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Long-term effects of overfeeding during lactation on insulin secretion — the role of GLUT-2

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Abstract

Overnutrition during critical developmental periods is believed to be a risk factor for the emergence of metabolic disorders in adulthood. The present study investigated the effects of pups overfeeding during lactation on offspring's insulin secretion. To study the consequences of overnutrition early in life in rats, litter size reduction has been shown to be an appropriate experimental model. To induce early postnatal overnutrition, litter size was reduced to three pups per litter at the third day following birth [overfed group (OG)]. In the control group (CG), the litter size was adjusted to 10 pups per litter. Metabolic parameters and glucose-stimulated insulin secretion were assessed. OG pups ingested more milk at 10 and 21 days and had an augmented food intake at 1 year compared to the CG. Consistently, body weight, body fat, and fasting plasma levels of insulin were higher in 1-year-old OG rats. In addition, OG rats exhibited enhanced insulin secretion, accompanied by elevated content of GLUT-2 in pancreatic islets compared to CG. These findings indicate that early postnatal overnutrition during a critical developmental period in life may program permanent alterations in glucose-stimulated insulin secretion.

Key words: Overnutrition; Insulin secretion; GLUT-2

1. Introduction

Adult offspring of mothers overnourished during the suckling period, despite a normal post-weaning diet, are susceptible to develop obesity [1]. The process by which nutritional disturbances lead to permanent changes in physiology, cell metabolism and molecular biology has been extensively studied [2]. For example, it has been shown that rats overfed during lactation develop hyperphagia and hyperinsulinemia and become overweight when adults [3–5]. Moreover, any change during the neonatal period may program the offspring's susceptibility to later development of chronic diseases including obesity and diabetes. Therefore, a balanced nutrition during lactation is of great importance to the proper maturation and physiology of different cells and tissues, including pancreatic islets, responsible for secreting insulin, a hormone which is directly involved with the emergence of obesity and diabetes [6].

Insulin plays a key role on animal development. For instance, it has been demonstrated that maternal malnutrition during pregnancy and lactation may induce the loss of proliferative capacity and decreases pancreatic islets' vascularization in the offspring, reducing insulin secretion and inducing glucose intolerance [7–11]. Insulin secretion depends essentially on glucose uptake by the pancreatic beta cells. This process is driven by the glucose transporter GLUT-2 [12–14]. The absence or deficiency in GLUT-2 expression may thus reduce insulin secretion. Glucose-unresponsiveness associated with GLUT-2 impairment is typically demonstrated in Type 2 diabetes [15–18]. Regarding molecular mechanisms involved in the proliferation and secretion of pancreatic beta cells, it has been suggested that protein kinase B (PKB), also know as Akt, is critically

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important in controlling the growth and energy distribution in these cells [19,20]. Interestingly, in Akt-deficient male mice, insulin resistance progresses to a severe form of diabetes accompanied by failure in insulin secretion [21,22]. Also, Akt may induce exocytosis of insulin-containing granules [23–25]. Therefore, Akt may play an important role in the development of pancreatic beta cells and in insulin secretion.

In the present study, we investigated the effects of early overnutrition on body weight and glucose-induced insulin secretion of the adult male offspring of Wistar rats. We studied GLUT-2 expression, as well as Akt and phospho-Akt (p-Akt) contents in pancreatic islets of overfed rats. Furthermore, insulin and leptin plasma levels as well as Akt phosphorylation in the liver and muscle were also determined. In summary, our data showed an increased GLUT-2 content in pancreatic islets of overfed rats compared to controls. This increase in GLUT-2 observed in pancreatic beta cells of overfed rats was not associated to an increase in Akt when compared with control animals.

2. Materials and methods

2.1. Animals

Female Wistar rats (90 days old) were mated, and the pregnant dams were randomly housed in individual cages at 23°C on a 12-h light/dark cycle. The animals were fed a standard laboratory chow ad libitum containing 22% protein during gestation and lactation. To induce early postnatal overnutrition, the litter size was adjusted to only three male pups per litter on the third day of life [overfed group (OG)] until the end of the lactation period (21 days). The control group had the litter size adjusted to 10 newborns (female and male) per litter [control group (CG)] a few hours after birth [3,4].

At the end of the lactation period, male pups (OG and CG) were separated and fed standard laboratory chow ad libitum containing 22% protein until the date of the experiments (at 1 year of age). After intragroup randomization, the total number of animals used was 17 per group, obtained from different litters. For the collection of plasma and tissues, the animals were anesthetized with thiopental and sacrificed by spinal cord displacement. The animals were treated in accordance with the Animal Care and Use Committee of the Biology Institute of the State University of Rio de Janeiro, which based their analysis on the principles described in the Guide for Care and Use of Laboratory Animals [26].

2.2. Milk consumption and maternal milk macronutrients

Milk intake was estimated in control and overfed rats at 10 and 21 days of life. Pups were weighed before and after a 24-h period of suckling. Subsequently, the pups were removed from dams to fast for 24 h, and body weight was measured again prior to and following the fasting period [27]. Milk consumption was estimated through the formula described below, where: V=milk volume consumed by the pups in grams, SP2=body weight of pups after 24 h of suckling, SP1=body weight of pups prior to 24 h of suckling and K=relative weight loss of pups after fasting.

Lactose (Lac), proteins (Ptn) and triglycerides (Tg) were measured in maternal milk. Lactose was measured by a colorimetric method using picric acid [28]; total protein was determined by Peterson's method [29] and plasma Tgs were determined by a colorimetric method using a Tg-PP commercial kit (Gold Analisa, Belo Horizonte, Brazil).

2.3. Body weight, body composition, Lee index and food intake

To verify if overfeeding early in life was able to induce abnormal weight gain, body weight and nasoanal length were measured in 1-year-old rats. Thereafter, we calculated the Lee index [30–32] to investigate weight gain against animal length and to verify if litter size reduction was able to induce the rats to become overweight [33]. The Lee index was calculated through the formula described below, where BW refers to body weight and NAL refers to nasoanal length: $BW^{0.03}$ (g)/NAL (mm).

Total body protein was assessed by the carcass method [34]. Visceral fat and food intake were determined by using an analytical scale (BD-600, Instrutherm, São Paulo, Brazil). One week prior to completing 1 year of age, animals were placed in individual cages and given access to 100 g of laboratory chow daily for 7 days. The remaining amount of chow in the cages after a 24-h period was weighed. Food intake was calculated subtracting the remaining chow after 24 h from the amount of food originally offered to the rats.

2.4. Blood sampling

One-year-old rats were anesthetized through an intraperitoneal administration of thiopental (30 mg/kg) and heparin (5000 U/kg), for the purpose of avoiding blood coagulation. Thereafter, blood samples (0.5 ml) were collected in syringes and centrifuged (14,000×g) for 10 min, and the plasma was separated for glucose, leptin and insulin assays. The samples were collected in an ice bath and kept at −20°C for the purpose of detecting glucose by the oxidase method (Gold Analisa, Belo Horizonte, Brazil), leptin by ELISA and insulin by radioimmunoassay (RIA) using monoiodated ^{125}I-labeled porcine insulin (MP Biomedicals, San Francisco, CA, USA) as a tracer, pig anti-insulin (Sigma-Aldrich, St. Louis, MO, USA) as an antibody and purified rat insulin (Novo Nordisk, Montes Claros, Brazil) as the standard. Iodine was measured by a gamma counter (DPC Medlab, Orlando, FL, USA). Leptin was measured using ELISA (Diagnostic System Laboratories, Webster, TX, USA).

2.5. Glucose tolerance test

After a 12-h fast, a glucose tolerance test was performed. Glucose (1g/kg of body weight) was injected intraperitoneally [35], and blood samples were collected

from the tail vein just prior to glucose administration (time 0) and at 30, 60 and 90 min following injection. Blood glucose was measured using a glucometer and ACCU CHECK-Active test strips (Roche, Grenzach-Wyhlen, Germany).

2.6. Muscle and liver collection after insulin stimulation

After a 12-h fast, 1-year-old animals were anesthetized through an intraperitoneal administration of thiopental (30 mg/kg), and an insulin bolus (0.5 ml, 2.0 IU/kg of body weight) was injected through a femoral catheter. After 10 min, samples of muscle (soleus) and liver were harvested and frozen in liquid nitrogen. Samples of muscle (soleus) and liver were also collected from control and overfed rats that were not stimulated with insulin. p-Akt content was determined by immunoblotting, and the ratio between p-Akt content without insulin stimulation and p-Akt content following insulin stimulation was calculated in both CG and OG groups.

2.7. Insulin secretion "in vitro"

Insulin secretion in vitro was evaluated in pancreatic islets isolated by the collagenase technique (Worthington, NJ, USA) [36]. A total of 100 islets obtained from 1-year-old animals (CG and OG) were placed on Millipore filters in a perfusion system with Krebs solution and a mixture of CO_2 (5%) and O_2 (95%). A peristaltic pump (Amersham, San Francisco, CA, USA) maintained a flow rate of 1 ml/min. Basal insulin levels were determined during 20 min by the infusion of 2.8 mmol/L glucose. After attaining the stable state, 16.7 mmol/L glucose was infused for 20 min to determine glucose-induced insulin secretion. The samples were collected in an ice bath and were kept at −20°C for insulin assay. Immunorreactive insulin was measured by RIA.

2.8. Western blotting analysis of GLUT-2, p-Akt and Akt

GLUT-2, p-Akt, Akt and actin contents were determined by immunoblotting in an extract of pancreatic islets obtained from 1-year-old control and overfed rats [37], and p-Akt content was determined by immunoblotting in extracts of muscle and liver, some following insulin stimulation and some without stimulation. A hundred pancreatic islets of each group and samples (100 mg) of muscle and liver obtained from each group were lysed in a buffer containing 50 mmol/L HEPES, 1 mmol/L $MgCl_2$, 10 mmol/L EDTA, 1% Triton X-100, 10 mg/ml aprotinin, 10 mg/ml leupeptin and 17.4 mg/ml PMSF (phenylmethanesulfonyl fluoride; Sigma-Aldrich). Homogenized samples were centrifuged for 3 min at 3000 rpm, and the supernatant was utilized in the experiments. The total protein content was determined by the Bradford method [38]. Samples (80 μg of pancreatic islets and 30 μg of muscle and liver) were subjected to 10% SDS-PAGE gels. The samples were loaded with a protein standard (Sigma-Aldrich), transferred to PVDF Hybond-P membrane (Amersham, Buckinghamshire, England) and blocked with Tween-TBS (20 mM Tris-HCl, pH: 7.5; 500 mM NaCL; 0.01% Tween-20) containing 2% bovine serum albumin (Merck, Darmstadt, Germany). Goat anti-GLUT2, rabbit anti-p-Akt, rabbit anti-Akt and goat anti-Actin 1:1000 (Santa Cruz, CA, USA) were used in Western blot analyses as primary antibodies. PVDF membranes were then incubated with an appropriate secondary antibody conjugated with biotin 1:1000 (Santa Cruz, CA, USA), followed by incubation with horseradish peroxidase-conjugated streptavidin 1:1000 for pancreatic islets and 1:5000 for muscle and liver (Zymed, CA, USA). Immunoreactive proteins from pancreatic islets were visualized by 3,3-diaminobenzidine (Sigma-Aldrich) staining, while immunoreactive proteins from muscle and liver were visualized by West Pico Chemiluminescent (Pierce, Rockford, IL, USA). The bands were quantified by densitometry, using Image J Software (NIH, Bethesda, MD, USA).

2.9. Statistical analysis

The results were expressed as mean±S.E.M. and were statistically analyzed by Mann–Whitney *U* test, using a significance level of $P \leq .05$.

3. Results

3.1. Milk ingestion and macronutrients in maternal milk

Fig. 1 shows milk consumption by the pups. Our data showed that rats raised in small litters ingested more milk than controls at both 10 and 21 days of life. Moreover, in Fig. 2, we show that maternal milk of female rats whose litters were reduced have increased Tg levels at 10 and 21 days following delivery and decreased amounts of protein at 21 days.

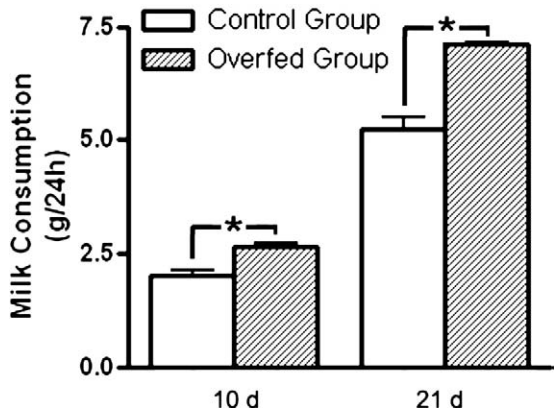

Fig. 1. Milk consumption. Values represent mean±S.E.M. of five pups per group. *Significant difference between overfed and control groups at 10 and 21 days of lactation was determined by Mann–Whitney *U* test, using a significance level set at $P \leq .05$.

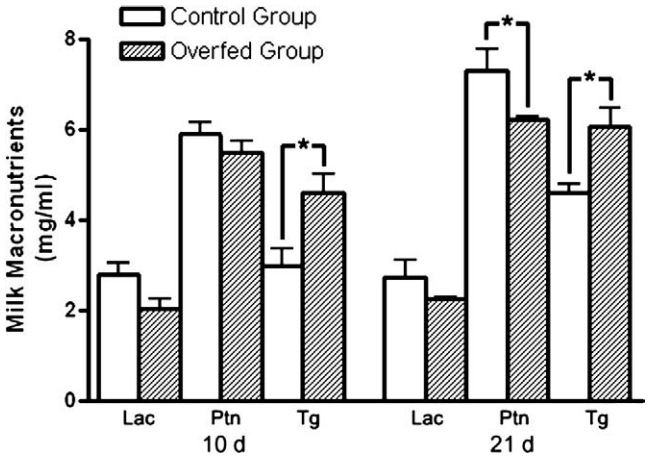

Fig. 2. Milk macronutrients. Values represent mean±S.E.M. of five pups each group. *Significant difference between overfed and control groups at 10 and 21 days of lactation was determined by Mann–Whitney *U* test using a significance level set at $P \leq .05$.

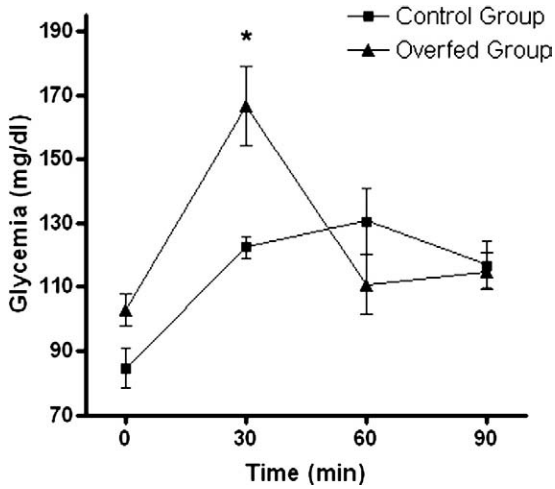

Fig. 3. Glucose tolerance test. Values represent mean±S.E.M. of five pups per group. *Significant difference between overfed and control group was determined by Mann Whitney *U* test using a significance level set at $P \leq .05$.

3.2. Body weight, body composition and plasma levels of glucose, lepton and insulin

Overfeeding during lactation induced hyperphagia at early ages, which persisted into adulthood. Adult (1-year-old) OG rats presented increased food intake, body weight and visceral fat when compared to age-matched controls. Body weight/body length ratio (Lee index) was also significantly higher in OG animals, indicating that this group was overweight. Fasting plasma level of leptin and insulin were higher in overfed rats when compared to controls, but no differences were found in plasma levels of glucose between the groups at fasting (Table 1).

3.3. Glucose tolerance tests

Glucose tolerance tests were performed in 1-year-old overfed rats and their age-matched controls. Thirty minutes after glucose administration, blood glucose level was higher in overfed rats relative to controls; however, at 60 and 90 min following glucose injection, glucose levels were similar in both groups (Fig. 3).

3.4. p-Akt in muscle and liver

Western blot analyses showed that there was no difference in p-Akt without insulin/p-Akt after insulin ratios either in muscle or in liver of overfed rats compared to controls at 1 year of age (Fig. 4). These data indicate that 1-year-old overfed rats do not display any difference compared to control rats regarding Akt phosphorylation following insulin stimulation in these tissues.

Table 1
Body weight, body length, body composition and plasma levels of insulin and glucose

1-year-old rats	Control (*n*=6)	Overfed (*n*=6)	*P* value
Body weight (g)	342±5.7	434±5.5	<.001
Nasoanal length (cm)	24.6±0.3	24.0±0.3	.2
Lee index	282±5.4	315±7.4	<.001
Food consumption	113.1±3.07	144.2±3.1	<.05
Body visceral fat (g)	2.4±0.1	5.9±0.3	<.001
Body protein (%)	23±1.2	22±0.5	.2
Fasting plasma glucose (mg/dl)	90±5.4	104±7.2	.1
Fasting plasma insulin (μIU/ml)	31±3.0	58±6.7	<.05
Fasting plasma leptin (pg/ml)	3.3±0.3	7.3±0.4	<.001

Values represent mean±SEM of 6 pups per group. Significant difference between overfed and control groups was determined by Mann–Whitney *U* test using a significance level set at $P \leq .05$.

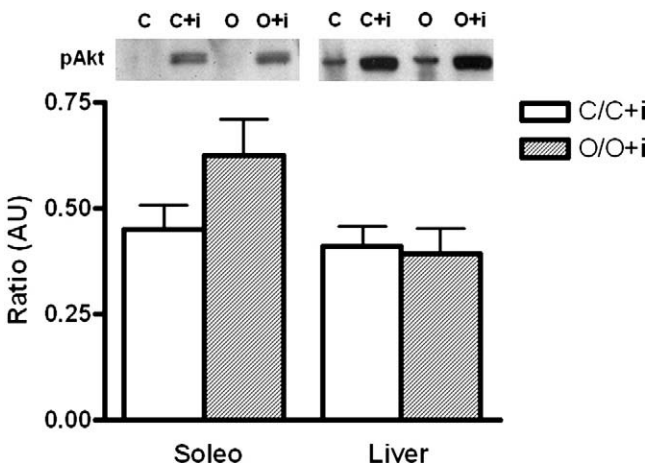

Fig. 4. Representative Western immunoblot of p-Akt content in soleo and liver. Values represent mean±S.E.M. of three pups per each group for p-Akt. *Significant differences between overfed and control groups were determined by Mann–Whitney test using a significance level set at $P \leq .05$.

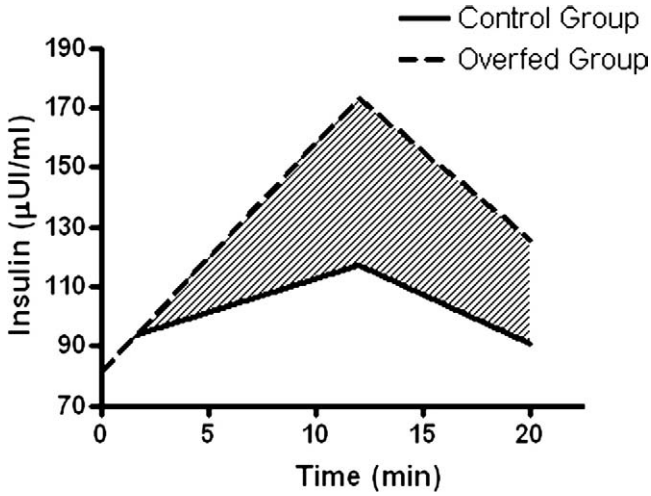

Fig. 5. AUC correspondent to insulin secretion in vitro. Values represent mean±S.E.M. of five pups per group. *Significant difference between overfed and control groups was determined by Mann–Whitney *U* test using a significance level set at $P \leq .05$.

3.5. Insulin secretion by pancreatic islets and total contents of GLUT2, p-Akt and Akt

The area under the curve (AUC) correspondent to insulin secretion in vitro during 20 min of 16.7 mM glucose stimulation was enhanced in overfed rats compared to controls at 1 year of age (Fig. 5). Western blot analyses showed that the increased insulin secretion was accompanied by an enhancement in GLUT-2 and in Akt contents in pancreatic islets of overfed rats at 1 year of age relative to their age-matched controls (Figs. 6 and 7). However, Akt phosphorylation, as indicated by p-Akt/Akt ratio, was decreased in overfed rats compared to controls (Fig. 7).

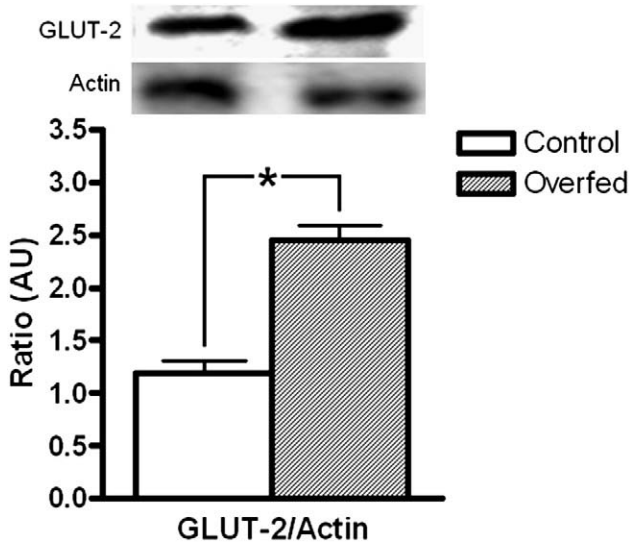

Fig. 6. Representative Western immunoblot of GLUT-2/actin content in pancreatic islets. Values represent mean±S.E.M. of four pups per group for GLUT-2. *Significant differences between overfed and control groups were determined by Mann–Whitney *U* test using a significance level set at $P \leq .05$.

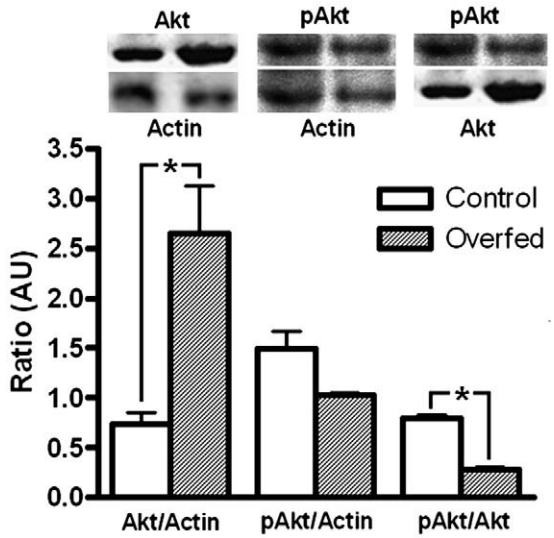

Fig. 7. Representative Western immunoblot of Akt/actin and p-Akt/actin ratios in pancreatic islets. Values represent mean±S.E.M. of six pups per each group for Akt/actin and three pups per group for p-Akt/actin. In addition, the p-Akt/Akt ratio in pancreatic islets was calculated. *Significant differences between overfed and control groups were determined by Mann–Whitney *U* test using a significance level set at $P \leq .05$.

4. Discussion

In the present study, we reinforce the concept of metabolic imprinting and programming, processes by which nutritional disturbances during early life can trigger metabolic and hormonal adaptations that will last into adulthood [2]. Specifically, our results emphasize the importance of an adequate nutrition during the suckling period [3,4,37] and suggest that overnutrition, mainly by a high-Tg maternal milk during lactation, may be considered pivotal to the establishment of deleterious effects to health later in life. It has been shown that exposure of β-cells to free fatty acids in vitro leads to enhanced rates of insulin secretion. The effects of fatty acids in pancreatic beta cells are still controversial, being described as cytotoxic [39] or cytoprotective [40]. Nevertheless, our data regarding the increased concentration of Tg in the maternal milk of dams whose litters were reduced suggest that this nutritional supply might induce the increase in insulin plasma level observed in overfed rats.

Our data showed that overnutrition by litter size reduction and, therefore, a greater access to milk, permanently altered feeding behavior of the progeny, inducing hyperphagia early in life which persisted into adulthood. Consistently, food intake, body weight and visceral fat were increased in adult overfed rats. We have previously demonstrated that, at 10 and 21 days of life, rats raised in small litters have increased plasma levels of insulin and leptin [41]. Interestingly, it has been suggested that increased insulin and leptin levels might

be involved in the mechanism responsible for modifying feeding behavior [42]. In addition, it has been shown that both hormones can stimulate the increase of galanin in the brain, a neuropeptide that induces hyperphagia and weight gain [4]. These hormones may also have a role in augmenting hypothalamus projections responsible for controlling hunger and satiety. Notably, such neuronal networks are extremely sensitive to food availability [43–45]. Although in the present study we have not investigated alterations in the brain, the hyperphagia observed in overfed rats might have been programmed by increased leptin and insulin plasma levels early in life [41]. The permanent hyperphagia observed in our model later in life might be the result of a central resistance to insulin and leptin, hormones with an increased level in the plasma of 1-year-old overfed rats. Moreover, the increased concentration of leptin found in overfed rats could induce Akt activation in pancreatic beta cells, as previously described [46].

Regarding metabolic alterations, glucose tolerance tests showed that overfed rats had impaired glucose tolerance at 30 min following glucose infusion, accompanied by an increased fasting plasma level of insulin. These data might indicate that overfed rats were insulin-resistant. However, we did not find any difference regarding Akt phosporylation following insulin stimulation in the liver and muscle of overfed rats compared to controls. Moreover, rats raised in small litters displayed increased glucose-stimulated insulin secretion in vitro. Our results corroborate with a previous study showing that overnutrition in the beginning of life leads to an enhancement in insulin secretion in vitro from 21 days of life into adulthood in male Wistar rats [47]. Our data suggest that overfed rats secreted higher amounts of insulin in order to maintain normal glucose levels. Therefore, the enhancement in insulin secretion might be an adaptation in the attempt to normalize blood glucose levels. The same was observed in Zucker diabetic fatty rats [48].

To investigate alterations that could be related to the enhancement in insulin secretion, we measured GLUT-2, p-Akt and Akt contents in pancreatic islets. We demonstrated an increase in GLUT-2 content in pancreatic islets of overfed rats, as shown by the GLUT-2/actin ratio. It was demonstrated that reduced glucose-stimulated insulin secretion in vitro observed in db/db mice is accompanied by decreased GLUT-2 expression [15]. However, restoration of GLUT-2 expression can normalize insulin secretion [16,18]. Moreover, it was also demonstrated that fatty acids decreased GLUT-2 expression by inducing its posttranslational degradation and, consequently, inhibited insulin secretion in pancreatic beta cells isolated from diabetic animals [49]. Therefore, we suggest that the enhancement in GLUT-2 expression in pancreatic islets could be related to the increased glucose-stimulated insulin secretion observed in overfed rats. On the other hand, some studies have also showed that weanling rats from dams fed high-fat diets during gestation and lactation periods showed an overexpressed protein level, despite an underexpressed mRNA level of GLUT-2 [50]. The authors suggested that GLUT-2 may have an increase in processing and stability such as the one observed in GLUT-8 protein regulation in the diabetic state [51]. In other words, hyperglycemia may induce changes in translational and or post-translational GLUT-2 protein processing [52].

It has been described that Akt-deficient mice exhibit hyperglycemia, hyperinsulinemia, impaired glucose tolerance, and insulin resistance [21,22]. It is noteworthy that Akt may also induce insulin secretion in pancreatic islets by acting on actin filaments involved in the exocytosis of insulin-containing granules [23–25]. Therefore, impaired Akt signaling might contribute to the pathogenesis of type 2 diabetes [23,53]. Our data demonstrated that rats raised in small litters have increased Akt content but decreased Akt phosphorylation in pancreatic islets of 1-year-old overfed rats compared to controls. Taken together, our data suggest that mechanisms other than Akt activation, including protein kinase A and *N*-ethylmaleimide-sensitive factor attachment receptor complex activation [54] might be potentially involved in glucose-stimulated insulin release by pancreatic beta cells in 1-year-old overfed rats.

In conclusion, our data demonstrated that overnutrition through litter size reduction programs the progeny to develop metabolic adaptations lasting into adulthood, such as hyperphagia, overweight, hyperleptinemia and hyperinsulinemia. These alterations were accompanied by increased glucose-stimulated insulin secretion by pancreatic islets. As a whole, our data strongly suggest that overfed rats maintain normal fasting glucose levels through a compensatory elevation in circulating insulin, which possibly occurs due to an increase in GLUT-2 content in pancreatic islets.

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References

[1] Armitage J, Poston L, Taylor P. Development origins and the metabolic syndrome: the role of maternal obesity. Obes Metab 2008;36:73–84.
[2] Waterland RA, Garza C. Potential mechanisms of metabolic imprinting that lead to chronic disease. Am J Clin Nutr 1999;69:179–97.
[3] Plagemann A, Heidrich I, Gotz F, Rohde W, Dorner G. Obesity and enhanced diabetes and cardiovascular risk in adult rats due to early postnatal overfeeding. Exp Clin Endocrinol 1992;99:154–8.
[4] Plagemann A, Harder T, Rake A, Voits M, Fink H, Rohde W, et al. Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome x-like alterations in adulthood of neonatally overfed rats. Brain Res 1999;836:146–55.
[5] Boullu-Ciocca S, Dutour A, Guillaume V, Achard V, Oliver C, Grino M. Postnatal diet-induced obesity in rats upregulates systemic and adipose

tissue glucocorticoid metabolism during development and in adulthood: its relationship with the metabolic syndrome. Diabetes 2005;54:197–203.
[6] Hill DJ, Duvillie B. Pancreatic development and adult diabetes. Pediatr Res 2000;48:269–74.
[7] Berney DM, Desai M, Palmer DJ, Greenwald S, Brown A, Hales CN, et al. The effects of maternal protein deprivation on the fetal rat pancreas: major structural changes and their recuperation. J Pathol 1997;183:109–15.
[8] Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001;414:799–806.
[9] Glass DJ. Skeletal muscle hypertrophy and atrophy signaling pathways. Int J Biochem Cell Biol 2005;37:1974–84.
[10] Pende M. mTOR, Akt, S6 kinases and the control of skeletal muscle growth. Bull Cancer 2006;93:39–43.
[11] Niswender KD, Baskin DG, Schwartz MW. Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. Trends Endocrinol Metab 2004;15:362–9.
[12] Thorens B, Guillam MT, Beermann F, Burcelin R, Jaquet M. Transgenic reexpression of GLUT1 or GLUT2 in Pancreatic beta Cells rescues GLUT2-null mice from early death and restores normal glucose-stimulated insulin secretion. J Biol Chem 2000;275:23751–8.
[13] Tal M, Liang Y, Najafi H, Lodish HF, Matschinsky FM. Expression and function of GLUT-1 and GLUT2 glucose transporter isoforms in cells of cultured rat pancreatic islets. J Biol Chem 1992;267:17241–7.
[14] Heimberg H, De Vos A, Pipeleers D, Thorens B, Schuit F. Differences in glucose transporter gene expression between rat pancreatic alpha- and beta-cells are correlated to differences in glucose transport but not in glucose utilization. J Biol Chem 1995;270:8971–5.
[15] Thorens B, Wu YJ, Leahy JL, Weir GC. The loss of GLUT2 expression by glucose-unresponsive beta cells of db/db mice is reversible and is induced by the diabetic environment. J Clin Invest 1992;90:77–85.
[16] Hughes SD, Quaade C, Johnson JH, Ferber S, Newgard CB. Transfection of AtT-20ins cells with GLUT2 but not GLUT-1 confers glucose-stimulated insulin secretion. Relationship to glucose metabolism. J Biol Chem 1993;268:15205–12.
[17] Ferber S, BeltrandelRio H, Johnson JH, Noel RJ, Cassidy LE, Clark S, et al. GLUT2 gene transfer into insulinoma cells confers both low and high affinity glucose-stimulated insulin release. Relationship to glucokinase activity. J Biol Chem 1994;269:11523–9.
[18] Tiedge M, Hohne M, Lenzen S. Insulin secretion, insulin content and glucose phosphorylation in RINm5F insulinoma cells after transfection with human GLUT2 glucose-transporter cDNA. Biochem J 1993;296:113–8.
[19] Dickson LM, Rhodes CJ. Pancreatic beta-cell growth and survival in the onset of type 2 diabetes: a role for protein kinase B in the Akt? Am J Physiol Endocrinol Metab 2004;287:192–8.
[20] Jetton TL, Lausier J, LaRock K, Trotman WE, Larmie B, Habibovic A, et al. Mechanisms of compensatory beta-cell growth in insulin-resistant rats: roles of Akt kinase. Diabetes 2005;54:2294–304.
[21] Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw 3rd EB, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 2001;292:1728–31.
[22] Garofalo RS, Orena SJ, Rafidi K, Torchia AJ, Stock JL, Hildebrandt AL, et al. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. J Clin Invest 2003;112:197–208.
[23] Bernal-Mizrachi E, Fatrai S, Johnson JD, Ohsugi M, Otani K, Han Z, et al. Defective insulin secretion and increased susceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet beta cells. J Clin Invest 2004;14:928–36.
[24] Cenni V, Sirri A, Riccio M, Lattanzi G, Santi S, de Pol A, et al. Targeting of the Akt/PKB kinase to the actin skeleton. Cell Mol Life Sci 2003;60:2710–20.
[25] Lang J. Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. Eur J Biochem 1999;259:3-17.
[26] Bayne K. Revised guide for the care and use of laboratory animals available. Physiologist 1996;39:208–11.
[27] Russell JA. Milk yield, suckling behaviour and milk ejection in the lactating rat nursing litters of different sizes. J Physiol 1980;303:403–15.
[28] Erb RE, Sitarz E, Malven PV. Blood plasma and milk prolactin, and effects of sampling technique on composition of milk from suckled ewes. J Dairy Sci 1977;60:197–203.
[29] Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977;83: 346–56.
[30] Bernardis LL, Patterson BD. Correlations between "Lee index" and carcass fat content in weanling and adult female rats with hypothalamic lesions. J Endocrinol 1968;40:527–8.
[31] Bernardis LL, Luboshitsky R, Bellinger LL, McEwen G. Nutritional studies in the weanling rat with normophagic hypothalamic obesity. J Nutr 1982;112:1441–55.
[32] Harder T, Rake A, Rohde W, Doerner G, Plagemann A. Overweight and increased diabetes susceptibility in neonatally insulin-treated adult rats. Endocr Regul 1999;33:25–31.
[33] Campos KE, Sinzato YK, Pimenta WP, Rudge MVC, Damasceno DC. Effect of maternal obesity on diabetes development in adult rat offspring. Life Sci 2007;81:1473–8.
[34] Leshner AI, Litwin VA, Squibb RL. A simple method for carcass analysis. Physiol Behav 1972;9:281–2.
[35] Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A, Thorens B. Heterogeneous metabolic adaptation of C57BL/6J mice to high-fat diet. Am J Physiol Endocrinol Metab 2002;282:834–42.
[36] Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1967;16:35–9.
[37] Thorens B, Sarkar HK, Kaback HR, Lodish HF. Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islet cells. Cell 1988;55:281–90.
[38] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[39] Azevedo-Martins AK, Monteiro AP, Lima CL, Lenzen S, Curi R. Fatty acid-induced toxicity and neutral lipid accumulation in insulin-producing RINm5F cells. Toxicol In Vitro 2006;20: 1106–13.
[40] Listenberger LL, Han X, Lewis SE, Cases S, Farese Jr RV, Ory DS, et al. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. Proc Natl Acad Sci U S A 2003;100:3077–82.
[41] Pereira RO, Moreira AS, de Carvalho L, Moura AS. Overfeeding during lactation modulates insulin and leptin signaling cascade in rats' hearts. Regul Pept 2006;136:117–21.
[42] Moura AS, Franco de Sa CC, Cruz HG, Costa CL. Malnutrition during lactation as a metabolic imprinting factor inducing the feeding pattern of offspring rats when adults. The role of insulin and leptin. Braz J Med Biol Res 2002;35:617–22.
[43] Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, et al. Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science 2004;304:110–5.
[44] McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 2005;85:571–633.
[45] Horvath TL, Bruning JC. Developmental programming of the hypothalamus: a matter of fat. Nat Med 2006;12:52–3.
[46] Park S, Hong SM, Sung SR, Jung HK. Long-term effects of central leptin and resistin on body weight, insulin resistance, and beta-cell function and mass by the modulation of hypothalamic leptin and insulin signaling. Endocrinol 2008;149:445–54.
[47] Srinivasan M, Laychock SG, Hill DJ, Patel MS. Neonatal nutrition: metabolic programming of pancreatic islets and obesity. Exp Biol Med 2003;228:15–23.
[48] Pi-Sunyer FX. The obesity epidemic: pathophysiology and consequences of obesity. Obes Res 2002;2:97-104.

[49] Gremlich S, Bonny C, Waeber G, Thorens B. Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. J Biol Chem 1997;272:30261–9.

[50] Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 1999;19:1720–30.

[51] Gorovits N, Cui L, Busik JV, Ranalleta M, Hauguel de-Mouzon S, Charron MJ. Regulation of hepatic GLUT8 expression in normal and diabetic models. Endocrionol 2003;144:1703–11.

[52] Cerf ME, Muller CJ, Du Toir DF, Louw J, Wolfe-Coote SA. Hyperglycaemia and reduced glucokinase expression in weanling offspring from dams maintained on a high-fat diet. Br J Nutr 2006;95:391–6.

[53] Weir GC, Bonner-Weir S. A dominant role for glucose in beta cell compensation of insulin resistance. J Clin Invest 2007; 117:81–3.

[54] Dubois M, Vacher P, Roger B, Huyghe D, Vandewalle B, Kerr-Conte I, et al. Glucotoxicity inhibits late steps of insulin exocytosis. Endocrinol 2007;48:1605–14.