Insulin Receptors on Monocytes of Young Healthy Persons Correlated with Glucose Tolerance and Insulin Sensitivity

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Summary. We have studied in normal man the interrelationships between insulin binding to monocytes, glucose tolerance and insulin sensitivity. In 25 young healthy persons we found a significant positive correlation between insulin binding and glucose disappearance rate both after glucose ($R = 0.68$, $p < 0.01$) and insulin ($R = 0.49$, $p < 0.02$) given intravenously.

Key words: Insulin receptors, monocytes, glucose tolerance, insulin sensitivity.

Several studies of the relation between insulin binding and the biological effects of insulin have been performed in vitro. Kono et al. [1] found in rat adipocytes an inhibitory effect of trypsin on both the specific insulin binding and the biological effect of insulin. Another strong argument for the relationship was advanced by Gliemann et al. [2, 3] and Freychet et al. [4, 5]. With a number of insulin analogues with altered binding affinity to isolated rat adipocytes and hepatocytes they found corresponding changes in the biological potency on these cells. Furthermore, Olefsky [6] has found that a decreased insulin binding in old, fat rats was coupled to a rightward shift in the insulin dose response curve for 2-deoxy-glucose uptake.

The purpose of the present study was to examine relations between the biological effect of insulin and insulin binding to monocytes in normal persons. The biological effect of insulin was estimated by the results of intravenous glucose tolerance tests and intravenous insulin sensitivity tests.

Insulin binding to monocytes is specific and insulin degradation is insignificant at 15°C [7]. It is sup-

posed that the monocyte receptors for insulin have the same characteristics as insulin receptors in muscle cells, adipocytes and hepatocytes [8].

Materials and methods

Subjects

The study comprised 25 apparently healthy 21–33 years old volunteers (84–113% of ideal body weight, Life Insurance Company, Geigy tables, 6th edition) randomly selected from the hospital staff. All ate a normal diet. Clinical data of the group are given in Table 1.

Plasma Insulin and Glucose

Plasma immunoreactive insulin was measured in duplicate by an alcohol precipitation method [9]. Plasma glucose was determined in duplicate using an o-toluidine method [10].

Cell Binding Studies

One hundred and seventyfive ml whole blood was drawn from an antecubital vein at 8 a.m. after an overnight fast and transferred to tubes containing EDTA (dipotassium salt). Mononuclear leucocytes were isolated by gradient centrifugation [11]. The cells were washed twice and incubated in Tris-HCl buffer (25 mmol/l, pH 8.0 at 15°C) at a concentration of about $70 \times 10^6/\text{ml}$ [12] for 100 min at 15°C with 125 I-insulin (Novo Research Institute, Copenhagen) at a concentration of 172 pmol/l. The specific activity of the tracer was about 25–30 $\mu\text{Ci}/\mu\text{g}$, with 0.1–0.2 iodine atoms per insulin

Table 1. Clinical and metabolic characteristics of the group studied

n	Sex	Age	Body weight	Fasting plasma glucose conc.	Fasting plasma insulin conc.	Specific cell binding fraction	IVGTT k-values	IVITT k-values
	M F	years	% of ideal	mmol/l	$\mu\text{U/ml}$	$\times 10^{-2}$	10^{-2} min^{-1}	10^{-2} min^{-1}
25	10 15	25 (21–33)	101 (84–113)	5.0 (4.5–5.9)	5.1 (0–15.1)	2.3 (1.3–4.4)	1.8 (1.0–3.0)	5.8 (3.1–7.0)
								median (range)

molecule [13]. At this low degree of iodination more than 95% of the iodinsulin is monoiodinated [14].

For competition studies native insulin in increasing concentrations was added to the incubation medium (see Fig. 5). At the end of the incubation period cell bound and free insulin was separated by centrifugation through silicone oil (density 1.04). "Specific cell binding" is defined as total binding fraction minus non-specific binding fraction. Radioactivity which remained bound in the presence of an excess of native insulin at 7000 nmol/l was considered "non-specific". This fraction averaged 25% of total binding. The monocytes were identified in cytocentrifuged smears stained with alpha naphthyl acetate esterase [15]. Monocytes comprised 14.5% of the mononuclear leucocytes. Median monocyte concentration was $9.6 \times 10^6/\text{ml}$ (range $6.2\text{--}15.4 \times 10^6/\text{ml}$). The specific cell binding fraction was adjusted to a cell number concentration of 10^7 monocytes per ml [7]. All binding studies were done in duplicate.

Intravenous Glucose Tolerance Test (IVGTT) and Intravenous Insulin Sensitivity Test (IVITT)

These tests were performed at the same occasions as the insulin receptor studies, i.e. in the morning after an overnight fast. A 30 min resting period was allowed between the blood sampling for receptor analysis and the intravenous injections. IVGTT was performed by injecting 25 g glucose during 4 min. Blood samples were taken for glucose and insulin determination at time -30 , -15 , 0 , 2 , 5 , 10 , 15 , 20 , 30 , 45 and 60 min. The half-time of the plasma glucose decay was estimated from the log-linear phase of the plasma glucose concentrations plotted as a function of time. The rate constant k_{IVGTT} was calculated from the formula: $0.693/T_{1/2}$ according to Lundbæk [16]. The median plasma glucose levels before and 60 min after the IV glucose load were not significantly different, whereas the plasma insulin concentration increased twofold. However, the rise in plasma insulin level caused by the glucose injection was negligible compared to the rise caused

by exogenous insulin. After 60 min 0.1 U crystalline insulin per kg body weight was injected intravenously. Blood samples for plasma glucose analyses were taken 5, 10, 15, 20, 25, 30, 35, 40 and 45 min after insulin injection. From 5 to 30 min after IV insulin the plasma glucose concentration declined linearly, and the k_{IVITT} was estimated over this time. The insulin sensitivity was expressed as the rate constant for plasma glucose disappearance, k_{IVITT} , estimated as k_{IVGTT} . The combined glucose tolerance test and insulin sensitivity test used has been discussed by Alford et al. [17] who found no significant difference between k_{IVITT} -values obtained after fasting and after a glucose load.

Insulin Secretion

The plasma insulin concentration after the glucose load was plotted as a function of time; the area below the resulting curve calculated by planimetry expressed in arbitrary units was taken as an estimate of insulin secretion. The area under the insulin secretion curve from 0 to 10 min was considered an estimate of the initial insulin response.

Statistical Methods

Wilcoxon's two-sample test was employed for comparison of values between groups. In correlation studies Spearman's Rho test was used.

Results

Values of fasting plasma glucose concentration, fasting plasma insulin concentration, k_{IVGTT} and k_{IVITT} and specific cell binding fraction are given in Table 1. Two persons had a $k_{\text{IVGTT}} = 1.0$, and are borderline cases for normal glucose tolerance. They were not excluded because our aim was to study relationships between the insulin binding and the glucose metabolism in a randomly selected group of young people with normal body weight.

The specific insulin binding at the tracer con-

Fig. 1. Correlation between the specific cell binding fraction at the tracer concentration (172 pmol/l) and the rate constant (k_{IVGTT}) for the glucose disappearance after a load of 25 g glucose IV.

Fig. 2. Correlation between the specific cell binding fraction at the tracer concentration and the rate constant (k_{IVITT}) for the glucose disappearance after IV injection of 0.1 U insulin/kg body weight.

Fig. 3. Correlation between the rate constants for the glucose disappearance after injection of 25 g glucose IV (k_{IVGTT}) and after injection of 0.1 U insulin IV per kg body weight (k_{IVITT}).

Fig. 4. The specific cell binding fraction at the tracer concentration as a function of the body weight expressed as percentage of ideal weight.

centration (172 pmol/l, which is within the physiological range), was positively correlated with glucose tolerance ($R = 0.68$, $p < 0.01$) and insulin sensitivity ($R = 0.49$, $p < 0.02$) (Figs. 1, 2). No significant relationship could be demonstrated between the fasting plasma insulin concentration and the insulin binding at tracer concentration ($R = 0.18$, $p > 0.1$). Furthermore there was no significant correlation be-

tween insulin secretion from 0 to 60 min after glucose IV and insulin binding ($R = -0.20$, $p > 0.1$).

Figure 3 shows a positive correlation between k_{IVGTT} and k_{IVITT} ($R = 0.44$, $p < 0.05$). The glucose tolerance was correlated with the initial insulin response after glucose IV ($R = 0.45$, $p < 0.05$) but unrelated to be total insulin response ($R = -0.02$, $p > 0.1$).

Fig. 5. The inhibiting effect of native insulin on the ^{125}I -insulin binding to monocytes from a 22 year old relatively insulin deficient male (○) and the total group of volunteers (●). Mononuclear leucocytes in a concentration of 70×10^6 per ml were incubated in a tris-HCL buffer (pH = 8) at 15°C for 100 min with ^{125}I -insulin in a concentration of 172 pmol/l and native insulin in increasing concentration. The specific cell binding fraction is corrected to a monocyte concentration of $10^7/\text{ml}$. Each point on the curve for the group represents mean $\pm$ SEM (1)

Body weight, expressed in percent of ideal weight, was negatively correlated with the specific cell binding fraction at the insulin tracer concentration ($R = -0.53$, $p < 0.01$) (Fig. 4).

Discussion

Insulin Binding and Glucose Metabolism

Luft and Cerasi [18] found that the insulin response to intravenous glucose challenge could be low in persons with normal glucose tolerance, and suggested that the glucose tolerance depends on both the insulin release and the insulin sensitivity.

In our present study there was a positive correlation between glucose tolerance and insulin sensitivity and between glucose tolerance and the initial insulin response after glucose IV. Moreover, we found insulin binding to be positively correlated with the glucose disappearance rate both after injection of glucose and insulin. Our data therefore suggest a close relation between the insulin receptor and the glucose disappearance rate in normal man. This is consistent with the reported animal experiments on insulin receptors and biological effects of insulin [1,

2, 3, 4, 5, 6]. Furthermore our results are in accordance with the recent report by Harrison et al. [19] who, in obese subjects, found a correlation between insulin binding to adipocytes and insulin sensitivity.

The present results add support to the concept that the monocyte receptor mirrors the insulin receptors of target tissues.

Insulin Binding and Plasma Insulin Concentration

Gavin et al. [20] found that in vitro high media insulin concentration led to decreased insulin receptor number on cultured human lymphocytes. An inverse correlation between insulin receptor concentration and fasting plasma insulin level has been repeatedly reported in chronic hyperinsulinaemic states [21, 22, 23]. Our results from young normals fail to demonstrate such a relationship between the hormone and its receptor, which in part may be due to the small range in plasma insulin concentration among persons with normal body weight. However, the daytime plasma insulin concentration is usually elevated considerably above the fasting level. Therefore we have searched for a correlation between insulin secretion and insulin binding, but could not find any association.

One of our subjects had a specific cell binding fraction of 4.4×10^{-2} , which is about twice as high as the median value of the group (Fig. 5). This person was insulin deficient. His fasting insulin was low ($2 \mu\text{U}/\text{ml}$) and did not increase significantly after glucose intravenously or after meals. In the fasting state he had slightly elevated concentrations of plasma free fatty acids and ketones. The insulin receptors of this person apparently compensate for a poor beta cell function of the pancreas, because he still had a normal glucose tolerance ($k_{\text{IVGTT}} = 1.4$).

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