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Copper deficiency inhibits Ca^{2+}-induced swelling in rat cardiac mitochondria

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Abstract

Cu deficiency disrupts the architecture of mitochondria, impairs respiration, and inhibits the activity of cytochrome *c* oxidase — the terminal, Cu-dependent respiratory complex (Complex IV) of the electron transport chain. This suggests that perturbations in the respiratory chain may contribute to the changes in mitochondrial structure caused by Cu deficiency. This study investigates the effect of Cu deficiency on Ca^{2+}-induced mitochondrial swelling as it relates to changes in respiratory complex activities in cardiac mitochondria of rats. Male weanling rats were fed diets containing either no added Cu (Cu0), 1.5 mg Cu/kg (Cu1.5), 3 mg Cu/kg (Cu3) or 6 mg Cu/kg (Cu6). The rate of Ca^{2+}-induced mitochondrial swelling in the presence of succinate and oligomycin was reduced, and the time to reach maximal swelling was increased only in the rats consuming Cu0 diet. Cytochrome *c* oxidase activity was reduced 60% and 30% in rats fed Cu0 and Cu1.5, respectively, while NADH:cytochrome *c* reductase (Complex I+ComplexIII) activity was reduced 30% in rats consuming both Cu0 and Cu1.5. Mitochondrial swelling is representative of mitochondrial permeability transition pore (MPTP) formation and the results suggest that Ca^{2+}-induced MPTP formation occurs in cardiac mitochondria of Cu-deficient rats only when cytochrome *c* oxidase activity falls below 30% of normal. Decreased respiratory complex activities caused by severe Cu deficiency may inhibit MPTP formation by increasing matrix ADP concentration or promoting oxidative modifications that reduce the sensitivity of the calcium trigger for MPTP formation.
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Keywords: Copper deficiency; Heart; Mitochondria; Permeability transition pore; Cytochrome *c* oxidase; Rats

1. Introduction

Histological studies have shown that Cu deficiency disrupts the architecture of cardiac mitochondria. The structural alterations caused by Cu deficiency include mitochondrial enlargement, fragmentation and disappearance of the cristae, pronounced vacuolization, and decreased matrical density [1–5]. In addition to structural changes, it has been reported that Cu deficiency also reduces cardiac mitochondrial respiration [6–8]. These findings suggest that impaired respiration contributes to the perturbations in mitochondrial architecture caused by Cu deficiency.

Mitochondrial respiration depends on the flow of electrons through four oligomeric Respiratory Complexes that comprise the electron transport chain. The energy released by electron flow through the respiratory complexes is conserved in an electrochemical potential consisting of a proton gradient and membrane potential ($\Delta\Psi_m$) produced by the coupled translocation of protons through the inner mitochondrial membrane at Complexes I, II and IV. Energy stored in the electrochemical potential is coupled to ATP synthesis by translocation of protons into the mitochondrial matrix through complex V (ATP synthase) [9]. Respiratory Complex IV, also known as cytochrome *c* oxidase, is a cuproenzyme whose activity is reduced in a variety of

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tissues, including the heart, by Cu deficiency [10–12]. Although the role of Cu as an essential cofactor most likely contributes to the inhibitory effect of Cu deficiency on cytochrome *c* oxidase activity, Medeiros and coworkers [13–15] reported that the contents of two nuclear encoded subunits of cytochrome *c* oxidase are reduced in cardiac mitochondria of Cu-deficient rats indicating that structural changes in the holoenzyme may also contribute to the loss of activity. Mederios et al. [13,16] have also reported that Cu deficiency alters ATP synthase by decreasing the content of the δ subunit. These findings indicate that Cu deficiency may exert effects at two loci in the electron transport chain and oxidative phosphorylation system.

It has been reported that inhibition of respiratory Complex I by rotenone [17,18] and cytochrome *c* oxidase by azide [19] leads to reductions in $\Delta\Psi_m$. Thus, a reduction in $\Delta\Psi_m$ may occur as a consequence of the inhibitory effect of Cu deficiency on cytochrome *c* oxidase. Although a definite association between reduced cytochrome *c* oxidase activity and $\Delta\Psi_m$ has not been established, reports showing that $\Delta\Psi_m$ is compromised in cardiac mitochondria of Cu-deficient rats [8] and Cu-deficient HL-60 cells [20] suggest that such an association is possible. A potential mechanism linking reduction in cytochrome *c* oxidase activity caused by Cu deficiency to reduced $\Delta\Psi_m$ may involve compromised permeability of the inner membrane resulting from alterations in the mitochondrial permeability transition pore (MPTP). The MPTP can exist in two conformations, a low conductance state that allows the diffusion of small ions across the inner membrane and a high conductance state that allows the unselective diffusion of large molecules [21]. Oxidative stress is a factor that can cause the transition of the MPTP from a low to a high conductance state [22,23]. Inhibition of cytochrome *c* oxidase can promote oxidative stress by increasing the mitochondrial production of reactive oxygen species (ROS). The redox state of the respiratory complexes is a major determinant of mitochondrial ROS production and is highest when the complexes are highly reduced [24]. Inhibition of cytochrome *c* oxidase may increase the reducing potential of the upstream respiratory complexes causing increased ROS production through single electron transfer to molecular oxygen. This has been demonstrated using flight muscles from houseflies where partial inhibition of cytochrome *c* oxidase increases mitochondrial hydrogen peroxide [25]. Cu deficiency has also been shown to increase hydrogen peroxide generation in hepatic mitochondria [26]. Thus, by inhibiting cytochrome *c* oxidase activity, Cu deficiency may produce sufficient oxidative stress to cause a transition of the MPTP from a low to a high conductance conformation. Because increased inner membrane permeability is associated with the high conductance state of the MPTP, the transition to a higher conductance conformation may also help explain the changes in cardiac mitochondrial structure caused by Cu deficiency. Ca^{2+} is an important determinant of the conductance state of the MPTP. Saturation of internal Ca^{2+} binding sites of the MPTP produces a conformation change that leads to the high conductance state [21]. It is possible that changes in the MPTP caused by Cu deficiency may also alter its sensitivity to the Ca^{2+}-induced switch from the low to the high conductance state. Accordingly, the purpose of this study was to investigate the effect of Cu deficiency on the Ca^{2+}-induced mitochondrial transition in rat heart mitochondria.

2. Materials and methods

2.1. Animals and diets

Male weanling Sprague–Dawley rats (Charles River, Wilmington, MA, USA) were housed in a room maintained at 22±2°C and 50±10% humidity with a 12-h light/dark cycle. The rats were divided into four treatment groups (11 rats/group) and fed AIN-93 G diet [27] formulated with $CuSO_4 \cdot 5H_2O$ to contain <1 mg Cu/kg (Cu0), 1.5 mg Cu/kg (Cu1.5), 3.0 mg Cu/kg, (Cu3) or 6.0 mg Cu/kg (Cu6). The analyzed Cu concentrations in the diets were: Cu0=0.457 mg/kg, Cu1.5=1.61 mg/kg, Cu3=3.22 mg/kg, and Cu6=6.39 mg/kg. Rats had free access to diets and demineralized water for 5 weeks. The study was approved by the Animal Care and Use Committee of the Grand Forks Human Nutrition Research Center, and the rats were maintained in accordance with the NIH guidelines for the care and use of laboratory animals.

2.2. Analyses

After 5 weeks of dietary treatment, the rats were anesthetized with ketamine/xylazine, and livers, hearts and blood were removed for analysis. Liver Cu and iron concentrations were measured by atomic absorption spectrophotometry [28]. Plasma ceruloplasmin was assayed in serum by its amine oxidase activity [29]. An electronic cell counter (Cell-Dyne 3500, Abbott Diagnostics, Abbott Park, IL, USA) was used to measure hemoglobin concentrations and hematocrits.

Individual hearts were homogenized in isolation buffer (1 g heart/ml buffer) containing 0.225 M mannitol, 0.075 M sucrose, 0.02 M HEPES and 0.01 mM EGTA, pH 7.4, to which bovine serum albumin (0.1% final concentration) and trypsin (2 mg/ml final concentration) were added. The homogenate was centrifuged at 9900*g* for 10 min, and the supernatant was discarded in order to limit exposure to trypsin. The pellet was resuspended in isolation buffer containing 0.1% bovine serum albumin and centrifuged at 500*g*. Mitochondria were obtained from the resulting supernatant by centrifuging at 9900*g* for 10 min. The pellet was collected and washed once with isolation buffer containing 0.1% bovine serum albumin, followed by a final wash in isolation buffer. The final mitochondrial pellet was suspended in 1 ml of isolation buffer. All isolation steps were performed at 4°C.