Soy isoflavones recover pancreatic islet function and prevent metabolic dysfunction in male rats

Tatiane Aparecida Ribeiro, Audrei Pavanello, Laize Peron Tófolo, Júlio Cezar de Oliveira, Ana Maria Praxedes de Moraes, Claudinéia Conationi da Silva Franco, Kelly Valério Prates, Isabela Peixoto Martins, Késia Palma-Rigo, Rosana Torrezan, Erica Yeo, Rodrigo Mello Gomes, Flávio Andrade Francisco, Paulo Cezar de Freitas Mathias and Ananda Malta

1Department of Biochemistry and Biomedical Science, McMaster University, Hamilton Ontario, Canada
2Department of Biotechnology, Genetics, and Cellular Biology, State University of Maringá, Maringá, Parana, Brazil
3Institute of Health Sciences, Federal University of Mato Grosso, Sinop, Mato Grosso, Brazil
4Department of Physiologic Science, State University of Maringá – Maringá, Parana, Brazil
5Laboratory of Neuroscience and Cardiovascular Physiology, Department of Physiological Sciences, Federal University of Goiás, Goiânia, Brazil

Correspondence should be addressed to A Malta: nandamalt@hotmail.com

Abstract

We tested whether chronic supplementation with soy isoflavones could modulate insulin secretion levels and subsequent recovery of pancreatic islet function as well as prevent metabolic dysfunction induced by early overfeeding in adult male rats. Wistar rats raised in small litters (SL, three pups/dam) and normal litters (NL, nine pups/dam) were used as models of early overfeeding and normal feeding, respectively. At 30 to 90 days old, animals in the SL and NL groups received either soy isoflavones extract (ISO) or water (W) gavage serving as controls. At 90 days old, body weight, visceral fat deposits, glycemia, insulinemia were evaluated. Glucose-insulin homeostasis and pancreatic-islet insulinotropic response were also determined. The early life overnutrition induced by small litter displayed metabolic dysfunction, glucose, and insulin homeostasis disruption in adult rats. However, adult SL rats treated with soy isoflavones showed improvement in glucose tolerance, insulin sensitivity, insulinemia, fat tissue accretion, and body weight gain, compared with the SL-W group. Pancreatic-islet response to cholinergic, adrenergic, and glucose stimuli was improved in both isoflavone-treated groups. In addition, different isoflavone concentrations increased glucose-stimulated insulin secretion in islets of all groups with higher magnitude in both NL and SL isoflavone-treated groups. These results indicate that long-term treatment with soy isoflavones inhibits early overfeeding-induced metabolic dysfunction in adult rats and modulated the process of insulin secretion in pancreatic islets.

Introduction

An increasing prevalence of obesity and related comorbidities such as type 2 diabetes has been reaching pandemic levels, especially among children and adolescents (Anderson 2018). Fetal and neonatal nutrition during developmental periods is suggested to play a key role in obesity and metabolic disease onset (Barker 1995,
Vickers et al. 2000). For instance, overnutrition, overweight or hormonal disturbances, like hyperinsulinemia and hyperglycemia, during early postnatal life were shown to favor the later development of metabolic dysfunction (Dorner & Plagemann 1994, Plagemann et al. 1999). Overall, exposure to environmental insults during childhood and adolescence may have long-lasting consequences on health (de Oliveira et al. 2013, Baird et al. 2017). Prevention and treatment strategies when initiated in these periods of life seem to be effective to prevent long-term obesity and related comorbidities (Templeman et al. 2015, Malta et al. 2016).

The use of dietary therapies is a potential strategy to prevent obesity and metabolic syndrome (Wing & Hill 2001, Torres et al. 2006). Isoflavonoids, such as genistein and daidzein, are a type of flavonoids predominantly found in soy products (Chi et al. 2016, Muller et al. 2016), common components in functional dietary supplements for human (Cederroth & Nef 2009), and well known for their beneficial effects on health (Torres et al. 2006, Panche et al. 2016, Clamp et al. 2018). Studies have shown that soy isoﬂavones exhibit anti-obesity and anti-diabetic effects (Kawser Hossain et al. 2016), by improving insulin secretion, insulin sensitivity, lipid profile, and reduction of body weight in both human and experimental rat model (Cederroth & Nef 2009, Silva et al. 2018). Obese Zucker rats treated with high-isoﬂavone soy protein showed improved insulin sensitivity (Tovar et al. 2005) and may in part be mediated by changes to insulin secretory capacity of pancreatic β-cells (Ascencio et al. 2004).

Pancreatic insulin secretion is primarily regulated by plasma glucose concentration. However, fine tuning of glucose-stimulated insulin secretion (GSIS) can also be modulated by amino acids, fatty acids, and hormones (Rorsman & Ashcroft 2018). Additionally, GSIS can be modulated by the autonomic nervous system (ANS). In particular, several neurotransmitters, such as acetylcholine (ACh) and noradrenaline, released by the parasympathetic (PNS) and sympathetic nerves system (SNS), respectively have the ability to potentiate and/or inhibit insulin secretion through the activation of muscarinic acetylcholine receptors (mAChR) and adrenoreceptors, present on the β-cell surface (Gautam et al. 2006). In rodent models of overweight and obesity, ANS imbalances have been suggested to be one cause of pancreatic β-cell dysfunction (Kiba 2004).

Interestingly, recent studies have shown that isoflavonoids can act on pancreatic β-cells to regulate insulin secretion through potentiation of GSIS (Noriega-López et al. 2007), by various intracellular mechanisms or through antioxidative actions (Kawser Hossain et al. 2016). However, the insulinotropic effect of isoflavonoids via the ANS has not yet been established.

Given the health benefits attributed to isoflavones and their possible effects in protecting against metabolic diseases, we hypothesize that chronic consumption of soy isoflavone initiated early postnatal life can prevent adult metabolic dysfunction induced by overfeeding during lactation through insulin secretion regulation. In this study, we investigated the effects of soy isoflavones treatment on body composition and glucose-insulin homeostasis in the early post-natal overfeeding rat model of metabolic dysfunction. In addition, we aimed to assess pancreatic-β-cell function under the action of autonomic-insulinotropic and -insulinostatic agents, as well as under the direct action of isoflavones.

Materials and methods

Ethical approval

All experiments were carried out in accordance with ARRIVE guidelines, Brazilian Association for Animal Experimentation (COBEA) and were approved by the ethical approval committee in Animal Research of the State University of Maringa, under protocol 9802290814.

The animals were supplied by the State University of Maringa central animal facility and maintained in the animal facility of the Laboratory of Secretion Cell Biology, under controlled conditions (temperature: 22 ± 2°C; photoperiod: 7:00–19:00 h), with water and standard diet (Nuvital®, Curitiba, Brazil) ad libitum throughout the experimental period. Experimental assays were performed in the Laboratory of Secretion Cell Biology.

Animals and treatments

Female Wistar rats at post-natal day 70 (P70) were mated with males Wistar rats at post-natal day 80 (P80). Upon confirmation of pregnancy by the presence of vaginal sperm, females were individually housed. At birth, all litters size was adjusted to nine pups per dam (preferentially male). A small litter animal model was used to induce an early overnutrition (Plagemann et al. 1992) and at post-natal day 3 (P3), the litter size was adjusted to three male pups per dam (small litter, SL group). Litters containing nine pups per dam served as controls (normal litter; NL group). At post-natal day 21 (P21), all the animals were weaned and housed as three males per cage. At post-natal day 30 (P30), the offspring from the SL and NL
was randomized to either normal litter isoflavone (NL-ISO) and small litter isoflavone (SL-ISO) that received daily gavage of 300 mg/kg soybean isoflavones stratum (Torrezan et al. 2008) until post-natal day 90 (P90) or normal litter water (NL-W) and small litter water (SL-W) that received daily gavage of water for the same period.

The soybean (Glycine Max; Fagron-Brasil®) stratum used in this protocol contains 42% of isoflavones, with a predominance of the biologically active aglycones, 32% daidzein, 9% daidzin, 0.14% genistein, 1% genistin, 0.52% glycitein and 0.54% glycitin.

**Body weight and fat pad stores measurement**

Body weight (bw) was measured at P30 and P90 (n = 10–19 rats; 6–7 litters per group, no litter was over-represented). At P90, the offspring was euthanized and fat pad stores (mesenteric and retroperitoneal) were removed and weighed to estimate adiposity. Fat pad stores were normalized with the bw (g/100 kg).

**Intravenous glucose tolerance test (ivGTT)**

At P90 (n = 10–18 rats; 6–7 litter per group, no litter was over-represented), rats from all groups underwent a surgical procedure under anesthesia (ketamine® (3 mg/100 g) and xylazine® (0.6 mg/100 g) to implant a silicone cannula into the right jugular vein, as previously described (Ribeiro et al. 2017). Rats were allowed to recover for 24 h post-surgery and were then fasted for 12 h and infused intravenously (iv) with a glucose load (1 g/kg). Blood samples were collected via silicone cannula at 0 (baseline), 5, 15, 30, and 45 min after glucose injection. Plasma obtained from the blood samples was stored at −20°C for subsequent determination of the plasma glucose and insulin concentrations (de Oliveira et al. 2013).

**Pancreatic islet function assessment**

Pancreatic islets of the four rats from four different litters for each group were isolated following the collagenase digestion of the pancreas, as described before (de Oliveira et al. 2011). After manual separation, four islets were placed in a 24 well cell culture plate with 1 mL of Krebs–Ringer buffer supplemented with glucose 5.6 mmol/L, and pre-incubated for 60 min at 37°C. After 60 min of pre-incubation, the islets were incubated with a fresh Krebs–Ringer buffer with different glucose concentrations (5.6, 8.3 or 16.7 mmol/L) for 1 h at 37°C. The cumulative insulin release over 60 min was quantified by RIA.

To study cholinergic function, following preincubation (glucose, 5.6 mmol/L), another batch of islets from each group was incubated for an additional 60 min in Krebs–Ringer solution containing either 8.3 mmol/L glucose or 8.3 mmol/L glucose plus 10 µmol/L ACh in the presence of 10 µmol/L neostigmine to block acetylcholinesterase action in the islets. The following cholinergic antagonists were also added to Krebs–Ringer solution containing 8.3 mmol/L glucose plus 10 µmol/L ACh in the presence of neostigmine: the non-selective mAChR antagonist atropine (Atr; 10 µmol/L), the mAChR subtype M1 antagonist pirenzepine (PZP; 100 µmol/L) and the mAChR subtype M3 antagonist 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP; 100 µmol/L). To calculate the insulinotrophic effects of ACh and cholinergic antagonists effects were expressed as a percentage, considering the GSIS in response to 8.3 mmol/L glucose and 8.3 mmol/L plus 10 µmol/L ACh as 100%, respectively.

To study adrenergic function, following the pre-incubation, a cohort of isolated pancreatic-islets from each group was stimulated with a high glucose concentration (16.7 mmol/L) in the presence of 1 µmol/L epinephrine (Epi) and either an α2-adrenoceptor antagonist, yohimbine (Yoh; 10 µmol/L) or a β2-adrenoceptor antagonist, propanolol (Pro; 1 µmol/L). To calculate the insulinostatic effect of Epi and adrenergic antagonists effects were expressed as a percentage, considering the GSIS in response to 16.7 mmol/L glucose and 16.7 mmol/L plus 1 µmol/L Epi as 100%, respectively.

To study the direct effect of isoflavone on insulin secretion, another cohort of four rats from four different litters for each group was used. After preincubation, islets were incubated for 60 min in Krebs solution containing either glucose at 5.6 mmol/L or 8.3 mmol/L plus different concentrations of soy isoflavone extract: 10, 50, 100, and 1000 µmol/L. Data are expressed as percentages, considering GSIS in response to 5.6 mmol/L or 8.3 mmol/L glucose as 100%.

The supernatants from all incubations were collected and stored at −20°C for further insulin measurements. The technical replicates (n = 10–20) were averaged to represent the islets insulin secretion in the graphs for each glucose and isoflavone concentrations, agonist/antagonist cholinergic, and adrenergic.

**Analysis of insulin concentrations**

Single insulin samples in both plasma and from pancreatic islet incubations were determined by RIA (Scott et al. 1981), in a gamma counter (Wizard2 Automatic Gamma Counter,
Isoflavones prevents metabolic diseases

T A Ribeiro et al.

84

The homeostasis model assessment of insulin resistance (HOMA-IR)

The HOMA IR was used as the physiological index of insulin resistance. This was assessed from fasting glucose and fasting insulin concentrations using the following formula: HOMA IR = (fasting insulin (ng/mL) × fasting glucose (mg/dL))/22.5 (Pacini & Mari 2003).

Statistical analysis

Results are shown as the means ± s.e.m. and subjected to a D’Agostino Pearson normality test to assess the Gaussian distribution. Exclusion of outlier data points was determined using an outlier calculator included in the GraphPad Prism version 6.1 for Windows (GraphPad Software Inc.) and excluded from RIA analyses. Data were subjected to variance analysis by two-way ANOVA. Differences between the means were evaluated by Tukey’s post hoc test. A P value < 0.05 was considered significant regarding the main effects of litter size (L), treatment (T), their interaction (I; litter size vs treatment), and the differences between groups. Tests were performed using GraphPad Prism indicated above.

Results

Effect of isoflavone treatment on biometric and fasting biochemical and HOMA-IR parameters

The effect of isoflavones supplementation on body weight and measures of adiposity are summarized in Table 1. Litter size reduction during the suckling phase caused an increase in the body weight at P30 in the SL animals compared with NL (118.4 ± 1.31 vs 94.92 ± 1.89; P < 0.001; Tukey’s post hoc test). At P90, body weight of SL-W was increased by 14.4% (P < 0.001), when compared with NL-W controls. Supplementation with isoflavones for 60 days decreased body weight in SL-ISO and NL-ISO when compared with water-treated controls (P < 0.01, two-way ANOVA). However, this reduction was greater in the SL-ISO compared with NL-ISO, indicating an interaction between effects of litter size and treatment (P < 0.05, two-way ANOVA).

Table 1 Effect of isoflavones treatment on body weight, adipose phenotype and fasting glucose/insulin. Data are expressed as mean ± s.e.m. (n = 10–19 rats; 6–7 litters per group).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NL-W</th>
<th>SL-W</th>
<th>NL-ISO</th>
<th>SL-ISO</th>
<th>Source of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight P90 (g)</td>
<td>365.9 ± 5.3</td>
<td>418.7 ± 8.8a,d</td>
<td>360.1 ± 6.2</td>
<td>383.6 ± 9.8b</td>
<td>I: P &lt; 0.05</td>
</tr>
<tr>
<td>Retroperitoneal fat pad (g/100 g)</td>
<td>1.27 ± 0.05</td>
<td>1.66 ± 0.08a,d</td>
<td>1.14 ± 0.06</td>
<td>1.30 ± 0.09b</td>
<td>I: P &lt; 0.01</td>
</tr>
<tr>
<td>Mesenteric fat pad (g/100 g)</td>
<td>0.72 ± 0.02</td>
<td>0.90 ± 0.10a,d</td>
<td>0.63 ± 0.04</td>
<td>0.67 ± 0.05b</td>
<td>I: ns T: P &lt; 0.01</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>82.47 ± 1.85</td>
<td>112.10 ± 1.68a,d</td>
<td>95.86 ± 2.37</td>
<td>104.90 ± 1.82b</td>
<td>I: P &lt; 0.001</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.36 ± 0.03</td>
<td>0.58 ± 0.12a,d</td>
<td>0.33 ± 0.03</td>
<td>0.40 ± 0.05b</td>
<td>I: P &lt; 0.001</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>1.69 ± 0.12</td>
<td>3.97 ± 0.30a,d</td>
<td>2.29 ± 0.31</td>
<td>2.47 ± 0.33b</td>
<td>I: P &lt; 0.001</td>
</tr>
</tbody>
</table>

Superscript letters indicate statistical difference (P < 0.05) against NL-W, SL-W or SL-ISO, based on Tukey multiple comparisons test. I, interaction between litter size and treatment factors based on two-way ANOVA; L, litter size factor; T, treatment factor.
At P90, the SL-W group exhibited an increase of 31% and 25% in retroperitoneal and mesenteric fat pad stores, respectively, compared with NL-W (P < 0.05). Treatment with isoflavones for 60 days also reduced fat weight in both SL-ISO and NL-ISO groups compared with water-treated controls (P < 0.01, two-way ANOVA), although the post hoc test shows no significant difference between the NL-W and NL-ISO. However, SL-ISO group exhibited a reduction of 21% in retroperitoneal fat pad (P < 0.01, two-way ANOVA) and 25.5% in mesenteric fat pad store (P < 0.05) compared with SL-W.

Regarding the effect of isoflavones supplementation on measures of glucose/insulin homeostasis (Table 1), as expected, SL-W fasting plasma glucose, fasting plasma insulin, and HOMA-IR index were increased by 36, 61, and 134% respectively, compared with NL-W (P < 0.05). Isoflavones treatment of rats from small litters (SL-ISO) resulted in decreased fasting plasma glucose, fasting plasma insulin, and HOMA IR index by 7, 31, and 62% respectively, compared with water-treated controls (SL-W) reflecting a significant interaction between litter size and treatment factors (P < 0.05, two-way ANOVA). Treatment with isoflavones in NL-ISO and SL-ISO resulted in different effects on insulin secretion compared to NL-W (P < 0.001), regardless of glucose concentrations. However, no differences were observed between NL-isoflavones treated and NL-W rats according to post hoc test (Fig. 2A).

Insulin secretion in response to mACHR agonist and antagonists is shown in Fig. 2B and C. Incubation with 10 μmol/L ACh showed that SL-W islets respond poorly to this specific cholinergic stimulation than NL-W islets (P < 0.05). Treatment with isoflavones in NL-ISO and SL-ISO groups resulted in increased insulin secretion in response to ACh as compared with NL-W and SL-W, respectively (P < 0.001, two-way ANOVA), but to a higher extent in the SL-ISO group, reflecting a significant interaction between litter size and treatment factors (Fig. 2B, P < 0.001, two-way ANOVA).

Atropine inhibited the cholinergic insulinotropic effect of isolated islets from all groups (Fig. 2C). However,
Figure 2
Pancreatic islet insulin secretion. Insulin secretion stimulated by different glucose concentrations (A). Insulin secretion stimulated by 8.3 mmol/L glucose and potentiated by 10 μmol/L ACh (B). Inhibition of the insulinotropic effect of ACh (8.3 mmol/L glucose plus 10 μmol/L ACh in the presence of neostigmine) by 10 μmol/L Atr, 100 μmol/L 4-DAMP and 100 μmol/L PZP (C). Insulin secretion stimulated by 16.7 mmol/L glucose and inhibited by 1 μmol/L Epi (D). Insulin secretion under the effect of 16.7 mmol/L glucose plus 1 μmol/L Epi in the presence of 10 μmol/L Yoh or 1 μmol/L Prop (E). Data are expressed as percentages, considering 8.3 mmol/L glucose (B), 8.3 mmol/L glucose plus 10 μmol/L ACh (C), 16.7 mmol/L glucose (D) and 16.7 mmol/L glucose plus 1 μmol/L Epi (E) as 100%. Bars represent the mean ± s.e.m. of insulin. Letters over the bars indicate a significant difference (P < 0.05) against NL-W, SL-W, NL-ISO or SL-ISO, based on Tukey multiple comparisons test. L, litter size factor; T, treatment factor and I, interaction between litter size and treatment factors based on two-way ANOVA. ns, no significant difference.
this inhibition was 53.6% in the SL-W group and 78% in the NL-W group (P < 0.001). NL-ISO and SL-ISO islets showed less inhibition in insulin secretion compared with their respective water-control groups, but to a lesser extent in the SL-ISO group, reflecting a significant interaction between litter size and treatment factors (P< 0.0001, two-way ANOVA).

Additionally, PZP inhibited the acetylcholine insulinotropic effect in all groups (Fig. 2C); however, SL-W and NL-ISO islets were inhibited to a lesser extent than NL-W (P < 0.05). SL-ISO islets showed a larger inhibition of insulin secretion compared SL-W islets indicating interaction between litter size and treatment factors (P< 0.0001, two-way ANOVA).

Addition of 4-DAMP inhibited the acetylcholine insulinotropic effect in islets from all groups (Fig. 2C). This inhibition was 25% in the SL-W group and 70% in the NL-W group (P < 0.0001). The inhibition of insulin secretion was lower in SL and NL isoflavones-treated animals compared with water-treated control groups, however, to a lesser extent in SL-ISO compared to SL-W, indicating interaction between litter size and treatment factors (P< 0.0001, two-way ANOVA).

We further investigated pancreatic islet insulin secretion in response to adrenergic agonists or antagonists (Fig. 2D and E). In the presence of 16.7 mmol/L glucose, Epi inhibited insulin secretion in all islet groups. However, SL-W showed reduced inhibition of insulin secretion compared to NL-W (P < 0.001). The inhibition of insulin secretion was lower in SL and NL isoflavones-treated animals compared with water-treated control groups, however, to a lesser extent in SL-ISO compared SL-W, indicating interaction between litter size and treatment factors (Fig. 2D, P< 0.0001, two-way ANOVA).

Yohimbine treatment prevented Epi-mediated inhibition of insulin secretion in all islet groups (Fig. 2E). Nonetheless, it was lower in islets from SL-W rats when compared with the NL-W rats (P < 0.01). Similarly, the inhibition of insulin secretion by epinephrine was blocked in both islet of isoflavones-treated groups when compared to their respective water groups, however, to a lesser extent in SL-ISO compared SL-W, indicating interaction between litter size and treatment factors (P< 0.0001, two-way ANOVA). When compared with insulin secretion following epinephrine treatment, propranolol reduced the insulin secretion level by 14% in NL-W, 5% in SL-W, 2% in NL-ISO and 10% in SL-ISO; however, no significant difference was observed between groups (Fig. 2E).

Pancreatic islets isolated from rats from each group under 5.6 mmol/L glucose were subjected to varying concentrations of soy isoflavones in vitro (Fig. 3A). Insulin secretion was stimulated in all groups in response to varying isoflavones concentrations. SL-W insulin secretion in response to soy isoflavone treatment was increased compared with NL-W, regardless of isoflavone concentration (P < 0.05). Furthermore, both groups supplemented with soy isoflavones, NL-ISO and SL-ISO, displayed increased insulin secretion in response to soy isoflavones concentrations above 10 µmol compared with their water-treated counterparts, indicating treatment effect (P< 0.0001, two-way ANOVA). In response to 10 µmol isoflavones, SL-ISO displayed decreased insulin secretion compared with SL-W (P < 0.0001).

We also investigated responses to the same concentrations of soy isoflavones under 8.3 mmol/L glucose (Fig. 3B). Soy isoflavones stimulated GSIS in islets from all groups, regardless of concentration. At concentrations above 50 µmol, insulin secretion of SL-W islets was decreased compared with NL-W (P < 0.05). Interestingly, islets from NL-ISO and SL-ISO were more responsive to all isoflavones incubations compared with water-treated controls, indicating an effect of treatment (P< 0.05, two-way ANOVA). Outlier was excluded from analyses.

Discussion

Extensive research over the past 10 years has provided important insight into the relationship between perinatal health and metabolic dysfunction in adulthood (Barker 2004). Early post-natal overfeeding induces insulin resistance, glucose intolerance, increases body weight, pancreatic β-cell dysfunction (Ribeiro et al. 2017) and has been associated with increased susceptibility to overweight and obesity as well as related comorbidities, such as type 2 diabetes (Morrison et al. 2008). In this study, we showed that soy isoflavones supplementation prevents adult metabolic dysfunction and recovery of pancreatic islet function in early postnatal overnourished rats.

Our results showed that supplementation with soy isoflavones can reverse the effects of early post-natal overnutrition on weight gain and white adipose deposition. Previous studies have shown that early post-natal overnutrition induces early malprogramming of hypothalamic energy circuits, leading to increased food intake and reduced thermogenic capacity by central leptin and insulin resistance in the arcuate nucleus (Plagemann et al. 1999), resulting in increased body weight and white adipose tissue. Furthermore, rats fed a high-fat diet...
supplemented with soy isoflavones display increased thermogenic capacity as measured by increased uncoupling protein-1 mRNA content contributing to attenuated body weight gain (Torre-Villalvazo et al. 2008). Though we have not directly measured thermogenic capacity in our cohort, decreased body weight gain and white adipose tissue may be attributed to improved thermogenic capacity in rats treated with soy isoflavones.

Body and adipose tissue weights are closely related to insulin, glucose, and lipid metabolism (Turpin et al. 2014) and further, bodyweight loss is associated with improving insulin resistance and glucose tolerance (Ferrannini et al. 2004). We observed that glucose tolerance and insulin resistance were improved in SL-ISO rats suggesting isoflavones treatment may reverse effects of early overnutrition on glucose/insulin metabolism.

Previous studies using the small-litter model of early overnutrition suggest that rats from small litters displayed hyperinsulinemia as early as during lactation (Malta et al. 2016). Early overfed rats had elevated non-insulin-dependent glucose transporter GLUT-2 content in pancreatic islets, which seemed to be related to increased insulin secretion (Cunha et al. 2009). Indeed, early hyperinsulinemia alone may program the development of adult obesity and diabetes (Templeman et al. 2017). Furthermore, the suppression of hyperinsulinemia in young high-fat diet-fed mice can attenuate obesity throughout adulthood (Templeman et al. 2015). Our experiments with isoflavones treatment in young SL rats improved hyperinsulinemia and protected against obesity. Studies conducted in vitro and in vivo showed that isoflavones can ameliorate hyperinsulinemia in obesity by decreasing GLUT-2 expression in pancreatic islets (Noriega-López et al. 2007). This may be a possible mechanism by which animals supplemented with soy isoflavones displayed lower insulin secretion in our current study.

SL rats treated with isoflavones showed improvement in glucose tolerance and insulin resistance by restoring HOMA-IR. Isoflavones seem to exert direct effects on insulin receptor signaling pathways in skeletal muscle and adipocyte cell cultures. This likely occurs through the modulation of insulin receptor substrate phosphorylation, activation of the phosphatidylinositol 3’-kinase (PI3K)/AKT pathway and AMP-activated protein kinase promoting non-insulin-dependent glucose transporter GLUT-4 expression and translocation to the cellular membrane (Lee et al. 2009, Wang et al. 2013).

Overnutrition in early life leads to long-term ANS imbalance, specifically, high vagal activity and modified muscarinic and adrenoceptors function and/or expression.
Isoflavones prevents metabolic diseases

(Xiao et al. 2007, Malta et al. 2016, Silva et al. 2018) ultimately impacting insulin secretion. As a consequence, this condition might exhaust the pancreatic β-cells and result in subsequent susceptibility to diabetic conditions. We observed that isolated pancreatic islets from SL-W rats had decreased insulin secretion in response to 10 μmol/L ACh, suggesting a down-regulation or a decreased sensitivity of M₃mAChR and M₄mAChR receptors in the pancreatic β-cell membrane.

The insulinotropic ACh effect is mainly driven by M₃mAChR activation (Gautam et al. 2006, Ribeiro et al. 2013); however, when binding to M₄mAChR may also potentiate insulin secretion (Wess et al. 2007). In addition, the M₃mACR and M₄mAChR subtypes have the same physiological contribution to buffer insulin secretion potentiated by ACh binding receptors M₃mAChR and M₄mAChR in pancreatic beta-cell (Miguel et al. 2002). Interestingly, the isoflavones treatment displayed a beneficial effect by reverting the impaired cholinergic response in the pancreatic islets from SL-ISO rats, preventing the possible development of diabetes.

Regarding adrenergic signaling in islets, SNS activation by α₂-adrenoceptor (α₂AR) inhibits insulin secretion, whereas, activation of the islet beta₂-adrenoceptor (β₂AR) stimulates insulin secretion (Borden et al. 2013). We observed reduced insulinostatic effects for epinephrine and insulinotropic effects for the α₂AR in animals treated with isoflavones. Together, these results suggest that isoflavones provide a beneficial effect on insulin secretion by maintaining the lowest response of adrenergic stimulation and avoiding the impaired cholinergic response in the islets of overnourished rats that converged on the improved glucose/insulin homeostasis which indicates a possible mechanism modulating autonomic nerve activity.

Several studies consistently have shown that soy isoflavones increase the GSIS (Jayagopal et al. 2002, Ascencio et al. 2004, Tovar et al. 2005, Talaei & Pan 2015) and prevent type 2 diabetes (Lee 2006, Gilbert & Liu 2013). Our in vitro studies showed that islets of all groups incubated with different concentrations of isoflavones showed a significantly increased insulin secretion. These results suggest that genistein has a positive effect on β-cell functions including the stimulation of insulin secretion (Jonas et al. 1995). Moreover, treatment with S-Equol, a metabolite of daidzein, and its glycoside daidzin, produced by intestinal bacteria, in isolated mice islets enhanced insulin secretion under glucose concentration through activating cAMP-PKA signaling in INS-1 pancreatic β-cells (Horiuchi et al. 2017). This same pathway is activated in pancreatic β-cells incubated with genistein (Liu et al. 2006).

Another interesting finding was that the islets of both isoflavones-treated groups had higher insulin secretion compared to untreated animals when incubated with isoflavones. This could partially be explained by the effect of isoflavones treatment, genistein, and daidzein, on increasing β-cell proliferation (El-Kordy & Alshahrani 2015, Horiuchi et al. 2017), considering that isoflavone treatment initiated in the peripuberal phase, another window for metabolic programming. However, the mechanisms whereby soybean isoflavones exert its beneficial effects on regulating insulin secretion were not investigated in this study.

In conclusion, our findings show that long-term treatment with soy isoflavones prevents early overfeeding-induced metabolic dysfunction in adult rats and regulates the process of insulin secretion in pancreatic islets. These effects may in part be modulated by the ANS. Puberty and young adulthood can be a potentially critical time to shaping future health, and our study demonstrates that, in rats, the consumption of isoflavones during this life stage can provide protection against later diabetes and obesity.

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
The current study was supported by the Brazilian Federal Foundation, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Author contribution statement
T A R, A P, A M and P C F M contributed to the study conception and design. T A R, A M, L P T and A P performed material preparation, data collection and analysis. J C O, A M P M, C C S F, K V P, I P M, K P R, R T, E Y, R M G, F A F contributed intellectually as well as reviewed, edited and approved the final version of this manuscript. All authors read and approved the final manuscript.

Acknowledgements
The authors thank Ms Maroly Pinto, Ms Marli Licero and Ms Leila Andreia Frota for helping to care for the rats in the animal facility.

References
Panche AN, Diwan AD & Chandra SR 2016 Flavonoids: an overview. Journal of Nutritional Science 5 e47. (https://doi.org/10.1017/jns.2016.41)


Templeman NM, Clee SM & Johnson JD 2015 Suppression of hyperinsulinemia in growing female mice provides long-term protection against obesity. Diabetologia 58 2392–2402. (https://doi.org/10.1007/s00125-015-3676-7)


Torres N, Torre-Villalvazo I & Tovar A 2006 Future directions in reducing hepatic lipotoxicity. Future Lipidology 1 331–341. (https://doi.org/10.2210/jn/138.3.462)


Received in final form 25 May 2021
Accepted 4 June 2021
Accepted Manuscript published online 8 June 2021