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Selenium and zinc protect brain mitochondrial antioxidants and electron transport chain enzymes following postnatal protein malnutrition

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ABSTRACT

Aims: Selenium (Se) and zinc (Zn) are trace elements required for optimal brain functions. Thus, the role of Se and Zn against protein malnutrition induced oxidative stress on mitochondrial antioxidants and electron transport chain (ETC) enzymes from rats' brain were investigated.
Main methods: Normal protein (NP) and low protein (LP) rats were fed with diets containing 16% and 5% casein respectively for a period of 10 weeks. Then the rats were supplemented with Se and Zn at a concentration of 0.15 mg L^{-1} and 227 mg L^{-1} in drinking water for 3 weeks after which the rats were sacrificed.
Key findings: The results obtained from the study showed significant ($p < 0.05$) increase in lipid peroxidation (LPO), ROS production, oxidized glutathione (GSSG) levels and mitochondrial swelling and significant ($p < 0.05$) reductions in catalase (CAT) and Mn-superoxide dismutase (Mn-SOD) activities, glutathione (GSH) levels, GSH/GSSG ratio and MTT reduction as a result of LP ingestion. The activities of mitochondrial ETC enzymes were also significantly inhibited in both the cortex and cerebellum of LP-fed rats. Supplementation with either Se or Zn restored the alterations in all the parameters.
Significance: The study showed that Se and Zn might be beneficial in protecting mitochondrial antioxidants and ETC enzymes against protein malnutrition induced oxidative stress.

1. Introduction

Low protein diet (LPD) is a major problem in developing countries that manifest itself in various clinical forms among children as well as pregnant and lactating women [4,44] with highest prevalence in sub-Saharan Africa and South Asia [57]. Protein is an important nutrient required for biological functions of intracellular antioxidants, neurotransmitters, synthesis of structural proteins, and enzymes. Its restriction may lead to increase in oxidative damage by diminishing antioxidant defenses of the tissue [9].

Mitochondria play a major role in several cellular functions including amino acid biosynthesis, fatty acid oxidation, apoptosis, calcium homeostasis and in aerobic ATP production [11,58]. A study has shown that low protein diet leads to altered rephosphorylation of ADP in the skeletal muscle [43] as well as impairment in the activity of mitochondrial electron transport chain (ETC) enzymes (complexes I–III) in the muscle of malnourished rats [10]. The consequences of low protein diet include altered cellular metabolism, impaired function, weakness and high risk of infection due to altered immunity [11].

Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue or organ caused by an imbalance between the production of reactive oxygen species (ROS) and biological systems' ability to scavenge the reactive intermediates or repair the resulting damage [60]. The most important source of free radicals is the respiratory chain where oxygen is converted into superoxide radicals ($O_2^{\bullet -}$). Superoxide radical is not so reactive but may react with hydrogen peroxide in the presence of trace metal ions to form more powerful hydroxyl radicals ($^{\bullet}OH$), which can cause damage to cells [72]. Reports have shown that mitochondrion is one of the sources of ROS production in neurons resulting in oxidative damage and have been implicated in neuronal apoptosis and cognitive dysfunction [31].

Selenium is an element with symbol Se and atomic number 34. It is necessary for cellular functions in many organisms, including animals. It is a component of the unusual amino acids selenocysteine and selenomethionine and also of the antioxidant enzymes glutathione peroxidase and thioredoxin reductase which indirectly reduce certain oxidized molecules [71]. Se deficiency is rare in healthy, well-nourished individuals. It can occur in patients with severely compromised intestinal functions, malnourished individuals, those undergoing total parenteral nutrition, and in those with advanced age (over 90) [55]. Its deficiency, as defined by low (<60% of normal) selenoenzyme activity in brain and endocrine tissues, occurs only when a low Se status is linked with an additional stress, such as high exposure to mercury [54] or as a result of increased oxidant stress [40]. Although Se is an

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essential trace element, it is toxic in large amounts and selenosis can occur if it exceeds the Tolerable Upper Intake Level (UL) of 400 μg per day [30]. Se is known for optimal regulation of brain functions through its incorporation into antioxidant selenoproteins [15,28]. The total amount of Se is comparatively low in brain and low Se status is associated with faster decline in cognitive functions and poor performance in coordination assessment and motor speed [64]. Evidences are abound that Se supplementation prevented cognitive declines, oxidative damages and restored functional deficits in many neurodegenerative diseases [1,73].

Moreover, zinc (Zn) with atomic number 30, is an essential trace element for both humans and animals [76]. In the brain, Zn is stored in specific synaptic vesicles by glutamatergic neurons [8] and can modulate brain excitability [23]. It plays a key role in synaptic plasticity and learning [45]. Zn plays important role in growth and development, synaptic transmission and neurological functions [16]. It has been reported that Zn is important in maintaining the blood brain barrier integrity and cognitive functions [61]. However, Zn has been called "the brain's dark horse" [8] because it also can be a neurotoxin, especially when it exceeds its daily UL, suggesting Zn homeostasis plays a critical role in normal functioning of the central nervous system [8]. Zn deficiency is usually due to insufficient dietary intake, but can be associated with malabsorption, acrodermatitis enteropathica, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses [51]. Animals with a diet deficient in Zn require twice as much food in order to attain the same weight as animals given sufficient Zn. Moreover, it is now recognized that milder Zn deficiency contributes to a number of health problems, especially common in children who live in developing countries [51]. Generally, several reports have shown that micronutrients deficiencies including Se and Zn occur after exposure to low protein diet [20,21,35,62].

Several studies have established that nutritional imbalance affects the mitochondrial electron transport chain enzymes [10,11,47]. However, there is paucity of information on the influence of Se and Zn on mitochondrial oxidative stress and ETC enzyme complexes from rat brain under protein-malnourished condition. Hence, the present study is aimed at investigating mitochondrial oxidative stress and the activities of the ETC enzymes following protein inadequacy and to evaluate the beneficial effect of Se and Zn on them.

2. Materials and methods

2.1. Chemicals

All the chemicals used in the present study were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Merck (Mumbai, India), Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) and Himedia Laboratories Pvt. Ltd (Mumbai, India).

2.2. Animals and diets

The male weanling Wistar rats (3–4 weeks old; 49.0 ± 2.0 g body weight) used for the study were obtained from the Central Animal House of the Panjab University, Chandigarh, India. The rats were acclimatized for five days and were housed in polypropylene cages, fed with commercial rat chow and water during this period before they were grouped randomly and fed with prepared diets. The normal protein (NP) diet contained sixteen per cent (16%) casein while the low protein diet (LP) contained five per cent (5%) casein (Table 1). The rats were maintained at 12 h light dark-cycles at room temperature. All procedures on animals care used in the present study complied with the Panjab University Animal Ethics Committee and were in accordance with the principles of *NIH Guidelines for Humane Use and Care of Laboratory Animals*.

Table 1
Composition of normal and low protein diets.

	Normal protein diet (16% casein) (g)	Low protein diet (5% casein) (g)
Casein	16.0	5.0
Starch	74.9	85.9
Fat (soya bean oil)	4.0	4.0
Vitamin mixture	1.1	1.1
Mineral mixture	4.0	4.0

2.3. Experimental protocols

Rats were grouped randomly to six (6) groups. The control groups (NP groups) and the low protein (LP) diet groups had six (6) rats and eight (8) rats respectively per group. All the rats (Groups I–VI) were fed with their respective diet for a period of ten (10) weeks before they were treated with either sodium selenite (Groups II and V) or zinc sulphate (Groups III and VI) for additional three (3) weeks as presented below:

Groups of rats	Treatments of rats
I: Normal protein (NP)	Fed with diet containing 16% casein only.
II: NP + Se	Fed with diet containing 16% casein and supplemented with Se at the end of 10th week.
III: NP + Zn	Fed with diet containing 16% casein and supplemented with Zn at the end of 10th week.
IV: Low protein (LP)	Fed with diet containing 5% casein only.
V: LP + Se	Fed with diet containing 5% casein and supplemented with Se at the end of 10th week
VI: LP + Zn	Fed with diet containing 5% casein and supplemented with Zn at the end of 10th week.

Concentrations of Se (0.15 mg L^{-1}) and Zn (227 mg L^{-1}) used in this study were as reported by Wedekind et al. [75] and Bhasin et al. [7] respectively. At the end of 3 weeks supplementation of Se and Zn, rats were sacrificed and brain regions (cortex and cerebellum) were dissected and stored at −80 °C until further analyses.

2.4. Sample preparation

Crude mitochondria were isolated from the cortex and cerebellum as described by Puka-Sundvall et al. [52]. The cortex and cerebellum were homogenized in ice-cold buffer A (10 mM Tris–HCl, pH 7.4, 0.44 M sucrose, 10 mM EDTA and 0.1% BSA) and centrifuged at 2100*g* for 15 min at 4 °C. The pellets were discarded and the supernatant was further centrifuged at 14,000*g* for 15 min at 4 °C. The crude mitochondrial pellets were separated and washed with buffer A and again spun at 7000*g* for 15 min at 4 °C. The final mitochondrial pellets were suspended in buffer B (0.44 M sucrose in 10 mM Tris–HCl, pH 7.4).

2.5. Biochemical estimations

2.5.1. Lipid peroxidation (LPO)

LPO levels were measured in the mitochondrial suspension by the method of Ohkawa et al. [46]. Equal volume of sample and Tris buffer (0.25 mL) was incubated at 37 °C for 2 h. Then, 0.5 mL of 10% ice cold TCA was added, mixed and centrifuged for 10 min at 2000*g*. 0.5 mL of supernatant was added to 0.5 mL of 0.67% TBA and were kept in water bath for 10 min for pink colour to develop. The level of malondialdehyde (MDA) formation was measured at 532 nm and the results were