

Effects of Borax and Sleeve Gastrectomy on mRNA Expression of Antioxidant Genes in Substantia Nigra Tissue of Obese Rats

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Abstract

Aim: To evaluate the effects of borax and sleeve gastrectomy on mRNA expression of antioxidant genes in substantia nigra tissue of obese rats.

Methods: Obese rats were fed with a high-fat diet containing 40% additional fat to the diet. Rats were allocated into four groups in random, which were normal rats (Group I) (n=14), obese rats subjected to SG (Group II) (n=14), obese rats subjected to borax (Group III) (n=14), and obese rats subjected to SG and borax (Group IV) (n=14). Catalase, superoxide dismutase (SOD) and glutathione S-transferase (GST) gene expressions were determined by polymerase chain reaction, real-time polymerase chain reaction (RT-qPCR) and western blotting.

Results: When normal rats (Group I), and obese rats subjected to SG (Group II) were compared, a decrease in expressions of catalase, SOD and GST genes was observed in Group II. When obese rats subjected to borax (Group III) were compared with Group I and Group II, a decrease in expressions of catalase, SOD and GST genes was observed in Group III. This phenomenon demonstrates that borax and SG both decrease expressions of catalase, SOD and GST genes. Furthermore, the most significant decrease in expressions of catalase, SOD and GST genes was observed in obese rats subjected to SG and borax (Group IV) when compared to other three study groups.

Conclusion: The borax decreases molecular obesity and consequently increases the expressions of Catalase, SOD and GST genes. These data show decrease of Catalase, SOD and GST genes in the substantia nigra tissue of obese rats, consistent with the possibility that these changes may contribute to disease pathogenesis.

Keywords: Borax; Obesity; Sleeve gastrectomy; Substantia nigra.

Introduction

Obesity is blamed for over 2.8 million annual deaths all over the world with increasing prevalence of related comorbidities, including metabolic (e.g. diabetes mellitus, hyperlipidemia, hypertension) and non-metabolic disorders (e.g., cancer, stroke, depression, polycystic ovary syndrome, fat liver disease, glomerulopathy, bone fragility etc.) [1,2]. Fontaine et al. revealed that a 25-year-old obese patient has a 22% decrease in life expectation [3]. Adams et al. revealed that compared with cases who were nonsmokers and had no pre-existing illnesses; there was a 20 2- to 3-fold greater risk of death among obese individuals [4].

Sleeve gastrectomy (SG) is a restrictive procedure that not only results in lasting weight loss but also improves insulin sensitivity and glucose tolerance before any significant weight loss has arisen [5]. However, it is unclear whether SG has effects in the central nervous system.

Boron is an essential element well-known to control a number of metabolic and physiological systems. In nature, boron does not exist by itself and combines to form boric acid. Borax (a salt of boric acid) has a role in the action of enzymes in animal biological systems and interacts with a number of micronutrients, steroid hormones, and plasma lipids [6,7]. Even though the precise role of boron has not been exactly revealed, nutritional intake in human was shown to activate numerous significant inflammatory modifications. Yet, there are still contradictory outcomes on metabolic outcomes both in humans and animals [8,9]. Kucukkurt et al. revealed that borax is able to scavenge free oxygen radicals [10]. Besides they revealed that oral supplementation with boron caused reduction of insulin in adipose tissue and a decrease in sensitivity of adipose tissue to insulin [10]. Boron limits oxidative

damage by enhancing the body's store of glutathione and its derivatives, or by inducing other reactive oxygen species neutralizing agents [11].

The aim of the present study was to evaluate the effects of borax and SG on mRNA expression of Catalase, superoxide dismutase (SOD) and glutathione S-transferase (GST) genes in substantia nigra tissue of obese rats.

Materials and methods

Experimental design: All animal studies were carried out with the approval of the Institutional Animal Care and Use Committee. Animals were housed at constant temperature (20-22°C) and humidity (50-60%) with a 12-h light and 12-h dark cycle. They were allowed free access to water and standard rat chow. Rats were allocated into four groups in random, which were normal rats (Group I) (n=14), obese rats subjected to SG (Group II) (n=14), obese rats subjected to borax (Group III) (n=14), and obese rats subjected to SG and borax (Group IV) (n=14). Obese rats were fed with a high-fat diet containing 40% additional fat to the diet. One of the limitations of this study is the short follow up of

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8 weeks and of monitor the body weight change with borax (Graph 1).

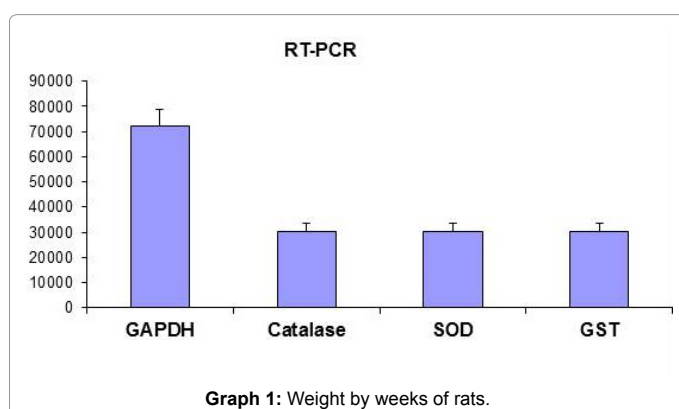
Surgical (sleeve gastrectomy) procedure: After overnight fasting, rats in the sleeve group were anesthetized with an intraperitoneal injection of 302 mg/kg chloral hydrate and placed in a supine position on a surgical board with the extremities immobilized. A sinister epigastric incision was used for the operation. The length was about 1.5-2 cm in total. The incision was kept open with a blade retractor and dissociates gastric omentum to disclose gastric cardium. The gastric cavity was closed with vascular clamps, and then gastric cavity and hemostating were cut off with a cauterizer. A gastric tube was created with 8-0 unabsorbable suture from the distal antrum (1.5-2 mm from the pylorus) to the Hiss angle. The fundus was completely removed (70-80% of total stomach). After the gastric tube was rebuilt, the peritoneal cavity was cleaned with saline, and then closed with 6-0 silk suture.

Application of Borax: For Group III and IV, 1 mg/L of boric acid was added in their drinking water.

PCR and RT-qPCR analysis: Total RNA was isolated from cultured cells by using TRIzol reagent (Sigma Chemical Co., St. Louis, MO, USA). Total RNA was isolated from exponentially substantia nigra tissues TRIzol Reagent (Sigma Chemical Co., St. Louis, MO, USA). Total RNA was treated with RQ1 DNase I (Promega, Madison, WI, USA). RT was performed according to the manufacturer's directions (Fermentas, Vilnius, Lithuania) using 1 unit of MMLV reverse transcriptase with 5 µg of total RNA and oligo dT22 primer. Similar amplification efficiencies of all gene targets and GAPDH were validated, permitting quantitative measurement of gene expression.

PCR gene expression of Catalase, SOD and GST were quantified using MyGenie96 Thermo BIONER PCR. The 25 µl PCR mixture contained were performed using 1 µL of cDNA template, 0.25 µL (5u/µL) of Taq polymerase, 2.5 µL (100 ng) of primers, 1.5 µL (25 mM) dNTP, 2.5 µL 10× PCR buffer and 1.5 µL MgCl₂. PCR reactions were performed: 94°C for 3 minutes, 30 cycles at 94°C for 45 seconds, 50-60°C for 45 seconds, and 72°C for 1 minutes, followed by a final extension at 72°C for 10 minutes.

Real-time PCR gene expression of Catalase, SOD and GST were quantified using the Roche Light Cycler real-time PCR analyzer according to the manufacturer's instructions (Roche Molecular Diagnostics, Mannheim, Germany). The 25 µl PCR mixture contained 12.5 µl of SYBR Green Universal PCR Master Mix (Perkin-Elmer Applied Biosystems, California, USA) 500 nmol/l of primers (forward and reverse), 100 nmol/l of labeled probe, and 1 µl of cDNA template. PCR was performed at 50°C for 2 min, 95°C for 10 min, and then run for 50 cycles at 95°C for 15 s and 60°C for 1 min.



Western Blotting

Protein precipitation with an ammonium sulfate is useful for purification of a total protein. Expression of the total protein was induced by the addition of 100 µM isopropyl β-D-thiogalactopyranoside and incubation was continued for a further 3 h at 37°C. Analysis of the purified proteins on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out with Coomassie brilliant blue staining. Proteins were transferred from the gel to a polyvinylidene fluoride filter (PVDF). For western blotting, the ProteoQwest colorimetric kit with TMB substrate was used with mouse monoclonal antibody and HRP-conjugated secondary antimouse antibody. Kaleidoscope pre-stained standards were used as molecular-mass markers.

Results

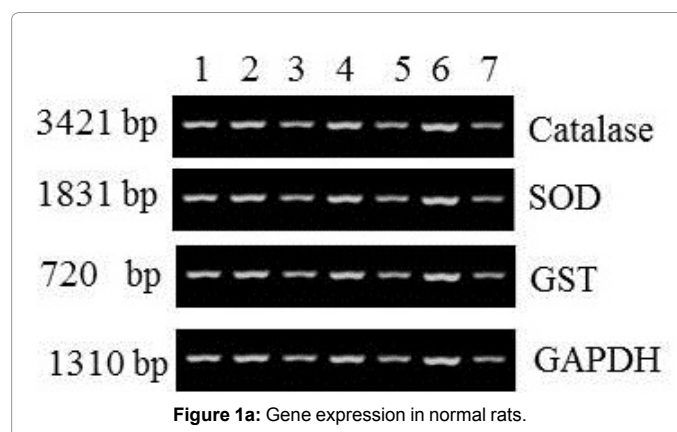
Within the context of this study, when normal rats (Group I), and obese rats subjected to SG (Group II) were compared, a decrease in expressions of Catalase, SOD and GST genes was observed in obese rats subjected to SG (Figures 1a-1d). When obese rats subjected to borax (Group III) were compared with both normal rats (Group I) and obese rats subjected to SG (Group II), a decrease in expressions of Catalase, SOD and GST genes was observed in obese rats subjected to borax (Figure 2a and 2b). This phenomenon demonstrates that borax and SG both decrease expressions of Catalase, SOD and GST genes. Furthermore, the most significant decrease in expressions of Catalase, SOD and GST genes was observed in obese rats subjected to SG and borax (Group IV) when compared to other three study groups (Figure 2c and 2d).

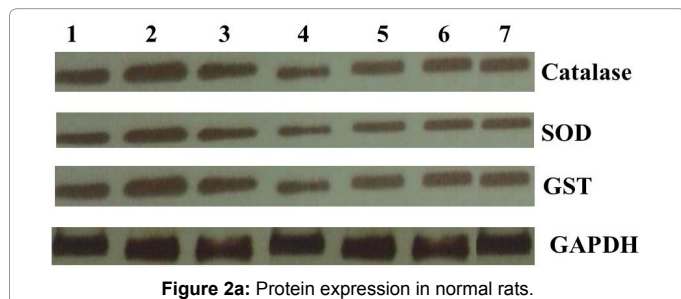
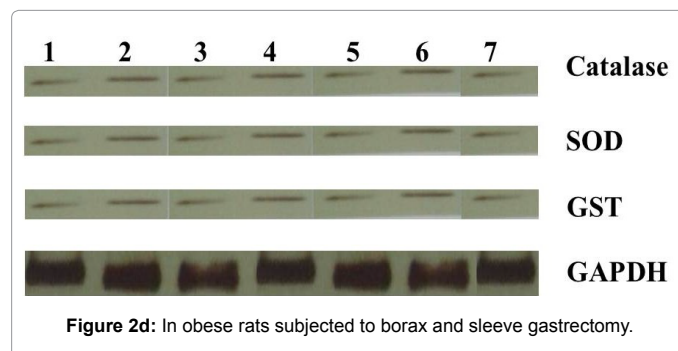
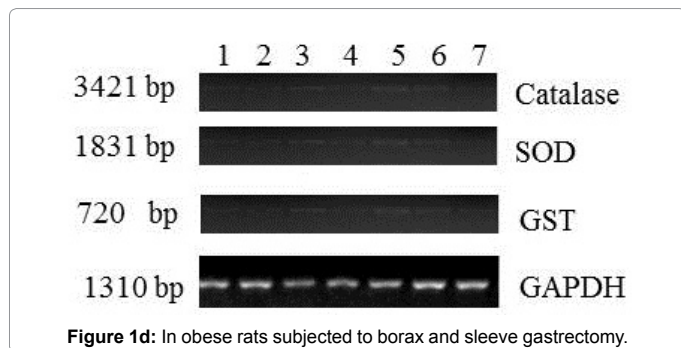
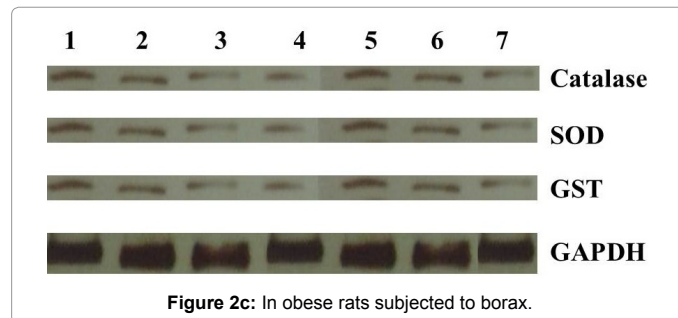
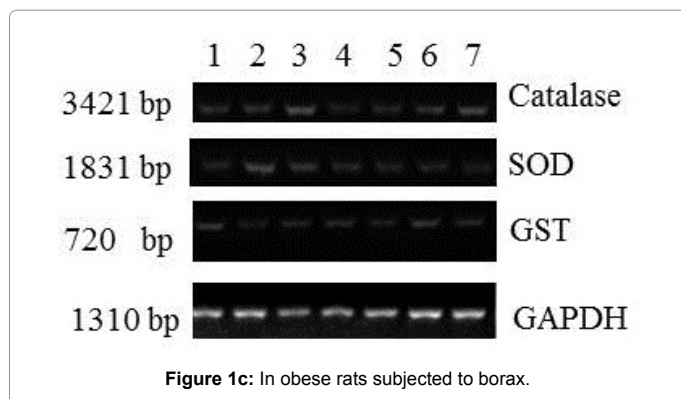
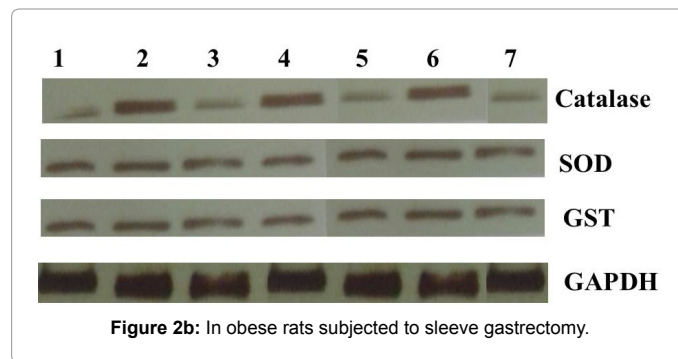
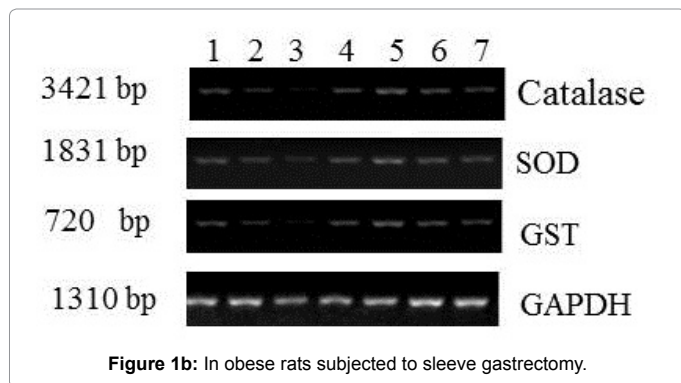
When groups were compared regarding genes, decreases in the expressions of Catalase, SOD and GST were observed secondary to obesity. When real-time PCR analysis was performed with the intention to quantitatively control the expressions of genes, decrease in Catalase, SOD and GST gene expressions was determined (Table 1).

Discussion

Here we aimed to reveal the effects of borax and sleeve gastrectomy on mRNA expression of antioxidant genes in substantia nigra tissue of obese rats. Our results showed that the most significant decrease in expressions of catalase, SOD and GST genes was observed in obese rats subjected to SG and borax, followed by obese rats subjected to borax, and obese rats subjected to SG.

Obesity is defined by a substandard inflammatory status, generating its consequences on numerous organs. Earlier literatures have found that weight loss is related with a decrease in inflammation markers (e.g.





		Group I	Group II	Group III	Group IV
	Before	226.95 g	226.95 g	226.95 g	226.95 g
1.	Week	212 g	267.5 g	258.7 g	222.1 g
2.	Week	215 g	266.3 g	262.0 g	220.4 g
3.	Week	220 g	270 g	270.0 g	207.2 g
4.	Week	226 g	272 g	284.9 g	212.1 g
5.	Week	226 g	285.4 g	288.9 g	215.8 g
6.	Week	228 g	297.9 g	302.9 g	200.0 g
7.	Week	224 g	295.6 g	302.4 g	189.9 g
8.	Week	226 g	301.9 g	313.4 g	199.3 g

Table 1: Quantitative real-time RT-PCR analysis for Catalase, SOD and GST.

obesity and the associated insulin resistance both take role in an exaggerated inflammatory reaction [15].

Bariatric surgery is currently the most noteworthy management of significant and sustained body weight loss in morbidly obese patients [16,17]. Hasani et al. showed that observed a significant difference for REE between obese patients after the surgery and controls as well as between prior-and post-surgery [18]. Recent evidence suggests that SG leads to a decreased food intake not only by mechanical restriction of the stomach but also by reducing a subject's appetite [19]. SG is supposed to modulate meal-related hormones of the gut-brain axis such

C-reactive protein) and pro-inflammatory cytokines (e.g. interleukin-6 and tumor necrosis factor) [12,13]. Oxidative stress results from an inequality concerning the total antioxidant defense of the tissue and the reactive species generated. The upsurge in fat-derived reactive oxygen species during the development of obesity to be the key contributory factor of the abnormal arrangement and reduced expression of adipose tissue antioxidative enzymes [14]. The pro-inflammatory effect of

as Ghrelin (signal = hunger) or peptide YY (slows gastric emptying) or GLP-1 (decreases food intake) [20]. In the present study, when normal rats, and obese rats subjected to SG were compared, a decrease in expressions of catalase, SOD and GST genes was observed in obese rats subjected to SG.

Boron has been shown to support the antioxidant defenses of tissue in animals and plants, via a unknown system that affects oxidative metabolism [21,22]. Garcia-Gonzalez et al. revealed that boron deficit increases the level of SOD, catalase, and peroxidase in heterocysts in the filamentous cyanobacterium *Anabaena PCC 7119* [23]. Turkez et al. showed that, low dose boron increases while high dose boron decreases both SOD and catalase activities in erythrocytes [24]. Pawa et al. administered borax orally to rats for three days followed by thioacetamide administration [25]. They showed that borax balanced the harmful effects of thioacetamide by modifying oxidative stress parameters and revealed that borax may keep oxidant/antioxidant balance. Oxidative stress shows up when antioxidant levels are decreased. Thus, antioxidant enzyme activities are essential in cell defense [26,27]. Inconsistent results regarding the effect of boron on body weight were showed in animal studies. Although the supplementation of boron reported to cause weight gain in some studies [6,9], conflicting results were also reported [8,10]. These results are believed to be the results of heterogeneity in type and dosage of the boron compound and duration of the exposure. In the present study, when obese rats subjected to borax were compared with both normal rats and obese rats subjected to SG, a decrease in expressions of Catalase, SOD and GST genes was observed in obese rats subjected to borax. Furthermore, the most significant decrease in expressions of Catalase, SOD and GST genes was observed in obese rats subjected to SG and borax when compared to other three study groups.

One of the limitations of this study is the short follow up of 8 weeks. However, this time period is the equivalent of 6 human years [28,29]. While we cannot exclude the potential for weight regain following sleeve gastrectomy, other investigators have demonstrated that weight loss is maintained at a 15-week time point following sleeve gastrectomy in rodents [30,31]. Although we still consider our results as preliminary, they warrant a larger comprehensive study, one which would include more rats in order to evaluate more precisely the role of antioxidant genes in obesity pathogenesis.

Conclusion

In conclusion, the authors showed decrease in expressions of Catalase, SOD and GST genes in substantia nigra tissue of obese rats, which may contribute to disease pathogenesis for the first time in the English literature. The borax decreases molecular obesity and consequently increases the expressions of Catalase, SOD and GST genes. We revealed that borax decreases molecular obesity and consequently increases expressions of Catalase, SOD and GST genes.

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