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Abstract

Insulin binding to erythrocytes was studied in diabetic patients. Insulin binding was lower in untreated diabetics and diabetic patients treated with diet or insulin than in normal subjects. Binding variation was mainly due to decreased binding site concentration in untreated and insulin-treated patients, and to lowered insulin binding site affinity in diet-treated patients. Several patients treated with hypoglycemic agents showed higher insulin binding due to increased binding site concentration. Insulin binding to erythrocytes may not always reflect the insulin binding status of insulin sensitive tissues.

KEYWORDS: insulin binding, diabetes mellitus, erythrocyte.

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— BRIEF NOTE —

INSULIN BINDING TO ERYTHROCYTES IN DIABETES MELLITUS

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Abstract. Insulin binding to erythrocytes was studied in diabetic patients. Insulin binding was lower in untreated diabetics and diabetic patients treated with diet or insulin than in normal subjects. Binding variation was mainly due to decreased binding site concentration in untreated and insulin-treated patients, and to lowered insulin binding site affinity in diet-treated patients. Several patients treated with hypoglycemic agents showed higher insulin binding due to increased binding site concentration. Insulin binding to erythrocytes may not always reflect the insulin binding status of insulin sensitive tissues.

Key words : insulin binding, diabetes mellitus, erythrocyte.

Recent studies on insulin binding in animal models revealed decreased insulin receptors in hyperinsulinemic states (1) and increased insulin receptors in hypoinsulinemic states (2) in insulin sensitive tissues. However, in humans, because of difficulties in obtaining insulin sensitive tissues for *in vitro* studies, most insulin binding studies are performed on monocytes which are believed to mirror the insulin binding status of the target organs for insulin (3). Decreased insulin binding by this cell type has been observed in non-insulin dependent diabetic patients (4,5).

Recently human peripheral erythrocytes were reported to have specific insulin binding sites (6,7). They were indistinguishable from insulin receptors in insulin sensitive tissue and were expected to be ideal cells for clinical evaluation of insulin rceptors. In fact, insulin binding studies conducted on erythrocytes from non-obese, non-insulin-dependent diabetics showed decreased insulin binding sites (8,9) as observed in monocytes (4,5).

As few studies have been performed on insulin binding in diabetic patients undergoing treatment (10) the present report describes the insulin binding status of peripheral erythrocytes from diabetic patients on treatment.

Materials and methods. The study group consisted of ten normal subjects and

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33 diabetic patients. Three non-insulin-dependent diabetics were studied in the untreated state. Six patients were treated with diet only, twelve patients with hypoglycemic agents and 13 patients with insulin. Five out of 13 insulin-treated patients were insulin-dependent diabetic patients. Peripheral venous blood was drawn into heparin (250 units/ml blood) after an overnight fast.

^{125}I-labeled porcine insulin with a specific activity of about 100 mCi/mg was obtained by a modified method (7) of Hunter and Greenwood (11). Binding studies were performed using the method of Gambhir *et al.* (6) modified in the following points (7): (a) the erythrocyte suspension was prepared by three successive sentrifugations of blood, removing the buffy coat and the upper portion of the erythrocyte pellet; (b) the reaction buffer contained 0.5 per cent of bovine serum albumin. Briefly 1.6x 10^9 erythrocytes were incubated with 0.4 ng ^{125}I-labeled insulin in the presence of 0 to 10^4ng/ml native insulin at 15 °C for 150 min in 0.5 ml of the reaction mixture. After the incubation, 0.2 ml duplicate aliquots were transferred into tubes containing dibutyl phthalate and buffer. After centrifugation at 4,000 x g for 5 min at 4 °C and counting total radioactivity, the buffer and dibutyl phthalate layers were aspirated and discarded, and the bound radioactivity of the excised pellets was counted. Radioactivity sedimented in the presence of 10^4 ng/ml of native insulin was considered nonspecific binding. Results were normalized to the per cent of specific binding of ^{125}I-labeled insulin per 4x 10^9 erythrocytes. The calculation of maximum binding capacity and average affinity of binding sites was performed as described by Scatchard (12) and De Meyts *et al.* (13).

The significance of differences between means was calculated by Student's t test (14).

TABLE 1. SPECIFIC PER CENT ^{125}I-LAVELED INSULIN BINDING, AND CONCENTRATION AND AFFINITY OF INSULIN BINDING SITES

Subjects	Number of patients	Specicific binding[a] (%)	Binding site Concentration[b] (Sites/Cell)	Binding site Affinity[b] $\overline{Ke}$[c] (x 10^8 M^{-1})	$\overline{Kf}$[c] (x 10^8 M^{-1})
Normal	10	12.10±1.13	230	1.49	0.16
Diabetics					
Untreated	3	9.95±1.30[d]	120	2.41	0.12
Treated with					
Diet	6	10.05±2.23[d]	270	1.03	0.18
Sulfonylurea	12	11.71±2.49	240	1.43	0.25
Insulin	13	10.58±1.77[d]	170	1.73	0.28

a Values are given as "mean ±SD" at 0.8 ng/ml of ^{125}I-labeled insulin.

b Values are calculated using mean values of specific per cent binding.

c $\overline{Ke}$ and $\overline{Kf}$ represent average bindingsite affinity at empty sites and filled sites respectively.

d $P<0.05$ compared with values for normal subjects.

Results and discussion. The results are summarized in Table 1. and Fig.1. Untreated non-insulin dependent diabetic patients showed significantly lower insulin binding than normal subjects (9.95±1.30 % versus 12.10±1.13 %, $P<0.05$). This observation is consistent with those previously reported for monocytes (4,5) and erythrocytes (8,9). Decreased insulin binding was associated with decreased binding site concentration and elevated average binding site affinity at empty sites.

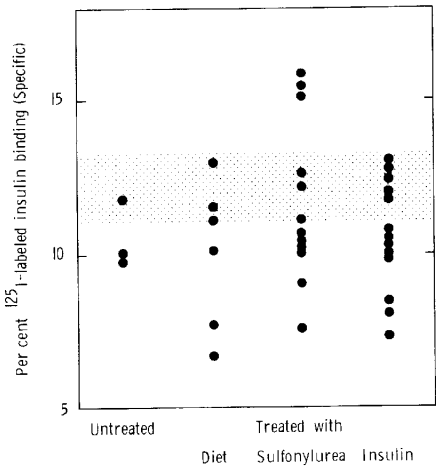

Fig. 1. Per cent specific ^{125}I-labeled insulin binding in diabetic patients. Insulin binding assay was performed at 0.8 ng/ml ^{125}I-labeled insulin described in the text. The shaded area represents the normal range (mean ± SD).

Diabetic patients treated with diet only or insulin showed decreased insulin binding as a group, but those treated with sulfonylurea did not. Though not confirmed in diabetic patients, chronic diet in obese patients restores decreased insulin binding by monocytes to normal (15). In the present study, we found nearly normal maximum binding capacity with decreased empty site affinity in diet-treated diabetic patients, which resulted in signifcantly decreased insulin binding (10.05±2.23 %, $P<0.05$).

Mean specific insulin binding did not differ between patients treated with sulfonylurea and normal subjects. Interestingly, three out of twelve sulfonylurea-treated patients had abnormally higher insulin binding than normal subjects (15.44±0.33 %, $P<0.001$). They showed increased maximum insulin binding capacity with almost normal binding affinity. Those with increased insulin binding did not seem to differ from other patients in oral hypoglycemic agents used, serum glucose or plasma insulin levels (data not shown). Hypernormal insulin binding in sulfonylurea treated diabetics has not been reported, though treatment with oral hypoglycemic agents is known to be associated with normalization of insulin binding by mononuclear leukocytes (10). There may be some regulatory mechanism specific for insulin binding sites on erythrocytes.

Insulin therapy restores increased insulin binding by hepatic plasma membranes to normal in streptozotocin-induced diabetic rats (16). Comparable observations in humans have not yet appeared. We observed significantly lower insulin binding in insulin-treated diabetic patients as a group (10.58±1.77 %, $P<0.05$). A decrease in insulin binding was secondary to a reduction in the number of binding sites per erythrocyte. There was no significant difference in

insulin binding between insulin-dependent and non-insulin-dependent diabetic patients (9.75±1.65 % versus 11.11±1.63 %). The patients with decreased insulin binding did not differ from the others in serum glucose and plasma insulin levels.

Down regulation, in which ambient insulin concentration regulates insulin binding by changing receptor concentration and/or affinity, has been demonstrated in vitro (17) and in vivo in the insulin sensitive tissues (3) and in circulating monocytes (3,4,15). However, othere did not find such a correlation (8,18). In the present study, we found no significant inverse relationship between insulin binding by erythrocytes and plasma insulin levels. Erythrocyte precursors may have insulin receptors (19) which are subjected to down regulation and also gene regulation (20). But after maturation, other regulatory mechanisms besides down regulation may modulate the insulin binding sites on peripheral erythrocytes.

In conclusion, mean insulin binding in diabetic patients either untreated or treated with diet or insulin, but not with oral hypoglycemic agents, was shown to be lower than in normal subjects. Insulin binding sites on peripheral erythrocytes may reflect indirectly the insulin receptors of insulin sensitive tissues in diabetic patients. However, care must be excised in deducing the status of insulin receptors of insulin sensitive tissues in individual diabetic patients from data on insulin binding by erythrocytes only.

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