JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1997, 48, 4, 839—849

T. RYCHLEWSKI[1], Ł. SZCZĘŚNIAK[1], P. DYLEWICZ[2], E. DESKUR[3], I. PRZYWARSKA[2], Z. KASPRZAK[1], J. KAROLKIEWICZ[1], L. KONYS[1]

THE INFLUENCE OF ORAL GLUCOSE INTAKE ON BINDING AND DEGRADATION OF ^{125}I-INSULIN BY RECEPTORS ON ERYTHROCYTES AS WELL AS ON INSULIN AND C-PEPTIDE SERUM LEVELS IN PATIENTS AFTER MYOCARDIAL INFARCTION AND HEALTHY INDIVIDUALS

[1]Chair of Physiology, Biochemistry and Hygiene of the University School of Physical Education, Poznań, Poland; [2]Department of the Cardiac Rehabilitation of the University School of Physical Education, Poznań, Poland; [3]Department of Cardiology, Institute of Internal Medicine of the University School of Medical Sciences, Poznań, Poland

In this study, we investigated the influence of glucose administration on binding and degradation of ^{125}I-insulin by receptors on erythrocytes as well as on insulin and C-peptide serum levels in 15 patients after myocardial infarction and in 15 age-matched healthy persons. Venous blood samples were taken directly before and at 30, 60 and 120 minutes after oral administration of 75g of glucose. In the collected blood samples serum glucose, insulin and C-peptide levels were determined. Binding and degradation of ^{125}I-insulin by specific receptors on red blood cells were evaluated using the method described by Gambhir and modified by the authors. Serum insulin and C-peptide levels were significantly higher while binding of ^{125}I-insulin to erythrocytes was decreased in patients after myocardial infarction. These results seem to support the hypothesis that insulin resistance and hyperinsulinism play a role in the pathogenesis of ischaemic heart disease. Impaired degradation of ^{125}I-insulin during the oral glucose tolerance test in the patients after myocardial infarction indicates that insulin resistance is located at the receptor level.

Key words: *oral glucose tolerance test, insulin resistance, ^{125}I-insulin, myocardial infarction*

INTRODUCTION

Hyperinsulinism and insulin resistance, defined as the decrease in insulin-induced glucose uptake by peripheral tissues, have recently been considered independent risk factors of ischaemic heart disease (1—3). The impaired response to insulin of glucose transport in skeletal muscle can be due to a decreased amount of available glucose transport protein (GLUT4) and/or to its diminished degree of translocation from the microsomal intracellular

compartment (4). Some authors also suggest decreased blood flow as a possible reason for reduced glucose uptake (5,6). Since the initial phase of insulin metabolic action is to bind to specific receptors located on the cell surface, the binding and degradation of ^{125}I-insulin by its receptors on erythrocytes in response to glucose intake is widely used to investigate the insulin resistance phenomenon. In the present study we attempted to investigate the insulin resistance phenomenon in patients after myocardial infarction. To this end we evaluated changes in serum glucose, insulin and C-peptide levels as well as in ^{125}I-insulin binding and degradation rate during a glucose tolerance test in these patients and compared the results to those found in normal subjects.

MATERIAL AND METHODS

The study group consisted of 15 male patients admitted to the Cardiac Rehabilitation Hospital in Poznań and the control group of 15 age-matched healthy volunteers. All the patients had in the previous 3—6 months suffered from uncomplicated Q-wave myocardial infarction documented by chest pain, evolving electrocardiogram changes and increases in plasma enzyme levels. Subjects who suffered from diabetes or hypertension, had effort angina or complex arrhythmias, low ejection fraction (<40% estimated by ultrasound echocardiography) or low exercise tolerance (<100 watts on the cycloergometer) were not included into the study. There was no significant difference in age, height, body weight and body mass index between the patients after myocardial infarction and the control group (*Table 1*). Consent to participate in this study, which was accepted by the local ethics committee, was obtained from all subjects.

Table 1. Age, height body weight and body mass index in patients after myocardial, infarction (MIC) and the control group (CG).

Feature	Patients after myocardial infarction			Control group		
	$\bar{x}$	min	max	$\bar{x}$	min	max
Age [years]	44.1	40.0	50.0	42.6	36.0	49.0
Height [cm]	172.6	165.0	181.0	170.6	165.0	180.0
Body weight [kg]	81.5	69.0	94.2	80.4	65.0	101.0
BMI [kg/m^2]	27.2	23.8	30.8	26.8	22.6	29.5

On admission the patients were subjected to an oral glucose tolerance test. Both in the patients and the control group the test was performed after an overnight fast. The patients were not given any drugs for 24 hours before the test. A standard load of 75g of glucose dissolved in 300ml of distilled water was administered over 5 minutes. Peripheral venous blood was taken directly before (fasting level) and at 30, 60 and 120 minutes after glucose administration. In the collected blood samples serum glucose level was determined using the Cormay test, serum insulin level was evaluated by the radio-immunological method of double antibodies with the RIA-INS test produced by ORiPi Otwock — Świerk and C-peptide concentration was measured by means of the Biodat — Serono test.

Binding and degradation of ^{125}I-insulin by specific receptors on red blood cells were evaluated using the method described by Gambhir (7) and modified by the authors. This modification

consisted in the use of constant concentrations of ^{125}I-insulin (0.9pg/0.1ml) and bovine insulin (2.4IU/0.1ml). Marked insulin produced by ORiPi Otwock — Świerk was used. The global activity equals about 10 μCi. The radioactivity of the samples was determined with a Scalar A-224 type gamma counter and the amount of ^{125}I-insulin was calculated on the assumption that one impulse corresponds to 2×10^{-4} pg of ^{125}I-insulin. As each measurement was done in triplicate, the mean values were calculated for further analysis. In order to illustrate the changes in the analyzed parameters at 30, 60 and 120 minutes following glucose intake, the percent values of the change with reference to initial value were calculated from the formula:

$$\frac{t^i - t^0}{t_0} * 100$$

where: t_i = value obtained at 30, 60 or 120 minutes after glucose intake

t_0 = value obtained before glucose administration (fasting level)

Statistical analysis was performed with use of Student-t test and repeated measures ANOVA.

RESULTS

Patients after myocardial infarction had significantly higher serum insulin and C-peptide levels at the beginning of the test as well as at 30, 60 and 120 minutes after glucose intake in comparison with the control group (*Table 2*). Also, the glucose level and the binding of ^{125}I-insulin to erythrocytes at 60 and 120 minutes after glucose administration differed significantly between these two groups. There was no significant difference in degradation of ^{125}I-insulin by erythrocytes during the glucose tolerance test , only value obtained before the test was significantly higher in the patients. Statistical analysis shows that all examined parameters (except ^{125}I-insulin degradation) were significantly different in the compared groups.

Table 3 presents the analysis of the Freedman — ANOVA variations for the changes in the level of the examined parameters in reference to t_0 during the OGGT- test among the groups. There was also a comparison between the groups done by the Student- t test. It shows that the increases of glucose insulin and C-peptide levels are statistically significant ($p < 0{,}01$) as far as the binding is concerned, statistically significant differences were noted for t_{30} and t_0, and t_{60} and t_0 . In the control group, significant increases at all time points were noted only in case of levels of insulin and C-peptide. Glucose concentration was significantly increased in reference to t_0 at 30 and 60 minutes. Insulin binding was significantly decreased at 30 minutes, and insulin degradation was increased at 120 minutes. A comparison of changes in analysed parameters in reference to t_0 between the two groups shows that changes in insulin levels were significantly different at all time points while there was no difference in insulin degradation. Changes in glucose concentration differed significantly at t_{60}-t_0 and t_{120}-t_0, in C-peptide levels at t_{60}-t_0, and in insulin binding at t_{120}-t_0.

Table 2. Serum glucose, insulin, C-peptide levels and ^{125}I-insulin binding and degradation before and at 30, 60 and 120 minute after oral glucose intake in patientsafter myocardial infarction (MIC) and control group (CG).

Feature	t_i	MIC	CG	Average difference	Student's t-test
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x}$ MIC $- \bar{x}$ CG	
Glucose [mg/dl]	0	77.6 ± 10.25	76.6 ± 7.96	1.00	0.24
	30	126.7 ± 21.68	111.8 ± 18.91	14.87	1.65
	60	131.1 ± 35.69	94.7 ± 13.02	36.33	2.93 **
	120	103.1 ± 32.42	76.8 ± 8.12	26.33	2.40 **
Test ANOVA		25.44 **	21.36 **		
Insulin [μlU/ml]	0	16.2 ± 11.34	7.7 ± 4.52	8.51	2.14 **
	30	84.1 ± 44.56	47.5 ± 18.43	36.65	2.34 **
	60	100.7 ± 51.21	36.7 ± 14.28	63.99	3.67 **
	120	53.2 ± 30.02	24.5 ± 13.02	28.68	2.70 **
Test ANOVA		27.73 **	23.16 **		
C-peptide [ng/ml]	0	2.9 ± 0.66	1.6 ± 0.49	1.3	4.82 **
	30	7.5 ± 1.35	6.7 ± 2.06	0.8	1.14
	60	9.9 ± 2.05	6.6 ± 1.27	3.3	4.26 **
	120	8.4 ± 3.08	5.2 ± 1.41	3.2	2.83 **
Teat ANOVA		28.99 **	20.23 **		
Binding [pg/10^{11}RBC]	0	0.68 ± 0.22	0.73 ± 0.19	−0.05	0.49
	30	0.50 ± 0.22	0.44 ± 0.70	0.06	0.66
	60	0.41 ± 0.15	0.68 ± 0.31	−0.27	2.15 *
	120	0.64 ± 0.17	0.93 ± 0.32	−0.27	2.65 *
Test ANOVA		19.06 **	15.24 **		
Degradation [pg/10^{11}RBC]	0	8.70 ± 2.67	5.05 ± 2.57	3.65	3.16 **
	30	7.84 ± 3.17	5.25 ± 2.43	2.59	2.04
	60	8.53 ± 4.06	6.34 ± 2.37	2.19	1.49
	120	9.72 ± 4.95	6.96 ± 2.64	2.76	1.53
Test ANOVA		0.87	6.60		

* $p < 0.05$

** $p < 0.01$

Table 3. Statistical analysis of changes of parameters with reference to t_0.

Feature	Δt	MIC		CG		Student't t-test $\Delta_{MIC} - \Delta_{CG}$
		$\Delta t_i - \Delta t_0$	t-test	$\Delta t_i - \Delta t_0$	t-test	
Glucose [mg/dl]	30–0	49.09	9.51 **	35.22	7.19 **	1.90
	60–0	53.48	6.00 **	18.15	4.51 **	3.27 **
	120–0	25.53	3.52 **	0.20	0.18	3.03 *
Insulin [μlU/ml]	30–0	67.94	6.59 **	39.80	8.08 **	2.24 *
	60–0	84.48	6.55 **	29.10	8.18 **	3.68 **
	120–0	36.96	4.93 **	16.80	4.89 **	2.22 *
C-peptide [ng/ml]	30–0	4.63	14.61 **	5.03	9.06 **	−0.66
	60–0	6.99	14.21 **	4.95	14.34 **	3.19 **
	120–0	5.46	7.20 **	3.60	8.37 **	1.96
Binding [pg/10^{11} RBC]	30–0	−0.19	−3.45 **	−0.28	−3.83 **	1.18
	60–0	−0.27	−3.32 **	−0.04	−0.46	−1.53
	120–0	−0.04	−0.58	0.20	2.14	−2.53 *
Degradation [pg/10^{11} RBC]	30–0	−0.86	−1.38	0.20	0.18	0.88
	60–0	−0.17	−1.24	1.29	1.82	−1.15
	120–0	1.02	0.80	1.91	2.33 **	−0.54

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

Fasting ^{125}I-insulin binding to its receptors on erythrocytes was not significantly lower in patients after myocardial infarction compared to the control group (*Fig. 1*). However, the kinetics of ^{125}I-insulin binding during the oral glucose tolerance test was different. In normal subjects, the ^{125}I-insulin binding decreased over the 30 minutes after glucose administration, then increased, and 120 minutes after glucose administration was even higher than at the beginning of the test. In contrast, in the study group the lowest insulin binding rate was observed 60 minutes after glucose intake and at 120 minutes it was equal to the initial value. Also, the kinetics of ^{125}I-insulin degradation by red blood cells was different in both groups (*Fig. 2*). The fasting degradation of ^{125}I-insulin was significantly higher in patients after myocardial infarction. In response to glucose intake, the degradation rate gradually increased in the control group while it did not change significantly in the study group.

It is of interest that there was an inverse relationship between the binding of ^{125}I-insulin and serum insulin level both in the control group and in the patients (*Fig. 3*). The alterations in C-peptide serum level during the test paralleled the changes in insulin concentration (*Fig. 4*). The changes in serum glucose concentration corresponded to the alterations in insulin level with the

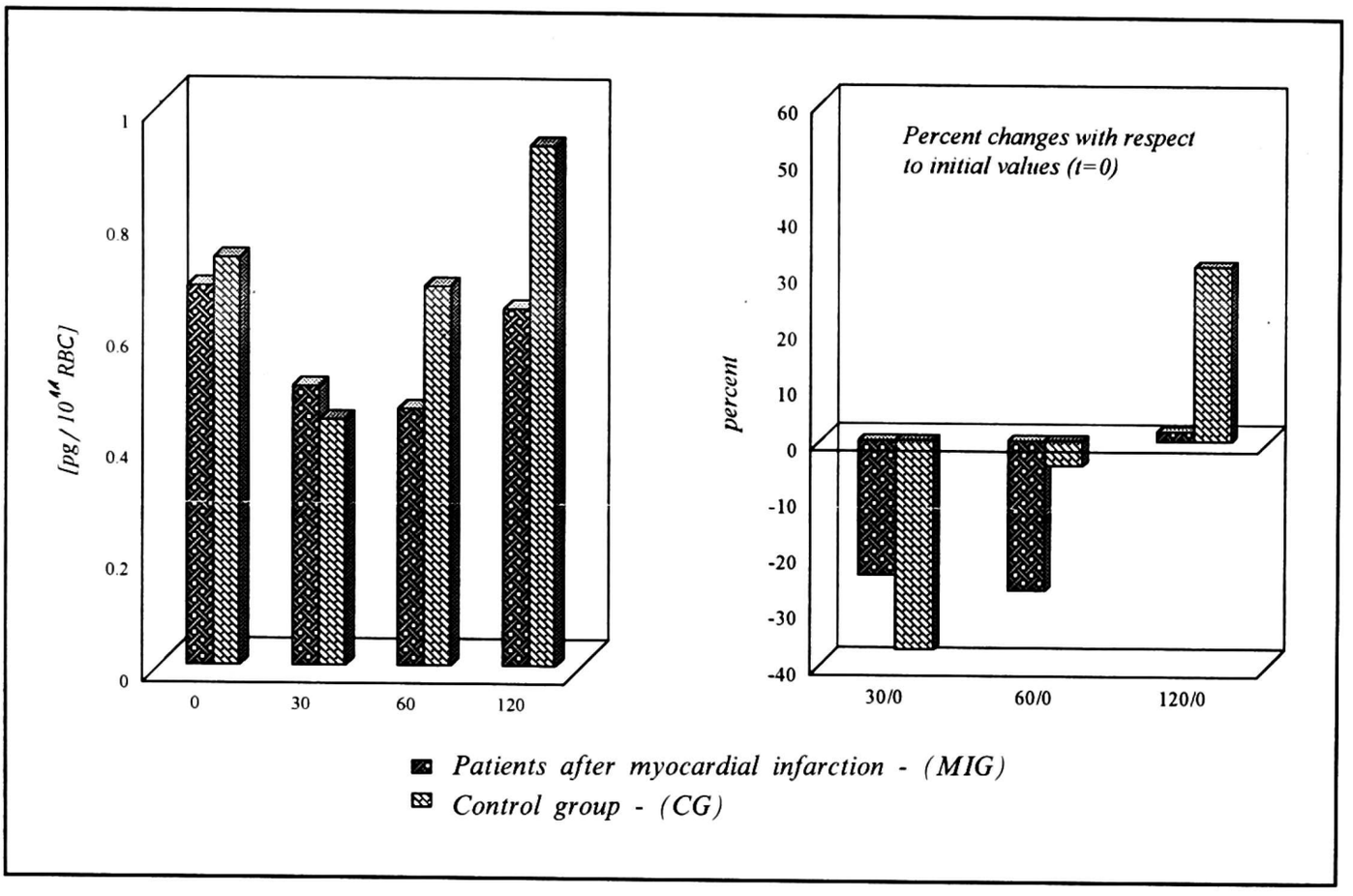

Fig. 1. Changes in ^{125}I-Insulin binding during oral glucose tolerance test

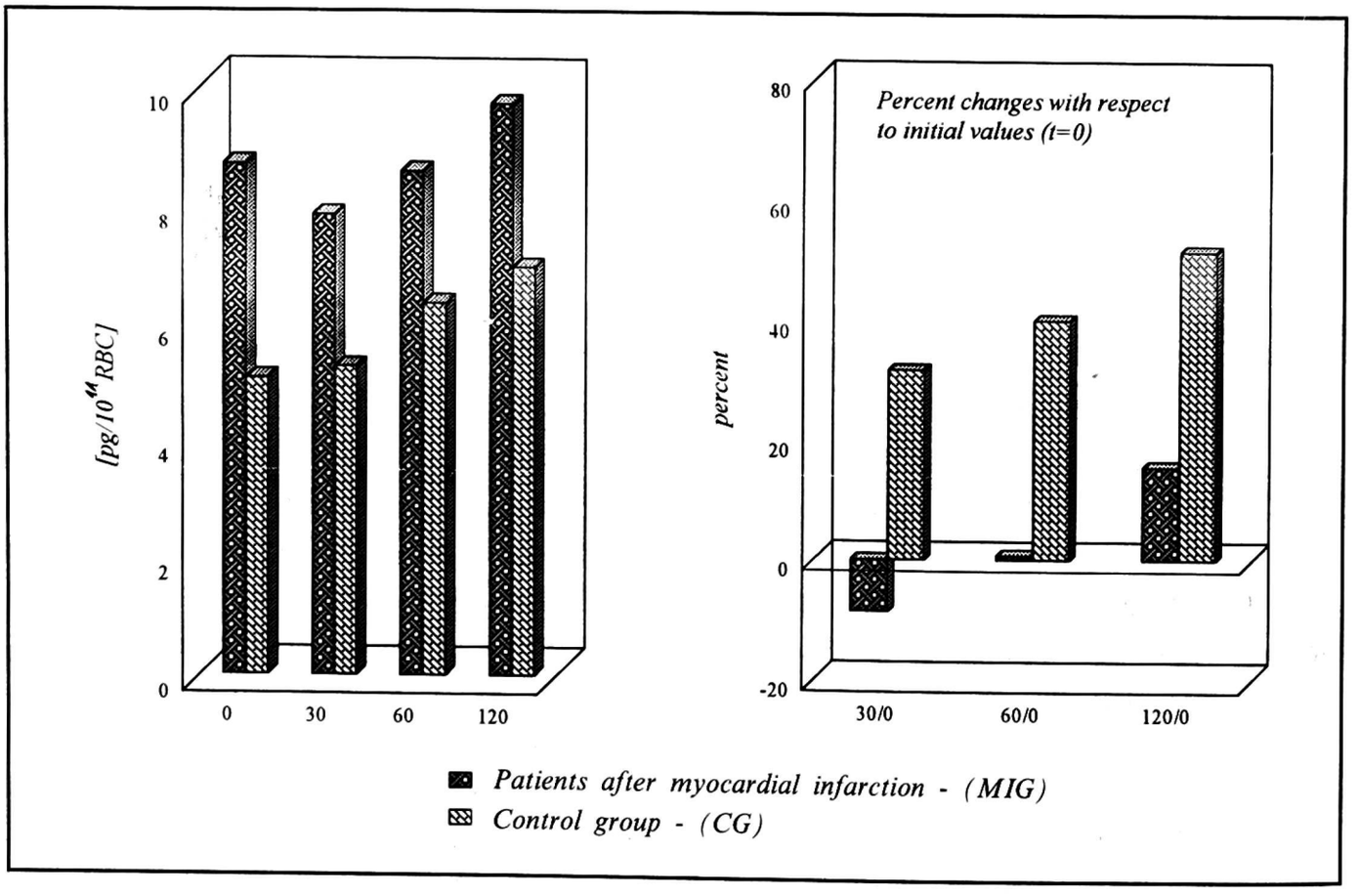

Fig. 2. Changes in degradation of ^{125}I-Insulin during oral glucose tolerance test

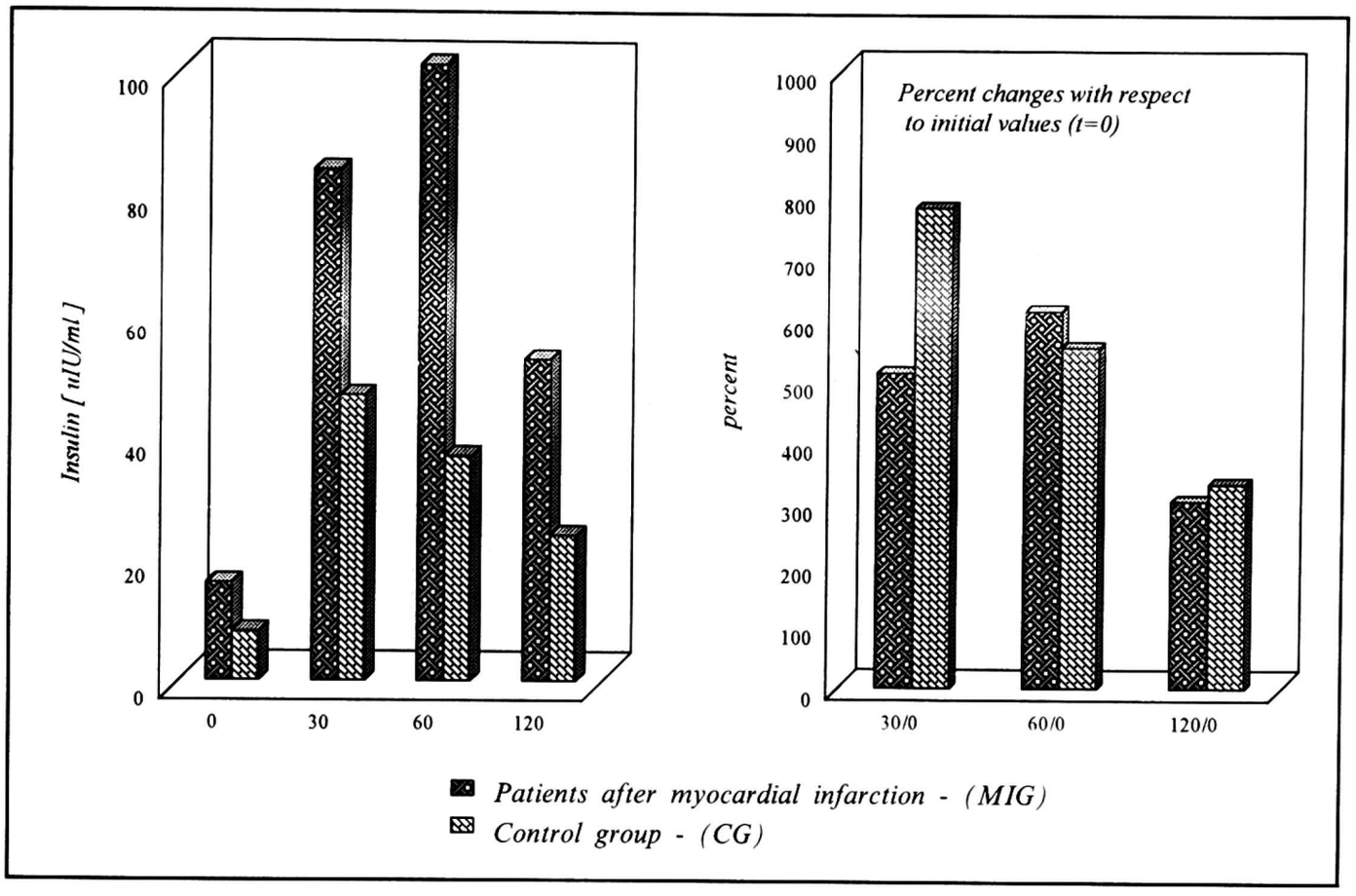

Fig. 3. Changes in Insulin concentration during oral glucose tolerance test

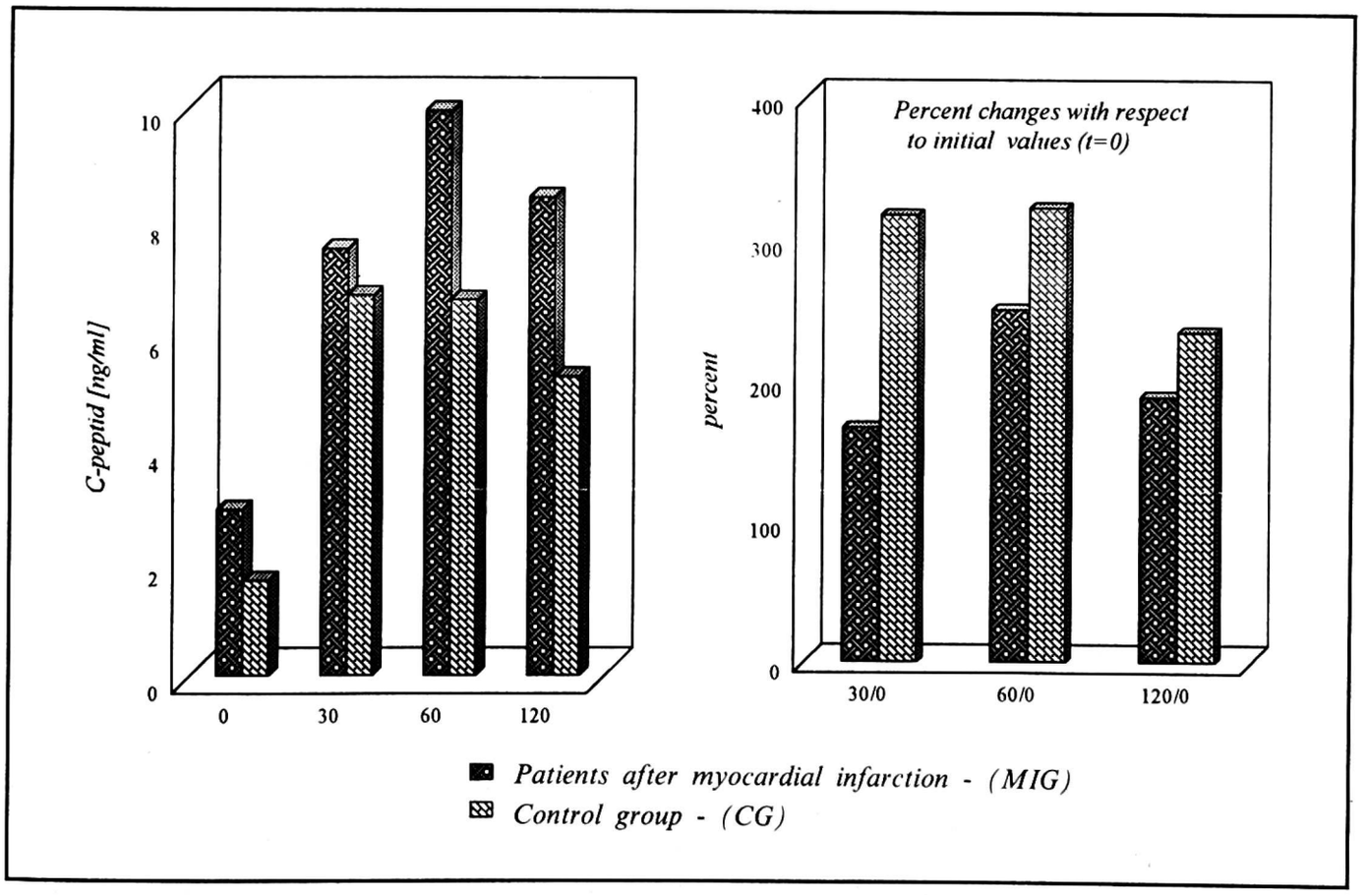

Fig. 4. Changes in C-peptide concentration during oral glucose tolerance test

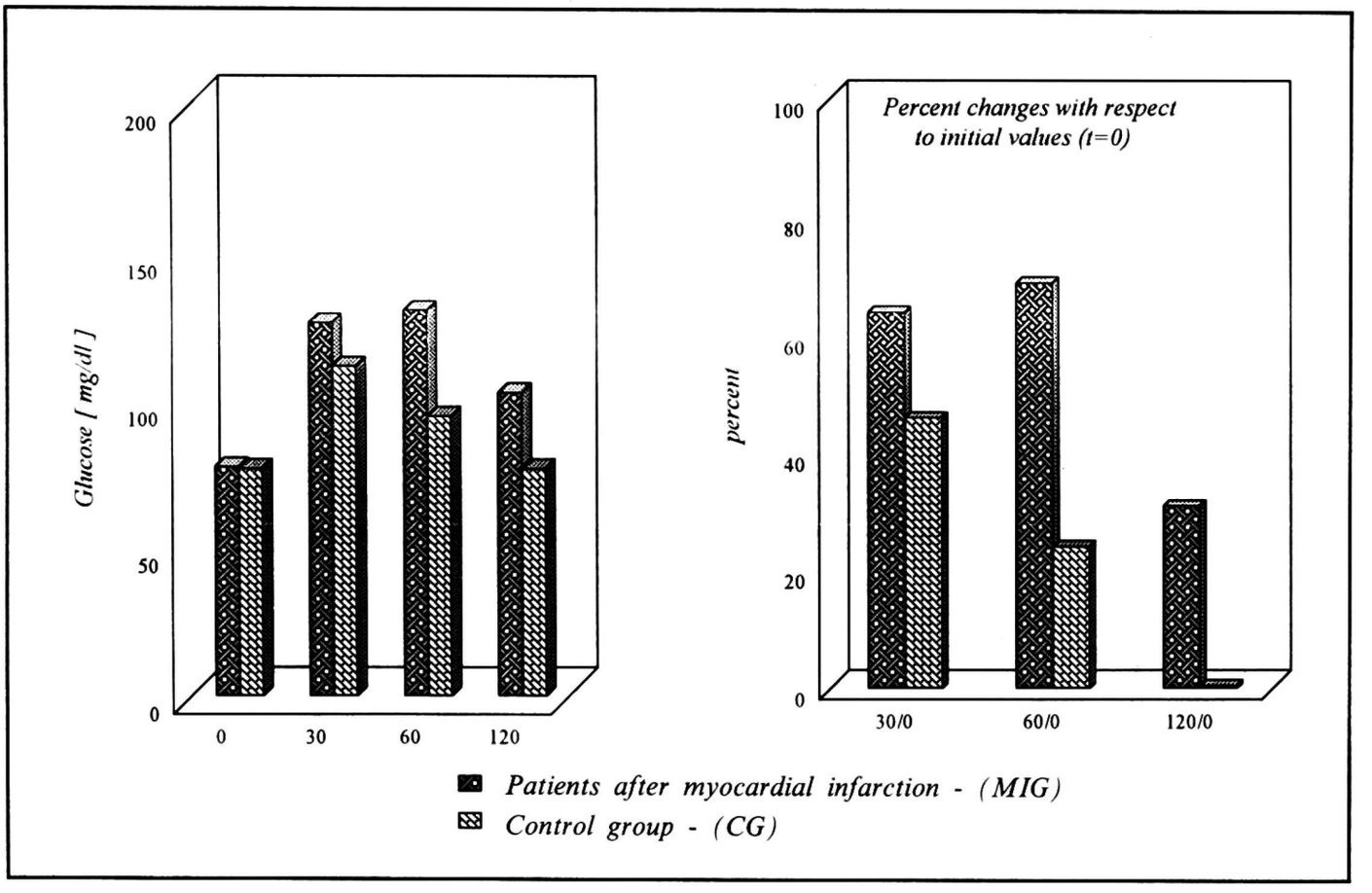

Fig. 5. Changes in glucose concentration during oral glucose tolerance test

difference that the fasting glucose level was equal in both compared groups (*Fig. 5*). The glucose curve was normal in the control group whereas in the study group it was shifted in time (maximum value at 60 minutes), while at 120 minutes glucose level was 40% higher than at the beginning, indicating glucose reduced glucose tolerance.

DISCUSSION

Our findings concerning serum insulin level and peripheral insulin resistance in patients after acute myocardial infarction agree with the results obtained earlier by other authors in studies of subjects with ischaemic heart disease (8—10) as well as with our own observations in essential hypertension (where the disturbances were even more pronounced) (11).

Insulin serum concentration two times as high in patients after myocardial infarction than in the control group (*Fig. 3*), accompanied by significantly higher C-peptide level, indicates increased insulin secretion by pancreatic islet cells and insulin resistance at the receptor level. Impaired ^{125}I-insulin binding to specific receptors on erythrocytes in comparison with healthy subjects supports the receptor defect hypothesis. There is an inverse correlation between insulin binding and insulin concentration, that is a higher insulin concentration

implicates a lower insulin binding rate. Also the achieved coefficient of correlation seem to prove that.

The negative correlation ($r = -0,64$) ($p<0,05$) between the ^{125}I-insulin binding and level of insulin serum in blood serum as well as positive correlation ($r = 0,65$) ($p<0,05$) between insulin and rate of degradation by the membrane enzyme were noted. Such correlations were not found in patients after myocardial infarction.

Kahn and co-workers (4) observed a reversible, during 8—12 weeks, decrease in binding of ^{125}I-insulin to platelets during acute myocardial infarction despite low insulin concentration has already been observed. This phenomenon could be explained by the increase in catecholamine concentration (12); however, a correlation between the catecholamine level and the ^{125}I-insulin binding rate has not been found. Decreased insulin binding to platelets resulted in platelet resistance to prostacycline PGI_2, which seemed to be of considerable importance to clot formation.

In the present study, we have found decreased fasting ^{125}I-insulin binding to erythrocytes accompanied by a two times as high insulin concentration in patients who had myocardial infarction 3—6 months earlier. After glucose intake (*Table 3*) in the group of patients after myocardial infarction, the difference in the insulin binding in t_{30} and t_{60} to t_0 is statistically significant, while in the control group a significant increase of insulin binding was observed only at 30 minutes. When comparing the both groups, significant difference was found exclusively at $t_{120} - t_0$. These results can be attributed to the impaired insulin receptor affinity or the "down regulation" phenomenon (13—15). There are no data indicating a decrease in the number of insulin binding sites on cell membranes in ischaemic heart disease. However, it has been found that insulin resistance in hypertensive patients is associated with the reduction in the number of insulin receptors on erythrocyte surface (16).

The diminished fasting ^{125}I-insulin binding to erythrocytes in patients after myocardial infarction was even more distinct after glucose intake (*Table 2, Fig. 1*). In healthy subjects the highest insulin concentration and the lowest insulin binding rate were found at 30 minutes after glucose administration ($r = -0,86$) ($p<0,05$). At 120 minutes the insulin binding was even higher than at the beginning ($r = -071$) ($p<0,05$). In contrast, in the study group the highest insulin concentration and the minimum binding level were noted at 60 minutes. It is worth noting that among the patients after myocardial infarction the high correlation ($p< 0,05$) between the glucose and insulin level in 30, 60 and 120 minutes (respectively $r = 0,64$; 0,57; 0,56) was noted.

The binding rate did not return to the initial value within 2 hours. We suppose this difference can be attributed to the dissimilar kinetics of ^{125}I-insulin degradation by receptors on erythrocytes in the two groups. ^{125}I-insulin degradation had decreased at 30 and 60 minutes after glucose intake in the

patients whereas in normal subjects it tended to increase in the course of the glucose tolerance test. Similar disturbances were found earlier in subjects with essential hypertension (11). Significant changes in binding of ^{125}I-insulin by receptors on erythrocytes without concomitant changes in ^{125}I-insulin degradation seem to support the hypothesis of the negative feedback between binding of insulin and insulin levels (13). Abel and co-workers (17) observed a decrease in the first-phase insulin release from pancreatic islet cells in response to glucose loading in patients with primary hypertension. They supposed this phenomenon was responsible for delayed maximum insulin and glucose concentrations and indicated insulin resistance in these patients.

The significant difference in the kinetics of ^{125}I-insulin binding between the control group and the patients after myocardial infarction is followed by different changes in glucose concentration (*Fig. 5*). This relationship indicates that insulin resistance at receptor level implicates glucose intolerance (4). The results could not be influenced by age-related glucose intolerance described by Chen (18), as the mean age did not differ between the two groups (*Table 1*).The influence of obesity on the receptor affinity to insulin, which was acknowledged in the previous papers (13—15), can be excluded because the mean body weight did not differ in both groups.

So, the picture of the changed glucose curve in patients after myocardial infarction as compared to the control group may prove the impairment glucose uptake by peripheral tissues. It may also show diminished recruitment of insulin dependent glucose transporters (19). It is noteworthy that systematic aerobic physical exercise in patients rehabilitated after acute myocardial infarction reduces glucose concentration and peripheral insulin resistance (20).

CONCLUSIONS

The results allow for the following conclusions:

1. Statistically significant higher level of glucose and insulin in patients after myocardial infarction during the OGGT- test as compared to healthy people shows the smaller glucose tolerance.
2. The results of insulin resistance at the erythrocytes level in patients after myocardial infarction is statistically significant lower value of the ^{125}I-insulin binding.

REFERENCES

1. Ferraninni E, Buzzigola G, Bonadonna R *et al.* Insulin resistance in essential hypertension. *N Engl J Med* 1987; 317: 350—357.
2. Shinozaki K, Suzuki M, Ikebucki M *et al.* Insulin resistance associated with compensatory hyperinsulinemia as an independent risk factor for vasospastic angina. *Circulation* 1995; 92 (7): 1749—1757.

3. Després J-P, Lamarche B, Mauriege P *et al.* Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* 1996; 334 (15): 952—956.
4. Kahn N N, Bauman W A, Sinha A K. Transient decrease of binding of insulin to platelets in acute ischemic heart diesease. *Am J Med Sci* 1994; 307 (1): 21—26.
5. Laakso M, Edelman S V, Brechtel G *et al.* Decreased effect of insulin to stimulated skeletal muscle blood flow in obese man. *J Clin Invest* 1990; 85: 1844—1882.
6. Nuutila P, Raitakari M, Laine H *et al.* Role of blood flow in regulating insulin — stimulated glucose uptake in humans. *J Clin Invest* 1996; 97 (7): 1741—1747.
7. Gambhir K K, Archer J A, Carter L. Insulin radioreceptor assay for human erythrocytes. *Clin Chem* 1987; 23: 1950—1959.
8. Zavaroni I, Bonora E, Pagliara M *et al.* Risk factors for coronary artery disease in healthly persons with hyperinsulinemia and normal glucose tolerance. *N J Med* 1989; 320 (11): 702—706.
9. Verstergaard H, Scott P, Steffensen R *et al.* Insulin resistant glucose metabolism in patients with microvascular angina — syndrome X. *Metabolism* 1995; 44 (7): 876—872.
10. Reaven G M. Pathophysiology of insulin resistance in human disease. *Physiol Ref* 1995; 75 (3): 473—486.
11. Szczęśniak Ł, Rychlewski T, Głuszek J *et al.* Result of oral intake of glucose by healthly subjects and patients with essential hypertension on the binding and degradation of ^{125}I-insulin by erythrocyte receptors. *J Physiol Pharmacol* 1996; 47 (2): 269—279.
12. Lederg-Meyer V, Herchuelz A, Valverde I *et al.* Mode of action of clonidine uppon islet function. Dissociated effects uppon the time course and magnitude of insulin release. *Diabetes* 1980; 29: 193—200.
13. Bar R S, Gordon P, Roth J *et al.* Fluctuation in the affinity and concentration of insulin receptors on circulating monocytes of obese patients. *J Clin Invest* 1976; 58: 1123—1149.
14. Archer J A, Gorden P, Roth J. Deffect in insulin binding to receptors in obese man. Amelioration with calorie restriction. *J Clin Invest* 1975; 55: 166—174.
15. Szczęśniak Ł, Rychlewski T, Kasprzak Z *et al.* Einfluß der kalorienarmen Diät und des aeroben Körpertrainings auf Bindung und Degradation von ^{125}I-insulin durch Rezeptoren von Erythrozyten bei Kindern mit einfacher Obesität. *Österreich J Sportmedizin* 1994; 3: 79—84.
16. Sanchez-Margalet V, Valle M, Labon J A *et al.* Diminished insulin receptors on erythrocyte ghosts in nonobese patients with essential hypertension independent of hyperinsulinemia. *J Cardiovasc Pharmacol* 1994; 24: 74—77.
17. Abel D E, Ledingham J G. Impaired glucose tolerance in hypertension is associated with impaired insulin release independently of changes in insulin sensitivity. *J Hypertens* 1994; 25: 1265—1273.
18. Chen M, Bergman R N, Pacini G. Pathogenesis of age -related glucose and responsiveness to insulin in forearm muscle. *J Am Geriatr Soc* 1980; 28: 304—307.
19. Constable S H, Favier R J, Cartee G D *et al.* Muscle glucose transport: interactions of *in vitro* contractions insulin and exercise. *J Appl Physiol* 1988; 64: 2329—2332.
20. Dylewicz P, Szczęśniak Ł, Przywarska I *et al.* The influence of diet and endurance training on exercise tolerance, insulin concentration and lipid profile in patients after myocardial infarction. International 14-th Puijo Symposium "Physical Activity, Diet and Cardiovascular Diseases- a Fresh Look Beyond Old Facts" 24—28, June 1996, Kuopio, Finland (Abstract).

R e c e i v e d: May 8, 1997
A c c e p t e d: September 9, 1997

Author's address: T. Rychlewski, Chair of Physiology, Biochemistry and Hygiene University School of Physical Education ul. Królowej Jadwigi 27/39, PL-61-871 Poznań, Poland