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# Biometric, nutritional, biochemical, and cardiovascular outcomes in male rats submitted to an experimental model of early weaning that mimics mother abandoning

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**Original Article**

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## Abstract

Literature describes breast milk as the best food for the newborn, recommending exclusive breastfeeding for up to 6 months of age. However, it is not available for more than 40% of children worldwide. Pharmacological and non-pharmacological models of 3-day early weaning were developed in rodents to investigate later outcomes related solely to this nutritional insult. Thus, the present work aimed to describe biometric, nutritional, biochemical, and cardiovascular outcomes in adult male rats submitted to 3-day early weaning achieved by maternal deprivation. This experimental model comprises not only nutritional insult but also emotional stress, simulating mother abandoning. Male offspring were physically separated from their mothers at 21st (control) or 18th (early weaning) postnatal day, receiving water/food ad libitum. Analysis performed at postnatal days 30, 90, 150, and 365 encompassed body mass and food intake monitoring and serum biochemistry determination. Further assessments included hemodynamic, echocardiographic, and cardiorespiratory evaluation. Early-weaned males presented higher body weight when compared to control as well as dyslipidemia, higher blood pressure, diastolic dysfunction, and cardiac hypertrophy in adult life. Animals early deprived of their mothers have also presented a worse performance on the maximal effort ergometer test. This work shows that 3-day early maternal deprivation favors the development of cardiovascular disease in male rats.

## Introduction

The research field named "Developmental Origins of Health and Disease" (DOHaD) aims to provide a clear perspective on how different life experiences modulate health and disease risks over a lifetime. This research field's history reached a milestone with David Barker's theory about the fetal origins of disease. After this, several studies, including animal research, confirmed the principle that early life affect cardiometabolic function in adulthood and elucidated some important underlying programming mechanisms.[1]

Literature describes breast milk as the best food for the newborn. It potentially reduces infant mortality and protects against infection and diseases.[2] As per several authors, such as Kramer and Kakuma, the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend exclusive breastfeeding up to 6 months of age. Exclusive breastfeeding excludes the consumption of any other food or drink besides breast milk, even water, throughout this period.[3] Despite this, it is estimated that exclusively breastfeeding is not available for no more than 40% of children worldwide along the first semester of life.[4] Literature also reports that many women cannot breastfeed their children during this time, mainly those from lower social classes.[5] Importantly, a growing body of evidence suggests that breastfeeding has a protective role against obesity, hypertension, dyslipidemia, and type 2 diabetes mellitus during adulthood.[6]

Different animal models of early weaning have already been developed in rodents. For example, there are pharmacological and non-pharmacological models that aim to suppress lactation 3 days earlier, considering standard weaning on postnatal day 21.[7] Bromocriptine administration in lactating female rats inhibits milk secretion, while bandages restrain the offspring's access to the dams' tits. As shared findings, these two experimental models have led to higher body mass and serum triglyceride (TG), as well as hyperleptinemia and central resistance to leptin in adulthood.[8–12] According to Quinn,[13] early weaning at postnatal day 18 mimics a child weaned at the age of 5 months.

However, unlike bromocriptine administration or breast bandages placement, early weaning achievement by maternal deprivation involves maternal milk restriction and maternal care

restriction, simulating mother abandoning.[7] In this scenario, it is crucial to evaluate the offspring outcomes upon the introduction of emotional stress besides nutritional insult. Thus, the present work aimed to describe biometric, nutritional, biochemical, and cardiovascular outcomes in male Wistar rats submitted to maternal deprivation at postnatal day 18. We hypothesize that the combination of nutritional insult and emotional stress raises cardiovascular risk and programs cardiovascular disease.

## Materials and methods

### Experimental design

The procedures have followed the National Research Council (US) Institute for Laboratory Animal Research. Ethics Committee of Fluminense Federal University (Niteroi, Brazil) approved the use of animals (CEUA UFF812/2016) following the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978). All rats received standard chow (Nuvilab®) and tap water ad libitum at controlled conditions (22 °C, 55–65% humidity, 12/12 h light/dark cycle).

The breeding laboratory of the university has provided Wistar rats used for mating (F0 generation). Male ($n = 8$) and female rats ($n = 16$) about 3 months of age and no kinship were mated (2 females for 1 male) for 5 days. Afterward, pregnant female rats placed in individual cages have spontaneously given birth to 10–12 puppies after 21 days of gestation, as expected (postnatal day 0). Litters mixing/adjustment (preferably six males/mother) and standard weaning occurred at postnatal days 1 and 21, respectively.[14] Accordingly to previous animal models of early weaning described by literature,[8-12] the dams (F0 generation) and consequently their respective offspring (F1 generation) were divided into two groups at postnatal day 18 by simple randomization:

Control – offspring weaned at postnatal day 21.
Early weaning – offspring weaned at postnatal day 18.

Standard and early weaning encompassed offspring–mother physical separation (two animals/cage). There was a total of 96 rats from the F1 generation:

Control – 34 males housed in 17 cages (14 females discarded) – 8 litters.
Early weaning – 38 males housed in 19 cages (10 females discarded) – 8 litters.

Offspring analysis occurred at postnatal days 30, 90, 150 and 365. Excepting for biochemical analysis, whenever possible, data were collected precisely from the same rats at different ages (eight males/group). The animals from each group were randomly distributed between biochemical and the other assays.

### Biometrical and nutritional analysis

Body weight was monitored twice a week until the end of the experimental period, always at the same hour. Nose-to-anus, abdominal, and thoracic length were measured using a measuring tape, allowing calculation of body mass index (BMI) and abdominal and thoracic lengths ratio. Daily food intake (g) was determined by subtracting the remaining chow by the total put in the cage the day before, dividing by the number of rats (two) per box. The summation of daily food intake throughout the interlude of interest gave the amount of food consumed in a given period.[15]

It was possible to record data from all animals euthanized at postnatal day 365 (16 animals/group).

### Echocardiographic and hemodynamic evaluation

Transthoracic echocardiography took place with anesthetized rats (ketamine 50 mg/kg plus xylazine 5 mg/kg intraperitoneally). The parameters recorded using a portable ultrasound (Siemens Accusion Cypress, Siemens AG, Munich, Germany) and a 10-MHz transducer to evaluate cardiac structure were interventricular septum (IVS) thickness, left ventricular posterior wall (LVPW) thickness, and left ventricular internal diameter (LVID) measured in diastole, as well as relative wall thickness (RWT), left ventricular mass (LVM), and left atrium to aorta ratio (LA/Ao). The parameters recorded to evaluate systolic and diastolic function were left ventricular ejection fraction and mitral deceleration time (MDT), respectively.[16]

Hemodynamic assessment occurred after 7 days of acclimation (10 min/day) in the morning. The animals submitted to the non-invasive tail-cuff method were awake (Insight, São Paulo, Brasil). Final values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) represent the average of six measurements successfully acquired and gave mean arterial pressure (MAP) through the formula: $MAP = SBP + 2(DBP/3)$.[17,18] Because of the assay's stress bias, it was not always possible to record all available animals' hemodynamic parameters. It was possible to achieve complete hemodynamic data from at least five animals per group.

### Maximum effort ergometer test

After 4 days of acclimation, animals were individually submitted to a maximum effort test using a treadmill (Imbrasport®, Brasília, Brasil). Initial speed was 0.9 km/h being followed by progressive increments of 0.3 km/h every 3 min. The end of the test was determined when animals remained still for at least 10 s is considered exhausted. Distance, time spent, and maximum speed developed were recorded and posteriorly analyzed.[19,20]

### Biochemical analysis

Serum samples were obtained by cardiac puncture after euthanasia using thiopental (100 mg/kg i.p) in fasting conditions and after 15 min of centrifugation at 1300×*g* (storage at −80 °C). Lipid profile was determined using a commercially available Labtest Brasil kit.[21] Friedewald equation[22] was applied to calculate low-density lipoproteins cholesterol (LDL-c) and very low-density lipoproteins cholesterol (VLDL-c) concentrations. The ratio of total cholesterol and high-density lipoproteins cholesterol (HDL-c) levels gave the Castelli I index. The ratio between LDL-c and HDL-c levels provided the Castelli II index.[23] As another atherogenic index, the ratio between TGs and HDL-c concentrations was also evaluated.[24] Serum leptin levels were also determined using a commercially available kit of Enzyme-Linked Immunoabsorbent Assay (Rat Leptin ELISA, Millipore, Billerica, MA, USA).[25]

Due to hemolysis, it was impossible to analyze all serum samples, allowing assays with at least five samples from each group.

### Statistical analysis

Data were analyzed using GraphPad Prism software version 7.0. Values are expressed as mean ± standard error of the mean. The Shapiro–Wilk test evaluated normality. Normally distributed data were analyzed applying Student's unpaired *t*-test comparing

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**Table 1.** Biometric parameters

| | Postnatal day 30 | | Postnatal day 90 | | Postnatal day 150 | | Postnatal day 365 | |
|---|---|---|---|---|---|---|---|---|
| | Control | Early weaning | Control | Early weaning | Control | Control | Early weaning | Control |
| Parameters | $n = 16$ | $n = 16$ | $n = 16$ | $n = 16$ | $n = 16$ | $n = 16$ | $n = 16$ | $n = 16$ |
| Body weight (g) | 92.5 ± 1.8 | 84.4 ± 0.7* | 367.5 ± 4.3 | 377.9 ± 3.5 | 440.4 ± 4.7 | 454.7 ± 5.0* | 525.1 ± 7.7 | 562.6 ± 562.6* |
| Abdominal/thoracic circumferences | 1.16 ± 0.03 | 1.13 ± 0.02 | 1.21 ± 0.01 | 1.21 ± 0.02 | 1.16 ± 0.02 | 1.17 ± 0.01 | 1.16 ± 0.02 | 1.21 ± 0.01* |
| Body mass index (g/cm$^2$) | 0.43 ± 0.01 | 0.39* ± 0.01 | 0.61 ± 0.01 | 0.63 ± 0.01 | 0.69 ± 0.01 | 0.68 ± 0.01 | 0.83 ± 0.02 | 0.86 ± 0.02 |

Data are represented as mean ± SEM.
*$p < 0.05$ between control and early weaning groups at the same postnatal day (Student's *t*-test).

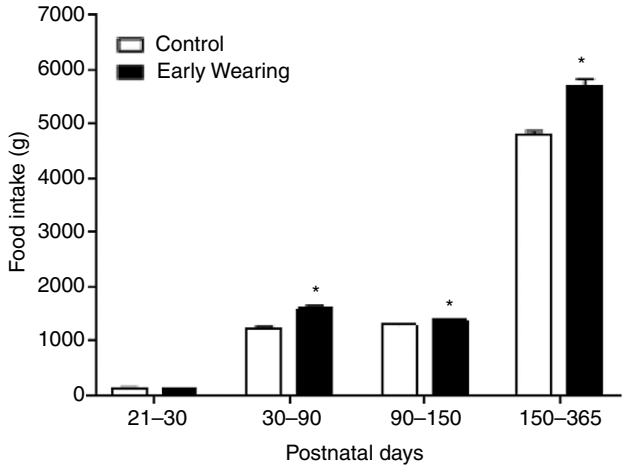


**Fig. 1.** Nutritional parameter. Food intake (g). Data are represented as mean ± SEM. *$p < 0.05$ between control and early weaning groups at the same period (Student's *t*-test). $n = 16$/group.

control and early weaning groups within the same postnatal day. Significance was accepted when the *p*-value was $< 0.05$.

## Results

### Biometrical and nutritional analysis

Table 1 shows that early-weaned rats at postnatal day 30 presented a lower body weight than their respective controls. In contrast, older animals presented a higher body weight, reflecting lower BMI in youth and a greater abdominal and thoracic circumferences ratio in the elderly. Food intake increased from postnatal day 90 upward (Fig. 1).

### Echocardiographic and hemodynamic evaluation

Table 2 shows data from echocardiographic studies and hemodynamic evaluation. There were differences between early-weaned rats and their respective controls at postnatal days 90, 150, and 365. IVS thickness and LVPW thickness, both measured in diastole, as well as LVM, were founded increased in early-weaned rats upward postnatal day 90. However, there were distinguished parameters in specific moments. Animals at postnatal day 90 presented higher left ventricular RWT, LA/Ao ratio, and DBP/mean blood pressure. Rats at postnatal day 365 also exhibited an increased LA/Ao ratio but accompanied by a superior SBP and reduced LVID and MDT. Animals at postnatal day 150 have presented higher values of SBP, DBP, and mean blood pressure. It was also observed higher values of DBP and mean blood pressure at postnatal day 90, while only SBP was increased at postnatal day 365.

### Maximum effort ergometer test

There were differences in exercise tolerance between groups only in elderly animals. Early-weaned rats at postnatal day 365 have presented a less lasting test, traveling a shorter distance and reaching an inferior maximum speed compared to respective controls (Table 3).

### Biochemical analysis

Serum levels of TGs and VLDL-c, as well as TG/HDL-c ratio, were found higher in early-weaned rats since postnatal day 30. Besides, the Castelli I index was increased in these animals compared to respective controls since postnatal day 150, while HDL concentration was decreased only at postnatal day 365 (Table 4).

There were also differences in leptin serum levels (Table 4). Its concentration was higher in early-weaned rats since postnatal 150.

## Discussion

The present study has shown an early weaning model achieved by maternal deprivation that combines nutritional insult and emotional stress. It was capable of raising cardiometabolic risk since youth, allowing cardiovascular disease development in adulthood/elderly. We found that early-weaned male rats presented higher body mass, SBP, and impaired serum lipid profile than controls. Besides, diastolic dysfunction associated with a suggestive pattern of cardiac hypertrophy, as well as exercise intolerance, was also observed in these animals.

Soon after early weaning, 30-day-old rats have presented lower body weight and BMI than controls, as reported in previous studies involving mother–offspring separation[26], bromocriptine administration,[9] and bandages.[12] Thus, these data validate the proposed experimental model of malnutrition during lactation.

Upward postnatal day 150, early-weaned rats have presented a slightly higher body weight than controls, accompanied by hyperphagia, since postnatal day 90. This observation may be due to 'catch-up growth' defined by Wit and Boersma[27] as an accelerated growth after a period of its inhibition determined by early undernutrition. In elderly animals, there was an increased abdominal/thoracic circumferences ratio. According to literature, this profile is related to a higher cardiometabolic risk.[28]

Dyslipidemic profile corroborates the increase in cardiovascular disease risk, worsened with aging. There were higher plasma levels of TG and VLDL-c, as well as increased TG/HDL-c ratio since postnatal day 30, followed by the rise of the Castelli

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**Table 2.** Echocardiographic and hemodynamic parameters

| | Postnatal day 30 | | Postnatal day 90 | | Postnatal day 150 | | Postnatal day 365 | |
|---|---|---|---|---|---|---|---|---|
| Parameters | Control | Early weaning | Control | Early weaning | Control | Early weaning | Control | Early weaning |
| Echocardiography | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ |
| LVEF (%) | 93.139 ± 0.769 | 93.999 ± 1.077 | 86.291 ± 1.536 | 89.708 ± 0.805 | 84.834 ± 3.278 | 84.709 ± 2.409 | 87.800 ± 2.035 | 88.500 ± 1.936 |
| LA/Ao | 0.937 ± 0.023 | 0.863 ± 0.035 | 1.094 ± 0.034 | 1.320 ± 0.041* | 1.317 ± 0.083 | 1.179 ± 0.051 | 0.898 ± 0.011 | 1.230 ± 0.098* |
| IVSd (cm) | 0.126 ± 0.003 | 0.127 ± 0.004 | 0.149 ± 0.005 | 0.189 ± 0.007* | 0.138 ± 0.005 | 0.160 ± 0.006* | 0.158 ± 0.006 | 0.185 ± 0.006* |
| LVPWd (cm) | 0.133 ± 0.003 | 0.135 ± 0.006 | 0.151 ± 0.005 | 0.188 ± 0.007* | 0.286 ± 0.011 | 0.311 ± 0.006* | 0.180 ± 0.003 | 0.195 ± 0.016* |
| LVIDd (cm) | 0.197 ± 0.025 | 0.513 ± 0.028 | 0.725 ± 0.031 | 0.719 ± 0.016 | 0.798 ± 0.026 | 0.808 ± 0.024 | 0.732 ± 0.006 | 0.695 ± 0.013* |
| RWT (cm) | 0.552 ± 0.027 | 0.553 ± 0.055 | 0.421 ± 0.025 | 0.530 ± 0.027* | 0.377 ± 0.019 | 0.422 ± 0.017 | 0.498 ± 0.016 | 0.548 ± 0.028 |
| LVM (g) | 0.872 ± 0.025 | 0.877 ± 0.015 | 1.168 ± 0.032 | 1.413 ± 0.043* | 1.23 ± 0.036 | 1.403 ± 0.073* | 1.308 ± 0.036 | 1.490 ± 0.065* |
| MDT (ms) | 70.50 ± 2.797 | 81.00 ± 4.573 | 70.50 ± 2.612 | 77.50 ± 2.228 | 78.50 ± 4.110 | 76.63 ± 3.775 | 89.60 ± 2.227 | 82.00 ± 2.160* |
| Hemodynamic evaluation | $n = 5$ | $n = 5$ | $n = 6$ | $n = 6$ | $n = 6$ | $n = 6$ | $n = 5$ | $n = 5$ |
| SBP (mmHg) | 149.1 ± 1.5 | 153.0 ± 1.0 | 144.7 ± 2.9 | 152.2 ± 3.0 | 131.8 ± 2.2 | 143.8 ± 2.3* | 152.6 ± 3.1 | 170.2 ± 3.7* |
| DBP (mmHg) | 99.0 ± 1.1 | 100.0 ± 2.1 | 109.2 ± 1.1 | 115.2 ± 1.5* | 89.7 ± 1.7 | 98.8 ± 1.9* | 109.6 ± 3.9 | 107.6 ± 2.4 |
| MAP (mmHg) | 116.0 ± 0.9 | 109.7 ± 5.1 | 121.2 ± 0.7 | 127.4 ± 1.0* | 93.2 ± 4.2 | 110.2 ± 4.8* | 123.8 ± 3.5 | 128.6 ± 2.9 |

LVEF, left ventricular ejection fraction; LA/Ao, left atrium to aorta ratio; IVSd, intraventricular septum thickness in diastole; LVPWd, left ventricular posterior wall thickness in diastole; LVIDd, left ventricular internal diameter in diastole; RWT, relative wall thickness; LVM, left ventricular mass; MDT, mitral deceleration time; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
Data are represented as mean ± SEM.
*$p < 0.05$ between control and early weaning groups at the same postnatal day (Student's *t*-test).

**Table 3.** Data from maximum effort ergometer test

| | Postnatal day 30 | | Postnatal day 90 | | Postnatal day 150 | | Postnatal day 365 | |
|---|---|---|---|---|---|---|---|---|
| | Control | Early weaning | Control | Early weaning | Control | Early weaning | Control | Early weaning |
| Parameters | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ | $n = 8$ |
| Maximum speed (km/h) | 2.53 ± 0.15 | 2.40 ± 0.06 | 2.03 ± 0.12 | 1.95 ± 0.08 | 1.99 ± 0.08 | 1.84 ± 0.07 | 1.93 ± 0.06 | 1.70 ± 0.05* |
| Distance (km) | 0.55 ± 0.06 | 0.51 ± 0.02 | 0.34 ± 0.04 | 0.32 ± 0.02 | 0.34 ± 0.02 | 0.29 ± 0.02 | 0.32 ± 0.02 | 0.25 ± 0.01* |
| Test duration (h) | 0.39 ± 0.02 | 0.38 ± 0.01 | 0.31 ± 0.02 | 0.30 ± 0.01 | 0.30 ± 0.01 | 0.29 ± 0.02 | 0.29 ± 0.01 | 0.25 ± 0.01* |

Data are represented as mean ± SEM.
*$p < 0.05$ between control and early weaning groups at the same postnatal day (Student's *t*-test).

**Table 4.** Biochemical parameters

| | Postnatal day 30 | | Postnatal day 90 | | Postnatal day 150 | | Postnatal day 365 | |
|---|---|---|---|---|---|---|---|---|
| | Control | Early weaning | Control | Early weaning | Control | Early weaning | Control | Early weaning |
| Parameters | $n = 6$ | $n = 6$ | $n = 5$ | $n = 5$ | $n = 6$ | $n = 6$ | $n = 8$ | $n = 8$ |
| TG (mg/dL) | 43.6 ± 5.1 | 77.0 ± 12.5* | 29.5 ± 6.9 | 64.8 ± 6.7* | 36.7 ± 6.6 | 56.8 ± 5.9* | 73.7 ± 6.9 | 125.6 ± 6.1* |
| LDL-c (mg/dL) | 27.1 ± 3.2 | 22.5 ± 8.1 | 1.8 ± 6.6 | 11.0 ± 10.2 | 5.37 ± 2.2 | 12.5 ± 3.6 | 11.9 ± 4.1 | 12.9 ± 4.4 |
| VLDL-c (mg/dL) | 8.7 ± 1.0 | 15.4 ± 2.5* | 5.9 ± 1.4 | 12.9 ± 1.3* | 7.4 ± 1.3 | 11.4 ± 1.2* | 14.8 ± 1.4 | 25.1 ± 1.2* |
| HDL-c (mg/dL) | 26.0 ± 2.1 | 22.5 ± 1.3 | 45.2 ± 4.1 | 36.0 ± 2.2 | 43.7 ± 4.2 | 35.4 ± 0.8 | 61.3 ± 5.2 | 45.3 ± 2.8* |
| Castelli I | 2.4 ± 0.1 | 2.5 ± 0.4 | 1.2 ± 0.2 | 1.6 ± 0.3 | 1.3 ± 0.1 | 1.7 ± 0.1* | 1.5 ± 0.1 | 1.9 ± 0.1* |
| Castelli II | 1.1 ± 0.1 | 0.9 ± 0.4 | 0.1 ± 0.2 | 0.4 ± 0.3 | 0.1 ± 0.1 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 |
| TG/HDL-c | 1.7 ± 0.2 | 3.1 ± 0.5* | 0.5 ± 0.2 | 1.3 ± 0.2** | 0.8 ± 0.1 | 1.6 ± 0.2** | 1.2 ± 0.1 | 2.8 ± 0.2* |
| Leptin (ng/mL) | 3.35 ± 0.73 | 4.65 ± 0.46 | 9.28 ± 2.04 | 7.55 ± 0.88 | 9.49 ± 0.98 | 15.54 ± 1.92* | 17.11 ± 1.82 | 24.43 ± 1.02* |

TG, triglyceride; LDL-c, low density lipoproteins cholesterol; VLDL-c, very low-density lipoproteins cholesterol; HDL-c, high density lipoproteins cholesterol.
Data are represented as mean ± SEM.
*$p < 0.05$ and **$p < 0.01$ between control and early weaning groups at the same postnatal day (Student's *t*-test).

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index and the decrease in HDL-c levels. Dyslipidemic profile was also observed in different models of early weaning.[10,12,29] Experimental studies have shown that hypertriglyceridemia is a risk factor for coronary heart disease, regardless of overweight. TG-rich lipoproteins, particularly VLDLs, have a direct atherogenic effect.[30-32] VLDLs are hydrolyzed in the liver by lipase and converted to LDL particles, a crucial trigger of atherosclerosis genesis that favors lipid deposition in vessels.[33-35] Besides, HDL-c has a protective role partly due to its ability to remove intracellular cholesterol and perform reverse transport, allowing its redistribution in the body and biliary excretion.[36] HDL-c presents antioxidant and anti-inflammatory properties, inactivating or preventing oxidation of phospholipids from LDL-c.[37,38] HDL-c also inhibits apoptosis of endothelial cells and is associated with a relevant antiatherogenic action.[35-37]. According to literature, the Castelli I index and TG/HDL ratio are atherogenic markers. They are useful instruments to predict coronary artery disease risk in daily practice, especially in people with other risk factors.[21,24,39]

Leptin serum levels have also increased in early-weaned male rats upward postnatal day 150. Usually, leptin increases the expression of neuropeptides associated with food intake inhibition. Proopimelanocortin, as well as cocaine and amphetamine-regulated transcripts, stimulates total energy expenditure via sympathetic activation. Besides, it decreases the expression of neuropeptide Y and agouti-related protein, associated with increased food intake and reduced energy expenditure.[39] Leptin plays an essential role in regulating energy homeostasis, decreasing appetite and increasing energy expenditure. Thus, the hyperphagia and the higher body weight suggest leptin hypothalamic resistance. Hyperphagia, higher body mass, and TG/leptin serum levels as well as leptin resistance outcomes were also observed in previous studies encompassing other animal models of early weaning.[10,11,29,41,42]

Early-weaned rats presented higher LVM, IVS thickness and LVPW thickness, both in diastole, compared to respective adult and elderly controls. RWT was higher in this group at postnatal day 90 but not 150 or 365. These data point to concentric remodeling that evolved into eccentric patterns commonly observed in patients with diastolic heart failure.[43] The observed increase in LA/Ao diameter ratio suggests pressure raise in LA and consequently dilatation of its cavity, possibly due to ventricular relaxation impairment during diastole.[44,45] MDT, intimately related to left ventricular compliance, was shorter in early-weaned animals characterizing diastolic dysfunction and left atrial dilatation does.[43]

The maximal effort ergometer test infers the cardiorespiratory capacity.[15] There is a linear relationship between maximum speed developed by the animal and its oxygen uptake rate.[46] Thus, a less lasting test accompanied by a shorter distance traveled and an inferior maximum speed described to elderly early-weaned rats suggest a diminished tolerance to exercise compared to respective controls. Diastolic dysfunction favors exercise intolerance.[47] It is noteworthy that maximum cardiac output during exercise is dependent on diastolic filling. If relaxation abnormalities accompany diastole, lower ventricular filling rates are achieved and become insufficient to supply cardiac output required during exercise sessions. Consequently, filling pressure increases and maximum capacity decreases; thus, as diastolic dysfunction progresses, the lower will become exercise capacity.[48,49]

Early-life stress is also a relevant factor involved in metabolic programming.[7] Despite this, biometric, nutritional, and biochemical outcomes here described are similar to those observed in studies using pharmacological and non-pharmacological models of early weaning that avoid emotional stress.[8-12,29,41,42]

According to Tractenberg *et al.*,[50] maternal deprivation protocols present methodological pitfalls due to different degrees of stress exposure in maternal separation-reared pups. Nevertheless, here we took care of important methodological issues to avoid bias, such as housing/husbandry conditions, animal/standard facility rearing, and litter controls.

Kikusui, Ichikawa, and Mori[51] observed that mice weaned on postnatal day 14 showed a prolonged increase in corticosterone compared to the littermates stayed with dam until postnatal day 21. The last ones have presented a transient increase. Moura *et al.*[10] also reported hypercorticosteronaemia and higher total catecholamine levels later in life. According to Huang *et al.*,[52] hypercorticosteronemia could explain the higher blood pressure seen because glucocorticoids increase blood pressure. For instance, hypertension is often associated with left ventricular hypertrophy and heart failure with preserved ejection fraction, that impair exercise tolerance, outcomes described in this study.[48,49,53,54]

Literature report that adaptation mechanisms may be different between sexes in early weaning models.[7] Nevertheless, our main objective was to describe outcomes in male rats submitted to an early weaning model encompassing an emotional stress, as widely performed with pharmacological and non-pharmacological models.

The measurement of corticosterone level would contribute to a better understanding of this model, although not compromise the relevance of our findings. Along with corticosterone measurement, sexual dimorphism also encompasses a perspective for future studies in this experimental model.

In conclusion, the present work demonstrates, for the first time, cardiovascular disease development in later life due to maternal deprivation at postnatal day 18. This early weaning model simulates mother abandoning, a social health challenge. As cardiovascular diseases are the leading death causes worldwide, public policies favoring exclusive breastfeeding until 6 months of age may constitute an important strategy to reduce their incidence, reducing direct and indirect health expenditures.

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**Conflict of interests.** The authors declare no conflict of interest.

## References

1. Penkler M, Hanson M, Biesma R, Müller R. DOHaD in science and society: emergent opportunities and novel responsibilities. *J DOHaD.* 2018; 10(3),268–273. doi: 10.1017/S2040174418000892
2. Victora CG, Smith, PG, Vaughan JP *et al.* Evidence for protection by breastfeeding against infant deaths from infectious diseases in Brazil. *Lancet (Br Edn).* 1987; 330: 319–322.
3. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev.* 2012; 8(CD003517), 1–95. doi: 10.1002/14651858.CD003517.pub2
4. WHO. Infant and Young Child Feeding. IOP Publishing WHO. 2017; http://www.who.int/mediacentre/factsheets/fs342/en/. Accessed 17 September 2019
5. UNICEF and WHO. GLOBAL BREASTFEEDING INVESTMENT CASE. IOP Publishing WHO. http://who.int/nutrition/publications/infantfeeding/global-bf-collective-investmentcase/en/. Accessed 12 December 2019
6. Kelishadi R, Farajian S. The protective effects of breastfeeding on chronic non-communicable diseases in adulthood: a review of evidence. *Adv Biomed Res.* 2014; 3, 3. doi: 10.4103/2277-9175.124629
7. 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; 1–11. doi: 10.1017/S2040174420000410

https://doi.org/10.1017/S2040174420000793 Published online by Cambridge University Press

8. Bonomo IT, Lisboa PC, Passos MC, Passos-Moura C, Reis AM, Moura EG. Prolactin inhibition in lactating rats changes leptin transfer through the milk. *Horm Metab Res.* 2005; 37(4), 220–225. doi: 10.1055/s-2005-861381
9. Bonomo IT, Lisboa PC, Pereira AR, Passos MC, de Moura EG. Prolactin inhibition in dams during lactation programs for overweight and leptin resistance in adult offspring. *J Endocrinol.* 2007; 192(2), 339–344. doi: 10.1677/joe.1.06952
10. Moura EG, Bonomo IT, Neto JF, *et al.* Maternal prolactin inhibition during lactation programs for metabolic syndrome in adult progeny. *J Physiol.* 2009; 587 (20), 4919–4929. doi: 10.1113/jphysiol.2009.176289
11. Bonomo IT, Lisboa PC, Passos MC, Alves SB, Reis AM, de Moura EG. Prolactin inhibition at the end of lactation programs for a central hypothyroidism in adult rat. *J Endocrinol.* 2008; 198, 331–337. doi: 10.1677/JOE-07-0505
12. Lima NS, Moura EG, Passos MC, *et al.* Early weaning causes undernutrition for a short period and programmes some metabolic syndrome components and leptin resistance in adult rat offspring. *Br J Nutr.* 2011; 105(9), 1405–1413. doi: 10.1017/S0007114510005064
13. Quinn R. Comparing rat's to human's age: how old is my rat in people years? *Nutrition.* 2005; 21(6), 775–777. doi: 10.1016/j.nut.2005.04.002
14. Koolhaas JM. The laboratory rat. In *The UFAW Handbook on the Care and Management of Laboratory Animals* (ed. Poole T), 2006; pp. 313–330. 7th ed. British: Blackwell Science.
15. Marques EB, Barros RBM, Rocha NN, Scaramello CBV. Aging and cardiac, biochemical, molecular and functional changes: experimental study. *Int J Cardiovasc Sci.* 2015; 1(1), 42–50. doi: 10.5935/2359-4802.20150007
16. 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. doi: 10.1016/j.echo.2005.10.005
17. Sousa JMA, Hermann JLV, Guimarães JB, Menezes PPO, Carvalho ACC. Avaliação das Pressões Sistólica, Diastólica e Pressão de Pulso como Fator de Risco para Doença Aterosclerótica Coronariana Grave em Mulheres com Angina Instável ou Infarto Agudo do Miocárdio sem Supradesnivelamento do Segmento ST. *Arq Bras Cardiol.* 2004; 82 (5), 426–429. doi: 10.1590/S0066-782X2004000500005
18. Fritz M, Rinaldi G. Influence of nitric oxide-mediated vasodilation on the blood pressure measured with the tail-cuff method in the rat. *J Biomed Sci.* 2007; 14(6), 757–765. doi: 10.1007/s11373-007-9191-1
19. Molnar AM, Servais S, Guichardant M, *et al.* Mitochondrial $H_2O_2$ production is reduced with acute and chronic eccentric exercise in rat skeletal muscle. *Antioxid Redox Signal.* 2006; 8(3–4), 548–558. doi: 10.1089/ars.2006.8.548
20. Wonders KY, Hydock DS, Hayward R. Time-course of changes in cardiac function during recovery after acute exercise. *Appl Physiol Nutr Metab.* 2007; 32(6), 1164–1169. doi: 10.1139/H07-127
21. Marques EB, Motta NAV, Kummerle AE, *et al.* In vivo effect of LASSBio-785, a lipid-lowering and anti-inflammatory agent, on cardiac $Ca^{2+}$-ATPases from hypercholesterolemic rats. *Int J Cardiol.* 2015; 201, 282–284. doi: 10.1016/j.ijcard.2015.08.091
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972; 18(6), 499–502.
23. Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA.* 1986; 256(20), 2835–2838. doi: 10.1001/jama.1986.03380200073024
24. Reaven G. Metabolic syndrome: pathophysiology and implications for management of cardiovascular disease. *Circulation.* 2002; 106(3), 286–288. doi: 10.1161/01.CIR.0000019884.36724.D9
25. Toste FP, de Moura EG, Lisboa PC, Fagundes AT, de Oliveira E, Passos MC. Neonatal leptin treatment programmes leptin hypothalamic resistance and intermediary metabolic parameters in adult rats. *Br J Nutr.* 2006; 95(4), 830–837. doi: 10.1079/BJN20061726
26. McIntosh J, Anisman H, Merali Z. Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects. *Dev Brain Res.* 1999; 113(1–2), 97–106. doi: 10.1016/S0165-3806(99)00005-X
27. Wit JM, Boersma B. Catch up growth: definition, mechanisms, and models. *J Pediatr Endocrinol Metab.* 2002; 15 (Suppl. 5), 1229–1241.
28. Huxley R, Mendis S, Zheleznyakov E, Reddy S, Chan J. Body mass index, waist circumference and waist:hip ratio as predictors of cardiovascular risk – a review of the literature. *Eur J Clin Nutr.* 2010; 64(1), 16–22. doi: 10.1038/ejcn.2009.68
29. Franco JG, Lisboa PC, Lima NS, *et al.* Resveratrol attenuates oxidative stress and prevents steatosis and hypertension in obese rats programmed by early weaning. *J Nutr Biochem.* 2013; 24(6), 960–966. doi: 10.1016/j.jnutbio.2012.06.019
30. Murad MH, Hazem A, Coto-Yglesias F, *et al.* The association of hypertriglyceridemia with cardiovascular events and pancreatitis: systematic review and meta-analysis. *BMC Endocr Disord.* 2012; 12, 2. doi: 10.1186/1472-6823-12-2
31. Borén J, Matikainen N, Adiels M, Taskinen MR. Postprandial hypertriglyceridemia as a coronary risk factor. *Clin Chim Acta.* 2014; 431, 131–142. doi: 10.1016/j.cca.2014.01.015
32. Havel RJ, Hamilton RL. Hepatocytic lipoprotein receptors and intracellular lipoprotein catabolism. *Hepatology.* 1988; 8(6), 1689–1704. doi: 10.1002/hep.1840080637
33. Brown MS, Goldstein JL. Biomedicine. Lowering LDL – not only how low, but how long? *Science.* 2006; 311(5768), 1721–1723. doi: 10.1126/science.1125884
34. Varbo A, Benn M, Smith GD, Timpson NJ, Tybjaerg-Hansen A, Nordestgaard BG. Remnant cholesterol, low-density lipoprotein cholesterol, and blood pressure as mediators from obesity to ischemic heart disease. *Circ Res.* 2015; 116 (4), 665–673. doi: 10.1161/CIRCRESAHA.116.304846
35. Klop B, Elte JW, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients.* 2013; 5(4), 1218–1240. doi: 10.3390/nu5041218
36. Rizzo M, Otvos J, Nikolic D, Montalto G, Toth PP, Banach M. Subfractions and subpopulations of HDL: an update. *Curr Med Chem.* 2014; 21(25), 2881–2891. doi: 10.2174/0929867321666140414103455
37. Gogonea V. Structural insights into high density lipoprotein: old models and new facts. *Front Pharmacol.* 2016; 6, 318. doi: 10.3389/fphar.2015.00318
38. Dobiásová M. Atherogenic index of plasma [log(triglycerides/HDL-cholesterol)]: theoretical and practical implications. *Clin Chem.* 2004; 50(7), 1113–1115. doi: 10.1373/clinchem.2004.033175
39. Sayın S, Kutlu R, Koçak A. The relationship between atherogenic index of plasma and major risk factors of cardiovascular disease in obese and non-obese individuals. *Eur Res J.* 2019; 5(4), 678–685. doi: 10.18621/eurj.414561
40. Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB. O tecido adiposo como centro regulador do metabolismo. *Arq Bras Endocrinol Metab.* 2006; 50(2), 216–229. doi: 10.1590/S0004-27302006000200008
41. 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. doi: 10.1055/a-0966-8784
42. Lima NS, Moura EG, Franco JG, *et al.* Developmental plasticity of endocrine disorders in obesity model primed by early weaning in dams. *Horm Metab Res.* 2013; 45(1), 22–30. doi: 10.1055/s-0032-1323703
43. Nagueh SF, Appleton CP, Gillebert TC, *et al.* Recommendations for the evaluation of left ventricular diastolic function by echocardiography. *Eur J Echocardiogr* 2009; 10(2), 165–193. doi: 10.1016/j.echo.2008.11.023
44. Hoss AJ, Petterson CHP, Scherer L. Miocardiopatia hipertrófica. Papel da ecocardiografia doppler no diagnóstico e na orientação terapêutica. *Arq Bras Cardiol* 1998; 70(4), 301–306. doi: 10.1590/S0066-782X1998000400012
45. Boon JA. *Manual of Veterinary Echocardiography*, 1998; pp. 478. Williams and Wilkins, Baltimore.
46. Rodrigues B, Figueroa DM, Mostarda CT, Heeren MV, Irigoyen MC, De Angelis K. Maximal exercise test is a useful method for physical capacity and oxygen consumption determination in streptozotocin-diabetic rats. *Cardiovasc Diabetol.* 2007; 6 (38). doi: 10.1186/1475-2840-6-38

47. Cohen-Solal A, Beauvais F, Tabet JY. Physiology of the abnormal response of heart failure patients to exercise. *Curr Cardiol Rep.* 2004; 6(3), 176–181. doi: 10.1007/s11886-004-0020-0
48. Otto MEB, Pereira MM, Beck ALS, Milani M. Correlação da função diastólica com a capacidade máxima de exercício ao teste ergométrico. *Arq. Bras. Cardiol.* 2011; 96(2), 107–113. doi: 10.1590/S0066-782X2011005000004
49. Little WC, Kitzman DW, Cheng CP. Diastolic dysfunction as a cause of exercise intolerance. *Heart Fail Rev.* 2000; 5(4), 301–306. doi: 10.1023/a:1026503028065
50. Tractenberg SG, Levandowski M, de Azeredo LA *et al.* An overview of maternal separation effects on behavioural outcomes in mice: evidence from a four-stage methodological systematic review. *Neurosci Biobehav Rev.* 2016; 68, 489–503. doi: 10.1016/j.neubiorev.2016.06.021
51. Kikusui T, Ichikawa S, Mori Y. Maternal deprivation by early weaning increases corticosterone and decreases hippocampal BDNF and neurogenesis in mice. *Psychoneuroendocrinology* 2009; 34(5), 762–772.
52. Huang BS, White RA, Ahmad M, Leenen F. Role of brain corticosterone and aldosterone in central angiotensin II-induced hypertension. *Hypertension.* 2013; 62, 564–571. http://dx.doi.org/10.1161/HYPERTENSIONAHA.113.01557
53. Dickstein K, Cohen-Solal A, Filippatos G, *et al.* ESC Committee for Practice Guidelines (CPG). ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the diagnosis and treatment of acute and chronic heart failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur J Heart Fail.* 2008; 10(10), 933–989. doi: 10.1016/j.ejheart.2008.08.005
54. Montera MW, Almeida RA, Tinoco EM *et al.* Sociedade Brasileira de Cardiologia. II Diretriz Brasileira de Insuficiência Cardíaca Aguda. *Arq Bras Cardiol.* 2009; 93(3 Suppl. 3), 1–65. doi: 10.1590/S0066-782X2009001900001