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Ł. SZCZĘŚNIAK, T. RYCHLEWSKI*, J. GŁUSZEK**, P. MICHOCKI, F. BANASZAK

RESULT OF ORAL INTAKE OF GLUCOSE BY HEALTHY SUBJECTS AND PATIENTS WITH ESSENTIAL HYPERTENSION ON THE BINDING AND DEGRADATION OF ^{125}I-INSULIN BY ERYTHROCYTE RECEPTORS

* Chair of Physiology, Biochemistry and Hygiene of the University School of Physical Education, Poznań, Poland
** Clinic of Essential Hypertension of the University of Medical Sciences, Poznań, Poland

The work presents the results of researches of binding and degradation of ^{125}I-Insulin by erythrocyte receptors in the patients with essential hypertension and healthy patients after glucose intake. In order to obtain full representation of the pattern of changes the serum IRI and glucose concentrations were assayed. Binding and degradation of ^{125}I-Insulin by erythrocyte receptors were determined with the method described by Gambhir (1977), modified by the authors. The modification consisted in usage of constant concentrations of iodized insulin (0.9 pg/0.1 ml) and bovine insulin (2.4 I.U./0.1 ml). Before administration of glucose and in 30, 60 and 120 minutes after, venous blood was collected from ulnar vein. All examined persons were in sitting position during the trial of glucose intake. Obtained results show, that blood insulin level in the patients with essential hypertension is statistically significantly higher than in healthy persons of similar anthropometric characteristics. Binding of ^{125}I-Insulin to erythrocyte receptors in fasting state is statistically significantly lower. Degradation after glucose intake in the patients shows decreasing tendency, while in healthy persons — growing tendency.

K e y w o r d s: *insulin receptor, insulin binding and degradation, essential hypertension, OGTT*

INTRODUCTION

The coexistence of hyperinsulinemia and insulin resistance in essential hypertension has been observed for years (1—3). Hyperinsulinemia in essential hypertension is a reflection of insulin resistance, the latter defined as a condition of reduced glucose transport to muscle tissue (3, 4). This transport

is accomplished by a protein carrier whose activity and membrane surface depends on the insulin itself (5). Since the first stage of insulin metabolic action is its binding to the receptor, the present research undertakes to determine the binding rate of ^{125}I-Insulin to erythrocyte receptors resulting from oral intake of glucose by patients with essential hypertension as well as by healthy subjects.

MATERIAL AND METHODS

Thirteen patients with essential hypertension and 10 healthy subjects of similar anthropometric features were investigated. Those with essential hypertension were not administered any hypotensive drugs within 30 minutes prior to the oral intake of glucose assay. The latter consited of 75 g of glucose dissolved in 300 ml of water administered over 5 minutes. Before the glucose intake (as well as 30, 60, and 120 minutes after it) venous blood was collected from the ulnar vein. Patients retained a sitting position during the glucose intake assay. The serum glucose level was measured by means of the Cormay test and the immunoreactive insulin level with the Świerk Isotope Research & Development Center's test. From blood collected with a view to heparin assay, blood cells were isolated by thrice repeated wasking with physiological saline and centrifugation. Blood cells, thus obtained, were suspended in a G-buffer (6). Binding and degradation of ^{125}I-Insulin by erythrocyte receptors was determined according to the method described by Gambhir (6) and modified by the authors. This modification entailed the use of constant concentrations of iodized insulin (0.9 pg/0.1 ml) and bovine insulin (2.4 j.m./0.1 ml). and bovine insulin (2.4 j.m./0.1 ml). Radioactivity was measured with a Scalar A-224 type gamma counter, and results, thus obtained, were calculated in pg of ^{125}I-Insulin with reference to 10^{11} blood cells. The results of the investigations were calculated in the form of average values at times t_0, t_{30}, t_{60}, t_{120}. In order to illustrate the changes of analyzed parameters in 30, 60 and 120 minutes following glucose intake, the percent values of the change were calculated with reference to values obtained during the fasting state according to the equation:

$$\frac{t_1 - t_0}{t_1} \times 100$$

where:

t_1 = average value obtained for the time 30, 60 or 120 minutes following glucose intake;
t_0 = average value obtained during the fasting state.

RESULTS

The results of investigations presented in the *Tables 1—3* and *Figures 1—4*. *Table 1* specifies average values of anthropometric features of 13 patients with essential hypertension (HT) and 10 healthy subjects (GK). The table includes, as well, average values of systolic and diastolic pressures. Pressure was measured prior to blood collection. *Table 2* shows average glucose values, immunoreactive insulin concentrations, binding and degradation of ^{125}I-Insulin by erythrocyte receptors at time t_0 and at 30, 60 and 120 minutes following glucose ingestion. As is shown in *Table 2*, the average values of analyzed physiological factors in examined groups demonstrate statistically

significant differences. This, however, excludes glucose concentrations in the fasting state and during insulin degradation within all of the above mentioned time ranges. Increases of glucose, immunoreactive insulin and changes in binding and degradation at times t_{30}, t_{60} and t_{120} in relation to t_0, are shown in *Table 3*. *Figures 1—4* present results in the form of average values and percent changes with relation to t_0.

Table 1. Anthropometric features and average values of systolic and diastolic blood pressures in subjects from examinated groups

Feature	Hypertensive group $\bar{x} \pm \sigma$	Control group $\bar{x} \pm \sigma$	Student's t-test
Age [years]	44.7±4.25	43.9±4.10	rs
Height [cm]	170.0±8.24	166.0±7.54	rs
Body weight [kg]	68.0±6.35	65.3±5.45	rs
BMI [kg/m^2]	24.4±3.4	23.3±3.2	rs
Systolic pressure	178±12.8	110.0±5.3	15.78 **
Diastolic pressure	100.0±7.4	84±6.4	5.42 **

Table 2. Average values of examined factors following oral intake of glucose in essential hypertension (HT) and control groups

Factor	t	HT group $\bar{x} \pm \sigma$	Control group $\bar{x} \pm \sigma$	Difference	Student's t-test
Glucose [mg/dl]	0	81.75±16.33	76.60± 7.95	5.15	0.88
	30	148±40.07	111.82±18.98	36.20	2.52 *
	60	150.69± 2.80	94.75±13.02	55.94	2.65 *
	120	109.02±53.37	76.80± 8.12	32.22	1.81
Insulin [μIU/ml]	0	13.69± 7.45	7.70± 4.52	5.99	2.14 *
	30	80.92±28.19	47.50±18.43	33.42	3.11 **
	60	86.31±26.39	36.70±14.28	49.61	5.13 **
	120	54.08±42.21	24.50±13.02	29.58	2.00
Binding ($pg^{125}I/10^{11}RBC$)	0	0.49±0.17	0.73±0.19	−0.24	2.95 **
	30	0.39±0.12	0.44±0.17	−0.05	0.78
	60	0.51±0.26	0.68±0.30	−0.17	1.35
	120	0.63±0.26	0.93±0.31	−0.30	2.33 **
Degradation ($pg^{125}I/10^{11}RBC$)	0	6.67±4.49	5.05±2.57	1.63	0.98
	30	4.80±2.39	5.25±2.43	−0.45	0.42
	60	5.31±2.96	6.34±2.36	−1.03	0.86
	120	5.38±2.10	6.96±2.63	−1.58	1.52

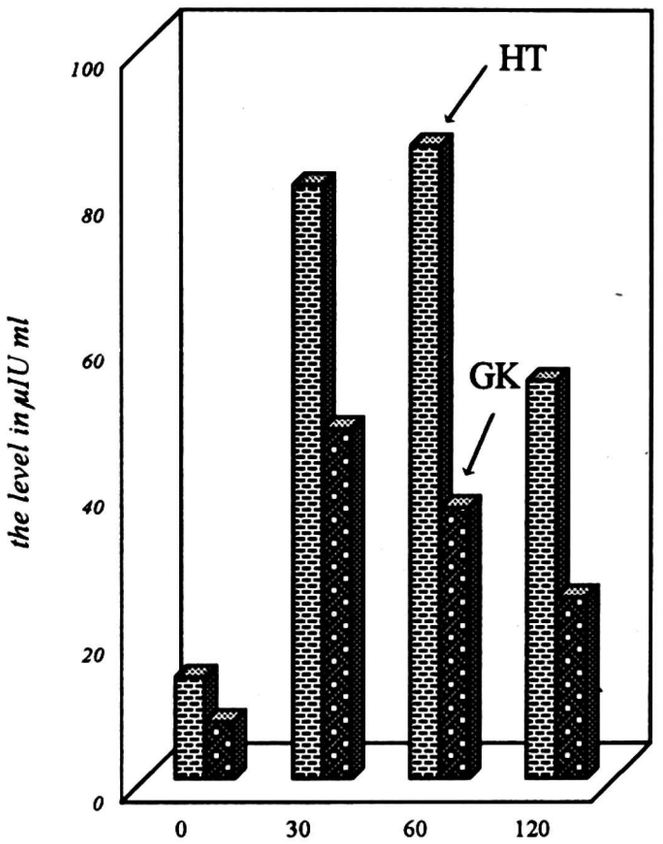
Table 3. Comparison of absolute increments of examined factors in the time t_{30}, t_{60} and t_{120} with reference to t_0.

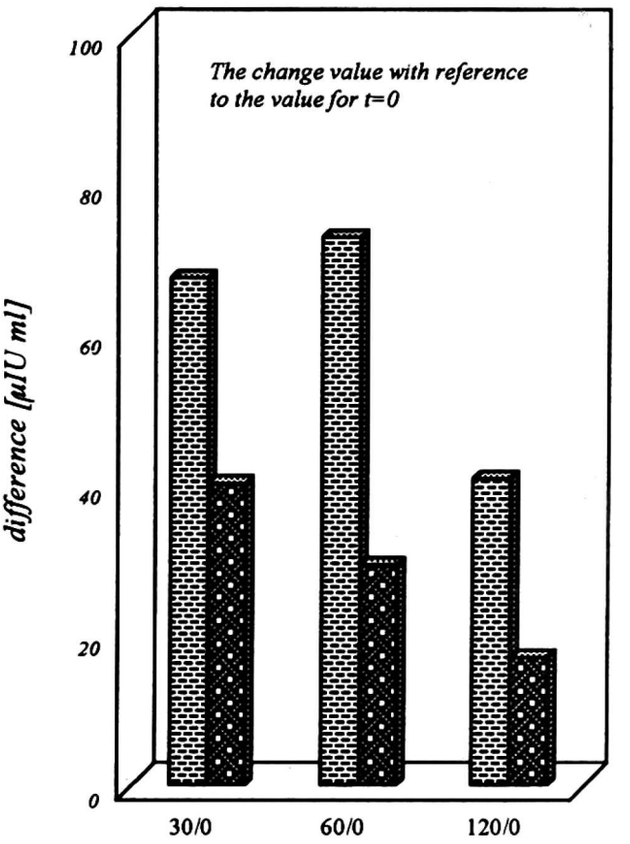
Group	Factor	Differences of average values					
		t_{30}/t_0	Student's t-test	t_{60}/t_0	Student's t-test	t_{120}/t_0	Student's t-test
HT	Glucose	66.279	7.80 **	68.938	4.67 **	27.269	2.28 *
	Insulin	67.231	9.66 **	72.615	11.61 **	40.385	3.60 **
	Binding	−0.098	1.33	0.024	0.23	0.146	2.79 *
	Degradation	−1.873	2.43	−1.362	1.17	−1.295	1.17
Control group	Glucose	35.220	7.19 **	18.500	4.51 **	0.200	0.18
	Insulin	39.800	8.09 **	29.000	8.18 **	16.800	4.90 **
	Binding	−0.287	3.84 **	−0.047	0.47	0.202	2.14
	Degradation	0.206	0.18	1.294	1.82	1.910	2.33 **

DISCUSSION

The investigations of many researches, as well as our own, have shown that, essential hypertension is often accompanied by hyperinsulinemia (7—9), a resistance to insulin (9—11). An increased level of insulin in patients suffering from ischaemic heart disease as well as a reverse correlation between insulin concentration and the HDL-cholesterol fraction (12—14) is known.

This resistance results from the insufficient action of insulin on target tissue due to receptor and postreceptor defect (14, 15). In our previous study (9), we have demonstrated that the binding of ^{125}I-Insulin to erythrocyte receptors in subjects burdened with essential hypertension is significantly lower, when compared to that in healthy individuals (8). The present research attempts to determine the binding of ^{125}I-Insulin to erythrocyte receptors following oral intake of glucose by patients with essential hypertension as well as by healthy subjects. Our results agree with earlier reports about hyperinsulinemia often accompanying original arterial hyperpressure. The role of hyperinsulinemia in arterial hyperpressure is, however, unclear. Consequently, this raises the significance of a closer investigation of insulin binding to the receptor and the degradation of insulin by cellular receptors. Both patients suffering from essential hypertension as well as healthy subjects with similar anthropometric features and examined by us have shown similar serum glucose levels in the fasting state. Statistically significant differences were related to concentration of immunoreactive insulin and the binding of ^{125}I-Insulin to blood cell receptors (*Table 2*). Burdening patients suffering from essential hypertension with





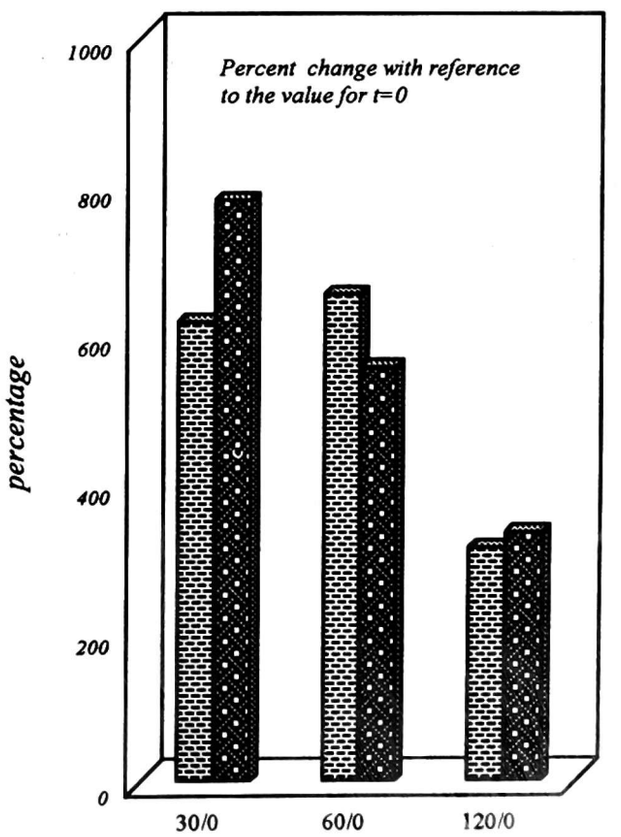
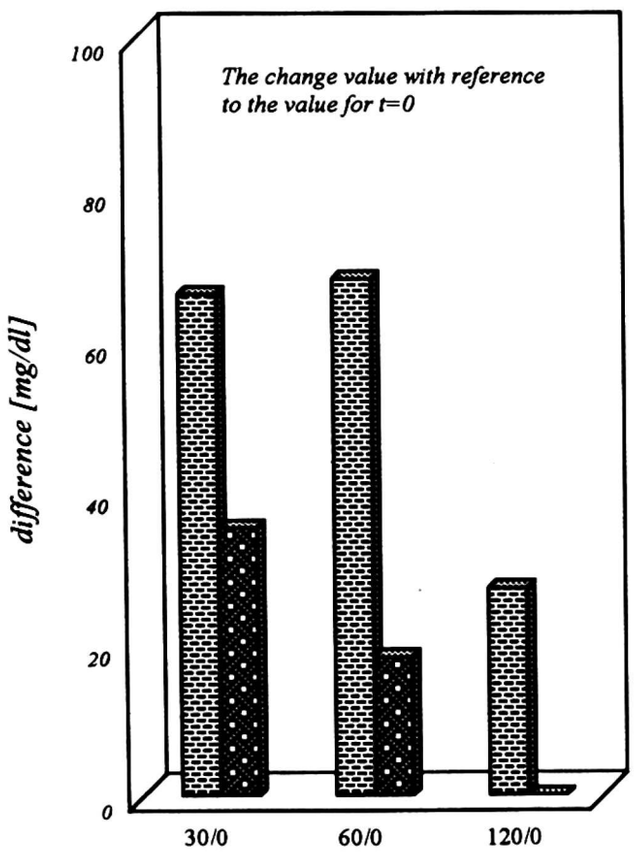
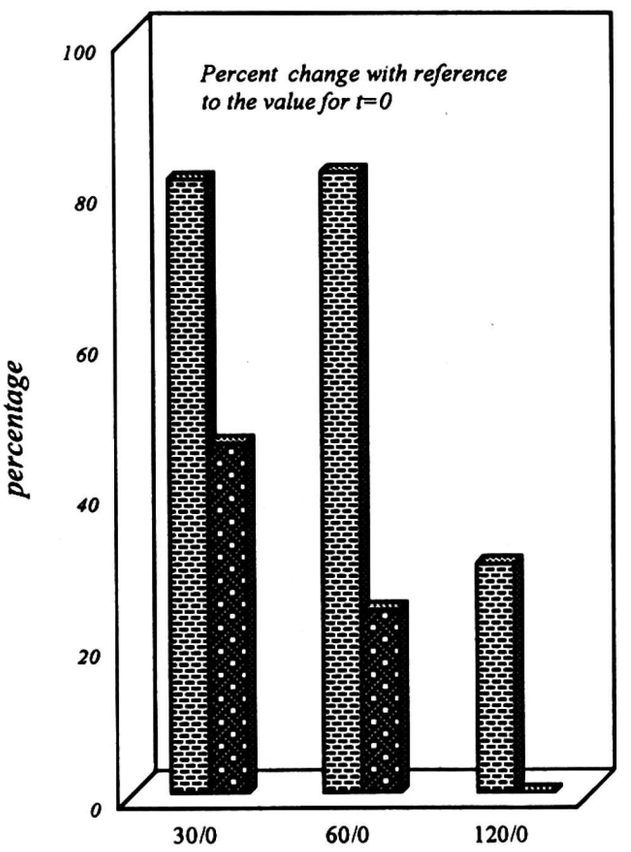


Fig. 1. Average values of serum IRI concentration and its changes the test of oral glucose intake.

HT - the group with essential hypertension
GK - control group





HT - the group with essential hypertension
GK - control group

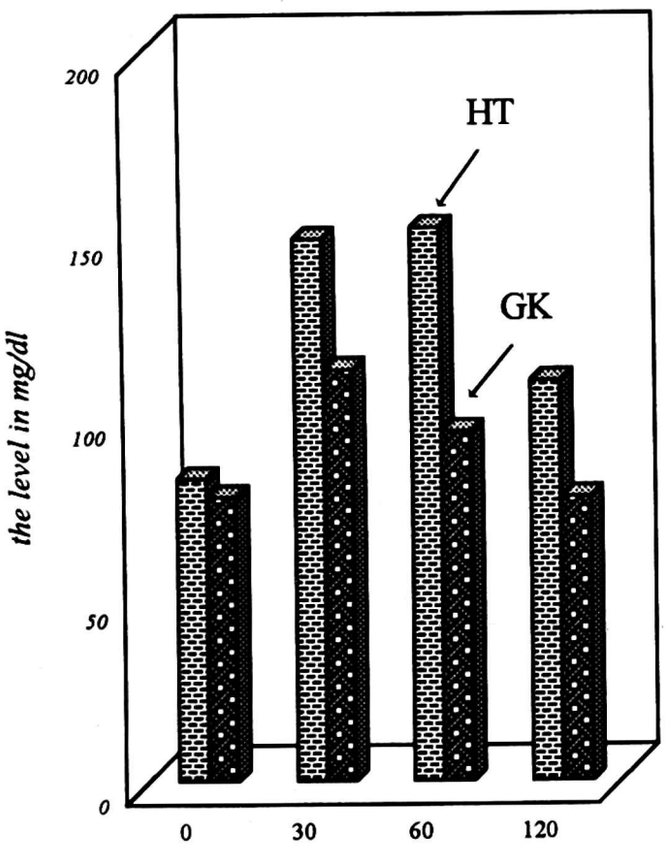
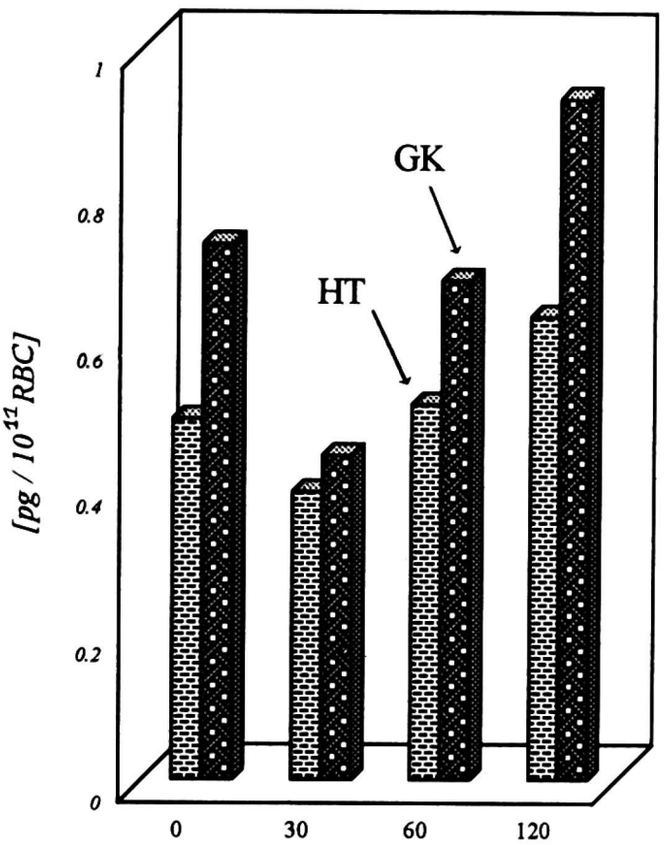
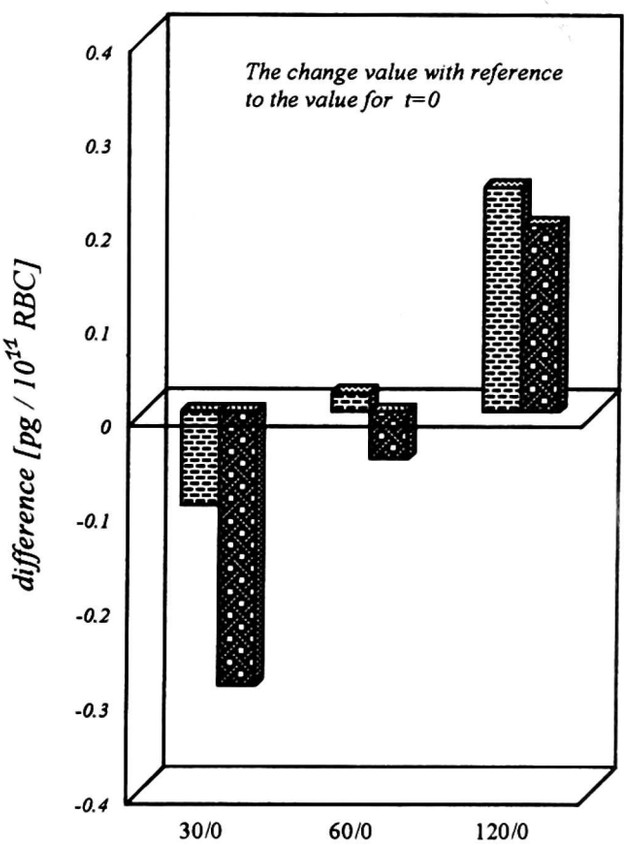


Fig. 2. Average values of serum glucose concentration and its changes during the test of oral glucose intake.





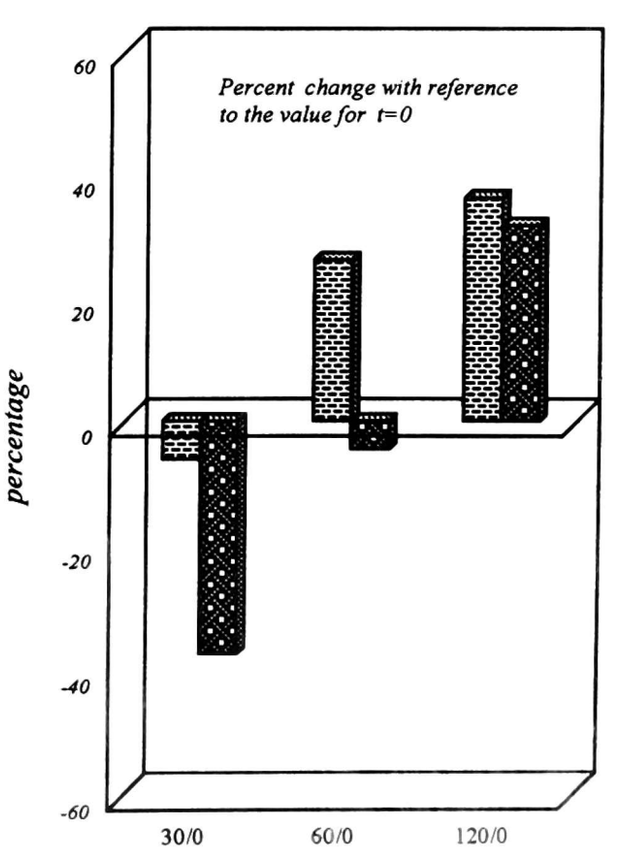
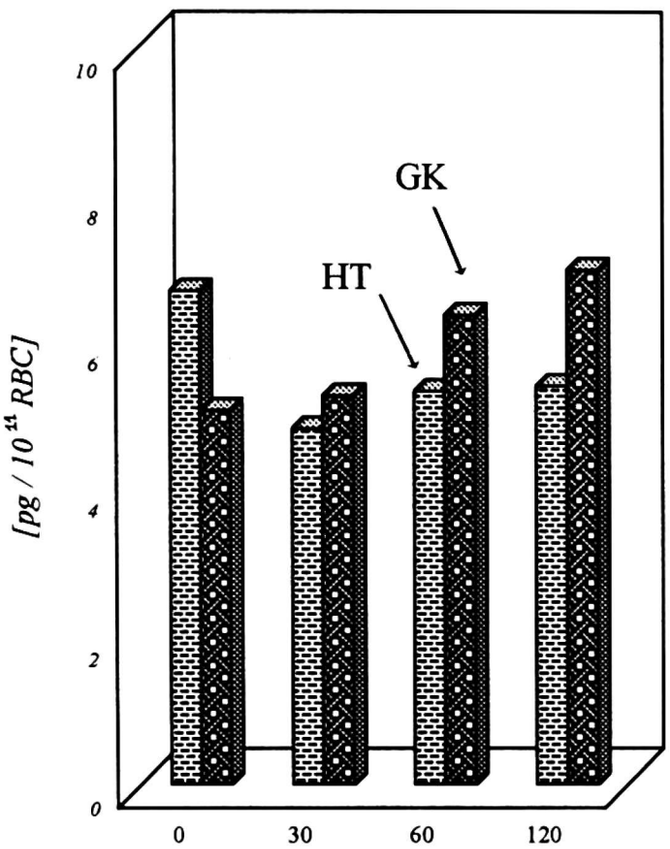
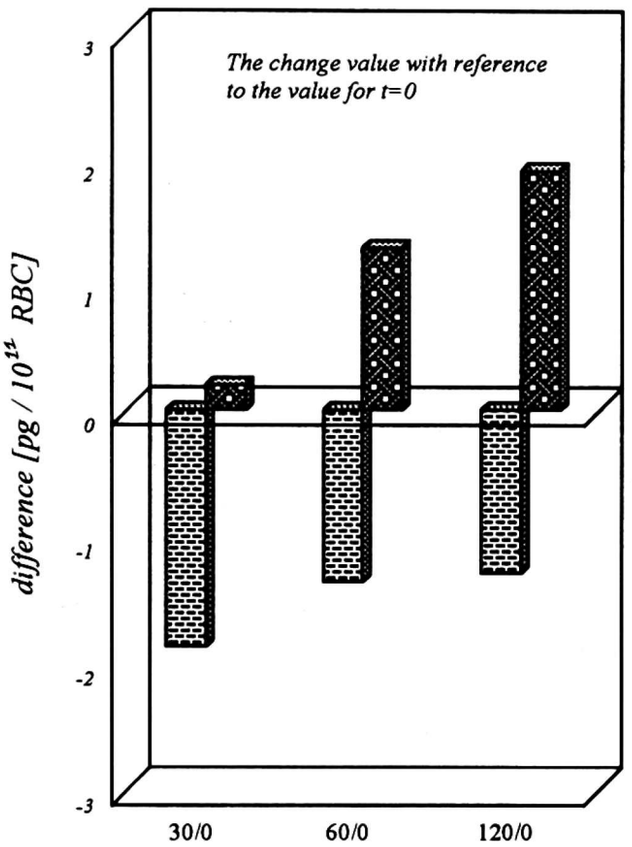


Fig. 3. Average values of binding of ^{125}I-Insulin and its changes the test of oral glucose intake.

HT - the group with essential hypertension
GK - control group





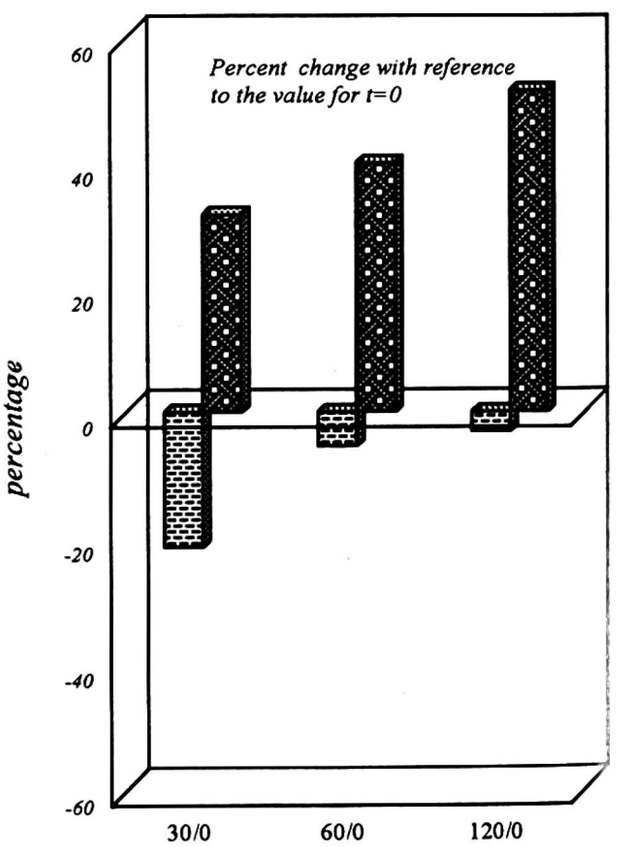


Fig. 4. Average values of degradation of ^{125}I-Insulin and its changes during the test of oral glucose intake.

HT - the group with essential hypertension
GK - control group

additional glucose intake resulted in a statistically significant higher serum glucose concentration as compared to that of the control group. It should be noted, furthermore, that a time of 120 minutes appeared insufficient to attain the level measured during the fasting state. As well, it should be noted that glucose intolerance ascribed to age by many researchers (16, 17) was eliminated as a factor in the present study, as neither the average age of hypertensive patients nor that of the control group subjects differed significantly (*Table 1*). The pattern of changes in the glycemic curve of patients with essential hypertension compared to those of the control group, evidences an impaired intake of glucose to the tissues (*Fig. 1*). This reflects either a decreased recruitment of insulin-dependent glucotransporters from their intracellular compartment to the membrane, or their fewer number (5).

The concentration pattern of immunoreactive insulin in serum appears to be almost parallel to the pattern of the glucose curve itself (*Fig. 2*). In healthy subjects, however, maximal growth falls within 30 minutes of glucose intake, whereas, in hypertensive patients — within 60 minutes of intake. This time shift and, above all, the significantly higher concentration of insulin, confirm the occurrence of insulin resistance (14).

In patients with essential hypertension and in healthy subjects, the binding of ^{125}I-Insulin to erythrocyte receptors showed a statistically significant difference in the fasting state. Glucose intake resulted in a considerable reduction of the binding in the 30th minute of the test both in patients with essential hypertension (about 25%) as well as in healthy subjects (40%). In the 60th and 120th minute of our investigation, we observed a slow increase of binding to erythrocyte receptors. In hypertensive patients, however, the binding values exceeded primary values as early as the 60th minute, whereas, in healthy subjects — only in the 120th minute of the assay. There is an inverse relation between the binding values and insulin concentration, i. e., within only the 30th minute of glucose administration, the higher the insulin concentration, the lower the binding. Later, however, a binding increase occurs, despite the high serum insulin concentration. Throughout the whole study, the binding of insulin to receptors in patients with essential hypertension was lower than that in healthy subjects, but the difference was statistically significant only in the final stage of the examination.

A reduced binding of ^{125}I-Insulin in patients with essential hypertension as well as in healthy subjects during the fasting state is probably a reflection of the smaller number of receptors on the blood cell surface or, perhaps, of the greater negative coupling between receptors of low and high affinity (18).

Sanchez-Margalet (19), found that lower binding of ^{125}I-Insulin to erythrocyte membranes in patients with essential hypertension is the result of fewer receptors. Although our own investigation does not allow us to confirm this observation, a reduced but parallel course of binding values obtained

during the oral intake of glucose, by patients with essential hypertension as well as by healthy subjects, allows us to accept such an interpretation. The absolute value of degradation of ^{125}I-Insulin by erythrocyte receptors in both the fasting state as well as following oral intake of glucose showed no differences in either group (*Table 2*). In healthy subjects, however, following glucose administration, an increased insulin degradation up to statistically significant values was observed during time t_{120}, whereas, in hypertensive patients, the value of insulin degradation showed a decreasing tendency. It appears, therefore, that the pattern of changes thus obtained in the degradation of ^{125}I-Insulin in patients with essential hypertension confirm the mechanism by which this hormone concentrates in the blood following glucose ingestion and supports the hypothesis that essential hypertension is a condition of insulin resistance.

The above results permit formulation of the following conclusions:

1. Insulin level in patients with essential hypertension is significantly higher when compared to healthy subjects of similar anthropometric features.
2. Binding of ^{125}I-Insulin to erythrocyte receptors in the fasting state is significantly lower in patients with essential hypertension as compared to healthy subjects.
3. Degradation of ^{125}I-Insulin following glucose ingestion by healthy subjects shows an increasing tendency, whereas, in hypertensive patients — a decreasing one.

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Author's address: Ł. Szczęśniak, Chair of Physiology, Biochemistry and Hygiene of the University School of Physical Education, 27/29 Królowej Jadwigi Str., 61-871 Poznań, Poland.