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## Insulin-like growth factor I (IGF-1) supplementation prevents diabetes-induced alterations in coenzymes Q<sub>9</sub> and Q<sub>10</sub>

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**Abstract** Diabetes, which causes enhanced oxidative stress, is a multifactorial disease that leads to deleterious effects in many organ systems within the body. Ubiquinones (coenzyme Q<sub>9</sub> and Q<sub>10</sub>) are amphipathic molecular components of the electron transport chain that function also as endogenous antioxidants and attenuate the diabetes-induced decreases in antioxidant defense mechanisms. Insulin-like growth factor 1 (IGF-1) is considered to be an “essential surviving factor”, the level and function of which are compromised in diabetes. This study investigated the impact of IGF-1 supplementation on ubiquinone levels in a rat model of type I diabetes. Adult male Sprague-Dawley rats were divided into four groups: control, control plus IGF-1, diabetic and diabetic plus IGF-1. Diabetic animals received a single intravenous injection of streptozotocin (STZ, 55 mg/kg). IGF-1 supplementation groups received a daily intraperitoneal dose of 3 mg IGF-1 per kilogram body weight for 7 weeks. Coenzyme Q<sub>9</sub> and Q<sub>10</sub> levels were assessed by ultraviolet detection on high pressure liquid chromatography. STZ

caused a significant reduction in body weight and an elevation in blood glucose level, which were not prevented by IGF-1 supplementation. In addition Q<sub>9</sub> and Q<sub>10</sub> levels in diabetic liver were significantly elevated. IGF-1 supplementation prevented liver alterations in Q<sub>10</sub> but not Q<sub>9</sub> levels. Q<sub>9</sub> and Q<sub>10</sub> levels in diabetic kidney were significantly depressed, and these deleterious effects were abolished by IGF-1 treatment. These data suggest that IGF-1 antagonizes the diabetes-induced alterations in endogenous antioxidants including coenzyme Q<sub>10</sub>, and hence may have a therapeutic role in diabetes.

**Key words** Insulin-like growth factor I · Insulin dependent diabetes mellitus · Coenzyme Q · Antioxidants

### Introduction

Accumulating evidence suggests that oxidative stress plays an important role in the pathogenesis of diabetes mellitus [1]. Although the mechanism behind the enhanced oxidative stress associated with diabetes is not well understood, impaired balance between pro-oxidants such as reactive oxygen species (ROS) and antioxidants such as superoxide dismutase and catalase is speculated to play a role in the exacerbated organ damage in diabetes [2]. Under normal conditions, ROS generated by mitochondrial respiratory metabolism may be efficiently neutralized by various antioxidant defense mechanisms. However, diabetes mellitus interrupts this balance, resulting in cellular dysfunction [3].

There is growing interest regarding the role of coenzyme Q (CoQ) as an endogenous antioxidant in the protection against oxidative stress (reviewed in [4]). Coenzyme Q<sub>10</sub> (ubiquinone) functions as an electron carrier and protein translocator; its metabolite ubiquinol is an antioxidant and is involved in regenerating vitamin E [4–6]. CoQ homologues

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are classified according to the number of isoprenoid units ( $Q_n$ ) attached at the 6-position on the benzoquinone ring of the coenzyme Q moiety. Coenzyme  $Q_9$  and coenzyme  $Q_{10}$  are the predominant coenzymes in rodents [7]. The content of coenzyme Q differs in organelles or subcellular fractions of cells, however a significant amount of coenzyme has been found in the mitochondria, functioning in concurrence with enzymes in cellular respiration to generate ATP. Ubiquinones, which are ubiquitous in nature, are found in certain mammalian species with variations in interspecies concentrations [8]. More specifically, there are concentration variations of  $Q_9$  and  $Q_{10}$  in brain, heart, liver, kidney, plasma serum and skin. In addition, intracellular organelles such as lysosomes, endoplasmic reticulum, nucleus, Golgi apparatus, cytosol and mitochondria vary in coenzyme concentration [9]. The levels of coenzymes  $Q_9$  and  $Q_{10}$  are decreased in the mitochondria of heart and liver in diabetes [10], thus increasing susceptibility towards injury caused by oxidative stress. Coenzyme  $Q_{10}$  has been shown to protect against ischemic and diabetic damage [11, 12], supporting its anti-oxidant property.

Insulin-like growth factor 1 (IGF-1), an “essential surviving factor” for cell proliferation and differentiation, is produced by many systems within the body and acts as both an autocrine and a paracrine hormone [13–17]. It stimulates growth and metabolism by binding to the IGF-1 receptor, thereby activating a protein tyrosine phosphorylation signal transduction cascade. The liver is the major source of IGF-1, and both hepatic and peripheral productions are mainly regulated by growth hormone [13–17]. Other hormonal, genetic, and nutritional factors may also be important determinants of intra- and inter-individual variability in IGF-1 [15–17]. Over 97% of IGF-1 is bound to members of a family of six proteins, the IGF-1 binding proteins (IGFBPs), which are believed to modulate the actions of IGF-1 in a cell-specific manner [16, 17]. IGF-1 possesses antioxidant properties in both the heart and other organ systems [14, 18]. Levels of IGF-1 and certain IGFBPs (e.g. IGFBP-3) are depressed in poorly controlled diabetic patients as well as in animals, potentially due to insufficient hepatic growth hormone or portal insulinization [19, 20]. In contrast, serum levels of IGFBP-1 and IGFBP-2 in diabetic patients are elevated due to lack of physiological suppression from insulin [21]. The elevation in IGFBPs may inhibit IGF-1's action and thus add further insult to diabetes. Since IGF-1 facilitates glucose transport and improve glycemic control in both type I and type II diabetes, it has been proposed as a treatment for diabetes, especially under insulin resistance [22, 23]. Both short- and long-term studies have confirmed the efficacy of IGF-1 treatment [22]. In light of the considerable interest in the possible therapeutic role of IGF-1 in the development of diabetic complications, this study was designed to examine the effect of recombinant IGF-1 administration on coenzyme content in liver and kidney from control and streptozotocin-induced diabetic rats.

## Materials and methods

### Experimental animals

Principles of laboratory animal care (NIH publication no. 83–25, revised 1985) were followed, as well as laws specified by the Animal Welfare Act (revised 1989). Briefly, 6-week-old male Sprague-Dawley (SD) rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were divided into control, control plus IGF-1, diabetic and diabetic plus IGF-1 groups with 5–7 animals per group. Experimental animals were made diabetic with a single intravenous tail vein injection of streptozotocin (STZ, 55 mg/kg) as described [24] and maintained for 7 weeks of untreated diabetes. A group of age-matched euglycemic rats received saline treatment. The diabetic state was assessed by measurement of the glucose concentration in whole blood collected at the time of organ removal (Accu-ChekII, model 792, Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). IGF-1 (Genentech, South San Francisco, CA, 3 mg/kg-day) was administered intraperitoneally for 7 weeks; saline used in the control groups. This dosage of IGF-1 is significantly higher than that used clinically ( $<0.5$  mg/kg-day) [21].

### Measurement of coenzyme $Q_9$ and $Q_{10}$ by high pressure liquid chromatography

Animals were euthanized by decapitation in the morning to avoid the influence of any diurnal fluctuation of the endogenous amines, enzymes and other antioxidant molecules. Livers and kidneys were removed, weighed and snap-frozen in liquid nitrogen. High pressure liquid chromatography (HPLC)-grade methanol, ethanol, and hexane were purchased from Fisher Chemicals (Hampton, NH, USA). Coenzyme  $Q_9$  was purchased from Sigma Chemicals (St. Louis, MO, USA) and coenzyme  $Q_{10}$  was a gift from Tishcon Corporation (Westbury, NY, USA). In the present study, an HPLC system (ISCO, 2350) consisting of two pumps, a universal injector and an ultraviolet variable wavelength absorbance detector (ISCO, V<sup>4</sup>) were used [8]. Coenzymes  $Q_9$  and  $Q_{10}$  were separated on a C-18 Hypersil-ODS silica-based column. Hypersil-ODS phases are based on completely porous spherical silica particles with a pore size of 120 Å. The column length was 125 mm and the diameter was 3 mm. The flow rate was 0.3 ml/min and UV detection was performed at 275 nm [8, 25]. The mobile phase consisted of a mixture of hexane and methanol [8]. Tissues were homogenized in ethanol (10% w/v) and then extracted with hexane (5 volumes hexane per 2 volumes ethanol) by shaking for 10 min in the dark. The hexane layer was removed by centrifugation and evaporated using a Meyer N-EVAP analytical evaporator. This layer was then re-dissolved in the mobile phase (methanol 75% and hexane 25%).

### Statistical analyses

Data are presented as mean and SEM. Statistical significance ( $p < 0.05$ ) for each variable was estimated by analysis of variance (ANOVA) or *t* test, where appropriate (SYSTAT, Evanston, IL). Dunnett's test was used for post hoc analysis.

## Results

After a period of 7 weeks, the streptozotocin-induced diabetic animals exhibited significantly lower body weights and higher blood glucose levels compared to the age-matched non-diabetic controls, irrespective of IGF-1 supplementation (Table 1).

Compared to control animals, Q<sub>10</sub> levels were significantly elevated in the liver of diabetic animals, and IGF-1 supplementation attenuated this response (Fig. 1a). In contrast, Q<sub>10</sub> levels were significantly decreased in the kidneys

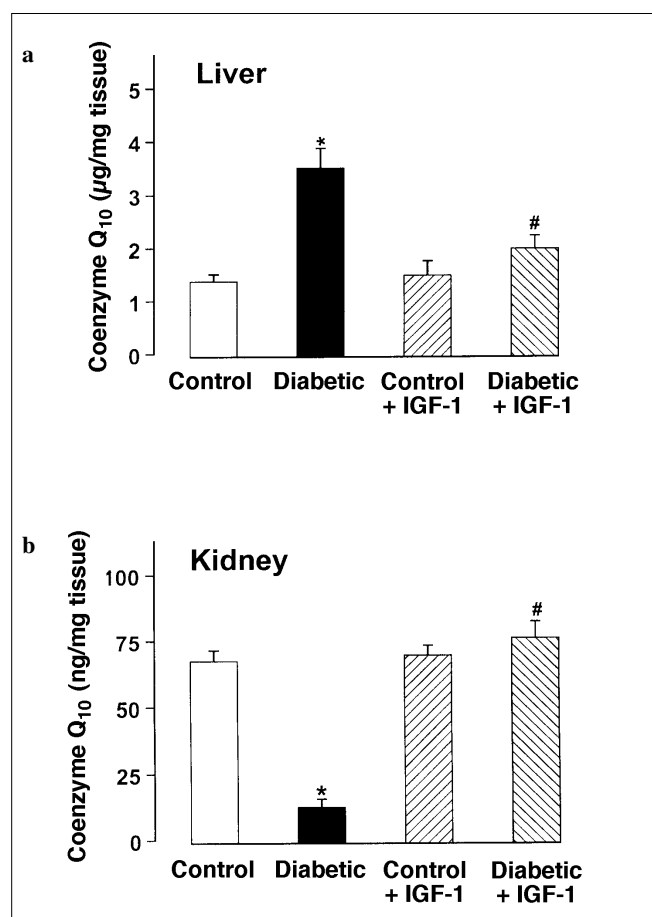
of diabetic animals, and this decrease was attenuated by IGF-1 supplementation (Fig. 1b). Supplementation with IGF-1 in control animals had no effect on Q<sub>10</sub> levels in either organ.

Compared to control animals, Q<sub>9</sub> levels were significantly elevated in the liver of diabetic animals, however IGF-1 supplementation was unable to correct this response (Fig. 2a). Q<sub>9</sub> levels were significantly decreased in the kidney of diabetic animals, and this decrease was abolished by IGF-1 supplementation (Fig. 2b). Supplementation with IGF-1 in control animals had no effect on Q<sub>9</sub> levels in either organ.

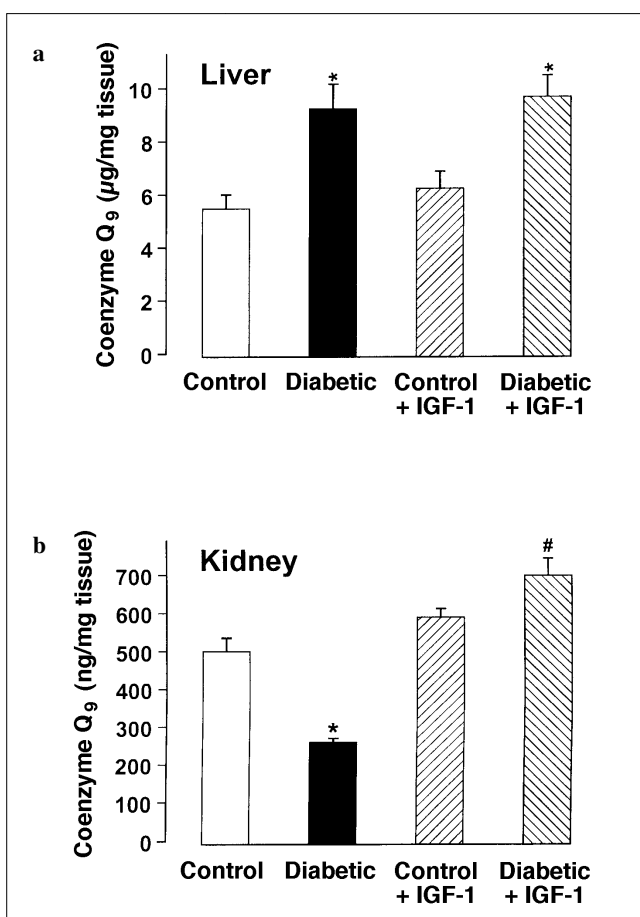
**Table 1** Body weight and plasma glucose in control and streptozotocin-induced diabetic rats, with or without supplementation with insulin-like growth factor-1 (IGF-1) at 3 mg/kg day for 7 weeks. Values are mean (SEM)

	Body weight, g	Blood glucose, mg/dl
Control (n=6)	409.3 (22.2)	91.2 (4.0)
Control + IGF-1 (n=5)	434.8 (9.9)	95.8 (13.9)
Diabetic (n=7)	221.3 (42.5)*	357.1 (27.6)*
Diabetic + IGF-1 (n=7)	237.0 (68.0)*	323.6 (50.9)*

\* $p < 0.05$  vs. control animals



**Fig. 1a, b** The effect of IGF-1 supplementation on the level of coenzyme Q<sub>10</sub> in control and streptozotocin-induced diabetic rats. **a** Liver Q<sub>10</sub> levels. **b** Kidney Q<sub>10</sub> levels. Values are expressed as mean and SEM. \* $p < 0.05$  vs. control; # $p < 0.05$  vs. diabetic



**Fig. 2a, b** The effect of IGF-1 supplementation on the level of coenzyme Q<sub>9</sub> in control and streptozotocin-induced diabetic rats. **a** Liver Q<sub>9</sub> levels. **b** Kidney Q<sub>9</sub> levels. Values are expressed as mean and SEM. \* $p < 0.05$  vs. control; # $p < 0.05$  vs. diabetic

## Discussion

The major finding of this study is that the levels of coenzyme Q<sub>9</sub> and Q<sub>10</sub> in liver and kidney are altered by diabetes and IGF-1 supplementation prevented these alterations to a great extent. Coenzyme Q<sub>10</sub> is an antioxidant and an indicator for oxidative stress [4, 26, 27]. However, the precise influence of various stressful and painful conditions on the status of coenzyme Q<sub>10</sub> needs to be delineated carefully [4]. In this study, we have shown that the level of coenzyme Q<sub>9</sub> is altered in diabetes mellitus, suggesting that ubiquinone may have a distinct antioxidant role in diabetes. The variation in the diabetes-induced responses between coenzyme Q<sub>9</sub> and Q<sub>10</sub> indicates a potential difference in their sensitivity to diabetes-induced oxidative stress.

The deleterious impact of streptozotocin on several biochemical and morphological parameters has been characterized in the kidney and liver of rat [2, 24]. The effect of streptozotocin is similar in liver and kidney with regard to decreased adenosine kinase activity [28], reduced expression of P450 2B-immunorelated protein [29], and alterations in the concentrations of nuclear T3 receptor and T3 bound to the receptor [30]. However, diabetic liver exhibited a significantly decreased activity of catalase, glutathione peroxidase, and superoxide dismutase along with lower levels of glutathione, while the diabetic kidney showed increased glutathione peroxidase activity [12].

Diabetes alters the levels of insulin-like growth factors and their serum-binding proteins, leading to alterations in organ function and morphology [19–21, 31]. Studies in diabetic rodents and humans have provided evidence that IGF-I may alleviate the diabetic state and insulin resistance [32]. Our present work is the first study examining the effect of IGF-1 supplementation on coenzyme Q<sub>9</sub> and Q<sub>10</sub> levels in the liver and kidneys of diabetic animals. Coenzyme Q<sub>10</sub> content changes significantly in various tissues following the onset of diabetes [10]. Thus, it increases the vulnerability of the tissue towards injury caused by oxidative stress. Coenzyme Q<sub>10</sub> administration protected against ischemic damage to the heart and diabetes-related complications [4, 11, 12]. The present study showed that Q<sub>9</sub> and Q<sub>10</sub> levels are significantly elevated in livers and decreased in kidneys of diabetic animals.

The antioxidant capacity of coenzyme Q<sub>9</sub> has been studied in hepatocytes by incubation with 2,2'-azobis (2-amidinopropane) dihydrochloride, an initiator of hydrophilic radicals. The levels of coenzyme Q<sub>9</sub> in rat hepatocytes decreased significantly after the addition of 2,2'-azobis (2-amidinopropane) dihydrochloride. The decrease in coenzyme Q<sub>9</sub> content may be due to its antioxidant ability to scavenge lipid peroxyl radicals produced by 2,2'-azobis (2-amidinopropane) dihydrochloride. Vitamin E, another well-established antioxidant, also nullified the effects of

peroxyl radicals [5]. It was concluded that coenzyme Q<sub>9</sub> together with vitamin E can act as potential antioxidants in hepatocytes [33].

Various studies have shown the presence of coenzymes Q<sub>9</sub> and Q<sub>10</sub> in addition to vitamins in various types of oils such as those from olive, corn and soya bean. These oils have the capacity to trap free radicals and exhibit their antioxidant properties. The total antioxidant capability of the oils was evaluated by measuring total radical-trapping antioxidant parameters in tert-butyl alcohol and using egg lecithin as the oxidizable substrate. Coenzymes Q<sub>9</sub> and Q<sub>10</sub> significantly contributed to the antioxidant effect of the oils [34]. Irradiation of T and B cells in spleens caused a decrease in immune response of T lymphocytes 48 days after onset of exposure. Chronic irradiation with a higher dose produced significant changes in the DNA of T lymphocytes. Coenzyme Q<sub>9</sub> administration partially eliminated damage to DNA structure and also restored the decrease in immune response in irradiated rats [35, 36]. This protective effect may be attributed to the antioxidant capacity of coenzyme Q<sub>9</sub>.

IGF-1 protects against oxidative stress and apoptosis induced by streptozotocin [37]. However, the mechanism of action behind the IGF-1-induced beneficial effect is not fully understood. Oxidative stress occurs due to a disturbance in balance between pro-oxidant and antioxidant molecules or enzymes, resulting in a pro-oxidant environment. This has been clearly established to be a pertinent factor in diabetes as well as in other pathological conditions, such as myocardial infarction and ovarian cancer. Oxidative stress is mainly mediated by free radical generation, resulting in protein and lipid damage, calcium ion accumulation, and ultimately cell death [38]. There are numerous studies showing that the pathogenesis of streptozotocin-induced diabetes mellitus involves oxidative stress [39]. Treatment with the lipophilic antioxidant coenzyme Q<sub>10</sub> protected against damage caused on liver glutathione peroxidase activity and kidney superoxide dismutase activity in diabetics. However, coenzyme Q<sub>10</sub> enhanced the increase in catalase activity in heart that was caused by diabetes [12].

The variation of coenzyme content in the diabetic model may be due to the difference in the species of animals [8], the dose of drug used to induce diabetes, the time at which the changes were observed, the method of extraction and the fraction of tissue used for analysis. In addition to diabetes, coenzyme Q levels are altered in aging, selenium deficiency and hyperthyroid rats [4]. Liver coenzyme Q<sub>9</sub> was significantly increased in hyperthyroid rats [40]. Interestingly, levels of Q<sub>9</sub> and Q<sub>10</sub> in the liver of selenium-deficient rats were severely depleted compared to control animals [41]. However serum, kidney and heart coenzyme Q<sub>9</sub> levels were not affected in selenium-deficient rats and in hyperthyroid rats [42]. The levels of coenzymes Q<sub>9</sub> and Q<sub>10</sub> were analyzed in skeletal muscles from tumor-bearing, exercising rats compared to sedentary tumor-bearers. Both tumor-bear-

ing groups had elevated contents of coenzymes Q<sub>9</sub> and Q<sub>10</sub> in the anterior tibial muscle. However, in the soleus muscle, only the tumor-bearing, exercising animals showed an increase in the levels of both coenzymes, whereas in cardiac muscle the tumor and exercise reduced the levels of coenzymes below that of sedentary controls [43]. Diabetes mellitus is also associated with impairment of testicular function, ultimately leading to reduced fertility. An earlier study showed the involvement of oxidative stress due to reduced mitochondrial antioxidant capacity. The enhanced susceptibility to oxidative stress in the diabetic rat led to an increase in testicular mitochondrial glutathione and coenzyme Q<sub>9</sub> contents [27]. The coenzyme Q concentrations were determined over the life span of the male laboratory rat in different tissues. Coenzyme Q decreased significantly at 25 months in the heart and kidney. The coenzyme Q concentration of liver increased over the life span, while it remained relatively constant in the brain and lung [44].

The varied patterns of coenzyme Q change in livers and kidneys may be the result of compensatory increases in enzyme activities or antioxidant molecules. These findings support the view that tissue antioxidant status may be a significant element in the etiology of diabetes and other complications. Our results suggest the presence of increased oxidative stress in diabetes as revealed by distinct alterations in coenzymes Q<sub>9</sub> and Q<sub>10</sub> concentrations. IGF-1 supplementation abolished this effect except for Q<sub>9</sub> levels in the liver, but had no effect on control animals. Therefore, IGF-I may be useful in preventing hyperglycemia-induced organ damage and oxidative stress in patients suffering from diabetic complications.

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