Antioxidant and Protective Effects of Selenium against Metabolic Syndrome Induced by High Fructose in Rats

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Abstract

High fructose diet fed rat model is known to develop the insulin-resistant syndrome with a very similar metabolic profile to the human X syndrome. The aim of the current study was to investigate the antioxidant and protective effect of selenium on insulin sensitivity induced by high fructose in rats. Forty male Wister rats were randomly divided into four groups (n=10): Healthy control rats, high fructose-fed rats, selenium treated rats fed high fructose and selenium fed control rats. Insulin resistance was induced in the second and third groups by administration of high-fructose diet for a period of five weeks. Fasting blood glucose (FBG) and insulin levels were higher in fructose-fed rats while oxidative stress parameters were lower than in control rats (p<0.05). Selenium administration can significantly improved the oxidative stress parameters and FBS levels in fructose-fed-selenium treated rats compared to fructose-fed rats. In summary, these results indicated that selenium has a hypoglycemic and hypoinsulinemic effects in the fructose-induced insulin resistant animals by its antioxidant effects. Moreover, our data suggests that selenium may be beneficial for people with decreased insulin sensitivity and increased oxidative stress, such as those with the metabolic syndrome or type 2 diabetes.

Key words: Fructose, metabolic syndrome, selenium, glucose, oxidative stress, insulin resistance, dehydroepiandrosterone (DHEAS).

Introduction

Metabolic Syndrome is a pathophysiological entity characterized by insulin resistance, hyperinsulinemia, dyslipidemia, hypertension, and obesity. The risk for developing diabetes type 2, cardiovascular disease, and renal disease is increased with increasing manifestations of the various components of the syndrome within any individual (8). The macronutrient content of the diet has been linked to the metabolic syndrome. Recently, consumption of dietary fructose has been suggested to be one of the environmental factors contributing to the development of obesity and the accompanying abnormalities of the metabolic syndrome. In fact, a well-known experimental model of metabolic syndrome is induced by high consumption of fructose; this model induces hypertension, hypertriglyceridemia, hyperinsulinemia, and insulin resistance in rats.
Fructose consumption is able to produce these effects because fructose is more lipogenic than glucose and usually causes greater elevations of triglycerides, which, in turn, increases intramyocellular triglyceride content in the skeletal muscle, causing insulin resistance (6).

These rat models develop an insulin resistant syndrome with a very similar metabolic profile to that of the human metabolic syndrome (21). In the metabolic syndrome, sources of oxidative stress are related to hyperglycemia, hyperinsulinemia, and inadequate antioxidant defenses. Alteration of insulin sensitivity leads to a higher rate of glucose oxidation and increased production of OH (22). Oxidative stress has been identified as a key factor in diabetes and atherosclerosis (20). Thus, uncontrolled free radical production might be one of the mechanisms underlying the development of comorbidities in individuals with the metabolic syndrome. In these patients, an increase in dietary antioxidant intake could represent a potential strategy to reduce the incidence of diabetes and cardiovascular disease (12).

The role of oxidative stress and free radical attack in the development of insulin resistance has been largely documented through the complications on lipids and proteins. Increased formations of lipid peroxidation end products and defects in free radical defense have been demonstrated in high fructose-fed rats (16).

Circulating levels of dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) decline with age and a relationship has been suggested between lowered DHEA levels and heart disease, cancer, diabetes, obesity, chronic fatigue syndrome, AIDS and Alzheimer's disease (13). DHEA and DHEAS are the most abundant circulating adrenal steroids in human. Apart from their roles as precursors of androgens and estrogens, their precise biological functions are still unknown. In addition, several studies have indicated that insulin resistance should induce a decrease in the levels of DHEA and its sulfate ester (DHEAS) in human. The relationship between lowered DHEA levels and diabetes, obesity and chronic fatigue syndrome has been suggested (12).

In a previous study we have shown that selenium supplementation could modulate lipid profile, liver and also kidney functions and the complications associated with fructose-induced metabolic syndrome in rats (14). This study was undertaken to evaluate the preventive effects of selenium as one of the most important antioxidant element on high fructose-induced insulin resistance (metabolic syndrome) in rats.

**MATERIALS AND METHODS**

**Chemicals**

Fructose was purchased from local markets. Selenium (Sigma-Aldrich, Castle Hill, Australia) was purchased from General Nutrition Centers (GNC) market, Jeddah, Saudi Arabia Shop Market. All chemicals used were of high analytical grade, product of Sigma (US), Merk (Germany) and BDH (England).

**Animals**

Adult male healthy rats weighing between 190–200 gm were acquired from the experimental Animal House of King Faisal Center. Rats were housed in standard cages and were maintained on standard pellet diet and tap water and kept at 25 ± 3°C.
temperature, 50–60% humidity, and a 12 h light-dark cycle. This acclimatization conditions lasts for one week before the commencement of the experiment. All animals received professional human care in compliance with the guidelines of the Ethical Committee of King Abdulaziz University- Jeddah-Kingdom of Saudi Arabia.

**Induction of metabolic syndrome**

Fructose-induced metabolic syndrome was induced in male Wister rats by adding fructose solution 20% (w/v) in tap water that was prepared every day (23).

**Experimental design**

Forty rats were randomly divided into 4 groups of 10 rats each as follows:
1. The first group acted as healthy control (C): Rats received standard rodent diet and tap water.
2. The second group acted as metabolic syndrome (F): Rats received standard rodent diet and tap water supplemented with 20% fructose.
3. The third group acted as treated fructose-fed (F-T): Rats received standard rodent diet and tap water supplemented with 20% fructose for 5 weeks. Selenium was given at the dose of 0.15 mg/0.5 ml distilled water/kg body weight per day by gavages.
4. The fourth group (C-S): Rats received standard rodent diet and tap water for 5 weeks. Selenium was administered during the third week till the end of the experiment. Body weight was measured every two weeks.

The experiment was carried out for 5 weeks. At the end of the treatment period and after an overnight fast (10 to 12 h), blood glucose concentrations were measured in tail vein blood using glucose meter (9), all animals were sacrificed under light ether anesthesia. Blood samples were collected for serum separation. Serum was separated by centrifugation at 3,000 rpm for 15 min and stored at -20°C for further analyses.

The serum parameters were analyzed spectrophotometrically by using double beam UV-Visible spectrophotometer (Shimadzu UV-Visible spectrophotometer, model 1700). Serum insulin levels were determined by ultra sensitive rat insulin kit (DRG), France), using double antibody enzyme-linked immunosorbent assay (ELISA). Serum DHEAS levels were determined by radioimmunometric assay, using commercial kit (Immunotech, France).

**Measurement of antioxidants**

Liver was quickly removed and washed in ice-cold saline. One hundred mg of liver tissue was homogenized in ice-cold trihydrochloride buffer (pH = 7.2). The homogenate was centrifuged at 800 rpm for 10 min, followed by centrifugation of the supernatant at 12,000 rpm for 15 min. The obtained supernatant was used for the estimation of nitric oxide (NO) according to the colorimetric method by Montgomery and Dymok (10), total antioxidant capacity (TAC) according to the colorimetric method described by Koracevic et al., (7) and glutathione peroxidase (GPx) by that of Rotruck et al. (15). Selenium levels were determined by the method described by Tinggi et al. (18).
Histopathology of selected organs: At the end of the high fructose induction, rats were sacrificed; dissected and major organs like liver and kidney were collected and fixed into 10% neutral buffered formalin for histological studies. Organs were processed and stained with hematoxylin and eosin (H &E) for histological examination.

Statistical analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± standard error (SE). The significant differences among values were analyzed using analysis of variance (one-way ANOVA) followed by Bonferroni as a post-ANOVA test by SPSS version 15. For all analyses, p values ≤ 0.05 were considered significant. Correlation of variables was tested by the Pearson test.

RESULTS

Effects on body weight, glucose, insulin and DHEAS levels.

Fructose-fed rats exhibited significant increase in body weight as compared to control normal rats. Treatment with selenium in fructose-fed rats reversed this increase in body weight (P< 0.01). As shown in Table 1, fructose-fed rats were significantly hyperglycemic and hyperinsulinemic compared with the control group (P<0.01). Fasting serum glucose and insulin level significantly decreased in the F-T group compared with the F group (P<0.01). Fructose-enriched diet also led to a decrease in serum DHEAS concentration in the F group compared with control animals (P<0.01), but selenium treatment prevented this decrease in serum DHEAS concentration in the F-T group compared with the F group (P<0.01).

Table (1): Fasting blood glucose, Body weight gain, Insulin levels and DHEAS levels in different experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n=10)</th>
<th>F (n=10)</th>
<th>F-T (n=10)</th>
<th>C-S (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (gm)</td>
<td>25.1±6.7</td>
<td>30.3±6.5</td>
<td>18.6±5.4 a,b</td>
<td>10.5±3.2 a,b</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>94.83±8.6</td>
<td>176±14.9 a</td>
<td>119±7.2 b</td>
<td>127±3.7 a,b</td>
</tr>
<tr>
<td>Serum Insulin (ng/ml)</td>
<td>0.5±0.03</td>
<td>1.18±0.07 a</td>
<td>0.58±0.02 b</td>
<td>0.51±0.01 b</td>
</tr>
<tr>
<td>DHEAS (µmol/L)</td>
<td>0.53±0.018</td>
<td>0.3±0.01 a</td>
<td>0.35±0.01 a</td>
<td>0.59±0.01 a,b</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE. aP < 0.01, compared to control rats. bP < 0.01, compared to fructose fed rats. Control: rats received standard diet, Fructose: rats received standard diet and 20% fructose in tap water, F-T: rats received standard diet + 20% fructose in tap water + selenium, C-S: rats received standard diet+ selenium.
The results illustrated in Table 2 demonstrated that there was a significant decrease in serum nitric oxide and total antioxidant capacity in untreated fructose group relative to control group (P< 0.05). Oral administration of selenium increased serum nitric oxide level and total antioxidant capacity level to be near the normal levels.

The activity of GPx in liver was significantly decreased in fructose-fed rats (Group 2) compared with the control rats (Table 2). Selenium-treated fructose fructose-fed rats (Group 3) showed significantly higher activities of the enzyme compared with those of fructose-fed rats (Group 2). The activities remained unaltered in control rats treated with selenium (Group 4). The concentration of the non-enzymatic selenium in liver was significantly decreased (P < 0.05) in fructose-fed rats compared with the control rats. The fructose-fed rats, when treated with selenium showed elevation compared with untreated fructose-fed rats. In control rats treated with selenium, there were no significant alterations (Table 2).

### Table (2): Oxidative status and selenium level in different experimental group

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control (n=10)</th>
<th>F (n=10)</th>
<th>F-T (n=10)</th>
<th>C-S (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO (mmol/L)</td>
<td>45±0.73</td>
<td>36.6±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.6±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>2.2±0.05</td>
<td>1.3±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48±0.06&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gpx (µg/min/mg protein)</td>
<td>5.8±0.17</td>
<td>4.2±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2±0.07&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>6.5±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Se (µmol/L)</td>
<td>0.68±0.04</td>
<td>0.3±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE. <sup>a</sup>P < 0.05, compared to control rats. <sup>b</sup>P < 0.05, compared to fructose fed rats. Control: rats received standard diet, Fructose: rats received standard diet and 20% fructose in tap water, F-T: rats received standard diet + 20% fructose in tap water + selenium, C-S: rats received standard diet+ selenium.

There were negative correlation between glucose and selenium (r=-0.44), GPx (r=-0.45) and insulin (r=-0.49), while there was a positive correlation between selenium and TAO (r=0.76), NO (r=0.75), DEAE (r=0.62), insulin (r= 0.72) and GPx (r=0.72) as shown in Table 3.

### Table (3): Correlation coefficient between parameters (*P=0.001).

<table>
<thead>
<tr>
<th></th>
<th>Se</th>
<th>Gpx</th>
<th>Insulin</th>
<th>DHEAS</th>
<th>Glucose</th>
<th>NO</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se</td>
<td>-----</td>
<td>0.72**</td>
<td>0.72**</td>
<td>0.62**</td>
<td>-0.44**</td>
<td>0.75**</td>
<td>0.76**</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.44*</td>
<td>-0.45**</td>
<td>-0.49**</td>
<td>NS</td>
<td>------</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Fig. 1. Histopathological changes in Liver of control and experimental rats.

A: Normal liver sections, B: liver section of fructose-fed rats, C: liver section of fructose treated rats, D: liver section of normal rats fed selenium.

Fig. 2. Histopathological changes in kidney of control and experimental rats.

A: Normal kidney sections, B: kidney section of fructose-fed rats, C: kidney section of fructose treated rats, D: kidney section of normal rats fed selenium.

Figure 1& 2 show liver and kidney sections of different experimental groups after 5 weeks of fructose administration. Light micrograph of liver tissue of fructose fed rats showing increase in the blood sinusoid in the central vein while in kidney tissue showing nearly normal distal convoluted tubule with diluted lumen low and nuclei in some proximal epithelial cells. Moreover, the experimental group rats showed similar kidney
histology as in normal group. No tubular foaming changes were observed. Besides, results from liver tissue of treated rats showing moderate blood sinusoid and many hepatocytes were binucleated.

Discussion

The benefits of using animal models in research works involved the ability to access its multifactorial genetics and complications. However, correlations between animal diseases and that in human kind are well established. The aim of this study was to investigate, in an animal model of insulin resistance, the effects of administration of selenium on blood oxidative stress parameters and insulin sensitivity.

It is interesting to observe that high fructose diet leads to an oxidative stress, as shown by the fall in the nitric oxide, total antioxidant capacity, glutathione peroxidase and selenium in the fructose fed rats group. In addition, high levels of dietary fructose have been reported to enhance oxidative damage in rats (4). In the present work, the fructose-fed animals exhibited decreased levels of oxidative stress parameters, compared with the control group. The underlying mechanisms for the detrimental consequences of a high fructose diet in animal models are not totally understood. A link between oxidative stress and insulin resistance has been demonstrated (2), and oxidative stress has been proposed as one of the underlying causes of the development of insulin resistance, beta-cell dysfunction, and impaired glucose tolerance. When selenium was added to the fructose diet, fasting glucose and insulin were decreased. This study reports the association between antioxidant and insulin-enhancing properties of selenium. Mechanisms for the induction of insulin resistance by oxidative stress have been proposed, and, conversely, high insulin is a leading cause of oxidative stress. In this study, the observed improvement in insulin sensitivity could be part of the antioxidant effects of selenium, because previous studies in fructose-fed rats have reported that a free radical scavenger, like vitamin E, improved insulin sensitivity (11). Similarly, an insulin sensitizer, metformin, has been shown to improve the free radical defense system (17).

Previous evidences have indicated that DHEA and DHEAS exert multiple antiatherogenic effects (3). Furthermore, many studies in human have shown that elevation of serum insulin level to its high physiological range inhibits adrenal production of DHEA levels. This is achieved by selective suppression of 17, 20-lyase steroidogenic enzyme activity (19). Hyperinsulinemia promotes macrovascular disease in part by reducing blood DHEA and DHEAS levels. Fructose feeding can induce free radical formation by causing downregulation of the key enzymes of the hexose monophosphate pathway that generate a reduced environment in the form of NADPH and NADH. Impaired regeneration of NADPH could result in an increased oxidative state of the cell (1).

Oxidative stress has been suggested to contribute to insulin resistance and plays a critical role in the pathogenesis of endothelial dysfunction (2). The significant decreased in the levels of malondialdehyde, glucose, insulin, and insulin resistance and increased in the levels of vitamins C and E, total antioxidant status, catalase, superoxide
dismutase, glutathione peroxidase, and nitric oxide in the supplemented groups as compared with hypertensive control indicated the role of oxidative stress in hypertension, insulin resistance, and endothelial dysfunction in this model. In this regard, the decreased nitric oxide level observed in hypertensive control might indeed reflect the impaired nitric oxide bioavailability. The possible link between insulin resistance and endothelial dysfunction is that binding of insulin to its receptor stimulates the production of nitric oxide at the endothelial level (5), and since nitric oxide constitutes one of the major vasodilator, the defect in insulin signaling pathway caused by insulin resistance appears to be closely associated with endothelial dysfunction.

The improved endothelial function and insulin sensitivity observed in the supplemented groups confirms the role of antioxidant vitamins in the management of metabolic syndrome. Thus, the exact molecular mechanisms underlying antioxidant effects of these vitamins on insulin sensitivity and endothelial function were not fully assessed in this model but could be attributed to their role in inhibiting NADPH oxidase activity, scavenging free radical, and stimulating the activity of nitric oxide synthase. Studies have shown that vitamins C and E and vitamin C (4) can stimulate the activity of endothelial nitric oxide synthase by increasing the intracellular availability of the endothelial nitric oxide synthase cofactor tetrahydrobiopterin, which could further increase nitric oxide synthesis.

In conclusion, our findings suggest that the elevation in insulin resistance, plasma levels of glucose and oxidative stress induced by a fructose diet can be improve by administration of selenium. The beneficial effects of selenium can be attributed to its antioxidant activity. The protective effects of selenium via alterations in the biochemical pathway will be further investigated.

References


