Nutrition 28 (2012) 691–697

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Dietary iron supplements may affect stress adaptation and aggravate stress hyperglycemia in a rat model of psychological stress

Yan Feng M.Sc. [a,b], Rui Qi M.Sc. [b], Manni Xu M.Sc. [b], Zhilei Shen M.Sc. [b], Min Li Ph.D. [b,*]

[a] Department of Nutrition, Zhejiang Province Tongde Hospital, Hangzhou, Peoples' Republic of China
[b] Department of Naval Medicine, Second Military Medical University, Shanghai, Peoples' Republic of China

ARTICLE INFO

Article history:
Received 11 May 2011
Accepted 18 September 2011

Keywords:
Iron
Stress hormones
Oxidative stress
Hyperglycemia

ABSTRACT

Objective: Iron supplementation is believed to decrease the risk of iron-deficiency anemia or low birth weight. In modern society, a majority of people are in a continual state of stress. Stress-induced hyperglycemia, known as *transient hyperglycemia*, may be a risk factor causing diabetes. To understand the role of iron in people under stress, it is necessary to evaluate the effect of iron supplementation on glucose or stress hyperglycemia.

Methods: The effect of a diet containing non-heme iron (80 or 320 mg/kg) on Sprague-Dawley rats and those under psychological stress was evaluated.

Results: Compared with control rats, a high-iron diet (320 mg/kg) increased blood glucose transiently in normal rats but induced hyperglycemia persistently in stressed rats throughout the experiment. Iron supplements further aggravated iron deposition and oxidative stress injury to the liver induced by the stress exposure. Glucose-related stress hormones were also affected by iron supplementation in stressed rats.

Conclusion: Oxidative stress may be one of the main reasons for insulin resistance. Moreover, changes in stress hormones indicate that high-iron supplements may affect stress adaptation. Both are primary reasons for the hyperglycemia induced by iron supplementation in stressed rats. Gaining an insight into the mechanisms and correlations of these changes may be beneficial to human health and is important for the prevention of pathologic glycemia-related diseases.

Introduction

Iron supplementation is considered to decrease the risk of iron-deficiency anemia and prevent low birth weight [1]. However, excess iron supplement use has been associated with an increased risk of type 2 diabetes [2–5], gestational diabetes [6,7], myelodysplastic syndrome [8], or even infection with the human immunodeficiency virus-1 [9].

The relation between iron and glucose is being widely debated. A prospective cohort study showed that heme iron intake from red meat sources rather than from non-red meat sources could increase the risk of type 2 diabetes [10]. A cross-sectional survey in China also reported that total iron could increase the risk of type 2 diabetes regardless of heme or non-heme iron [11].

Iron is a pro-oxidant that catalyzes some cellular reactions to produce reactive oxygen species, causing oxidative stress injury [12–14]. Increased oxidative stress has been proposed to contribute to an increased risk of type 2 diabetes [15–18], and iron overload may affect levels of stress hormones [19]. Perhaps for all these reasons, hyperglycemia is induced.

With the development of society and the acceleration in the pace of life, people are typically in a constant state of stress. It is known that a stress state can increase glucagon, adrenaline, growth hormone, and glucocorticoid levels and induce stress hyperglycemia, thus increasing the risk of diabetes [20–22]. All these changes could be detrimental to patients or even individuals with normal stress.

Our previous study reported that the iron content in the liver was increased and serum iron was decreased significantly after psychological stress exposure [23,24]. Some studies have shown that iron deposition in the liver may affect levels of stress hormones [25].

Indeed, the relation between the iron state in the body and the risk of diabetes is not clearly understood. Another

This work was supported by grant 30872120 from the National Natural Foundation of China.

* Corresponding author. Tel./fax: +86-021-8187-1120.
E-mail address: linlimin115@hotmail.com (M. Li).

0899-9007/$ - see front matter Crown Copyright © 2012 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.nut.2011.09.014

controversial issue is whether iron supplementation is necessary during stress to improve the decreased serum iron level induced by psychological stress exposure, or whether excess iron storage in the liver might increase the risk of diabetes.

We therefore investigated the relation between the iron state in the body and blood glucose, especially during stress, in an attempt to provide laboratory evidence for the development of a recommended dietary allowance of iron in stressed people (including soldiers and athletes), which might elucidate the pathogenesis and prevention of pathologic glycemia-related diseases.

Materials and methods

Animals

Adult male Sprague-Dawley (SD) rats (Shanghai-BK Co., Ltd., Shanghai, China), weighing 120 ± 5 g, were acclimated in individual cages at 24 ± 1°C and 40% to 60% humidity in an alternating 12-h light/12-h dark cycle, with the light on 08:00, for 7 d with free access to diet (iron content 80 mg/kg) and deionized water and then randomly divided into a 2-d group, a 7-d group, and a 14-d group. Each group was subdivided into four subgroups consisting of eight rats each: a normal control (NC) group (iron content 80 mg/kg), a normal iron plus psychological stress (NP) group, a high-iron control (HC) group (iron content 320 mg/kg), and a psychological stress exposure plus supplementation with a high-iron diet (HP) group.

Animals were cared for in accordance with the institutional animal care guidelines. All animal studies were approved by the animal research committee of the Second Military Medical University, Shanghai, China.

Psychological stress exposure

Using a communication box system, a psychological stress model was established as described previously [26,27]. Ten rats were put into the box to receive foot shocks. Rats to be psychologically stressed were put into adjacent compartments to receive emotional stimulations including jumping up and crying from the animals receiving foot shocks. Rats in the NP and HP groups were exposed to the psychological stress for 30 min every day.

Blood glucose test

A drop of blood was drawn from the caudal vein of the rats in the 2-, 7-, and 14-d groups within 60 min after the psychological stress exposure. Blood glucose values were determined by test strips (Roche Diagnostics, Mannheim, Germany) at an alternate-day interval throughout the experiment.

Enzyme-linked immunosorbent assay and radioimmunoassay analyses

Plasma glucagon (North China Institute of Biology, Beijing, China) and corticosterone (China Institute of Atomic Energy, Beijing, China) were determined by ^{125}I radioimmunoassay kits. The levels of insulin (Mercodia, Upsala, Sweden) and epinephrine (EPI; R&D System, Minneapolis, MN, USA) were measured by enzyme-linked immunosorbent assay kits.

Iron analysis and oxidative stress injury to the liver

The rat liver was digested with mixed acids (perchloric acid:thick nitric acid 1:4) and incubated at 60°C for 24 h until the liquids became transparent [28]. The content of iron in the liver was measured by iron flame or graphite atomic absorption spectrophotometry (Z-8100, Hitachi, Tokyo, Japan) [29]. To guarantee a coefficient of the standard curve higher than 0.999, the recovery rate of the dialysis system was controlled in the range of 90% to 110%. All samples were handled in the same manner and under the same condition.

Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) were used as markers of oxidation stress injury to the liver. According to the kit instructions (Jiancheng Bioengineering Institute, Nanjing, China), antioxidative stress enzymes and MDA levels in the liver homogenate were detected. The result was expressed as nanomoles or units per milligram of protein.

Statistical analysis

Data are expressed as mean ± standard deviation. Repeated-measurement data were used to analyze differences in blood glucose and stress hormones among the multiple groups by analysis of variance. The indices between the normal control groups and stress groups were compared by two-sample t test. Statistical analyses were performed with SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Effects of iron diet supplementation on mean body weight gain in stressed rats

There was no significant difference in food intake among the four groups during the entire experimental session. After psychological stress exposure, the mean body weight gain decreased by 13.52% and 16.79% in the normal and high-iron groups, respectively (Fig. 1).

Effects of iron supplements on plasma corticosterone and EPI concentrations in stressed rats

Compared with their corresponding control groups, the plasma corticosterone concentrations in the stressed groups significantly increased by 33.88% and 19.65%, respectively, in the normal and high-iron groups on day 2 and by 16.84% and 38.31% on day 7 ($P < 0.05$), but on day 14, the blood glucose value increased significantly only in the HP group (27.99%) compared with the HC group ($P < 0.05$). After the 14-d psychological stress exposure, the plasma corticosterone concentration in the psychologically stressed group with normal iron decreased to the normal level. Only on day 2 did the high-iron diet supplementation increase the plasma corticosterone level significantly compared with the normal control group for the normal SD rats ($P < 0.05$). Compared with the NC group, the corticosterone level in the HP group increased by 45.29%, 45.58%, and 37.60%, respectively, in the 2-, 7-, and 14-d groups, and the level also exceeded that in the NP group in the latter two groups ($P < 0.05$; Fig. 2A).

The same trend was observed with EPI. Compared with their corresponding control groups, the EPI levels in the stressed groups were significantly increased on days 2, 7, and 14 ($P < 0.01$). In SD rats compared with the control group, the EPI concentration was increased by 68.25% after administration of the high-iron supplement only on day 2 in our experiment ($P < 0.05$). The EPI concentration in the HP group was increased significantly in the 7-d and 14-d groups compared with the NC and NP groups ($P < 0.01$), but there was no significant difference from the NP group on day 2 ($P > 0.05$; Fig. 2B).

Effects of iron supplement on plasma glucagon and insulin concentrations in stressed rats

In stressed rats, plasma glucagon increased significantly on day 2 compared with their corresponding control group values (Fig. 3A), but this trend did not appear in the 7-d and 14-d groups. There was no significant difference among the four groups on days 7 and 14 ($P > 0.05$).

Compared with their corresponding control groups, plasma insulin concentrations were decreased after stress exposure throughout the experiment ($P < 0.05$). High-iron supplementation increased the insulin level significantly on days 2 and 7 in the experimental session ($P < 0.01$). High-iron supplementation to stressed rats increased the insulin level significantly compared with the NP 2-d and 7-d groups, but there was no significant difference between the NP and HP 14-d groups ($P > 0.05$; Fig. 3B).

Effects of iron supplement on blood glucose in stressed rats

The blood glucose level significantly increased on days 2 and 4 after the psychological stress exposure in the normal iron groups ($P < 0.05$) but decreased from day 6. Compared with their corresponding control groups, there was no significant