RESEARCH ARTICLE

Overweight during lactation and its implications for biometric, nutritional and cardiovascular parameters of young and adult male and female rats

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Abstract

Litter size reduction can induce early overnourishment, being an attractive experimental model to study short- and long-term consequences of childhood obesity. Epidemiological data indicate sex differences regarding cardiometabolic disorders and hypertrophic cardiomyopathy. The present study aimed to describe biometric, nutritional and cardiovascular changes related to neonatal overweight promoted by litter size reduction in young and adult Wistar rats of both sexes. Litter adjustment to eight or four pups/mother (1:1 male-to-female ratio) gave, respectively, control and overweight groups. Body mass, food intake, haemodynamic and echocardiographic parameters and cardiorespiratory capacity were evaluated at postnatal days 30 and 150. Diminished litters were correlated with higher body mass and weight gain (12%) during lactation, validating the experimental model of neonatal overweight. Soon after weaning male (16%) and female (25%) offspring of these litters presented a lower food intake than their respective control, without differences in body mass. Adult males from reduced litters presented higher abdominal circumference (7%), systolic blood pressure (10%), interventricular septum thickness (15%) and relative wall thickness (15%) compared with their respective control. Rats’ performance on the maximal effort ergometer test was not affected by neonatal overweight. Data suggest the occurrence of catch-down growth and hypophagia in male and female rats submitted to neonatal overweight. However, only male rats presented haemodynamic and cardiac structural changes. These findings are crucial to personalised/gender medicine.

Key words: Lactation: Child development: Overweight: Cardiovascular system: Sex characteristics

Obesity/overweight is a major global health problem that leads to increased mortality. This condition in early life may be related to postnatal nutrition and can evoke metabolic disorders and several co-morbidities, increasing cardiovascular risk and favouring CVD in adulthood. Estimates of deaths related to CVD increased about 14% between 2006 and 2016[1–4].

Studies investigating the relationship between events in early life, as nutritional insults, and functional status in the future belong to a new research field named ‘developmental origin of health and disease’ (DOHaD). The history of DOHaD as a research field reached a milestone with David Barker’s theory encompassing the programming of diseases with fetal origins[5]. The understanding that the environment and individual lifestyle directly interact with the genome to influence epigenetic changes is growing fast[6]. These changes alter homeostasis through the remodelling of organs and tissues[7]. As the heart is not entirely developed soon after birth, nutritional insults in early life may contribute to the occurrence of cardiac diseases in adulthood also through direct effects[8].

Animal models comprise an interesting strategy to evaluate future outcomes related to nutritional insults in early life and

Abbreviations: AC, abdominal circumference; IVS, interventricular septum thickness; IVSd, interventricular septum thickness diastole; IVSs, interventricular septum thickness systole; LVID, left ventricle internal diameter; LVIDd, left ventricle internal diameter diastole; LVPW, left ventricle posterior wall thickness; LVPWd, left ventricle posterior wall thickness diastole; LVPWs, left ventricle posterior wall thickness systole; NAL, nose-to-anus length; TC, thoracic circumference.

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developmental plasticity. Studies with male animals (mice and rats) report that overnourishment during lactation induces metabolic and haemodynamic heart impairment during adulthood. In general, experimental models of neonatal overfeeding encompass litter size reduction that allows milk supply increase to the offspring. This experimental model is cheap and effective to investigate short- and long-term consequences of neonatal overweight\textsuperscript{10–12}. However, such evidence is not available for female animals.

Despite the accumulating evidence that sex leads to differences in biology, for several reasons, the variable sex has been largely ignored in biomedical research\textsuperscript{13}. In humans, there are sex differences regarding CVD. The literature points out sex differences in cardiometabolic disorders and differences between men and women with hypertrophic cardiomyopathy\textsuperscript{14,15}. Individualised medicine must consider sex and gender to initiate personalising care, allowing the improvement of the outcomes. For this, evidence supporting sex-specific decisions also needs to be provided by basic scientists\textsuperscript{16}. Thus, the present study aimed to evaluate biometric, nutritional and cardiovascular outcomes related to neonatal overweight/overnourishment in young and adult Wistar rats of both sexes.

Materials and methods

Animals and experimental model

The Ethics Committee of Fluminense Federal University (Niteroi, Brazil) approved the use of animals (Comissão De Ética No Uso De Animais (CEUA) UFF812/2016) following the Guide for the Care and Use of Laboratory Animals (National Institutes of Health (NIH) publication no. 8023, revised 1978). All rats received standard chow (Nuvilab\textsuperscript{86}) and water \textit{ad libitum} at controlled conditions (22°C, 55–65% humidity, 12 h light–12 h dark cycle). The breeding laboratory of the University provided Wistar rats used for mating (F0 generation). Male (\(n = 10\)) and female rats (\(n = 20\)) about 3 months of age and no kinship were mated (two females for one male) for 5 d. Pregnant rats placed in individual cages gave birth to ten to twelve pups after 21 d of gestation. The offspring (F1 generation) were divided into two groups at postnatal day 1 to minimise stress by simple randomisation\textsuperscript{17}.

- Control – eight pups per mother (four males and four females);
- Overweight – four pups per mother (two males and four females).

There was a total of seventy-two rats from the F1 generation:

- Control – thirty-two animals (sixteen males and sixteen females) – four litters;
- Overweight – forty animals (twenty males and twenty females) – ten litters.

Offspring analysis occurred at postnatal days 30 and 150, being considered young and adult animals\textsuperscript{18}. Whenever possible, data were collected precisely from the same rats at both ages. Euthanasia happened at the end of the experimental period after administrating a lethal dose of thiopental intraperitoneally.

Biometric and nutritional analyses

Body mass was monitored from birth to postnatal day 150, while food intake monitoring began upon weaning at postnatal day 21, allowing biometric and nutritional analysis\textsuperscript{19,20}.

Feed efficiency was estimated between postnatal days 21–30, 30–150 and 21–150, using the formula: (final body mass − initial body mass)/\(\Sigma\)food intake.

It was possible to record other biometric parameters of anaesthetised rats using a tape measure: nose-to-anus length (NAL), abdominal circumference (AC) and thoracic circumference (TC) (cm). BMI was calculated through the formula: body mass/NAL\textsuperscript{2}.

It was possible to achieve complete biometric and nutritional data from eight animals/group at both ages.

Echocardiography studies

The analyses of cardiac structure and function were performed through transthoracic echocardiography using a portable ultrasound system equipped with a 10 MHz transducer (Siemens Accuson Cypress). Previously the animals were anaesthetised with ketamine plus xylazine (50 mg + 5 mg/kg intraperitoneally). The assays were performed according to the American Society of Echocardiography\textsuperscript{21} and all parameters were measured at least three times per animal. The parameters recorded to address cardiac structure were left ventricular internal diameter (LVVID), interventricular septum thickness (IVS) and left ventricular posterior wall thickness (LVPW), measured in systole and diastole, as well as relative wall thickness, left ventricle mass, and left atrium/aorta ratio. Systolic volume, ejection fraction and fractional shortening, related to functional parameters, were calculated through algorithms of the equipment software. The parameter recorded to evaluate diastolic function was mitral deceleration time. It was possible to achieve complete echocardiographic data from at least ten animals per group at both ages.

Haemodynamic evaluation

Haemodynamic evaluation was performed by indirect measurement of systolic blood pressure and heart rate through the tail-cuff method\textsuperscript{22,23}. The assays occurred in the morning after 3 d of acclimatisation using the ADInstruments ML125 NIBP (Non-Invasive Blood Pressure) system connected to the ADInstruments PowerLab/400 digital-analogue converter. The signal was analysed using LabChart 6 Pro software (ADInstruments). Final systolic blood pressure and heart rate values of each animal were calculated by taking the average of six successful separate measurements obtained in the absence of spontaneous tail movement in awake rats.

Thus, because of the assay’s stress bias, it was not possible to record haemodynamic parameters of all animals submitted to echocardiography. It was possible to achieve complete haemodynamic data from eight animals/group, preferably at both ages.

Maximal effort ergometer test

After 3 d of acclimatisation, responsive animals (non-sedentary) were also submitted to a maximum effort
ergometer test (day 4). Non-responsive animals (sedentary) were discarded from this test. Thus, it was not possible to evaluate all rats submitted to previous assays. Data were achievable from at least five animals per group, preferably at both ages.

The protocol comprised a treadmill (Imbrasport®), without inclination and initial speed of 0.9 km/h, followed by progressive increments of 0.3 km/h every 3 min until animals were considered to be exhausted. The end of the test was determined when the animals remained still for at least 10 s. The parameters recorded were distance travelled, time spent and maximum speed developed in the test (24, 25).

Statistical analysis
The Kolmogorov–Smirnov test was applied to verify normality and data were expressed as mean values and standard deviations. Body mass recorded throughout lactation was analysed using a two-way ANOVA. The tested factors were litter size \( v. \) time. As the interaction was significant, the simple effects were analysed by Bonferroni’s post hoc test for multiple comparisons between control and overweight groups within the same sex. The unpaired \( t \) test was used to compare data obtained from these groups after weaning at the same age as well as weight gain during lactation. Statistical analyses were performed using Prism Software (Graph Pad Prism 7.0). A value of \( P < 0.05 \) was considered statistically significant.

Results
Body and nutritional analysis
Figs 1(a) and 1(b) show the body mass of male and female offspring throughout lactation. Reduced litters presented higher body mass during lactation and increased weight gain (Fig. 1(c) and 1(d)). Similar values of body mass, NAL, TC and BMI were seen between groups within the same sex at postnatal days 30 and 150. Nevertheless, adult males from reduced litters presented higher AC and AC:TC ratio than those from normal ones (Tables 1 and 2).

Despite no differences in feed efficiency, food intake was found lower in rats from reduced litters compared with those from regular litters soon after weaning. In the same period, females from reduced litters presented lower weight gain than their respective controls (Tables 3 and 4).

Haemodynamic and echocardiographic parameters
Tables 5 and 6 show haemodynamic and echocardiographic parameters from male and female animals, respectively. Male rats from reduced litters presented higher systolic blood pressure and structural changes in youth (as higher IVSd, IVSs, LVPWd, LVPWs and LVM) and adulthood (higher IVSd and relative wall thickness, lower LVIDd) than from regular ones (Table 5). Curiously, adult female rats from reduced litters presented lower systolic blood pressure compared with their respective controls. They also presented structural changes characterised by an increased IVSd, LVPWd and relative wall thickness in youth without functional alterations (Table 6). All animals presented ejection fraction superior to 80% and similar values of mitral deceleration time.

Performance on maximal effort ergometer test
Overweight and control groups of male and female offspring presented similar performance on the maximal effort ergometer test (Fig. 2).
BM (g) 129
⋅
BM (g) 165
⋅
overweight, according to the literature, and could be addressed
achieved, distinct outcomes may be seen in male and female rats.
related
parameters later in life. The literature has previously reported
favoured differences in haemodynamic and echocardiographic
or adulthood. Despite this, the early nutritional insult has
has led to overweight in the neonatal period but not in youth
Litter size reduction soon after birth and throughout lactation
As expected, the reduction of litter size leads to neonatal
overweight, according to the literature, and could be addressed
by the higher weight gain. Thus, this useful experimental
model was validated in the present study, allowing the investi-
gation of short- and long-term consequences of overfeed-
ing(26–28). Studies have reported that litter size reduction may
increase maternal milk availability to the offspring, leading to
higher body weight(12,29–34). As the hypothalamic area related
to food intake and satiety is not entirely structured at the
beginning of the lactation period, milk intake seems to be limi-
ted only by gastrointestinal tract capacity(35,36).
Litter size may modulate milk content. The literature has
reported that the TAG content of the milk from dams submit-
ted to litter reduction increases between the 10th and 21st days

| Table 1. Biometric parameters of male offspring | Postnatal day 30 | Postnatal day 150 |
|-----------------------------------------------|------------------|------------------|
|                                               | Control          | Overweight       | Control          | Overweight       |
|                                               | Mean±SD          | Mean±SD          | Mean±SD          | Mean±SD          |
| n                                             | 8                | 8                | 8                | 8                |
| BM (g)                                        | 165±10           | 158±9            | 165±5            | 448±43           |
| NAL (cm)                                      | 17.3±0.8         | 16.9±0.6         | 25.8±1.1         | 458±56           |
| BMI (g/cm²)                                   | 0.55±0.05        | 0.56±0.03        | 0.67±0.03        | 0.70±0.07        |
| AC (cm)                                       | 15.3±1.1         | 15.3±0.9         | 20.5±1.4         | 22.1±1.3         |
| TC (cm)                                       | 13.7±0.8         | 13.7±0.5         | 17.8±1.3         | 18.2±0.6         |
| AC:TC                                         | 1.15±0.05        | 1.12±0.02        | 1.15±0.04        | 1.21±0.06        |

BM, body mass; NAL, nose-to-anus length; AC, abdominal circumference; TC, thoracic circumference.

*P < 0.05 v. respective control group. Data were analysed using the unpaired t test.

| Table 2. Biometric parameters of female offspring | Postnatal day 30 | Postnatal day 150 |
|-----------------------------------------------|------------------|------------------|
|                                               | Control          | Overweight       | Control          | Overweight       |
|                                               | Mean±SD          | Mean±SD          | Mean±SD          | Mean±SD          |
| n                                             | 8                | 8                | 8                | 8                |
| BM (g)                                        | 129±14           | 138±13           | 248±19           | 260±31           |
| NAL (cm)                                      | 16.5±0.6         | 16.6±0.4         | 21.7±0.9         | 22.3±0.7         |
| BMI (g/cm²)                                   | 0.48±0.04        | 0.50±0.04        | 0.53±0.03        | 0.53±0.06        |
| AC (cm)                                       | 14.0±0.8         | 14.8±0.8         | 18.0±0.6         | 18.6±0.9         |
| TC (cm)                                       | 12.8±0.9         | 13.1±0.6         | 15.3±0.8         | 15.4±0.8         |
| AC:TC                                         | 1.09±0.04        | 1.13±0.05        | 1.18±0.04        | 1.21±0.05        |

BM, body mass; NAL, nose-to-anus length; AC, abdominal circumference; TC, thoracic circumference.

| Table 3. Nutritional parameters of male offspring | Postnatal days 21–30 | Postnatal days 30–150 | Postnatal days 21–150 |
|-----------------------------------------------|----------------------|----------------------|----------------------|
|                                               | Control              | Overweight           | Control              | Overweight           | Control              | Overweight           |
|                                               | Mean±SD              | Mean±SD              | Mean±SD              | Mean±SD              | Mean±SD              | Mean±SD              |
| n                                             | 8                    | 8                    | 8                    | 8                    | 8                    | 8                    |
| Weight variation (g)                          | 46.9±14.8            | 336.9±20.7           | 345.0±64.6           | 382.8±24.0           | 385.7±57.3           |
| Food intake (g)                               | 131.3±16.4           | 2540±232.5           | 2663±168.2           | 2672±216.5           | 2773±148.7           |
| Feed efficiency                               | 0.350±0.066          | 0.043                | 0.133±0.014          | 0.129±0.019          | 0.145±0.018          | 0.139±0.015          |

*P < 0.05 v. respective control group. Data were analysed using the unpaired t test.

Discussion
Litter size reduction soon after birth and throughout lactation has led to overweight in the neonatal period but not in youth or adulthood. Despite this, the early nutritional insult has favoured differences in haemodynamic and echocardiographic parameters later in life. The literature has previously reported related findings in adult male rats submitted to neonatal overfeeding. However, none of the studies investigated the outcomes of the same insult in female rats. According to the results here achieved, distinct outcomes may be seen in male and female rats.

As expected, the reduction of litter size leads to neonatal overweight, according to the literature, and could be addressed...
of lactation. Thus, neonatal overweight may also be induced by the higher energy content of maternal milk.\(^\text{20,37}\). Differences regarding food intake are also in agreement with the literature that describes hypophagia in young animals submitted to overfeeding during lactation.\(^\text{38}\). Although the consequence over body mass is controversial, the similarity about feed efficiency and body weight here observed suggests the occurrence of catch-down growth, a phenomenon also reported by other studies encompassing similar animal models.\(^\text{39–43}\).

The literature has correlated anthropometric markers of adiposity, systolic blood pressure and cardiovascular risk, not only in humans but also in rats.\(^\text{20,44,45}\). According to the relationship ascribed, data indicate that adult male rats from reduced litters presented increased cardiovascular risk compared with regular ones. Abdominal fat deposition is related to pathological conditions and may favour atherosclerosis and acute myocardial infarction.\(^\text{46}\). Although the literature has already reported the increase of blood pressure in adult male rats due to neonatal overfeeding,\(^\text{26,47–50}\), the same analysis has not included female rats. Thus, data from the present study suggest that the reduction in litter size does not affect the cardiovascular risk of female animals as described for males.

Higher levels of systolic blood pressure, as seen in young and adult male rats submitted to litter size reduction, predispose to diastolic dysfunction and structural remodelling of the left ventricle, a central change in the pathogenesis of cardiac dysfunction. Indeed, echocardiographic data of the present study suggest the occurrence of myocardial hypertrophy and concentric remodelling of the left ventricle in these animals. These structural alterations may eventually lead to ventricular dilation and systolic dysfunction in heart failure progression.\(^\text{51–58}\). Although changes regarding echocardiographic parameters in this animal model have not been described previously, the literature reports that overnourishment during lactation may increase cardiac sensitivity to insulin.
and leptin. The consequent improvement of glucose uptake and energy supply would favour cardiac hypertrophy in male rats. Despite no preliminary signs of cardiovascular risk increase in female rats from reduced litters, there were differences regarding echocardiographic parameters. Data suggest the occurrence of cardiac structural changes in young females. The lack of cardiac hypertrophy inferences in adulthood may be discussed, taking sexual maturation into account. Female rats reach puberty around postnatal day 30 and reproductive senescence occurs between 15 and 20 months of age. An oestrogen-cardioprotective effect throughout the

### Table 6. Haemodynamic and echocardiographic data of female offspring (Mean values and standard deviations)

| Postnatal day 30 | Postnatal day 150 |
|-----------------|------------------|
|                 |                  |
|                  | Control | Overweight | Control | Overweight |
|                  | Mean    | SD       | Mean    | SD       |
|                  | Mean    | SD       | Mean    | SD       |
| Systolic blood pressure (mmHg) | 101.5 ± 7.3 | 106.6 ± 3.9 | 140.5 ± 19.6 | 124.8 ± 15.3 |
| Heart rate (bpm) | 406.2 ± 36.5 | 362.8 ± 62.6 | 350.6 ± 41.3 | 353.9 ± 49.4 |
|                  | n        |           | n        |           |
|                  | 8        |           | 11       |           |
|                  | 10       |           | 10       |           |
|                   | 0.116 ± 0.006 | 0.128* ± 0.005 | 0.158 ± 0.019 | 0.177 ± 0.032 |
| IVSd (cm)        | 0.217 ± 0.037 | 0.212 ± 0.014 | 0.302 ± 0.020 | 0.315 ± 0.037 |
| IVSs (cm)        | 0.358 ± 0.059 | 0.350 ± 0.024 | 0.535 ± 0.059 | 0.504 ± 0.062 |
| LVId (cm)        | 0.149 ± 0.076 | 0.127 ± 0.024 | 0.191 ± 0.063 | 0.160 ± 0.039 |
| LVPWd (cm)       | 0.115 ± 0.006 | 0.128* ± 0.005 | 0.165 ± 0.022 | 0.179 ± 0.030 |
| LVPWs (cm)       | 0.212 ± 0.029 | 0.214 ± 0.013 | 0.300 ± 0.024 | 0.315 ± 0.032 |
| RWT (cm)         | 0.654 ± 0.088 | 0.733* ± 0.038 | 0.629 ± 0.135 | 0.721 ± 0.155 |
| LVM (g)          | 0.725 ± 0.039 | 0.750 ± 0.041 | 1.000 ± 0.074 | 1.050 ± 0.144 |
| LA:Ao            | 1.075 ± 0.108 | 1.074 ± 0.129 | 0.960 ± 0.112 | 1.049 ± 0.154 |
| LVEF (%)         | 95.18 ± 1.85 | 93.96 ± 3.87 | 93.91 ± 4.46 | 94.91 ± 3.52 |
| S (%)            | 65.88 ± 4.13 | 64.04 ± 6.19 | 65.12 ± 7.19 | 66.12 ± 9.23 |
| Mitral DT (ms)   | 62.50 ± 7.15 | 67.75 ± 8.07 | 87.92 ± 8.31 | 90.00 ± 4.63 |

IVSd, interventricular septum thickness diastole; IVSs, interventricular septum thickness systole; LVId, left ventricle internal diameter diastole; LVlDs, left ventricle internal diameter systole; LVPWd, left ventricle posterior wall thickness diastole; LVPWs, left ventricle posterior wall thickness systole; RWT, relative wall thickness; LVM, left ventricle mass; LA:Ao, left atrium:aorta ratio; LVEF, left ventricle ejection fraction; FS, fractional shortening; mitral DT, mitral deceleration time.

* $P < 0.05$ vs. respective control group. Data were analysed using the unpaired $t$ test.

![Fig. 2](https://journals.cambridge.org/jns)
reliable and reproducible method that yields important outcomes on cardiopulmonary exercise tests that constitute an accurate, on the maximum effort ergometer test. Exercise intolerance, may explain the similar performance noticed for the animals and O2 consumption (69).

Thus, it is not possible to precisely explain why the reduction of milk consumption, secretion and content during lactation.

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