

ERYTHROCYTE INSULIN RECEPTOR ABNORMALITIES

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SUMMARY

Using circulating erythrocytes as the cellular mode, insulin binding was studied in 8 newly diagnosed non-obese male NIDDM subjects and an equal number of matched non-diabetic controls. Mean specific binding of insulin was significantly lower in patients than in controls ($p < 0.001$). This was due to a significant decrease in mean insulin receptor concentration per cell as well as decreased receptor affinity ($p < 0.001$). Compensation of diabetes with a diet and oral drug therapy significantly improved specific insulin binding to receptors, with receptor number and affinity increasing ($p < 0.001$) to near normal levels, thus reflecting increased peripheral insulin sensitivity in these diabetic subjects.

INTRODUCTION

A number of recent studies have shown that human erythrocytes can be used as a cellular model for assessing the status of insulin receptors in diabetes^{1,2}. Erythrocytes have specific surface receptors which have binding characteristics similar to those of the insulin receptors found in classical target tissues for insulin action². Since erythrocytes can be easily obtained in sufficient numbers for serial studies, they have been used as a model for insulin binding. In the present study, we have demonstrated abnormalities in erythrocyte insulin binding in subjects with non-insulin dependent diabetes mellitus.

MATERIAL AND METHODS

Eight newly diagnosed, untreated, non-insulin dependent, male diabetic patients³, and an equal number of age-matched, healthy, non-diabetic male subjects were studied. All subjects were non-obese and of ideal body weight. None of the subjects had any complications of diabetes or associated illnesses.

All patients were put on a high carbohydrate, high fibre (HCHF) diet containing 65% carbohydrate (in complex form), 20% protein and 15% fat. Four principal feeds consisting of 20%, 35%, and 30% of the total 1800-2200 calories were given. Oral drugs given were a combination of glibenclamide (1.25-5 mg) and metformin given together in two divided doses 30 min before breakfast and dinner. All patients were reassessed after achieving optimal glycaemic regulation. This period ranged between 10 and 20 days (mean 15).

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Materials: Mono (125_I) - Tyr-A14 porcine insulin of specific activity, 360 μ ci/ μ g (Hoechst, Frankfurt) purified porcine insulin, ELISA kit for assaying plasma IRI and Ficoll-Hypaque (Pharmacia, Uppsala) were gifted by M/s Boehringer Mannheim, West Germany.

Preparation of Cells: Fasting and post-prandial blood samples were collected during the initial visit as well as after control of diabetes. The fasting samples were used for insulin binding studies. Whole blood was centrifuged at 400g for 10 min at 25 °C. Plasma was separated and stored at -20 °C for insulin assay.

Erythrocytes were fractionated by passing twice through Ficoll-Hypaque gradients. The final cell suspension in HEPES buffer consisted of 4-4.5 $\times 10^9$ cells/ml; 98% of the cells were viable. Cell concentration was determined using the haemocytometer and by haematocrit.

Insulin binding studies: Erythrocytes ($4-4.5 \times 10^9$ cells/ml) were incubated at 15 °C with (125_I) - insulin (20 pg in 50 μ l) with or without varying amounts of unlabelled insulin (0 to 0.5×10^5 ng) in a total volume of 0.5 ml. After 210 min of incubation, duplicate samples were placed in pre-chilled microfuge tubes along with the buffer and dibutylphthalate. Cell bound and free insulin were separated by centrifugation at 7000 g at 4 °C for 10 min. The radioactivity in the cell pellet and supernatant was determined in a gamma counter (ECIL, Hyderabad). Non-specific binding is defined as the amount of radioactive insulin that remains bound in the presence of 10^5 ng/ml of unlabelled porcine insulin. All binding data were corrected for the non-specific binding to represent specific cell binding for purposes of comparison.

Plasma glucose was estimated by the orthotoluidine method⁵. Plasma IRI was assayed using the ELISA kit.

Data Analysis : The data on insulin binding studies were analysed by competition curve, scatchard plot and average affinity profiles⁴.

Statistical Analysis : All values are expressed as Mean \pm SD. P values were obtained using student's *t* test.

RESULTS

The clinical characteristics of the study group are shown in Table 1. Mean fasting and post prandial plasma glucose values decreased significantly ($p < 0.001$) on control of diabetes. The mean fasting IRI value was higher (marginally) than in controls initially, and decreased slightly after therapy. The mean stimulated plasma IRI concentration was low in comparison to controls ($P < 0.001$) and did not change significantly after therapy. There were no significant changes in the BMI of patients during the short period of therapy.

Table 2 summarizes the data on insulin binding to erythrocyte receptors. The per cent maximal specific binding of tracer insulin was decreased in diabetic subjects (before therapy) when compared with non-diabetic subjects ($P < 0.001$). This decrease was caused mainly by a decreased concentration of insulin receptors. The follow-up study after control of diabetes showed an increase in both the per cent maximal specific binding of tracer insulin ($P < 0.001$) and a significant increase in the insulin receptor number ($P < 0.01$).

Changes in affinity : The mean concentration of native insulin required to inhibit the maximal specific cell binding of tracer insulin by 50% (Table 2) indicated changes in the affinity of the erythrocyte insulin receptors. This was substantiated by the analysis of the data by average affinity profiles. Initially, in uncompensated diabetes, there was a decrease in the affinity constant K_e (empty site) at near-zero occupancy of the receptor. This increased to near normalcy after stabilization of diabetes.

DISCUSSION

Diabetes mellitus is a metabolic disorder of heterogeneous aetiology. Defects in insulin action at the target tissue may be prominent in the pathophysiology of the disease⁶. The abnormalities in the target tissue may be due to defects at the level of insulin binding to its receptor or at the post-binding level⁷.

Many studies have shown decreased insulin binding in insulin resistant states such as non-insulin dependent diabetes mellitus^{6,7}. Using the erythrocyte as the study tool, we have shown that in NIDDM patients with severe uncontrolled hyperglycaemia, there is decreased specific binding of insulin to erythrocyte insulin receptors. This appears to be mainly due to a significant decrease in the receptor concentration per cell and also due to a marginal decrease in the affinity of the receptor.

TABLE 1 : Clinical characteristics of study subjects

	Age (years)	BMI	Plasma Glucose (mmol/l)		HbA1 %	Plasma IRI (μ U/ml)	
			Fasting	Post prandial		Fasting	Postprandial
Non-diabetic subject (n = 8)	41 \pm 6	21.0 \pm 0.4	4.3 \pm 0.2	6.1 \pm 0.2	6.8 \pm 0.7	11 \pm 3	60 \pm 8
NIDDM subjects (n = 8)							
(i) Before treatment	45 \pm 10	22.5 \pm 0.8	9.5 \pm 1.6*	16.1 \pm 3.5*	9.9 \pm 0.7*	18 \pm 11	31 \pm 6*
(ii) After treatment	—	22.1 \pm 0.6	6.0 \pm 0.3**	8.0 \pm 1.5**	8.9 \pm 0.7*	12 \pm 5	45 \pm 16

BMI : Body mass index : weight in kg \div height in square metres.
 Non-diabetics versus NIDDM before treatment : * $P < 0.001$.
 Before treatment versus after treatment : $P < 0.05$; ** $P < 0.001$

TABLE 2: Summary of analysis of data on insulin binding

	Maximal specific binding of tracer insulin (%)	Receptor sites per cell	Insulin for 50% inhibition of binding (ng/ml)	Average affinity constant (K_e) $\times 10^7 M^{-1}$
Non-diabetic subjects (n = 8)	11.3 \pm 1.2	1700 \pm 110	4.3	1.3 \pm 0.1
NIDDM subjects (n = 8)				
(i) Before treatment	6.8 \pm 0.3*	810 \pm 65*	8.4	1.0 \pm 0.08*
(ii) After treatment	11.0 \pm 1.4**	1400 \pm 70**	5.3	1.2 \pm 0.1**

Non-diabetic subjects versus NIDDM before treatment : * $P < 0.001$.
 Before versus after treatment : ** $P < 0.001$.

The peripheral concentration of insulin may be normal, low or high depending on the degree of insulin resistance and the beta cell secretory defects. In the present study, we found that all diabetic subjects had significantly lowered mean stimulated IRI response. All patients with hyperglycaemia have abnormalities of insulin binding, the severity of which may be variable. This study shows that in newly diagnosed NIDDM patients, defects exist both in the insulin receptor affinity as well as in the receptor number in erythrocytes.

Identification of these abnormalities in diabetic subjects may help to plan treatment strategies. It has been shown that both diet⁸ and exercise improve insulin binding. Similarly, oral hypoglycaemic agents, especially sulphonylurea compounds, improve insulin receptor status⁹. In the present study, using conventional therapeutic measures such as diet and a combination of oral hypoglycaemic drugs, improvement in insulin binding to isolated erythrocyte insulin receptors has been observed. This increased insulin binding appears to be observed even in the absence of any demonstrable changes in peripheral IRI concentration, thus reflecting the high degree of peripheral insulin sensitivity achieved.

The factors responsible for such a rapid improvement in insulin action *in vivo* may be multiple. Further studies are needed to evaluate the effect of a combination of an HCHF diet, glibenclamide and metformin on insulin binding to receptors on target tissues. Studies on insulin receptors in a wide spectrum of diseases involv-

ing insulin resistance may, hopefully, lead to a better understanding of the receptor abnormalities in these conditions and help in optimizing therapeutic measures to correct such defects.

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QUIZ

State whether the following statements are True or False

Question: 1

The following are typical manifestations of carbon dioxide retention

- A drowsiness
- B cold extremities
- C low blood pressure
- D flapping tremor
- E muscle twitching

Question: 2

Contraindications to cervical manipulation for neck pain include

- A sensory loss or paraesthesiae in the upper limbs
- B pyramidal tract signs in the lower limbs
- C minor radiological changes of cervical spondylosis
- D pain radiating over the occiput
- E cervical subluxation on X-ray

Question: 3

In Behcet's syndrome

- A peak incidence is in elderly females
- B oral and genital ulcers are the usual presenting symptoms
- C sterile pustules develop on the skin
- D topical steroids promote healing of skin lesions
- E central nervous system involvement may mimic multiple sclerosis

Question: 4

Characteristic findings on rectal biopsy occur in

- A amoebiasis
- B giardiasis
- C Crohn's Disease
- D schistosomiasis
- E lymphogranuloma venereum