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Asymptomatic Cardiomyopathy in Children and Adolescents with Type 1 Diabetes Mellitus: Association of Echocardiographic Indicators with Duration of Diabetes Mellitus and Metabolic Parameters

Erdal Adal[1], Güven Koyuncu[2], Ahmet Aydın[2], Ahmet Çelebi[3], Gülsev Kavunoğlu[2] and Halit Çam[2]

[1]Departments of Pediatric Metabolism and Nutrition and Pediatric Endocrinology, SSK Bakırköy Childhood and Maternity Education Hospital and [2]Departments of Pediatric Metabolism and Nutrition and [3]Department of Pediatric Cardiology, Medical Faculty of Cerrahpaşa, University of Istanbul, İstanbul, Turkey

ABSTRACT

This study was designed to determine the relationship of dimensions, wall thickness and function of the left ventricle with diabetes duration, fasting blood glucose, lipid profile, β-OH-butyrate, free fatty acids (FFA) and carnitine levels in children and adolescents with type 1 diabetes mellitus (DM1) who had no cardiovascular complications. Thirty-five patients with DM1 (18 F/17 M, mean age: 12.0 years) and age matched control children (n = 24) were enrolled in the study. Patients with DM1 were subdivided into Group I (mean DM1 duration 3.5 years, n = 14), and Group II (mean DM1 duration 8.2 years, n = 21). Dimensions, wall thickness and systolic functions of the left ventricle were normal in all patients with DM1. Diastolic functions were normal in Group I. In Group II, peak A wave velocity (AVEL) (p = 0.004), velocity-time integral of A wave (AVTI) (p = 0.007) and isovolumetric relaxation time corrected by heart rate (cIVRT) (p = 0.048) were high, and peak E wave velocity (EVEL) and velocity-time integral of E wave (EVTI) were normal. E/A (p <0.0001) and EVTI/AVTI (p = 0.001) were low in this group. In Group I, systolic and diastolic blood pressure, HDL-cholesterol and FFA values were normal; total cholesterol (p = 0.047), LDL-cholesterol (p = 0.017), β-OH-butyrate (p = 0.003), and acetyl carnitine (p = 0.006) levels were high. In Group II, diastolic blood pressure (p = 0.008), total cholesterol (p <0.0001) and LDL-cholesterol (p <0.0001) were increased; and total carnitine (p = 0.019), free carnitine (p = 0.002) and HDL-cholesterol (p = 0.039) were decreased. Correlations were detected between total carnitine and AVEL and HR; free carnitine and AVEL, E/A and HR; HbA_{1c} and EVTI/AVTI and cIVRT; LDL-cholesterol and E/A, EVTI/AVTI ratios and cIVRT; HDL-cholesterol and AVEL; FFA and LVDD, IVSD, LVPWD, LVmass and CO; metabolic parameters and DM1 duration and echocardiographic findings such as AVEL, EVEL, EVTI, VmaxAV and CO. In conclusion, left ventricular dimensions, wall thickness and systolic functions were normal in children and adolescents with DM1 who had no obvious cardiovascular complications. Left ventricular diastolic functions were abnormal in patients of Group II. Left ventricular diastolic function abnormalities were associated with glycemic control, free and total carnitine, and LDL- and HDL-cholesterol levels.

KEY WORDS

adolescent, child, type 1 diabetes mellitus, diabetic cardiomyopathy, echocardiography, carnitine

Reprint address:
Erdal Adal
Nevbahar M. Ahmet Hikmet S. Yayla Apt. No: 21/9
Fındıkzade, İstanbul, Turkey
e-mail: erdaladal@yahoo.com

INTRODUCTION

Diabetes mellitus is a complex disease which, unless well controlled, impairs the structure and function of many organs in the body. Thus, it predominates among the most important causes of mortality and morbidity throughout the world. Premature death among patients with diabetes mellitus is most often due to cardiovascular disorders and renal insufficiency[1,2].

In the Framingham Heart Study, in which 5,209 individuals were followed for 18 years, it was detected that diabetes mellitus could cause cardiac disorders in the absence of factors such as hypertension, obesity, hypercholesterolemia, and coronary artery disease[1]. Cardiac disorders which develop independently of atherosclerosis are termed diabetic cardiomyopathy. This diabetic cardiomyopathy has also been confirmed histopathologically (perivascular collagen accumulation, fibrosis)[3-5].

Many echocardiographic studies conducted in patients with diabetes mellitus have been published previously. Asymptomatic elderly diabetic patients have also been included in these studies; because factors such as age, ischemia, and hypertension have also been known to affect left ventricular diastolic functions in particular in this group, echocardiographic studies have focused on young patients[6-16].

It has been shown in various investigations that the onset of diabetic complications, such as cardiomyopathy, dates back to childhood and adolescence[17-20]. The length and quality of the patient's life may be increased through recognition of myocardial disorders before clinical symptoms appear (subclinical diabetic cardiomyopathy), and application of necessary steps to prevent progression of the disease[21]. For the detection of cardiac disorders in children with diabetes mellitus in whom atherosclerotic changes have not yet developed, it is important to demonstrate that diabetic cardiomyopathy is an independent entity.

Results of studies in children with diabetes mellitus performed using M-mode echocardiography are conflicting. In these studies, myocardial contractility, that is, systolic function, has been reported to be low[22-24], normal[25-27], or high[28-30]. Diastolic functions have been found to be impaired in some of these studies[31-33], but were normal in the remainder[29-34].

Although the pathogenesis of diabetic cardiomyopathy is not well known, it seems that the fact that the heart cannot use glucose as an energy source, but depends totally on the oxidation of fatty acids, is an important factor. Oxidation of fatty acids upon entry into the mitochondria and their use as an energy source depend on an adequate amount of free carnitine in the environment. If carnitine is not present in sufficient amounts in the environment, free fatty acids (FFA) and related metabolites increase. Both the toxic effects of these accumulating substances and the decrease in energy production (ATP) may impair cardiac performance, resulting in diabetic cardiomyopathy[35-36].

It has been shown in several investigations in animals[37-40], adults[41-44], and children[45-47] that free carnitine is decreased in diabetes mellitus. A number of studies have demonstrated that carnitine therapy in diabetic animals has beneficial effects on impaired cardiac functions[48-51].

The present study had two main objectives:

- To demonstrate echocardiographically (M-mode and Doppler) whether early asymptomatic diabetic cardiomyopathy occurs in children and adolescents with diabetes mellitus;
- To investigate whether systolic and diastolic cardiac functions are associated with the duration of diabetes mellitus, degree of diabetic control, carnitine fractions, and other metabolic indicators.

PATIENTS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki. Thirty-five patients diagnosed with type 1 diabetes mellitus (DM1) and followed up in the Paediatrics Department, Medical Faculty of Cerrahpaşa, University of Istanbul, were included in the study. Patients with hypertension, persistent microalbuminuria, retinopathy, clinical neuropathy, and ketoacidosis episodes within the previous 2 months were excluded from the study. Of the children and adolescents in the study group, 18 (51%) were females and 17 (49%) were males, with an age distribution ranging from 3.6 to 20.7 years (mean 12.0 ± 4.2). Duration of DM1 varied from 2 to 16 years, with an average of 6.3 ± 3.2

years. Insulin was administrated as two daily doses in 11 patients (31%) and as multiple doses (0.5-2 U/kg/day) in 24 patients (69%). The control group included age- and sex-matched children and adolescents. Of the 24 subjects in this group, 11 (46%) were female and 13 (54%) were male. Their age distribution ranged from 3.6 to 17.5 years, averaging 11.0 ± 4.1 (Table 1). There was no statistically significant difference in terms of gender between study and control groups (χ^2 = 0.178, p = 0.67).

Patients with DM1, were divided into two groups according to the time from the onset of with DM1: 14 patients with DM1 duration shorter than 5 years (mean 3.55 years) comprised Group I, and 21 patients with DM1 duration above 5 years (mean 8.16 years) formed Group II.

Systolic and diastolic blood pressures were measured on the day that echocardiography was recorded. Body surface area [BSA = 0.0001 x 71.84 x (height)$^{0.725}$ x (weight)$^{0.425}$] and body mass index [BMI = weight/(height)^2] were calculated; Glycosylated haemoglobin (HbA_{1c}) levels were mesasured.

For the echocardiographic examination, a transducer with a frequency ranging from 2.5 to 4 mHz in a Ving-Med CFM 800 device was used, and ECG recordings were obtained simultaneously. All measurements were repeated at least three times and their averages were taken. In M-mode examinations, LVDd (left ventricle diastolic diameter), LVDs (left ventricle systolic diameter), IVSDd, (diastolic diameter of interventricular septum), IVSDs (systolic diameter of interventricular septum), LVPWDd (left ventricle posterior wall diastolic diameter), LVPWDs (left ventricle posterior wall systolic diameter), LADs (left atrium diastolic diameter), Ao (Aorta) and LA/Ao (left atrium/aorta ratio) were measured according to the recommendations of the American Society of Echocardiography[52]. From these, FS (fraction of shortening), EF (ejection fraction), and LVmax values were obtained according to the formulae of Teicholz *et al.*[53] and Deveroux and Recihek[54] found in the device software.

In order to assess systolic functions, aortic flow samples were also obtained by pulsed Doppler just distal to the aortic valve in apical 5 space images. VmaxAV (maximum velocity of AV), PmaxAV, LVET (left ventricular ejection time), VTI, RR and HR (heart rate) were determined from this flow sample.

Aortic flow sample was obtained by placing the cursor just distal to the aortic valve at the apical part in five different spaces by low pulsed Doppler, the graph was drawn as recommended, and the measured value of the aortic valve opening just when the aortic valve was open in systole was entered on the parasternal long axis; CO (cardiac output) and CI (cardiac index) were calculated using the formula in the device software. Mean circumferential fiber contraction velocity (mVcf) was calculated by dividing the contraction fraction (FS) obtained in M-mode echocardiography by the left ventricular ejection time (LVET) obtained by Doppler echocardiography. From this $mVcf_c$, $LVET_c = LVET/\sqrt{RR}$ was derived[55].

Doppler flow samples from the mitral valve for examination of diastolic functions were obtained by placing the cursor parallel to the flow just above the mitral valve by PW Doppler at the apical four space image[56]. In particular, transmitral flow samples in expirium were recorded while measurements were being made. At least three, and at most five, measurements were made successively, and their averages were taken. The following measurements were made in the left ventricular diastolic flow samples: E_{max} (maximum velocity of early diastolic wave), A_{max} (maximum velocity of late diastolic wave), E/A ratio, EVTI (E velocity time integral), AVTI (A velocity time integral), EVTI/AVTI ratio and IVRT (isovolumic relaxation time).

Venous blood samples for blood glucose (normal [N]: 75-115 mg/dl), cholesterol (N: 115-220 mg/dl), triglycerides (N: 35-185 mg/dl), HDL-cholesterol (N: 35-85 mg/dl), LDL-cholesterol (N: 50-170 mg/dl), VLDL-cholesterol (N: 2-26 mg/dl), β-OH-butyrate, FFA, HbA_{1c} and total carnitine levels were obtained during the week when echocardiography was carried out in the patients; these samples were taken when patients had been fasting in the morning and were obtained from the forearm by squeezing very slightly without applying a measurable pressure. Microalbumin/creatinine ratio was measured in the urine 2-4 times at 3-month intervals. Patients exceeding the upper limit (25

mg/g) twice were considered as having persistent microalbuminuria, and excluded from the study. HbA_{1c} levels were measured from blood samples with a Bayer DCA 2000 analyzer using the HbA_{1c} kit for the device. Values were recorded as percentages (N: 4.3-5.7%). FFA (N: 0.1-0.6 mEq/l) were determined in mEq/l by the enzymatic-calorimetric method by spectrophotometry using Wako WFA C test kits in Bausch and Lomb Spectronic 100. Serum β-OH-butyrate levels were determined using the enzymatic method described by Gau[57]. Total and free carnitine in serum were determined by the method described by Cejka and Kithier[58]; values were recorded in μmol/l.

For statistical calculations in the two groups, Student's t-test was used for comparison of the mean parametric values, chi-squared test for comparison of non-parametric values, and Pearson correlation test for correlation analyses.

RESULTS

Fasting blood glucose, total cholesterol, LDL-cholesterol, FFA and β-OH-butyrate levels were found to be higher in patients with DM1 than in controls, but the HDL-cholesterol level was lower than in controls. There was no difference between VLDL-cholesterol and triglyceride levels in the two groups. In patients with DM1 total and acetylated carnitine levels were normal, whereas free carnitine levels were clearly low and AC/FC ratio was high (Table 1). While systolic and diastolic blood pressures were normal in Group I patients, diastolic blood pressures were increased in Group II patients (65 vs 72 mm Hg; $p = 0.008$).

In M-mode echocardiography examinations, no statistically significant difference was found between patients with DM1 and controls in terms of left ventricular and left atrial diameters, IVS and left ventricular posterior wall thickness, and left ventricular mass (Table 2). There was no impairment detected in left ventricular systolic functions of patients with DM1 with either M-mode or Doppler examinations (Tables 2 and 3). There was, however, a trend towards impairment in diastolic functions (Table 3).

Diastolic functions were normal in Group I patients with DM1 duration below 5 years (Table 4), but various disorders in diastolic function were detected in Group II in which DM1 duration was above 5 years (Table 5). In Group II, peak A wave velocity (AVEL) (0.73 vs 0.63 m/sec, $p = 0.004$), velocity-time integral of A wave (AVTI) (5.18 vs 4.33, $p = 0.007$) and isovolumetric relaxation time corrected by heart rate (cIVRT) (0.084 vs 0.075 sec, $p = 0.048$) were higher; on the other hand, peak E wave velocity (EVEL) and E wave velocity-time integral (EVTI) were normal. In patients of this group, E/A (1.34 vs 1.58, $p < 0.0001$) and EVTI/AVTI (2.08 vs 2.58, $p = 0.001$) rates were lower.

Important correlations were found between echocardiographic findings and metabolic parameters (Table 6).

DISCUSSION

Congestive heart failure predominates among the most important factors which determine the life expectancy of patients with DM1[1,2]. The length and quality of patient life may be increased through recognition of myocardial disorders before clinical symptoms appear (subclinical diabetic cardiomyopathy), and application of necessary steps to prevent progression of the disease. For this purpose, a number of echocardiographic studies have been performed in childhood and adolescence using M-mode and Doppler[6-16].

Results of echocardiographic studies by M-mode in children with DM1 are conflicting. In these studies, myocardial contractility, or systolic function, has been reported to be low[22-24], normal[25,26] or high[28-30].

Cardiac functions were first examined in 1981 by Lababidi *et al.* in children with DM1 using M-mode echocardiography. In this study, myocardial contractility was detected to be decreased along with septal hypertrophy (increase in the average diameters of left atrium and both ventricles) in 101 children and adolescents with DM1. These changes, however, were not found to be associated with DM1 duration and degree of control[22].

In an M-mode echocardiography study in 33 children with DM1, Friedman *et al.* found that the left ventricular end-diastolic volume was high, and mean ejection fraction, minor axis shortening and circumferential fiber contraction velocity were

TABLE 1

Characteristics of patients with type 1 diabetes mellitus and healthy controls (means ± SD) (range)

	Diabetic patients (n = 35)	Control group (n = 24)	t	p
Age (years)	12.0 ± 4.2 (3.6-20.1)	11.0 ± 4.1 (3.6-17.5)	0.95	0.35
Diabetes duration (years)	6.3 ± 3.2 (2.0-16.0)	-	-	-
Weight (kg)	39.3 ± 12.8 (15.0-64.0)	40.2 ± 16.7 (16.5-71.0)	0.23	0.82
Height (cm)	142 ± 18 (95-171)	143 ± 22 (107-175)	0.27	0.79
Body surface area (m^2)	1.24 ± 0.28 (0.62-1.75)	1.26 ± 0.36 (0.70-1.86)	0.22	0.82
BMI (kg/m^2)	18.94 ± 2.68 (13.6-25.1)	18.53 ± 2.89 (14.1-23.2)	0.55	0.58
Systolic pressure (mm Hg)	107 ± 10 (90-130)	105 ± 7 (90-115)	0.67	0.50
Diastolic pressure (mm Hg)	70 ± 10 (50-90)	64 ± 6 (50-75)	2.59	**0.012***
Total carnitine (μmol/dl)	23.16 ± 7.88 (9.8-41)	25.24 ± 6.09 (15.0-37.0)	1.09	0.28
Free carnitine (FC) (μmol/dl)	12.94 ± 5.90 (3.00-24.97)	17.44 ± 5.93 (7.68-31.70)	2.88	**0.006***
Acetyl carnitine (AC) (μmol/dl)	10.23 ± 6.82 0.24-28.0	7.80 ± 4.54 0.80-17.0	1.64	0.11
AC/FC	1.21 ± 1.48 (0.03-7.88)	0.52 ± 0.35 (0.04-1.33)	2.63	**0.012***
Glucose (mg/dl)	313 ± 110 (95-485)	99 ± 8 (87-118)	11.40	**<0.0001***
Total cholesterol (mg/dl)	187 ± 48 (119-324)	144 ± 35 (102-214)	3.75	**<0.0001***
HDL-cholesterol (mg/dl)	34.7 ± 15.1 (11.0-70.4)	42.2 ± 7.4 (30.8-55.0)	2.53	**0.014***
LDL-cholesterol (mg/dl)	128.3 ± 42.4 (50.5-223.8)	81.6 ± 33.7 (40.8-165.4)	4.50	**<0.0001***
VLDL-cholesterol (mg/dl)	24.2 ± 12.4 (15.0-82.8)	20.6 ± 6.5 (13.6-33.4)	1.32	0.19
Triglycerides (mg/dl)	121 ± 62 (75-414)	103 ± 33 (68-167)	1.32	0.19
HbA_{1c} (%)	9.9 ± 2.4 (5.6-14.0)	-	-	-
β-OH-Butyrate (mmol/l)	0.39 ± .30 (0.6-1.6)	0.20 ± 0.18 (0.02-0.50)	2.66	**0.010***
Free fatty acids (mEq/l)	0.77 ± 0.45 (0.10-1.87)	0.57 ± 0.26 (0.20-1.15)	2.19	**0.032***

* Significant p values are shown in bold.

TABLE 2

Comparison of M-mode echocardiography parameters of patients with type 1 diabetes mellitus and control group

	Diabetic patients (n = 35)	Control group (n = 24)	t	p
Thickness, diameter and mass				
Left ventricle				
Left ventricle diastolic diameter (LVDd) (cm)	4.05 ± 0.45	4.23 ± 0.60	1.38	0.17
Diastolic diameter of interventricular septum (IVSDd) (cm)	0.67 ± 0.14	0.66 ± 0.16	0.30	0.76
Left ventricle posterior wall thickness (LVPWDd) (cm)	0.68 ± 0.12	0.68 ± 0.17	0.03	0.98
Left ventricle mass (LV Mass) (g)	83.82 ± 31.14	94.48 ± 53.95	0.87	0.39
Left atrium				
Left atrium diastolic diameter (LADs) (cm)	2.89 ± 0.35	2.93 ± 0.32	0.45	0.65
Left atrium/aorta ratio (LA/Ao)	1.26 ± 0.20	1.26 ± 0.19	0.02	0.99
Systolic functions				
Left ventricle ejection fraction (LVEF) (%)	73.9 ± 4.7	73.9 ± 5.1	0.01	0.99
Fraction of shortening (FS) (%)	36.3 ± 3.8	36.4 ± 4.2	0.06	0.95
Corrected mean velocity of circular fiber shortening ($mVcf_c$)	1.16 ± 0.15	1.17 ± 0.14	0.27	0.79

TABLE 3

Comparison of Doppler echocardiography parameters in patients with type 1 diabetes mellitus and controls

	Diabetic patients (n = 35)	Control group (n = 24)	t	p
Diastolic functions				
A wave velocity (AVEL) (m/sec)	0.69 ± 0.11	0.63 ± 0.11	1.91	0.06
E wave velocity (EVEL) (m/sec)	0.95 ± 0.11	0.98 ± 0.13	0.93	0.36
E/A ratio	1.42 ± 0.25	1.60 ± 0.32	2.45	**0.017***
A velocity time integral (AVTI) (cm)	4.89 ± 1.03	4.27 ± 0.88	2.41	**0.019***
E velocity time integral (EVTI) (cm)	10.38 ± 1.74	10.57 ± 2.12	0.38	0.71
EVTI/AVTI	2.18 ± 0.45	2.53 ± 0.49	2.77	**0.008***
Isovolumic relaxation time (IVRT) (sec)	0.065 ± 0.013	0.063 ± 0.010	1.02	0.31
Corrected isovolumic relaxation time (cIVRT) (sec)	0.079 ± 0.017	0.074 ± 0.010	1.51	0.14
Systolic functions				
Maximum velocity of AV (VmaxAV) (m/sec)	1.35 ± 0.18	1.31 ± 0.13	1.01	0.32
Cardiac output (CO) (l/min)	4.18 ± 0.95	4.45 ± 1.34	0.90	0.37
Cardiac index (CI)	3.43 ± 0.63	3.56 ± 0.45	0.88	0.39
Velocity time integral of AV (VTIAV) (cm)	25.14 ± 4.14	24.46 ± 2.83	0.71	0.48
Heart rate (HR) (l/min)	89 ± 11	88 ± 11	0.54	0.59

* Significant p values are shown in bold.

TABLE 4

Comparison of Doppler echocardiographic and metabolic parameters in patients with type 1 diabetes mellitus with diabetes duration shorter than 5 years

	Diabetic patients (n = 14)	Control group (n = 22)	t	p
Diastolic functions				
A wave velocity (AVEL) (m/sec)	0.62 ± 0.12	0.62 ± 0.10	0.10	0.92
E wave velocity (EVEL) (m/sec)	0.93 ± 0.14	0.98 ± 0.12	1.13	0.27
E/A ratio	1.53 ± 0.32	1.62 ± 0.33	0.75	0.46
A velocity time integral (AVTI) (cm)	4.45 ± 0.94	4.22 ± 0.73	0.83	0.41
E velocity time integral (EVTI) (cm)	10.13 ± 1.61	10.53 ± 2.08	0.62	0.54
EVTI/AVTI	2.33 ± 0.48	2.53 ± 0.50	1.19	0.24
Isovolumic relaxation time (IVRT) (sec)	0.060 ± 0.010	0.605 ± 0.009	0.14	0.89
Corrected isovolumic relaxation time (cIVRT) (sec)	0.073 ± 0.012	0.073 ± 0.010	0.13	0.90
Systolic functions				
Maximum velocity of AV (VmaxAV) (m/sec)	1.29 ± 0.15	1.30 ± 0.14	0.33	0.74
Cardiac output (CO) (l/min)	3.59 ± 0.71	4.20 ± 1.09	1.87	0.07
Cardiac index (CI)	3.35 ± 0.77	3.52 ± 0.44	0.75	0.46
Velocity time integral of AV (VTIAV) (cm)	23.58 ± 3.28	24.32 ± 2.91	0.70	0.49
Heart rate (HR) (1/min)	88 ± 14	88 ± 11	0.07	0.94
Metabolic parameters				
Total carnitine (µmol/dl)	26.17 ± 8.80	24.76 ± 6.61	0.57	0.57
Free carnitine (µmol/dl)	13.35 ± 7.18	17.42 ± 6.16	1.81	0.08
Acetyl carnitine (µmol/dl)	12.81 ± 7.00	7.34 ± 4.25	2.93	**0.006***
β-OH-Butyrate (mmol/l)	0.44 ± 0.26	0.20 ± 0.17	3.26	**0.003***
Free fatty acids (mEq/l)	0.83 ± 0.48	0.60 ± 0.24	1.68	0.11
Total cholesterol (mg/dl)	171 ± 36	146 ± 36	2.06	0.047*
HDL-cholesterol (mg/dl)	35.6 ± 15.8	41.6 ± 7.4	1.34	0.20
LDL-cholesterol (mg/dl)	114.5 ± 40.3	82.7 ± 34.7	2.52	**0.017***
VLDL-cholesterol (mg/dl)	20.8 ± 3.0	21.2 ± 6.5	0.24	0.81
Triglycerides (mg/dl)	105 ± 15	106 ± 32	0.24	0.81

* Significant p values are shown in bold.

TABLE 5

Comparison of Doppler echocardiographic and metabolic parameters in patients with type 1 diabetes mellitus with diabetes duration more than 5 years

	Diabetic patients (n = 21)	Control group (n = 20)	t	p
Diastolic functions				
A wave velocity (AVEL) (m/sec)	0.73 ± 0.09	0.63 ± 0.11	3.06	**0.004***
E wave velocity (EVEL) (m/sec)	0.97 ± 0.09	0.99 ± 0.13	3.06	0.50
E/A ratio	1.34 ± 0.17	1.58 ± 0.23	3.97	**<0.0001***
A velocity time integral (AVTI) (cm)	5.18 ± 1.01	4.33 ± 0.89	2.85	**0.007***
E velocity time integral (EVTI) (cm)	10.55 ± 1.83	10.99 ± 2.05	0.72	0.48
EVTI/AVTI	2.08 ± 0.41	2.58 ± 0.46	3.65	**0.001***
Isovolumic relaxation time (IVRT) (sec)	0.068 ± 0.014	0.063 ± 0.010	1.32	0.19
Corrected isovolumic relaxation time (cIVRT) (sec)	0.084 ± 0.018	0.075 ± 0.011	2.04	**0.048***
Systolic functions				
Maximum velocity of AV (VmaxAV) (m/sec)	1.39 ± 0.20	1.28 ± 0.13	2.16	**0.037***
Cardiac output (CO) (l/min)	4.58 ± 0.89	4.77 ± 1.22	0.58	0.56
Cardiac index (CI)	3.49 ± 0.53	3.53 ± 0.45	0.28	0.78
Velocity time integral of AV (VTIAV) (cm)	26.18 ± 4.39	24.51 ± 2.93	1.43	0.16
Heart rate (HR) (1/min)	90 ± 9	84 ± 8	2.06	**0.046***
Metabolic parameters				
Total carnitine (μmol/dl)	21.16 ± 6.69	26.02 ± 5.98	2.45	**0.019***
Free carnitine (μmol/dl)	12.66 ± 5.05	18.30 ± 5.94	3.28	**0.002***
Acetyl carnitine (μmol/dl)	8.50 ± 6.29	7.71 ± 4.83	0.45	0.66
β-OH-Butyrate (mmol/l)	0.35 ± 0.33	0.19 ± 0.17	1.91	0.06
Free fatty acids (mEq/l)	0.73 ± 0.43	0.54 ± 0.26	1.69	0.10
Total cholesterol (mg/dl)	198 ± 52	146 ± 33	3.82	**<0.0001***
HDL-cholesterol (mg/dl)	34.1 ± 15.0	42.2 ± 8.1	2.13	**0.039***
LDL-cholesterol (mg/dl)	137.5 ± 42.2	82.0 ± 32.3	4.71	**0.0001***
VLDL-cholesterol (mg/dl)	26.6 ± 15.5	21.4 ± 6.8	1.36	0.18
Triglycerides (mg/dl)	133 ± 78	107 ± 34	1.36	0.18

* Significant p values are highlighted.

TABLE 6

Correlation of metabolic and echocardiographic parameters

Metabolic parameter	Echocardiographic parameter	r	p
Free carnitine	**A velocity**	-0.30	0.020
	E/A	0.28	0.032
	Heart rate	-0.29	0.025
Total carnitine	**A velocity**	-0.30	0.018
	Heart rate	-0.26	0.044
LDL-cholesterol	**E/A**	-0.38	0.003
	EVTI/AVTI	-0.41	0.001
	cIVRT	0.45	<0.0001
HDL-cholesterol	**A velocity**	-0.27	0.050
β-OH-Butyrate	**LV wall thickness**	-0.29	0.030
	Aorta valve opening	-0.29	0.028
	Cardiac output	-0.31	0.019
Free fatty acids	**LV diastolic diameter**	-0.27	0.040
	LV septum thickness	-0.41	0.002
	LV wall thickness	-0.49	<0.0001
	LV mass	-0.43	0.001
	Aorta valve opening	-0.35	0.070
	Cardiac output	-0.37	0.040
HbA_{1c}	**EVTI/AVTI**	-0.38	0.026
	cIVRT	0.40	0.017
Duration of diabetes	**A velocity**	0.40	0.015
	E velocity	0.35	0.040
	EVTI	0.45	0.007
	V_{max} AV	0.37	0.030
	Cardiac output	0.51	<0.0001

low[23]. Similar to the above studies, Juhasz *et al.* reported that myocardial contractility was reduced[24].

In contrast to the above-mentioned studies, Thusen *et al.* found that myocardial contractility (high velocity in fractional contraction and fiber contraction) was increased in children with DM1[28]. Similar results were also found by Gøtzsche *et al.*[29]. Kimball *et al.* found in 39 adolescents with DM1 that left ventricular mass, performance, contractility, and systolic and diastolic blood pressures were increased compared with controls[30].

Indices regarding left ventricular contractility in children and adolescents with DM1 were found to be normal by Hausdorf *et al.*[25], Borow *et al.*[26], and Schwingshandl *et al.*[27].

In our study, there was neither any disorder in left ventricular systolic functions nor myocardial mass increase, consistent with the results of recent researchers. The varying results obtained in these studies may be due to inappropriate echocardiography technology, and difference in age and DM1 duration of the diabetic groups.

Results of Doppler echocardiography studies in children are also controversial. Riggs and Transue investigated diastolic functions in 20 children using Doppler echocardiography for the first time. They demonstrated that the early phase (E) of diastolic filling was reduced, whereas its active phase (A), associated with atrial contraction, was normal. No relationship was shown between diastolic parameters and DM1 duration or degree of diabetic control[31]. Nevertheless, in a study by Gøtzsche *et al.* in 23 children with DM1 who had no diabetic microvascular complications, no diastolic function disorder was detected with Doppler echocardiography[29].

In a study by Salazar *et al.* in 31 children with DM1 aged between 4 and 20 years with DM1 duration ranging from 1 to 16 years who had no cardiovascular risk factors, no impairment in systolic and diastolic function was detected by Doppler echocardiography[34].

In 50 diabetic children with an average age of 13 years who carried no cardiovascular risk factors and who had mean DM1 duration of 5.9 years, Cerutti *et al.* studied systolic and diastolic functions using M-mode and Doppler echocardiography. In this study, the mean time to pressure half-life, an indicator of the early diastolic phase, was high, and a relationship was shown between diastolic parameters and DM1 duration and degree of diabetic control[33].

Ragonese *et al.* demonstrated that the velocity of A was increased and E/A ratio was decreased in 82 children and young adults with an average age of 17 years who did not have cardiovascular risk factors [32]. Lo *et al.* showed in a study on 40 identical twins with DM1 (mean age: 26 years) that the E/A ratio was reduced and IVRT was prolonged, and these parameters were associated with DM1 duration[15].

In our study, diastolic functions in patients with DM1 duration below 5 years (mean: 3.5 years) were not different from controls. On the other hand, we found that in patients with an average age of 13.5 years with DM1 duration over 5 years (mean: 8.2 years), the active phase of diastolic filling associated with atrial contraction (AVEL) was increased, early passive phase (EVEL) was normal, AVTI was elevated, and E/A and EVTI/AVTI ratios were reduced. Patients with a history of with DM1 longer than 5 years had higher heart rates than controls, and left ventricular filling patterns were different (Table 5).

In our study, the isovolumetric relaxation time (IVRT), which was one of the sensitive criteria indicating diastolic function disorder, was not different in patients with DM1 compared with controls, but we compared IVRT corrected by heart rate, since patients with DM1 with a diabetes history of more than 5 years had a higher mean heart rate than the matched control group. Patients with DM1 with a history of diabetes longer than 5 years were found to have significantly higher cIVRT (Table 5).

If diastole is divided into four phases (isovolumetric relaxation, rapid filling, diastase, atrial contraction), we can say that at least two (isovolumetric relaxation, atrial contraction) of these phases have a tendency towards impairment.

Results of investigations examining the effects of DM1 duration and degree of control on left ventricular function are conflicting. It was determined in our study that, as the degree of diabetic control decreased (as HbA_{1c} increased), EVTI/

AVTI ratio declined and cIVRT was prolonged (Table 6). In the investigations by Cerruti *et al.*[33] and Lo *et al.*[15], a relationship was also shown between diastolic parameters and DM1 duration and degree of control. This relationship, however, was not detected in the study conducted by Riggs and Transue[31].

In several studies in adult patients with type 1 and type 2 diabetes mellitus, an elevated A wave, normal or elevated E wave, reduced E/A ratio, and prolonged IVRT values were detected, suggesting diastolic dysfunction[6-10,14,15,21,59]. These studies all show that left ventricular relaxation (IVRT) is prolonged and the active phase associated with atrial contraction is augmented in diabetes mellitus.

As the number of investigations increased, the concept that diastolic dysfunction develops prior to systolic dysfunction in patients with diabetes mellitus has been postulated; the reason is that diastolic dysfunction develops before systolic dysfunction in many cases, such as congestive heart failure, hypertension and coronary artery disease. Raev carried out an investigation in a series of 157 patients with DM1. Of the patients, 43 (27.4%) had diastolic dysfunction and 18 (11.5%) had systolic dysfunction. Thus, the incidence of diastolic dysfunction was twice as high. Prolongation of DM1 duration caused the frequency of diastolic and systolic dysfunction to increase considerably. Diastolic dysfunction started 8 years after the onset of DM1, whereas systolic dysfunction began 18 years after onset[14].

The results of Raev[14] are consistent with our study because in our study the group with a mean DM1 duration of 3.5 years had normal systolic and diastolic functions (Table 4); but the group with mean DM1 duration of 8.2 years had normal systolic functions while showing a tendency towards impairment in diastolic functions (Table 5).

In our study, patients with DM1 were found to have high levels of fasting blood glucose, total cholesterol, LDL-cholesterol, FFA and β-OH-butyrate; low levels of HDL-cholesterol; and normal levels of triglycerides and VLDL-cholesterol (Table 1). A negative correlation between total cholesterol and LDL-cholesterol with E/A and EVTI/AVTI was detected. There was a negative correlation between β-OH-butyrate and left ventricular posterior wall thickness, aortic valve opening and cardiac output. Negative correlations were detected between FFA and left ventricular diastolic diameter, interventricular septum thickness, left ventricular posterior wall thickness, aorta root, aortic valve opening, and cardiac output (Table 6). The relationship between diastolic functions and these metabolic parameters has not previously been investigated as far as we know.

Although the pathogenesis of diabetic cardiomyopathy is not well known, it is seemingly an important factor that the cardiac muscle does not use glucose as an energy source, but depends totally on the oxidation of fatty acids. Oxidation of fatty acids upon entry into mitochondria and their use as the energy source depend on an adequate amount of free carnitine in the environment. If carnitine does not exist in sufficient amounts in the environment, FFA and related metabolites increase. The toxic effects of these accumulating substances and decrease in energy production (ATP) may both impair cardiac performance, resulting in diabetic cardiomyopathy.

In our study, in children with DM1 the total and acetylated carnitine levels were normal, and free carnitine levels were low. Total carnitine levels were also low in those whose duration of DM1 was above 5 years. These results are consistent with the results of other investigations on this topic, which were conducted in animals[37-40,60], adult humans[41-44] and children[45-47].

Several investigations have been made in diabetic animals on the favorable effects of carnitine on the heart[48-51]. Lopaschuck *et al.* showed that carnitine therapy in diabetic animals prevented the defects related to the elevation of long-chain acetylated-CoA and calcium storage in the sarcoplasmic reticulum[49]. Paulson *et al.* demonstrated that carnitine therapy in diabetic animals maintained the carnitine levels, decreased serum lipids, glucose and ketone bodies, and increased the resistance of the heart to ischemia[51]. Pieper and Murray established that carnitine therapy reduced ATP loss and prevented the increase in long-chain acetylated-CoA in diabetic animals[50].

Rodrigues *et al.* administered carnitine to some of the mice to which they had given streptozotosin to make them diabetic. When diabetes mellitus

developed in the group which was not given carnitine, left ventricular pressure, ventricular relaxation rate and cardiac contractility decreased and lipids were elevated. In the group given carnitine, no cardiac disorders occurred, and lipid and glucose levels were lower. These findings suggest that carnitine decreases the severity of diabetes mellitus and corrects the impaired cardiac performance in diabetic animals[48].

In our study, a negative correlation between total and free carnitine with heart rate and A velocity, which is one of the left ventricular diastolic parameters, and a positive correlation between free carnitine with E/A ratio were found (Table 6). As far as we know, such a relationship has not been investigated in any human research to date. This finding may be an important factor in the clarification and treatment of the pathogenesis of diabetic cardiomyopathy.

One of the most important handicaps in our study is that the diastolic dysfunction detected could not be demonstrated histologically. However, it is clear that obtaining a cardiac biopsy from a patient who does not show any symptoms of cardiac failure would not be ethical. Heart biopsy has not been used in any of the echocardiography studies except those in animals.

One of the weak points in our study is that we do not know whether the low serum carnitine levels detected reflect the carnitine levels in heart tissue, but it was shown in animal studies that blood carnitine levels were similar to tissue levels[48,61,62].

The results of our study suggest that there might be a relationship between low levels of free carnitine and diastolic functions. However, it has not been shown whether this disorder could be corrected by administering carnitine to patients. Investigating this topic in another study, we wish to establish definitely that the use of carnitine therapy in diabetes mellitus might be beneficial.

In conclusion, we have demonstrated that diastolic functions tend to deteriorate early depending on diabetes mellitus duration and the degree of diabetic control, and these disorders are associated with low levels of free carnitine.

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