $n = 15$	p
IVSs (cm)	0.22 ± 0.03	0.24 ± 0.04	*0.1882*	0.26 ± 0.05	0.27 ± 0.03	*0.9880*
IVSd (cm)	0.12 ± 0.01	0.12 ± 0.01	*0.6748.*	0.14 ± 0.03	0.14 ± 0.02	*0.8502*
LVPWs (cm)	0.22 ± 0.03	0.24 ± 0.03	*0.3415*	0.26 ± 0.05	0.27 ± 0.03	*0.6650*
LVPWd (cm)	0.12 ± 0.01	0.12 ± 0.01	*0.7622*	0.15 ± 0.03	0.15 ± 0.01	*>0.9999*
LVIDs (cm)	0.14 ± 0.03	0.21 ± 0.06*	*0.0051*	0.21 ± 0.04	0.28 ± 0.09*	*0.0270*
LVIDd (cm)	0.43 ± 0.07	0.49 ± 0.08	*0.0509*	0.50 ± 0.13	0.60 ± 0.10	*0.0916*
RWT (cm)	0.58 ± 0.09	0.52 ± 0.14	*0.2682*	0.62 ± 0.25	0.51 ± 0.11	*0.3699*
LMV (g)	0.77 ± 0.05	0.83 ± 0.07	*0.0935*	0.93 ± 0.14	1.00 ± 0.12	*0.1588*
LA/Ao (cm)	1.02 ± 0.11	1.04 ± 0.07	*0.5832*	1.00 ± 0.10	1.03 ± 0.11	*0.5297*
SV (ml)	0.11 ± 0.02	0.12 ± 0.03	*0.8163*	0.19 ± 0.07	0.20 ± 0.06	*0.6097*
LVEF (%)	94.74 ± 2.03	90.58 ± 4.19*	*0.0111*	90.55 ± 3.50	86.62 ± 6.39	*0.0856*
FS (%)	63.96 ± 5.68	58.07 ± 6.81*	*0.0405*	57.30 ± 5.60	52.40 ± 9.67	*0.1875*
Mitral DT (ms)	66.00 ± 8.72	63.20 ± 7.36	*0.4672*	70.30 ± 10.22	66.31 ± 6.00	*0.2387*
Systolic blood pressure (mmHg)	99.03 ± 8.31	103.70 ± 5.93	*0.1221*	119.00 ± 12.98	127.40 ± 9.12	*0.1289*

d, diastole; FS; fractional shortening; IVS, interventricular septum thickness; LVPW, left ventricle posterior wall thickness; LVID, left ventricle internal diameter; RWT, relative wall thickness; LVM, left ventricle mass; LA/Ao, left atrium-to-aorta ratio; SV, systolic volume; LVEF, left ventricle ejection fraction; Mitral DT, mitral deceleration time; s, systole.
Values are expressed as mean ± standard deviation. Data were analyzed using unpaired *t*-test or Mann–Whitney test.
*$P < 0.05$ Leptin versus Control group.

the Leptin group presented lower food intake (−13.8%, $p = 0.0003$) and higher feed efficiency (+28%, $p = 0.0058$).

Biometric parameters

Body weight and nose-to-anus length were similar between prepubertal and adulthood females from Leptin and Control groups (Table 2).

Echocardiographic and Hemodynamic analysis

Table 3 highlights some differences between Leptin and Control groups regarding echocardiographic but not hemodynamic data. Prepubertal female rats submitted to leptin administration presented high values of LVIDs (+50%, $p = 0.0051$) and remained higher in these animals (+33.3%, $p = 0.0270$) in adulthood. In addition, prepubertal animals from the Leptin group presented lowers values of LVEF (−4.4%, $p = 0.0111$) and FS (−9.2%, $p = 0.0405$).

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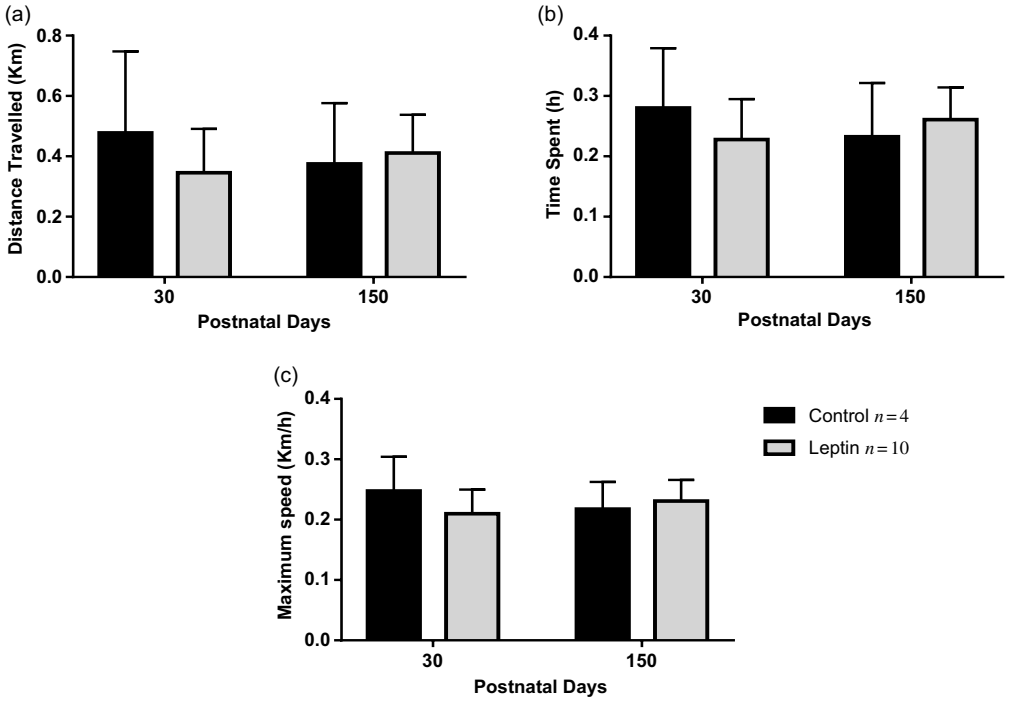

Fig. 1. Data from maximal effort ergometer test (Control $n = 4$, Leptin $n = 10$). (a) Distance traveled, (b) time spent, and (c) maximum speed developed. Values are expressed as mean ± standard deviation. Data were analyzed using unpaired *t*-test or Mann–Whitney test.
*$P < 0.05$ Leptin versus Control group.

Maximal effort ergometer test

Within the same age, Leptin and Control groups presented a similar performance on the maximal effort ergometer test in all parameters analysed (Fig. 1a–c).

Discussion

In this work, leptin administration led to hypophagia and higher feed efficiency in prepubertal animals. Echocardiographic data showed a slightly reduced LVEF and FS in youth. However, these data do not reflect functional impairment. Independently of the age, the LVIDs was higher in the Leptin group. No differences were observed regarding systolic blood pressure and performance on the maximal effort ergometer test. It is essential to highlight that these outcomes are different from those reported for male rats submitted to the same experimental protocol[16].

The nutritional profile suggests that neonatal leptin administration altered the metabolism of prepubertal female rats but not adult ones[33]. The early decrease in food consumption seems to be accompanied by a reduction in energy expenditure. Toste et al.[15] reported higher serum levels of insulin due to neonatal leptin administration. The literature describes a role for leptin and insulin on energy balance and food intake, highlighting the interaction between these hormones' signaling pathways in the hypothalamus[12,34–36]. Nevertheless, unlike male rats[15], data does not indicate the development of leptin's central resistance in adult female rats in this experimental model. According to the literature, male rats seem to be more susceptible to obesity than females. This observation may be explained by estrogen's role in energy homeostasis and neuropeptides secretion, increasing anorexigenic neuropeptides, and decreasing orexigenic neuropeptides levels[37–41].

According to the literature, leptin has a stimulating effect over the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Besides, this adipokine can enhance the secretion of luteinizing (LH) and follicle-stimulating (FSH) hormones by the anterior pituitary, acting directly on estrogens synthesis[42]. However, Pietrobon et al.[23] have observed that the relationship between leptin and estrogen levels depends on the experimental model of metabolic programming. While non-pharmacological early weaning promoted hyperphagia and hyperleptinemia without differences regarding estradiol levels in adult females, pharmacological early weaning determined normophagia, normoleptinemia, and reduced plasma estradiol concentrations.

In agreement to literature, body mass and nose-to-anus length can be used as biometric parameters to discuss adiposity in male and female rats[43–45]. No cutoff points were established for female rats. However, we have observed body mass and nose-to-anus length values similar to Araújo et al.[31] Thus, according to this reference, prepubertal and adult female rats submitted to neonatal leptin administration did not present an increase in cardiometabolic risk related to an adiposity increment. These data are different from those observed by Toste et al.[15] who studied male rats submitted to neonatal leptin administration.

Besides similar biometric parameters, no difference regarding systolic blood pressure was seen within female Leptin and Control groups independently of the age. These findings may be due to leptin levels/activity. Previously, hyperleptinemia has been related to higher values of systolic blood pressure and heart rate in adult male rats of the same experimental model[46]. Trevenzoli et al.[13] suggested that hyperleptinemia increases adrenal medullary function through sympathetic nervous system activation. The high leptin levels on lactation program the sympathoadrenal system's activity in adulthood and it may contribute to the development of adult chronic diseases such as hypertension[13]. Leptin signaling seems to be necessary for the increased systolic blood pressure induced by obesity[47]. However, a recent study has dissociated hypertension development in obese individuals and leptin presence[48].

Echocardiographic data shows an increased LVIDs in female rats of the Leptin group suggesting decreased ventricular compliance. This abnormality may be a sign of dilated cardiomyopathy[49]. This structural change was not accompanied by functional injury

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as the differences seen between prepubertal groups regarding LVEF and FS were insufficient to address systolic dysfunction. The values recorded to Control and Leptin groups are similar to those attributed to normal function by literature[50,51]. In contrast, Marques et al.[16] have shown that leptin administration during lactation programmed cardiac structural and functional changes both in young and adult male rats. These observations may also explain the differences noticed between sex regarding the maximum effort ergometer test. While young and adult male rats submitted to neonatal leptin administration have traveled a shorter distance during a shorter test, developing a lower maximal velocity[16], no differences were noticed between females concerning these parameters. An important symptom of diastolic dysfunction is exercise intolerance, which can be assessed by cardiopulmonary exercise tests[52]. Maximal effort ergometer tests have already been successfully applied previously to assess cardiorespiratory capacity in rats[16,31]. Literature provides a linear relationship between maximum speed and oxygen consumption[53].

Estrogen has been recognized as a cardioprotective hormone due to its direct and indirect actions on myocardial cells and blood vessels. In animal models of CVD, adult females exhibited lower mortality and vascular injury, cardiac function preservation, and slower progression to decompensated heart failure than males. Estrogen deprivation mitigated this cardioprotection[54–57]. On the other hand, estrogen reposition was able to increase cardiomyocytes' survival in a murine model of infarction, prevent hypertrophy in cardiomyocytes' culture, and improve cardiac function in the isolated hearts of gonadectomized rats[58,59]. According to literature, the activation of membrane-bound receptor G protein-coupled estrogen receptor (GPER) and estrogen receptor beta (ERβ) modulates Ca^{2+} homeostasis. The increased expression of sarco/endoplasmic reticulum Ca^{2+}−ATPase and phospholamban improves cardiomyocytes contractility[60,61]. In addition, GPER activation by specific agonists may reduce infarct size after myocardial ischemia-reperfusion, preserving cardiac function through phosphatidylinositol 3-kinase-dependent signaling pathways[62].

All data together suggest sex dimorphism concerning different outcomes related to neonatal leptin administration. The measurement of leptin and estrogen levels should contribute to a better understanding of the mechanisms underlying these findings. Although the lack of these data constitutes limitations, it does not compromise the relevance of them. Besides, cardiac outcomes were similar comparing female Leptin and Control groups independent of the age. Literature also reports that sex differences may be related to sex chromosomes, products of genes located on the X and Y chromosomes, not only to gonadal hormones[63–66].

In conclusion, this study suggests that female Wistar rats are less susceptible to cardiac programming due to neonatal leptin administration than male rats, contributing to a neglected research area named Gender Medicine. Further studies are welcome to investigate better sex differences and the underlying cardiac structure and function preservation mechanism in female rats.

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Conflicts of interest. None.

Ethical standards. The authors declare that all proceedings adopted in this study were under the approval of the Ethics Committee of Fluminense Federal University (protocol number CEUA/UFF812-16).

References

1. Barker DJ. The origins of the developmental origins theory. *J Intern Med*. 2007; 261(5), 412–417.
2. Reynolds CM, Gray C, Li M, Segovia SA, Vickers MH. Early life nutrition and energy balance disorders in offspring in later life. *Nutrients*. 2015; 7(9), 8090–8111.
3. Wells JC. The thrifty phenotype: An adaptation in growth or metabolism? *Am J Hum Biol*. 2011; 23(1), 65–75.
4. Langley-Evans SC. Nutrition in early life and the programming of adult disease: a review. *J Hum Nutr Diet*. 2015; 28(1), 1–14.
5. Crispi F, Crovetto F, Gratacos E. Intrauterine growth restriction and later cardiovascular function. *Early Hum Dev*. 2018; 126, 23–27.
6. Zebrowski DC, Jensen CH, Becker R, *et al.* Cardiac injury of the newborn mammalian heart accelerates cardiomyocyte terminal differentiation. *Sci Rep*. 2017; 7(1), 8362.
7. Senyo SE, Lee RT, Kühn B. Cardiac regeneration based on mechanisms of cardiomyocyte proliferation and differentiation. *Stem Cell Res*. 2014; 13(3 Pt B), 532–541.
8. Schuster S, Hechler C, Gebauer C, Kiess W, Kratzsch J. Leptin in maternal serum and breast milk: association with infants' body weight gain in a longitudinal study over 6 months of lactation. *Pediatr Res*. 2011; 70(6), 633–637.
9. Fields DA, George B, Williams M, *et al.* Associations between human breast milk hormones and adipocytokines and infant growth and body composition in the first 6 months of life. *Pediatr Obes*. 2017; 12 Suppl 1, 78–85.
10. Casabiell X, Pinheiro V, Tome MA, *et al.* Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. *J Clin Endocr Metab*. 1997; 82, 4270–4273.
11. Teixeira CV, Passos MCF, Ramos CF, Dutra SCP, Moura EG. Leptin serum concentration in rats whose mothers were submitted to malnutrition during lactation. *J Nutr Biochem*. 2002; 13, 493–498.
12. Elias CF, Purohit D. Leptin signaling and circuits in puberty and fertility. *Cell Mol Life Sci*. 2013;70(5), 841–862.
13. Trevenzoli IH, Pinheiro CR, Conceição EP, *et al.* Programming of rat adrenal medulla by neonatal hyperleptinemia: adrenal morphology, catecholamine secretion, and leptin signaling pathway. *Am J Physiol Endocrinol Metab*. 2010; 298(5), E941–E949.
14. Granado M, Fuente-Martín E, García-Cáceres C, Argente J, Chowen JA. Leptin in early life: a key factor for the development of the adult metabolic profile. *Obes Facts*. 2012; 5(1), 138–150.
15. Toste FP, de Moura EG, Lisboa PC, *et al.* Neonatal leptin treatment programmes leptin hypothalamic resistance and intermediary metabolic parameters in adult rats. *Br J Nutr*. 2006; 95, 830–837.
16. Marques EB, Rocha NN, Dos santos MC, Nascimento JH, Scaramello CBV. Cardiac programming in rats submitted to leptin treatment during lactation. *Int J Cardiol*. 2015; 181C, 141–143.
17. Marques EB, Pinto LMO, Nascimento JH, Scaramello CBV. Spontaneous and Isoprenaline-Evoked response of isolated heart preparations from rats submitted to leptin treatment during lactation. *Int J Cardiol*. 2015; 195, 48–50.
18. World Health Organization cardiovascular disease risk charts: revised models to estimate risk in 21 global regions. *Lancet Glob Health*. 2019.
19. Ventura-Clapier R, Dworatzek E, Seeland U, *et al.* Sex in basic research: concepts in the cardiovascular field. *Cardiovasc Res*. 2017; 113, 711–724.
20. Posa A, Szabó R, Kupai K, *et al.* Cardioprotective effect of selective estrogen receptor modulator raloxifene are mediated by heme oxygenase in estrogen-deficient rat. *Oxid Med Cell Longev*. 2017; 2017, 2176749.
21. Mehta LS, Beckie TM, DeVon HA, *et al.* Acute myocardial infarction in women a scientific statement from the american heart association. *Circulation*. 2016; 133, 916–947.
22. Souza LL, de Moura EG, Lisboa PC. Does early weaning shape future endocrine and metabolic disorders? Lessons from animal models. *J Dev Orig Health Dis*. 2020; 3, 1–11.

https://doi.org/10.1017/S2040174420001312 Published online by Cambridge University Press

23. Pietrobon CB, Bertasso IM, Silva BS, *et al.* Body adiposity and endocrine profile of female wistar rats of distinct ages that were early weaned. *Horm Metab Res*. 2020; 52(1), 58–66.
24. Journal of the Brazilian Society of Laboratory Animal Science. *Brazilian Society of Laboratory Animal Science (SBCAL)*. 2018; 6, 1.
25. Percie du Sert N, Hurst V, Ahluwalia A, *et al.* The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol*. 2020; 18(7), e3000410.
26. Numan M. Maternal behavior. In *The physiology of reproduction* (eds. Knobil E, Neill J), 1988; 1569–1645. Raven Press, Ltd, New York.
27. Li N, Guenancia C, Rigal E, *et al.* Short-Term moderate diet restriction in adulthood can reverse oxidative, cardiovascular and metabolic alterations induced by postnatal overfeeding in mice. *Sci Rep*. 2016; 28(6), 308–317.
28. Bailoo JD, Reichlin TS, Würbel H. Refinement of experimental design and conduct in laboratory animal research. *ILAR Journal*. 2014; 55(3), 383–391.
29. Heijning BJM, Oosting A, Kegler D, van der Beek EM. An increased dietary supply of medium-chain fatty acids during early weaning in rodents prevents excessive fat accumulation in adulthood. *Nutrients*. 2017; 9, 631.
30. Quinn, R. Comparing rat's to human's age: how old is my rat in people years? *Nutrition*. 2005; 21(6), 775–777.
31. Araújo GA, Farias RS, Pedro SS, *et al.* Overweight during lactation and its implications for biometric, nutritional and cardiovascular parameters of young and adult male and female rats. *J Nutr Sci*. 2020; 9(27), 1–9.
32. Lang RM, Bierig M, Devereux RB, *et al.* Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005; 18(12), 1440–1463.
33. Wideman CH, Murphy HM. Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats. *Nutr Neurosci*. 2009; 12(5), 233–240.
34. Hermsdorff HHM, Vieira MAQM, Monteiro JBR. Leptin and its influence in the pathophysiology of eating disorders. *Rev Nutr*. 2006; 19(3), 369379.
35. Mendonça LS, Moreira JAR. The influence of hormones leptin and insulin in localized fat. *Revista Científica da FHO|UNIARARAS*. 2015, 3(2), 47–56.
36. Maior AS. Hormonal regulation of food intake: a brief review. *Revista de Medicina, Ribeirão Preto*. 2012; 45(3), 303–309.
37. Litwak SA, Wilson JL, Chen W, *et al.* Estradiol prevents fat accumulation and overcomes leptin resistance in female high-fat diet mice. *Endocrinology*. 2014, 155(11), 4447–4460.
38. Acharya KD, Gao X, Bless EP, Chen J, Tetel MJ. Estradiol and high fat diet associate with changes in gut microbiota in female ob/ob mice. *Scientific RepoRtS*. 2019; 9, 20192.
39. Hong J, Stubbins RE, Smith RR, Harvey AE, Núñez NP. Differential susceptibility to obesity between male, female and ovariectomized female mice. *Nutr J*. 2009; 8, 11.
40. Clegg DJ. Minireview: the year in review of estrogen regulation of metabolism. *Mol Endocrinol*. 2012; 26(12), 1957–1960.
41. Sharma G, Prossnitz ER. G-Protein-Coupled Estrogen Receptor (GPER) and sex-specific metabolic homeostasis. *Adv Exp Med Biol*. 2017; 1043, 427–453.
42. Matysková R, Zelezná B, Maixnerová J, *et al.* Estradiol supplementation helps overcome central leptin resistance of ovariectomized mice on a high fat diet. *Horm Metab Res*. 2010; 42(3), 182–186.
43. Leopoldo AS, Lima-Leopoldo AP, Nascimento AF, *et al.* Classification of different degrees of adiposity in sedentary rats. *Braz J of Med Biol Res*. 2016; 49(4), e5028.
44. Kleinert M, Clemmensen C, Hofmann SM, *et al.* Animal models of obesity and diabetes mellitus. *Nat Rev Endocrinol*. 2018; 4, 140–162.
45. Fouda YB, Tom ENL, Atsamo AD, Bonabe C, Dimo T. Effects of stem bark aqueous extract of Fagara tessmannii Engl (Rutaceae) on cardiovascular risks related to monosodium glutamate-induced obesity in rat: In vivo and in vitro assessments. *J of Ethnophar*. 2020; 260(5), 112972.
46. Trevenzoli IH, Valle MM, Machado FB, *et al.* Neonatal hyperleptinaemia programmes adrenal medullary function in adult rats: effects on cardiovascular parameters. *J Physiol*. 2007; 580(2), 629–637.
47. Simonds SE, Pryor JT, Ravussin E, *et al.* Leptin mediates the increase in blood pressure associated with obesity. *Cell*. 2014; 159(6), 1404–1416.
48. von Schnurbein J, Manzoor J, Brandt S, *et al.* Leptin is not essential for obesity-associated hypertension. *Obes Facts*. 2019; 12(4), 460–475.
49. Lakdawala NK, Winterfield JR, Funke BH. Dilated cardiomyopathy. *Circ Arrhythm Electrophysiol*. 2013; 6(1), 228–237.
50. Souza NS, Dos-Santos RC, Silveira ALB, *et al.* Effects of autonomic balance and fluid and electrolyte changes on cardiac function in infarcted rats: a serial study of sexual dimorphism. *Clin Exp Pharmacol Physiol*. 2016; 43(4), 476–483.
51. Yu Y, Shun-Guang W, Weiss RM, Felder RB. Sex differences in the central and peripheral manifestations of ischemia-induced heart failure in rats. *Am J Physiol Heart Circ Physiol*. 2018; 316(1), H70–H79.
52. Kitzman DW, Groban L. Exercise intolerance. *Heart Fail Clin*. 2008; 4, 99–115.
53. Rodrigues B, Figueroa DM, Mostarda CT, *et al.* Maximal exercise test is a useful method for physical capacity and oxygen consumption determination in streptozotocin-diabetic rats. *Cardiovasc Diabetol*. 2007; 13, 6–38.
54. Wang F, Keimig T, He Q, *et al.* Augmented healing process in female mice with acute myocardial infarction. *Gend Med*. 2007; 4, 230–247.
55. Javeshghani D, Schiffrin EL, Sairam MR, Touyz RM. Potentiation of vascular oxidative stress and nitric oxide-mediated endothelial dysfunction by high-fat diet in a mouse model of estrogen deficiency and hyperandrogenemia. *J Am Soc Hypertens*. 2009; 3, 295–305.
56. Dent MR, Tappia PS, Dhalla NS. Gender differences in apoptotic signaling in heart failure due to volume overload. *Apoptosis*. 2010; 15, 499–510.
57. Lagranha CJ, Deschamps A, Aponte A, Steenbergen C, Murphy E. Sex differences in the phosphorylation of mitochondrial proteins result in reduced production of reactive oxygen species and cardioprotection in females. *Circ Res*. 2010; 106, 1681–1691.
58. Patrizio M, Marano G. Gender differences in cardiac hypertrophic remodeling. *Ann Ist Super Sanità*. 2016, 52(2), 223–229.
59. Baka T, Hodosy J, Krajcirovicova K, *et al.* 17β-Estradiol treatment reversed left ventricular dysfunction in castrated male rats: an echocardiographic study. *Can J Physiol Pharmacol*. 2018, 96(8), 850–854.
60. Schuster I, Mahmoodzadeh S, Dworatzek E, *et al.* Cardiomyocyte-Specific overexpression of oestrogen receptor beta improves survival and cardiac function after myocardial infarction in female and male mice. *Clin Sci*. 2016, 130, 365–376.
61. Alencar AK, da Silva JS, Lin M, *et al.* Effect of age, estrogen status, and late-life GPER activation on cardiac structure and function in the fischer344xbrown norway female rat. *J Gerontol A Biol Sci Med Sci*. 2017, 72, 152–162.
62. Deschamps AM, Murphy E. Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. *Am J Physiol Heart Circ Physiol*. 2009; 297: H1806–H1813.
63. Wang J, Bingaman S, Huxley VH. Intrinsic sex-specific differences in microvascular endothelial cell phosphodiesterases. *Am J Physiol Heart Circ Physiol*. 2010; 298(4), H1146–H1154.
64. Boutin-Ganache I, Picard S, Deschepper CF. Distinct gene-sex interactions regulate adult rat cardiomyocyte width and length independently. *Physiol Genomics*. 2002; 12, 61–67.
65. Decano JL, Pasion KA, Black N, *et al.* Sex-Specific genetic determinants for arterial stiffness in Dahl salt-sensitive hypertensive rats. *BMC Genet*. 2016; 17, 19.
66. Ngun T, Ghahramani N, Sánchez FJ, Bocklandt S, Vilain E. The genetics of sex differences in brain and behavior. *Front Neuroendocrinol*. 2011; 32(2), 227–246.

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