Nutrition, Metabolism & Cardiovascular Diseases (2016) **26**, 310–317

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Microcirculatory effects of zinc on fructose-fed hamsters

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Received 27 March 2015; received in revised form 6 November 2015; accepted 11 November 2015
Available online 7 December 2015

KEYWORDS
Microcirculation;
Zinc;
Fructose;
Hamster cheek pouch

Abstract *Background and aims:* Fructose is a major dietary component directly related to vascular dysfunction and diseases such as obesity, diabetes, and hypertension. Zinc is considered a non-pharmacological alternative for treating diabetes due to its antioxidant and hyperglycemia-lowering effects in diabetic animals. Therefore, the aim of this study was to evaluate the effects of dietary zinc supplementation on the microcirculatory parameters of fructose-fed hamsters.

Methods and Results: Male hamsters (*Mesocricetus auratus*) were fed drinking water substituted by 10% fructose solution for 60 days, whereas control animals were fed drinking water alone. Their microcirculatory function was evaluated using cheek pouch preparation, as well as their blood glucose and serum insulin levels. Their microcirculatory responses to acetylcholine (ACh, an endothelium-dependent vasodilator) and to sodium nitroprusside (SNP, an endothelium-independent vasodilator) as well as the increase in macromolecular permeability induced by 30 min of ischemia/reperfusion (I/R) were noted. Endothelium-dependent vasodilation was significantly increased in control animals with high zinc supplementation compared to the groups without zinc supplementation. Zinc was able to protect against plasma leakage induced by I/R in all control and fructose-fed groups, although the microvascular permeability was higher in animals fed drinking water substituted by 10% fructose solution compared to those fed filtered drinking water alone.

Conclusion: Our results indicate that dietary zinc supplementation can improve microvascular dysfunction by increasing endothelial-dependent dilatation and reducing the increase in macromolecular permeability induced by I/R in fructose-fed animals.

Introduction

Over the past 30 years, the ingestion of fructose has increased worldwide due to its addition in industrial food [1]. The replacement of filtered drinking water by fructose solution has also been reported to induce alterations in vascular reactivity [2], microvascular hemodynamics [3,4], and endothelial dysfunction [5].

It is also well established that high fructose intake leads to an increase in de novo lipogenesis, blood triglyceride levels, and hepatic insulin resistance [6]. All of these factors play an important role in the initiation of metabolic syndrome, an important risk factor for cardiovascular diseases, the main cause of death worldwide [7].

Endothelium-dependent vascular dysfunction is considered a cardiovascular risk factor that affects both

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http://dx.doi.org/10.1016/j.numecd.2015.11.006

macroscopic and microscopic blood vessels, which is related to an imbalance in the production of endothelial mediators that regulate different vascular processes, such as vessel tone, the most widely studied aspect of endothelial function [8]. As reviewed by Abdullah et al. (2011), several studies show that animals fed fructose-rich diets with induced metabolic syndrome present vascular dysfunction with increased vasoconstrictor sensitivity and impaired endothelium-dependent relaxation, probably due to an insulin-resistant/hyperinsulinemic state [9].

Antioxidant micronutrients are widely studied for the prevention of glucose intolerance and insulin resistance induced by high-fat diets [10–13]. One of these micronutrients is zinc, the second most prevalent trace element in the body, involved in the structure and function of >300 enzymes and essential for normal metabolism and cell function. It interacts with glucose metabolism via different targets including insulin secretion and enzyme activity implicated in glucose metabolism [14].

Several studies show the association of zinc with cardiovascular diseases. The blood zinc levels are reduced in patients with ischemia/myocardial infarction, congestive heart failure, conduction abnormalities, and heart transplant, resulting in poor outcomes [15,16]. In addition, the administration of zinc during reperfusion has been shown to improve myocardial recovery in rodents, which recover by almost 100% from ischemia. This process appears to be associated with the prevention of the degradation of protein kinase C (PKC) isoforms, suggesting the role of zinc as a second messenger [14,17].

Our study investigated the effects of a diet with zero, normal, and threefold higher zinc content on the microcirculatory parameters of hamsters with metabolic syndrome induced by high fructose intake.

Methods

The methods are described in detail in the Online Supplement.

Experimental animals

This study was approved by the State University of Rio de Janeiro Committee for Animal Experimentation (CEA/215/2007) and conducted according to the *Guide for Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996), and in line with rules established by the Brazilian College of Animal Experimentation.

A total of 152 male golden hamsters (*Mesocricetus auratus*) weighing approximately 100 g and aged 4–5 months were obtained from Anilab (Paulinea, São Paulo, Brazil) and housed individually in the laboratory in a 12:12 h light/dark facility at a controlled temperature (21.5 ± 0.5 °C), with provision of experimental chow and water ad libitum.

Experimental chow

Three types of chows were prepared according to the following parameters: zero, normal, and high content of zinc (threefold higher).

Experimental design

The animals were randomly assigned to six groups: (1) those fed filtered water (CTRL, $n = 27$) and (2) 10% fructose solution (FRU, $n = 24$) and rodent chow without zinc; (3) those fed filtered water (CTRL + Zn, $n = 25$) and (4) 10% fructose solution (FRU + Zn, $n = 25$) and rodent chow with normal levels of zinc; and (5) those fed filtered drinking water (CTRL + HiZn, $n = 27$) and (6) 10% fructose solution (FRU + HiZn, $n = 24$) and rodent chow with threefold higher levels of zinc. All diets were given for 60 days.

For microcirculatory analysis, the animals from each group were equally divided into three smaller groups: I/R followed by topical application of (1) ACh and histamine, (2) SNP and histamine, and (3) histamine (Fig. 1A and B).

Animal preparation and blood collection

On the day of the experiment, anesthesia was induced by an intraperitoneal injection of 0.1–0.2 ml of sodium pentobarbital (Pentobarbital Sodique, 60 mg/ml, Sanofi Santé Animale, Paris, France) and maintained with intravenous α-chloralose (100 mg/kg body weight (Sigma Chemicals, St. Louis MO, USA)).

One hour after the anesthesia, blood was withdrawn by periorbital puncture and the non-fasting glycemic state was analyzed with a glucometer.

Cheek pouch preparation

The cheek pouch of each hamster was everted and mounted in an experimental chamber, as previously described by Duling [18] and modified by Bouskela and Grampp [19].

Increase in macromolecular permeability induced by I/R

To quantify the increase in macromolecular permeability, 30 min after stabilization of the cheek pouch preparation, fluorescein isothiocyanate (FITC)–dextran of molecular weight 150,000 Da (Bioflor HB, Uppsala, Sweden, 25 mg/100 g body weight, 5% solution) was administered intravenously. The macromolecular permeability was quantified by counting the number of leaky sites (leaks) in the prepared area (1 cm^2) using an ultraviolet (UV)–light microscope (40 × magnification). Leaks are defined as visible extravascular spots (diameter ≥ 40 μm) of FITC–dextran in postcapillary venules seen under fluorescent light. The number of leaks was counted at baseline and during reperfusion, after 30 min of ischemia, induced by placing an air-inflatable tourniquet around the neck of