Poor pubertal protein nutrition disturbs glucose-induced insulin secretion process in pancreatic islets and programs rats in adulthood to increase fat accumulation

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Abstract

Similar to gestation/lactation, puberty is also a critical phase in which neuronal connections are still being produced and during which metabolic changes may occur if nutrition is disturbed. In the present study we aimed to determine whether peripubertal protein restriction induces metabolic programming. Thirty-day-old male rats were fed either a low protein (LP group) diet (4% w/w protein) or a normal protein (NP group) diet (23%) until 60 days of age, when they received the NP diet until they were 120 days old. Body weight (BW), food intake, fat tissue accumulation, glucose tolerance, and insulin secretion were evaluated. The nerve electrical activity was recorded to evaluate autonomous nervous system (ANS) function. Adolescent LP rats presented hypophagia and lower BW gain during the LP diet treatment (P<0.001). However, the food intake and BW gain by the LP rats were increased (P<0.001) after the NP diet was resumed. The LP rats presented mild hyperglycemia, hyperinsulinemia, severe hyperleptinemia upon fasting, peripheral insulin resistance and increased fat tissue accumulation and vagus nerve activity (P<0.05). Glucose-induced insulin secretion was greater in the LP islets than in the NP islets; however, the cholinergic response was decreased (P<0.05). Compared with the islets from the NP rats, the LP islets showed changes in the activity of muscarinic receptors (P<0.05); in addition, the inhibition of glucose-induced insulin secretion by epinephrine was attenuated (P<0.001). Protein restriction during adolescence caused high-fat tissue accumulation in adult rats. Islet dysfunction could be related to an ANS imbalance.

Key Words
- adolescence
- low-protein diet
- pancreatic islets
- insulin secretion

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Introduction

Such insults as hyper- or hypo-nutrition during early life induce serious health risks, including obesity, type 2 diabetes and cardiovascular diseases among others in adult individuals. It has been shown that protein malnutrition during gestation and/or lactation provokes alterations in metabolism when animals or humans reach adulthood. Those observations have supported Barker’s or the ‘Thrifty phenotype’ hypothesis, which postulates that undernutrition during pregnancy imposes metabolic changes in the fetus that will be shifted to a permanent economic metabolism, including in adult life (Hales & Barker 2001); however, if the baby is exposed to a nutritional abundance after birth, the metabolic syndrome is generated, as reported for several experimental models (Vickers et al. 2005, Velkoska et al. 2008, Bol et al. 2009) and epidemiological data from humans who underwent famine early in life (Ravelli et al. 1976, Huang et al. 2007). This phenomenon is also known as metabolic programming or metabolic imprinting.

There are strong associations between metabolic programming and functional and structural changes in the brain. Hypothalamic neuron dense areas related to body weight (BW) control are changed in adult rats that were malnourished during pregnancy (Mokler et al. 2007). Similar results were obtained in rats from dams that were protein restricted during gestation and lactation (Plagemann et al. 2000a,b). Pregnancy and lactation have been shown to be crucial for brain development; additionally, the developing brain hypothalamus of rodents is extremely dependent on the milk suckling phase, unlike in human beings. Compared with humans, rats showed a delay in brain development that involved lactation, as recently observed (Morgane et al. 2002, Delahaye et al. 2008). Therefore, this stage of life is particularly vulnerable to an energy metabolism imbalance when offspring reach adulthood (Velkoska & Morris 2011). Protein restriction early in life programs the adult metabolism toward several alterations through imprinting in the CNS (Resnick et al. 1979) and in relation to the peripheral system/tissues, such as the hypothalamus–pituitary–adrenal (HPA) axis (Lesage et al. 2006), skeletal muscle (Sampaio de Freitas et al. 2003), liver (Ozanne 1999) and endocrine pancreas (Reusens & Remacle 2006). The pancreatic β-cell mass and/or function suffer a great impact from metabolic imprinting caused by perinatal calorie/protein malnourishment (Fagundes et al. 2007, Inoue et al. 2009, de Oliveira et al. 2011).

Puberty is another phase when new neuron connections are being produced in the brain (Blakemore et al. 2010). The increase of sexual hormones during puberty is also a cause of structural and functional changes in the hypothalamus and HPA axis, which allows for modulation of BW control and metabolism when the individual reaches adulthood (Lurzel et al. 2011). A stressful stimulus during this very vulnerable phase can induce permanent changes in physiological functions (Pervanidou & Chrousos 2012). The goal of the present work was to test whether adolescence can be another critical window to provoke metabolic programming, including pancreatic islet dysfunction.

Materials and methods

Animals and protein restriction

Because the adolescence period in rats has been reported to last from either postnatal days 21–59 and/or from 28 to 42 (Spear 2000, Tirelli et al. 2003), in the present study, we chose the period of 30–60 days of age to cover the entire pubertal and adolescent periods.

Thirty-day-old male Wistar rats from 16 different litters were randomly chosen for the study protocol and were distributed into two groups (8 l/group). One group of rats were fed a normal protein (NP; 23% w/w protein) diet ad libitum (Nuvital, Curitiba, PR, Brazil) throughout the experimental period; these experiments. Throughout the experimental period, the rats (five rats per cage) were maintained under controlled conditions of temperature (22±2 °C) and photoperiod (0700–1900 h), with water and food provided ad libitum.

The Ethical Committee for Animal Experiments of the State University of Maringá, which adheres to the Brazilian Federal Law, approved the protocol.

BW gain and food intake

Since the beginning of the treatment (when the rats are 30 days old), the chow consumption and BW of the rats were recorded every 2 days. The food intake values were
calculated as the difference between the amount of diet remaining and the total provided, which was divided by the number of days and the number of rats in the cages (Vicente et al. 2004). The BW gain was also calculated by subtracting the BW values at 30 days of age (beginning of diet treatment) from each daily BW value of each rat from both experimental groups throughout the diet treatment. The area under the curve (AUC) for the entire observation period (30–120 days) was also calculated.

Intravenous glucose tolerance test

At 120 days old, rats underwent a surgical procedure to perform the intravenous glucose tolerance test (ivGTT) as previously described (de Oliveira et al. 2011). Subtraction of the fasting plasma glucose and insulin concentration was used to obtain the glycemia ($\Delta_{\text{glycemia}}$) and insulinemia increments ($\Delta_{\text{insulinemia}}$) for each time period of the ivGTT. Increases in total $\Delta_{\text{glycemia}}$ and $\Delta_{\text{insulinemia}}$ were calculated with the glycemia and/or insulinemia AUC for the 45 min of the ivGTT.

The blood samples collected previously for the ivGTT were used to assess the fasting glycemia, insulinemia and leptinemia. Plasma obtained from the blood samples was stored at −20 °C for the subsequent determination of the following: the glucose concentration by the glucose oxidase method with a commercial kit (Gold Analisa; Belo Horizonte, MG, Brazil); the insulin concentration by an RIA with a gamma counter (Wizard2 Automatic Gamma Counter, 2470 Model; PerkinElmer, Shelton, CT, USA) with human insulin as a standard, an anti-rat insulin antibody (Sigma–Aldrich), and 125I-labeled recombinant human insulin (PerkinElmer); and leptinemia by a commercial ELISA kit (Enzo Life Sciences, Plymouth Meeting, PA, USA). The intra- and interassay coefficients of variation were respectively 12.2 and 9.8% for insulin and 5.9 and 7.2% for leptin.

Insulin sensitivity index

Because the insulin sensitivity index (ISI) performed by Matsuda & DeFronzo (1999) generates a reasonable approximation of the whole-body insulin sensitivity, we used this index with a slight modification to measure the whole-body insulin sensitivity in our experimental model. To calculate the ISI, we performed the following calculation: $\text{ISI} = 10^4/((\text{fasting glycemia} \times \text{fasting insulinemia}) \times (\text{AUC}_{\text{glycemia}} \times \text{AUC}_{\text{insulinemia}})).$

Electrical activity of the vagus nerve and sympathetic superior cervical ganglia

The rate of vagus and sympathetic nerve firing of rats for each group was recorded as previously reported (de Oliveira et al. 2011).

Pancreatic islet isolation

Pancreatic islets were isolated by a collagenase technique as previously described (Gravena et al. 2002) with several adaptations. Rats at 120 days of age were decapitated, and the abdominal wall was cut open. Next, 8 ml of Hanks buffered saline solution (HBSS; (mmol/l): NaCl, 136.9; KCl, 5.4; MgSO4·7H2O, 0.81; Na2HPO4, 0.34; KH2PO4, 0.44; CaCl2·2H2O, 1.26; NaHCO3, 4.16; glucose, 0.06; BSA, 15 and ((v/v); 95% O2, 5% CO2, mixed)/10 min, pH 7.4) containing ((w/v) 0.1% collagenase type XI, 5% BSA and 0.6% N-(2-hydroxyethylpiperazine)-N’-(2-ethanesulphonic acid; HEPES; Sigma–Aldrich) was injected into the rats’ common bile duct. The pancreas, which was swollen with the collagenase solution, was quickly excised and incubated in Becker glass for 17–18 min (NP rats) or 11–12 min (LP rats) at 37 °C. The suspension was then thrown away and the pancreas was washed with HBSS for three continuous washings. Islets were collected with the aid of a stereomicroscope. At least three rats each from three different litters for each experimental group were used for each experimental procedure.

Insulin secretion stimulation

To adapt isolated islets to a baseline glucose concentration (5.6 mmol/l), they were preincubated for 60 min in 1 ml of normal Krebs–Ringer solution ((mmol/l): NaCl, 115; NaHCO3, 24; KCl, 1.6; MgCl2·6H2O, 1; CaCl2·2H2O, 1; BSA, 15; pH 7.4) containing 5.6 mmol/l glucose. This solution was gassed with (v/v) 95% O2, 5% CO2 (mixed) to maintain pH 7.4. After the preincubation, the islets were incubated with different glucose concentrations (5.6, 8.3, 11.1, 16.7, 20.0 and 24.0 mmol/l) for an additional 60 min. The supernatants from the incubations were collected and stored for further insulin measurements.

To study the muscarinic acetylcholine receptor (mACHr) function, the islets were incubated after preincubation for a further 60 min in the Krebs–Ringer solution containing either 8.3 mmol/l glucose or 8.3 mmol/l glucose plus 10 μmol/l acetylcholine in the presence of 10 μmol/l neostigmine to avoid acetylcholinesterase action in the islets. Additionally, a nonselective
mAChR antagonist, 10 μmol/l atropine, was also used. To block the mAChR subtype M₁, M₂ and M₃ function, their antagonists were used as follows: 100 μmol/l pirenzepine, 1 μmol/l methoctramine and 100 μmol/l 4-diphenyl-acetoxy-N-methylpiperidine methiodide (4-DAMP) respectively. The doses of antagonists were previously tested, and concentrations were chosen that induced at least 20% inhibition or that potentiated the insulinotropic effect of 10 μmol/l acetylcholine in islets incubated with 8.3 mmol/l glucose in the presence of 10 μmol/l neostigmine.

To study the adrenoceptors function, other batches of islets from both groups were preincubated with 5.6 mmol/l glucose and then stimulated with a high glucose concentration (16.7 mmol/l), either in the presence of 1 μmol/l epinephrine and an α₂ adrenoceptor antagonist, yohimbine (10 μmol/l) or in the presence of epinephrine and a β₂ adrenoceptor antagonist, propranolol (1 μmol/l). Similar to the muscarinic receptor studies, the doses of antagonists were previously tested, and concentrations that induced at least 50% inhibition or potentiated the insulin secretion stimulated with 16.7 mmol/l glucose were chosen.

All of the drugs described above for studying the muscarinic and adrenergic functions were purchased from Sigma–Aldrich.

**Fat pad store measurement**

After all the experimental procedures, the rats were killed after an anesthetic charge (thiopental, 45 mg/kg BW) by decapitation. The fat pad stores (retroperitoneal, periepithelial, visceral and inguinal) were removed and weighed to assess the state of obesity.

**Statistical analyses**

Results are given as the means±S.E.M. and were subjected to Student’s t-test. P<0.05 was considered statistically significant. The tests were performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

**Results**

**Effects of an LP diet treatment during adolescence on BW gain, food intake, fat pad store, and plasma parameters**

Protein restriction during adolescence caused a 10% decrease in the BW of 120-day-old rats compared with adult animals that were well nourished with protein during adolescence (P<0.001, n=32–40); however, the body length was not affected (P=0.299, n=20–29) as shown in Table 1. The ISI of the LP rats was 11.1% (P<0.01, n=10) lower than NP ones, and the fasting plasma glucose, insulin and leptin concentrations from the treated rats were increased by 7, 32 and 128% respectively compared with the untreated rats (P<0.05, n=5–19), as shown in Table 1.

Figure 1A shows that the poor-protein diet that was provided to rats between 30 and 60 days old caused no BW changes; however, when the treatment was finished, the BW increased. After calculating the AUC, the BW of the LP group was 35% lighter than the BW of the NP rats (P<0.001, n=20–35), as shown in the inset of Fig. 1A. When the BW gain was calculated for 60- to 120-day-old rats, the protein restriction provoked a twofold increase compared with the untreated rats (P<0.001, n=20–35; Fig. 1B including the inset, which shows the respective AUCs).

During the protein malnourishment from 30 to 60 days of age, the rats ate 30% less than the rats that were treated with the NP diet (P<0.001, n=5–7 l; Fig. 2A including the respective AUC in the inset); however, after the treatment at 60–120 days of age, the LP rats ate 28% more than the NP animals (P<0.001, n=5–7 l), as shown in Fig. 2B, which includes insets showing the respective AUCs.

Figure 3 shows that an LP treatment during adolescence caused an increase in the fat tissue accumulation. The upper left panel shows that the retroperitoneal fat pad increased 32% in the LP rats (P<0.001, n=30).

**Table 1** The effects of an LP diet treatment during adolescence on adult rats. The data represent the means±S.E.M. from five to eight different litters

<table>
<thead>
<tr>
<th>Biometric and biochemistry parameters</th>
<th>NP</th>
<th>n</th>
<th>LP</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>416.5±6.36</td>
<td>32</td>
<td>375.9±4.92</td>
<td>40</td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>23.25±0.13</td>
<td>20</td>
<td>23.06±0.12  (NS)</td>
<td>29</td>
</tr>
<tr>
<td>Fasting glycemia (mmol/l)</td>
<td>4.95±0.13</td>
<td>13</td>
<td>5.30±0.06*</td>
<td>16</td>
</tr>
<tr>
<td>Fasting insulinemia (pmol/l)</td>
<td>48.22±3.44</td>
<td>14</td>
<td>63.71±5.17*</td>
<td>19</td>
</tr>
<tr>
<td>Fasting leptinemia (pmol/l)</td>
<td>135.62±29.38</td>
<td>5</td>
<td>310.00±69.38*</td>
<td>5</td>
</tr>
<tr>
<td>ISI</td>
<td>123.9±3.09</td>
<td>10</td>
<td>110.1±2.99†</td>
<td>10</td>
</tr>
</tbody>
</table>

Student’s t-test was used between the groups. *P<0.05, †P<0.01, and ‡P<0.001. NS, no significant difference.
The periepididymal tissue from the LP animals accumulated 26% more fat than the NP ones ($P<0.01$, $n=30$), as shown in the upper right panel. The lower left panel shows that the fat from the inguinal tissue increased 31% in the LP rats compared with the NP animals ($P<0.01$, $n=30$), while the LP visceral tissue was augmented 21% compared with the NP group ($P<0.01$, $n=30$), as shown in the lower right panel.

Plasma glycemia and insulinemia during the ivGTT

During the entire ivGTT, when the glycemia and insulinemia increments were performed, the only significant point was in the peak of glycemia, as well as in the last point of the insulinemia (Fig. 4); however, the AUC, which is shown in the inset of Fig. 4A, revealed that the glycemia was reduced by 14% compared with the NP animals ($P<0.05$, $n=12–15$). As shown in the inset of Fig. 4B, in the same test, the plasma insulin concentration was 19% higher in the LP animals than in the NP rats ($P<0.05$, $n=12–15$).

Glucose-induced insulin secretion and muscarinic/adrenergic regulation on this process in isolated pancreatic islets

Glucose induced the insulin secretion in a dose-dependent manner in islets from both animal groups; however, the pancreatic islets from the LP rats had a shift in the curve to the left compared with the curve of the islets from the NP animals. The LP islets that were stimulated by high glucose doses secreted twice as much insulin compared with the NP islets ($P<0.001$, $n=12–16$), as shown in Fig. 5.

Figure 6 shows the insulinotropic effect of acetylcholine on the glucose-induced insulin secretion. Glucose (8.3 mmol/l) stimulated 28% more insulin secretion from islets from the LP rats than from the NP rats ($P<0.05$,
Acetylcholine potentiated the glucose-induced insulin secretion in both islet groups; however, the LP islets showed only 24% of the effect \( (P<0.05, n=14) \), while the NP islets exhibited more than 88% \( (P<0.01, n=14; \text{Fig. } 6A) \). The acetylcholine insulinotrophic effect on the islets from the LP rats was 55% lower than that from the NP rats \( (P<0.001, n=14; \text{Fig. } 6B) \).

A nonselective mAChR antagonist, atropine, inhibited the acetylcholine insulinotrophic effect on the 8.3 mmol/l glucose-stimulated insulin secretion in both islet groups (32% for NP and 36% for LP; \( P<0.001, n=14 \); \text{Fig. } 7). Using a selective M₃mAChR subtype antagonist, pirenzepine, the inhibition of the acetylcholine insulinotropic effect showed a larger magnitude for the NP islets (45%) than for the LP islets (37%, \( P<0.001, n=14 \)).

A more effective inhibition to the cholinergic insulino-tropic effect was obtained with a selective M₃mAChR subtype antagonist, 4-DAMP, on both groups (50% for NP and 55% for LP islets; \( P<0.001, n=14 \); \text{Fig. } 7). Alternatively, the selective M₃mAChR subtype antagonist, methoctramine, enhanced the acetylcholine insulinotropic effect by 22% in islets from the NP rats, while it induced a 20% inhibition of the potentiating effect by acetylcholine in islets from the LP rats \( (P<0.001, n=14; \text{Fig. } 7) \).

As shown in Fig. 8A, compared with isolated islets from the NP rats, the islets from the LP rats presented a high insulin secretion \( (P<0.01, n=14) \) when stimulated with a high glucose concentration (16.7 mmol/l), and the epinephrine treatment was able to inhibit this effect in both islet groups. However, the insulino-static effect of epinephrine was 40% lower in islets from the LP rats compared with the 55% decrease in islets from the NP rats \( (P<0.001, n=14; \text{Fig. } 8A) \). The magnitude of the insulino-static effect of epinephrine was 27% lower in islets from the LP rats compared with the NP rats \( (P<0.001, n=14; \text{Fig. } 8B) \).

Using the selective \( \alpha_2 \)-adrenoceptor antagonist, yohimbine (10 \( \mu \)mol/l), the inhibition of insulin secretion by epinephrine was blocked in both islet groups \( (P<0.001, n=14) \), although it was 43% lower in islets from the LP rats when compared with the NP rats \( (P<0.001, n=14; \text{Fig. } 9) \). However, the use of a selective \( \beta_2 \)-adrenoceptor antagonist, propranolol (1 \( \mu \)mol/l), blocked the insulin secretion to both islet groups with the same magnitude, which was
similar to the effect observed with epinephrine alone ($P<0.001, n=14$; Fig. 9).

**Effect of an LP diet treatment during adolescence on autonomous nervous system activity in obese programmed adult rats**

While there were no significant differences ($P=0.82, n=12–13$) in the electrical activity of the sympathetic nerve between the animal groups, the superior vagus nerve from the LP rats presented 31% more activity than from the NP group ($P<0.05, n=13–14$; Fig. 10).

**Discussion**

A 30-day reduction in protein in the diet (4%) from 30 to 60 days of age led the 120-day-old male adult rats to develop high fat tissue accumulation, similar to what occurs with the same nutritional impairment during gestation (Vickers et al. 2005), which might suggest that metabolism was programmed to save energy, and even the intake of an NP (23%) diet after the period of LP diet provokes excess fat accumulation. It has been shown that protein and/or calorie restriction during pregnancy results in rat pups that are underweight, which present with the onset of obesity when they reach adulthood (Vickers et al. 2005). A similar observation has been obtained for human beings who were malnourished during their fetal life (Roseboom et al. 2001). Although presenting with a high fat tissue accumulation, moderate fasting hyperglycemia, hyperinsulinemia, severe hyperleptinemia and peripheral insulin resistance, the adult rats that were subjected to the protein restriction during adolescence were not overweight. Other animal models of obesity do not show an excess of BW, including a neonatal treatment with monosodium L-glutamate (MSG; Mozes et al. 2004) and certain diet-induced obese animals models (Boozer et al. 1995, Smith et al. 1998).

It should be argued about which phase of adolescence was sensitive to the LP treatment and whether it was around or during the pubertal phase. It has been shown that during the increase of sexual hormones, changes are observed in the brain neuron connections (Andersen et al. 2000, Cunningham et al. 2002). Changes in metabolism including peripheral insulin resistance, particularly if a stress has occurred during that stage of life, have also been observed (Pervanidou & Chrousos 2012). These observations suggest that protein malnourishment targets only the pubertal phase for metabolic programming; however, we cannot discard the possibility that the target could be prior to the pubertal time. It has been observed that a maternal LP diet during different intervals of lactation, including the last third of

![Figure 5](image)

**Figure 5**

Insulinotropic effect of different glucose concentrations. Symbols represent the means ± S.E.M. of the insulin release that was stimulated by glucose (5.6, 8.3, 11.1, 16.7, 20.0 and 24.0 mmol/l), which were obtained from the pancreatic islets of 12–16 rats from four different litters of each experimental group. The statistical differences between the LP and NP groups for each glucose concentration were found by Student’s $t$-test; *$P<0.05$, **$P<0.01$ and ***$P<0.001$. Glu, glucose.

![Figure 6](image)

**Figure 6**

The *in vitro* effect of the muscarinic receptor agonist and antagonists on glucose-induced insulin secretion. Bars represent the means ± S.E.M. of insulin secretion from the pancreatic islets of 14 rats that were obtained from four different litters. (A) Insulin secretion that was stimulated by 8.3 mmol/l Glu and potentiated by 10 µmol/l ACh. (B) The percentage of insulin release potentiated by 10 µmol/l ACh, with the line from 0 representing 100% of the glucose-induced insulin release throughout the 60 min of incubation in both the NP and LP islets. The symbols over the bars refer to the significance levels as follows: *$P<0.05$ and **$P<0.001$ between the NP and LP groups; *$P<0.05$ for the LP and **$P<0.01$ for the NP group treated with 10 µmol/l ACh compared with 8.3 mmol/l Glu based on Student’s $t$-test. ACh, acetylcholine; Glu, glucose.
The lactation period, also induces metabolic imprinting when the pups reach adulthood (Barbosa et al. 1999). It is known that at the end of the suckling phase, the rat pups start to eat chow, which push the youngest toward weaning (Blass & Teicher 1980).

In addition to the observation that the brain development of rodents reaches its peak during lactation (Morgane et al. 2002), the neuronal connections do not finish at the end of the suckling phase (Sisk & Zehr 2005), which indicate that a nutritional injury, such as protein restriction during the first third of lactation, might change the brain structure and function (de Oliveira et al. 2011). It is also possible that after the burst of sexual hormone release at 45–48 days of age (Lewis et al. 2002), the brain is still developing neuronal connections, which allows for a nutritional maneuver to interfere with the metabolic programming when the youngest reaches adulthood. It has been shown that a stressful stimulation around the rats’ pubertal period permanently changes the brain function (Tsoory et al. 2010). Independent of the adolescence phase, which was the target of the treatment, data in the present work show for the first time that protein restriction during adolescence is able to induce the development of high-fat tissue accumulation in adult rats or make them highly prone to later obesity onset.

Metabolically programmed obesity-prone rats, due to an LP intake during adolescence, also presented an imbalance in the autonomous nervous system (ANS) activity, which showed a high vagal activity. Numerous data from animal models and humans have sustained the hypothesis that obesity presents a direct relationship with ANS dysfunctions because obesity has been related to a high parasympathetic activity and a low sympathetic activity (Inoue et al. 1991, Lee et al. 1993, Atef et al. 1995).

It has been shown that perinatal nutritional manipulation (either under- or overnutrition) programs long-lasting impaired effects on the ANS. Modified adrenoreceptors subtypes ratio and/or reduced sympathetic outflow activity, as well as reduced innervations of adult offspring peripheral tissues other than pancreatic islets, such as enteric sympathetic nervous system and white adipose tissue noradrenergic innervations (Philipp & Pirke 1987, Santer & Conboy 1990, Xiao et al. 2007,
There is much evidence that indicates a close relationship between metabolic programming and pancreatic β-cell dysfunction (Reusens & Remacle 2006, Inoue et al. 2009, Branco et al. 2012). Adult rats programmed by an LP intake during adolescence presented with high fasting plasma insulin concentrations and mildly high insulinemia during an ivGTT, which supports the low glycemia in the LP rats. It is remarkable that insulin resistance was observed. Pancreatic islets from these obesity-prone rats, when stimulated by different glucose concentrations, oversecrete insulin, which may suggest a β-cell dysfunction. Glucose-stimulated islets that were isolated from other obese rats and mice secrete large amounts of insulin (Ahren 2000). Several mechanisms underlying this pancreatic malfunction have been proposed, including a high vagal tonus that allows increased acetylcholine release in the parasympathetic terminals of β-cells. The extra charge of the vagal neurotransmitter potentiates the glucose-stimulated insulin secretion (Ahren 2000). The programmed obese rats that were fed a poor-protein diet during adolescence presented a high vagal activity. In addition, the islets from LP rats showed a
low acetylcholine insulino tropic effect, which might suggest that a high vagal tonus provoked a downregulation of mAChR or an upregulation of adrenoceptors on the β-cell membrane.

Similarly, we showed that an MSG-induced obese animal model with vagal hyperactivity had disrupted mAChR function in their islets (Grassioli et al. 2007); however, other obese animal models have shown a strong adrenergic sensitivity in addition to a weak cholinergic response in the pancreatic islets (Campfield et al. 1986). Regarding the adrenergic response, the islets from LP animals release less insulin than those from NP rats. The weak cholinergic insulino tropic effect of the LP islets cannot be attributed to a potent adrenergic inhibitory response. Our data also suggest that the LP islets did not show an upregulation of the adrenergic receptors, which could be related to the unchangeable electrical activity from the sympathetic nerve of the LP rats. Alternatively, to justify the weak cholinergic response of the pancreatic islets, we could not discard the changes in the composition of the mAChR subfamily.

It has been shown that an insulin-secreting β-cell line presents four mAChR subtypes, M₁, M₂, M₃ and M₄ (Miguel et al. 2002). The acetylcholine insulino tropic response has been principally attributed to M₃mAChR (Gautam et al. 2006); however, when binding to M₁mAChR, this response also potentiates glucose-induced insulin secretion (Renuka et al. 2006). Alternatively, our group has demonstrated that the M₂ and M₃mAChR subtypes have an inhibitory effect on the islets stimulated by acetylcholine (Miguel et al. 2002, Grassioli et al. 2007). After using a selective mAChR antagonist to M₁ and M₃mAChR, the data indicate that there was no evidence to conclude a downregulation of the β-cell mAChR; however, the inhibitory response of M₃mAChR from the LP islets was slightly greater than the NP ones. Surprisingly, the M₂mAChR from LP islets increased the cholinergic response instead of inhibiting it. Interestingly, it was suggested that the persistent activation of β-cell’s M₃mAChR could be a mechanism that explains an increased glucose-induced insulin release in obese animals (Gautam et al. 2006). Transgenic mice that expressed Q490L mutant M₃mAChR in β-cell are resistant to impairment of glucose homeostasis when exposed to a high-fat diet. Isolated islets from these mutant mice, when stimulated by glucose, secreted more insulin than the wild type littermate. This effect is a cholinergic ligand-independent signal (Gautam et al. 2010), which can indicate that the high glucose insulino tropic effect is associated with high activity of M₃mAChR. It was shown that M₃mAChR overexpression in β-cells causes increased insulin plasma concentrations in fasting and fed states of mutant mice (Gautam et al. 2006).

Overall, the data obtained from the isolated islets in the present work suggest that an LP diet administrated to adolescent rats induced β-cell dysfunction, which was linked to changes in the activity of mAChR subtypes. Changes in the mAChR activity were also observed in islets isolated from MSG-induced obese rats (Rohner-Jeanrenaud et al. 1983, Grassioli et al. 2007). In addition to other mechanisms involved in the adolescent metabolic programming, the β-cell dysfunction associated with an imbalanced ANS–muscarinic receptor composition might be important and should be a target for preventive and therapeutic methods to reduce the consequences of metabolic diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

P C L, E G M and P C F M designed the research; J C O, L F B, R A M, A M and T A S R, conducted the research; R T and C G provided essential reagents and materials; C C S F analyzed the data; J C O and P C F M wrote the manuscript; P C F M had primary responsibility for final content. All authors read and approved the final manuscript.

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