Lakomkin V. L., Abramov A. A., Lukoshkova E. V., Studneva I. M., Prosvirnin A. V., Kapelko V. I.
National Medical Research Center of Cardiology, Moscow, Russia

Normalisation of diabetic heart pump function at decreased functional load

Aim	To study left ventricular (LV) hemodynamics in presence of decreased blood inflow to the heart as well as changes in myocardial content of energy metabolites in diabetic rats.
Material and methods	Diabetic cardiomyopathy is characterized by impaired heart contractility and by transition of cardiomyocyte energy metabolism fatty acids exclusively as a source of energy. This reduces the efficiency of energy utilization and increases the heart vulnerability to hypoxia. This study was performed on rats with type 1 diabetes mellitus induced by administration of streptozotocin (60 mg/kg). The LV pump function was studied with a catheter that allows simultaneous measurement of LV pressure and volume in each cardiac cycle.
Results	Blood glucose was approximately sixfold increased at 2 weeks. Heart failure was detected with decreases in ejection fraction by 27%, minute volume by 39%, and stroke work by 41%. Systolic dysfunction was based on a decrease in LV peak ejection velocity by more than 50%. Furthermore, the LV developed pressure and contractility index were within the normal range, while 1.5 times increased arterial stiffness was the factor that hampered ejection. The sum of adenine nucleotides was decreased by 21%, the ATP content was decreased by 29%, and also creatine phosphate formation was reduced in the myocardium of diabetic rats. Lactate content in the diabetic myocardium was increased almost threefold, which indicated mobilization of aerobic glycolysis. With the reduced preload, equal diastolic volume (0.3 ml), and equal blood pressure (60 mm Hg), the diabetic heart pump function did not differ from the control.
Conclusion	In type 1 diabetes mellitus, decreases in functional load and oxygen consumption normalize the myocardial pump function with disturbed energy metabolism.

Keywords	Diabetes mellitus; heart; contractility; pressure-volume; inflow; energy metabolism
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Corresponding author	Lakomkin V.L. E-mail: v.lakomkin@yandex.ru

Type 1 diabetes mellitus related to pancreatic injury may be induced experimentally by administering alloxan or streptozotocin. The latter approach is more common due to the renal and hepatic toxicity of alloxan [1, 2]. Rapidly developing hyperglycemia affects blood vessels and modifies the myocardial energy metabolism, switching it to the use of fatty acids as the only source of energy [3]. However, this reduces energy efficiency [4] and makes the myocardium more susceptible to hypoxia. Decreasing oxygen demand together with reducing functional load can improve energy supply for myofibrils. Diabetic cardiomyopathy is characterized by slowing of the heart rate, decreasing blood pressure (BP), the maximum left ventricular (LV) pressure and the rate of its development, as well as slowing down relaxation [5, 6]. It has been shown recently that myocardial relaxation can be accelerated by decreasing the heart blood supply [7]. This was accompanied by a decrease in LV diastolic pressure and improved LV filling. Thus, we used this technique to improve myocardial contractility in diabetic animals.

Objective

To investigate LV hemodynamics under decreased heart blood supply and measure the levels of energy metabolites in the myocardium of diabetic rats.

Materials and Methods

Forty male Wistar rats weighing 306–388 g were used in the study, which was conducted following Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The animals were divided into groups: streptozotocin (STR, intraperitoneally 60 mg/kg) in citrate buffer (pH 4.5) was injected in one group (n=10), while the other group was injected with citrate buffer 0.5 mL (n=10). The rat hearts were snap-frozen in situ in liquid nitrogen in separate series intended for biochemical tests (10 rats in the STR and the control groups). Energy metabolites were determined 2 weeks after the streptozotocin injection at the time of starting vivisections [8].

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Cardiac contractility was measured by LV catheterization through the right carotid artery under Zoletil using a standard FTH-1912B-8018 PV catheter and an ADV500 transducer amplifier (Transonic, Canada). Signals measured at baseline in a fragment with multiple (100 to 400 times) records of parameters were used to automatically calculate the mean values of the parameters characterizing cardiac function in LabChart 8.1 (ADInstruments, Australia). Although BP was not measured in these experiments, its changes could be evaluated according to the LV pressure at its maximum rate of rise P (dP/dt max), which almost coincides with the time of the aortic valve opening. In addition to the indicators calculated using in software, a contractility index was calculated, which represents a quotient of the maximum rate of pressure rise divided by P (dP/dt max). Right ventricle supply was restricted by transient (2–3 s) contraction of the inferior vena cava. After making an incision 2.5 cm long from the end of the xiphoid process along the white line, a ligature was inserted under the inferior vena cava between the liver and the diaphragm in order to allow the ends to emerge and be freely accessible. The ligature provided several heart cycles with a gradually decreasing LV filling. Measurement of the area of the LV pressure-volume curve reflected LV energy consumption in each cardiac cycle and, thus, the oxygen intake.

Fasting glucose levels was measured in blood collected from the tail vein with a OneTouch Select Plus Flex meter. Baseline glucose levels of 5.1–5.5 mmol/L remained unchanged in the control animals at 1–2 weeks. They increased in the STR group to 27±0.8 mmol/L in 1 week and to 31.2±1.5 mmol/L in 2 weeks. During this period, rats gained a mean of 42 g in the control group and lost a mean of 68 g in STR group.

The results are expressed as the means and standard errors of the mean (M±SEM). The data obtained were processed using a two-tailed Student's t-test.

Results

Energy metabolism

As can be seen from Table 1, total adenine nucleotide content was significantly reduced in the myocardium of diabetic rats. This difference can be primarily attributed to ATP (–29%). The formation of phosphocreatine was also impaired as confirmed by a more than 2-fold decrease in the ratio of phosphocreatine to free creatine: 9±3% in diabetes and 22±4% in the control group ($p<0.05$). Lactate levels were increased in the diabetic myocardium (5.9±0.8 μmol/g vs. 2.1±1.0 μmol/g of dry tissue weight; $p<0.001$). There was a negative correlation between the levels of lactate and phosphocreatine ($r=–0.70$; $p<0.02$).

Table 1. Adenine nucleotides (AN) in the myocardium of diabetic rats

Group	ATP	ADP	AMP	Total AN
Control (n=10)	13.5±1.1	5.9±0.3	1.8±0.2	21.1±0.8
Diabetes mellitus, (n=8)	9.6±0.3**	5.8±0.3	1.3±0.1*	16.7±0.2***

* – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ compared to the control.
The metabolite levels are expressed in μmol/L of the tissue dry weight.

Contractility

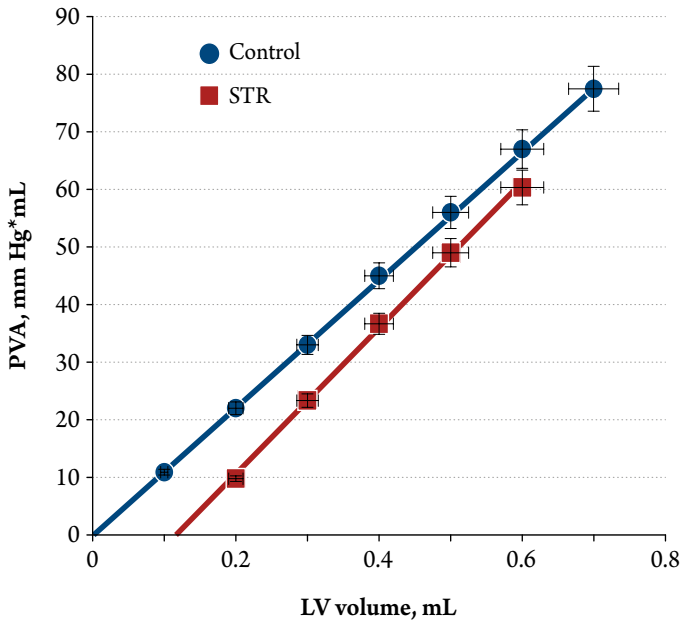
The baseline measurement of cardiac hemodynamics and contractility in the STR group revealed heart failure with reduced left ventricular ejection fraction, minute volume, and stroke output by 27%, 39%, and 41%, respectively (Table 2). These changes are due to a more than 2-fold reduction in the peak LV ejection rate. The rise in LV pressure and P (dP/dt max), which is almost the same as BP, were normal; the same was true of the contractility index. A 1.5-time increase in arterial rigidity was identified as the factor that hampered the ejection (see Table 2).

A momentary decrease in heart supply was accompanied by a gradual decrease in the left ventricular end-diastolic volume (LVEDV) and the area of the volume-pressure curve per cardiac cycle (PVA). Although the ratio of these parameters was comparable to linear in all the experiments, the corresponding regression line always crossed the volume axis near zero in the control experiments; the crossing point was close to 0.1 mL, while the slope of the regression line was slightly steeper in the diabetic rat experiments than in the control group (Figure 1). Although the difference in the slope coefficients of the corresponding regression lines (control – 119±3.3, SRT – 139±10) was not statistically significant ($p=0.099$), this difference may show a more severe decrease in the rate of oxygen intake by the diabetic heart with restricted blood supply.

Some of these changes can be attributed to LV diastolic volume reduced by 15% (see Table 2, column 2), i.e., less pronounced stretching of myocardial fibers. For this reason, all indicators were measured during the supply restriction to the same volume of 0.3 mL in each experiment (see Table 2). Although the decrease in pumping function was almost the same in the diabetes and the control groups, the peak LV ejection rate even increased compared to the baseline due to significantly reduced BP. However, myocardial contractility was reduced with low blood pressure in the diabetes group: the contractility index, maximum rise of LV pressure, and rising LV pressure were significantly lower by 28%, 37%, and 19%, respectively (see Table 2, column 4).

To equalize the groups in BP, the same indicators were determined in the control group, but with a BP of 62 mm Hg, the same as in the diabetes group (see Table 2, column 5). Comparison of the data showed that all

Figure 1. Dependence of PVA (area of the pressure-volume curve per cardiac cycle) on the LV end- diastolic volume in the control rats (n=8) and diabetic rats subjected to SRT injection (n=8)



indicators of function were higher in the control group than in the diabetes group (see Table 2, column 4); however, the difference was insignificant except for accelerated relaxation and reduced LV diastolic pressure. Thus, the hearts of diabetic rats differed only in diastolic parameters with the same vascular bed resistance and approximately the same LV filling. Attention should be paid to the changes of the arterial elasticity index in the control group, which increased as blood pressure decreased (see Table 2, columns 1, 3, and 5). Thus, there is a self-regulatory system response aimed at maintaining coronary blood flow when BP is decreased.

Discussion

The findings showed that systolic dysfunction in diabetic rats is combined with a decrease in myocardial energy reserves of ATP and phosphocreatine. Continuously low levels of phosphocreatine were observed along with normal ATP levels [9, 10] despite mitochondria having reduced oxygen consumption in the diabetic hearts [11]. Similar changes have been observed in patients with type 1 diabetes mellitus, i.e., the phosphocreatine/ATP ratio is typically

Table 2. Hemodynamics of the heart in diabetes at baseline and with decreased diastolic volume and blood pressure

Parameter	Control-1	Diabetes-1	Control-2	Diabetes-2	Control-3
	baseline		DV 0.3 mL		BP 60 mm Hg
Columns	1	2	3	4	5
Number of experiments	8	8	8	8	8
Parameters reflecting performance and energy consumption of the heart					
Minute volume, mL/min	138±6	84±4***	97±5	64±4***	76±9
HR, bpm	351±5	331±5*	357±6	332±3*	351±2
LVEF, %	73±3	53±3***	84±3	67±5*	74±4
Cardiac performance, mm Hg·mL	47,4±1,7	27,9±1,5***	29,0±2,0	16,3±1,7***	19,8±2,1
Peak ejection rate, mL/sec	11,5±1,8	5,3±0,2***	8,5±0,7	6,5±0,5*	7,9±0,3
PVA, mm Hg×mL	62±3	43±4***	41,3±5,3	27,1±3,0*	25,4±2,8
Parameters reflecting preload and afterload of the heart					
LVEDP, mm Hg	4,3±1,2	6,8±1,0	3,2±1,1	4,5±1,1#	1,0±0,8
Ea, mm Hg/μL	0,27±0,01	0,40±0,05**	0,35±0,04	0,42±0,03	0,39±0,06
LVEDV, mL	0,55±0,03	0,47±0,02*	0,31±0,02	0,30±0,02	0,30±0,02
Systolic parameters					
LV peak pressure, mm Hg	128±6	120±3	111±5	90±5*	95±2
Maximum rate of LV pressure rise, mm Hg/sec	10960±602	9340±769	9860±810	6260±970*	7760±378
Contractility index, sec-1	126±5	111±8	134±7	117±10	121±5
P (dP/dt max), mmHg	87±4	84±2	73±4	63±4	62±1
Diastolic parameters					
Peak LV filling velocity, mL/sec	12,6±1,3	8,1±0,5**	6,9±0,5	5,9±0,6	6,9±0,6
Maximum rate of LV pressure drop, mm Hg/sec	9300±351	7370±409**	6660±432	5300±581	6370±113
Relaxation time constant (tau), msec	6,6±0,4	8,6±0,4**	6,1±0,2	7,9±0,5*#	5,8±0,2
Minimum LV pressure, mm Hg	0,5±1,3	4,3±1,1	–0,7±1,0	2,5±1,1#	–2,7±0,8

* – p<0.05, ** – p<0.01, *** – p<0.001 versus the corresponding control; # – p<0.05 versus column 5. DV – diastolic volume; BP – blood pressure; HR – heart rate; LVEF – left ventricular ejection fraction; PVA – area under the volume-pressure curve per cardiac cycle; LVEDP – left ventricular end-diastolic pressure; LVEDV – left ventricular end-diastolic volume; LV – left ventricle.

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Превенар 13

Сделайте шаг к защите
от пневмококковой инфекции

Единственная пневмококковая конъюгированная вакцина для детей от 2 месяцев и взрослых всех возрастов*

[redacted]

reduced [12, 13]. Decreased levels of phosphocreatine result from the reduced creatine phosphokinase activity, which is not stimulated by creatine [11, 13] moreover, the flow did not increase through creatine kinase with increasing performance [14]. In our experiments, the hearts of diabetic rats formed increased levels of lactate despite the metabolism of cardiomyocytes having switched to using fatty acids. Glycolysis appears to be slow, with lactate being formed from pyruvate in the lactate dehydrogenase reaction when pyruvate dehydrogenase is inhibited.

Baseline reduced pumping function of the diabetic hearts was registered under the conditions of slower diastolic LV volume and increased arterial elasticity: both factors can reduce LVEF. When the conditions of blood supply and resistance (diastolic volume, BP, and arterial elasticity) are the same, all indicators of the pumping function differed insignificantly from the control group, the difference remained only between diastolic indicators. These data suggest that the myocardium of diabetic rats can function normally under low load and limited oxygen consumption; moreover, that this level of performance causes systolic dysfunction only when in vivo conditions of cardiac performance are created.

Individual attention should be paid to the elasticity of arteries, which was increased 1.5-fold in diabetic rats at baseline and remained the same with lower blood supply and BP. Since such continuously increased rigidity increased in control experiments only with when BP decreased to maintain adequate coronary blood flow, it is likely to be typical of diabetes mellitus. Diabetes-related vascular damage is due to the effect of high-concentration glucose on the vascular wall, which increases arterial tone, along with sharply increased production of active forms of oxygen and nitrogen [15]. Due to the constancy of this factor, vascular rigidity increases and stabilizes, thus producing an additional load to the heart. The result is the calcification of coronary vessels, which become less reactive [16] resulting in decreased coronary reserve [13].

Along with the hemodynamic causes of systolic dysfunction, the impaired energy supply of cardiac contractility should also be taken into consideration in diabetes mellitus: maintaining heartbeat requires much more energy than achieving pressure rise. Thus, all indicators of LV ejection were reduced in our work; moreover, the indicators of LV pressure rise were within the normal range.

Our findings showed that a decrease in the functional load and oxygen consumption normalizes myocardial contractility in diabetic rats with energy metabolism disorders. Therefore, treatment strategy should be based on preventing increased load that requires more oxygen consumption.

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