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Overnourishment during lactation induces metabolic and haemodynamic heart impairment during adulthood

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Abstract *Aim:* In this study, the effects of postnatal overfeeding on heart energy homoeostasis and cardiac haemodynamics in adult male *Swiss* mice were examined.
Methods and Results: During the suckling period, the mice were divided into four groups of control or overfed pups in combination with baseline or ischaemia/reperfusion treatments (control group baseline, CGBL; overfed group baseline, OGBL; control group ischaemia/reperfusion, CGIR; and overfed group ischaemia/reperfusion, OGIR). End diastolic pressure (EDP), heart contraction speed (Max d*P*/d*t*), relaxation speed (Min d*P*/d*t*), isovolumetric relaxation time (Tau) and frequency by beats per minute (BPM) were measured. During baseline and ischaemia/reperfusion, key proteins such as AKT1, AKT2, AKT3, pAKT, adenosine monophosphate-activated protein kinase (AMPK), pAMPK, insulin receptor beta (IRβ), protein tyrosine phosphatase 1B (PTP1B), insulin receptor substrate 1 (IRS1), fatty acid binding protein (FABP), CD36, phosphoinositide 3-kinase (PI3K) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) were studied. The expression of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), carnitine palmitoyltransferase 1 (CPT1) and uncoupling protein 3 (UCP3) was studied as a marker of cardiac hypertrophy and energetic metabolism. Cardiac fibrosis was analyzed by quantifying collagen deposition, which is increased in the OGBL and OGIR groups compared with the control groups.
Conclusions: The OGBL group showed reduced EDP compared with the CGBL group and high Max d*P*/d*t* compared with the OGBL group. Ischaemia/reperfusion increased EDP and Min d*P*/d*t* in the intragroup comparison. By contrast, Tau and frequency were not significantly different among groups. The OGIR mice showed significant alterations in heart metabolism proteins, including AKT2, pAKT/AKT1, pAKT/AKT2, AMPK, pAMPK/AMPK, PTP1B, IRS1, FABP and CD36. Furthermore, alterations in ANP, BNP, CPT1 and UCP3 messenger RNA (mRNA) expression indicated hypertrophy and reduction in their efficiency, such that exclusive overnutrition in childhood induces a long-term effect on haemodynamics, metabolism and heart remodelling.

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Introduction

Cardiovascular disease is a health-related effect of obesity [12,22]. In addition to the genetic determinants of heart disease and obesity, nutritional changes during specific life moments can also induce the disease. For instance, the authors and others have recently shown that overfeeding of mice and rats during early life is associated with adult obesity and impairment of several morphologic and physiological organ changes [6,7,15,21]. Among these organs, the heart has an expressive metabolic plasticity in response to nutritional changes [1,16,20]. The heart undergoes morphological and physiological changes with increases in collagen deposition that ultimately result in haemodynamic impairment and increase susceptibility to post-ischaemic injury [7,18]. For instance, we recently demonstrated that several key proteins in the insulin signalling pathway, such as protein kinase B (AKT), adenosine monophosphate-activated protein kinase (AMPK), insulin receptor substrate 1 (IRS1) and protein tyrosine phosphatase 1B (PTP1B), were changed in adult hearts due to early postnatal overfeeding [9,16]. These data support the hypothesis that metabolic changes induced by early overnutrition leads to impaired energy use and conversion mechanisms in the cardiomyocytes of adults [10].

Different nutrients have distinct functions in modulating energy production, and these signalling processes are likely dependent on age and previous nutritional history [23]. More particularly, during heart reperfusion, it has been suggested that nutrition plays a key role during the transition from anaerobic to aerobic respiration, which occurs in cardiomyocytes by modifications of AMPK, AKT, PTP1B, IRβ (insulin receptor beta), FABP (fatty acid-binding protein), CD36, CPT1 (carnitine palmitoyltransferase 1) and UCP3 (uncoupling protein 3) expression [11,17,19,27,30]. In addition to these factors, several hormones modulate the metabolism of the mitochondrial substrate to generate energy for the cardiomyocytes. For instance, in cardiomyocytes and plasma of mice overfed during lactation, insulin and leptin hormones and their respective receptors are more highly expressed. Increased hormone levels are associated with hypertension, atherosclerosis, left ventricle (LV) hypertrophy and cardiac adaptive response to improve glucose and/or fatty acid uptake, perhaps in an attempt to improve cardiac energy supply [13,15,20,28]. In addition, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are polypeptide hormones synthesized in the heart, specifically in the atria and ventricles, respectively, which is their primary site of action. These hormones have been associated with metabolic syndromes, as they may increase visceral fat mass and develop insulin resistance in humans [3,24].

In this study, the effect of overfeeding during lactation in adult hearts (120 days of life) during ischaemia/reperfusion and its consequences on cardiac haemodynamics, molecular biology and gene expression of key proteins as well as the hormone peptides involved in glucose metabolism and the development of vasodilation and hypertrophy are reported. Our results demonstrated marked associations among early overnutrition, impairment of heart haemodynamics, insulin and leptin resistance, molecular profiles of key proteins involved in energy production and peptide markers of hypertrophy in adulthood.

Methods

Animals and treatments

Virgin female *Swiss* mice were time-crossed at 3 months of age. During pregnancy and lactation, they were singly housed in individual cages, with access to water and a standard commercial diet (Nuvilab®) ad libitum. To induce early postnatal overnutrition, the number of pups per dam was adjusted at postnatal day 3 to three male mice per litter to form the overfed group (OG), whereas male mice litters containing six pups per mother formed the control group (CG).

After weaning at postnatal day 21, mice of both groups were separated from the dams and fed water and standard laboratory chow in a temperature-controlled room. All mice, breeders and offspring were housed at the Animal Research Facility of Biology Institute, UERJ, under pathogen-free conditions with a reverse daily cycle of 12-h light: 12-h darkness. The mice were fasted for 12 h, and then injected with heparin (5,000 UI/kg, i.p.) and anaesthetized intraperitoneally with pentobarbital sodium (Hypnol 3%, 50 mg/kg, i.p.) until total loss of nociceptive reflexes (verified by paw pinching). The animals were sacrificed in the morning at the same time and weighed at 120 days of age.

The animals studies and experimental procedures were approved by the Ethics Committee for the Care and Use of Experimental Animals of the Biology Institute, State University of Rio de Janeiro (Permit number: CEUA/055/2011), based on the principles described in the Guide for Care and Use of Laboratory Animals [2].

Glycaemia

After a 12-h fast, blood glucose concentration was measured from blood droplets removed from the tail vein of OG and CG mice with a glucometer (Accu-Chek, Roche, São Paulo, Brazil).

Cardiac contractile function

The heart was rapidly excised within ∼1 min, placed in Krebs–Henseleit buffer at room temperature and then positioned on a Langendorff apparatus (ADInstruments, Oxford, UK) with the aorta cannulated. The heart was retrogradely perfused with Krebs–Henseleit buffer containing (in millimoles per litre) 110.0 NaCl, 4.6 KCl, 1.2 $MgSO_4 \cdot 7H_2O$, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 2.0 pyruvate and 10 glucose equilibrated with 95% O_2 and 5% CO_2 at 37 °C.

A catheter with a balloon polyvinyl chloride (PVC) film was inserted into the LV and connected to a pressure transducer to measure the end diastolic pressure (EDP),