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High selenium impairs hepatic insulin sensitivity through opposite regulation of ROS

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HIGHLIGHTS

- High Se induces hepatic insulin resistance.
- High Se activates selenoprotein and depletes chromium, leading to lipolysis.
- High Se increases "bad" ROS generation and weakens "good" ROS signal.

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ABSTRACT

Insulin resistance is the hallmark of type 2 diabetes. As an essential trace element, selenium (Se) is recommended worldwide for supplementation to prevent Se-deficient pathological conditions, including diabetes and insulin resistance. However, recent evidence has shown that supra-nutritional Se intake is positively associated with the prevalence of diabetes. In the present research, we examined the effect of high Se on insulin sensitivity, and studied possible mechanisms in rats and in rat hepatocytes. Insulin sensitivity and glucose/lipid metabolism were determined by glucose/insulin tolerance test, western blot, immunofluorescence, specific probes and other biochemical assays. We show that high Se activates selenoproteins, including glutathione peroxidase and selenoprotein P, and depletes chromium, leading to a common metabolic intersection—lipolysis in adipose tissue and influx of fatty acids in liver. Fatty acid β-oxidation generates acetyl-CoA, which is metabolized in trichloroacetic acid cycle, supplying excessive electrons for mitochondrial oxidative phosphorylation and leading to increased "bad" reactive oxygen species (ROS) production in mitochondria and final disturbance of insulin signaling. Furthermore, high Se-activated selenoproteins also weaken insulin-stimulated "good" ROS signal generated by NAD(P)H oxidase, leading to attenuation of insulin signaling. Taken together, these data suggest that excessive intake of Se induces hepatic insulin resistance through opposite regulation of ROS.

1. Introduction

Diabetes has become a major public health problem in the whole world and about 285 million people worldwide suffer from diabetes (International Diabetes Federation, 2009). More than 90% of the diabetes patients suffer from type 2 diabetes mellitus (T2DM) (Wild et al., 2004). Insulin resistance is the hallmark in the development of T2DM. Once insulin resistance occurs, insulin action is impaired, leading to increase of hepatic glucose production and reduction of glucose uptake in peripheral tissues, and final hyperglycemia. In light of the important role of liver in the regulation of glucose/lipid metabolism, hepatic insulin resistance plays a fundamental role in systematic insulin resistance (Leclercq et al., 2007).

As an essential trace element, selenium (Se) has a major metabolic significance for human beings. Se deficiency causes several disorders, including cardiovascular and neurodegenerative diseases, aging, and impairment of the immune system. Therefore,

Abbreviations: T2DM, type 2 diabetes mellitus; Se, selenium; IPGTT, intraperitoneal glucose tolerance test; IPITT, intraperitoneal insulin tolerance test; AUC, area under the curve; Akt, protein kinase B; PEPCK, phosphoenolpyruvate carboxykinase; G6P, glucose-6-phosphate; GPx, glutathione peroxidases; SeP, selenoprotein P; MSA, mercaptosuccinic acid; CrPic, chromium picolinate; HSL, hormone-sensitive lipase; PPARγ, peroxisome proliferator-activated receptor; CREB, cAMP response element binding protein; FFAs, free fatty acids; SS, sodium salicylate; FAO, fatty acid oxidation; ROS, reactive oxygen species; CPT1, carnitine palmitoyltransferase 1; TCA, trichloroacetic acid; OXPHOS, mitochondrial oxidative phosphorylation; MitoQ, MitoQuinone; Rot, rotenone; CCCP, carbonyl cyanide 3-chlorophenylhydrazone; Oligo, oligomycin; ETO, etomoxir; DPI, diphenyliodonium.

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consumption of high amounts of Se-enriched dietary supplements is prevalent worldwide, especially in developed countries. Traditional view considers that Se deficiency contributes to insulin resistance and T2DM (Kljai and Runje, 2001) and at dietary levels of intake, individuals with higher Se levels are at lower risk for T2DM (Park et al., 2012). However, allowing for narrow therapeutic window of Se, supra-nutritional Se intake may exert adverse health effects (Whanger et al., 1996). Recent experimental and clinical evidences have substantially challenged the beneficial view of Se. The most astonishing findings come from a Nutritional Prevention of Cancer trial: T2DM incidence in participants who received 200 μg Se daily over 12 years were increased twofold compared with that of placebo group (Stranges et al., 2007). After that, several epidemiological studies demonstrated that high serum Se levels were positively associated with the prevalence of diabetes (Bleys et al., 2007; Stranges et al., 2010a) and adverse blood lipid profile (Bleys et al., 2008; Laclaustra et al., 2010; Stranges et al., 2010b, 2011) in both Se-replete US and Se-lower EU population. Recently, Zeng et al. (2012) reported that high intake of dietary Se affected insulin sensitivity in gestating rats and their offspring. However, the causative effect of high Se on insulin signaling is not clear and the underlying molecular mechanism is far from completely understood.

In this study, we have examined the effect of high Se on insulin sensitivity and possible mechanisms. We show that high Se induces hepatic insulin resistance through either promotion of lipolysis-induced ROS injury or attenuation of insulin-stimulated ROS signal.

2. Materials and methods

2.1. Animal experiments

All animal experiments were approved by the Institutional Animal Care and Research Advisory Committee of the Fourth Military Medical University. 60 rats were randomly allocated into indicated groups. Rats were given intragastric administration of 219 μg/kg (equal to 100 μg/kg Se) or 438 μg/kg (equal to 200 μg/kg Se) sodium selenite daily for 6 weeks with or without intraperitoneal injection of 10 mg/kg MSA, 0.5 mg/kg CrPic, or 10 mg/kg SS in the last 10 days. Saline was used as substitute in the control group.

2.2. Cell experiments

The rat normal hepatocyte cell line BRL-3A was cultured in RPMI 1640 supplemented with 10% FBS (Gibco BRL, Rockville, MD, USA). After growing into confluence, cells were exposed to indicated concentrations (1 μM or 10 μM) of sodium selenite for 36 h. For some experiments, cells were treated by 10 μM of sodium selenite in the presence or absence of MSA (0.05 mM), CrPic (1 μM), SS (2 μg/ml), Rot (2 μM), CCCP (2 μM), Oligo (2 μg/ml), Eto (2 μM), MitoQ (500 nM) and DPI (2 μM) for 36 h. For some experiments, cells were treated by 10 μM of sodium selenite in the presence or absence of MSA (0.05 mM) and DPI (2 μM) for 36 h, and then exposed to 100 nM insulin or not for 10–15 min. At the end, cells were harvested for the detection of western blot and other analyses.

2.3. Fasted and refed blood glucose

Rats were fasted for 12 h overnight and then refed for 4 h. Tail blood samples were drawn and analyzed immediately for glucose content using HemoCue B-Glucose Analyzer (HemoCue, Lake Forest, CA).

2.4. IPGTT and IPITT

The intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed. Prior to each test, rats were fasted for 6 and 4 h, respectively. Then, a baseline blood sample was taken from their tail and each rat received either i.p. glucose, 1 g/kg body weight or i.p. insulin, 0.75 U/kg body weight (Novolin R by Novo Nordisk Pharmaceuticals, Princeton, NJ). Tail blood samples were drawn at, 30, 60, and 120 min after the injection and were analyzed immediately for glucose content using HemoCue B-Glucose Analyzer (HemoCue, Lake Forest, CA).

2.5. Western blot

Briefly, liver and cell lysates were prepared by incubation on ice with lysis buffer (50 mM Tris–Cl (pH 7.5), 20 mM NaCl, 5 mM EDTA, 1% TX-100, 0.1% SDS, 5% glycerol + protease inhibitors), and centrifuged at 20,000 × g. The supernatant was collected and protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo) with bovine serum albumin as a standard control. The supernatant was mixed with equal volume of sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 5% mercaptoethanol, 1% bromophenol blue, and 25% glycerol). Then the mixture was boiled for 5 min and centrifuged at 10,000 × g for 10 min. The supernatants were used for immunoblotting. Protein extractions were separated by using SDS-PAGE on 10% polyacrylamide gels, and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). After blocking for 1 h with 8% skimmed milk in TBS buffer (10 mM Tris, 150 mM NaCl), the membrane was incubated with indicated primary antibodies overnight at 4 °C. After the membrane was washed four times for 15 min each with TBST buffer (10 mM Tris, 150 mM NaCl, and 0.1% Tween-20), it was incubated in the appropriate HRP-conjugated secondary antibody (diluted 1:5000 in TBST) at 37 °C for 30 min. The protein bands were visualized using chemiluminescent reagents according to the manufacturer's instructions and quantified using an image analyzer Quantity One System (Bio-Rad, Richmond, CA, USA). All protein quantifications were adjusted for the corresponding β-actin level, which was not consistently changed under the different treatment conditions.

2.6. Glycogen determination

Glycogen levels were measured in cells incubated for 3 h in the presence of 1 nM insulin (Usbio) using a glycogen assay kit (Biovision).

2.7. Chromium determination

Chromium (III) levels were measured using a commercial chromium assay kit (BioAssay) according to the manufacture's instruction.

2.8. Determination of mitochondrial superoxide anion

At the end, cells and frozen sections of livers were incubated with MitoSOX (500 nM) for 30 min at 37 °C, and then observed under a confocal microscopy (Olympus).

2.9. Determination of intracellular ROS

At the end, cells were incubated with CellROX Deep Red Reagent (5 μM) for 30 min at 37 °C, and then observed under a confocal microscopy (Olympus).

2.10. Determination of products of lipolysis

For FFAs in livers, detection was conducted according to the manufacturer's instructions. For FFAs in cells, cells were incubated with BODIPY D-3835 (a fatty acid probe, 3.5 ng/ml) for 30 min at 37 °C, and then observed under a confocal microscopy (Olympus).

2.11. Statistical analyses

Statistical analyses were performed using one-way ANOVA followed by a SNK-q test for multiple comparisons. Data were presented as the mean ± SD; $p < 0.05$ was considered significant.

3. Results

3.1. High Se induces hepatic insulin resistance

In our study, rats were orally administrated with 219 μg/kg sodium selenite (equal to 100 μg/kg Se) (low Se, LSe) or 438 μg/kg (equal to 200 μg/kg Se) (high Se, HSe) sodium selenite daily for 6 weeks. Fasted and refed blood glucose was increased by HSe (Fig. 1A). Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were conducted to evaluate the effect of high Se on glucose and insulin tolerance. Fig. 1B showed that, following glucose load, the blood glucose of rats treated with Se increased compared with control. Additionally, Se significantly increased the area under the curve (AUC) of IPGTT and thus resulted in impaired glucose tolerance (Fig. 1C). Following insulin load, the blood glucose of rats treated with Se were higher than that of control (Fig. 1D), which was confirmed by AUC (Fig. 1E), indicating impaired insulin tolerance.

Liver plays a central role in glucose/lipid metabolism and thus is a main determinant of insulin sensitivity in the whole body. In the study, several rats were injected with 10 U/kg insulin 30 min before sacrifice to investigate the insulin signaling transduction in liver. Western blot analysis has shown that insulin-stimulated phosphorylation of protein kinase B (Akt) was inhibited by Se in rats (Fig. 1F).