

Ł. SZCZEŚNIAK, J. KAROLKIEWICZ, \*E. DESKUR, T. RYCHLEWSKI, L. KONYS,  
K. STANKIEWICZ

## THE INFLUENCE OF EXERCISE-INDUCED OXIDATIVE STRESS ON BINDING AND DEGRADATION OF $^{125}\text{I}$ -INSULIN BY THE RECEPTORS ON ERYTHROCYTES

Chair of Physiology, Biochemistry and Hygiene of the University School of Physical Education,  
Poznań, Poland

\*Department of Cardiology and Intensive Therapy, Institute of Internal Medicine of the University  
School of Medical Sciences, Poznań, Poland

In the present work we investigated the influence of oxidative stress induced by strenuous exercise on the affinity of a specific insulin receptors on human erythrocytes. 13 male members of the national basketball staff performed a maximal treadmill exercise test. In the blood samples collected before and directly after the test acid-base equilibrium parameters, lactic acid concentrations, glucose and insulin serum levels as well as  $^{125}\text{I}$ -insulin binding and degradation by receptor on erythrocytes were measured. As markers of oxidative stress, plasma thiobarbituric acid-reactive substance levels (TBARS) and red blood cells glutathione content (GSH) were determined. After the exercise test TBARS levels increased significantly and GSH concentrations decreased indicating that oxidative stress occurred. Binding of  $^{125}\text{I}$ -insulin to the receptors on erythrocytes decreased significantly during the test, while there was only insignificant reduction in  $^{125}\text{I}$ -insulin degradation. Correlation analysis and multiple linear regression revealed that changes in insulin degradation by receptors on erythrocytes during exhaustive exercise are determined by oxidative stress, probably via oxidation of sulfhydryl groups of certain enzymes. The affinity of receptors for insulin seems to depend mainly on glucose concentrations.

Key words: *physical exercise, erythrocyte, binding and degradation of insulin, oxidative stress*

### INTRODUCTION

There is a large body of evidence derived from clinical studies demonstrating that moderate physical activity enhances binding of  $^{125}\text{I}$ -insulin to the receptors on red blood cells (1—4). In contrast, exhaustive exercise is associated with the impairment of insulin binding to erythrocytes (5—7). As insulin binding was shown to correlate well with the lactic acid concentrations

(LA), pH and base excess (BE) (7—9), changes in the acid-base balance have been proposed to play a pivotal role in the regulation of insulin receptor affinity.

Strenuous physical exercise is associated with significant changes in acid-base balance and marked metabolic acidosis. Dissociation of lactic acid leads to the generation of great amounts of protons and thus evokes changes in the NAD/NADH ratio (10). Under these conditions, the sequential, univalent reduction of molecular oxygen is favored resulting in the release of free radicals (11—12). These highly reactive derivatives may oxidize proteins, lipids and nucleic acids causing damage to surrounding structures (13—15). This situation is referred to as oxidative stress.

The aim of the present study was to investigate the possible relationships between exercise-induced oxidative stress and insulin binding and degradation by the specific receptors on erythrocytes. As direct detection of oxygen free radicals is difficult because of their highly reactive and transient nature, we evaluated indirect markers of oxidative stress: thiobarbituric acid-reactive substance levels (TBARS) as markers of lipid peroxidation, and the red blood cell reduced glutathione concentration (GSH), before and directly after a maximal exercise test.

## MATERIAL AND METHODS

The study group consisted of 13 male members of the national basketball team at the time of preparations for the European Championship 1997. The mean age was  $24.7 \pm 2.20$  years and the mean body mass index was  $24.8 \pm 1.41 \text{ kg/m}^2$ .

All subjects performed a maximal treadmill exercise test with oxygen consumption and carbon dioxide production measurement (CardiO<sub>2</sub> System equipped with CPX-D-Medical-Graphics-Corporation program, USA). The initial speed of 10km/h was increased by 2km/h every three minutes.

Venous and capillary blood samples were collected before and directly after the treadmill exercise test. In the capillary blood samples the acid-base equilibrium parameters were evaluated (analyzer AVL 995 Hb, AVL List GmbH Graz, Austria), the lactic acid levels were measured using the enzymatic method (Boehringer Mannheim, Germany), and the glucose levels were determined (Cormay, Poland). Serum insulin concentrations were evaluated by the radioimmunological method of double antibodies (ORiPi, Otwock-Świerk, Poland; the sensitivity threshold was 0.1μIU/ml). Red blood cells were centrifuged, washed thrice and suspended in G-buffer (16). Binding and degradation of <sup>125</sup>I-insulin by specific receptors on erythrocytes were evaluated using the method described by Gambhir (16) and modified by the authors. This modification consisted of the use of constant concentrations of bovine insulin (2.4μ IU/0.1ml) and <sup>125</sup>I-insulin (0.9pg/0.1ml). Samples were incubated for two hours at the room temperature ( $21 \pm 1^\circ\text{C}$ ). The radioactivity was then determined with a Scaler A-224 type gamma and the amount of <sup>125</sup>I-insulin was calculated assuming that one impulse corresponds to  $2 \times 10^{-4}$ pg of <sup>125</sup>I-insulin. The results are presented in pg/10<sup>11</sup> erythrocytes. The red blood cells GSH content was determined by the Beutler method

(17) and the TBARS plasma levels were measured according to the method described by Rice-Evans (18).

Consent to participate in this study, which was accepted by the local ethics committee, was obtained from all subjects

### *Statistical analysis*

Results are presented as a mean average  $\pm$ SD. Differences between the results obtained before and after exercise were assessed using the Wilcoxon matched pairs test. Correlations between  $^{125}\text{I}$ -insulin binding and degradation by receptors on erythrocytes and other parameters were performed. Non-linear regression analysis showed that all regressions were linear. The influence of investigated parameters (glucose and endogenous insulin serum levels, lactic acid concentrations, pH, base excess, RBC glutathione content, TBARS plasma levels, exercise duration and run distance) on  $^{125}\text{I}$ -insulin binding and degradation at rest and after strenuous exercise was assessed using multiple linear regression. All subset regression was performed to select the best multiple regression equation defined as the one in which the average error of a square had the least value (19).

## RESULTS

The mean duration of the treadmill exercise test was  $467 \pm 76.0$  s and the mean run distance was  $1245.5 \pm 186\text{m}$ .

Exercise was associated with disturbances in the acid-base homeostasis as evidenced by significant changes in the lactic acid levels (LA), pH and the base excess (BE) (*Table 1*).

*Table 1.* Exercise — induced changes in acid — base balance parameters glucose and insulin concentrations

Parameters	before exercise test $\bar{x} \pm \text{SD}$	after exercise test $\bar{x} \pm \text{SD}$	Wilcoxon matched pairs test
pH	$7,370 \pm 0,015$	$7,170 \pm 0,059$	0,0014 **
BE (mmol)	$-1,23 \pm 0,978$	$-14,76 \pm 2,880$	0,0014 **
LA (mmol)	$2,00 \pm 0,294$	$13,5 \pm 2,540$	0,0014 **
Gluc. (mg/dl)	$75,2 \pm 6,620$	$94,6 \pm 8,987$	0,0018 **
Ins. ( $\mu\text{IU/ml}$ )	$17,0 \pm 7,596$	$35,4 \pm 14,015$	0,0021 **

\*\* $p < 0,01$

Glucose and endogenous insulin serum levels increased significantly after the exercise levels compared with the results obtained at rest (*Table 1*).

Binding of  $^{125}\text{I}$ -insulin to the receptors on erythrocytes decreased significantly from  $1.16 \pm 0.336$  before to  $0.77 \pm 0.275$   $\text{pg}/10^{11}\text{RBC}$  after the exercise test ( $p = 0.0014$ ), while there was only a non-significant reduction in  $^{125}\text{I}$ -insulin degradation by red blood cells ( $6.93 \pm 2.189$  vs.  $5.97 \pm 1.603$   $\text{pg}/10^{11}\text{RBC}$ ,  $p = 0.2488$ ) (Fig. 1).

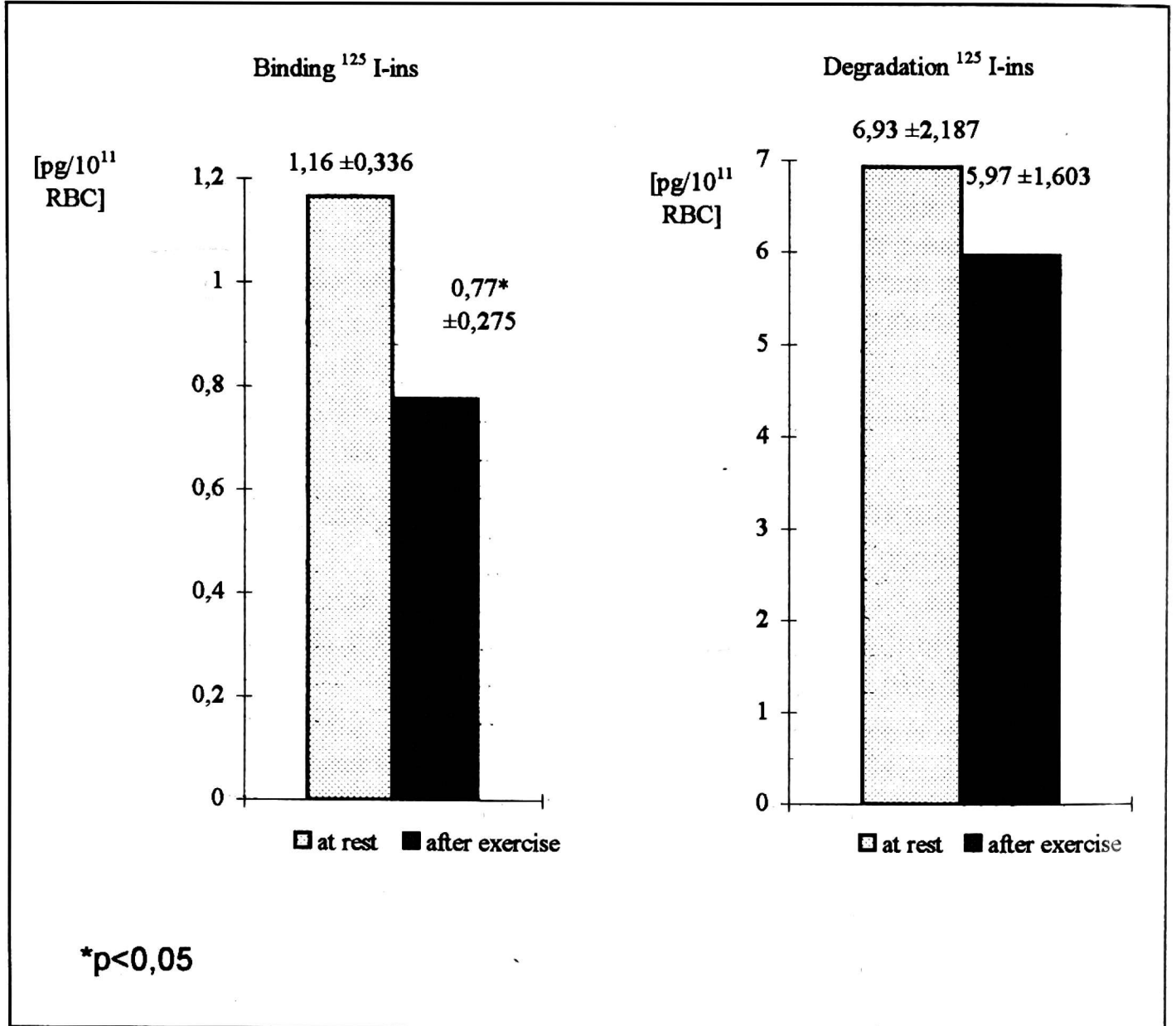
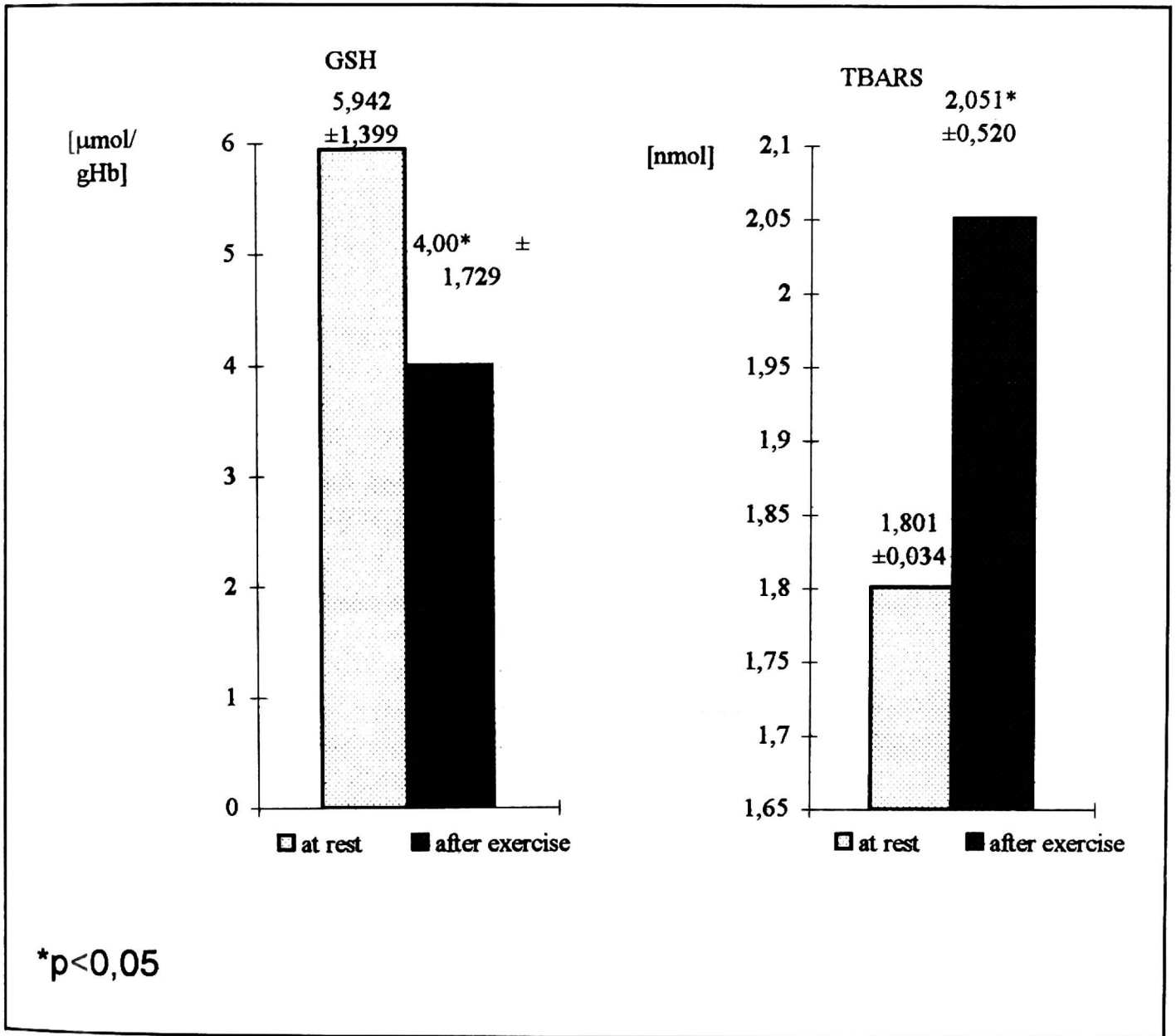


Fig. 1. Changes in  $^{125}\text{I}$ -insulin binding and degradation by receptors on erythrocytes during maximal tread exercise test.

The red blood cells reduced glutathione content decreased during the treadmill exercise test ( $5.492 \pm 1.399$  before vs.  $4.000 \pm 1.729$  after the test,  $p = 0.0464$ ), while the thiobarbituric acid-reactive substances concentration increased ( $1.801 \pm 0.0345$  vs.  $2.052$ ,  $p = 0.0330$ ) (Fig. 2).

In order to find out whether oxidative stress influences binding and degradation of  $^{125}\text{I}$ -insulin, correlation analysis and all subset multiple

regressions were performed. After the exercise test, degradation of  $^{125}\text{I}$ -insulin correlated positively with TBARS plasma levels (*Fig. 3*) and negatively with GSH concentrations (*Fig. 4*). No such correlation was found at rest. There was also a negative correlation between  $\Delta$  difference after-before exercise in  $^{125}\text{I}$ -insulin degradation and  $\Delta$  after-before exercise in RBC glutathione content (*Fig. 5*).



*Fig. 2.* Changes in RBC glutathione concentrations and plasma TBARS levels during exercise test.

In case of  $^{125}\text{I}$ -insulin binding, we found a negative correlation with TBARS plasma concentrations at rest (*Fig. 6*).

*Table 2* presents selected, by means of multiple regression subsets of parameters and single variables, the best predicting  $^{125}\text{I}$ -insulin and degradation by receptors on erythrocytes. Correlation coefficients are given for all subsets of parameters and a single variables.

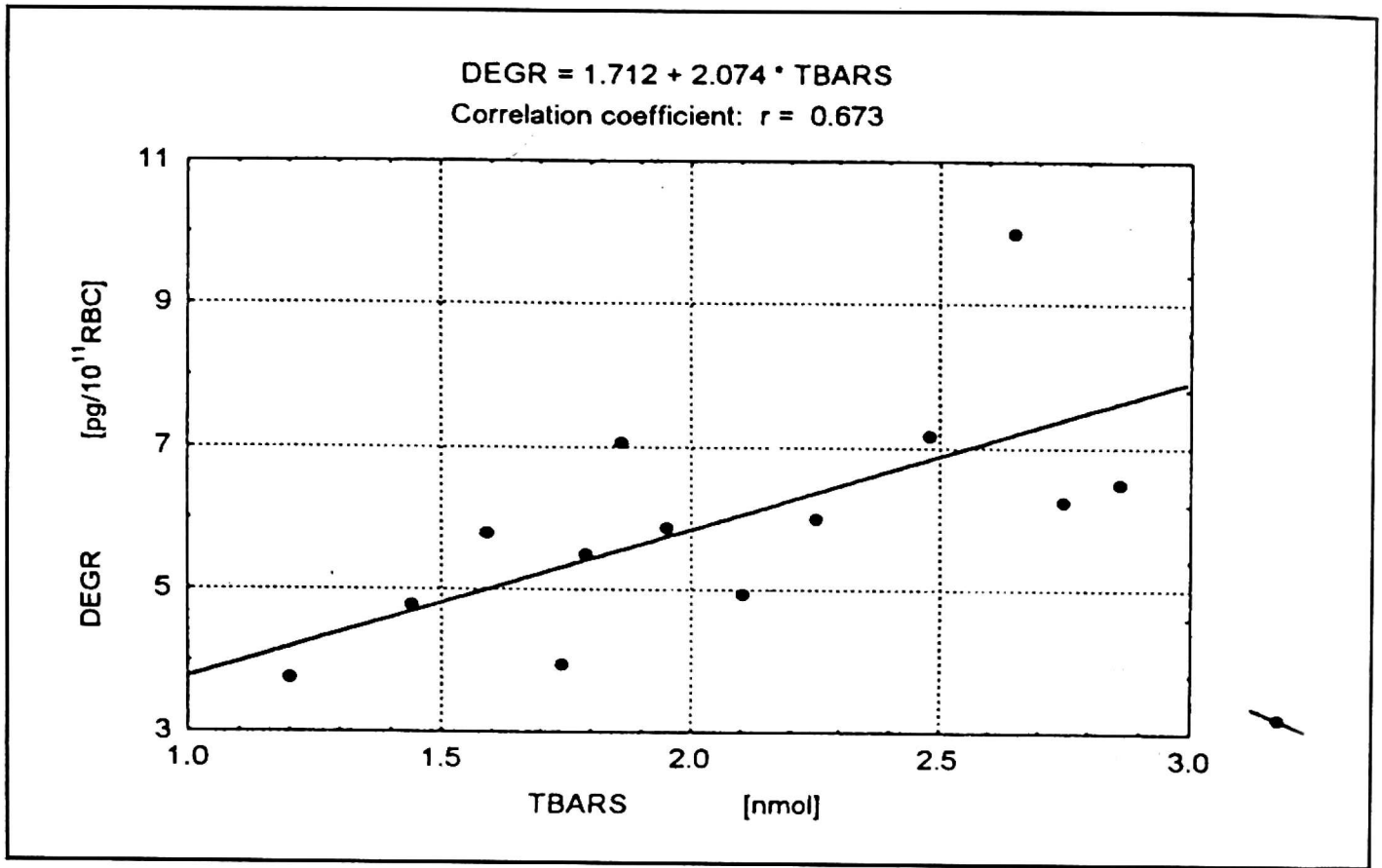


Fig. 3. Correlation between  $^{125}\text{I}$ -insulin degradation by receptors on erythrocytes and TBARS plasma concentrations after exercise test.

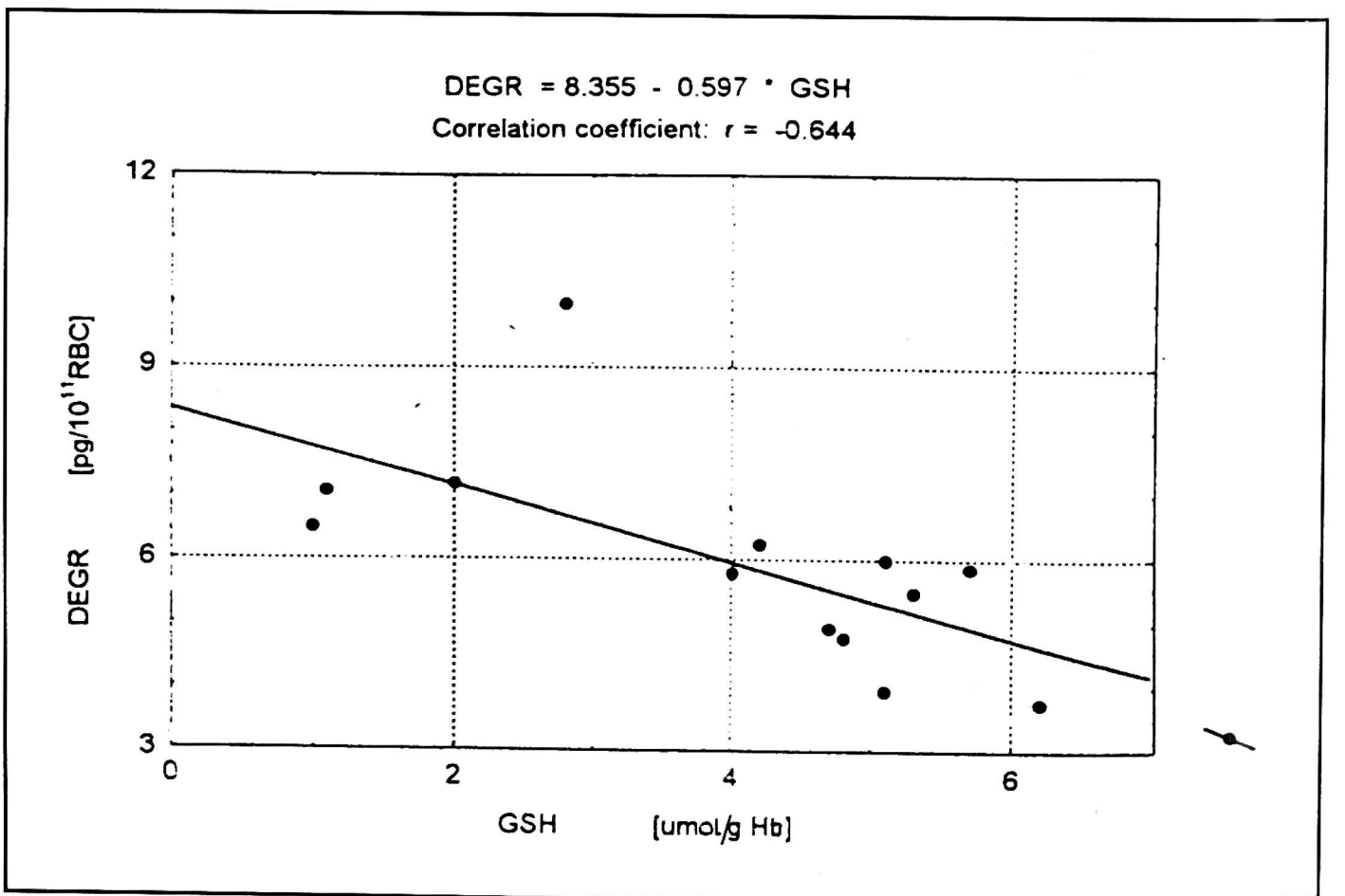


Fig. 4. Correlation between  $^{125}\text{I}$ -insulin degradation by receptors on erythrocytes and RBC glutathione content after exercise test.

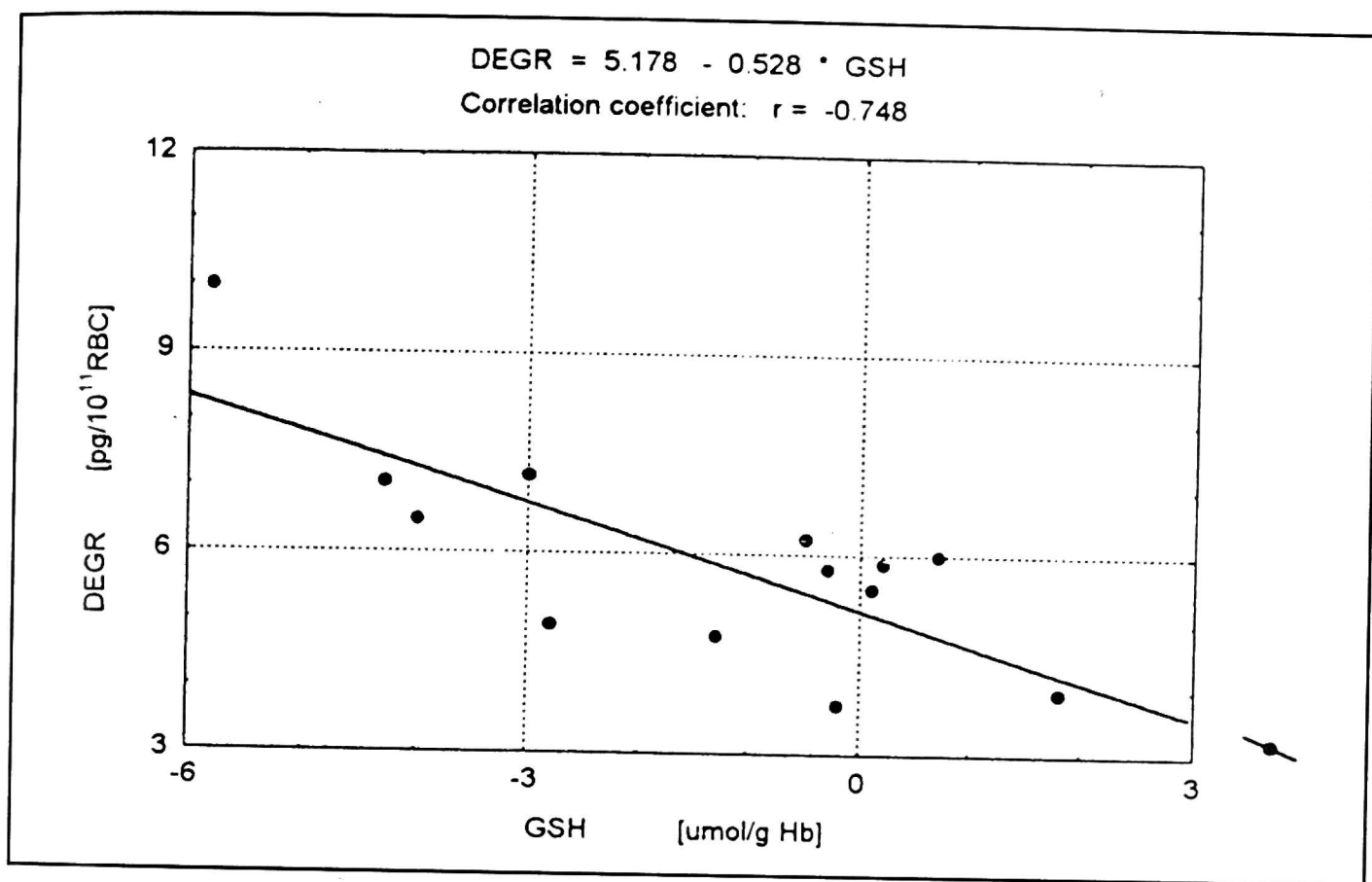


Fig. 5. Correlation between increments in  $^{125}\text{I}$ -insulin degradation during exercise test and increments GSH concentrations.

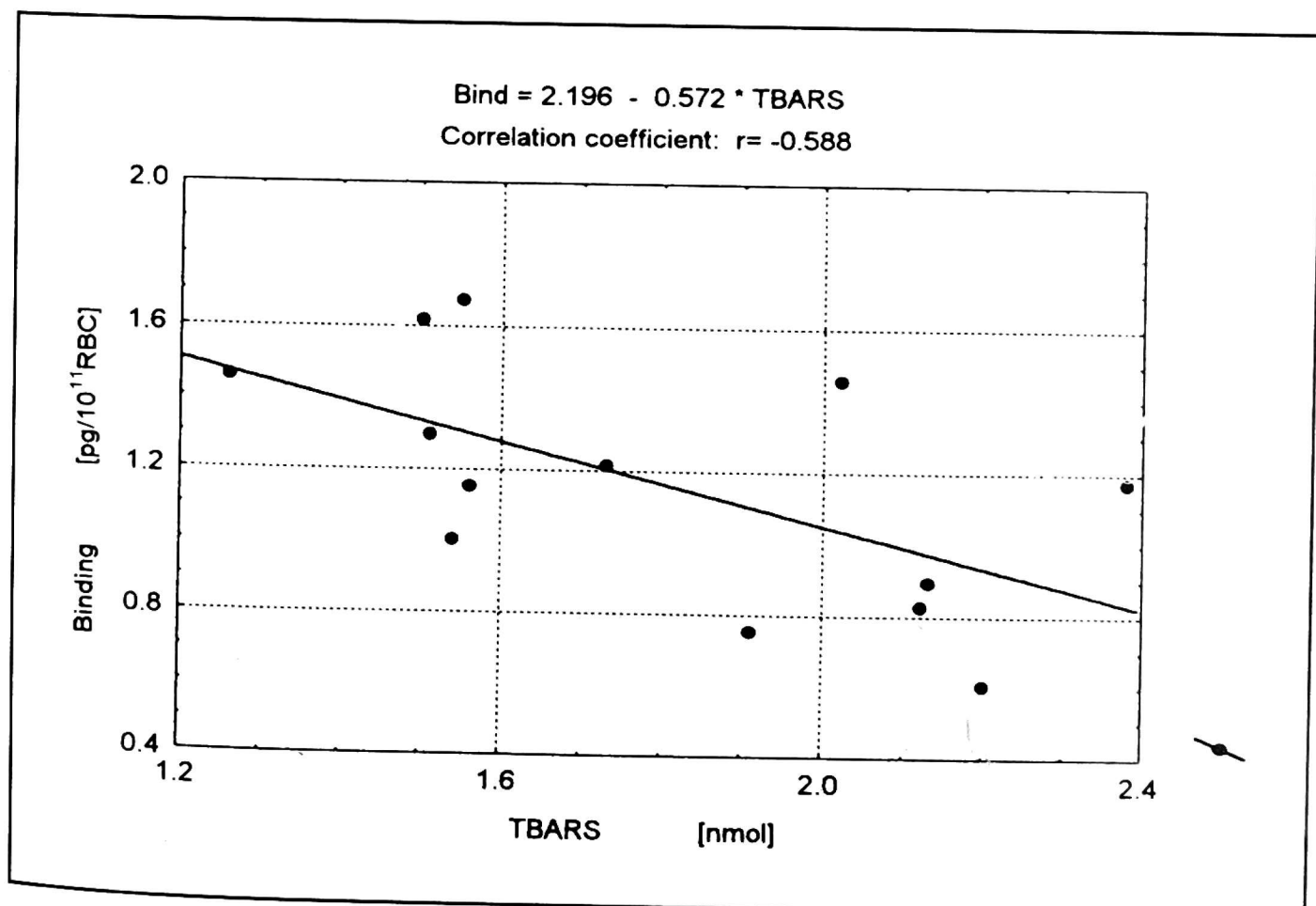


Fig. 6. Correlation between  $^{125}\text{I}$ -insulin binding by receptors on erythrocytes and TBARS plasma concentrations before exercise test.

Table 2: Subsets of parameters and single variables the best predicting  $^{125}\text{I}$ -insulin binding and degradation by receptors on erythrocytes (multiple linear regression analysis)

		all parameters	D	the best subsets of parameters	D	the best single parameter	D
before exercise test	$^{125}\text{I}$ -insulin-binding	glucose insulin pH BE LA	92,13%	glucose insulin BE GSH TBARS	87,94%	TBARS	34,57%
	$^{125}\text{I}$ -insulin degradation	GSH TBARS	76,10%	insulin pH BE GSH	53,43%	insulin	14,26%
after exercise test	$^{125}\text{I}$ -insulin-binding	ex. duration run distance glucose insulin pH BE	93,38%	ex. duration run distance glucose pH BE	89,90%	glucose	52,30%
	$^{125}\text{I}$ -insulin degradation	LA GSH TBARS	91,76%	ex. duration run distance pH BE TBARS	89,19%	TBARS	45,34%
A difference after-before exercise test	$^{125}\text{I}$ -insulin-binding	ex. duration run distance glucose insulin pH BE	71,49%	ex. duration run distance glucose insulin TBARS	58,89%	TBARS	6,03%
	$^{125}\text{I}$ -insulin degradation	LA GSH TBARS	92,55%	ex. duration pH BE TBARS	82,65%	ex. duration	52,43%

D – determination coefficient

## DISCUSSION

In our research we discovered that strenuous physical activity with an intensity increasing up to the maximum causes oxidative stress, as reflected by a decrease of GSH concentrations in erythrocytes and an increase of TBARS levels in plasma (Fig. 2). This is confirmed by a negative correlation between the duration of activity and run distance and the GSH concentrations. This means that GSH concentrations decrease with an increase of activity load, which may directly influence the amount of substances reacting with TBE and the damage which results from it. The results of our study are similar to the



results of other authors (20—22) showing that the degree of damage caused by reactive forms of oxygen depends on the intensity of the exercise. The activity induced changes in the balance between the processes of oxydation and reduction seem to influence the degree of  $^{125}\text{I}$ -insulin degradation by a membrane associated enzyme. As shown in *Table 2*, at rest  $^{125}\text{I}$ -insulin degradation depended on the insulin levels in blood serum, acid-based equilibrium parameters and GSH levels. After exhausting exercise the TBARS levels were the most important factors. Other factors included in the best subset were: the duration of physical activity, run distance, the pH of blood and base deficit. Our results are similar to the results obtained by Duckworth *et al.* (23) and Kuo *et al.* (24) who showed that insulin degradation decreases with decreasing pH and correlates to the increase of lactate dehydrogenase activity during the condition of oxydative stress. In our research, blood pH following exercise was decreased by an average of 0.200 ( $p < 0.01$ ) and lactic acid increased by an average of 11.5 mmol ( $p < 0.01$ ).

A similar group of factors were noted to be associated with changes of exercise-rest insulin degradation levels. After maximum physical activity, the degradation of insulin correlated positively with TBARS plasma levels (*Fig. 3*) and negatively with GSH concentrations (*Fig. 4*). This means that a decrease of the basic endogenous antyoxydant content in erythrocytes may play a role in the decrease of the amount of insulin being degraded by a membrane associated enzyme. A negative correlation was also seen between effort-rest differences in the amount of degraded insulin and the difference in GSH levels ( $\Delta$  after-before effort =  $5.178 - 0.528 * \text{GSH}$ ) (*Fig. 5*).

The peroxydation-oxydation reaction with favored oxydation or reaction of oxydation of oxyhemoglobin to methemoglobin is a source of a highly reactive form of oxygen in red blood cells (2). Superoxide anion radical formed in this reaction in the presence of  $\text{Fe}^{2+}$  ions undergoes dismutation to form hydrogen peroxide. The glutathione and selenium dependent glutathione peroxidase are the main factors which remove hydrogen peroxide (7, 15). When this defense mechanism is insufficient the concentration of superoxide anion radical ( $\text{O}_2^-$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) increase which favor the Fenton reaction and the formation of highly reactive hydroxyl radicals ( $\text{OH}^-$ ). These radicals react not only with polyunsaturated lipids of cell membranes causing their peroxydation but also with protein causing their oxydation. Oxydative damage of proteins resulting from sulfhydryl group oxydation leads to a loss of their biological value. This fact seems to explain the decrease in  $^{125}\text{I}$ -insulin degradation by membrane associated enzyme following physical activity. Insulin proteinase EC 3.4.23.5 is an enzyme containing metal dependent on thiol groups (3).

Other authors already informed that the efficiency of the antyoxydative system depends on the cytoplasmic NAD(P)/NAD(P)H ratio (9,14).

Confirmation of the above, in our research, is the correlation coefficients obtained at rest between GSH concentrations and lactate levels ( $r = -0.74$ ;  $p < 0.05$ ) and base deficit ( $r = 0.63$ ;  $p < 0.05$ ).

The maximum physical effort also participated in lowering  $^{125}\text{I}$ -insulin binding by red cell insulin receptors (*Fig. 1*). In our previous papers (25,26) we stated that during the oral glucose tolerance test, glucose and insulin levels in blood serum determined the affinity of specific receptors to insulin. In our paper we discovered that the glucose level is the most important factor effecting the magnitude of binding after intensive physical effort (determination coefficient = 52.30%) (*Table 2*). Other parameters included in the so called best subset (exercise duration, run distance, base deficit and pH) formed the determination coefficient in (ca) 30%. During rest conditions binding of  $^{125}\text{I}$ -insulin was dependent on glucose, insulin, BE, GSH and TBARS concentrations ( $D = 87.94\%$ ) but the most important factor were the TBARS levels ( $D = 34.57\%$ ).

We also noted a negative correlation between TBARS concentrations and  $^{125}\text{I}$ -insulin binding (*Fig. 6*). This indicates that substances reacting with TBA acid may react most probably with thiol groups of insulin receptors. The obtained research results and multiple regression analysis confirm previous reports (23—27) that binding of  $^{125}\text{I}$ -insulin depends on many factors including glucose insulin levels, acid-based equilibrium parameters and oxidative stress.

## CONCLUSION

The results of our study indicate that changes in insulin degradation by receptors on erythrocytes during maximal treadmill exercise tests are determined by oxidative stress, while the affinity of the insulin receptor depends mainly on glucose serum concentrations.

## REFERENCES

1. Koivisto VA, Soman VR, Defronzo R, Felig P. Effects of acute exercise and training on insulin binding to monocytes and insulin sensitivity *in vivo*. *Acta Paediatr Scand Suppl* 1980; 238: 70—78.
2. Landt KW, Campaigne BN, James FW, Sparling MA. Effects of exercise training on insulin sensitivity in adolescents with type I diabetes. *Diabetes Care* 1985; 8: 461—465.
3. Przywarska I, Dylewicz P, Szczeńniak Ł *et al.* The effects of endurance training on binding and degradation of the  $^{125}\text{I}$ -insulin by erythrocyte receptors in patients rehabilitated after myocardial infarction. *Kardiol Pol* 1997; 46: 403—409.

4. Szcześniak Ł, Rychlewski T, Kasprzak Z, Banaszak F. Einfluß der kalorienarmen Diät des aeroben Körpertrainings auf Bindung und Degradation von <sup>125</sup>I-insulin durch Rezeptoren von Erythrozyten bei Kindern mit einfacher Obesität. *Österr J Sportmed* 1994; 3: 79—84.
5. Koivisto VA, Soman VR, Conrad P, Hendler R, Nadel E, Felig P. Insulin binding to monocytes in trained athletes: changes in the resting state and after exercise. *J Clin Invest* 1979; 64: 1011—1015.
6. Okuno Y, Fujii S, Okada K, Tabata T, Tanaka S, Wada M. Effects of acute exercise on insulin binding to erythrocytes in normal men. *Horm Metab Res* 1983; 15: 366—369.
7. Szcześniak Ł, Banaszak F, Rychlewski T. Binding and degradation of <sup>125</sup>I-insulin by erythrocyte receptors in the persons practicing tennis, subjected to the test of maximal power and the exercise of 75% VO<sub>2</sub>max (abstract). International Congress of Applied Research in Sports. 9—11 August 1994, Helsinki.
8. Gambhir KK, Archer JA, Bradley CJ. Characteristics of human erythrocyte insulin receptors. *Diabetes* 1978; 27: 701—708.
9. Igarashi M, Yamatani K, Fukase N *et al.* Effect of acidosis on insulin binding on glucose uptake in isolated rat adipocytes. *J Exp Med* 1993; 169: 205—213.
10. Stanley WC, Gertz EW, Wisneski JA, Morris DL, Neese RA, Brooks GA. Systemic lactate kinetics during graded exercise in men. *Am J Physiol* 1985; 249 (6Pt 1): E595—E607.
11. Duthie GG, Robertson JD, Maughan RJ, Morrice PC. Blood antioxidant status and erythrocyte lipid peroxydation following distance running. *Arch Biochem Biophys* 1990; 282: 78—83.
12. Lovlin R, Cottle W, Pyke I, Kavanagh M, Belcastro AN. Are indices of free radical damage related to exercise intensity? *Eur J Appl Physiol* 1987; 56: 313—316.
13. Clemens MR, Waller HD. Lipid peroxydation in erythrocytes. *Chem Phys Lipids* 1987; 45: 251—268.
14. Kappus H, Sies H. Toxic drug effects associated with oxygen metabolism: Redox cycling and lipid peroxydation. *Experientia* 1981; 37: 1233—1241.
15. Mena P, Maynar M, Gutierrez JM, Timon J, Campillo JE. Erythrocyte free radical scavenger enzymes in bicycle professional racers. Adaptation to training. *Int J Sports Med* 1991; 12: 563—566.
16. Gambhir KK, Archer JA, Carter L. Insulin radioreceptor assay for human erythrocytes. *Clin Chem* 1977; 23: 1590—1595.
17. Beutler G. Red Cell Metabolism. New York, Grune and Stratton Inc. 1984.
18. Rice-Evans CA, Diplock AT, Symons MCR. Techniques in Free Radical Research. Amsterdam, Elsevier, 1991.
19. Konys L, Mejza S. Wybór najlepszego równania regresji wielokrotnej liniowej. *Rocz Nauk AR* (Poznań) 1981; 10: 67—82.
20. Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 1982; 107: 1198—1205.
21. Gohil K, Viguie Ch, Stanley WC, Brooks GA, Packer L. Blood glutathione oxydation during human exercise. *J Appl Physiol* 1988; 64: 115—119.
22. Jenkins RR. Free radical chemistry. Relationship to exercise. *Sports Med* 1988; 5: 156—170.
23. Duckworth WC, Kitabchi AE. Insulin metabolism and degradation. *Endocr Rev* 1981; 2: 210—223.
24. Kuo WL, Gehm BD, Rosner MR. Regulation of insulin degradation: Expression of an evolutionarily conserved insulin-degrading enzyme increases degradation via an intracellular pathway. *Mol Endocrinol* 1991; 5: 1467—1476.
25. Rychlewski T, Szcześniak Ł, Dylewicz P *et al.* The influence of oral glucose intake on binding and degradation of <sup>125</sup>I-insulin by receptors of erythrocytes as well as on insulin and C-peptide

serum levels in patients after myocardial infarction and healthy individuals. *J Physiol Pharmacol* 1997; 48: 839—849.

26. Szczęśniak Ł, Rychlewski T, Głuszek J, Michocki P, Banaszak F. Result of oral intake of glucose by healthy subjects and patients with essential hypertension on the binding and degradation of  $^{125}\text{I}$ -insulin by erythrocyte receptors. *J Physiol Pharmacol* 1996; 47: 269—279.
27. Waelbroeck M. The pH dependence of insulin binding. *J Biol Chem* 1982; 257: 8284—8291.

Received: April 30, 1998

Accepted: July 9, 1998

Autor's address: Prof. Łucja Szczęśniak, University School of Physical Education, Chair of Physiology, Biochemistry and Hygiene, ul. Królowej Jadwigi 27/39, Poznań, Poland