JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1629–1641

Received April 13, 2007
Accepted August 20, 2007
DOI 10.1211/jpp.59.12.0004
ISSN 0022-3573

Cod liver oil supplementation improves cardiovascular and metabolic abnormalities in streptozotocin diabetic rats

Aslı Ceylan-Isık, Tuğba Hünkar, Esin Aşan, Fugen Kaymaz, Nuray Arı, Tülin Söylemezoğlu, Nurten Renda, Halim Soncul, Musa Bali and Çimen Karasu (Antioxidants in Diabetes-Induced Complications) The ADIC Study Group

Department of Pharmacology, Faculty of Pharmacy, Ankara University, Ankara, Turkey

Aslı Ceylan-Isık, Nuray Arı

Institute of Forensic Medicine, Ankara University, Ankara, Turkey

Tuğba Hünkar, Tülin Söylemezoğlu

Department of Histology and Embryology, Hacettepe University, Ankara, Turkey

Esin Aşan, Fugen Kaymaz

Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Nurten Renda

Department of Nephrology, Faculty of Medicine, Gazi University, Ankara, Turkey

Halim Soncul

Department of Cardiovascular Surgery, Faculty of Medicine, Gazi University, Ankara, Turkey

Musa Bali

Department of Medical Pharmacology, Faculty of Medicine, Gazi University, Ankara, Turkey

Çimen Karasu

Correspondence: Ç. Karasu, Department of Medical Pharmacology, Faculty of Medicine, Gazi University, 06510, Besevler, Ankara, Turkey. E-mail: karasu@gazi.edu.tr

Acknowledgement: The partial support of this project by CANSIN Medical Turkey is gratefully acknowledged.

Abstract

Abnormalities in the metabolism of essential fatty acids and the results of increased oxidative stress have been implicated in cardiovascular disorders observed in diabetes mellitus. This study, therefore, aimed to investigate the effects of cod liver oil (CLO, Lysi Ltd, Iceland), which comprises mainly an antioxidant vitamin A, n:3 polyunsaturated fatty acids (n:3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on cardiovascular abnormalities in streptozotocin (STZ)-diabetic rats. Two days after single STZ (55 mg kg^{-1}, i.p.) or vehicle injection, diabetes was verified by increased blood glucose, and non-diabetic and diabetic rats were left untreated or treated with CLO (0.5 mL kg^{-1} daily, by intragastric probing) for 12 weeks. Plasma glucose, triacylglycerol and cholesterol concentrations were significantly elevated in 12-week untreated-diabetic rats; CLO provided better weight gain, entirely prevented the plasma lipid abnormalities, but partially controlled the glycaemia in diabetic rats. In isolated aorta rings, diabetes resulted in increased phenylephrine-induced vasoconstriction and isoprenaline-induced vasorelaxation, impaired endothelium-dependent vasodilatation and unchanged responsiveness to sodium nitroprusside. CLO treatment completely prevented endothelial deficiency, partly corrected the phenylephrine-induced vasoconstriction and did not affect the responses to isoprenaline and sodium nitroprusside in diabetic aorta. Diabetes also produced a marked decrease in the rate of spontaneously beating right atria and a significant increase in basal contractile force of left ventricular papillary muscle. The responsiveness of right atria to the positive chronotropic effect of isoprenaline was significantly decreased in diabetic rats, and was increased in CLO-treated diabetic rats. The positive chronotropic effect of noradrenaline was markedly increased in diabetic atria, but prevented by CLO treatment. Diabetes also resulted in an increased positive inotropic response of papillary muscle to both noradrenaline and isoprenaline, which were prevented by CLO treatment. CLO treatment also resulted in lower tissue sensitivity (pD_2) to these agonists in diabetic papillary muscle. Ventricular hydroxyproline content was found to be unchanged among the experimental groups. The ultrastructure of diabetic myocardium displayed various degenerations (i.e. intracellular oedema, myofibrillar fragmentation, condensed pleomorphic mitochondria, thick capillary irregular basement membrane, swollen endothelial cells), which were partially prevented by CLO treatment. We conclude that the supplementation with CLO is effective in preventing cardiovascular disorders observed in experimental diabetes.

Introduction

Cod liver oil (CLO) is a particularly rich source of polyunsaturated fatty acids (n:3 PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and vitamin A. It is well documented that, as major constituents of membrane lipids, EPA and DHA are vital to the proper functioning of various tissues, including vessels and heart, and facilitate normal growth, development and function of tissues (Simopoulos 1999; Das 2000). They are known to lower lipids, increase HDL, and have anti-thrombotic, anti-inflammatory and

vasodilatory properties, reduce erythrocyte deformability, provide protection against free-radical-induced damage and arteriosclerosis and also prevent high blood pressure and arrhythmias (Nosari et al 1994; Axelrod 1998; Bechoua et al 1999; Das 2000). In diabetic patients, treatment with CLO has been shown to decrease thromboxane production (Beitz et al 1986), improve endogenous antioxidant defence (Hünkar et al 2002) and largely inhibit oxidative stress (D'Aquino et al 1999). CLO also has a protective effect against Type 1 diabetes in women (Stene et al 2000).

Several clinical and experimental studies have revealed that diabetes mellitus leads to endothelial dysfunction, atherosclerosis, abnormal vascular reactivity, hypertension and impaired cardiac performance (Altan et al 1989; Karasu et al 1997a, b; Koçak et al 2000; Zobali et al 2002a; Avagaro et al 2006; Reusch & Draznin 2007). It is well established that atherosclerosis is a major underlying cause of cardiovascular disease observed in diabetes mellitus (Avagaro et al 2006; Wang et al 2006; Reusch & Draznin 2007), and impaired tissue responsiveness to catecholamines and adrenoceptor agonists is associated with diabetes-induced cardiovascular abnormalities (Karasu et al 1990; Kamata et al 1997; Dinçer et al 1998). In the diabetic state, chronic hyperglycaemia leads to an increase in non-enzymatic glycation of circulating and structural proteins, together with a glucose-generated oxidative and carbonyl stress, known as glycoxidation. There is a simultaneous glycoxidation of both protein and phospholipid components that increases oxidative stress and promotes lipoxidation in diabetes. Lipid peroxidation in the vascular wall leads to the local production of reactive carbonyl species that mediate recruitment of macrophages, cellular activation and proliferation, and chemical modification of vascular proteins. The pathology of diabetic macro- and micro-angiopathy and cardiomyopathy has been focused on the aspects of an increased oxidative stress and carbonyl modification of proteins by autoxidation products of carbohydrates, lipids and amino acids (carbonyl stress) (Baynes 1991; Miyata et al 2003). On the other hand, previous studies have shown that tissue contents of polyunsaturated fatty acids (PUFAs) are decreased in diabetic patients and animals (Mohan & Das 2000), and there is a defective conversion of the main dietary essential fatty acid, linoleic acid, to gamma-linolenic acid (GLA), which causes alterations in the fatty-acid profile. In diabetes there is an impaired activity of Delta(6)- and Delta(5)-desaturases (Horrobin 1993; Ghebremeskel et al 2002). A defect in the activity of D6 and D5 desaturases has been shown to decrease the formation of GLA, dihomo-DGLA (DGLA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from dietary linoleic acid (LA) and α-linolenic acid (ALA) (Das 2007). This, in turn, leads to inadequate formation of prostaglandin E1, prostacyclin (PGI2), PGI3, lipoxins, resolvins, neuroprotectin D1, nitric oxide (NO) and nitrolipids that have anti-inflammatory and platelet anti-aggregatory actions, inhibit leucocyte activation and augment wound healing and resolve inflammation and, thus, lead to the initiation and progression of atheroslcerosis (Das 2007).

According to these findings, the authors have reported that n:3 PUFAs prevent the cytotoxic action of diabetogenic agents on pancreatic beta-cells by enhancing the antioxidant status, suppressing production of cytokines and activating PPARs (Mohan & Das 2001). Oral feeding of oils rich in n:3 EPA and DHA and n:6 gamma-linolenic acid and arachidonic acid has been shown to prevent the development of alloxan-induced diabetes mellitus in experimental animals (Suresh & Das 2003). In this respect, an essential-fatty-acid-rich diet (i.e. EPA) has been shown to increase aorta arachidonic acid levels (Takahashi et al 1988) and leads to amelioration in some functional deficits in the microcirculation of diabetic animals (Karasu et al 1995; Pieroni et al 2007). Similarly, long-term consumption of modified diet with n-3 PUFAs improves blood lipids and vascular function in an animal model of insulin resistance and Type 2 diabetes mellitus (Mustad et al 2006), and DHA/EPA attenuates endothelial dysfunction in Type 2 diabetic patients (Hilpert et al 2007). Although a large number of investigations have been conducted with different fish oils, the effects of CLO on vascular reactivity and cardiac performance, endothelial function and myocardial ultra-structure, as well as general metabolism, have not been examined extensively in a diabetic model. Moreover, we have already shown that antioxidants, including vitamins E (Karasu et al 1997a) and A (Zobali et al 2002a), probucol (Karasu 1998), alpha-lipoic acid (Koçak et al 2000), stobadine (Pekiner et al 2002) and some lipid lowering drugs, including gemfibrozil (Ozansoy et al 2001; Ceylan et al 2004), have some capacity to prevent or reverse cardiovascular abnormalities in STZ-diabetes.

Thus, the aim of this study was to assess the effects of CLO supplementation on vasoconstriction, endothelial function, heart rate, cardiac contractility, myocardial ultra-structure, hydroxyproline production, lipid profile and some vital requirements of STZ-diabetic rats.

Materials and Methods

Drugs

All chemicals, except CLO, were purchased from Sigma Chemical (St Louis, MO). CLO was supplied by Cansin Medical (a company in Turkey, which distributes the products of Lysi Co. from Iceland). Specifications of CLO: vitamin A $\geq$ 1000 IU g^{-1}, vitamin D $\geq$ 100 IU g^{-1}, eicosapentaenoic acid (EPA) $\geq$ 8.0%, docosahexaenoic acid (DHA) $\geq$ 9.0%, polyunsaturated fatty acid (PUFA) $\geq$ 20%.

Induction of diabetes and the treatment protocols

Male Wistar rats, 250–300 g were fed a standard rat chow diet and had free access to water. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 55 mg kg^{-1}) to rats fasted overnight. Blood samples for biochemical measurements were taken from the tail vein. Those rats with a blood glucose concentration of 250 mg dL^{-1} or more were considered to be diabetic. The experimental groups comprised control and diabetic untreated or treated with cod liver oil (CLO; Lysi Ltd, Iceland; 0.5 mL kg^{-1} daily by intragastric probing). The dose regimen of the CLO was chosen according

to a previous study (Hünkar et al 2002). Rats were treated for a period of 12 weeks, beginning 48 h after STZ injection. Food and water consumptions of rats was measured daily over a 24-h period for each cage of three rats, but data were transformed to give values/rat/day. This study was approved by the Ethics Committee of Ankara University. The principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were observed.

Blood and tissue analysis

Blood glucose concentration and body weight were determined weekly. At the end of the treatment period, the rats were anaesthetized with urethane and blood and tissue samples were immediately collected for the measurement of plasma triacylglycerol and cholesterol concentrations and biochemical tissue analysis. Blood glucose concentrations were measured by an Ames glucometer (Glucometer III; Bayer Diagnostics, France); plasma triacylglycerol and cholesterol concentrations were measured using a commercially available enzyme kit (Wako, Osaka, Japan).

Myocardial hydroxyproline concentrations were determined according to a previously described method (Chiariello et al 1986). Protein levels in the myocardium were measured as described by Lowry et al (1951).

Isolation of cardiac tissue preparations and cardiac performance experiments

Hearts from untreated control, untreated diabetic and CLO-treated diabetic rats were removed and immediately perfused with Krebs-Henseleit (KH) solution of following composition (in mM): 118.0 NaCl, 25.0 $NaHCO_3$, 4.8 KCl, 1.2 $MgSO_4$, 1.4 $CaCl_2$ and 11.1 glucose. This solution was buffered to pH 7.4 by saturation with 95% O_2–5% CO_2, and the temperature was maintained at 37°C. After all blood was washed from the hearts, entire right atria were isolated and allowed to beat spontaneously in a temperature-controlled (37°C) tissue bath (20 mL) containing the KH solution described above. One end of the atrial tissue was attached to a tissue holder and the other to an isometric transducer (Ugo Basile, No. 7004) connected to the recorder (Ugo Basile, No. 7050) (Varese, Italy). The tissues were allowed to equilibrate for 60 min under a resting tension of 1 g (Karasu et al 1990). The left ventricular papillary muscles were dissected and mounted horizontally onto a tissue holder with two platinum electrodes in a temperature-controlled bath. The other end of the tissue was attached to an isometric transducer, which was connected to a recorder. Resting tension in the left papillary muscle was maintained at 2 g throughout the experimental period. Each preparation was allowed to equilibrate in KH solution for 60 min while electrically driven by a Grass stimulator (S-88) at frequency of 1.6 Hz, 0.4–8.0 V and duration of 5 ms (Savage et al 1995).

After initial incubation, the basal chronotropic response of atria and basal inotropic response of papillary muscle were measured. Positive chronotropic and inotropic effects of isoprenaline (10^{-10} to 10^{-5} M) or noradrenaline (10^{-10} to 10^{-5} M) in right atria and papillary muscles were recorded.

Isolation of aortic rings and vascular reactivity studies

Thoracic aorta was quickly excised and placed in cold KH buffer. The aortic segments were carefully cleaned of fat and loose connective tissue and sectioned into 3-mm-long rings. In all rings, extreme care was taken to avoid stretching and contact with the luminal surface to avoid damage to the endothelium during isolation. Aortic rings were suspended between parallel hooks in 5-mL tissue baths filled with KH solution, which were thermoregulated at 37°C. Changes in isometric tension were recorded on a Ugo Basile recorder via a Ugo Basile-7004 transducer. The rings were equilibrated for 60 min under a resting tension of 2 g before experiments were begun. During this period, the rings were washed every 15 min.

At the end of the equilibration period, concentration–response curves to increasing concentrations of phenylephrine were performed on each ring. After generating phenylephrine concentration–response curves, each ring was serially washed to baseline and equilibrated. Rings were then contracted with a submaximal concentration of phenylephrine, which produced approximately 80% of the maximum response. This concentration was usually 1 μM but was occasionally varied between 10^{-6} M and 3×10^{-6} M to obtain equieffective agonist activity. After reaching a plateau of contraction, cumulative concentration–response curves to acetylcholine (10^{-8} to 10^{-5} M) or isoprenaline (10^{-8} to 10^{-4} M) and then sodium nitroprusside (10^{-10} to 10^{-6} M) were obtained. The tissue was washed three times in the next 15 min and then allowed to recover for at least another 20 min before the next drug addition (Karasu et al 1997 a, b).

Electron microscopy

Myocardial tissue was processed for electron microscope examination according to previously described techniques (Warley et al 1995). After isolation, ventricular samples were cut into small pieces, fixed at 4°C for 2 h in 2.5% buffered glutaraldehyde and postfixed for 1.5 h with osmium tetraoxide. Tissues were dehydrated in ethyl alcohol followed by propylene oxide and embedded in araldite. Ultra-thin circumferential sections (at least five sections were taken from three different levels of each specimen) were cut using a diatome knife (Agar Scientific, UK), then mounted on a 3.05-mm, 20-mesh copper grid and contrast stained with saturated aqueous uranyl acetate for 30 min and Reynolds lead citrate for 5 min. Sections were examined using an electron microscope (Carl-Zeiss, Oberkochen, Germany).

Statistical analysis

Data are expressed as mean ± s.e.m. They were first subjected to Bartlett's test for homogeneity of variances and were given a log transformation if necessary. One-way analysis of variance was then performed, followed by the Student–Newman–Keul's test to estimate the significance of differences for individual between-group comparisons. Agonist pD_2 values (apparent agonist affinity constants; –log EC50) were calculated from each agonist concentration–effect curve by linear regression

analysis of the linear portion of the curve and taken as a measure of the sensitivity of the tissues to each agonist.

Results

General characteristics and the ventricular hydroxyproline levels of rats

As expected, food and water intake of untreated diabetic rats was greater than control rats (Figure 1A, B). As shown in Figure 1, CLO treatment reduced the food and water intake of diabetic rats, at least in initial weeks of the treatment. However, there was no significant difference between control and diabetic rats when considering food or water consumption at the end of the treatment period. CLO led to an increase in food and water consumption of the control rats (Figure 1A, B).

The initial body weights were similar in all groups. One week after STZ injection, the body weights of rats was significantly decreased; this reduction was continued until the end of the experiments (Table 1). Although CLO treatment exerted beneficial effects on weight gain of diabetic rats, these rats maintain low body-weight status until their sacrifice when compared with control rats. CLO treatment did not significantly affect the weight-gain profile of normal rats during the treatment period (Table 1).

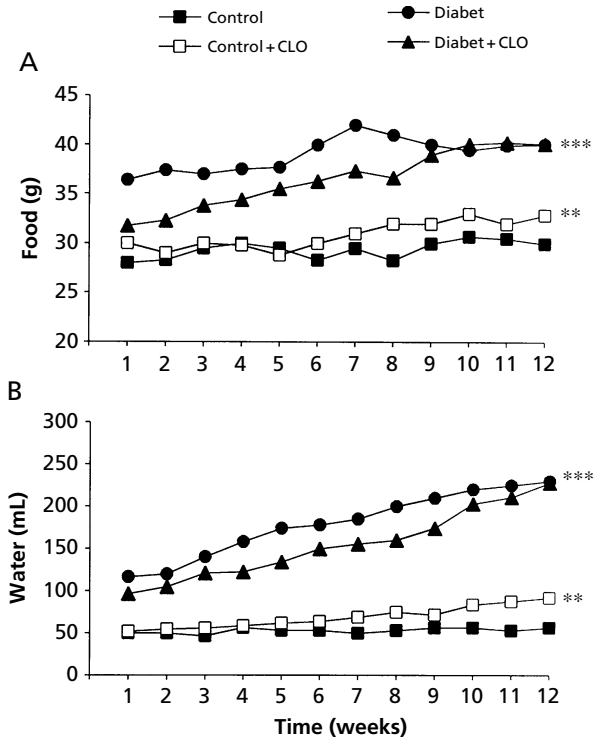

Figure 1 Food and water consumption of control and diabetic (Diabet) rats treated or untreated with cod liver oil (CLO). Data (mean ± s.e.m.) were analysed by analysis of variance and between groups differences for each variable were tested using Newman–Keul's test. $^{**}P < 0.01$, $^{***}P < 0.001$ vs untreated control.

Blood glucose concentrations were significantly higher in diabetic rats than in control rats throughout the experiments (Table 1). No significant difference was found between blood glucose concentrations of untreated and CLO-treated control rats. Although CLO was significantly effective in controlling blood glucose levels, diabetic rats were found to be still hyperglycaemic at the end of the treatment (Table 1).

Plasma triacylglycerol and cholesterol concentrations markedly increased in untreated diabetic rats compared with controls (Table 1). CLO treatment completely prevented these abnormalities in plasma lipids (Table 1).

Table 1 also shows myocardial hydroxyproline concentrations. Both total protein and hydroxyproline concentrations were unchanged in diabetic rats treated or untreated with CLO compared with untreated control rats, indicating unchanged myocardial collagen synthesis in diabetes.

Vascular reactivity and endothelial function

In comparison with controls, the maximum contractile response to phenylephrine was significantly increased in rings from untreated diabetic rats (Figure 2). Apparent affinity (pD_2 value) calculated for the contractile effect of phenylephrine, however, was found to be unchanged when compared with controls (Table 2). CLO-treatment significantly inhibited the contractile effect of phenylephrine in both control and diabetic rats, although the pD_2 value remained unchanged. The maximum contractile response to phenylephrine was found to be similar in rings from CLO-treated control and CLO-treated diabetic rats (Figure 2).

The endothelium-dependent vasodilator effect of acetylcholine partly decreased in aortic rings from untreated diabetic rats (Figure 3A). The sensitivity of diabetic tissue to acetylcholine was unchanged (Table 2). CLO treatment completely prevented the endothelium-dependent vascular response to acetylcholine in diabetic rats. As shown in cumulative concentration–response curves for acetylcholine (Figure 3A), the maximum relaxations were no different in CLO-treated diabetic rings from those observed in rings from untreated or CLO-treated control rats.

The relaxations in response to isoprenaline were significantly increased in untreated-diabetic rats compared with controls (Figure 3B). The concentration–response curve for isoprenaline was shifted to the left for aortic rings from untreated diabetic rats, and the pD_2 value was increased (Table 2). The responsiveness of rings to isoprenaline did not significantly change in control rats, but partly ameliorated in diabetic rats when the they were treated with CLO (Figure 3B).

Concentration–response curves (not shown) and pD_2 values (Table 2) for sodium nitroprusside were similar in ring preparations from all experimental rats, suggesting that the activity of vascular smooth muscle guanylate cyclase was unaltered by diabetes or by the treatment. Sodium nitroprusside (10^{-10} to 10^{-6} M) produced 100% relaxation in rings from all rats.

Cardiac performance

Right atria from untreated diabetic rats were observed to have a significantly lower basal spontaneous rate of contraction

Table 1 Metabolic parameters and the general characteristics of animals and tissues

	Untreated control (n = 6)	CLO-treated control (n = 6)	Untreated diabetic (n = 6)	CLO-treated diabetic (n = 10)
Final body weight (g)	350 ± 9$^{\#}$	365 ± 11$^{\#}$	206 ± 7*	278 ± 8*$^{\#}$
Final blood glucose (mg dL^{-1})	110 ± 5$^{\#}$	100 ± 7$^{\#}$	412 ± 21*	330 ± 12*$^{\#}$
Plasma cholesterol (mg dL^{-1})	64 ± 4$^{\#}$	58 ± 5$^{\#}$	112 ± 6*	67 ± 6$^{\#}$
Plasma triacylglycerol (mg dL^{-1})	85 ± 4$^{\#}$	62 ± 3$^{\#}$	238 ± 19*	97 ± 13$^{\#}$
Weight of aorta rings (mg)	5.31 ± 0.27‡	5.23 ± 0.23$^{\#}$	4.01 ± 0.17†	5.30 ± 0.18$^{\#}$
Weight of right atria (mg)	38 ± 2.94	ND	32 ± 1.37	31 ± 3.68
Weight of left ventricular papillary muscle (mg)	31.6 ± 0.08	ND	28.9 ± 0.37	31.2 ± 1.85
Ventricular hydroxyproline levels (μg (mg tissue)$^{-1}$)	0.75 ± 0.020	ND	0.66 ± 0.023	0.75 ± 0.037
Ventricular total protein levels (μg (mg tissue)$^{-1}$)	25.67 ± 0.60	ND	25.86 ± 0.95	26.65 ± 0.67

Data are reported as means ± s.e.m. †$P < 0.01$, *$P < 0.001$ vs untreated control; $^{\ddagger}P < 0.01$, $^{\#}P < 0.001$ vs untreated diabetic. ND, not determined.

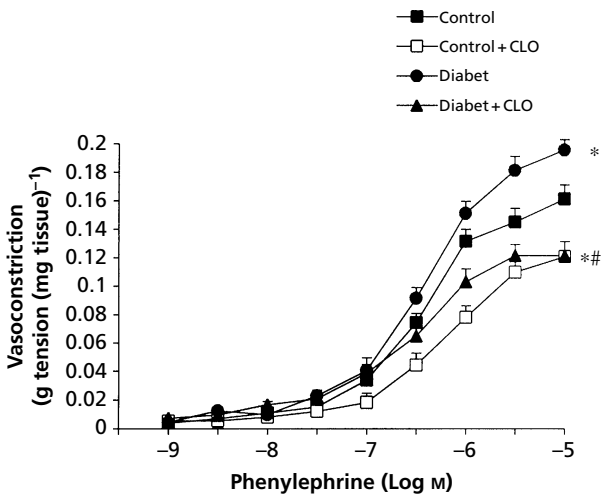

Figure 2 Vasoconstrictor effect of phenylephrine in control and diabetic (Diabet) rats treated or untreated with cod liver oil (CLO). Data (mean ± s.e.m.) were analysed by analysis of variance and between groups differences for each variable were tested using Newman–Keul's test. *$P < 0.001$ vs untreated control; #$P < 0.001$ vs untreated diabetic.

when compared with the spontaneous rate of right atria from control rats (Table 3). CLO treatment normalized basal rate of right atria in diabetic rats. Noradrenaline produced a cumulative increase in the heart rate of right atria from both diabetic and control rats (Figure 4A). Right atria from untreated diabetic rats exhibited a significantly lower response to noradrenaline when compared with the atrial response of control rats (Figure 4A). However, the pD_2 value for noradrenaline in untreated diabetic right atria was not statistically different from the non-diabetic controls (Table 3). Isoprenaline produced concentration-dependent increases in the contraction rate of atria from control and diabetic rats (Figure 4B). Although, there was a significant decrease in chronotropic response to isoprenaline in atria from untreated diabetic rats, the pD_2 value was not significantly changed when compared with controls. Treatment with CLO increased the maximum chronotropic response of diabetic atria to isoprenaline; this increase was significantly higher when compared with the effects of isoprenaline in atria from non-diabetic control rats (Figure 4B). The pD_2 value for the chronotropic response to isoprenaline were found to be similar among the groups (Table 3).

As shown in Table 3, papillary muscle from untreated diabetic rats had a significantly increased basal contractile force compared with papillary muscle from control rats. CLO treatment of diabetic rats resulted in nearly normal basal contractility in papillary muscle (Table 3). The positive inotropic response to noradrenaline in diabetic preparations was significantly increased with an increased sensitivity (pD_2) of tissues to this agonist (Figure 5A, Table 3). CLO treatment significantly decreased the sensitivity (pD_2), which was statistically different from the sensitivity of both non-diabetic and untreated diabetic papillary muscles to noradrenaline. Statistically significant increases were observed in the positive inotropic effect of isoprenaline on diabetic papillary

Table 2 pD_2 values calculated from concentration–response curves for agonists in rat aorta rings

Agonists	Untreated control (n = 6)	CLO-treated control (n = 6)	Untreated diabetic (n = 6)	CLO-treated diabetic (n = 10)
Phenylephrine	6.48 ± 0.22	6.20 ± 0.14	6.50 ± 0.10	6.44 ± 0.32
Acetylcholine	6.89 ± 0.12	6.51 ± 0.20	6.85 ± 0.11	6.64 ± 0.12
Isoprenaline	5.52 ± 0.16	5.88 ± 0.15	6.38 ± 0.10¶	6.22 ± 0.21¶
Sodium nitroprusside	8.78 ± 0.18	8.74 ± 0.11	8.82 ± 0.22	8.76 ± 0.20

$^{\P}P < 0.05$ vs untreated control.

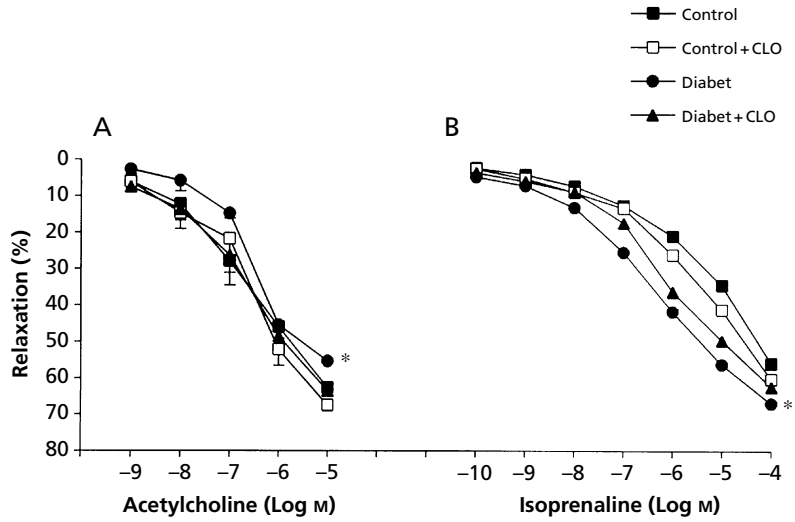

Figure 3 Effect of treatment with cod liver oil (CLO) on vascular relaxation to acetylcholine (A) and isoprenaline (B) in isolated rings of aorta in control and diabetic (Diabet) rats. Data (mean ± s.e.m.) were analysed by analysis of variance and between groups differences for each variable were tested using Newman–Keul's test. $*P < 0.001$ vs untreated control.

Table 3 Basal chronotropic response of rat right atria and basal inotropic responses of left ventricular papillary muscle, and pD_2 values calculated from concentration–response curves for noradrenaline and isoprenaline

	Untreated control (n = 6)	Untreated diabetic (n = 6)	CLO-treated diabetic (n = 10)
Right atria			
Basal chronotropy heart rate (beats/min)	129 ± 5	95 ± 7*	120 ± 6*f*
Noradrenaline	8.25 ± 0.21	7.52 ± 0.31	8.11 ± 0.33
Isoprenaline	9.24 ± 0.32	9.58 ± 0.34	8.66 ± 0.22
Papillary muscle			
Basal inotropy (g)	0.127 ± 0.007	0.193 ± 0.006*	0.133 ± 0.009*f*
Noradrenaline	7.77 ± 0.24	8.68 ± 0.18†	6.82 ± 0.18†#
Isoprenaline	7.88 ± 0.18	8.25 ± 0.36	7.18 ± 0.24*f*

$\dagger P < 0.01$, $*P < 0.001$ vs untreated control; $fP < 0.05$, $\#P < 0.001$ vs untreated diabetic.

muscle, whereas apparent affinities calculated for its effects were found to be unchanged (Figure 5B, Table 3). CLO-treated diabetic rats exhibited a significant amelioration in inotropic response of papillary muscles to isoprenaline and showed a significantly decreased sensitivity (pD_2) when compared with untreated diabetic rats (Figure 5B, Table 3).

Morphology of ventricular tissue

The ultrastructure of papillary muscle and ventricular muscle appeared to be greatly regular in the control group. In control tissues, myofibrils were highly packed and separated by rows of mitochondria (Figure 6). The myofibrillar sarcomeres exhibited clear Z-lines. The distribution of cytoplasmic organelles, including mitochondria and myofilaments, were regular in control tissues (Figure 6).

In untreated diabetic rats, myocytes varied in size — some appeared smaller in diameter while others showed considerable increase in size when compared with control. Large myocytes often exhibited intracellular oedema. Disruption in myofilament bundle organization, including myofibrillar fragmentation, was seen. Thicker closely spaced Z lines, no H zones and short sarcomeres in small cells appeared. Condensed pleomorphic mitochondria with no detectable cristae were randomly distributed between the disorganized myofilament bundles. No interstitial fibrosis, but accumulation of flocculent material in the interstitium, was evident (Figure 7A, B). In untreated diabetic rats, the microvasculature showed variable ultrastructural deformations: in focal areas degeneration of endothelial cells and complete disintegration of capillaries; endothelial cell swelling and associated luminal narrowing, even sometimes complete closure, of the lumen; thickening in basal membrane, variable; many pericytes with long cytoplasmic extensions circularly surrounding the endothelial cells forming a multilayered pericyte investment; some capillary branches increased their diameter (Figure 7C, D).

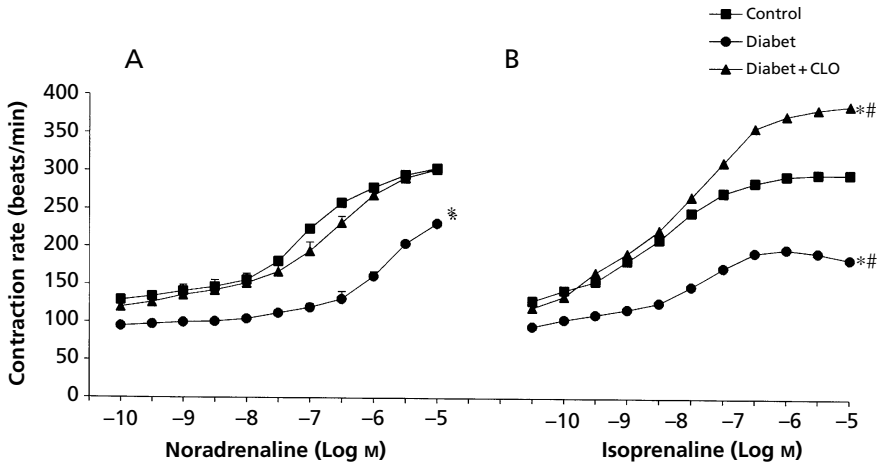

Figure 4 Effect of treatment with cod liver oil (CLO) on positive chronotropic effects of noradrenaline (A) and isoprenaline (B) in isolated right atria in diabetic (Diabet) rats. Data (mean ± s.e.m.) were analysed by analysis of variance and between groups differences for each variable were tested using Newman–Keul's test. *$P < 0.001$ vs untreated control; #$P < 0.001$ vs untreated diabetic.

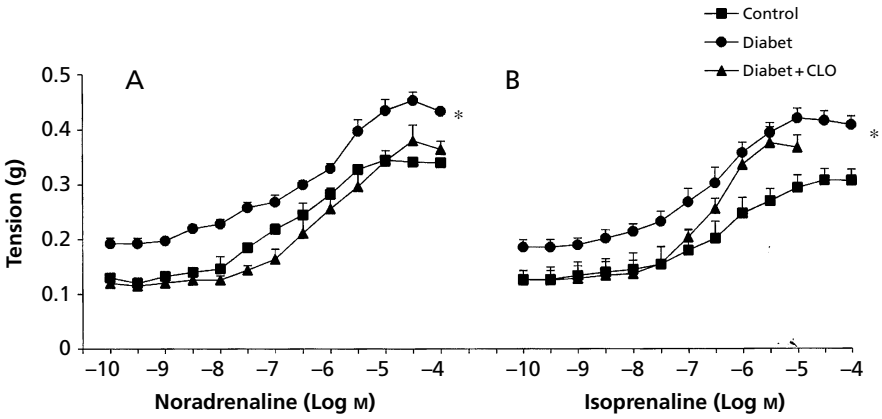

Figure 5 Effects of treatment with cod liver oil (CLO) on positive inotropic effects of noradrenaline (A) and isoprenaline (B) in isolated left ventricular papillary muscle in diabetic (Diabet) rats. Data (mean ± s.e.m.) were analysed by analysis of variance and between groups differences for each variable were tested using Newman–Keul's test. *$P < 0.001$ vs untreated control.

In the CLO-treated group, structural deformities were partially restored, myofibrillar organisation was quite regular and thick Z lines and clear H zones were seen in higher magnifications. Mitochondria formed parallel rows between the myofilament bundles. The electron density of mitochondrial matrix was normal but the cristae were irregular. Small lipid droplets, some of which were extracted during specimen preparation, were seen in the cytoplasm of cardiac muscle cells in a close association with mitochondria. The intensity of these droplets seemed to be less than that observed in cardiac muscle of untreated diabetic rats. Interstitial space contained some flocculent material. Although some capillaries exhibited structural irregularities, most of them gained their usual morphology. No basal membrane thickening and no multilayered pericyte investment was encountered. In the interstitium around the blood vessels, and also within the peripheral cytoplasm of myocytes, irregular electron-dense myelin figures were evident. Normal-appearing capillary, lined by thin endothelial cells containing erythrocytes in the lumen, was observed (Figure 8A–D).

Discussion

The main findings of this study indicate that supplementation with CLO improves the general status of diabetic rats, with an effect on food and water intakes, body weight gain, hyperglycaemia and dyslipidaemia, protecting endothelial functions and vasomotor activity, helping to maintain baseline myocardial mechanics, and tending to ameliorate chronotropic and inotropic responses of diabetic heart to β-adrenoceptor agonists. The results of our previous study, showing an ameliorating effect of CLO on hyperglycaemia, dislipidaemia, advanced lipoxidation end-products and antioxidant status in

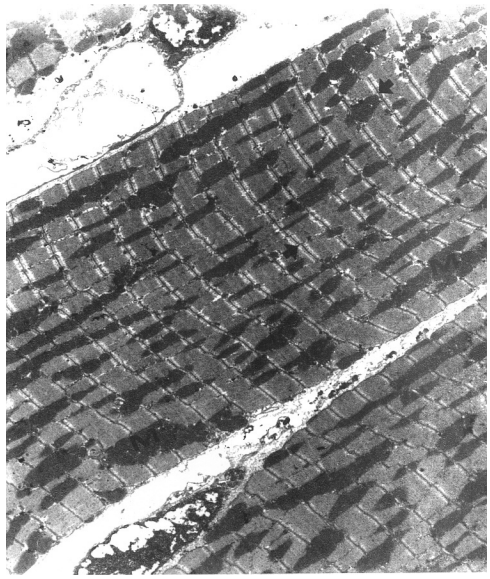

Figure 6 Ultrastructure of nondiabetic rat cardiac muscle. Regularly spaced sarcomeres, Z lines (arrow), H zones and parallel bundles of mitochondria (M) are seen. Uranyl acetate – lead citrate, ×3600.

diabetic rats (Hünkar et al 2000), supports our new findings, and may explain the beneficial effects of CLO in tissue functions of diabetic rats. The glucose- and lipid-lowering effects of essential fatty acids, which are the main component of CLO, are widely known (Beitz et al 1986; Takahashi et al 1988; Linn et al 1989; Hun et al; 1999; Mohan & Das 2001), and n:3 PUFAs can protect the pancreas against the cytotoxic action of diabetogenic agents by enhancing the antioxidant status and suppressing production of cytokines (Mohan & Das 2001; Suresh & Das 2003).

Increased oxidative stress has been shown to contribute to enhanced contractile response of diabetic vascular smooth muscle through various cellular mechanisms, including enhanced activity of DAG-PKC (Kunusaki et al 1994), stimulated IP_3-induced Ca^{2+} release (Yuichiro et al 1992), increased phosphoinositide turnover and altered voltage-operated calcium-channel activity in diabetic vessels (Chang et al 1993). Increased oxidative stress also leads to impaired EDRF/NO availability, which may be another explanation for enhanced vasoconstriction in diabetic vessels since spontaneously release of EDRF/NO has been shown to act as functional antagonist of α-adrenoceptor-mediated contractions (Karasu et al 1997a, b; Koçak et al 2000). In this study, we demonstrated that diabetes produces a deficient relaxation of aorta in response to endothelial stimulation. It is well known that the counter-regulatory role of the endothelium is markedly depressed in diabetes due to increased production of reactive oxygen species (Chang et al 1993; Karasu et al 1997a, b; Pieper et al 1999); this confirms the present findings. In this study, we also showed that there is an increase in the relaxations and the sensitivity of diabetic aorta to isoprenaline, which is parallel with the findings of our previous study (Karasu et al 1997b), but is contrary to those of other investigators (Kamata et al 1989). The mechanism(s) of increased relaxation to isoprenaline may be linked with alterations in the density or affinity of β-adrenoceptors and post-receptor events on vascular smooth muscle (Karasu et al 1997b). As is well known, the peroxidation of lipids in lipoproteins in the vascular wall leads to local production of reactive carbonyl species, which mediates some changes in membrane fluidity and membrane-associated processes, such as carrier-mediated transport, passive diffusion and ligand–receptor interactions, by advanced lipoxidation end-products (Yuichiro et al 1992; Chang et al 1993; Kunusaki et al 1994).

The results of this study also confirm our early report showing that spontaneously beating right atria from diabetic rats have a decreased basal rate, but an increased basal contractile force, compared with atria from control rats (Karasu et al 1990). In this study, we also evaluated the responsiveness of right atria to the positive chronotropic effects of noradrenaline and isoprenaline, and found that untreated diabetic rat atria were less responsive to both β-adrenoceptor agonists while the sensitivity was unchanged. Our findings obtained with diabetic atria are in agreement with the data of previous reports by us and others (Karasu et al 1990; Booth & Hodgson 1993; Dinçer et al 1998). It has been established that the diabetic heart is generally characterized by diminished responsiveness to β-adrenoceptor stimulation in association with decreased β-adrenoceptor density and alterations in the β-adrenoceptor signal transduction pathway (Karasu et al 1990; Booth & Hodgson 1993; Kamata et al 1997; Dinçer et al 2001). In addition to abnormal β-adrenoceptor density or population, alterations in catecholamine turnover and metabolism (Latifpour & McNeill 1984) as well as the intracellular signalling pathways have also been reported as the mechanisms for diabetes-induced abnormal heart rate (Dinçer et al 1998, 2001). In fact, the cardiac content of noradrenaline and noradrenaline turnover, uptake, synthesis and release have been demonstrated to all be enhanced in diabetic cardiomyopathy (Ganguly et al 1987), which might lead to down-regulation of cardiac β-adrenoceptors, resulting in a deficiency in β_1-adrenoceptor-mediated stimulation. As reported previously, we also found that diabetes results in an increased responsiveness of papillary muscle to noradrenaline and isoprenaline (Austin & Chess-Williams 1992; Howarth et al 2000). The mechanisms of contrary responsiveness of diabetic atria and papillary muscle to β-adrenoceptor agonists are not known, but similar controversial findings were reported previously (Dinçer et al 1998). Investigators have reported that β_1-adrenoceptor- but not β_2-adrenoceptor-mediated chronotropic responses are reduced in the right atria while no significant differences in the inotropic responses to noradrenaline and isoprenaline occur (Dinçer et al 2001). It has also been reported that the abnormal responsiveness of diabetic hearts to stimulation with β-adrenoceptor agonists might be due to a decrease in β_1-adrenoceptor and an increase in β_2-adrenoceptor expression (Dinçer et al 2001) and that β-adrenoceptor-mediated responses are not always parallel with a decrease in β-adrenoceptor density (Beenen et al 1997). Although collagen accumulation and decreased cardiac compliance are general features of diabetic cardiomyopathy (Pitel et al 1998), we did not find a significant difference in myocardial hydroxyproline levels among the experimental groups. As described previously, diabetes-induced ventricular dysfunction can

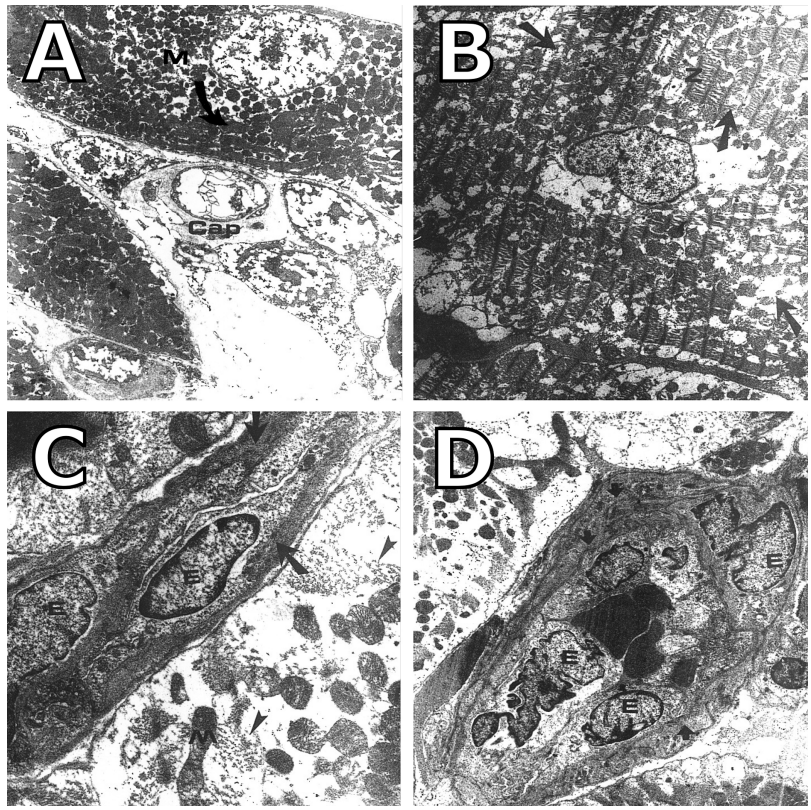

Figure 7 Electron micrographs from the untreated diabetic rat myocardium. A. Relatively small cardiac muscle cell containing condensed mitochondria (M), and disrupted myofilament bundles (arrow). In the intermyocardial connective tissue a degenerated capillary (Cap) is also seen. Uranyl acetate–lead citrate, ×3600. B. Slightly larger cardiac muscle cell displaying intracellular oedema, myofibrillar fragmentation (arrow) and thick closely spaced Z lines (Z). Uranyl acetate–lead citrate, ×3600. C. A capillary lined by swollen endothelial cells (E), completely closing the lumen of the vessel; thick irregular basement membrane (arrow) is evident. Adjacent to this capillary cross section of a cardiac muscle cell displaying condensed pleomorphic mitocondria (M) and cross sections of irregular myofilament bundles (arrow head) are seen. Uranyl acetate–lead citrate, ×9600. D. Another capillary lined by swollen endothelial cells (E) surrounding the endothelial cells are many irregular multilayered pericyte processes (arrow). Uranyl acetate–lead citrate, ×3600.

occur without influencing the accumulation of myocardial collagen (Woodiwiss et al 1996). The biochemical finding with hydroxyproline also supports our morphological findings, demonstrating no interstitial fibrosis but accumulation of flocculent material in the interstitium of diabetic myocytes.

We also carried out morphological studies to investigate whether progressive ultrastructural degeneration might underlie the contractile abnormalities observed in diabetic myocytes. The ultrastructure of diabetic ventricle displayed some characteristics, including disrupted myofilament bundle organization, myofibrillar fragmentation, condensed pleomorphic mitochondria, focal areas of contracted sarcomeres and capillary basal laminar thickening, that are consistent with those described in previous reports (McGrath & McNeill 1986; Warley et al 1995). The ultrastructural deformities regarding the contractile elements and energy-providing mitochondria strongly suggest that there is important impairment in the contractile property of diabetic cardiac muscle. Photographs also indicate the existence of impaired relaxation since most of the cells appeared in a fully contracted state, which is in agreement with the findings of increased basal and stimulated contractility of papillary muscle in untreated diabetic rats. In addition, degenerative endothelial cells observed in myocardial microvasculature are in accordance with endothelial deficiency in response to acetylcholine in diabetic aorta, and supports the contracted state of diabetic papillary muscle. Although ultrastructural evaluation of aorta was not achieved in this study, we previously demonstrated that STZ-diabetic aorta shows many pathological changes related to increased lipoxidation (Karasu et al 1997a; Karasu 1998; Koçak et al 2000).

We found that the increased vascular contractility is partially, while endothelial vasorelaxant function is entirely, protected by the treatment with CLO. However, CLO treatment did not produce a pronounced effect on increased responses to isoprenaline. There is reasonable evidence to suggest that PUFAs, especially GLA, DGLA, AA, EPA and DHA, are necessary for endothelial health and normal function (Das 2007). Our laboratory and others demonstrated that CLO or other fish oils inhibit peroxidative stress (Bechoua et al 1999; D'Aquino et al 1999; Mori et al 2000; Hünkar et al 2002), and induces an antioxidative defence against reactive oxygen species by changing the activity of catalase and glutathione-dependent enzymes (D'Aquino et al 1999; Hossain et al 1999;

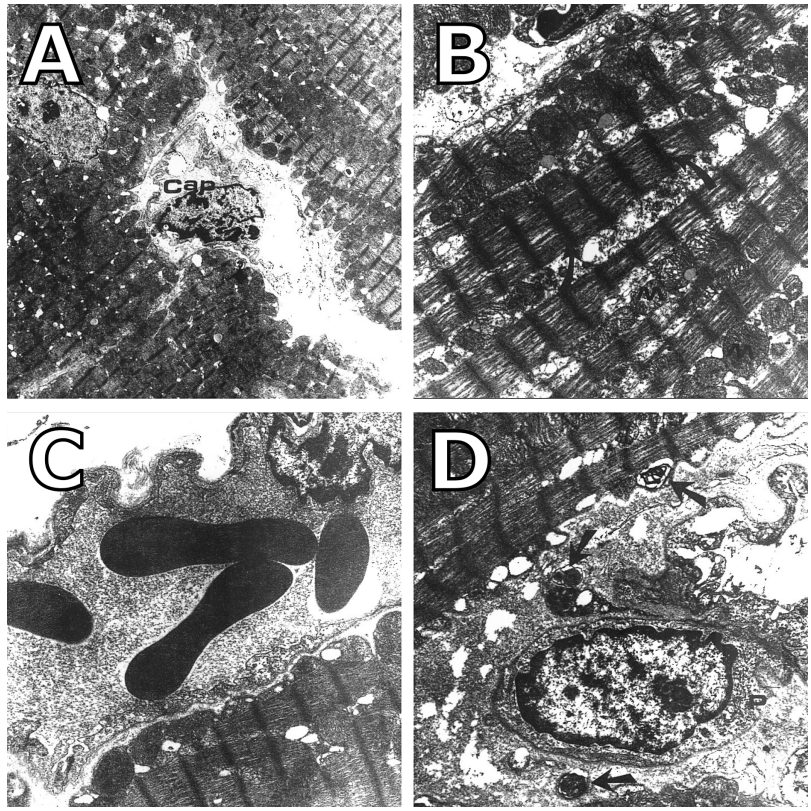

Figure 8 Ultrastructure of cod liver oil (CLO)-treated diabetic rat myocardium. A. Low-power electron micrograph, displaying cardiac muscle cells and a normal-appearing capillary (Cap) in the intermyocardial area. Uranyl acetate–lead citrate, ×3600. B. Details of the cytoplasm of cardiac muscle cells; Z lines (arrow) slightly thicker, myofibrillary organization seemed to be restored, mitochondria (M) with well defined cristae between contractile elements. Uranyl acetate–lead citrate, ×9600. C. Normal-appearing capillary, lined by thin endothelial cells containing erythrocytes in the lumen. Uranyl acetate–lead citrate, ×9600. D. Intermyocardial area, containing a flocculent material, part of the endothelial lining of degenerated blood vessels and associated normal appearing pericytes (P). Electron-dense structures, often exhibiting lamellar myelin-like formations (arrow), are seen both in the interstitium and in the peripheral cytoplasm of cardiac muscle cells. Uranyl acetate–lead citrate, ×9600.

Hünkar et al 2002). The modulating effect on calcium signalling might be another major contributing factor associated with the preventive effect of CLO on abnormal vascular reactivity. Dietary supplementation with n:3 PUFAs has been shown to suppress proliferation of vascular smooth muscle (Terano et al 1996) and inhibits store-operated calcium influx (Triboulat et al 2001). The short-term intake of n:3 PUFAs has been shown to improve endothelial cell markers, such as thrombomodulin, von Willebrand factor, tissue plasminogen activator antigen and soluble forms of the cell adhesion molecules E-selectin, P-selectin and vascular cell adhesion molecule-1 (Seljeflot et al 1998). On the other hand, long-term administration of EPA ethyl ester improved endothelial dysfunction and stimulated EDRF/NO production in different model of diabetes mellitus (Brown & Hu 2000; Nishimura et al 2000; Nobukata et al 2000). Studies have shown that fish oil or EPA/DHA has a pronounced inhibitory effect on the expression of cytokines in endothelial cells, inhibits leucocyte–endothelial cell interactions, down-regulates inflammatory processes and inhibits early atherosclerotic lesions (DeCaterina et al 1994) and the progression of atherosclerosis in diabetes (Ni et al 1994; Das 2007). The above-mentioned factors might also account for the cardioprotective effect of CLO. The beneficial effects of CLO on basal cardiac mechanics and the responses to β-adrenoceptor agonists may largely depend on tissue protection against free-radical-mediated oxidative and carbonyl stress. Furthermore, n:3 fatty acids increase heart rate (Das 2000), decrease cardiac noradrenaline in diabetic rats (Nishimura et al 2000), prevent calcium overload and modulate L-type calcium channels in the heart (Hallaq et al 1992).

On the other hand, vitamin A, a component of CLO, is a powerful antioxidant, has been shown to protect the cardiovascular system through controlling hyperglycaemia, inhibiting dyslipidaemia and peroxidative stress and maintaining antioxidant tissue defence in diabetic rats (Zobali et al 2002a, b). We previously showed that the treatment of diabetic rats only with insulin does not achieve an adequate inhibition in oxidative, especially peroxidative, stress, but the antioxidant vitamin A alone is able to do so, and use of vitamin A together with insulin provides a better metabolic control and more benefits than use of insulin alone in the reduction of vascular complications in an animal model of diabetes (Zobali et al 2002a, b). In a recent clinical study, it has been shown that neither normalization of glycaemia nor vitamin C treatment alone is able to normalize endothelial dysfunction

or oxidative stress. However, a combination of insulin and vitamin C normalized endothelial dysfunction and decreased oxidative stress to normal levels (Ceriello et al 2007).

Conclusion

Our study showed that CLO can protect vasculature and myocardium against experimental diabetes-induced oxidative modifications that may have significance for diabetic patients in the management of metabolic and cardiovascular disorders, but the study needs to be conducted in human diabetics. On the other hand, as an adjunct to insulin, Type-1 diabetics have to mostly use other compounds, such as an antihypertensive, antioxidant (i.e. vitamins), lipid-lowering agents and other agents that inhibit platelet aggregation, stimulate nitric oxide production and modulate cardiac, renal and neuronal functions to control diabetes-induced complications. CLO represents the majority of these mentioned benefits, most probably because of its rich content, including vitamins, antioxidants, DHA, PUFA and EPA.

References

Altan, V. M., Karasu, C., Ozuari, A. (1989) The effects of type-1 and type-2 diabetes in endothelium-dependent relaxation in rat aorta. *Pharmacol. Biochem. Behav.* **33**: 519–522

Austin, C. E., Chess-Williams, R. (1992) Transient elevation of cardiac beta-adrenoceptor responsiveness and receptor number in the streptozotocin-diabetic rat. *J. Autonomic Pharmacol*. **12**: 205–214

Avogaro, A., Fadini, G. P., Gallo, A., Pagnin, E., de Kreutzenberg, S. (2006) Endothelial dysfunction in type 2 diabetes mellitus. *Nutr. Metab. Cardiovasc. Dis*. **1** (Suppl.): S39–S45

Axelrod, L. (1998) Omega-3 fatty acids in diabetes mellitus. Gift from the sea? *Diabetes* **38**: 539–543

Baynes, J. W. (1991) Role of oxidative stress in development of complications in diabetes. *Diabetes* **40**: 405–412

Bechoua, S., Dubois, M., Dominguez, Z., Goncalves, A., Nemoz, G., Lagarde, M., Prigent, A. F. (1999) Protective effect of docosahexaenoic acid against hydrogen peroxide-induced oxidative stress in human lymphocytes. *Biochem. Pharmacol*. **57**: 1021–1030

Beenen, O. H., Batink, H. D., Pfaffendorf, M., van Zwieten, P. A. (1997) Beta-adrenoceptors in the hearts of diabetic-hypertensive rats: radioligand binding and functional experiments. *Blood Pressure* **6:** 44–51

Beitz, J., Schimke, E., Liebaug, U., Block, H. U., Beitz, A., Honigmann, G., Sziegoleit, W., Muller, G., Mest, H. J. (1986) Influence of a cod liver oil diet in healthy and insulin-dependent diabetic volunteers on fatty acid pattern, inhibition of prostacyclin formation by low density lipoprotein (LDL) and platelet thromboxane. *Klin. Wochenschr*. **64**: 793–799

Booth, R. J., Hodgson, W. C. (1993) Effects of aldose reductase inhibition with epalrestat on diabetes-induced changes in rat isolated atria. *Clin. Exp. Pharmacol. Physiol*. **20**: 207–213

Brown, A. A., Hu, F. B. (2000) Dietary modulation of endothelial function: implications for cardiovascular disease. *Am. J. Clin. Nutr*. **73**: 673–686

Ceriello, A., Kumar, S., Piconi, L., Esposito, K., Giugliano, D. (2007) Simultaneous control of hyperglycemia and oxidative stress normalizes endothelial function in type 1 diabetes. *Diabetes Care* **30**: 649–654

Ceylan, A., Karasu, C., Aktan, F., Ozansoy, G. (2004) Simvastatin treatment restores vasoconstriction and the inhibitory effect of LPC on endothelial relaxation via affecting oxidizing metabolism in diabetic rats. *Diabetes Nutr. Metab*. **17**: 203–210

Chang, K. C., Chung, S. Y., Chong, W. S., Suh, J. S, Kim, S. H., Noh, H. K., Seong, B. W., Ko, H. J., Chun, K.W. (1993) Possible superoxide radical-induced alteration of vascular reactivity in aortas from streptozotocin-treated rats. *J. Pharmacol. Exp. Ther*. **266**: 992–1000

Chiariello, M., Ambrosio, G., Cappelli-Bigazzi, M., Perrone-Filardi, P., Brigante, F., Sifola, C. (1986) A biochemical method for the quantitation of myocardial scarring after experimental coronary artery occlusion. *J. Mol. Cell. Cardiol*. **18**: 283–290

D'Aquino, M., Benedetti, P. C., Di Felice, M., Gentili, V., Tomassi, G., Maiorino, M., Ursini, F. (1999) Effect of fish oil and coconut oil on antioxidant defence system and lipid peroxidation in rat liver. *Free Radic. Res. Commun*. **12–13**: 147–152

Das, U. N. (2000) Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? *Prostaglandins Leukot. Essent. Fatty Acids* **63**: 351–362

Das, U. N. (2007) A defect in the activity of Delta6 and Delta5 desaturases may be a factor in the initiation and progression of atherosclerosis. *Prostaglandins Leukot. Essent. Fatty Acids* **76**: 251–268

DeCaterina, R., Cybulsky, M. I., Clinton, S. K., Gimbrone, M. A., Libby, P. (1994) The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler. Thromb*. **14**: 1829–1836

Dincer, U. D., Onay, A., Ari, N., Ozcelikay, A. T., Altan, V. M. (1988) The effects of diabetes on beta-adrenoceptor mediated responsiveness of human and rat atria. *Diabetes Res. Clin. Pract*. **40**: 113–122

Dincer, U. D., Bidasee, K. R., Guner, S., Tay, A., Ozcelikay, A. T., Altan, V. M. (2001) The effect of diabetes on expression of beta1-, beta2-, and beta3-adrenoreceptors in rat hearts. *Diabetes* **50**: 455–461

Ganguly, P. K., Beamish, R. E., Dhalla, K. S., Innes, I. R., Dhalla, N. S. (1987) Norepinephrine storage, distribution, and release in diabetic cardiomyopathy. *Am. J. Physiol*. **252**: E734–E739

Ghebremeskel, K., Bitsanis, D., Koukkou, E., Lowy, C., Poston, L., Crawford, M. A. (2002) Liver triacylglycerols and free fatty acids in streptozotocin-induced diabetic rats have atypical n-6 and n-3 pattern. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*. **132**: 349–354

Hallaq, H., Smith, T. W., Leaf, A. (1992) Modulation of dihydropyridine-sensitive calcium channels in heart cells by fish oil fatty acids. *Proc. Natl Acad. Sci. USA* **89**: 1760–1764

Hilpert, K. F., West, S. G., Kris-Etherton P. M., Hecker, K. D., Simpson, N. M., Alaupovic, P. (2007) Postprandial effect of n-3 polyunsaturated fatty acids on apolipoprotein B-containing lipoproteins and vascular reactivity in type 2 diabetes. *Am. J. Clin. Nutr*. **85**: 369–376

Horrobin, D. F. (1993) Fatty acid metabolism in health and disease: the role of delta-6-desaturase. *Am. J. Clin. Nutr*. **57** (Suppl. 5): 732S–736S

Hossain, M. S., Hashimoto, M., Gamoh, S., Masumura, S. (1999) Antioxidative effects of docosahexaenoic acid in the cerebrum versus cerebellum and brainstem of aged hypercholesterolemic rats. *J. Neurochem*. **72**: 1133–1338

Howarth, F. C., Qureshi, M. A, Lawrence, P., Adeghate, E. (2000) Chronic effects of streptozotocin-induced diabetes on the ultrastructure of rat ventricular and papillary muscle. *Acta Diabetol*. **37**: 119–124

Hun, C. S., Hasegawa, K., Kawabata, T., Kato, M., Shimokawa, T., Kagawa, Y. (1999) Increased uncoupling protein2 mRNA in white adipose tissue, and decrease in leptin, visceral fat, blood glucose, and cholesterol in KK-Ay mice fed with eicosapentaenoic and docosahexaenoic acids in addition to linolenic acid. *Biochem. Biophys. Res. Commun*. **259**: 85–90

Hünkar, T., Aktan, F., Ceylan, A., Karasu, C. (2002) Effects of cod liver oil on tissue antioxidant pathways in normal and streptozotocin-diabetic rats. *Cell Biochem. Funct*. **20**: 297–302

Kamata, K., Miyata, N., Kasuya, Y. (1989) Involvement of endothelial cells in relaxation and contraction responses of the aorta to isoproterenol in naive and streptozotocin-induced diabetic rats. *J. Pharmacol. Exp. Ther*. **249**: 890–894

Kamata, K., Satoh, T., Tanaka, H., Shigenobu, K. (1997) Changes in electrophysiological and mechanical responses of the rat papillary muscle to alpha- and beta-agonist in streptozotocin-induced diabetes. *Can. J. Physiol. Pharmacol*. **75**: 781–788

Karasu, C. (1998) Acute probucol treatment partially restores vasomotor activity and abnormal lipid metabolism whereas morphological changes are not affected in aorta from long-term STZ-diabetic rats. *Exp. Clin. Endocrinol. Diabetes* **106**: 189–196

Karasu, C., Ozturk, Y., Altan, N., Yildizoglu-Ari, N., Ikizler, C., Altan, V. M. (1990) Thyroid hormones mediated effect of insulin on alloxan diabetic rat atria. *Gen. Pharmacol*. **21**: 735–740

Karasu, C., Dewhurst, M., Stevens, E. J., Tomlinson, D. R. (1995) Effects of anti-oxidant treatment on sciatic nerve dysfunction in streptozotocin-diabetic rats; comparison with essential fatty acids. *Diabetologia* **38**: 129–134

Karasu, C., Ozansoy, G., Bozkurt, O., Erdogan, D., Omeroglu, S. (1997a) Antioxidant and triglyceride-lowering effects of vitamin E associated with the prevention of abnormalities in the reactivity and morphology of aorta from streptozotocin-diabetic rats. Antioxidants in Diabetes-Induced Complications. (ADIC) Study Group. *Metabolism* **46**: 872–879

Karasu, Ç., Ozansoy, G., Bozkurt, O., Erdogan, D., Omeroglu, S. (1997b) Isoprenaline-induced endothelium-dependent and -independent relaxation of aorta in long-term STZ-diabetic rats: reversal effect of dietary vitamin E. *Gen. Pharmacol*. **29**: 561–567

Koçak, G., Aktan, F., Canbolat, O., Özoğul, C., Elbeğ, Ş., Yıldızoğlu-Arı, N., Karasu, Ç. (2000) Alpha-lipoic acid treatment ameliorates metabolic parameters, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes (The ADIC Study Group) *Diab. Nutr. Metab*. **13**: 308–319

Kunusaki, M., Bursell, S. E., Umeda, F., Nawata, H., King, G. L. (1994) Normalization of diacylglycerol-protein kinase C activation by vitamin E in aorta of diabetic rats and rat smooth muscle cells exposed to elevated glucose levels. *Diabetes* **43**: 1372–1377

Latifpour, J., McNeill, J. H. (1984) Cardiac autonomic receptors: effect of long-term experimental diabetes. *J. Pharmacol. Exp. Ther*. **230**: 242–249

Linn, T., Noke, M., Woehrle, M., Kloer, H. U., Hammes, H. P., Litzlbauer, D., Bretzel, R. G., Federlin, K. (1989) Fish oil-enriched diet and reduction of low-dose streptozocin-induced hyperglycemia. Inhibition of macrophage activation. *Diabetes* **38**: 1402–1411

Lowry, O. H., Rosenbrough, N. J., Farr, A. L, Randall, R. F. (1951) Protein measurement with folin phenol reagent. *J. Biol. Chem*. **193**: 265–275

McGrath, G. M., McNeill, J. H. (1986) Cardiac ultrastructural changes in streptozotocin-induced diabetic rats: effects of insulin treatment. *Can. J. Cardiol*. **2**: 164–169

Miyata, T., Ishikawa, N., van Ypersele de Strihou, C. (2003) Carbonyl stress and diabetic complications. *Clin. Chem. Lab. Med.* **41**: 1150–1158

Mohan, I. K., Das, U. N. (2000) Effect of L-arginine-nitric oxide system on the metabolism of essential fatty acids in chemical-induced diabetes mellitus. *Prostaglandins Leukot. Essent. Fatty Acids* **62**: 35–46

Mohan, I. K., Das, U. N. (2001) Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. *Nutrition* **17**: 126–151

Mori, T. A., Puddey, I. B., Burke, V., Croft, K. D., Dunstan, D. W., Rivera, J. H., Beilin, L. J. (2000) Effect of omega-3 fatty acids on oxidative stress in humans: GC-MS measurement of urinary F_2-isoprostane excretion. *Redox Report* **5**: 45–46

Mustad, V. A., Demichele, S., Huang, Y. S., Mika, A., Lubbers, N., Berthiaume, N., Polakowski, J., Zinker, B. (2006) Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse. *Metabolism* **55**: 1365–1374

Ni, J. S., Wu, J. X., Xiao, R. Q. (1994) The preventive and curative action of fish oil compound on early atherosclerotic lesions in the aortic of diabetic rats. *Zhonghua Bing Li Xue Za Zhi* **23**: 31–33

Nishimura, M., Nanbu, A., Komori, T., Ohtsuka, K., Takahashi, H., Yoshimura, M. (2000) Eicosapentaenoic acid stimulates nitric oxide production and decreases cardiac noradrenaline in diabetic rats. *Clin. Exp. Pharmacol. Physiol*. **27**: 618–624

Nobukata, H., Ishikawa, T., Obata, M., Shibutani, Y. (2000) Long-term administration of highly purified eicosapentaenoic acid ethyl ester improves the dysfunction of vascular endothelial and smooth muscle cells in male WBN/Kob rats. *Metabolism* **49**: 1588–1591

Nosari, I., Cortinovis, F., Lepore, G., Maglio, M. L., Pagani, G. (1994) Use of omega-3 in diabetic patients. *Clin. Ther*. **144**: 213–221

Ozansoy, G., Akin, B., Aktan, F., Karasu, Ç. (2001) Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does not alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats. *Mol. Cell. Biochem*. **216**: 59–63

Pekiner, B., Ulusu, N. N., Das-Evcimen, N., Sahilli, M., Aktan, F., Stefek, M., Stolc, S., Karasu, Ç. (2002) In vivo treatment with stobadine prevents lipid peroxidation, protein glycosylation and calcium overload but does not ameliorate Ca^{+2}-ATPase activity in heart and liver of streptozotocin-diabetic rats: comparison with vitamin E. *Biochim. Biophys. Acta* **1588**: 71–78

Pieper, G. M. (1999) Enhanced, unaltered and impaired nitric oxide-mediated endothelium-dependent relaxation in experimental diabetes mellitus: importance of disease duration. *Diabetologia* **42**: 204–213

Pieroni, G., Vague, P., Coste, T. C. (2007) At low doses, a gamma-linolenic acid-lipoic acid conjugate is more effective than docosahexaenoic acid-enriched phospholipids in preventing neuropathy in diabetic rats. *J. Nutr*. **137**: 368–372

Pitel, S., Raccah, D., Gerbi, A., Reddi, A. S. (1998) Collagen metabolism in the myocardium of normal and diabetic rats. *Exp. Mol. Pathol*. **48**: 236–243

Reusch, J. E., Draznin, B. B. (2007) Atherosclerosis in diabetes and insulin resistance. *Diabetes Obes. Metab*. **9**: 455–463

Savage, A. O., Dunbar, J. C., Brown, R. A. (1995) Effects of acetaldehyde on the isolated papillary muscle of diabetic rats. *J. Cardiovasc. Pharmacol*. **26**: 251–258

Seljeflot, I., Arnesen, H., Brude, I. R., Nenseter, M. S., Drevon, C. A., Hjermann, I. (1998) Effects of omega-3 fatty acids and/or antioxidants on endothelial cell markers. *Eur. J. Clin. Invest.* **28**: 629–635

Simopoulos, A. P. (1999) Essential fatty acids in health and chronic disease. *Am. J. Clin. Nutr*. **70** (Suppl.): 560S–569S

Stene, L. C., Ulriksen, J., Magnus, P., Joner, G. (2000) Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. *Diabetologia* **43**: 1093–1098

Suresh, Y., Das, U. N. (2003) Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus. Effect of omega-3 fatty acids. *Nutrition* **19**: 213–228

Takahashi, R., Morse, N., Horrobin, D. F. (1998) Plasma, platelet, and aorta fatty acids composition in response to dietary n-6 and n-3 fats supplementation in a rat model of non-insulin-dependent diabetes. *J. Nutr. Sci. Vitaminol. (Tokyo)* **34**: 413–421

Terano, T., Shiina, T., Tamura, Y. (1996) Eicosapentaenoic acid suppressed the proliferation of vascular smooth muscle cells through modulation of various steps of growth signals. *Lipids* **31** (Suppl.): S301–S304

Triboulat, C., Hichami, A., Denys, A., Khan, N. A. (2001) Dietary (n-3) polyunsaturated fatty acids exert antihypertensive effects by

modulating calcium signaling in T cells of rats. *J. Nutr*. **131**: 2364–2369

Wang, J., Song, Y., Wang, Q., Kralik, P. M., Epstein, P. N. (2006) Causes and characteristics of diabetic cardiomyopathy. *Rev. Diabet. Stud*. **3**: 108–117

Warley, A., Powell, J. M., Skepper, J. N. (1995) Capillary surface-area is reduced and tissue thickness from capillaries to myocytes is increased in the left-ventricle of streptozotocin-diabetic rats. *Diabetologia* **38**: 413–421

Woodiwiss, A. J., Kalk, W. J., Norton, G. R. (1996) Habitual exercise attenuates myocardial stiffness in diabetes mellitus in rats. *Am. J. Physiol*. **271**: H2126–H2133

Yuichiro, J. S., Ford, G. D. (1992) Superoxide stimulates IP3-induced Ca^{2+} release from vascular smooth muscle sarcoplasmic reticulum. *Am. J. Physiol*. **262**: H114–H116

Zobali, F., Besler, T., Ari, N., Karasu, C. (2002a) Hydrogen peroxide-induced inhibition of vasomotor activity: evaluation of single and combined treatments with vitamin A and insulin in streptozotocin-diabetic rats. *Int. J. Exp. Diabet. Res*. **3**: 119–130

Zobali, F., Besler, T., Ari, N. & Karasu, C. (2002b) Hydrogen peroxide-induced inhibition of vasomotor activity: evaluation of single and combined treatments with vitamin A and insulin in streptozotocin-diabetic rats. *Int. J. Exp. Diabet. Res*. **3**: 119–130