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Original article

Magnesium-deficient high-fat diet: Effects on adiposity, lipid profile and insulin sensitivity in growing rats

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SUMMARY

Background & aims: To determine if magnesium deficiency aggravates the effects of a high-fat diet in growing rats in terms of obesity, lipid profile and insulin resistance.
Methods: The study population comprised 48 newly weaned male Wistar Hannover rats distributed into four groups according to diet, namely, control group (CT; $n = 8$), control diet provided *ad libitum*; pair-feeding control group (PF; $n = 16$), control diet but in the same controlled amount as animals that received high-fat diets; high-fat diet group (HF; $n = 12$), and magnesium-deficient high-fat diet group ($HFMg^-$; $n = 12$). The parameters investigated were adiposity index, lipid profile, magnesium status, insulin sensitivity and the phosphorylation of proteins involved in the insulin-signaling pathway, i.e. insulin receptor β-subunit, insulin receptor substrate 1 and protein kinase B.
Results: The HF and $HFMg^-$ groups were similar regarding gain in body mass, adiposity index and lipid profile, but were significantly different from the PF group. The $HFMg^-$ group exhibited alterations in magnesium homeostasis as revealed by the reduction in urinary and bone concentrations of the mineral. No inter-group differences were observed regarding glucose homeostasis. Protein phosphorylation in the insulin-signaling pathway was significantly reduced in the high-fat groups compared with the control groups, demonstrating that the intake of fat-rich diets increased insulin resistance, a syndrome that was aggravated by magnesium deficiency.
Conclusions: Under the experimental conditions tested, the intake of a magnesium-deficient high-fat diet led to alterations in the insulin-signaling pathway and, consequently, increased insulin resistance.

1. Introduction

Magnesium deficiency is often correlated with alterations in carbohydrate homeostasis[1–4] in both adults[4,5] and children.[6,7] Since type 2 diabetes mellitus (DM2), a condition primarily associated with adults, is currently very prevalent among children, it is important to establish the role of magnesium in cell processes and, most especially, its function as a cofactor of enzymes involved in energy metabolism.[8,9] A key problem that requires further attention is whether hyperglycemia induces magnesium deficiency or, conversely, whether the deficiency of magnesium provokes hyperglycemia and increases the risk of chronic non-communicable diseases.

Some physiological conditions, such as DM2, that give rise to hyperglycemia and insulin resistance may also induce magnesium deficiency in individuals with inadequate mineral intake or osmotic dieresis.[8] However, when kidney function is preserved, the probability of magnesium deficiency is low since reabsorption of the mineral is increased.[4] On the other hand, glycemic control in DM2 patients and in obese individuals seems to be influenced by magnesium status and the level of dietary intake of the mineral.[4,6,7]

Abbreviations: Akt, protein kinase B; ANOVA, analysis of variance; CT, *ad libitum* control group; GLUT4, glucose transporter 4; HF, high-fat group; $HFMg^-$, magnesium-deficient high-fat group; HDL-c, high-density lipoprotein-cholesterol; HOMA-β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; IR-β, insulin receptor β-subunit; IRS-1, insulin receptor substrate 1; K_{ITT}, rate constant of insulin tolerance test; p-Akt, phosphorylated protein kinase B; p-IR-β, phosphorylated insulin receptor β-subunit; p-IRS-1, phosphorylated insulin receptor substrate 1; PF, pair-feeding control group; DM2, type 2 diabetes mellitus; VLDL-c, very low-density lipoprotein-cholesterol.

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Various reports have described the dietary inadequacy of magnesium in the population worldwide resulting from the ingestion of foods that are poor in mineral sources.[9] Moreover, a number of studies involving humans and experimental animals have demonstrated a relationship between magnesium intake and metabolic, oxidative and inflammatory disorders.[3,10–14] Based on such observations, we have suggested that insufficient intake of magnesium may aggravate the effects of a high-fat diet.[15–17] The objective of the present study was, therefore, to determine if the dietetic inadequacy of magnesium in growing rats aggravates the effects of a high-fat diet, particularly with regard to obesity, lipid profile and insulin resistance.

2. Materials and methods

Details of the project were submitted to and approved by the Ethical Research Committee of the Faculdade de Ciências Farmacêuticas, Universidade de São Paulo (protocol # 2009/221) and the procedures were carried out in accordance with the guidelines issued by the Colégio Brasileiro de Experimentação Animal.

2.1. Animals and experimental design

The study population comprised 48 genetically heterogeneous, newly weaned male Wistar Hannover rats (*Rattus novergicus*. *var. albinus*), each weighing 52.0 ± 3.6 g, obtained from the Biotério de Produção e Experimentação of the Faculdade de Ciências Farmacêuticas and the Instituto de Química, Universidade de São Paulo (São Paulo, SP, Brazil). The animals were acclimatization for seven days with standard feed AIN-93G, following which they were randomly distributed into four groups according to diet provided, namely: control group (CT; $n = 8$) who received, *ad libitum*, a control diet (standard AIN-93G) containing 500 mg magnesium per 100 g of feed; pair-feeding control group (PF; $n = 16$) who received the CT diet, but in controlled amount matching the day to day ingestion of the corresponding experimental high-fat groups; high-fat group (HF; $n = 12$) who received, *ad libitum*, a high-fat diet (modified AIN-93G) containing 25 g lard and 500 mg magnesium per 100 g of feed; and magnesium-deficient high-fat group ($HFMg^{-}$; $n = 12$) who received a high-fat low-magnesium diet (modified AIN-93G) containing 25 g lard and 150 mg magnesium per 100 g of feed (Table 1).

Table 1
Contents of experimental diets.

Component (g/100 g of diet)	CT/PF	HF	$HFMg^{-}$
Casein	20.00	20.00	20.00
L-Cysteine	0.30	0.30	0.30
Maize starch	39.66	7.86	7.92
Maltodextrin	13.20	20.00	20.00
Sucrose	10.00	10.00	10.00
Cellulose	5.00	5.00	5.00
Soybean oil	7.00	7.00	7.00
Lard	–	25.00	25.00
Saline mixture[A]	3.50	3.50	3.50
Magnesium oxide	0.085	0.085	0.026
Vitamin mixture[B]	1.00	1.00	1.00
Choline Bitartrate	0.25	0.25	0.25
tert-Butyl hydroquinone (antioxidant)	0.0014	0.0014	0.0014

All diets were prepared by Harlan Teklad Laboratories (Madison, WI, USA).
CT/PF: control diet: TD.110294; 7 g soybean oil and 500 mg of magnesium per 100 g feed; HF: high-fat diet: TD.110297; 25 g lard, 7 g soybean oil and 500 mg of magnesium per 100 g feed; $HFMg^{-}$: magnesium-deficient high-fat diet: TD.110299; 25 g lard, 7 g soybean oil and 150 mg of magnesium per 100 g feed.
[A] Based on AIN-93G-MX[21] but with modifications in the concentration of magnesium, which was added later according to the specifications of the diets.
[B] Based on AIN-93G-VX[21] without modification.

Animals were housed individually in stainless steel semi-metabolic cages and maintained under a 12 h photoperiod (7:00 to 19:00 h) at 22 ± 2 °C and 50–60% relative humidity. During the 32-day experimental period, demineralized water was supplied *ad libitum*, water and feed were monitored daily, and the animals were weighed three times per week. Inspections were carried out daily in order to detect possible symptoms associated with magnesium deficiency, including hyperemia of ears and paws, irritability, convulsions, alopecia, skin lesions and edema.

In the week preceding euthanasia, each animal was submitted to an insulin resistance test and transferred to a metabolic cage for a 24 h period to allow the collection of urine and feces for magnesium analysis. At the end of the experimental period, animals were fasted for 12–14 h and submitted to euthanasia under anesthesia induced by intraperitonial injection (0.2 mL/kg body weight) of xylazine solution (25 mg/kg; Virbaxil; Virbac, São Paulo, SP, Brazil) and ketamine (10 mg/kg; Ketaset; Fort Dodge Animal Health, Fort Dodge, IA, USA) in the proportion of 1:2 (v/v). Absence of a plantar reflex was verified and the abdomen was opened through a midline incision. Fragments of liver were excised and transferred immediately to liquid nitrogen to enable subsequent quantification of components of the insulin-signaling pathway. In order to analyze the activation of such proteins, 0.75 U of insulin/kg was injected into the vena cava followed by the removal of further liver fragments after 30 s. Blood was collected through the aorta and transferred to tubes containing 30% sodium citrate solution (Synth®, São Paulo, SP, Brazil) as anticoagulant (10 μg/mL of blood) for the quantification of magnesium, and to tubes without anticoagulant for other serum analyses.

Following these procedures, intracardiac perfusion of the organs was carried out using 85% sodium chloride solution until complete exsanguination, after which the liver, tibias, gastrocnemius muscles, and right epididymal and retroperitoneal fat deposits were dissected and all adhering tissues removed. The organs were weighed, frozen in liquid nitrogen and stored at a −80 °C. Finally, the gastrointestinal tract was cleaned and returned, together with the fat deposits, to the carcass for storage at −20 °C until the assessment of body composition.

2.2. Experimental diets

All diets were prepared in pellet form by Harlan Teklad Laboratories (Madison, WI, USA) on the basis of formulations suggested by the American Institute of Nutrition[18] and modified, as necessary, by the authors to provide high-fat diets in which starch was replace by lard. The basic mineral mixture was prepared without magnesium salt, and this component was added subsequently according to the concentration intended for each specific diet.

Chemical analyses of the diets were performed according to the guidelines of the Association of Official Analytical Chemists.[19] Moisture was evaluated by the loss-on-drying technique in which the sample was weighed before and after endpoint drying in an oven at 105 °C, total lipids were estimated according to the Soxhlet method, and proteins were assessed according to the micro-Kjeldahl method. Wet-ashing was achieved by digestion of the sample in nitric acid:hydrogen peroxide (5:1; v/v) and, following dilution with 1% nitric acid (v/v), the mineral content was assessed using a Perkin Elmer (Norwalk, CT, USA) AAnalyst 100 atomic absorption spectrometer. For the determination of calcium and magnesium, 5% lanthanum (III) oxide (Merck, Darmstadt, Germany) solution was added to the sample to yield a final concentration of lanthanum equivalent to 0.1% (w/v). Calibration curves were constructed using standard solutions of calcium chloride, magnesium chloride, iron (III) chloride, zinc chloride and copper (II) chloride (Titrisol, Merck) The levels of phosphorus were estimated