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

Júlio Cezar de Oliveira, Patrícia Cristina Lisboa¹, Egberto Gaspar de Moura¹, Luiz Felipe Barella, Rosiane Aparecida Miranda, Ananda Malta, Claudinéia Conatoni da Silva Franco, Tatiane Aparecida da Silva Ribeiro, Rosana Torrezan², Clarice Gravena and Paulo Cezar de Freitas Mathias

Laboratory of Secretion Cell Biology, Department of Cell Biology and Genetics, State University of Maringá, Block H67, Room 19, Colombo Avenue 5970, 87020-900 Maringá, Paraná, Brazil

¹Department of Physiological Sciences, Roberto Alcântara Gomes Biology Institute, State University of Rio de Janeiro, Rio de Janeiro, Paraná, Brazil

²Department of Physiological Sciences, State University of Maringá, Maringá, Paraná, Brazil

Correspondence
should be addressed to
P C de Freitas Mathias
Email
pmathias@uem.br

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

Journal of Endocrinology
(2013) 216, 195–206

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 protocol (NP group), while the other group of rats received a low protein (LP; 4% w/w protein) diet with the same amount of calories as the normal group (LP group) as described previously (de Oliveira *et al.* 2011) during 30–60 days of life. Because gender differences in insulin concentration and glucose tolerance have been observed in some early poor-protein feeding studies (Lopes Da Costa *et al.* 2004), only male offsprings were used in these experiments. Throughout the experimental period, the rats (five rats per cage) were maintained under controlled conditions of temperature ($22 \pm 2^\circ\text{C}$) 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 (Δ_{Glycemia}) and insulinemia increments ($\Delta_{\text{Insulinemia}}$) for each time period of the ivGTT. Increases in total Δ_{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 ^{125}I -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: $\text{ISI} = 10^4 / \sqrt{(\text{fasting glycemia} \times \text{fasting insulinemia}) \times (\text{AUC}_{\Delta_{\text{Glycemia}}} \times \text{AUC}_{\Delta_{\text{Insulinemia}}})}$.

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; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.81; Na_2HPO_4 , 0.34; KH_2PO_4 , 0.44; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.26; NaHCO_3 , 4.16; glucose, 0.06; BSA, 15 and ((v/v; 95% O_2 + 5% CO_2 , 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; NaHCO_3 , 24; KCl, 1.6; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1; BSA, 15; pH 7.4) containing 5.6 mmol/l glucose. This solution was gassed with (v/v) 95% O_2 + 5% CO_2 (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 $\mu\text{mol/l}$ acetylcholine in the presence of 10 $\mu\text{mol/l}$ neostigmine to avoid acetylcholinesterase action in the islets. Additionally, a nonselective

mAChR antagonist, 10 $\mu\text{mol/l}$ atropine, was also used. To block the mAChR subtype M_1 , M_2 and M_3 function, their antagonists were used as follows: 100 $\mu\text{mol/l}$ pirenzepine, 1 $\mu\text{mol/l}$ methoctramine and 100 $\mu\text{mol/l}$ 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 $\mu\text{mol/l}$ acetylcholine in islets incubated with 8.3 mmol/l glucose in the presence of 10 $\mu\text{mol/l}$ 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 $\mu\text{mol/l}$ epinephrine and an α_2 -adrenoceptor antagonist, yohimbine (10 $\mu\text{mol/l}$) or in the presence of epinephrine and a β_2 -adrenoceptor antagonist, propranolol (1 $\mu\text{mol/l}$). 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, periepididymal, visceral and inguinal) were removed and weighed to assess the state of obesity.

Statistical analyses

Results are given as the means $\pm$ 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 ($P < 0.001$, $n = 32\text{--}40$); however, the body length was not affected ($P = 0.299$, $n = 20\text{--}29$) 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 ($P < 0.05$, $n = 5\text{--}19$), 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 ($P < 0.001$, $n = 20\text{--}35$), 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 ($P < 0.001$, $n = 20\text{--}35$; 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 ($P < 0.001$, $n = 5\text{--}7$ 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 ($P < 0.001$, $n = 5\text{--}7$ 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 $\pm$ S.E.M. from five to eight different litters

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

Student's *t*-test was used between the groups. * $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$. NS, no significant difference.

Figure 1

BW progression and BW gain. The BW (A) from 30 to 120 days of age. The data are given as means $\pm$ S.E.M. of 20–35 rats from four to seven different litters. The BW gain (B) of 60- to 120-day-old rats (post-LP diet treatment). The inset in the upper left panel in each figure represents the AUC of the BW and/or BW gain. * $P < 0.001$ based on Student's *t*-test.

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 ($P < 0.05$,

Figure 2

Food intake. The data represent the means $\pm$ S.E.M. of the food intake measurements during the LP diet treatment (30–60 days old; A) and after the diet treatment period (60–120 days old; B), which were obtained from five to seven different litters. The inset in the upper right panel in each figure represents the food intake AUC. * $P < 0.001$ by Student's *t*-test.

Figure 3

Effect of an LP diet during adolescence on the fat pad stores. Bars represent the means $\pm$ s.e.m. of the retroperitoneal, periepididymal, visceral, and inguinal fat pad stores from 30 rats of six different litters. * $P < 0.01$ and ** $P < 0.001$ by Student's *t*-test.

$n = 14$; Fig. 6A). 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% ($P < 0.01$, $n = 14$; Fig. 6A). The acetylcholine insulinotropic effect on the islets from the LP rats was 55% lower than that from the NP rats ($P < 0.001$, $n = 14$; Fig. 6B).

A nonselective mAChR antagonist, atropine, inhibited the acetylcholine insulinotropic effect on the 8.3 mmol/l glucose-stimulated insulin secretion in both islet groups (32% for NP and 36% for LP; $P < 0.001$, $n = 14$; Fig. 7). Using a selective M_1 mAChR 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 insulinotropic effect was obtained with a selective M_3 mAChR subtype antagonist, 4-DAMP, on both groups (50% for NP and 55% for LP islets; $P < 0.001$, $n = 14$; Fig. 7). Alternatively, the selective M_2 mAChR 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 ($P < 0.001$, $n = 14$; 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 insulinostatic effect of epinephrine was 40% lower in islets from the LP rats compared with the 55% decrease in islets from the NP rats ($P < 0.001$, $n = 14$; Fig. 8A). The magnitude of the insulinostatic effect of epinephrine was 27% lower in islets from the LP rats compared with the NP rats ($P < 0.001$, $n = 14$; Fig. 8B).

Using the selective α_2 -adrenoceptor antagonist, yohimbine (10 μ 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 ($P < 0.001$, $n = 14$; Fig. 9). However, the use of a selective β_2 -adrenoceptor antagonist, propranolol (1 μ mol/l), blocked the insulin secretion to both islet groups with the same magnitude, which was

Figure 4

(A) Plasma glucose and (B) insulin increments during the ivGTT. Symbols represent the means $\pm$ s.e.m. for 12–15 rats from four to six different litters of each experimental group. The inset in the upper right panel of each figure represents the AUC of the glycemia and/or insulinemia increment during the ivGTT. * $P < 0.05$ based on Student's *t*-test.

Figure 5

Insulintropic effect of different glucose concentrations. Symbols represent the means $\pm$ 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.

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 (Boozar *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 6

The *in vitro* effect of the muscarinic receptor agonist and antagonists on glucose-induced insulin secretion. Bars represent the means $\pm$ 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 μ mol/l ACh. (B) The percentage of insulin release potentiated by 10 μ mol/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 are as follows: **P* < 0.05 and ***P* < 0.001 between the NP and LP groups; #*P* < 0.05 for the LP and Ω *P* < 0.01 for the NP group treated with 10 μ mol/l ACh compared with 8.3 mmol/l Glu based on Student's *t*-test. ACh, acetylcholine; Glu, glucose.

Figure 7

Effect of muscarinic receptor antagonists on glucose-induced insulin secretion from isolated pancreatic islets. Bars represent the means $\pm$ S.E.M. of the insulin secretion from the pancreatic islets of 14 rats that were obtained from four different litters. The line from 0 represents 100% of the glucose-induced insulin release potentiated by 10 μmol/l acetylcholine in both the NP and LP groups. The bars above or below the line from 0 represent the percentage of rise or fall that was provoked by the various treatments (μmol/l), nonselective antagonist (atropine, 10) and antagonists selective to M₁mAChR, pirenzepine, 100; M₂mAChR, methoctramine, 1 and M₃mAChR, 4-DAMP, 100) on the glucose-induced insulin release potentiated by 10 μmol/l acetylcholine throughout the 60 min of incubation in both the NP and LP groups. The symbols over the bars refer to the significance levels are as follows: $\Phi P < 0.001$ for the NP or $\Omega P < 0.001$ for the LP group for each indicated muscarinic antagonist compared with the 10 μmol/l acetylcholine treatment; $*P < 0.001$ between the NP and LP groups for each respective muscarinic antagonist treatment by Student's *t*-test. ACh, acetylcholine; Atr, atropine; 4-DAMP, 4-diphenylacetoxy-*N*-methylpiperidine methiodide; PZP, pirenzepine; MTT, methoctramine.

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 adrenoceptors 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,

Figure 8

Effect of epinephrine on the glucose-induced insulin secretion in isolated pancreatic islets. The bars represent the means $\pm$ S.E.M. of the insulin secretion from the pancreatic islets of 14 rats that were obtained from four different litters. (A) Insulin secretion stimulated by 16.7 mmol/l glucose and blocked by 1 μmol/l epinephrine. (B) The percentage of insulin secretion that was decreased by 1 μmol/l epinephrine, with the line from 0 representing 100% of the glucose-induced insulin release throughout the 60 min of incubation for both the NP and LP islets. The symbols over the bars refer to the significance levels are as follows: $*P < 0.01$ and $**P < 0.001$ between the NP and LP groups; $\Phi P < 0.001$ for the LP and $\Omega P < 0.001$ for the NP group treated with 1 μmol/l epinephrine compared with 16.7 mmol/l glucose based on Student's *t*-test. Epi, epinephrine; Glu, glucose.

Figure 9

Effect of adrenoceptor antagonists on the glucose-induced insulin secretion in isolated pancreatic islets. The bars represent the means $\pm$ S.E.M. of the insulin secretion from pancreatic islets of 14 rats obtained from four different litters. The line from 0 represents 100% of the insulin secretion that was stimulated by 16.7 mmol/l glucose in the presence of 1 µmol/l epinephrine for both the NP and LP groups. The bars above the line from 0 represent the percentage of the increase that was provoked by the various treatments ((µmol/l), selective antagonist for α_2 -adrenoceptors (yohimbine, 10) and β_2 -adrenoceptors (propranolol, 10)) on the insulin secretion that was stimulated by 16.7 mmol/l glucose in the presence of 1 µmol/l epinephrine throughout the 60 min of incubation from both the NP and LP groups. The symbols over the bars refer to significance levels are as follows: $^{\Phi}P < 0.001$ for the NP and $^{\Omega}P < 0.001$ for the LP islets treated with 10 µmol/l yohimbine compared with the 10 µmol/l epinephrine treatment; $*P < 0.001$ between the NP and LP groups treated with 16.7 mmol/l glucose in the presence of 1 µmol/l epinephrine based on Student's *t*-test. Epi, epinephrine; Pro, propranolol; Yoh, yohimbine.

Garcia *et al.* 2011), might affect fat cell metabolism leading to obesity. As reported, our group recently used two different animal models to show that the vagal electrical activity is enhanced in an obesity state (Scomparin *et al.* 2011, Barella *et al.* 2012). Obesity-prone rats that were obtained by protein restriction during the peripubertal phase did not show any changes in the electrical activity of the sympathetic superior ganglia; however, the present results indicate an ANS imbalance based on the high vagal activity. The literature indicates large discrepancies among studies that report low, unchanged and high activity of the sympathetic nervous system during obesity in both subjects and experimental animal models (Tentolouris *et al.* 2006). It has been claimed that the paradoxical sympathetic activity in obese individuals is tissue/organ specific (Davy & Orr 2009), but this phenomenon is not completely understood. Alternatively, it is known that parasympathetic overactivity has a strong association with hyperinsulinemia, insulin resistance, and the development of obesity (Rohner-Jeanraud *et al.* 1983, Tentolouris *et al.* 2006), which are in agreement with our data.

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

Figure 10

Sympathetic and parasympathetic electrical activity from the cervical superior nerves. The bars represent the means $\pm$ S.E.M. of the firing rates from A the vagus nerves and B the sympathetic nerves from 12 to 14 rats that were obtained from four different litters for each experimental group. The representative records of each nerve discharge, which illustrate the data for each experimental group, are given in the lower panels. $*P < 0.05$ by Student's *t*-test. NS, no significant difference.

low acetylcholine insulintropic 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 insulintropic 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, M_1 , M_2 , M_3 and M_4 (Miguel *et al.* 2002). The acetylcholine insulintropic response has been principally attributed to M_3 mAChR (Gautam *et al.* 2006); however, when binding to M_1 mAChR, this response also potentiates glucose-induced insulin secretion (Renuka *et al.* 2006). Alternatively, our group has demonstrated that the M_2 and M_4 mAChR 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 M_1 and M_3 mAChR, the data indicate that there was no evidence to conclude a downregulation of the β -cell mAChR; however, the inhibitory response of M_3 mAChR from the LP islets was slightly greater than the NP ones. Surprisingly, the M_2 mAChR from LP islets increased the cholinergic response instead of inhibiting it. Interestingly, it was suggested that the persistent activation of β -cell's M_3 mAChR 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 M_3 mAChR 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 insulintropic effect is associated with high activity of M_3 mAChR. It was shown

that M_3 mAChR 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-Jeanraud *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.

Funding

This work was supported by the Brazilian Federal Foundation, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Paraná Science Foundation (Fundação Araucária) and the Carlos Chagas Filho Research Foundation of the State of Rio de Janeiro (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, FAPERJ).

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.

References

- Ahren B 2000 Autonomic regulation of islet hormone secretion – implications for health and disease. *Diabetologia* **43** 393–410. (doi:10.1007/s001250051322)
- Andersen SL, Thompson AT, Rutstein M, Hostetter JC & Teicher MH 2000 Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse* **37** 167–169. (doi:10.1002/1098-2396(200008)37:2<167::AID-SYN11>3.0.CO;2-B)
- Atef N, Ktorza A & Penicaud L 1995 CNS involvement in the glucose induced increase of islet blood flow in obese Zucker rats. *International Journal of Obesity and Related Metabolic Disorders* **19** 103–107.
- Barbosa FB, Medina AR, Balbo SL & de Freitas Mathias PC 1999 Low protein diets administered to lactating rats affect in a time-dependent manner the development of young. *Research Communications in Molecular Pathology and Pharmacology* **106** 63–76.

- Barella LF, de Oliveira JC, Branco RC, Camargo RL, Gomes RM, Mendes FC, Miranda RA, Gravena C, Torrezan R, Grassioli S *et al.* 2012 Early exposure to a high-fat diet has more drastic consequences on metabolism compared with exposure during adulthood in rats. *Hormone and Metabolic Research* **44** 458–464. (doi:10.1055/s-0032-1306300)
- Blakemore SJ, Burnett S & Dahl RE 2010 The role of puberty in the developing adolescent brain. *Human Brain Mapping* **31** 926–933. (doi:10.1002/hbm.21052)
- Blass EM & Teicher MH 1980 Suckling. *Science* **210** 15–22. (doi:10.1126/science.6997992)
- Bol VV, Delattre AI, Reusens B, Raes M & Remacle C 2009 Forced catch-up growth after fetal protein restriction alters the adipose tissue gene expression program leading to obesity in adult mice. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **297** R291–R299. (doi:10.1152/ajpregu.90497.2008)
- Boozar CN, Schoenbach G & Atkinson RL 1995 Dietary fat and adiposity: a dose–response relationship in adult male rats fed isocalorically. *American Journal of Physiology* **268** E546–E550.
- Branco RC, de Oliveira JC, Grassioli S, Miranda RA, Barella LF, Gomes RM, Bataglini LA, Torrezan R, Gravena C & de Freitas Mathias PC 2012 Maternal protein malnutrition does not impair insulin secretion from pancreatic islets of offspring after transplantation into diabetic rats. *PLoS ONE* **7** e30685. (doi:10.1371/journal.pone.0030685)
- Campfield LA, Smith FJ & Larue-Achagiotis C 1986 Temporal evolution of altered islet neurotransmitter sensitivity after VMH lesion. *American Journal of Physiology* **251** R63–R69.
- Cunningham MG, Bhattacharyya S & Benes FM 2002 Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence. *Journal of Comparative Neurology* **453** 116–130. (doi:10.1002/cne.10376)
- Davy KP & Orr JS 2009 Sympathetic nervous system behavior in human obesity. *Neuroscience and Biobehavioral Reviews* **33** 116–124. (doi:10.1016/j.neubiorev.2008.05.024)
- Delahaye F, Breton C, Risold PY, Enache M, Dutriez-Casteloot I, Laborie C, Lesage J & Vieau D 2008 Maternal perinatal undernutrition drastically reduces postnatal leptin surge and affects the development of arcuate nucleus proopiomelanocortin neurons in neonatal male rat pups. *Endocrinology* **149** 470–475. (doi:10.1210/en.2007-1263)
- Fagundes AT, Moura EG, Passos MC, Oliveira E, Toste FP, Bonomo IT, Trevenzoli IH, Garcia RM & Lisboa PC 2007 Maternal low-protein diet during lactation programmes body composition and glucose homeostasis in the adult rat offspring. *British Journal of Nutrition* **98** 922–928. (doi:10.1017/S0007114507750924)
- Garcia AP, Palou M, Sanchez J, Priego T, Palou A & Pico C 2011 Moderate caloric restriction during gestation in rats alters adipose tissue sympathetic innervation and later adiposity in offspring. *PLoS ONE* **6** e17313. (doi:10.1371/journal.pone.0017313)
- Gautam D, Han SJ, Hamdan FF, Jeon J, Li B, Li JH, Cui Y, Mears D, Lu H, Deng C *et al.* 2006 A critical role for β cell M3 muscarinic acetylcholine receptors in regulating insulin release and blood glucose homeostasis *in vivo*. *Cell Metabolism* **3** 449–461. (doi:10.1016/j.cmet.2006.04.009)
- Gautam D, Ruiz de Azua I, Li JH, Guettier JM, Heard T, Cui Y, Lu H, Jou W, Gavrilova O, Zawulich WS *et al.* 2010 Beneficial metabolic effects caused by persistent activation of β -cell M3 muscarinic acetylcholine receptors in transgenic mice. *Endocrinology* **151** 5185–5194. (doi:10.1210/en.2010-0519)
- Grassioli S, Gravena C & de Freitas Mathias PC 2007 Muscarinic M2 receptor is active on pancreatic islets from hypothalamic obese rat. *European Journal of Pharmacology* **556** 223–228. (doi:10.1016/j.ejphar.2006.11.022)
- Gravena C, Mathias PC & Ashcroft SJ 2002 Acute effects of fatty acids on insulin secretion from rat and human islets of Langerhans. *Journal of Endocrinology* **173** 73–80. (doi:10.1677/joe.0.1730073)
- Hales CN & Barker DJ 2001 The thrifty phenotype hypothesis. *British Medical Bulletin* **60** 5–20. (doi:10.1093/bmb/60.1.5)
- Huang JS, Lee TA & Lu MC 2007 Prenatal programming of childhood overweight and obesity. *Maternal and Child Health Journal* **11** 461–473. (doi:10.1007/s10995-006-0141-8)
- Inoue S, Nagase H, Satoh S, Saito M, Egawa M, Tanaka K & Takamura Y 1991 Role of the efferent and afferent vagus nerve in the development of ventromedial hypothalamic (VMH) obesity. *Brain Research Bulletin* **27** 511–515. (doi:10.1016/0361-9230(91)90151-9)
- Inoue T, Kido Y, Asahara S, Matsuda T, Shibutani Y, Koyanagi M & Kasuga M 2009 Effect of intrauterine undernutrition during late gestation on pancreatic β cell mass. *Biomedical Research* **30** 325–330. (doi:10.2220/biomedres.30.325)
- Lee HC, Curry DL & Stern JS 1993 Tonic sympathetic nervous system inhibition of insulin secretion is diminished in obese Zucker rats. *Obesity Research* **1** 371–376.
- Lesage J, Sebaai N, Leonhardt M, Dutriez-Casteloot I, Breton C, Deloof S & Vieau D 2006 Perinatal maternal undernutrition programs the offspring hypothalamo-pituitary–adrenal (HPA) axis. *Stress* **9** 183–198. (doi:10.1080/10253890601056192)
- Lewis EM, Barnett JF Jr, Freshwater L, Hoberman AM & Christian MS 2002 Sexual maturation data for Crl Sprague-Dawley rats: criteria and confounding factors. *Drug and Chemical Toxicology* **25** 437–458. (doi:10.1081/DCT-120014794)
- Lopes Da Costa C, Sampaio De Freitas M & Sanchez Moura A 2004 Insulin secretion and GLUT-2 expression in undernourished neonate rats. *Journal of Nutritional Biochemistry* **15** 236–241. (doi:10.1016/j.jnutbio.2003.12.004)
- Lurzel S, Kaiser S, Kruger C & Sachser N 2011 Inhibiting influence of testosterone on stress responsiveness during adolescence. *Hormones and Behavior* **60** 691–698. (doi:10.1016/j.yhbeh.2011.09.007)
- Matsuda M & DeFronzo RA 1999 Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* **22** 1462–1470. (doi:10.2337/diacare.22.9.1462)
- Miguel JC, Abdel-Wahab YH, Mathias PC & Flatt PR 2002 Muscarinic receptor subtypes mediate stimulatory and paradoxical inhibitory effects on an insulin-secreting β cell line. *Biochimica et Biophysica Acta* **1569** 45–50. (doi:10.1016/S0304-4165(01)00232-X)
- Mokler DJ, Torres OI, Galler JR & Morgane PJ 2007 Stress-induced changes in extracellular dopamine and serotonin in the medial prefrontal cortex and dorsal hippocampus of prenatally malnourished rats. *Brain Research* **1148** 226–233. (doi:10.1016/j.brainres.2007.02.031)
- Morgane PJ, Mokler DJ & Galler JR 2002 Effects of prenatal protein malnutrition on the hippocampal formation. *Neuroscience and Biobehavioral Reviews* **26** 471–483. (doi:10.1016/S0149-7634(02)00012-X)
- Mozes S, Sefickov Z, Lenhardt L & Racek L 2004 Effect of adrenalectomy on the activity of small intestine enzymes in monosodium glutamate obese rats. *Physiological Research* **53** 415–422.
- de Oliveira JC, Scomparin DX, Andreazzi AE, Branco RC, Martins AG, Gravena C, Grassioli S, Rinaldi W, Barbosa FB & Mathias PC 2011 Metabolic imprinting by maternal protein malnourishment impairs vagal activity in adult rats. *Journal of Neuroendocrinology* **23** 148–157. (doi:10.1111/j.1365-2826.2010.02095.x)
- Ozanne SE 1999 Programming of hepatic and peripheral tissue insulin sensitivity by maternal protein restriction. *Biochemical Society Transactions* **27** 94–97. (doi:10.1042/bst027a007a)
- Pervanidou P & Chrousos GP 2012 Metabolic consequences of stress during childhood and adolescence. *Metabolism* **61** 611–619. (doi:10.1016/j.metabol.2011.10.005)
- Philipp E & Pirke KM 1987 Effect of starvation on hypothalamic tyrosine hydroxylase activity in adult male rats. *Brain Research* **413** 53–59. (doi:10.1016/0006-8993(87)90153-3)
- Plagemann A, Harder T, Rake A, Melchior K, Rohde W & Dörner G 2000a Hypothalamic nuclei are malformed in weanling offspring of low protein malnourished rat dams. *Journal of Nutrition* **130** 2582–2589.
- Plagemann A, Waas T, Harder T, Rittel F, Ziska T & Rohde W 2000b Hypothalamic neuropeptide Y levels in weaning offspring of low-

- protein malnourished mother rats. *Neuropeptides* **34** 1–6. (doi:10.1054/npep.1999.0778)
- Ravelli GP, Stein ZA & Susser MW 1976 Obesity in young men after famine exposure in utero and early infancy. *New England Journal of Medicine* **295** 349–353. (doi:10.1056/NEJM197608122950701)
- Renuka TR, Robinson R & Paulose CS 2006 Increased insulin secretion by muscarinic M1 and M3 receptor function from rat pancreatic islets *in vitro*. *Neurochemical Research* **31** 313–320. (doi:10.1007/s11064-005-9022-6)
- Resnick O, Miller M, Forbes W, Hall R, Kemper T, Bronzino J & Morgane PJ 1979 Developmental protein malnutrition: influences on the central nervous system of the rat. *Neuroscience and Biobehavioral Reviews* **3** 233–246. (doi:10.1016/0149-7634(79)90011-3)
- Reusens B & Remacle C 2006 Programming of the endocrine pancreas by the early nutritional environment. *International Journal of Biochemistry & Cell Biology* **38** 913–922. (doi:10.1016/j.biocel.2005.10.012)
- Rohner-Jeanrenaud F, Ionescu E & Jeanrenaud B 1983 The origins and role of efferent vagal nuclei in hyperinsulinemia in hypothalamic and genetically obese rodents. *Journal of the Autonomic Nervous System* **9** 173–184. (doi:10.1016/0165-1838(83)90139-X)
- Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ & Bleker OP 2001 Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Twin Research* **4** 293–298. (doi: 10.1016/S0303-7207(01)00721-3)
- Sampaio de Freitas M, Garcia De Souza EP, Vargas da Silva S, da Rocha Kaezer A, da Silva Vieira R, Sanchez Moura A & Barja-Fidalgo C 2003 Up-regulation of phosphatidylinositol 3-kinase and glucose transporter 4 in muscle of rats subjected to maternal undernutrition. *Biochimica et Biophysica Acta* **1639** 8–16. (doi:10.1016/S0925-4439(03)00096-6)
- Santer RM & Conboy VB 1990 Prenatal undernutrition permanently decreases enteric neuron number and sympathetic innervation of Auerbach's plexus in the rat. *Journal of Anatomy* **168** 57–62.
- Scomparin DX, Grassioli S, Gomes RM, Torrezan R, de Oliveira JC, Gravena C, Pera CC & Mathias PC 2011 Low-intensity swimming training after weaning improves glucose and lipid homeostasis in MSG hypothalamic obese mice. *Endocrine Research* **36** 83–90. (doi:10.3109/07435800.2010.534750)
- Sisk CL & Zehr JL 2005 Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology* **26** 163–174. (doi:10.1016/j.yfrne.2005.10.003)
- Smith BK, Kelly LA, Pina R, York DA & Bray GA 1998 Preferential fat intake increases adiposity but not body weight in Sprague-Dawley rats. *Appetite* **31** 127–139. (doi:10.1006/appe.1998.0162)
- Spear LP 2000 The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews* **24** 417–463. (doi:10.1016/S0149-7634(00)00014-2)
- Tentolouris N, Liatis S & Katsilambros N 2006 Sympathetic system activity in obesity and metabolic syndrome. *Annals of the New York Academy of Sciences* **1083** 129–152. (doi:10.1196/annals.1367.010)
- Tirelli E, Laviola G & Adriani W 2003 Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. *Neuroscience and Biobehavioral Reviews* **27** 163–178. (doi:10.1016/S0149-7634(03)00018-6)
- Tsoory MM, Guterman A & Richter-Levin G 2010 “Juvenile stress” alters maturation-related changes in expression of the neural cell adhesion molecule L1 in the limbic system: relevance for stress-related psychopathologies. *Journal of Neuroscience Research* **88** 369–380. (doi:10.1002/jnr.22203)
- Velkoska E & Morris MJ 2011 Mechanisms behind early life nutrition and adult disease outcome. *World Journal of Diabetes* **2** 127–132. (doi:10.4239/wjd.v2.i8.127)
- Velkoska E, Cole TJ, Dean RG, Burrell LM & Morris MJ 2008 Early undernutrition leads to long-lasting reductions in body weight and adiposity whereas increased intake increases cardiac fibrosis in male rats. *Journal of Nutrition* **138** 1622–1627.
- Vicente LL, de Moura EG, Lisboa PC, Monte Alto Costa A, Amadeu T, Mandarim-de-Lacerda CA & Passos MC 2004 Malnutrition during lactation in rats is associated with higher expression of leptin receptor in the pituitary of adult offspring. *Nutrition* **20** 924–928. (doi:10.1016/j.nut.2004.06.014)
- Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, Breier BH & Harris M 2005 Neonatal leptin treatment reverses developmental programming. *Endocrinology* **146** 4211–4216. (doi:10.1210/en.2005-0581)
- Xiao XQ, Williams SM, Grayson BE, Glavas MM, Cowley MA, Smith MS & Grove KL 2007 Excess weight gain during the early postnatal period is associated with permanent reprogramming of brown adipose tissue adaptive thermogenesis. *Endocrinology* **148** 4150–4159. (doi:10.1210/en.2007-0373)

Received in final form 24 October 2012

Accepted 12 November 2012

Accepted Preprint published online 14 November 2012