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Coenzyme Q_{10} improves endothelial dysfunction of the brachial artery in Type II diabetes mellitus

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Abstract

Aim/hypothesis. We assessed whether dietary supplementation with coenzyme Q_{10} improves endothelial function of the brachial artery in patients with Type II (non-insulin-dependent) diabetes mellitus and dyslipidaemia.
Methods. A total of 40 patients with Type II diabetes and dyslipidaemia were randomized to receive 200 mg of coenzyme Q_{10} or placebo orally for 12 weeks. Endothelium-dependent and independent function of the brachial artery was measured as flow-mediated dilatation and glyceryl-trinitrate-mediated dilatation, respectively. A computerized system was used to quantitate vessel diameter changes before and after intervention. Arterial function was compared with 18 non-diabetic subjects. Oxidative stress was assessed by measuring plasma F_2-isoprostane concentrations, and plasma antioxidant status by oxygen radical absorbance capacity.
Results. The diabetic patients had impaired flow-mediated dilation [3.8 % (SEM 0.5) vs 6.4 % (SEM 1.0), $p = 0.016$], but preserved glyceryl-trinitrate-mediated dilation, of the brachial artery compared with non-diabetic subjects. Flow-mediated dilation of the brachial artery increased by 1.6 % (SEM 0.3) with coenzyme Q_{10} and decreased by –0.4 % (SEM 0.5) with placebo ($p = 0.005$); there were no group differences in the changes in pre-stimulatory arterial diameter, post-ischaemic hyperaemia or glyceryl-trinitrate-mediated dilation response. Coenzyme Q_{10} treatment resulted in a threefold increase in plasma coenzyme Q_{10} ($p < 0.001$) but did not alter plasma F_2-isoprostanes, oxygen radical absorbance capacity, lipid concentrations, glycaemic control or blood pressure.
Conclusion/interpretation. Coenzyme Q_{10} supplementation improves endothelial function of conduit arteries of the peripheral circulation in dyslipidaemic patients with Type II diabetes. The mechanism could involve increased endothelial release and/or activity of nitric oxide due to improvement in vascular oxidative stress, an effect that might not be reflected by changes in plasma F_2-isoprostane concentrations. [Diabetologia (2002) 45: 420–426]

Keywords Coenzyme Q_{10}, endothelial function, nitric oxide, diabetes.

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Abbreviations: CoQ, Coenzyme Q_{10}; FMD, flow-mediated dilatation; NMD, glyceryl-trinitrate-mediated dilatation; ORAC, oxygen radical absorbance capacity

In Type II (non-insulin-dependent) diabetes mellitus the pathogenesis of vascular disease, its most common complication, remains unclear [1]. Endothelial dysfunction reflects the disordered physiology of several endothelium-derived vasoactive factors, in particular nitric oxide [2]. Endothelial dysfunction occurs commonly in diabetes and is an early feature of vasculopathy [3, 4]. Increased oxidative stress due to the effects of hyperglycaemia and its sequelae is a recognized feature of diabetes [5]. It might cause endothelial dysfunction through the inactivation and

decreased synthesis of nitric oxide by reactive oxygen species [6] such as superoxide.

While epidemiology suggests that conventional antioxidant vitamins can benefit vascular disease, the evidence from controlled clinical trials is less secure [7], especially in diabetes [8]. Vitamin E supplementation has not consistently been shown to improve endothelial-dependent vasodilator tone in diabetic patients [9, 11]. Moreover, with vitamin C, the only study reported in Type II diabetes was not placebo-controlled and involved acute intra-arterial administration of this antioxidant [12].

Coenzyme Q_{10} (CoQ) is a critical intermediate of the mitochondrial electron transport chain that regulates cytoplasmic redox potential and it can inhibit superoxide generation by endothelial cells [13, 14]. CoQ is a more powerful antioxidant than vitamin E, inhibiting its pro-oxidant activity [15, 16]. Its deficiency can occur in diabetes in relation to impaired mitochondrial substrate metabolism [17] and increased oxidative stress [6]. Mitochondrial CoQ deficiency could be involved in the pathogenesis of Type II diabetes by impairing beta-cell function [18]. Low serum CoQ concentrations have been negatively correlated with poor glycaemic control and diabetic complications [19, 20]. Accordingly, some clinical trials have shown that CoQ could improve glycaemic control and blood pressure in diabetes [14, 18, 21, 22]. Hence, CoQ could have a potential role in the treatment of diabetes and its complications [14, 18, 23]. However, to date no studies have reported the effects of CoQ on vascular dysfunction or cardiovascular disease in diabetes.

We report a placebo-controlled observation of the effect of CoQ supplementation on vascular function of the peripheral circulation in Type II diabetic patients. Endothelial function was quantitated as postischaemic flow-mediated dilatation of the brachial artery using a new edge-detection software system that increases the precision of measurements [24]. We also measured potential changes in oxidative stress by measuring plasma F_2-isoprostanes and measuring the oxygen radical absorbance capacity of plasma.

Subjects and methods

Subjects. A total of 40 patients with Type II diabetes diagnosed by standard criteria and with dyslipidaemia were recruited from the community. Dyslipidaemia was defined as a fasting serum triglyceride of greater than 1.8 mmol/l or HDL cholesterol of less than 1.0 mmol/l with total cholesterol of less than 6.5 mmol/l and a total cholesterol-to-HDL cholesterol ratio of more than 4. Patients were excluded based on the following criteria: age older than 75 years, BMI greater than 40 kg/m^2, history of myocardial infarction or stroke, insulin therapy, smoking, macroalbuminuria, serum creatinine greater than 150 μmol/l, liver abnormalities, use of antioxidants or lipid-regulating therapy, uncontrolled hypertension (> 160/90 mmHg), and treatment with angiotensin-converting enzyme inhibitors, calcium antagonists or aspirin. Volunteers underwent a clinical examination, urinalysis and a 12-lead ECG. The vascular function in the diabetic patients was compared with 18 healthy, non-diabetic normolipidaemic subjects of similar age [mean age 54 years (SD 12.0); cholesterol 5 mmol/l (SD 0.4), HDL cholesterol 1.6 (0.3), triglyceride 0.9 (95% CI 0.8, 1.1)].

Study design. This study is part of a larger study examining the effects of CoQ and lipid-regulating therapies on vascular function of peripheral arteries measured by several techniques. We report on the effect of CoQ monotherapy on postischaemic dilation of the brachial artery. Eligible patients entered a run-in period of 6 weeks during which they were instructed to consume an isocaloric fat-modified diet of constant antioxidant composition. They then underwent the brachial artery reactivity test described below, after which they were randomized double-blind to treatment with either CoQ (Blackmores, Sydney, NSW, Australia) or matching placebo in a trial of 12 weeks duration. Coenzyme Q_{10} (200 mg) was taken as two 50 mg capsules orally twice a day. Volunteers were interviewed every 2 weeks to assess their compliance with therapeutic units and brachial artery reactivity was re-studied 12 weeks after randomization. The Ethics Committee of the Royal Perth Hospital approved the study and all volunteers gave their written consent.

Laboratory methods. Venous blood was collected after a 12 h fast at baseline and at 12 weeks. Serum total cholesterol, triglyceride and HDL cholesterol were measured using enzymatic, colorimetric methods (Boehringer Mannheim, Mannheim, Germany) on a Hitachi 917 biochemical analyser (Hitachi, Tokyo, Japan). High-density lipoprotein cholesterol was measured after precipitation of apolipoprotein B-100 (apoB) containing lipoproteins with dextran sulphate. Low-density lipoprotein cholesterol was estimated by the Friedewald formula and by a direct assay when triglycerides were more than 3.5 mmol/l. The particle size of LDL was estimated by non-denaturing gel electrophoresis. Glycated haemoglobin (HbA_{1c}) was measured by high performance liquid-chromatography (HPLC, BioRad Laboratories, Sydney, Australia). Plasma glucose and insulin were assayed using an enzymatic method (Boehringer) and an automated immuno-enzymometric assay (Tosoh, Kyobashi, Tokyo, Japan), respectively. Serum and urinary creatinine were measured by the modified Jaffe reaction. Total serum CoQ concentration was assayed by reverse-phase high performance liquid-chromatography using electrochemical detection [25]. Plasma F_2-isoprostanes were analysed using gas-chromotography mass-spectrometry with electron capture negative chemical ionization [26]. Plasma oxygen radical absorbance capacity (ORAC) was measured using a fluorescent assay using a Trolox standard [27].

Brachial artery ultrasonography. Brachial artery ultrasonography was carried out [28]. Briefly, a 12-megahertz transducer connected to an Acuson Aspen ultrasound (Acuson, Mountain View, Calif, USA) and fixed in position by a stereotactic clamp 5 to 10 cm proximal to the ante-cubital crease was used to image the brachial artery. Images were recorded before and after vasodilatory stimuli and recorded on s-VHS videotape (Sony MQSE 180). Continuous ECG monitoring was done in all studies. Reactive hyperaemia of the brachial artery was induced after release of a pneumatic tourniquet placed around the left forearm and inflated to 50 mmHg above systolic blood pressure for 5 min. Pulse wave Doppler flow velocities were used to derive flow rate (ml/min) pre-reactive and post-reac-

Table 1. Clinical, biochemical and vascular characteristics of the patients in the placebo and CoQ groups at baseline

Characteristics	Placebo group	CoQ group
n (male/female)	13/2	18/2
Age (years)	54.1 (10.4)	52.7 (6.2)
BMI (kg/m^2)	31.3 (5.4)	29.9 (3.3)
SBP (mmHg)	139.1 (15.1)	128.0 (18.4)
DBP (mmHg)	81.0 (5.8)	75.8 (9.3)
Glucose (mmol/l)	6.9 (2.1)	8.2 (2.7)
HbA_{1c} (%)	6.2 (0.8)	6.9 (1.4)
Insulin (mU/l)	15.1 (7.4)	12.2 (5.4)
Cholesterol (mmol/l)	5.3 (0.6)	5.3 (0.9)
Triglyceride (mmol/l)	2.5 (2.1, 3.0)	2.0 (1.7, 2.5)
HDL-cholesterol (mmol/l)	1.00 (0.08)	0.95 (0.15)
LDL-cholesterol (mmol/l)	3.2 (0.7)	3.2 (0.9)
LDL size (nm)	25.3 (0.8)	25.0 (0.8)
Coenzyme Q_{10} (mmol/l)	1.2 (0.3)	1.5 (0.3)*
ORAC activity (μmol/l)	3638 (3270, 4046)	3723 (3275, 4233)
Plasma F_2-isoprostanes (pmol/l)	1297 (1023, 1643)	1102 (892, 1361)
Brachial artery: Baseline diameter (mm)	4.3 (0.6)	4.2 (0.5)
Resting blood flow (ml/min)	186.6 (72.8)	205.9 (113.7)
Reactive hyperaemia (%)	462.2 (239.8)	431.9 (254.7)
Flow-mediated dilatation (%)	4.5 (3.0)	2.8 (3.0)
Nitrate-mediated dilatation (%)	16.9 (6.4)	16.3 (5.2)

Values are means (SD) or geometric means and 95% CI
*$p = 0.03$ vs placebo group

tive hyperaemia. After the brachial artery diameter returned to baseline 400 μg glyceryl trinitrate was administered sublingually to assess endothelium-independent vasodilatory response. All images obtained were assessed blindly by two independent observers and only pairs of scans that were of consistently acceptable quality were included in this analysis. Analysis of flow-mediated dilatation (FMD) and glyceryl-trinitrate-mediated dilatation (NMD) of the brachial artery was carried out using a semi-automated edge detection software system [24] and operated by an experienced observer, who was blinded to the treatment group assignment. Responses were calculated as percentage change in brachial artery diameter from baseline. The analytical (intra-observer) coefficient of variation of the computerized technique is 6.7% compared with 32.5% using ultrasonic callipers, a more conventional visual estimation. The resolving power of the method tested on 'phantom arteries' is 8.3 μm.

Statistical methods. Data were analysed parametrically after logarithmic transformation of variables where appropriate. Discrete variables were compared by Chi-square test. Treatment effects were analysed using general linear models with adjustments for baseline values and resting brachial artery diameter. Association between variables were examined by linear regression methods. Statistical significance was defined at a p value of less than 0.05.

Results

The diabetic patients were mostly middle-aged men and were on average, overweight, normotensive, in good glycaemic control and had typical diabetic dyslipidaemia. Of the 40 patients randomized, 39 completed the study and one withdrew due to an incidental illness. High quality ultrasound images were obtained in all patients randomized to CoQ. However, satisfactory image quality was not obtained in ultrasound scans from four patients randomized to placebo. Thus, our analysis refers to the residual 35 diabetic patients. Their characteristics did not differ from those withdrawn from the study.

Plasma F_2-isoprostanes were not different between the diabetic patients and the non-diabetic control subjects [1245 μmol/l (95% CI 1075, 1442) vs 1310 (1085, 1583), $p = 0.670$]. However, plasma ORAC was lower in the diabetics patients than in the control subjects [3766 μmol/l (95% CI 3476, 4081) vs 4729 (4551, 4914) $p = < 0.001$].

Compared with the control subjects, the diabetic patients overall had lower post-ischaemic FMD of the brachial artery [3.8% (SEM 0.5) vs 6.4 (1.0), $p = 0.016$] but similar NMD responses [16.5% (SEM 0.9) vs 18.5 (1.7), $p = 0.291$].

No differences were shown in the characteristics (Table 1) between patients randomized to placebo and CoQ treatment ($p > 0.05$), except for higher baseline plasma CoQ concentrations ($p = 0.03$) in the CoQ group. Only four patients in the study took metformin, one in the placebo and three in the CoQ group. The plasma glucose immediately before ultrasonography did not differ ($p = 0.20$) between the groups (Table 1).

The changes in baseline diameter, reactive hyperaemia, FMD and NMD of the brachial artery in the CoQ were compared with the placebo group (Table 2). There was an improvement in FMD of the brachial artery in the CoQ compared with the placebo group, without changes or differences in baseline arterial diameter, reactive hyperaemia or NMD. In an analysis with absolute FMD (mm) at 12 weeks as the dependent variable, there was still a favourable treatment effect of CoQ ($p = 0.002$) after adjusting for baseline brachial artery diameter (mm) at 12 weeks and pre-randomization percentage FMD response. This analysis together with the lack of significant change in resting brachial artery diameter (Table 2) demonstrates that the favourable effect of CoQ on percentage FMD response was independent of changes in basal brachial artery tone. There were no differences between the groups in the plasma glucose concentration immediately before ultrasonography post-intervention [8.2 mmol/l (SEM 0.8) for CoQ vs 7.3 mmol/l (0.7) for placebo, $p = 0.43$].

Treatment with CoQ was associated with an increase in plasma CoQ concentrations, from

Table 2. Changes in baseline diameter, reactive hyperaemia, flow-mediated dilatation (FMD) and nitrate-mediated dilatation (NMD) of the brachial artery in the diabetic patients treated with placebo or CoQ supplementation for 12 weeks

Variable	Placebo group	CoQ group	*p* value
Change in baseline artery diameter (mm)	0.05 (0.09)	–0.02 (0.08)	0.611
Change in resting blood flow (ml/min)	–43.3 (19.7)	20.6 (30.4)	0.136
Change in reactive hyperaemia (%)	85.0 (38.6)	–27.3 (84.5)	0.316
Change in FMD (%)	–0.4 (0.5)	1.6 (0.3)	0.005
Change in NMD (%)	–0.1 (1.4)	0.4 (1.2)	0.771

Values are means ± SEM

1.3 mmol/l (SEM 0.1) to 4.8 (0.4), $p < 0.001$; however, there were no alterations ($p > 0.05$) in plasma F_2-isoprostanes, plasma ORAC activity, glucose, HbA_{1C}, plasma lipids, blood pressure or other variables (Table 1). Changes in plasma CoQ concentrations were not correlated with the changes in plasma F_2-isoprostanes. The FMD of the brachial artery increased with CoQ alone from 2.8% (SEM 0.7) to 4.4% (0.5), $p < 0.001$ but the post-treatment response tended to stay lower ($p = 0.07$) than the values for the non-diabetic control group. There was no correlation between the change in FMD and change in other variables in the CoQ group. In data pooled from both patient groups, improvement in FMD was only correlated with treatment group assignment to CoQ.

Discussion

Our randomized, double-blind study shows a favourable effect of oral CoQ supplementation on endothelial dysfunction of the peripheral circulation in patients with Type II diabetes. Coenzyme Q_{10} improved abnormal endothelium-dependent vasodilator tone of the brachial artery without altering the vasodilatory response to the endothelium-independent agonist glyceryl trinitrate. This favourable effect of CoQ was independent of changes in resting brachial artery diameter. The improvement in endothelial function also occurred in the presence of dyslipidaemia and was not related to changes in plasma F_2 -isoprostanes, glycated haemoglobin or blood pressure.

That oxidative stress is increased in diabetes is well supported by experimental and clinical observations [5, 6, 11, 29, 30]. Oxidative stress occurs in diabetes as a consequence of several mechanisms related to hyperglycaemia [5, 6]. These include accumulation of AGE, activation of the polyol pathway and stimulation of protein kinase C activity. Diabetes-induced generation of reactive oxygen species, in particular superoxide, decreases the expression of nitric oxide synthase and inactivates nitric oxide [6, 31]. Vascular oxidative stress could explain why our patients had impaired FMD of the brachial artery with preserved vasodilation to glyceryl trinitrate. Unexpectedly, our patients did not show evidence of increased oxidative stress, as measured by plasma concentrations of F_2-isoprostanes. Plasma F_2-isoprostanes reflect non-enzymic, free radical induced lipid peroxidation and might not be sensitive to increases in oxidative stress at the vascular wall. Previously reported increases in plasma F_2-isoprostanes and their diminution in diabetes with antioxidant vitamins referred to patients with poorer glycaemic control than our study group [29, 30]. We found that plasma antioxidant capacity was decreased in our patients in agreement with previous reports showing that diabetic patients have decreased plasma concentrations of antioxidant vitamins [32]. However, low plasma ORAC was not corrected by CoQ supplementation, suggesting that CoQ might not contribute to plasma antioxidants measured by this assay. A dissociation between changes in endothelial function and plasma F_2-isoprostane concentrations was found using antioxidant vitamin supplementation in non-diabetic subjects [33]. Our patients were dyslipidaemic and it is possible that increased plasma concentrations of lipoprotein remnants, small-dense LDL and low HDL cholesterol might have contributed to endothelial dysfunction in the absence of a systemic increase in oxidative stress [6].

There is evidence in Type II diabetes for and against improvement in vasodilator function of forearm resistance arteries in response to the muscarinic agonist acetylcholine with vitamin E supplementation [10, 11]. One positive study using a vitamin E analogue was not placebo controlled but did show reduction in plasma F_2-isoprostanes in a small number of patients [11]. Vitamin E supplementation was reported to improve forearm microcirculatory function in a larger sample of Type II diabetic patients in a well-controlled trial [10]. Improvement in methacholine-mediated vasodilator function of forearm resistance vessels was also reported in Type II diabetes following the intra-arterial administration of vitamin C [12]. However, that study used a small sample size, involved an acute intervention, and did not use a placebo arm. A recent report has shown that intra-arterial administration of the powerful antioxidant α-lipoic acid improved forearm blood flow responses to acetylcholine to the same extent as ascorbic acid in patients with Type II diabetes [34]. The greatest benefit was seen in patients with low-plasma concentration of CoQ, supporting an important role of CoQ in vascular endothelial dysfunction in Type II diabetes. We

have extended previous reports by investigating intervention with the antioxidant CoQ in a larger sample of patients and in a peripheral conduit artery. In contrast to forearm microcirculatory blood flow responses to acetylcholine, post-ischaemic FMD of the brachial artery has been shown to be a surrogate for the coronary circulation and to predict coronary events in patients with angina [35, 36].

The beneficial properties of CoQ could relate not only to its antioxidant effect [15, 16], but also to improvements in glycaemic control [18] and blood pressure [14]. Coenzyme Q_{10} is a powerful antioxidant that might decrease superoxide generation from endothelial cells [13, 14]. Since our patients remained dyslipidaemic, their increased plasma concentration of small-dense LDL and low HDL would have contributed to endothelial dysfunction [6]. By decreasing vascular oxidative stress, CoQ could decrease the oxidative modification of LDL and HDL in the sequestered environment of the arterial wall, thereby increasing the synthesis and/or action of endogenously derived nitric oxide [6, 37, 38]. This effect might particularly extend to triglyceride-rich lipoproteins in the post-prandial phase, when oxidative stress could be maximal in diabetes [39]. Coenzyme Q_{10} supplementation has been reported to improve glycaemic control [14, 18, 21, 22] and blood pressure [22] in patients with diabetes. However, along with other studies [40, 41], we found no evidence to support this finding. Other potential mechanisms whereby CoQ could have improved endothelial function in the brachial artery involve reduction in the cellular levels of asymmetric dimethyl-arginine and AGEs [31, 42], as well as an increase in the bioavailability of tetrahydrobiopterin [43] and glutathione [44]. Normalization of mitochondrial superoxide production could be central to these mechanisms and to an anti-inflammatory effect of CoQ [45].

We did not rigorously study the mechanism of action of CoQ with pharmacological agents, such as acetylcholine with and without N^G-monomethyl-L-arginine. Post-ischaemic FMD of human conduit arteries has predominately been shown to be mediated by nitric oxide, although studies to date have mostly referred to the radial artery [46]. We cannot infer that the vascular benefit of CoQ extends to microcirculatory function, where the mediators of shear-stress induced increase in blood flow could be different from conduit vessels [4]. Because NMD of the brachial artery was tested maximally with a high dose of GTN, we cannot strictly exclude that our diabetic patients had non-endothelial vascular dysfunction, and that this abnormality improved with CoQ. Acute hyperglycaemia has been shown to impair FMD of the brachial artery [47] and we did not study patients at isoglycaemia. However, at the time of ultrasound, patients were on average near-normoglycaemic and there was no difference in blood glucose concentrations before and after intervention. Metformin has also been reported to improve brachial artery endothelial function in diabetes [48], but the number of patients included in our study on this agent were small and did not differ between intervention and control groups. The clinical relevance of the small but significant 1.6% increase in FMD in our patients with CoQ supplementation is not clear, given that FMD is only weakly correlated with coronary responses and that NMD did not change with treatment. We were able to detect a small improvement in FMD, however, because of the enhanced precision of our computerized method for assessing luminal diameter changes.

If our findings reflect the favourable effect of CoQ on the bioavailability and action of nitric oxide, they have implications for the prevention and reversal of atherogenesis, procoagulopathy and myocardial dysfunction in diabetic patients [2, 4, 6]. That a benefit in endothelial function was seen in the presence of diabetic dyslipidaemia without complete restoration of FMD to normal, raises the possibility of further investigating the synergistic effects of CoQ with other agents that could improve vascular function in diabetes, such as angiotensin-converting enzyme inhibitors, fish-oils and lipid regulators [4, 6].

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