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GLUCOSAMINE IN SERUM OF PATIENTS AFTER MYOCARDIAL INFARCTION SUBJECTED TO REHABILITATION TRAINING

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This paper presents results of 3 weeks physical training on glucosamine level in serum of male patients after myocardial infarction (MI) aged between 38 and 61. Patients were randomised in two groups: the training group (n = 21), staying in Cardiac Rehabilitation Department and the control group (n = 11), discharged home for 3 weeks. Each group received indentical dietary instructions. The training group performed exercises every day: on bicycle ergometer during 30 minutes (5 times a week), overall-conditioning exercises for 30 minutes daily and 30 to 60 minutes of walking each day. Before administering of the therapy and 3 weeks later all MI patients performed the bicycle ergometer exercise test until the ventilatory threshold was reached. Before that test and 3 minutes after its termination capillary and venous blood samples were drawn. In the capillary blood samples indices of acid-base balance, lactate level, and glucose level were determined. In venous blood samples the serum levels of immunoreactive insulin, C-peptide and glucosamine were determined as well as binding of ^{125}I-insulin to erythrocyte receptors. Obtained results show that administered therapy increased physical fitness and decreased of glucosamine concentration, insulinaemia and insulin resistance.

Key words: *glucosamine, insulin resistance, myocardial infarction.*

INTRODUCTION

Glucosamine, a product of glucose metabolism *via* the hexosamine pathway, becomes incorporated into macromolecules such as gangliosides, glycolipids, glycoproteins and proteoglycans. The first and rate-limiting step for glucose entry into this pathway is catalysed by glutamine: fructose--6-phosphate transferase (GFAT), which uses the amino group of glutamine to convert fructose-6-phosphate (F-6-P) into glucosamine-6-phosphate (G-6-P). The hexosamine biosynthetic pathway is hypothesized to mediate some of the toxic effects of hyperglycemia. The results of *in vitro* studies suggest that

increased flux of glucose through the hexosamine biosynthesis pathway contributes to glucose — induced insulin resistance (1—3), defined as a decrease in insulin-induced glucose uptake by peripheral tissues. Marshall *et al.* (3) demonstrated that the induction of insulin resistance by glucose required glutamine and could be inhibited by glutamine analogues.

Hyperinsulinaemia and insulin resistance have recently been considered an independent risk factors of ischaemic heart disease (4—6). The purpose of this study was to determine the glucosamine level in serum of patients after myocardial infarction and to find out whether rehabilitation training influenced serum glucosamine concentration.

MATERIAL AND METHODS

The study was performed on 32 male patients after myocardial infarction (MI) aged between 38 and 61 years and 17 healthy male subjects. The patients were normotensive without diabetes mellitus and heart failures. They were admitted at the Cardiac Rehabilitation Department of the Rehabilitation Hospital in Poznań-Kiekrz on the average 3.6 months after MI (range of 1.5 to 6 months). After preliminary examination, the screening bicycle ergometer cardiopulmonary exercise test (ECG) was performed. The test was symptom limited. The patients who were qualified to the program met the following criteria: exertion tolerance > 100 W, ejection fraction (EF) > 40%, no symptoms of heart ischemia and severe cardiac arhythmia. Then the patients were randomised into 2 groups: the training group (n = 21) and the control group (n = 11). The average anthropometric parameters of the patients are presented in *Table 1.* The following day, the secondary exercise ECG test was performed with measurement of O_2 uptake and CO_2 elimination using computer set Cardio O_2 by Medical Graphics USA. Exercise was initiated at 25 W and it was increased by 25 W every 3 minutes. The test was continued until the ventilatory threshold was reached. Before the test and 3 minutes after it, capillary blood samples from the finger tip and venous blood samples from the ulnar vein were drawn.

Table 1. Average anthropometric characteristics of both groups of patients after MI and healthy subjects.

	Training Group		Control Group		Healthy	
	arithmetic means	standard deviation	arithmetic means	standard deviation	arithmetic means	standard deviation
AGE (years)	47.15	5.12	45.91	5.07	45.57	3.97
HEIGHT (cm)	175.85	6.12	177.36	4.73	172.21	10.21
BODY MASS (kg)	85.04	9.67	81.50	9.31	82.05	13.68
BMI (kg/m^2)	27.69	2.57	25.82	3.07	27.66	2.37

The following variables in the capillary blood samples were measured: the acid-base balance parameters using AVL 995 Hb analyser, lactate level by Boehringer Mannheim (Germany) reagent tests and the glucose level by tests from Cormay Company (Poland). The samples of venous blood

serum were analysed for the insulin level by radioimmunoassay with double antibodies using RIA-INS sets from Research and Development Center for Isotopes in Otwock-Świerk, the C-peptide level by Biodata-Serono (Italy) test and the glucosamine level by the Elson-Morgan spectrophotometric method modified by Sobocinski (7). Erythrocytes were isolated from heparinizied blood and binding of ^{125}I-insulin by erythrocyte receptors was determined according to the method described by Gambhir *et al.* (8), with our own modification (9). Radioactivity measurements were done on the Scaler A-224 gamma counter. The results of biochemical analyses were compared to those in healthy male 17 subjects. Their blood samples were drawn in the same conditions as from MI patients. The anthropometric parameters of healthy subjects are shown in *Table 1*. After the initial investigation, patients from control group were discharged home for 3 weeks. Both the training and the control group received identical dietary instructions according to European Atherosclerosis Society recommendations (10). The first stage of training consisted mainly of continued endurance exercise on a bicycle ergometer. The training sessions started not ealier than 2 hours after a meal and were preceded by a medical examination every day. The training program included 15 training sessions during 3 weeks (5 times a week). The training session lasted 30 minutes and included: warming up for 5 minutes (bicycle pedalling without load), exercise with a load for 20 minutes and active recovery for 5 minutes (bicycle pedalling without load). Blood pressure and heart rate were systematically recorded during each training. The training load was set at the ventilatory threshold which was calculated on the basis of cardiopulmonary exercise test. Moreover, patients participated in overall-conditioning exercises for 30 minutes daily and additionally walked 30 to 60 minutes each day. After 3 weeks, in both groups, the analysed parameters were examined again according to the same protocol as before training. The exercise ECG test was discontinued at the same work load as in the first examination. At least 24 hours before initial and final examinations the patients did not receive any medications, however during the study period the same drug treatment as before admission to hospital was continued. Both the training and the control group were given the same medications: aspirin (90% of all the patients from the training group and 100% from the control group), β-blocker (70% and 67%, respectively), ACE-inhibitor (7% and 20%), trimetazidin (7% of both groups) and syncumar (10% of the patients only from the training group). This investigation was conducted after receiving the approval of patients and the Regional Ethic Board of Committee for Science and Research. The presented results were analysed by Student's t-test.

RESULTS

Table 1 shows that there were no significant differences in anthropometric parameters between the patients from the training and the control group and also between then and the healthy subjects. The average work performed during the initial test by patients from the training group was 47.6 ± 2.7 kJ and 46.5 ± 4.6 kJ by patients from the control group. After the rehabilitation, a significant decrease in exercise heart rate (from 138.2 ± 17.7 to 128.0 ± 16.3 beats/min, $p < 0.05$), and the rate pressure product (RPP) attained during exercise (from 24598.1 ± 367.8 to 20980.2 ± 391.4, $p < 0.01$) were observed in the patients from the training group. In the patients from the control group the mean exercise heart rate and RPP in the second examination did not significantly differ from the results in the initial test (HR decreased from 137.5 ± 18.4 beats/min to 134.8 ± 18.1 beats/min; RPP increased from

22730.9 ± 370.9 to 22916.3 ± 553.8). A reduction in exercise lactic acid concentration from 3.79 ± 0.98 to 3.12 ± 1.16 mmol ($p < 0.05$), an increase in exercise blood pH (from 7.333 ± 0.022 to 7.342 ± 0.022) and an increase in exercise base eccess (BE) from −5.19 ± 1.52 to −4.78 ± 1.30 mmol were found in the training group. In the control group there were not statistically significant differences in blood acid-base balance parameters and lactate between the initial and final test (*Table 2*).

Table 2. Average values of blood acid-base balance parameters and lactic acid level of both groups of patients after MI before and after effort during initial and final test.

	INITIAL TEST											
	TRAINING GROUP						CONTROL GROUP					
	Before effort		After effort		Difference after--before	Student's t-test	Before effort		After effort		Difference after--before	Student's t-test
	$\bar{x}$	σ	$\bar{x}$	σ			$\bar{x}$	σ	$\bar{x}$	σ		
pH	7.371	0.016	7.333	0.022	−0.038	−9.828**	7.362	0.026	7.330	0.031	−0.038	−4.463**
BE (mmol)	−1.85	0.99	−5.19	1.52	−3.34	−13.460**	−1.55	1.37	−4.95	2.73	−3.40	−5.610**
LA (mmol)	1.29	0.35	3.79	0.98	2.50	11.787**	1.27	0.27	3.55	1.63	2.28	4.815**
	FINAL TEST											
pH	7.371	0.021	7.342	0.022	−0.029	−7.011**	7.379	0.020	7.327	0.016	−0.052	−9.247**
BE (mol)	−2.00	1.50	−4.78	1.30	−2.78	−7.740**	−1.40	1.30	−4.92	1.88	−3.52	−7.816**
LA (mmol)	1.33	0.27	3.12	1.16	1.79	6.972**	1.33	0.18	3.57	1.23	2.24	6.883**

Table 3 presents comparison of the resting (pre-exercise) concentration of glucosamine, insulin, C-peptide and erythrocyte insulin binding before effort in two terms of examination. There were significant decreases in the serum glucosamine concentration (defference between 1st and 2 nd examination was 14.14 mg%, $p < 0.01$), insulin level ($\Delta = 0.62$ ng/ml, $p < 0.05$) in the training group, whereas in the control group only glucosamine level decreased but the difference was smaller than in the training group ($\Delta = 7.2$ mg%, $p < 0.05$). However, the difference of glucosamine concentration decrease between both groups of MI patients was not statistically significant. The glucosamine concentration after exercise did not differ from that obtained before the effort in both tests (*Fig. 1*). Fig. 2 presents average glucosamine concentration of MI patients from the control and training groups divided into subgroups according to similar period from heart infarction to examination. There was

not statistically significant correlation between glucosamine concentration and lapse of time from heart infarction to the initial test, although there was a downward tendency observed. A comparison of biochemical parameters between healthy subjects and MI patients before rehabilitation (*Table 4*) shows the presence of hyperinsulinaemia, hyperglycemia and high glucosamine concentration in the patients.

Table 3. Average values of biochemical indices measured before the effort during initial and final examination in patients after MI.

	TRAINING GROUP				CONTROL GROUP			
	Test I	Test II	Difference II-I	Student's t-test	Test I	Test II	Difference II-I	Student's t-test
	$x \pm \sigma$	$x \pm \sigma$			$x \pm \sigma$	$x \pm \sigma$		
Glucosamine (mg%)	100.43±15.52	86.29±13.44	−14.14	−5.293**	103.74±18.12	96.54±15.23	−7.20	−2.947*
Glucose (mg/dl)	95.22±16.57	92.65±18.76	−2.57	−1.294	99.74±39.36	94.20±15.31	−5.54	−0.679
Insulin (μlU/ml)	14.91±8.74	8.42±3.38	−6.49	−7.184	15.35±9.77	11.00±6.36	−4.35	−0.968
Binding (pg/10^{11}RBC)	0.660±0.213	0.732±0.310	0.072	1.088	0.704±0.226	0.665±0.190	−0.039	−0.708
C-peptide (ng/ml)	2.70±1.56	2.08±0.98	−0.62	−2.432*	2.77±0.42	2.83±1.63	0.06	0.131

**$p < 0.01$
*$p < 0.05$

Table 4. Average values of serum biochemical indices in healthy subjects (H) and patients after MI (TG — the training group, CG — the control group) measured before the effort during initial test.

	H $x \pm \sigma$	Difference TG-H	Student's t-test	Difference CG-H	Studen's t-test
Glucosamine (mg%)	74.41±8.46	26.02	6.040**	29.33	4.562**
Glucose (mg/dl)	76.72±7.96	18.60	4.135**	23.12	2.263**
Insulin (μIU/ml)	7.74±4.52	7.17	2.151*	7.61	2.032*
Binding (pg/10^{11}RBC)	0.731±0.191	−0.071	−1.043	−0.030	−0.328
C-peptide (ng/ml)	1.62±0.49	1.08	2.673*	1.15	6.175**

**$p < 0.01$
*$p < 0.05$

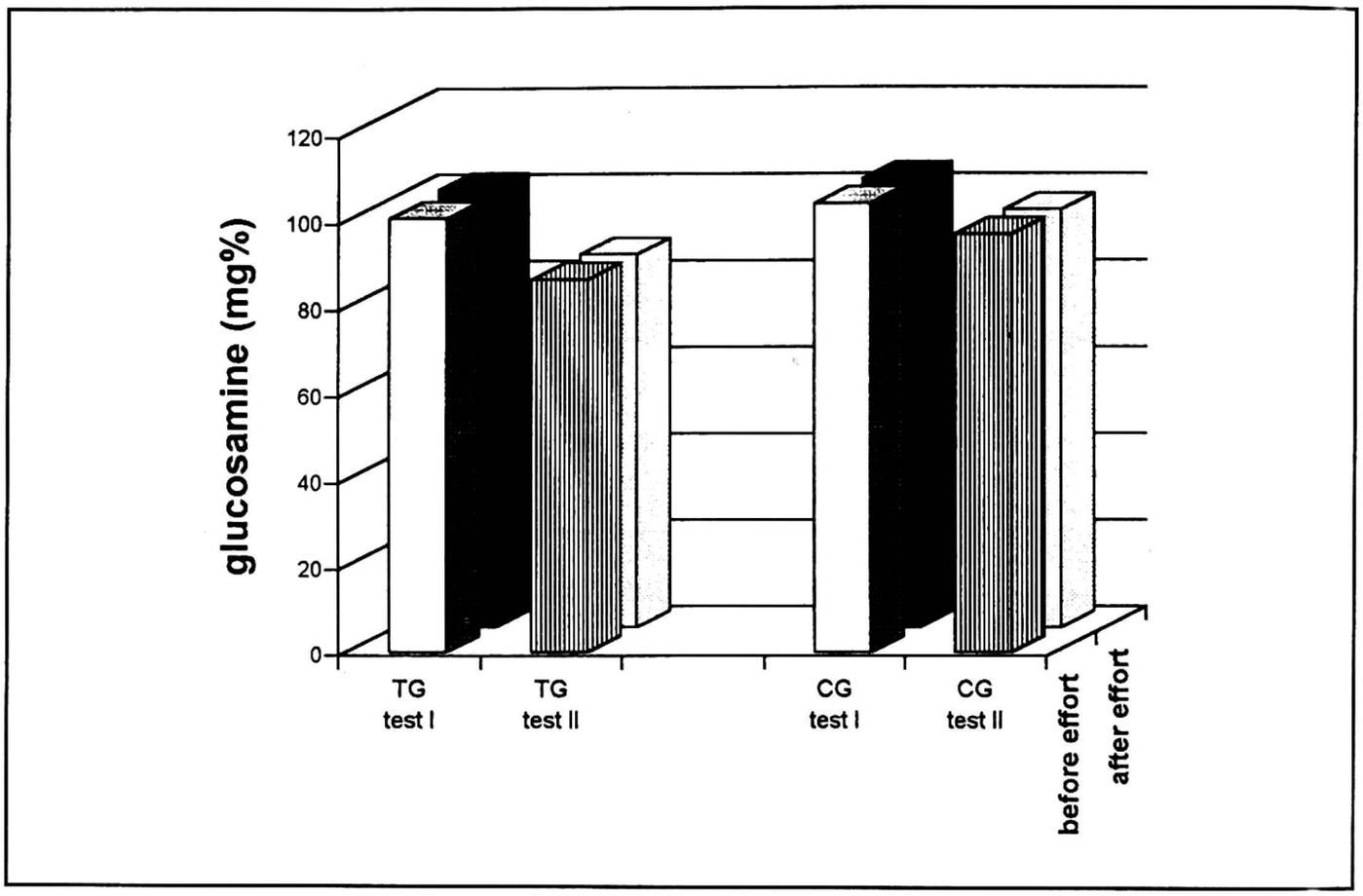

Fig. 1. The comparison of average values of glucosamine concentration before and after effort for the training group (TG) and the control group (CG) after MI in both measurement periods.

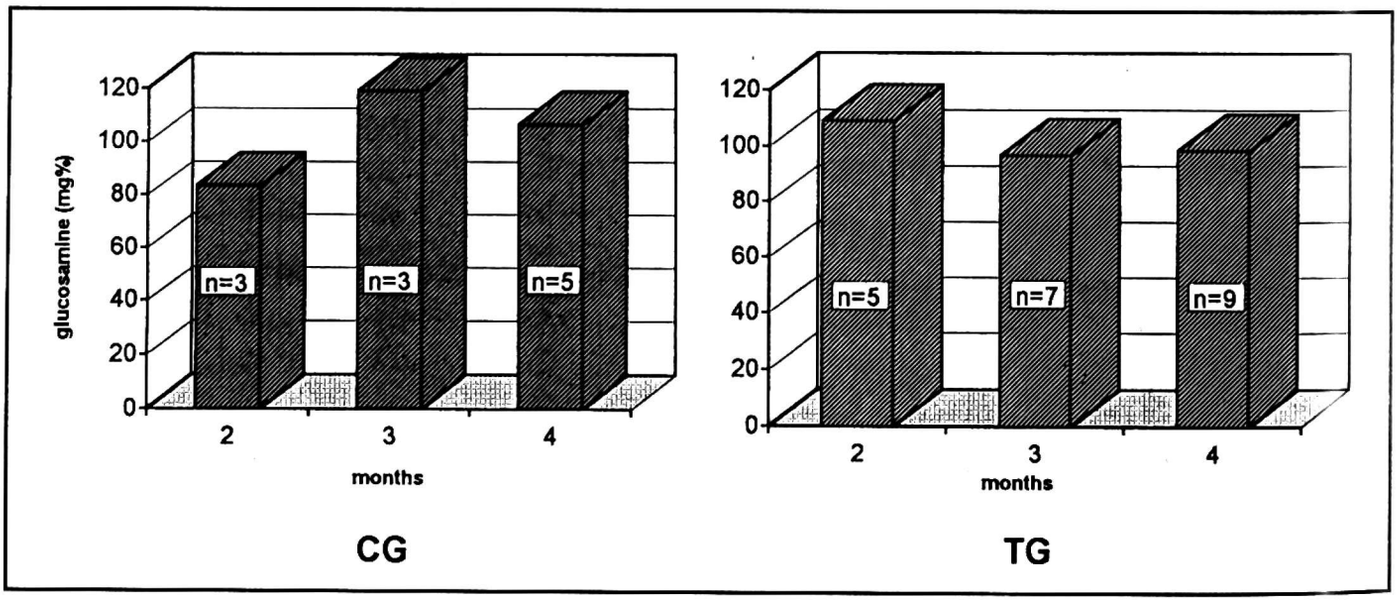

Fig. 2. Average glucosamine concentration of MI patients from the control group (CG) and training group (TG) in relation to lapse of time from heart infarction to the initial test. Patients are divided into subgroups according to quantity of months passaged from heart infarction.

DISCUSSION

The present study showed increase insulin and glucose concentration in blood accompanied with higher C-peptide level in MI patients indicating increased insulin secretion by pancreatic islet cells and insulin resistance at the

receptor level. Our findings concerning serum insulin level and peripheral insulin resistance of MI patients agree with the results obtained ealier by other authors in studies of subjects with ischaemic heart disease (11—13) as well as with our previous observations (14, 15). Impaired ^{125}I-insulin binding to specific receptors on erythrocytes which we found in MI patients in comparison to healthy subjects supports the receptor defect hypothesis. There is an inverse correlation between insulin binding and insulin concentration, high insulin concentration implicates, therefore lower insulin binding rate (16). Khan and co-workers (17) reported that the impaired response of glucose transport to insulin in skeletal muscle can be due to decreased amount and sensitivity of available glucose transport protein (GLUT-4). Robinson *et al.* (18) observed that glucosamine pretreatment inhibited basal and, to a greater degree, insulin-stimulated glucose transport in muscles in a time- and dose-dependent manner. Insulin receptor number, tyrosine kinase activity, and GLUT-4 protein expression were unaltered, suggesting that glucosamine pretreatment inhibited the distribution of GLUT-4 to the cell membrane. In this study there was observed a significantly higher ($p < 0.01$) glucosamine level in serum of MI patients in comparison with healthy subjects. The presence of increased glucosamine concentration simultaneously with hyperinsulinaemia and impaired ^{125}I-insulin binding to erythrocytes suggests that this hexosamine can be involved in insulin resistance mechanism.

There is a complex and unclear epidemiologic linkage between hyperglycemia and atherosclerosis (19, 20). Vascular smooth muscle cell proliferation is an early event in the pathogenesis of atherosclerosis, and growth factors were hypothesied to play an important role in the control of this process (21). McClain and co-workers (22) investigated the regulation of the expression of two growth factors α (TGFα) and basic fibroblast growth factor (bTGF). They showed that glucosamine was more potent stimulant of growth factors expression than glucose, leading to a 6-fold increase in TGFα mRNA. TGFα protein levels were also increased by glucosamine treatment, and predominant species present was the membrane-bound precursor from of TGFα. We suppose that increased flux of glucose to glucosamine could contribute to vascular complications of patients with cardiac disease.

The present investigation showed that physical effort performed systematically leads to an improvement of physical capacity of MI patients indicated by decrease in exercise HR and RPP and diminishing acid-base balance difference disturbances induced by the effort. In the patients from the training group there was also a significant decrease in glucosamine concentration after rehabilitation. The diminished glucosamine level in patients from the control group, a little lower than in the training group, suggests that not only training but also a restricted life style may influence hexosamine concentration. Downward tendency of glucosamine concentration in both

groups of MI patients in relation to lapse of time from the heart infarction to initial test may suggests the influence of drugs on that parameter. No difference in the glucosamine concentration were found during a single bout of exercise. This indicated that a decrease in hexosamine level results from improved glucose metabolism over a longer period. A decrease of hyperinsulinaemia in patients from the training group after rehabilitation accompanied with a towards increase of ^{125}I-insulin binding to erythrocytes is the evidence of decreased insulin resistance. An increase of ^{125}I-insulin binding to erythrocytes after systematic physical effort was reported in our previous papers (9, 23). Nelson and co-workers (24) observed that glutamine: fructose-6-phosphate amidotransferase (GFAT) activity was not affected by exercise. The lower glucosamine concentration after rehabilitation of patients with heart disease in our study can be probably attributed to the improvement of glucose uptake by tissues due to the greater tissue insulin sensitivity to insulin and lesser glucose flux into glucosamine biosynthetic pathway.

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