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Original article

Swimming training prevents metabolic imprinting induced by hypernutrition during lactation

Stefani Valeria Fischer [a, *], Cláudia Regina Capriglioni Cancian [a], Elisangela Gueiber Montes [a], Nayara de Carvalho Leite [b], Sabrina Grassiolli [a]

[a] Department of General Biology, State University of Ponta Grossa, Ponta Grossa, Parana, Brazil
[b] Department of Structural and Functional Biology, Institute of Biology, UNICAMP, Campinas, São Paulo, Brazil

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SUMMARY

Background & aims: Reduction in litter size during lactation induces hypernutrition of the offspring culminating with altered metabolic programming during adult life. Overnourished rats present alterations in the endocrine pancreas and major predisposition to the development of type 2 diabetes. Our study evaluated the impact of swimming training on insulin secretion control in overnourished rats.
Methods: At postnatal day 3 male rat pup litters were redistributed randomly into Small Litters (SL, 3 pups) or Normal Litters (NL, 9 pups) to induce early overfeeding during lactation. Both groups were subjected to swimming training (3 times/week/30 min) post-weaning (21 days) for 72 days. At 92 days of life pancreatic islets were isolated using collagenase technique and incubated with glucose in the presence or absence of acetylcholine (Ach, 0.1–1000 μM) or glucagon-like peptide 1 (GLP1, 10 nM). Adipose tissue depots (white and brown) and endocrine pancreas samples were examined by histological analysis. Food intake and body weight were measured. Blood biochemical parameters were also evaluated.
Results: Swimming training prevented metabolic program alteration by hypernutrition during lactation. Exercise reduced obesity and hyperglycemia in overnourished rats. Pancreatic islets isolated from overnourished rats showed a reduction in glucose-induced insulin secretion and cholinergic responses while the insulinotropic action of GLP1 was increased. Physical training effectively restored glucose-induced insulin secretion and GLP1-stimulated action in pancreatic islets from overnourished rats. However, swimming training did not correct the weak cholinergic response in pancreatic islets isolated from overnourished rats.
Conclusions: Swimming training avoids obesity development, corrects glucose-induced insulin secretion, as well as, GLP1 insulinotropic response in overnourished rats.

1. Introduction

Changes in the nutritional environment such as high lipid and carbohydrate consumption associated with a sedentary modern lifestyle are central elements of the obesity epidemic particularly during critical periods of development including the prenatal and neonatal periods. These phases are considered windows into early development processes during which alterations in the nutritional state could have lifelong consequences for the development of major diseases during adult life [1,2].

There are numerous studies showing that under- or over-nutrition during gestation and/or lactation in rats affects the metabolic programming of the energetic metabolism and modulates insulin action and secretion from pancreatic islets in the adult life. These precocious dietary interventions are accompanied by adult-onset diseases in the offspring particularly obesity and type 2 diabetes (T2D) [3]. The definitive diagnosis of T2D is directly dependent upon the association of two conditions: insulin resistance and beta cell dysfunction. Beta cell failure in T2D involves

* Corresponding author. Comandante Paulo Pinheiro Schimidht Street, 366, Uvaranas, Ponta Grossa, Paraná, Brazil. Tel.: +55 42 99725626.
E-mail address: stefaniduda@gmail.com (S.V. Fischer).

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reduction of overall beta cell mass and insulin secretory function [4]. Several studies have shown that nutritional intervention during critical developmental periods of fetal and early post-natal life induce adverse effects on beta-cell development and glucose-induced insulin secretion [1,5].

Experimental studies in rats have demonstrated that modifications in the litter size during lactation can induce early postnatal overnutrition. Animals reared in small litters (SL) are considered 'overnourished' and are more susceptible to obesity and metabolic disorders during adult life. These effects are attributed to large milk volume and greater energy intake during lactation. Rats from SL exhibit hyperphagia, obesity, elevated triacylglycerols, hyperleptinemia, hyperinsulinemia and impaired glucose tolerance during adulthood [2].

It has been shown that regular physical training reduces adipose tissue content, increases insulin sensitivity, and improves glucose homeostasis. However, the effects of physical training on the endocrine pancreas are contradictory [6,7]. In islets isolated from lean-exercised rats a reduction in glucose-induced insulin secretion was frequently observed. In contrast, islets isolated from obese and/or diabetic exercised models exhibited variable effects on insulin secretion control [8,9]. In this study we investigated the impact of swimming training on obesity induced by overnutrition during lactation.

2. Material and methods

2.1. Animals

All animal protocols were approved by the Ethics Committee for Experimental Animals at the State University of Ponta Grossa (CEUA number 03482/2012) which based their analysis on the principles adopted and promulgated by Brazilian Law.

Thirty virgin Wistar rats (age 70 ± 10 days) were mated with male rats using the harem system at a proportion of 3 females to 1 male. After mating, each female was placed in an individual cage with unlimited access to water and food. The day after delivery, excess pups in each litter were removed to retain 10 pups per dam. At postnatal day 3, male rat pup litters were redistributed randomly into Small Litters (SL, 3 pups) or Normal Litters (NL, 9 pups) to induce early overfeeding during lactation. According described by several authors (Babicky et al., 1973; Fiorotto et al., 1991; Plagemann et al., 1992), neonatal over-nutrition can be easily induced by a reduction of the number of pups of the litter during lactation. Thus, SL rat pups experience quantitative as well as qualitative over-nutrition during neonatal life once that, this manipulation results in a surplus of milk for each offspring accompanied by changes in the composition of the milk with increased caloric and fat content. A total of 15 litters were evaluated for each experimental group. Offspring were weaned at 21 days of age and were allowed free access to stock diet and water thereafter. After weaning, 6 rats per cage were maintained. To eliminate potential sex-related outcome variance, only male offspring were studied. All groups were maintained at a controlled temperature (21 ± 3 °C) with a 12–12 h light-dark cycle, and food and water were provided *ad libitum*.

2.2. Swimming training

After 21 days, SL and NL groups were subdivided into exercised (EXE) or sedentary (SED) animals. Swimming training was performed according to the protocol previously described by Gobatto et al [10]. and modified by Leite et al [8]. Briefly, exercised groups swam 3 times/week for 30 minutes for a period of 72 days. Water temperature was maintained at 32 ± 2 °C to eliminate cold-induced stress. According study of the Gobatto et al. the intensity of the swimming training can be determined by of the amount of load weight attached to the tail [10]. Using Maximal Lactate Steady State (MLSS) these authors have demonstrated that the use of loads higher than 6% of the body weight, in long lasting exercise sessions, at the beginning of a training period, could lead to undesirable effects of intense physical exercise. In this sense, Rocha et al., 2012 recently have demonstrated that higher intensity of swimming training could promotes cardiac lesions. Thus, load weight equivalent of 5% of the body weight was attached to the base of the tail to avoid acclimation and to guarantee a low at moderate intensity of exercise. In addition, Shima et al., 1993 showed that exercise training once every 2 and 3 days prevented the development of NIDDM in OLETF rats (a model of spontaneous non-insulin dependent diabetes mellitus) [11]. Contrary at other studies our swimming program was started precociously (21 days) and maintained until adult life (92 days). Scomparin et al., 2006 using similar swimming training program have demonstrated that fat reduction and metabolic adjusts are more accented when swimming training started after weaning [12]. Thus, 4 experimental groups were studied: NL-SED, NL-EXE, SL-SED and SL-EXE. A total of 15-20 rats were exercised according to their group assignment and were subjected to experimental procedures described in the next sections. All experimental protocols were performed 48 h after the last swimming session.

2.3. Islet isolation and incubation

At 93 days old, 4–6 rats from each group were sacrificed and pancreatic islets were isolated with collagenase as previously described by Lacy and Kostianovsky [13], with a few adaptations. After anesthesia (xylazine and ketamine; 0.6 mg and 3 mg/100 g body weight, respectively), the abdominal wall was opened and 10 mL of Hank's buffered saline solution (HBSS) containing collagenase type V (1.0 mg/mL) was injected into the rat's common bile duct. The pancreas was quickly excised and incubated for 15 min at 37 °C, and the suspension was subsequently filtered and washed with HBSS [0.1% bovine serum albumin fraction V (BSA)]. Islets were collected with the aid of a microscope. Batches of 4 islets were pre-incubated for 60 min in 1 mL of normal Krebs–Ringer solution containing 120 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 24 mM $NaHCO_3$ and 5.6 mM glucose. This solution was gassed with O_2/CO_2 (95/5%) to maintain a pH of 7.4 and supplemented with BSA (0.1%). After adaptation to a low glucose concentration (5.6 mM) solution, islets were incubated for 60 additional min in the presence of stimulatory glucose concentrations of 8.3 mM, 16.7 mM, 20.0 mM and 28 mM. After pre-incubation, other islet batches were incubated for 60 min in Krebs solution containing glucose (8.3 mM) in the presence of acetylcholine at different concentrations (0.1, 1, 10, 100 and 1000 μM). Neostigmine (10 μM), an inhibitor of acetylcholinesterase activity, was used to avoid acetylcholine degradation. The effects of glucagon-like 1 peptide (GLP1) on glucose-induced insulin secretion were also investigated. In this experiment, islets were incubated with 1 mL of Krebs solution containing 5.6 mM or 16.7 mM glucose in the presence or absence of GLP1 (10 nM). Samples of incubation media were obtained and frozen until measurements for secreted insulin could be performed by radioimmunoassay (RIA).

2.4. Blood biochemical analyses

After 12 h of fasting other groups of rats ($n = 10$/group) were killed by decapitation and total blood collected in heparinized tubes. Plasma glucose, cholesterol and triglyceride levels were measured by enzymatic procedures using commercial kits (Gold Analisa®, Belo Horizonte – MG, Brazil) and read by means of an