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Splenectomy attenuates obesity and decreases insulin hypersecretion in hypothalamic obese rats

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ABSTRACT

Objective. Obesity-induced abnormalities, such as insulin resistance, dyslipidemia and hypertension, are frequently correlated with low-grade inflammation, a process that may depend on normal spleen function. This study investigated the role of the spleen in the obesity induced by monosodium glutamate (MSG) treatment.

Materials/Methods. MSG-obese and lean control (CON) rats were subjected to splenectomy (SPL) or non-operated (NO).

Results. MSG-NO rats presented a high adipose tissue content, insulin resistance, dyslipidemia and islet hypersecretion, accompanied by hypertrophy of both pancreatic islets and adipocytes when compared with CON-NO rats. In addition, changes in nitric oxide response were found in islets from the MSG-NO group without associated alterations in inducible nitric oxide synthase (iNOS) or IL1β expression. MSG-NO also presented increased leukocyte counts and augmented LPS-induced nitric oxide production in macrophages. Splenectomy of MSG-obese animals decreased insulin hypersecretion, normalized the nitric oxide response in the pancreatic islets, improved insulin sensitivity and reduced hypertrophy of both adipocytes and islets, when compared with MSG-NO rats.

Conclusion. Results show that splenectomy attenuates the progression of the obesity modulating pancreas functions in MSG-obese rats.

Abbreviations: AUC, area under the curve; BSA, bovine albumin serum; bw, body weight; CON, control; CRP, C-reactive protein; GK, glucokinase; H&E, hematoxylin and eosin; Hct, hematocrit; IL1β, interleukin 1 beta; IL6, interleukin 6; iNOS, inducible oxide synthase; ITT, insulin tolerance test; ivGTT, intravenous glucose tolerance test; Kitt, rate constant of plasma glucose disappearance; LI, Lee index; L-NAME, *N*-nitro-L-arginine methyl ester; LPS, lipopolysaccharide; MSG, monosodium glutamate; NK cells, natural killer cells; NO, non-operated; NOS, nitric oxide synthase; PMA, phorbol myristate acetate; RBC, red cell concentrations; RIA, radioimmunoassay; ROS, reactive oxygen species; SNS, sympathetic nervous system; SPL, splenectomy,T2D, Type 2 diabetes; TNFα, tumoral necrosis factor alpha; WBC, white blood cell count.

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1. Introduction

Obesity is a complex metabolic disease characterized by chronic systemic low-grade inflammation, an event that is denominated "sterile inflammation", i.e., inflammation of a non-infectious origin [1,2]. This metabolic profile, typical of adulthood obesity, has also recently been linked to low-grade inflammation in childhood obesity [3]. The association between inflammatory processes and obesity abnormalities may constitute an underlying mechanism for increased risk of developing chronic disease in adult life, particularly type 2 diabetes (T2D).

Several inflammatory mediators, such as interleukin 1 beta (IL1β), tumoral necrosis factor alpha (TNFα) and interleukin 6 (IL6) are altered in obesity, contributing to the insulin resistance as well as to pancreatic beta-cell dysfunction. Both processes play an important role in the pathogenesis of T2D onset [4,5]. The deleterious action of pro-inflammatory cytokines, in particular IL1β, in the endocrine pancreas involves increases in nitric oxide production [6], and chronic low-grade inflammation modulates glucose homeostasis, deteriorating beta cell function and reducing insulin sensitivity [7].

The spleen is a crucial regulator of immune homoeostasis via its ability to link innate and adaptive immunity, in turn granting protection against infections [8]. The spleen not only contains up to 15% of the body's fixed macrophages, as well as substantial numbers of T lymphocytes and natural killer cells (NK cells), but also produces various cytokines during critical states [9]. Several alterations in the immune system occur in the obese state in humans and rodents, including splenic-immune dysfunctions [10]. For instance, alterations in the spleen volume (as a stable index of chronic inflammation) alongside activation of the immune system and elevated concentration of high sensitivity C-reactive protein (CRP), characterize young adult obese subjects with non-alcoholic fatty liver disease [11,12]. Thus, it is possible that immune and/or metabolic responses originating in the spleen are involved in obesity and T2D development.

High doses of monosodium glutamate (MSG) administered during the neonatal period in rats induce several metabolic abnormalities that result in extreme adiposity, accompanied by insulin resistance, glucose intolerance, dyslipidemia and insulin hypersecretion [13–15]. These characteristics are frequently clustered as metabolic syndrome, a major cause of T2D onset in humans [16]. Alterations in immune function have also been observed in MSG-treated obese rats, including splenic dysfunctions [17,18]. Since recent studies suggest that the spleen participates in metabolic and immunological abnormalities present in obesity [17,19,20], the aim of the present study was to evaluate the effect of splenectomy in the hypothalamic obesity induced by neonatal MSG treatment.

2. Methods

2.1. *MSG Obesity Model*

Obesity was induced in neonatal male Wistar rats by the administration of subcutaneous injections of MSG (4 g/kg body weight, bw) on each of the five first days after birth (n = 40 rats). Control (CON) rats were injected with NaCl at an equimolar concentration (n = 40 rats). At 21 days of age, animals were weaned and allowed access to standard rodent chow (Nuvital, Curitiba, Brazil) and water ad libitum. For the entire experiment, the rats were housed in controlled conditions of luminosity (12 h light–dark) and temperature (21 ± 2 °C). All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the Ethics Committee for Experimental Animals at the State University of Ponta Grossa approved the present protocol (CEUA 17), which was written in accordance with the federal laws of Brazil.

2.2. *Splenectomy*

At 60-days-old, half of the CON and MSG animals were subjected to splenectomy (SPL). Rats were anesthetized with an intramuscular injection of xylazine (0.2 mg/g) and ketamine (0.5 mg/g), v:v. The surgical site was shaved and sterilized, and a small incision was made in the upper left abdominal quadrant. The spleen was mobilized and isolated, the splenic artery and vein were ligated with 4-0 silk, and the spleen was removed from the abdomen. Sterile sutures were used to close the wound. For the Sham group, the abdomen was opened, but the spleen was not removed. The CON and MSG groups that did not undergo any operation (non-operated; NO) maintained intact spleens. Thus, after surgery, there were a total of 6 groups, defined as follows: CON-NO (n = 15); CON-SHAM (n = 5); CON-SPL (n = 20); MSG-NO (n = 15); MSG-SHAM (n = 5) and MSG-SPL (n = 20). For 30 days after surgery, the bw and food and water intake of all experimental groups were measured. As the sham operation did not affect any of these parameters, the CON-SHAM and MSG-SHAM groups were excluded from the experimental procedures described in the following sections.

2.3. *Insulin Resistance and Glucose Tolerance Test*

At 90 days of life and after 6 h of fasting, 8 rats from each group underwent an intraperitoneal insulin tolerance test (ITT). Samples for blood glucose measurements were collected at 0 (basal), 5, 10, 15, 20, 25 and 30 min after insulin injection (1 U/kg bw). Thereafter, the rate constant of plasma glucose disappearance (Kitt) was calculated from the slope of the least square analysis of the plasma glucose concentrations during the linear phase of the decline as indicated in a previous report [21]. At 90 days of life other animal groups were submitted at intravenous glucose tolerance test (ivGTT) according protocol previously established with modifications [22]. Briefly, a silicone catheter was inserted in right jugular vein (n = 5–8 rats/group) and after overnight fasting the rats received glucose load (1 g/kg b.w.). The blood samples were collected before glucose infusion (time: 0') and after glucose infusion (times: 5', 15' and 30' minutes). The plasma obtained from ivGTT was used for dosage of glucose by glucose oxidase method and insulin by radioimmunoassay (RIA) technique. The results obtained in individual times of the ivGTT were used to calculate area under curve (AUC).

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