Effect of maternal dyslipidaemia on the cardiorespiratory physiology and biochemical parameters in male rat offspring

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Abstract

The present study evaluated the effects of maternal dyslipidaemia on blood pressure (BP), cardiorespiratory physiology and biochemical parameters in male offspring. Wistar rat dams were fed either a control (CTL) or a dyslipidaemic (DLP) diet during pregnancy and lactation. After weaning, both CTL and DLP offspring received standard diet. On the 30th and 90th day of life, blood samples were collected for metabolic analyses. Direct measurements of BP, respiratory frequency (RF), tidal volume (VT) and ventilation (VE) under baseline condition, as well as during hypercapnia (7% CO₂) and hypoxia (KCN, 0.04%), were recorded from awake 90-d-old male offspring. DLP dams exhibited raised serum levels of total cholesterol (TC) (+4-fold), TAG (2-fold), VLDL + LDL (7-fold) and reduced HDL-cholesterol (2-fold). In the spectral analysis, the DLP group showed augmented low-frequency (LF) power and LF:high-frequency (HF) ratio under baseline condition, as well as during hypercapnia and hypoxia. In conclusion, the present data show that maternal dyslipidaemia alters cardiorespiratory physiology and may be a predisposing factor for hypertension at adulthood.

Key words: Maternal dyslipidaemia: Biochemical parameters: Hypertension: Chemosensitivity

Arterial hypertension and metabolic disorders remain the main risk factors for cardiovascular diseases and are associated with high morbidity and mortality rates worldwide(1,2). This indicates that a better understanding of the underlying mechanisms causing hypertension and metabolic impairment is essential for effective interventions and prevention strategies.

The excessive consumption of fat and sugar, usually described as the westernised lifestyle, has contributed to the growing incidence of metabolic disorders such as dyslipidaemia and type 2 diabetes(3). It has been established that metabolic disorders, especially during pregnancy and early life, enhance susceptibility to the development of cardiometabolic diseases(4-7). For example, offspring from dams exposed to a high-fat diet during pregnancy exhibit hypertension and metabolic alterations in adult life(8,9).

Maternal dyslipidaemia is a serious metabolic disorder with a limited therapeutic approach. It has been described as an important risk factor for fetal death, preterm birth and with adverse effects on offspring development(10,11). Increasing evidence suggests that a maternal dysmetabolic condition, such as maternal dyslipidaemia, may compromise placental function and impair the fetus–placental supply through elevated oxidative stress(12), pro-inflammatory markers(13) and altered lipid and glucose handling(14).

Abbreviations: ECG, electrocardiogram; HF, high frequency; HR, heart rate; ITT, insulin tolerance test; LF, low frequency; MAP, mean arterial pressure; MDA, malondialdehyde; OGTT, oral glucose tolerance test; RF, respiratory frequency; TC, total cholesterol; VE, ventilation; VT, tidal volume.

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Previous studies have shown that the increase in pro-inflammatory markers and imbalance in oxidative stress can alter the mechanisms of blood pressure (BP) control, such as: (i) decrease in baroreflex sensitivity(15), (ii) respiratory chemosensitivity dysregulation(16–18) and (iii) sympathetic hyperactivity(19,20).

It has been also established that elevated sympathetic activity is involved in the generation of hypertension(21,22) and is associated with metabolic disorders(23–25). It is reported that the sympathetic nervous system may be modulated via central and peripheral respiratory chemoreceptors, evoking reflex responses of sympatho-excitation and tachypnoea(26,27). However, it remains unknown whether a sympathetic hyperactivity, in part modulated by raised respiratory chemosensitivity, could be a potential mechanism underlying maternal dyslipidaemia-induced hypertension and metabolic disorders.

Thus, in the present study, we examined the effects of maternal dyslipidaemia during pregnancy and lactation on the cardiorespiratory physiology and glucose-lipid biochemical parameters in male offspring. Our hypothesis was that a sympathetic hyperactivity associated with higher respiratory chemosensitivity could be a potential hidden basis for the development of arterial hypertension and metabolic disorder in adult male offspring from dams exposed to a dyslipidaemic (DLP) diet during pregnancy and lactation.

**Methods**

Experimental procedures were performed in accordance with the revised guide for the care and use of laboratory animals(28). The experimental protocol was submitted and approved by the institutional animal care and use committee (CEU-UFPB protocol 01/2016) of the Federal University of Paraíba, Brazil.

**Animals and experimental design**

In all, ten virgin female Wistar rats (Rattus norvegicus) were maintained at room temperature (22 ± 1°C) with a controlled light–dark cycle (dark 06.00–18.00 hours). A standard laboratory chow diet (52% carbohydrate, 21% protein and 4% lipids; Purina Agriband) and water were given *ad libitum*. At 90 d of age, rats were subjected to dietary manipulations: control (CTL) and DLP experimental groups were formed with two rats per litter to ensure standardised nutrition until weaning. The CTL group, n = 5) and DLP diets (DLP group, n = 5) were offered *ad libitum* during 1 week. Then, the rats were mated in a group, and female and male offspring were housed separately (four per cage) and had free access to a normal diet until the beginning of the experimental phase. The CTL and DLP experimental groups were formed with two rats from each litter. We first examined the impact of maternal dyslipidaemia on serum glucose and lipid parameters in 30-d-old rats. Then, the male rats were maintained in the protocol to access the long-term effect of maternal dyslipidaemia on biochemical parameters and cardiorespiratory physiology at day 90. Female offspring were not included in the present study.

**Effects of the dyslipidaemic diet during pregnancy and lactation in dams**

From the 1st day of pregnancy to the end of the lactation, the body weight and food intake of each dam were measured daily using a digital scale (model AS-1000; Marte) having an error range of 0.1 g. Food intake was monitored by measuring the difference between the amount of food put in the cage and the amount remaining after 24 h. The results of body weight and food consumption were compiled and expressed as weekly consumption.

At 24 h after weaning, the dams were fasted overnight and then an oral glucose tolerance test (OGTT) was performed. Then, 24 h after the OGTT, the dams were again fasted overnight and an insulin tolerance test (ITT) was performed after an intraperitoneal injection of insulin (0.75 IU/kg body weight). Blood glucose concentrations were measured before (0 min) and after (30, 60, 90 and 120 min). Last, 48 h after the ITT, the dams were fasted overnight and euthanised by decapitation. The dams had remained on a DLP diet during these four days after weaning. Serum samples were collected for quantification of total cholesterol (TC), TAG and HDL with commercial kits (Biodin). The LDL and VLDL values were calculated by the use of previously described formulas(31).

The livers from both groups were collected for analysis of lipid accumulation. Liver tissue was excised, washed in saline solution (0.9% NaCl) and fixed in buffered formalin (10%) for 48 h. Then, the tissues were sectioned in microtome to obtain 8-µm-thick slices. A total of ten sections were stained with Oil Red O (Sigma) for 10 min. Sections were examined under light microscopy (4× and 20×) and representative photos were acquired for each group(32). Another ten different sections were also stained with haematoxylin–eosin and analysed under a conventional light microscope (Motic BA 200; Scientific Instrument Company).

**Measurement of somatic parameters in male offspring**

The body weight was recorded at birth and on the 21st, 30th, 60th and 90th days of age with an appropriate balance

The liver of all male offspring was collected after 24 h of weaning and the total lipid concentrations in liver were measured. Liver lipid extraction was performed according to the procedure of Folch et al.(33). Liver lipids were separated by thin-layer chromatography and quantified using a densitometer (Altima 2000; Biorad). The liver of all male offspring was collected after 24 h of weaning and the total lipid concentrations in liver were measured. Liver lipid extraction was performed according to the procedure of Folch et al.(33). Liver lipids were separated by thin-layer chromatography and quantified using a densitometer (Altima 2000; Biorad).

**Table 1. Nutritional composition of the diets (g/100 g diet)**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control diet (AIN-93G)</th>
<th>Dyslipidaemic diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (85%)*</td>
<td>20</td>
<td>19.86</td>
</tr>
<tr>
<td>Dextrin maize starch</td>
<td>13</td>
<td>15.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Maize starch</td>
<td>39.7</td>
<td>33.6</td>
</tr>
<tr>
<td>Soyabean oil</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Animal fat (lard)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Non-hydrolysed vegetable fat</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Gallic acid (Sigma)</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Cholesterol (Sigma)</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Tent-butylated hydroxytoluene</td>
<td>0.014</td>
<td>0.014</td>
</tr>
</tbody>
</table>

* Casein showed 85% purity (85 g protein for each 100 g casein).
(model AS-1000). The body length of the pups was recorded by the measurement of external surfaces (length from nose-to-anus) with callipers (0.01 mm accuracy). The body weights and body lengths of pups were used to determine the anthropometrical parameter of the Lee index (cube root of body weight (g)/nose-to-anus length (cm))\(^4\).

Glucose and insulin tolerance test

At 30 and 80 d, the rats were fasted overnight for the OGTT and ITT tests. The OGTT was performed after administration of an oral glucose load (2 g/kg) via gavage. Blood samples were taken from the tail veins before glucose administration and, subsequently, at 15, 30, 60 and 90 min. At 24 h after OGTT, ITT was performed after an intraperitoneal injection of insulin (0.75 IU/kg body weight) and blood glucose concentrations were measured before (0 min) and after (30, 60, 90 and 120 min). All measurements of blood glucose concentration were performed with an Accu-Check glucometer (Bayer).

Biochemistry parameters and oxidative stress assay

At 30 and 80 d, offspring from both groups were anaesthetised with ketamine (80 mg/kg) and xylazine (10 mg/kg), and blood samples (approximately 2 ml) were collected by plexus retro-orbital disruption and centrifuged at 300 \( \times \) g, 25°C, during 15 min\(^4\). This experiment was performed 48 h after the glucose homeostasis experiments. Serum samples were collected for the quantification of TC, HDL, VLDL, LDL, TAG and glucose with commercial kits. The concentration of malondialdehyde (MDA), an end product of lipid peroxidation, was measured as thiobarbituric acid to produce a red-coloured complex. Briefly, 400 \( \mu \)l of perchloric acid (7%) was added to 250 \( \mu \)l of serum, mixed and centrifuged at 600 \( \times \) g, 4°C, during 20 min. The supernatant was collected, added to 400 \( \mu \)l of thiobarbituric acid (0.6%), heated at 100°C during 1 h and read at 532 nm. A standard curve of MDA was constructed and the results were expressed as nmol of MDA/ml\(^3\).

Surgical procedure for blood pressure record in male rats at 90-d-old

At least 4 d after the plexus retro-orbital disruption experiments, BP measurements were performed as previously described\(^4\). For this experiment, eight rats used in each group come from four different litters. In brief, male (CTL, \( n = 8 \); DLP, \( n = 8 \)) rats at 90 d old were anaesthetised with ketamine (80 mg/kg) and xylazine (10 mg/kg) to insert polyethylene catheters in the femoral artery and vein. The catheters were tunneled through the back of the neck and ketoprofen (5 mg/kg) was injected subcutaneously. The offspring underwent a period of surgical recovery of 24 h until the BP record experiments began. Arterial pressure (AP) and heart rate (HR) were recorded in conscious animals connecting the arterial catheter to a pressure transducer (LabChart 7 Pro; ADInstruments).

Measurements and analysis of arterial blood pressure, ventilation and sympathetic tonus

The pulsatile AP was recorded for 40–50 min under basal conditions, and the values of the systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and HR were calculated by using the whole-body plethysmographic method\(^5\). Air flow was suspended for short periods (3 min), and breathing was captured by a pressure differential transducer connected to a signal amplifier (ML141 Spirometer, PowerLab and ADInstruments).

After recording the baseline ventilation measurements, the respiratory and cardiovascular responses to CO\(_2\) were induced by flushing a hypercapnic gas mixture (7% CO\(_2\), 21% O\(_2\) and N\(_2\) balance; Linde Gas) into the plethysmographic chamber, as previously described by our research group\(^6\). \( R_F \), tidal volume (\( V_T \)) and ventilation minute (\( V_{VT} \)) were determined in an air-conditioned room (control baseline) before and after the hypercapnic challenge. A period of 10 s was selected for the determination of mean \( R_F \). \( V_T \) was calculated using the formula described previously by Malan\(^5\) and \( V_{VT} \) acquired through product \( R_F \times V_T \). The cardiovascular analysis was performed during the flow of the hypercapnic gas mixture. A period of 30 s of BP and HR was selected at 1, 2, 3, 4 and 5 min of gas flow. The cardiorespiratory reflex was assessed by the difference between the baseline and response observed after hypercapnia stimulus (\( \Delta \)).

A period of 10 min after hypercapnic stimuli, a peripheral chemoreflex was activated through the intravenous injection of potassium cyanide (KCN; 40 mg/100 ml per rat; Merck) in accordance with previous reports\(^26,30\). The difference between MAP, HR and \( R_F \) baseline and the peak increased after the stimulus (\( \Delta MAP \), \( \Delta HR \) and \( \Delta R_F \)) was assessed for, respectively, sympathetic, parasympathetic and respiratory components.

Last, 10 min after the peripheral chemoreflex activation, the contribution of the sympathetic vascular tone to cardiovascular system was evaluated by an intravenous injection of the ganglionic blocker (hexamethonium; 30 mg/kg; Sigma-Aldrich\(^8\)). The sympathetic tonus was calculated by the difference between the MAP after the blocker and the baseline MAP.

All of the data were analysed offline with the use of appropriate software (LabChart 7 Pro; ADInstruments), and at the end of the research period the animals were euthanised with 0.5 ml overdose of ketamine (endovenously). All protocols performed for the BP and respiratory parameter experiments are shown in Fig. 1.

Electrocardiogram analysis

ECG recordings were processed by computer software (ECG analysis module for LabChart Pro; ADInstruments) for automatic detection of the R waves and beat-by-beat calculation of RR interval. For detection of ECG, 12 ms was used to determine typical QRS width and for R waves at least 100 ms apart. For ECG analysis, 70 ms was used for the maximum PR and 90 ms for maximum RT.
Spectral analysis

The cardiovascular autonomic evaluation was performed using the frequency domain analysis of the SAP and pulse interval (PI) by an appropriate software program (CardioSeries-v.2.4; www.danielpenteado.com). The spectra were integrated in the low-frequency (LF, 0-0.08 Hz) and the high-frequency (HF) bands (0.08-0.8 Hz). To assess the sympathovagal index, the LF: HF ratio of the variability was calculated. In addition, the spontaneous baroreflex sensitivity was calculated through a sequence method.

Statistical analysis

Each experimental group included at least one or two animals from each litter. Bartlett’s test was performed to evaluate data homogeneity as to respiratory, cardiovascular and biochemical parameters, and statistical results supported the use of unpaired Student’s t tests. Two-way ANOVA and Bonferroni’s post hoc test were used for analysis of food intake, body weight, OGTT and ITT during pregnancy and lactation. Statistical analysis was conducted with GraphPad Prism 5 program for Windows (GraphPad Software®, Inc.). The values are presented as means with their standard errors. Values of $P < 0.05$ were considered as statistically significant.

Results

Food intake, body weight and metabolic profile in dams exposed to a dyslipidaemic diet during pregnancy and lactation

As observed in Fig. 2, after weaning, DLP dams presented significantly raised serum levels of TC (4-fold), TAG (2-fold), VLDL+LDL (7-fold) and reduced HDL-cholesterol (2.4-fold) when compared with CTL dams. We also observed that the atherogenic indexes TAG/HDL, TC/HDL and VLDL+LDL/HDL were all increased in the DLP dams ($P < 0.05$, Fig. 2).

After weaning, the DLP dams exhibited higher hepatic lipid accumulation than the CTL dams (Fig. 2). We measured the food intake and body weight during pregnancy and lactation. Despite the reduced food intake in the last 2 weeks of lactation, there were no differences in the body weight and total weight gain between the CTL and DLP dams (online Supplementary Fig. S1).

In order to evaluate whether the DLP diet during pregnancy and lactation would alter the regulation of glucose homeostasis, we conducted OGTT and ITT at the end of lactation.

As shown in Fig. 3, the DLP dams exhibited higher serum glucose in 15 (0-7 (SEM: 1.7) v. 6.2 (SEM: 0.4) mmol/l, $P < 0.05$) and 30 min (9.2 (SEM: 0.8) v. 6.3 (SEM: 0.5) mmol/l, $P < 0.05$) of OGTT when compared with CTL. In agreement, the DLP dams presented greater AUC 90 min after OGTT ($P < 0.05$, Fig. 3).

Similarly, the DLP dams also exhibited higher serum glucose after intraperitoneal insulin injection in 30 (3.4 (SEM: 0.16) v. 2.2 (SEM: 0.21) mmol/l, $P < 0.05$) and 120 min (3.12 (SEM: 0.56) v. 1.5 (SEM: 0.19) mmol/l, $P < 0.05$) and consequently greater AUC 120 min after the injection of insulin ($P < 0.05$, Fig. 3).

Together, these results show that excessive consumption of saturated trans-fatty acids and cholesterol during pregnancy and lactation induce a maternal dysmetabolic condition characterized by dyslipidaemia and altered glucose tolerance and insulin sensitivity.

Short-term effect of maternal dyslipidaemia on somatic growth and biochemical parameters in juvenile male offspring

Fig. 4 shows the somatic growth curves from birth to 90 days. Body weight in the rats exposed to maternal dyslipidaemia was lower at birth (5.2 (SEM: 0.08) v. 5.6 (SEM: 0.1) g, $P < 0.05$, Fig. 4) and weaning (39.5 (SEM: 0.7) v. 44.5 (SEM: 0.6) g, $P < 0.05$, Fig. 4) when compared with the CTL rats. From weaning to 60 d, the body weight was similar between groups. However, at 90 d, the DLP rats exhibited significantly higher body weight (362 (SEM: 4.7) v. 336 (SEM: 4.9) g, $P < 0.05$, Fig. 4) in comparison with the CTL rats. Despite the changes in body weight, no change was observed in body length ($P > 0.05$; Fig. 4) and Lee index ($P > 0.05$; Fig. 4).

Regarding the short-term effects of maternal dyslipidaemia on glucose handling in male offspring (30 d after birth), despite the fact that the DLP offspring exhibited increased glucose at 15 min (4.3 (SEM: 0.3) v. 3.5 (SEM: 0.19) mmol/l, $P < 0.05$, Fig. 5) and a tendency for increased AUC of OGTT ($P = 0.06$; Fig. 5), the fasting serum glucose concentration was similar between groups ($P > 0.05$, Fig. 5). Serum lipid profiles of the male offspring reveal that serum TC and VLDL cholesterol concentrations were increased in DLP rats ($P < 0.05$; Fig. 5). The TAG and HDL-cholesterol concentrations were not different between CTL and DLP offspring (Fig. 5).

Long-term effect of maternal dyslipidaemia on the biochemical and cardiorespiratory parameters in male rat offspring

Investigating the long-term effects of maternal dyslipidaemia on glucose handling (80 d of age), insulin sensitivity and the serum
Fig. 2. Fasting serum total cholesterol (TC) (a), TAG (b), VLDL + LDL (c) and HDL (d), atherogenic index (e), hepatic (f) and intestine (g) lipid accumulation in dams that received a control (CTL) or dyslipidaemic (DLP) diet during pregnancy and lactation. Groups: CTL group (n=5) and DLP group. Values are means with their standard errors represented by vertical bars. * P<0.05 v. CTL using unpaired Student’s t test.

Fig. 3. Oral glucose tolerance test (a) and insulin tolerance test (b) in dams that received a control (CTL) or dyslipidaemic (DLP) diet during pregnancy and lactation. Groups: CTL group (n=5, – – – – ) and DLP group (n=5, – – – – ). Values are means with their standard errors represented by vertical bars. * P<0.05 v. CTL using two-way ANOVA, followed by Bonferroni’s post hoc test or Student’s t test.
Maternal dyslipidaemia induces hypertension in male offspring

Lipid profile, no difference was observed between CTL and DLP male offspring \((P>0.05); \text{Fig. 6}\).

On examining male offspring at 90 d, we found that the DLP rats displayed an increase in SAP, DAP and MAP in comparison with the CTL group \((P<0.05); \text{Table 2}\). The arterial hypertension observed in our model was not associated with tachycardia, as evidenced by the similar baseline HR between the CTL and DLP groups \((P>0.05); \text{Table 2}\). Although BP and HR are interrelated components of the cardiovascular system, resting HR and BP do not necessarily increase at the same rate in hypertensive condition. The inverse proportion between BP and HR may be due to increased stiffness of the large arteries, such as the aorta and large elastic arteries. Spectral analysis using baseline BP recordings demonstrated that the LF range of SAP and the LF:HF ratio of the PI were augmented in the DLP group \((P<0.05); \text{Table 2}\). In relation to spontaneous baroreflex sensitivity, both the CTL and DLP rats exhibited similar baroreflex gain \((P>0.05); \text{Table 2}\). In addition, all ECG measures analysed, such as RR, PR, QRS and QT intervals and P duration, were not different between the CTL and DLP groups \((P>0.05); \text{Table 2}\).

Representative baseline recordings of pulsatile arterial pressure (PAP), MAP and HR in male rat offspring are shown in the online Supplementary Fig. S2.

Under the baseline condition, \(R_0\), \(V_T\) and \(V_E\) values were similar between the CTL and DLP male offspring \((P>0.05); \text{Fig. 7}\). Hypercapnia (\(\text{CO}_2\ 7\%\)) produced an increase in \(R_0\), \(V_T\) and \(V_E\) values in both the CTL and DLP groups (online Supplementary Fig. S2). In comparison with CTL offspring, the DLP rats exhibited higher values of \(\Delta R_0\) (Fig. 6) and \(\Delta V_E\) (Fig. 7), but no difference in \(\Delta V_T\) (Fig. 7) values after hypercapnia. Breathing recordings of representative rats from the CTL and DLP groups during the basal and hypercapnic conditions are shown in the online Supplementary Fig. S3. In addition, during the 5 min of hypercapnic gas flushing, the MAP (Fig. 7) was augmented in the DLP group when compared with the CTL group, but no difference in HR was observed (Fig. 7).

Peripheral chemoreflex activation produced pressor, bradycardic and tachypnoeic responses in both groups (Fig. 8). The increases in MAP \((P<0.05); \text{Fig. 8}\) and \(R_0\) \((P<0.05); \text{Fig. 8}\) were significantly greater in the DLP group than in the CTL group. In contrast, the magnitude of the decrease in HR showed a tendency towards reduction in the DLP rats \((P=0.06); \text{Fig. 8}\). Together, these results suggest that the DLP offspring exhibited increased sensitisation of cardiorespiratory chemoreceptors.

To test whether sympathetic tonus was augmented in DLP rats at 90 days, we evaluated the changes in MAP (\(\Delta \text{MAP}\)) after administration of a pharmacological ganglionic blocker in both groups. After the blockade, the DLP rats showed increased \(\Delta \text{MAP}\) \((P<0.05); \text{Fig. 9}\) when compared with CTL rats.
The quantification of serum MDA is summarised in Fig 10. MDA concentrations in DLP rats were increased at 30 d (936 K. S. L. Guimarães) and 90 d of age (P < 0.05; Fig. 10) when compared with the CTL rats.

### Discussion

Previous studies have reported that high dietary fat, cholesterol and sugar intake during pregnancy (14,35) or lactation (36) impair lipid and glucose metabolism in mouse and rat dams.

To the best of our knowledge, here we have demonstrated for the first time that dams fed a DLP diet during pregnancy and lactation periods exhibit high levels of cholesterol, TAG, atherogenic index, compromised glucose tolerance, insulin sensitivity and hepatic steatosis at weaning. These findings suggest that an excessive consumption of a diet rich in saturated trans-fatty acids and cholesterol during pregnancy and lactation may be a useful experimental model for studying the effects of dyslipidaemia in dams and in their offspring.

Similarly to previous studies, we have observed that pups from dams fed a DLP diet had low weight at birth and weaning, but recovered normal body weight at 30 d of age (14,35). Interestingly, a recent meta-analysis of clinical trials found that maternal dyslipidaemia during pregnancy was associated with an increased risk of preterm birth (10). Together, this evidence reveals that high levels of maternal lipid may increase the risk of low birth weight.

In adult life, DLP rats exhibited higher body weight in comparison with the CTL offspring. Both groups, however, presented a similar Lee index. Body fat composition was not analysed, so it is not possible to state that DLP offspring displayed obesity. Future studies investigating possible development of obesity will be focused in our next research.

### Table 2. Baseline parameters of blood pressure in male offspring at 90 d old from control and dyslipidaemic dams (Mean values with their standard errors)

<table>
<thead>
<tr>
<th></th>
<th>Male offspring</th>
<th>DLP group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTL group</td>
<td>DLP group</td>
</tr>
<tr>
<td><strong>Cardiovascular parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>127</td>
<td>155*</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>74</td>
<td>103*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>90</td>
<td>121*</td>
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<tr>
<td>HR (bpm)</td>
<td>381</td>
<td>346</td>
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<tr>
<td><strong>Spectral analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF (mmHg^2)</td>
<td>4.8</td>
<td>9.0*</td>
</tr>
<tr>
<td>HF (mmHg^2)</td>
<td>5.9</td>
<td>3.0</td>
</tr>
<tr>
<td>LF:HF</td>
<td>0.13</td>
<td>0.29*</td>
</tr>
<tr>
<td>BRS (ms/mmHg)</td>
<td>1.18</td>
<td>1.21</td>
</tr>
<tr>
<td><strong>ECG analysis</strong></td>
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<td></td>
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<tr>
<td>RR interval (s)</td>
<td>0.165</td>
<td>0.171</td>
</tr>
<tr>
<td>PR interval (s)</td>
<td>0.049</td>
<td>0.047</td>
</tr>
<tr>
<td>P duration (s)</td>
<td>0.030</td>
<td>0.028</td>
</tr>
<tr>
<td>QRS interval (s)</td>
<td>0.033</td>
<td>0.034</td>
</tr>
<tr>
<td>QT interval (s)</td>
<td>0.085</td>
<td>0.083</td>
</tr>
</tbody>
</table>

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per min; LF, low-frequency band; HF, high-frequency band; BRS, spontaneous baroreflex sensitivity; ECG, electrocardiogram.

* P < 0.05.
In our study, serum TC and LDL-cholesterol were maintained at a high level in DLP offspring at 30 d of age. It has been shown that impaired glucose tolerance associated with high serum levels of cholesterol, TAG and fatty liver are observed in pups from dams fed a high-fat or high-cholesterol diet during pregnancy or lactation\(^\text{36,37}\). These alterations possibly occur as a consequence of high cholesterol and fat content in the dams’ milk\(^\text{36}\).

In agreement with other studies that used maternal high-fat diet as a model\(^\text{9}\), our study showed that male offspring exposed to maternal dyslipidaemia during pregnancy and lactation developed arterial hypertension at 90 d of age. It should be noted, however, that another study reported that offspring from dams exposed to hypercholesterolaemia during pregnancy did not exhibit hypertension in adulthood\(^\text{89}\). However, in that study, BP was measured in anaesthetised rats, limiting the comparison with our results.

It is well accepted that the autonomic dysfunction, verified by excessive sympathetic tone and vagal withdrawal, alters the vascular reactivity and contributes to the development of hypertension in both hypertensive experimental models and hypertensive patients\(^\text{27,30,39,40}\).

The LF and HF ranges or the LF:HF ratio represent more accurately the sympathetic activity and autonomic modulation\(^\text{41}\). In addition to these methods for estimating sympathetic activity, we used an experimental protocol involving a ganglionic sympathetic blockade with hexamethonium. We observed in our study that LF power, LF:HF ratio and delta for arterial pressure variation (ΔMAP) after hexamethonium infusion were increased in the DLP offspring. Together, our results support the hypothesis that the arterial hypertension observed in male rats from dams exposed to dyslipidaemia during pregnancy and lactation is associated with sympathetic hyperactivity. It is interesting to note that increased LF power, LF:HF index and great delta arterial pressure variation after hexamethonium infusion are observed in hypertensive rats\(^\text{41}\) and onset hypertension in men\(^\text{42}\).
It has been reported that a baroreflex dysfunction could lead to a sympathetic over-activity and subsequent development of hypertension\(^{(43)}\). In addition, sympathetic outflow and BP have been shown to be modulated by the rhythmicity of the respiratory nervous system\(^{(44)}\). Part of this modulatory effect is influenced by the peripheral and central respiratory chemoreceptors. Activation of these chemoreceptors produces a powerful activation of the cardiorespiratory neuronal network and enhances the sympathetic and respiratory outflow\(^{(45)}\).

On analysing spontaneous baroreflex sensitivity, we found no differences between the groups, suggesting that baroreflex control, at least within the narrow range of the physiological variations of BP, is not altered in adult rats exposed to maternal dyslipidaemia. This result differed from another study showing a reduction in the baroreflex control in male offspring from dams fed a westernised diet\(^{(9)}\).

The critical window relevant for the control of breathing and chemosensory consists of that period when respiratory control

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**Fig. 8.** Representative recording of mean arterial pressure (MAP) and heart rate (HR) during chemoreflex activation (a) and evaluation of variation in MAP (b), HR (c) and respiratory frequency (d) during chemoreflex activation (KCN, 0.04%) in male offspring at 90 d old from dams exposed to maternal dyslipidaemia during pregnancy and lactation. Groups: control group (CTL, n 10) and dyslipidaemic group (DLP, n 10). Values are means with their standard errors represented by vertical bars. *P < 0.05 v. CTL using Student’s t test. bpm, Beats per min.

**Fig. 9.** Representative tracings of pulsatile arterial pressure (PAP) and mean arterial pressure (MAP) after hexamethonium (a) and evaluation of delta change of the MAP (b) in male offspring at 90 d old from dams exposed to maternal dyslipidaemia during pregnancy and lactation. Groups: control group (CTL, n 10) and dyslipidaemic group (DLP, n 10). Values are means with their standard errors represented by vertical bars. *P < 0.05 v. CTL using Student’s t test.
is still too immature and incapable of mounting an effective defence against acute respiratory challenges(46). A number of environmental factors such as stress stimuli or oxygen levels experienced during early life can elicit, in the short or long term, developmental plasticity in the respiratory control system, which may be exemplified through changes in baseline ventilation or the hypoxic/hypercapnic ventilatory response(46,47).

Our research group has convincingly demonstrated that nutritional insults during pregnancy and lactation can have an impact on the development of the respiratory phenotype, such as causing changes in baseline ventilation and hypoxic and hypercapnic ventilatory response(49). Here, we have demonstrated that cardiovascular and ventilatory reflex responses to hypercapnia and hypoxia intensified significantly in male rats exposed to dyslipidaemia during pregnancy and lactation.

Clinical and experimental studies have shown that high levels of inflammatory markers (e.g. IL-6 and TNF) associated with high reactive O₂ species (ROS) are found in the plasma and placenta from pregnant women with metabolic disorders or obesity(48). Together, pro-inflammatory cytokine and ROS could impair placental functioning and induce adverse effects on the fetus(13,49).

A recent study showed that inflammation-induced discharge of PGE₂ alters breathing patterns and increases ventilatory response to CO₂(50). Interestingly, Samarasinge and colleagues demonstrated that the ventilatory responses to hypoxia and hypercapnia were intensified in mouse offspring exposed to prenatal inflammation in the final days of gestation(51). It was also shown that oxidative stress augments chemoreflex sensitivity in rats exposed to chronic intermittent hypoxia(17). Our finding was that MDA levels, a biomarker used to assess oxidative stress, were increased in the serum of DLP offspring.

Considering the complementary approaches used in the present study, we propose, on the basis of our results, that environmental stress caused by maternal dyslipidaemia increases chemoreceptor sensitivity, which is a primary contribution to sympathetic hyperactivity. Then, these changes may reinforce the development of hypertension in maternal dyslipidaemia-induced hypertension model. It is, however, important to note that we investigated only male offspring. A growing number of studies have related that onset of hypertension evidenced in offspring from maternal nutritional insults dams is sex-dependent. The findings related that female offspring exposed to a nutritional insult during fetal life can be resistant to the development of hypertension in young adulthood(48,52). Our study was limited by the lack of pro-inflammatory cytokine and stress oxidative marker measurements in dams and of investigation of the molecular mechanisms associated with our physiological findings. These gaps will be investigated in future studies.

In conclusion, the present study shows that male offspring from DLP dams exhibit hypertension in adult life combined with sympathetic hyperactivity and autonomic responses to hypoxia and hypercapnia.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517003014

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