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Modification of myosin isozymes and SR Ca^{2+}-pump ATPase of the diabetic rat heart by lipid-lowering interventions

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Abstract

To define metabolic influences on cardiac myosin expression and sarcoplasmic reticulum (SR) Ca^{2+}-stimulated ATPase, streptozotocin-diabetic rats were treated for 9–10 wk with etomoxir, an inhibitor of carnitine palmitoyl transferase I (CPT-1) and fatty acid synthesis, or an antilipolytic drug, acipimox. Etomoxir reduced myosin V_3 of diabetic rats but did not normalize it. However, the high serum triglyceride, free-fatty acid and cholesterol concentrations in diabetic animals were greatly reduced. After bypassing the CPT-1 inhibition with a medium-chain fatty acid (miglyol) diet, the V_3 contents and serum lipids were still reduced in the etomoxir-treated diabetic rats; V_3 was also reduced in diabetic rats fed miglyol or treated with acipimox. Since low serum insulin or triiodothyronine concentrations in diabetic rats were not improved by these interventions but changes in V_3 were correlated with those in triglyceride, free-fatty acid and cholesterol concentrations, it is likely that myosin may be influenced by some metabolic factors. To assess the role of adrenergic influences, diabetic rats (7–8 wk) were treated with an antisympathotonic drug, moxonidine, a β-adrenoceptor blocking drug, propranolol, and a bradycardic drug, tedisamil. Myosin V_3 was not reduced significantly in moxonidine-treated or propranolol-treated rats in comparison to untreated diabetic rats. Serum thyroid hormones and insulin were not altered, whereas triglycerides were reduced but not significantly by these antiadrenergic agents. Lowering serum lipids in diabetic rats by treatment with etomoxir, miglyol and acipimox increased the depressed SR Ca^{2+}-stimulated ATPase activity. On the other hand, in diabetic rats treated with moxonidine, propranolol or tedisamil, the ATPase activity was not increased significantly. These results suggest that normalization of blood lipids is important for improving subcellular organelle function in diabetic hearts with impaired glucose utilization. (Mol Cell Biochem **132:** 69–80, 1994)

Key words: diabetic heart, myosin isozymes, sarcoplasmic reticulum Ca^{2+}-stimulated ATPase, blood lipids

Introduction

Diabetes mellitus is known to impair the function of various subcellular organelles of heart muscle [1]. The expression of myosin heavy chain (MHC) genes is shifted in favor of β-MHC leading to a reduced speed of diabet-

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ic cardiac muscle shortening [2, 3]. Among structures involved in the Ca^{2+} homeostasis of the myocyte, the activity of the Ca^{2+}-pump of sarcoplasmic reticulum (SR) is depressed which contributes to the delayed relaxation of diabetic heart [4]. Although it has been shown that these alterations can be reversed by treating diabetic rats with insulin [2, 4], the cellular signals remain poorly understood. Circulating thyroid hormones are depressed in diabetes [2–4] and are thus considered to contribute to the changes in MHC expression [5]. However, normalization of thyroid hormones in diabetic animals failed to prevent the increased β-MHC expression [4, 6]. Since the adrenergic activity during the initial stages of diabetes is increased [7] and the fuel utilization is deranged [3] it is possible that metabolic changes as a consequence of altered adrenergic activity and fuel utilization play an important role in the genesis of subcellular alterations in diabetic heart. To define metabolic influences on MHC expression and SR Ca^{2+}-pump, rats were made diabetic with streptozotocin [3, 4].

To assess the effects of the adrenergic nervous system, diabetic rats were treated with the centrally acting antisympathotonic drug, moxonidine [8, 9], the β-adrenoceptor blocking drug, propranolol [10] and the bradycardic drug, tedisamil [11, 12]. Changes in MHC expression were examined at the protein level by determining the myosin isozyme population and alterations in the SR Ca^{2+}-pump were monitored by measuring the Ca^{2+}-stimulated ATPase activity. In diabetes, the hormone-sensitive triglyceride lipase of adipose tissue becomes activated because the balance between the inhibitory action of insulin and the stimulatory action of catecholamines as well as glucagon is deranged due to the depressed insulin production [13]. Since high blood free-fatty acids (FFA) result in an enhanced synthesis of triglycerides in the heart, diabetic rats were treated with various drugs that are expected to reduce the elevated levels of blood lipids and, thereby, the increased fatty acid utilization in the diabetic heart [14]. In the first approach, diabetic rats were treated with etomoxir which inhibits mitochondrial carnitine palmitoyltransferase 1 (CPT-1) and thus reduces the fatty acid oxidation [15, 16]. Because etomoxir also inhibits the *de novo* fatty acid synthesis [17, 18], the CPT-1 independent effect of etomoxir was assessed. In this case the etomoxir-treated diabetic rats were fed a diet containing the medium-chain fatty acids, octanoate and decanoate ('miglyol'), which can penetrate the mitochondrial membrane independently of the CPT-1 shuttle [14]. It was previously shown that the cardiac growth due to CPT-1 inhibition is prevented by such a diet [19] and any effect of etomoxir in the etomoxir-treated rats fed miglyol, can thus be seen due to reduced fatty acid supply to the heart. To examine the possible direct effect of the elevated medium-chain fatty acids, diabetic rats were fed the miglyol diet. Furthermore, diabetic rats were also treated with the antilipolytic drug acipimox [20].

Materials and methods

Animals and materials

Male Sprague-Dawley rats (8 weeks old) were purchased from Charles River (Montreal, Canada). The rats had free access to food and tap water. The regular chow (5% fat) and a 'fat-free' (less than 1% fat) diet were purchased from Agway (Syracuse, NY). Miglyol 812 (50–65% octanoate, 30–45% decanoate) was obtained from Huels AG (Marl, Germany). The fat-free diet was supplemented with miglyol to a fat content of 5%. Enantiomeric etomoxir (Ethyl (R)-(+)-2-[6-(2-chlorophenoxy)hexyl]oxirane-2-carboxylate, B 877-44) was provided by Dr. H.P.O. Wolf of Byk-Gulden (Konstanz, Germany), acipimox by Dr. K.J. Krüger of Farmitalia Carlo Erba (Freiburg, Germany), moxonidine (PHYSIOTENS®) by Dr. U. Kühl of Kali Chemie-Pharma (Hannover, Germany) and tedisamil (KC 8857) by Dr. D. Thormählen of Kali Chemie-Pharma. DL-propranolol was from Sigma (St. Louis, MO). Diabetes mellitus was induced in rats by intrafemoral injection of 65 mg/kg body wt streptozotocin [3, 4] and various treatments were started the following day. After decapitation, the hearts were removed and 10–20 mg portions of the left and right ventricles were frozen in liquid nitrogen for determination of myosin isozymes whereas SR vesicles were prepared from the fresh ventricles. The first 5–7 ml of blood from the trunk was collected and the serum was used for determination of lipids and hormones. Animal care and experimental procedures were according to the institutional guidelines.

Etomoxir- and acipimox-treatments

Sixteen diabetic rats were fed a regular diet and half of these animals were daily administered by gavage 15 mg/kg body wt etomoxir [15, 16] for 9–10 wk. Sixteen diabetic rats were fed the miglyol diet and half of these animals were daily administered by gavage 15 mg/kg body wt

etomoxir for 9–10 wk. Eight diabetic rats were fed the regular diet and were given 50 mg/kg body wt acipimox [20] daily in the drinking water for 9–10 wk. The acipimox intake was calcualted from the water consumption of the rats. Eight untreated normal rats served as controls for the etomoxir-, miglyol- or acipimox-treated group of diabetic rats.

Moxonidine-, propranolol- and tedisamil-treatments

Seven diabetic rats were treated with 8 mg/kg body wt moxonidine [8, 9], seven rats with 50 mg/kg body wt propranolol and seven diabetic rats with 35 mg/kg body wt tedisamil [11, 12]. All drugs were mixed with the powdered regular diet. Drug doses were calculated based on the food intake. Seven diabetic rats and seven control rats received the same powdered diet as the treated rats. Treatment duration was 7–8 wk.

Myosin isozymes

The proportion of myosin isozymes was determined by non-dissociating polyacrylamide gel electrophoresis in the presence of pyrophosphate [21]. The myosin isozymes were stained with Coomassie brilliant blue R250 and the gels were scanned using an Ultroscan laser densitometer (LKB, Bromma, Sweden). The isozymes were quantitated by measuring peak heights. If an isozyme was less than 5%, the isozyme profile was fitted to an envelope consisting of three Gaussian curves that were integrated [21]. For classifying the pattern of MHC distribution, the isozymes were plotted in a ternary diagram and were compared with an MHC distribution assuming assortative pairing of MHC [21]. The theoretical distribution was derived from the following equations: $V_1 = (\alpha\text{-MHC})^2 + c(\alpha\text{-MHC})\,(\beta\text{-MHC})$, $V_2 = 2(1\text{-}c)(\alpha\text{-MHC})(\beta\text{-MHC})$, $V_3 = (\beta\text{-MHC})^2 + c(\alpha\text{-MHC})(\beta\text{-MHC})$, whereby c denotes the proportion of MHC that does not form dimers randomly. The parameter c was estimated as 0.37 [21].

Sarcoplasmic reticulum

Fragmented SR was isolated as described [3]. The total ATPase activity was assayed in a medium containing 100 mM KCl, 5 mM NaN_3, 5 mM $MgCl_2$, 0.01 mM free Ca^{2+}, 5 mM ATP and 20 mM Tris-Cl, pH 6.8. The basal activity measured in the presence of 2 mM EGTA was subtracted from the total activity to obtain Ca^{2+}-stimulated ATPase activity.

Serum parameters

The concentrations of triiodothyronine (T_3) and thyroxine (T_4) were determined by fluoroimmunoassays (Delfia; Pharmacia, Fairfield, NJ). Glucose was measured using the Sigma kit no. 16-UV, triglycerides using the Sigma kit no. 336 and total cholesterol using the Sigma kit no. 352. High-density lipoprotein (HDL) cholesterol was determined in the supernatant after precipitation of the low- and very low-density lipoproteins using the heparin-manganese chloride technique [22]. Nonesterified free-fatty acids (FFA) were measured using the NEFA-C enzymatic colorimetric assay (Wako, Dallas, Tx). Insulin was determined using a rat insulin RIA kit (Linco Research, St. Louis, MO).

Heart rates

Hearts rates were derived from the electrocardiogram of conscious control and diabetic rats treated with moxonidine, propranolol or tedisamil as described previously [23]. Essentially, the R waves of a rat sitting on small steel plates (2 rows of 6 plates sized 5 × 9 cm) attached to the bottom of the rat cage were monitored using a storage oscilloscope (Tektronix 5111A, Tektronix, Barrie, Ont).

Statistical evaluation

The equality of variances was checked by Cochran's C test. In case of unequal variances, a Box-Cox transformation was performed before analysis of variance (STATGRAPHICS of STSC, Rockville, Md). Multiple comparisons were made by Duncan's new multiple range test. Values are mean ± SD; statistical significance was assumed at $P < 0.05$.

Results

Growth and endocrine characteristics

The rats injected with 65 mg/kg body wt streptozotocin

Table 1. Effects of etomoxir and acipimox on growth characteristics and serum parameters in diabetic rats

	BW (g)	LVW (mg)	RVW (mg)	TG (mM)	FFA (μEq/l)	Cholesterol (mM)	HDL (mM)
Control	481 ± 36	918 ± 84	259 ± 34	2.6 ± 0.9	310 ± 84	2.4 ± 0.2	1.5 ± 0.2
Diabetic	353 ± 36**	772 ± 65**	206 ± 35	15.7 ± 4.2**	703 ± 276**	5.1 ± 2.6**	1.5 ± 0.4
Diabetic + etomoxir	353 ± 26	869 ± 99*	241 ± 27	3.6 ± 0.8*	332 ± 53*	2.8 ± 0.3*	2.1 ± 1.2
Diabetic + etomoxir + miglyol	337 ± 33	756 ± 95	213 ± 41	5.8 ± 4.1*	523 ± 159*	2.8 ± 0.9*	1.2 ± 0.2
Diabetic + miglyol	341 ± 52	709 ± 75	202 ± 34	9.0 ± 5.3*	590 ± 97	3.4 ± 0.9*	1.5 ± 0.1
Diabetic + acipimox	354 ± 39	779 ± 92	207 ± 29	9.5 ± 4.1*	702 ± 184	3.5 ± 0.7*	1.7 ± 0.2

** $P < 0.05$ between untreated diabetic and control rats; * $P < 0.05$ between drug-treated and untreated diabetic rats. Number of rats was eight in each group. Abbreviations: BW, body wt; LVW, left ventricular wt; RVW, right ventricular wt; TG, triglycerides; FFA, free-fatty acids; HDL, high-density lipoprotein cholesterol.

exhibited a markedly reduced body wt and ventricular wt after 9–10 wk (Table 1). The treatment of diabetic rats with 15 mg/kg body wt etomoxir did not affect body wt but significantly increased left ventricular wt; right ventricular weight was not increased significantly (Table 1). When etomoxir-treated diabetic rats were fed the miglyol diet, the increase in ventricular wt was prevented (Table 1). The miglyol diet on its own or acipimox had no

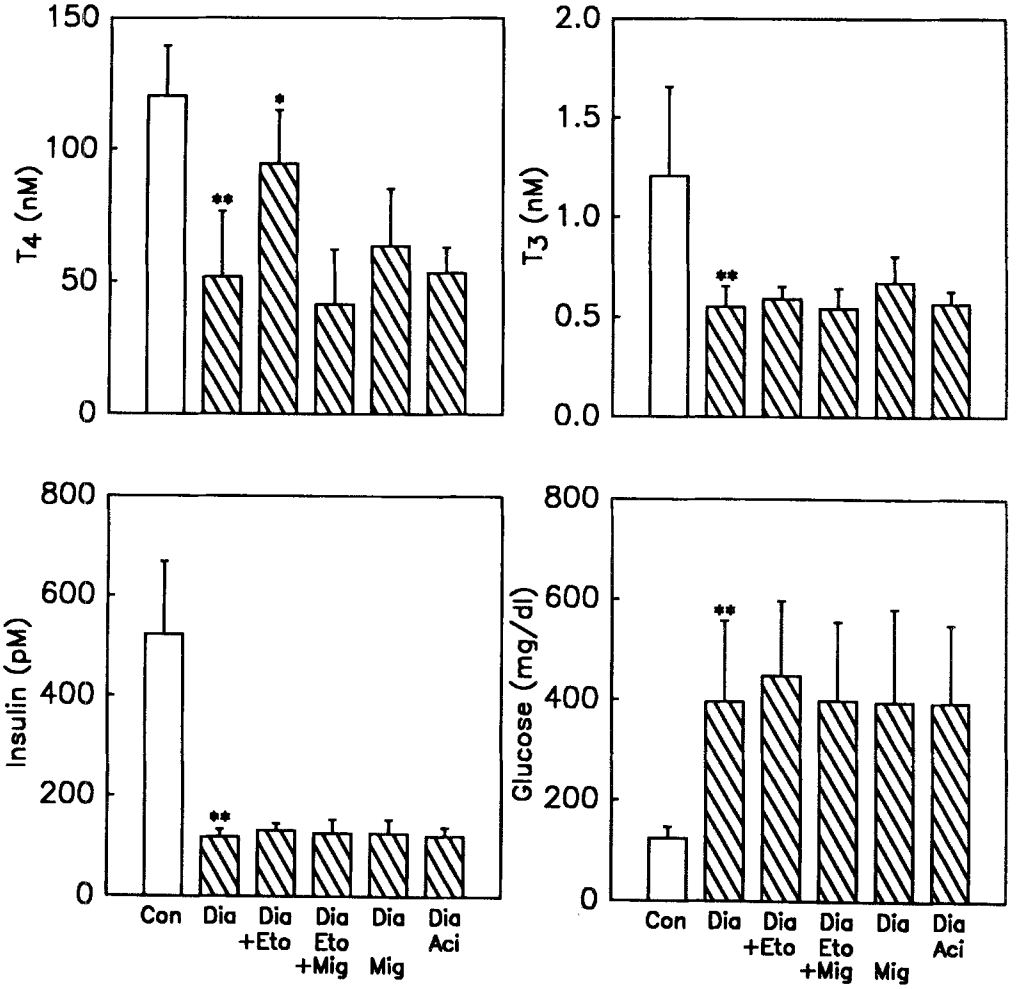

Fig. 1. Serum concentrations of T_4, T_3, insulin and glucose of control rats (Con), untreated diabetic rats (Dia), diabetic rats treated with etomoxir (Eto), diabetic rats treated with etomoxir and miglyol (Eto + Mig), diabetic rats fed miglyol (Mig) and diabetic rats treated with acipimox (Aci). ** $P < 0.05$ between untreated diabetic and control rats; * $P < 0.05$ between treated diabetic rats and untreated diabetic rats. Number of rats was eight in each group.

Table 2. Effects of moxonidine, propranolol and tedisamil on serum parameters in diabetic rats

	Glucose (mg/dl)	Insulin (pM)	T_3 (nM)	T_4 (nM)	TG (mM)	FFA (μEq/l)	Cholesterol (mM)
Control	121 ± 21	487 ± 94	1.2 ± 0.2	93 ± 5	1.4 ± 0.5	276 ± 75	2.1 ± 0.5
Diabetic	358 ± 81**	46 ± 41**	0.4 ± 0.1**	47 ± 19**	8.5 ± 2.6**	480 ± 98**	4.0 ± 0.9**
Diabetic + moxonidine	436 ± 96	48 ± 34	0.4 ± 0.1	40 ± 14	5.1 ± 4.6	334 ± 213	3.9 ± 1.4
Diabetic + propranolol	378 ± 69	48 ± 15	0.5 ± 0.1	60 ± 18	5.2 ± 3.4	373 ± 125	3.0 ± 1.0
Diabetic + tedisamil	332 ± 92	34 ± 26	0.5 ± 0.2	41 ± 15	6.1 ± 4.1	438 ± 161	3.8 ± 1.4

** $P < 0.05$ between untreated diabetic and control rats. Number of rats was seven in each group. Abbreviations: T_3, triidothyronine; T_4, thyroxine; TG, triglycerides; FFA, free-fatty acids.

significant influence on body wt or ventricular wt (Table 1). These treatments had no influence on the reduced serum insulin or increased glucose concentrations of diabetic rats (Fig. 1). Also the low serum T_3 concentrations were not increased (Fig. 1). In diabetic rats treated with etomoxir, the T_4 concentration was significantly increased but was significantly lower than that in the control rats (Fig. 1); the other treated diabetic rats did not exhibit significantly different serum T_4 concentrations compared with untreated diabetic rats (Fig. 1). Thus, these treatments did not affect established endocrine factors known to influence MHC expression or SR Ca^{2+}-stimulated ATPase activity [2, 3, 5]. In a second series of experiments, diabetic rats were treated for 7–8 wk with moxonidine, propranolol or tedisamil which had also no significant effect on serum insulin, glucose or T_3 concentrations (Table 2). Only in the case of propranolol, the serum T_4 concentration was increased but it was not significant (Table 2). These drug treatments also did not affect the diabetes-induced changes in body wt and ventricular wt (data not shown).

The drugs had distinct effects on the increased serum triglyceride, FFA and total cholesterol concentrations of diabetic rats (Table 1). In etomoxir-treated diabetic rats, triglyceride, FFA and cholesterol concentrations were greatly normalized (Table 1). When etomoxir-treated rats were fed the miglyol diet, the reduction of triglycerides and FFA was less pronounced (Table 1). The miglyol diet alone and the acipimox treatment reduced serum triglyceride and cholesterol concentrations in diabetic rats significantly (Table 1). The HDL-cholesterol concentration was neither affected by diabetes nor by etomoxir, miglyol or acipimox treatments (Table 1). The treatment of diabetic rats with moxonidine, propranolol or tedisamil did not reduce the serum triglyceride concentration significantly (Table 2).

Myosin isozymes

The diabetic rats exhibited a marked shift in the cardiac myosin isozyme population (Fig. 2). The proportion of V_3 increased from 8 ± 5% to 77 ± 15% and the proportion of V_1 decreased from 76 ± 14% to 7 ± 5% (Fig. 2). The etomoxir-treated diabetic rats exhibited a greatly reduced proportion of V_3 which was, however, significantly higher than that in the control rats (Fig. 2). The effect of etomoxir could not be prevented by feeding the miglyol diet (Fig. 2). In diabetic rats fed miglyol but not treated with etomoxir, the proportion of V_3 was also significantly reduced but it was significantly higher than that in the etomoxir-treated rats (Fig. 2). Also acipimox reduced significantly the proportion of V_3 but had a significantly smaller effect than etomoxir (Fig. 2).

Compared with left ventricles, the right ventricles of normal rats exhibited an increased proportion of V_1 and a

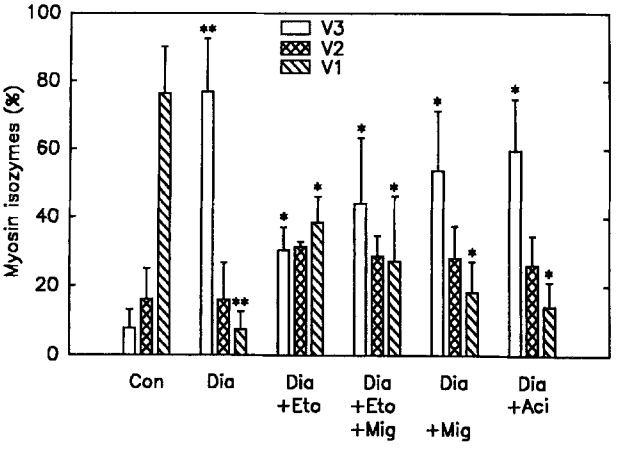

Fig. 2. The proportion of myosin isozymes V_3, V_2 and V_1 in left ventricles of control rats (Con), untreated diabetic rats (Dia), diabetic rats treated with etomoxir (Eto), diabetic rats treated with etomoxir and miglyol (Eto + Mig), diabetic rats fed miglyol (Mig) and diabetic rats treated with acipimox (Aci). ** $P < 0.05$ between untreated diabetic and control rats; * $P < 0.05$ between treated diabetic rats and untreated diabetic rats. Number of rats was eight in each group.

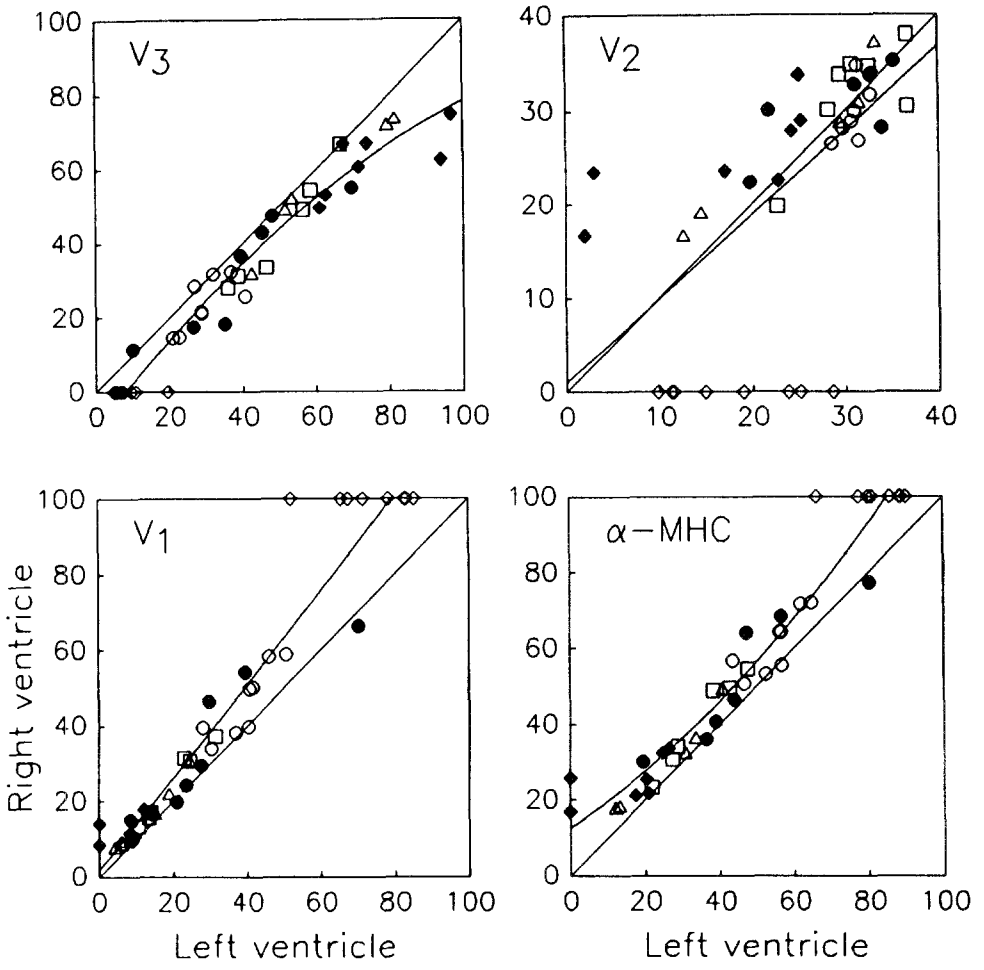

Fig. 3. Left ventricular vs. right ventricular myosin isozymes V_3, V_2, V_1 and α-MHC of control rats (◇), untreated diabetic rats (◆), diabetic rats treated with etomoxir (○), diabetic rats treated with etomoxir and miglyol (●), diabetic rats fed miglyol (□) and diabetic rats treated with acipimox (△). The following regression curves were calculated: For V_3: $y = -9.8 + 1.3x - 0.004x^2$, R = 0.97; for V_2: $y = 1.01 + 0.90x$, R = 0.64; for V_1: $y = 1.85 + 1.21x + 0.0004x^2$, R = 0.96; for α-MHC: $y = 12.6 + 0.66x + 0.004x^2$, R = 0.97 (R, square root of the coefficient of determination). For comparison, lines of identity which assume no differences in left and right ventricular isozymes are given. Number of rats was eight in each group.

reduced proportion of V_3 corresponding to an increased α-MHC (0.5 % V_2 + % V_1) proportion (Fig. 3). A higher α-MHC proportion was also observed in right ventricles of untreated or treated diabetic rats (Fig. 3) showing that chamber specific influences on MHC expression were preserved in diabetic hearts. The proportions of V_1, V_2 and V_3 of untreated and treated diabetic rats (Fig. 4) also followed a relationship established previously [21] for normal and hypertensive rats. This demonstrates that the pattern of MHC distribution at the time of sacrifice was typical of normal rats differing in age and is consistent with the view that the increase in cardiac mass of etomoxir-treated diabetic rats did not induce myofilament compartments primarily composed of α-MHC [21, 23].

To examine whether a link existed between MHC expression and serum lipids, the proportion of V_3 was plotted versus the serum triglyceride concentration of untreated or treated diabetic rats (Fig. 5A). The proportion of Vzi3 was approximately 30% if the triglyceride concentration was nearly normalized. Higher triglyceride concentrations were associated with an increased proportion of V_3 (Fig. 5A). This correlation (R = 0.84) appeared not to depend on the type of drug used for lowering serum triglycerides. Equidirectional correlations (Figs. 5B and 5C) were observed for serum FFA (R = 0.68) and serum total cholesterol concentrations (R = 0.81). No correlation was observed for serum HDL-cholesterol concentrations (Fig. 5D). It is noteworthy that the treatment of diabetic rats with moxonidine, propranolol or tedisamil affected the MHC expression in the same direction. In the moxonidine- and propranolol-treated diabetic rats, the V_3 proportion was reduced by 13% and 16% respectively, but these changes were not significant (Table 3).

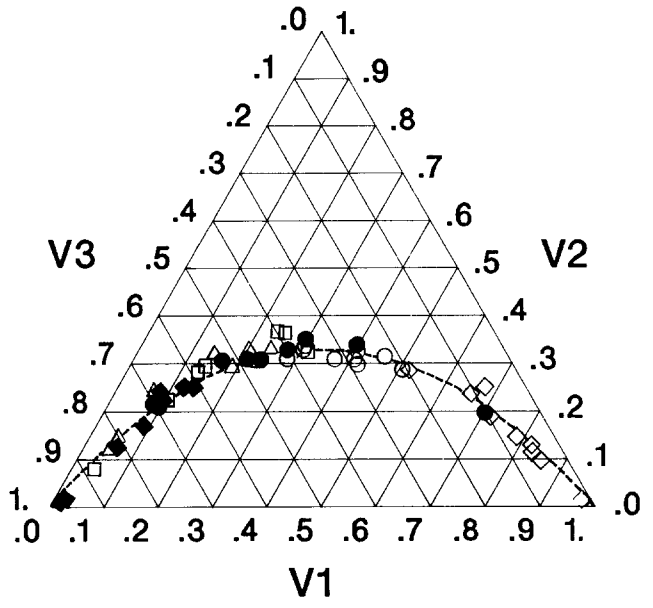

Fig. 4. Myosin isozyme populations of control rats (◇), untreated diabetic rats (◆), diabetic rats treated with etomoxir (○), diabetic rats treated with etomoxir and miglyol (●), diabetic rats fed miglyol (□) and diabetic rats treated with acipimox (△). Number of rats was eight in each group. The broken line corresponds to isozyme populations calculated for α-MHC proportions arising from a transition from V_1 to V_3 and assuming that 37% of the MHCs form preferentially homodimers (Rupp and Dietz, 1991). This calculated relationship can account for the isozyme populations of normal and spontaneously hypertensive rats of varying ages.

SR Ca^{2+}-stimulated ATPase activity

The diabetic rats exhibited a significantly reduced Ca^{2+}-stimulated ATPase activity of the SR Ca^{2+}-pump (Fig. 6). In diabetic rats treated with etomoxir, the ATPase activity was significantly increased compared with the untreated diabetic rats (Fig. 6). In the etomoxir-treated rats fed the miglyol diet, the increase was not significant. The ATPase activity however, did not differ significantly from that of control rats. In rats fed the miglyol diet or treated with acipimox, the ATPase activities were also significantly increased (Fig. 6). The treatment with moxonidine, propranolol or tedisamil resulted in an insignificant increase of the ATPase activity (Table 3).

Discussion

The aim of the present study was to examine endocrine influences on subcellular organelles of the diabetic heart. It was previously shown that insulin injections normalized changes in subcellular organelles as well as the endocrine disturbances and general growth characteristics in diabetic rats [2, 4]. Since circulating thyroid hormones are reduced in diabetic rats, these were initially thought to play an importnat role in the altered MHC expression [2]. However, because supraphysiological thyroid doses were required for normalization of the MHC expression, it was suggested that the thyroid hormone responsiveness was reduced or that additional signals were involved [6]. Also the rate of SR Ca^{2+} uptake could not be normalized by substituting thyroid hormones [4]. Thus it became apparent that the most likely influences affecting MHC expression could arise from altered metabolic [3] or increased catecholamine [23, 24] influences.

To define the relative contribution of metabolic influences, diabetic rats were treated with drugs that are expected to reduce the high blood lipid concentrations. In contrast to insulin substitution, the drugs employed were not expected to normalize the general diabetic state. Etomoxir is known to inhibit mitochondrial CPT-1, reduce myocardial fatty acid oxidation [15, 16] and, in a compensatory manner, increase glucose oxidation [25]. Besides this well documented effect of agents belonging to the group of oxirane-2-carboxylates [14], etomoxir has also been shown to inhibit the *de novo* fatty acid synthesis [17, 18]. Such an effect can account for the finding that a reduced fatty acid utilization was associated with reduced serum FFA and triglyceride concentrations. When etomoxir was administered acutely, the increased blood FFA and triglyceride concentrations in diabetic animals were not affected [26]; however, the chronic etomoxir treatment is considered not only to inhibit mitochondrial fatty acid oxidation but also to reduce the fatty acid supply to the heart. To further define the two actions of etomoxir, the CPT-1 inhibition was bypassed by feeding a medium-chain fatty acid diet. An octanoate/decanoate mixture (Miglyol) was added to a diet containing less than 1% fat. The total fat content was adjusted to 5% to match that of the regular rat chow. Although the uptake of long-chain fatty acids would be inhibited, medium-chain fatty cids can penetrate the mitochondrial membranes and are, therefore, utilized [14]. In the miglyol-fed rats treated with etomoxir, any effect of etomoxir on subcellular organelles had, therefore, to arise from lowered blood lipids. Miglyol by itself is expected to have a lipid-lowering action [27]. Diabetic rats were also treated with acipimox which reduces lipolysis, the FFA flux to the liver and the synthesis of very low- and low-density lipoproteins [20].

To characterize additional effects of an increased adrenergic activity during the initial stages of diabetes

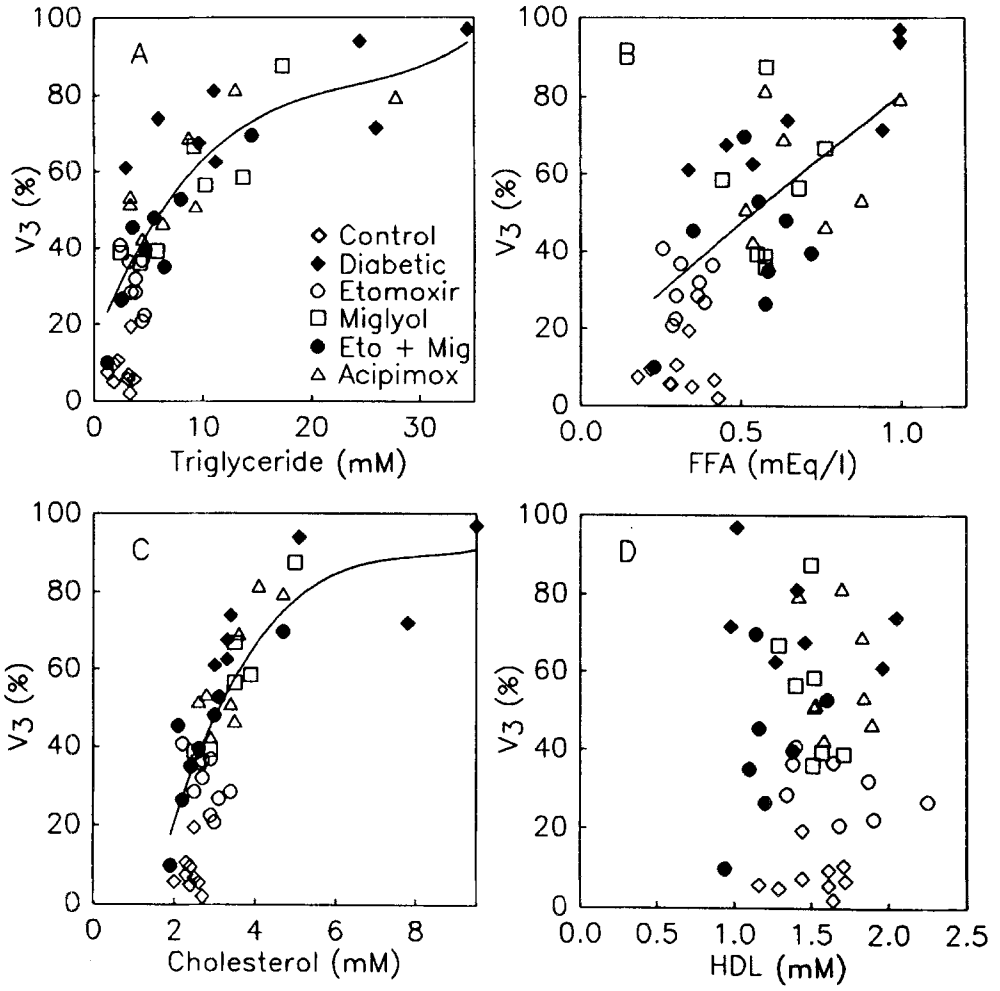

Fig. 5. The relationship between myosin isozyme V_3 and serum triglyceride concentration (A), free fatty acid concentration (FFA) (B), total cholesterol concentration (C) and HDL-cholesterol concentration (D). The symbols represent the values of individual rats as defined in the inset of (A). The following regression curves were calculated, whereby the control rats were not considered (A): $y = 15.1 + 7.1x - 0.27x^2 + 0.004x^3$, $R = 0.84$; for (B): $y = 10.8 + 75.5x - 5.6x^2$, $R = 0.68$; for (C): $y = -64.0 + 54.4x - 6.5x^2 + 0.27x^3$, $R = 0.81$. Number of rats was eight in each group.

[7], diabetic rats were treated with the non-selective β-adrenoceptor blocking drug, propranolol. This drug is expected to reduce the adrenergic influence on heart muscle and to reduce a β_1-adrenoceptor-mediated increase in lipolysis of adipose tissue [10]. The second generation centrally acting antihypertensive drug, moxoni-

Table 3. Effects of moxonidine, propranolol and tedisamil on growth chracteristics, myosin isozymes and SR Ca^{2+}-pump ATPase

	Heart rate (beats/min)	BW (g)	LVW (mg)	V_1 (%)	V_2 (%)	V_3 (%)	SR Ca^{2+}-ATPase (nmol/mg/min)
Control	438 ± 27	473 ± 28	875 ± 75	100 ± 0	0 ± 0	0 ± 0	160 ± 39
Diabetic	340 ± 73**	302 ± 61**	666 ± 143**	15.9 ± 13.0**	27.9 ± 10.1**	56.2 ± 21.7**	97 ± 28**
Diabetic + moxonidine	363 ± 25	239 ± 39	528 ± 25	21.5 ± 7.6	35.2 ± 6.8	43.3 ± 13.4	113 ± 46
Diabetic + propranolol	345 ± 32	281 ± 36	616 ± 64	26.1 ± 11.3	33.3 ± 6.5	40.6 ± 16.8	112 ± 24
Diabetic + tedisamil	368 ± 15	250 ± 30	586 ± 105	17.8 ± 11.6	29.3 ± 10.9	52.9 ± 21.6	116 ± 22

** $P < 0.05$ between untreated diabetic and control rats. Number of rats was seven in each group. Abbreviations: BW, body wt; LVW, left ventricular wt.

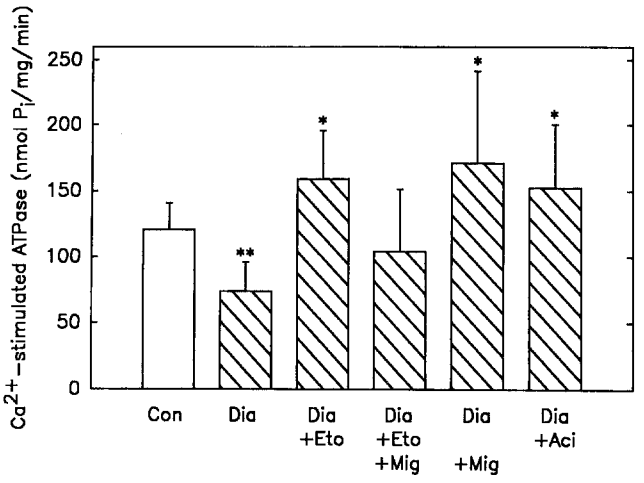

Fig. 6. The Ca^{2+}-stimulated ATPase of the SR Ca^{2+}-pump of control rats (Con), untreated diabetic rats (Dia), diabetic rats treated with etomoxir (Eto), diabetic rats treated with etomoxir and miglyol (Eto + Mig), diabetic rats fed miglyol (Mig) and diabetic rats treated with acipimox (Aci). ** $P < 0.05$ between untreated diabetic and control rats; * $P < 0.05$ between treated diabetic rats and untreated diabetic rats. Number of rats was eight in each group.

dine which interacts with imidazoline receptors of the ventrolateral medulla and thus reduces sympathetic outflow [8, 9] was also employed. The bradycardic drug, tedisamil, was chosen because it reduces the beating rate of normal hearts but has no negative inotropic action [11, 12]. To assess whether these drugs have the same effect in diabetic rats as in normal rats, heart rates were determined in conscious rats. The diabetic hearts exhibited a reduced heart rate (Table 3) confirming findings on chronically instrumented diabetic rats [28]. In contrast to normal rats (data not shown), the treatment with propranolol or tedisamil had no further effect on heart rate of diabetic rats. This would be in accordance with the observation that the number of adrenergic receptors in the diabetic hearts is reduced [29].

The diabetic rats exhibited markedly reduced growth characteristics. In the etomoxir-treated rats, the left ventricular wt was significantly increased although the body wt was not affected. Because the miglyol diet prevented the increase in ventricular wt, it can be indicated that the CPT-1 block was bypassed by the present miglyol feeding [19]. The cardiac growth stimulus appears thus to be closely linked to the reduced fatty acid utilization arising from CPT-1 inhibition. The finding that etomoxir did not increase cardiac growth of diabetic rats to match that of control rats can be attributed to the prevailing catabolic state of the diabetic rats.

The proportion of V_3 was greatly increased in the untreated diabetic rats; this change in MHC expression was greater than that observed previously [2, 3]. In diabetic rats treated with etomoxir, the proportion of V_3 was reduced and V_1 was increased but the isozyme populations were partially normalized. The present data confirm the findings that methyl palmoxirate, which also inhibits CPT-1, can reduce the proportion of V_3 in diabetic rats [30]. The etomoxir treatment did not completely normalize MHC expression, most probably because serum insulin and T_3 concentrations remained reduced. The increase in serum T_4 is not expected to have a marked influence because T_3 has a much higher affinity for nuclear thyroid receptors than T_4 [31].

The novel finding is that the etomoxir-treated diabetic rats fed miglyol still exhibited a reduced proportion of myosin V_3. Because the miglyol feeding prevented the cardiac growth by bypassing the mitochondrial CPT-1 inhibition, it can be suggested that CPT-1 inhibition may not be a prerequisite for the reduction of the V_3 proportion. Since serum T_3 and T_4 in the etomoxir-treated miglyol fed rats were not different from untreated diabetic rats, these hormones, therefore, may not account for the reduced β-MHC expression. Because serum triglyceride and FFA concentrations were reduced in the etomoxir-treated miglyol fed rats and because rats with high triglyceride, FFA and cholesterol concentrations exhibited higher proportions of V_3, processes associated with increased lipid levels appear to have a major role in the increased β-MHC expression. This conclusion is supported by the finding that miglyol-fed and acipimox-treated diabetic rats exhibited a lower V_3 proportion and reduced levels of serum triglyceride and cholesterol concentrations. In accordance, the β-MHC expression was correlated with serum triglyceride, FFA and cholesterol concentrations, whereby the mechanism by which serum lipids were altered, does not seem to be important. It can thus be suggested that the effect of etomoxir on serum lipids may be the major mechanism involved in the reduced β-MHC expression. Because an earlier study did not report data on blood lipids in the methyl palmoxirate-treated diabetic rats [30], it is difficult to decide as to what extent the reported reduction of the V_3 proportion was due to the lowered lipid influences. Although one might expect a better correlation with FFA compared with triglycerides, it is pointed out that circulating FFA are subject to short-term variations arising for example, from animal handling. The finding that the V_3 proportion was correlated with total cholesterol reflects the fact that triglyceride concentration is related to total cholesterol concentration [22]. In this study we have also shown that moxonidine and propranolol,

which reduce the influence of the adrenergic nervous system and thus would be expected to result in an increased proportion of V_3 in normal rats [23, 24], did not show any effect in diabetic rats. These drugs are, therefore, not likely to act on MHC expression by reducing an enhanced adrenergic activity. This conclusion is supported by the observation that heart rates were not reduced upon treating diabetic rats with these drugs.

The study does not implicate a direct effect of blood triglycerides, FFA or cholesterol on MHC expression but shows that these lipid parameters are indicative of metabolic influences on cardiac MHC expression. According to the well established glucose-fatty acid cycle, increased blood FFA concentrations are associated with an increased myocardial fatty acid oxidation and concommitantly a reduced glucose oxidation [32–34]. The operation of this fuel competition has been demonstrated for the fasted and diabetic state [32–34]. Thus, cardiac glucose oxidation of the diabetic rats would be expected to be depressed not only due to the impaired insulin production but also due to the increased blood FFA concentrations which arise primarily from activation of the hormone-sensitive triglyceride lipase of adipose tissue [13]. This shift in fuel utilization appears to provide a signal for an increased β-MHC expression. In accordance, the interventions which reduce the serum FFA and thus most probably the fatty acid oxidation of heart [25] also reduce the proportion of V_3. The apparent correlation with triglyceride and total cholesterol concentrations reflects the fact that high FFA concentrations lead to an increased synthesis of these lipids. The intriguing finding concerning a small potentiating effect of CPT-1 inhibition on β-MHC expression can most probably be explained on the basis of low serum insulin concentrations which prevented a further increase of glucose utilization although fatty acid utilization was most probably reduced further. The data therefore, suggest that a reduction in β-MHC expression is primarily related to processes associated with an increased glucose utilization rather than a reduced fatty acid utilization. In support of this hypothesis are the findings that an increased carbohydrate intake of food-restricted rats [3, 35] or rats with pressure overloaded hearts [36] reduced the proportion of V_3. Also etomoxir-treated rats with normal or pressure overloaded hearts exhibited a reduced β-MHC expression [37, 38], an increased SR Ca^{2+}-stimulated ATPase activity [37] and an increased rate of SR Ca^{2+} uptake [38]. Because a moderate increase in serum triglyceride concentrations arising from a high saturated fat intake did not induce an increased proportion of V_3 in normal rats [38], it can be concluded that an important prerequisite for the triglyceride-dependent increase of V_3 is a depressed glucose utilization of the heart. The present data shown in Fig. 5 also demonstrate that the altered MHC expression of diabetic hearts can be separated into influences apparently depending on blood lipids and influences operating in the absence of altered lipids. If serum triglycerides were not increased, the V_3 proportion of diabetic hearts was about 30%; however, it could reach 100% whenever the triglyceride concentration was increased markedly. The increased V_3 proportion of diabetic rats with normal blood lipids arises most probably from reduced thyroid and insulin influences.

The data in this study show that the depressed ATPase activity of the SR Ca^{2+} pump can be normalized by reducing serum lipid concentrations in diabetic rats. Although the results on cardiac steady state mRNA levels of the Ca^{2+}-ATPase are controversial suggesting either no change [39] or a reduction [40] of the Ca^{2+}-ATPase gene expression, the present study focussing on changes detectable at the protein level show that the reduced Ca^{2+}-pump ATPase of diabetic hearts can be increased apparently in parallel with an increased α-MHC expression. As regards to the mechanisms affecting the Ca^{2+}-pump ATPase, it appears that the phosphorylation of phospholamban is not increased by etomoxir (Vetter R, Rupp H, unpublished) but instead its action is at the transcriptional/posttranscriptional level of the Ca^{2+}-pump ATPase expression (Zarain-Herzberg A, Rupp H, Elimban V, Dhalla NS, unpublished).

Although heart function was not assessed in this study, the greatly normalized subcellular organelles are expected to be associated with an improved cardiac performance. In support of this contention would be the finding that treatment of diabetic rats with the vasodilator hydralazine also lowered blood lipids improved heart performance [41]. Moreover, treatment of diabetic rats with etomoxir markedly increased the hemodynamic performance of the *in situ* diabetic heart [25]. The present data also provide the important evidence that the unfavourable changes in MHC expression are not related to hyperglycemia but to increased blood lipids. In this respect it should be noted that hypertriglyceridemia proved to be a risk factor for cardiovascular disease [42]. Hypertriglyceridemia is not only an inherited disorder but can be induced by fat- and sugar-rich diets which also impair insulin sensitivity [43]. The current findings are, therefore, also of relevance for the rational design of diets required for the prevention of various cardiovascu-

lar disorders [43]. Our data show for the first time that besides well established effects of increased blood lipids on the cardiovascular system [42], they can also be associated with an unfavourable shift in the gene expression of heart muscle. The possibility that increased blood lipids affect gene expression of various membrane components involved in cellular ion homeostasis deserves further investigation.

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References

1. Nagano M, Dhalla NS: The Diabetic Heart. Raven Press, New York, 1991
2. Dillmann WH: Diabetes mellitus induces changes in cardiac myosin of the rat. Diabetes 29: 579–582, 1980
3. Rupp H, Elimban V, Dhalla NS: Diabetes-like action of intermittent fasting on sarcoplasmic reticulum Ca^{2+}-pump ATPase and myosin isoenzymes can be prevented by sucrose. Biochem Biophys Res Commun 164: 319–325, 1989
4. Ganguly PK, Pierce GN, Dhalla KS, Dhalla NS: Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am J Physiol 244: E528–E535, 1983
5. Morkin E, Bahl JJ, Markham BE: Control of cardiac myosin heavy chain gene expression by thyroid hormones. In: LH Kedes, FE Stockdale (eds) Cellular and Molecular Biology of Muscle Development. Alan R. Liss., New York, 1989, pp 381–389
6. Dillmann WH: Influence of thyroid hormone administration on myosin ATPase activity and myosin isoenzyme distribution in the heart of diabetic rats. Metabolism 31: 199–204, 1982
7. Ganguly PK, Dhalla KS, Innes IR, Beamish RE, Dhalla NS: Altered norepinephrine turnover and metabolism in diabetic cardiomyopathy. Circ Res 59: 684–693, 1986
8. Ernsberger P, Damon TH, Graff LM, Schäfer SG, Christen MO: Moxonidine, a centrally acting antihypertensive agent, is a selective ligand for I_1-imidazoline sites. J Pharmacol Exp Ther 264: 172–182, 1993
9. Ollivier JP, Christen MO, Schäfer SG: Moxonidine: a second generation of centrally acting drugs. An appraisal of clinical experience. J Cardiovasc Pharmacol 20 (suppl 4): S31–S36, 1992
10. Presta E, Leibel RL, Hirsch J: Regional changes in adrenergic receptor status during hypocaloric intake do not predict changes in adipocyte size or body shape. Metabolism 39: 307–315, 1990
11. Kühl UG, Buschmann G: Cardiovascular profile of KC 8857 in comparison with other cardioactive agents. J Mol Cell Cardiol 19 (suppl III): 50, 1987
12. Grohs JG, Fischer G, Raberger G: Cardiac and hemodynamic effects of the selective bradycardic agent KC 8857 during exercise-induced myocardial ischemia. Eur J Pharmacol 161: 53–60, 1989
13. Harris RA, Crabb DW: Metabolic interrelationships. In: TM Devlin (ed.) Textbook of Biochemistry. John Wiley, London, 1986, pp 531–557
14. Selby PL, Sherratt HSA: Substituted 2-oxiranecarboxylic acids: a new group of candidate hypoglycaemic drugs. Trends Pharmacol 10: 495–500, 1989
15. Eistetter K, Wolf HPO: Etomoxir. Drugs of the Future 11: 1034–1036, 1986
16. Wolf HPO: Aryl-substituted 2-oxirane carboxylic acids: A new group of antidiabetic drugs. In: CJ Bailey, PR Flatt (eds) New Antidiabetic Drugs. Smith-Gordon, London, 1990, pp 217–229
17. Agius L, Alberti KG: Regulation of flux through pyruvate dehydrogenase and pyruvate carboxylase in rat hepatocytes. Effects of fatty acids and glucagon. Eur J Biochem 152: 699–707, 1985
18. Vaartjes WJ, De Haas CGM, Haagsman HP: Effects of sodium 2-[5-(4-chlorophenyl)-pentyl]-oxirane-2-carboxylate (POCA) on intermediary metabolism in isolated rat liver cells. Biochem Pharmacol 35: 4267–4272, 1986
19. Lee SM, Bahl JJ, Bressler R: Prevention of the metabolic effects of 2-tetradecylglycidate by octanoic acid in the genetically diabetic mouse (db/db). Biochem Med 33: 104–109, 1985
20. Tornvall P, Walldius G: A comparison between nicotinic acid and acipimox in hypertriglyceridaemia – effects on serum lipids, lipoproteins, glucose tolerance and tolerability. J Internal Med 230: 415–421, 1991
21. Rupp H, Dietz K: Mathematical models of myosin heterodimer formation in the rat heart during thyroid hormone alterations. Circ Res 68: 27–37, 1991
22. Bauer JD: Clinical Laboratory Methods. C.V. Mosby, St. Louis, 1982, pp 554–555
23. Rupp H, Berger HJ, Pfeifer A, Werdan K: Effect of positive inotropic agents on myosin isozyme population and mechanical activity of cultured rat heart myocytes. Circ Res 68: 1164–1173, 1991
24. Gupta MP, Gupta M, Stewart A, Zak R: Activation of alpha-myosin heavy chain gene expression by cAMP in cultured fetal rat heart myocytes. Biochem Biophys Res Commun 174: 1196–1203, 1991
25. Rösen P, Schmitz FJ, Reinauer H: Improvement of myocardial function and metabolism in diabetic rats by the carnitine palmitoyltransferase inhibitor etomoxir. In: M Nagano, S Mochizuki, NS Dhalla (eds) Cardiovascular Disease in Diabetes. Kluwer Academic Publ., Boston, 1992, pp 361–372
26. Reavan GM, Chang H, Hoffman BB: Additive hypoglycemic effects of drugs that modify free-fatty acid metabolism by different mechanisms in rats with streptozotocin-induced diabetes. Diabetes 37: 28–32, 1988
27. Leveille GA, Pardini RS, Tillotson JA: Influence of medium-chain triglycerides on lipid metabolism in the rat. Lipids 2: 287–294, 1967
28. Doursout M-F, Kashimoto S, Chelly JE: Cardiac function in the diabetic conscious rat. In: M Nagano, NS Dhalla (eds) The Diabetic Heart. Raven Press, New York, 1991, pp 11–19
29. Heyliger CE, Pierce GN, Singal PK, Beamish RE, Dhalla NS: Cardiac alpha- and beta-adrenergic receptor alterations in diabetic cardiomyopathy. Basic Res Cardiol 77: 610–618, 1982

30. Dillmann WH: Methyl palmoxirate increases Ca^{2+}-myosin ATPase activity and changes myosin isoenzyme distribution in the diabetic rat heart. Am J Physiol 248: E602–E606, 1985
31. Morkin E, Flink IL, Goldman S: Biochemical and physiologic effects of thyroid hormone on cardiac performance. Progr Cardiovasc Disease 25: 435–464, 1983
32. Randle PJ, Tubbs PK: Carbohydrate and fatty acid metabolism. In: RM Berne (ed.) Handbook of Physiology Section 2: The Cardiovascular System, Volume I. The Heart, American Physiological Society, Bethesda, 1979, pp 805–844
33. Weiss RG, Chacko VP, Gerstenblith G: Fatty acid regulation of glucose metabolism in the intact beating rat heart assessed by carbon-13 NMR spectroscopy: the critical role of pyruvate dehydrogenase. J Mol Cell Cardiol 21: 469–478, 1989
34. Neely JR, Morgan HE: Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Ann Rev Physiol 36: 413–459, 1974
35. Morris GS, Surdyka DG, Haddad F, Baldwin KM: Apparent influence of metabolism on cardiac isomyosin profile of food-restricted rats. Am J Physiol 258: R346–R351, 1990
36. Rupp H, Elimban V, Dhalla NS: Sucrose feeding prevents changes in myosin isoenzymes and sarcoplasmic reticulum Ca^{2+}-pump ATPase in pressure-loaded rat heart. Biochem Biophys Res Commun 156: 917–923, 1988
37. Rupp H, Elimban V, Dhalla NS: Modification of subcellular organelles in pressure-overloaded heart by etomoxir, a carnitine palmitoyltransferase I inhibitor. FASEB J 6: 2349–2353, 1992a
38. Rupp H, Wahl R, Hansen M: Influence of diet and carnitine palmitoyltransferase I inhibition on myosin and sarcoplasmic reticulum. J Appl Physiol 72: 352–360, 1992b
39. Dillmann WH: Diabetes-induced changes in specific proteins and mRNAs in the rat heart. In: M Nagano, NS Dhalla (eds) The Diabetic Heart. Raven Press, New York, 1991, pp 263–270
40. Russ M, Reinauer H, Eckel J: Diabetes-induced decrease in the mRNA coding for sarcoplasmic reticulum Ca^{2+}-ATPase in adult rat cardiomyocytes. Biochem Biophys Res Commun 178: 906–912, 1991
41. Rodrigues B, Goyal RK, McNeill JH: Effects of hydralazine on streptozotocin-induced diabetic rats: prevention of hyperlipidemia and improvement in cardiac function. J Pharmacol Exp Ther 237: 292–299, 1986
42. Castelli WP: The triglyceride issue: a view from Framingham. Am Heart J 112: 432–437, 1986
43. Rupp H: Insulin resistance, hyperinsulinemia, and cardiovascular disease. The need for novel dietary prevention strategies. Basic Res Cardiol 87: 99–105, 1992