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Supplementation of curcumin and vitamin E enhances oxidative stress, but restores hepatic histoarchitecture in hypothyroid rats

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ABSTRACT

Aims: In the present study, the effects of vitamin E and curcumin on hepatic dysfunction, mitochondrial oxygen consumption as well as hyperlipidemia in hypothyroid rats are reported.
Main methods: Adult male rats were rendered hypothyroid by administration of 0.05% 6-n-propyl-2-thiouracil (PTU) in their drinking water, while vitamin E (200 mg/kg body weight) and curcumin (30 mg/kg body weight) were supplemented orally for 30 days.
Key findings: Hypothyroidism-induced elevation in serum aspartate aminotransferase activity was found to decline in vitamin E and curcumin treated rats. Nevertheless, distorted histoarchitecture revealed in hypothyroid rat liver was alleviated to normal by vitamin E and curcumin treatment. Regulation of hypothyroidism induced decrease in complexes I and II mediated mitochondrial respiration by vitamin E and curcumin was found to be different. Administration of curcumin to hypothyroid rats alleviates the decreased state 4 respiration and increased respiratory control ratio (RCR) level in complex I mediated mitochondrial oxygen consumption, whereas complex II mediated respiration was not influenced by exogenous antioxidants. Although, increase in serum concentration of total cholesterol was not modified by exogenous antioxidants, increased level of non-high-density lipoprotein cholesterol (non-HDL-C) in serum of hypothyroid rats was further enhanced by vitamin E and curcumin. Moreover, a significant elevation in mitochondrial lipid peroxidation and protein carbonylation was noticed in hypothyroid groups treated with vitamin E and curcumin.
Significance: The present study suggests that supplementation of curcumin and vitamin E enhances oxidative stress parameters and hyperlipidemia; nevertheless, it protects hypothyroid-induced altered rectal temperature, serum transaminase activity and hepatic histoarchitecture.

Introduction

Mitochondria are the important sites for production of reactive oxygen species (ROS) due to incomplete reduction of molecular oxygen to water as a consequence of electron leakage in electron transport chain (Boveris and Chance, 1973; Nohl et al., 2004). Cellular processes that increase oxygen consumption and oxidative phosphorylation result in increased production of ROS (Videla, 2000; Yen, 2001; Venditti and Di Meo, 2006). Earlier, we have reported that hyperthyroidism is associated with oxidative stress, as thyroid hormone profoundly promotes mitochondrial oxygen consumption (Subudhi et al., 2008). Although hypothyroidism is generally a hypometabolic state, generation of free radicals is not proportionately lower in this dysfunction in comparison to normal individuals, as revealed by flow cytometry analysis (Sarkar et al., 2006). Hypothyroidism is associated with hyperlipidemia which is a major cause of cardiovascular disease (Pekkanen et al., 1990). Furthermore, higher levels of plasma lipids are reported to be associated with increased production of ROS (Ohara et al., 1993).

Various reports suggest that administration of T_3 or T_4 (levothyroxine) to hypothyroid rats restores the change in lipid profile (Ruggiero et al., 1987; Kiya et al., 2006). However, Mogulkoc et al. (2005a,b) reported that thyroxine administration to hypothyroid rats resulted in oxidative stress in several tissues. Therefore, administration of exogenous antioxidant is a preferable therapeutic agent against hypothyroid induced oxidative stress. In an earlier investigation, it has been demonstrated that intraperitoneal administration of vitamin E acts as a protective agent against oxidative stress in 6-n-propyl-2-thiouracil (PTU) induced hypothyroid rats (Sarandol et al., 2005). However, information on the oral supplementation of exogenous antioxidants singly or in combination against hypothyroidism induced oxidative stress is wanting. In general, oral supplementation is a better option for administration of antioxidants as therapeutic molecules.

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Recently, we have reported that oral administration of vitamin E and curcumin alleviates the enhanced mitochondrial oxygen consumption and oxidative stress in L-thyroxine induced hyperthyroid rat liver mitochondria (Subudhi et al., 2008). Vitamin E and curcumin are low molecular mass antioxidants. Vitamin E is a potent lipid soluble antioxidant in biological system, while curcumin is a naturally occurring phenolic compound isolated as a yellow pigment from turmeric (*Curcuma longa*). The compound has been reported to possess a variety of biological and pharmacological activities including, antioxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic and anti-angiogenic (Duvoix et al., 2005). Moreover, these compounds are remarkably free of toxicity, as shown by the fact that the dry curcuma rhizome (turmeric) has been widely used as a food condiment for human consumption (Ammon and Wahl, 1991).

With this background, the present investigation has been designed to determine the effect of oral administration of vitamin E and curcumin on serum aspartate aminotransferase (AspAT), alanine aminotransferase (AlaAT) activities, hepatic histoarchitecture, complexes I and II mediated mitochondrial respiration, oxidative stress parameters such as lipid peroxidation and protein carbonylation. In addition, serum lipids such as; total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and non-high-density lipoprotein cholesterol (non-HDL-C) have also been examined to evaluate the role of vitamin E and curcumin on hyperlipidemia in hypothyroid rats.

Materials and methods

Animals and experimental design

Experiments were carried out on 130±10 days old adult male Wistar strain rats (*Rattus norvegicus*) supplied by the National Institute of Nutrition (Hyderabad, India). Animals were maintained at 25 °C± 2 °C under standard conditions in the animal room. Animal care, maintenance and experiments were performed under the supervision of the Institutional Animal Ethics Committee (IAEC). Rats were divided into five groups: Group I; euthyroid, Group II; hypothyroid, Group III; hypothyroid with vitamin E, Group IV; hypothyroid with curcumin and Group V; hypothyroid with vitamin E and curcumin. Hypothyroidism was achieved by administering 0.05% PTU in their drinking water for 30 days (Ladenson et al., 1986). Hypothyroid rats were supplemented with 200 mg/kg body weight of vitamin E and 30 mg/kg body weight of curcumin orally for 30 days in respective groups (Subudhi et al., 2008). All the chemicals used were of analytical grade.

Tissue preparation

Animals were sacrificed after 30 days of treatment by decapitation under ether anaesthesia. Blood samples were collected and serum levels of T_3, T_4 and TSH were analyzed using commercially available ELISA kits (Monobind Inc, USA) in a microplate reader (BIO-RAD model 550). Immediately after sacrifice, liver samples were washed in cold normal saline solution, pet dried, frozen in liquid nitrogen and stored at −80 °C for oxidative damage analysis. For liver mitochondrial oxygen consumption and histological analysis fresh tissue samples were processed as described below.

Activity of serum enzymes and lipid profile

The activities of AspAT and AlaAT were determined in serum sample of different groups using commercially available kits (Merck Specialities Pvt. Ltd., India). The levels of TC and HDL-C were measured according to manufacturer's instruction (Span Diagnostics Ltd., India). The non-HDL-C levels were calculated as TC–HDL-C.

Histological analyses

For histological studies, liver tissues fixed in freshly prepared sublimate formol, dehydrated in graded ethanol series, cleared in xylene, embedded in paraffin wax were sectioned (5 μm) and stained with Hematoxylin and Eosin. Sections were observed under light microscope for qualitative and quantitative characterization. Hepatocyte nuclei with evident nucleoli were recorded by coupling a calibrated ocular micrometer grid to a 40× objective while observing histological sections of liver tissue. The resulting crude counts that represented both whole and sectioned nuclei were corrected to true counts using Abercrombie's correction factor (Abercrombie, 1946).

Mitochondria isolation

For mitochondrial oxygen consumption, mitochondria were isolated using the method described elsewhere (Subudhi et al., 2008). Immediately after sacrifice, liver tissue was excised, properly cleaned, freed of connective tissue and kept in ice. A 10% (w/v) homogenate was prepared in homogenization buffer (210 mM mannitol, 10 mM sucrose, 5 mM HEPES and 1 mM EGTA, pH 7.4) with three strokes at 250 rpm in a Potter Elvehjem homogenizer at 4 °C. The homogenate

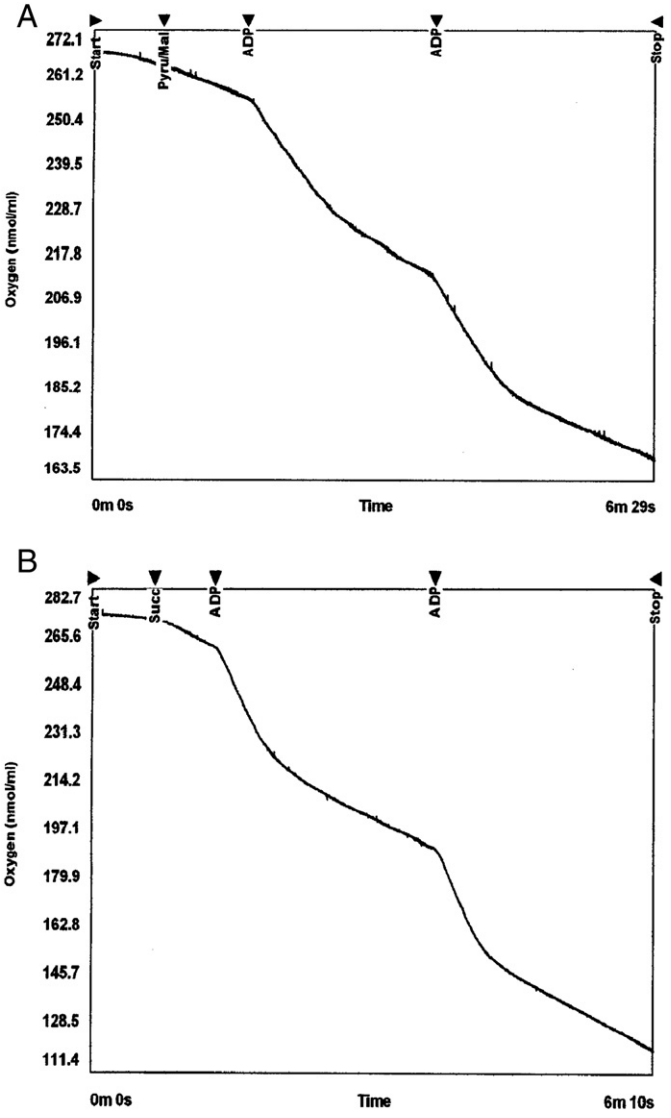

Fig. 1. A representative oxygraph showing state 3 and state 4 respiration in hepatic mitochondria with 2.5 mM pyruvate/malate each and 250 nmol ADP (A) or 5 mM succinate and 250 nmol ADP (B).