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Erythrocyte Insulin Receptors in Non-Insulin -Dependent Diabetics before and after Treatment

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ABSTRACT

On 40 non-insulin-dependent diabetes mellitus (NIDDM) patients who were hospitalized for the purpose of control and education of diabetes mellitus, erythrocyte insulin receptor assay was conducted with the used of ^{125}I-insulin at the time of admission and the results obtained were compared with the results obtained from 98 normal subjects.

After an average of 20 days of treatment, erythrocytes insulin receptor assay was repeated and the effect of diabetes mellitus treatment on insulin receptor was examined.

The mean specific ^{125}I-insulin binding to erythrocytes in untreated NIDDM patients was significantly decreased (5.64 ± 0.30%, mean ± SEM) when compared with that of normal subjects (7.35 ± 0.20 %, $p<0.001$). The average native insulin concentration required for half maximum binding (HMB) of normal subjects and NIDDM patients before treatment were 3.1 and 5.4 ng/ml, respectively, and the mean affinity constants of the receptors of high affinity site ($\overline{K}e$) were 1.49 ± 0.05 and 0.75 ± 0.05 $\times 10^9 M^{-1}$ ($p<0.001$), respectively. The insulin receptor number of high affinity site in untreated NIDDM patients was 33 ± 1.2 sites/cell, being significantly higher ($p<0.001$) than that of normal subjects (22 ± 0.9 sites/cell).

After treatment of these patients, a marked decrease of blood glucose and a slight decrease of total cholesterol and triglycerides were observed with a concomitant significant increase of ^{125}I-insulin binding to erythrocytes (from 5.64 ± 0.30% before to 6.68 ± 0.29% after treatment, $p<0.001$). The average native insulin concentration required for HMB after treatment decreased to 4.2 ng/ml and $\overline{K}e$ significantly increased to 1.12 ± 0.07 $\times 10^9 M^{-1}$ ($p<0.001$). The mean concentration of receptors of high affinity site decreased to 28 ± 1.5 sites/cell after treatment.

The changes observed in erythrocytes insulin receptors by diabetes aimed at normalization of blood glucose level were similarly observed in the insulin (n = 13), sulfonylurea (n = 16), and diet treatment groups (n = 11).

No difference in the mean fasting plasma immunoreactive insulin (IRI) concentration was observed between normal subjects and untreated NIDDM patients and also no significant relationship could be demonstrated between fasting plasma IRI and specific ^{125}I-insulin binding (%) to erythrocytes. A significant inverse correlation was observed between $\overline{K}e$ and plasma lipids (total cholesterol $r = -0.301$, $p<0.02$ and triglyceride $r = -0.291$, $p<0.05$), but no direct significant correlation could be demonstrated between $\overline{K}e$ and fasting blood glucose ($r = -0.220$, p = NS).

These results indicate that: 1. Even in the absence of hyperinsulinemia in untreated NIDDM patients, there is a decrease in specific ^{125}I-insulin binding to erythrocytes, which was found to be attributable to reduced affinity of the insulin receptors. 2. The number of erythrocyte insulin receptors of high affinity site was increased in untreated NIDDM patients, which was considered to be a phenomenon to compensate for decrease in affinity. 3. The affinity of the insulin receptors of NIDDM patients improved following treatment and the receptor number of high affinity site also approached that of normal level, suggesting reversibility. 4. The effect of treatment on erythrocytes insulin receptors of NIDDM patients was similarly observed in the insulin, sulfonylurea and diet treatment groups, indicating that it could not be a direct effect of the drugs. 5. Normalization of intracellular metabolism of the post receptor level after treatment may have served as an important factor in the improvement of erythrocyte insulin receptors of NIDDM patients.

Since the first step in insulin action is binding to its specific receptor on the plasma membrane, estimation of receptors in the diabetic state may be important in assessing the mechanism of insulin resistance, especially in non-insulin -dependent diabetics. In human, insulin receptors have been studied on monocytes[29,42,56,57,59,60], adipocytes[6,8,21,40,41,45,46], fibroblasts[18,35], liver cells[1], and placental cells[19,48]. Of these cells, monocytes, adipocytes and fibroblasts are frequently used in clinical investigations. In 1977, Gambhir et al[13] introduced a method using erythrocytes for insulin receptor studies, which required only about 10 ml of blood and these cells have been employed by other investigators[3,18,22,26,52,53] as the ideal cells for clinical insulin receptor studies. Insulin binding to liver cells in obese non-diabetics[1], monocytes[2,5] and adipocytes[41] in non-insulin-dependent diabetes mellitus (NIDDM) patients have been reported to be decreased primarily due to reduction in insulin receptor concentration and treatment of diabetes associated with restoration of insulin receptor concentration to the normal level.

Insulin binding to erythrocytes in NIDDM patients has been reported to be also decreased[26,37], but the mechanisms of binding defects in uncontrolled state and mechanisms of binding changes after treatment remain unresolved. moreover, although erythrocytes have been documented to have insulin receptors and binding characteristics similar to other cells[3,26,52], a number of evidence indicate that binding changes[21,46] or mechanisms of binding changes[57] do not always reflect those of other cells. Insulin binding to erythrocytes in NIDDM patients under various clinical conditions has been rarely studied.

The aim of this study was to investigate the insulin receptors to erythrocytes in normal subjects and in NIDDM patients before and after treatment with insulin, sulfonylurea and diet alone. The mechanism of binding changes was investigated by evaluating insulin binding before and after treatment and by evaluating with respect to known plasma factors.

MATERIALS AND METHODS

Materials.

Crystalline porcine insulin was obtained from Ely Lilli Co. (Indianapolis, Indiana). ^{125}I-monoiodinated porcine insulin was purchased from New England Nuclear (Boston, Massachusetts), bovine serum albumin fraction V from Sigma. Glucose, CaC12, Tris, Hepes and di-n-butylphthalate were purchased from Nakarai Chemical Ltd (Tokyo, Japan). Ficoll 400 and Sephadex G-50 were obtained from Pharmacia Fine Chemicals (Upsala, Sweden) and Conray from Daiichi Seiyaku (Tokyo, Japan). Insulin radioimmunoassay kit was purchased from Amersham (Tokyo, Japan).

Subjects.

The study groups consisted of 98 normal subjects (43 males and 55 females, 30-70 years of age with mean of 52.00 ± 1.01 years), all having normal glucose tolerance test and 40 NIDDM patients (18 males and 22 females, 27-74 years of age with mean of 53.35 ± 2.04 years). These patients were hospitalized at Hiroshima University medical School Hospital for education and control of diabetes. Thirteen

Table 1. Clinical and metabolic characteristics of the study groups

Groups	Age (yrs)	Sex M	Sex F	BMI (%)	FBS mg/dl	F-IRI μU/ml	T. Chol. mg/dl	Triglycerides mg/dl
Insulin	52.90± 3.51	4	9	21.56± 0.92	215.78± 16.94***	9.80± 2.21	239.50± 17.20	198.71± 40.35
Sul-fonylurea	55.64± 3.57	10	6	22.18± 1.08	187.13± 10.33***	11.40± 2.14	223.73± 14.56	169.42± 19.80
Diet alone	51.50± 3.15	4	7	23.20± 0.73	130.45± 13.96***	8.83± 1.00	224.55± 19.95	165.33± 19.62
Mean	53.35± 2.04	18	22	22.31± 0.60	177.79± 9.46***	10.28± 1.20	227.96± 9.58	172.64± 14.73
Normal subjects	52.00± 1.01	43	55	24.51± 1.87	79.88± 0.84	9.68± 0.40	221.83± 4.39	141.17± 9.64

The values are expressed as mean ± SEM.
*** $p<0.001$ compared with normal subjects.
BMI: Body mass index (weight kg/(height m)^2).
F-IRI: Fasting immunoreactive insulin.

out of 40 NIDDM were treated with insulin, 16 with sulfonylurea, and 11 with diet therapy alone (25—30 kcal/kg standard body weight/day).

Erythrocyte insulin receptor assessments were carried out within a week after admission and were repeated to all patients before discharge. Clinical and metabolic characteristics of the study groups are given in Table 1.

Preparation of Buffer G.

The composition of Buffer G[13] was as follows: 50 mM Hepes, 50 mM Tris, 10 mM Glucose, 2 mM EDTA, 10 mM CaC12, 50 mM NaC1, 5 mM KC1, 10 mM MgC12, and 0.2% bovine serum albumin with pH adjusted to 7.9 at room temperature.

Purification of ^{125}I-monoiodinated porcine insulin.

To obtain pure ^{125}I-insulin for binding studies, monoiodinated porcine insulin was filtrated on a gel chromatography using Sephadex G-50 (column 50 × 2.5 cm) eluted in Buffer G with pH 7.9 in a cold room. All fractions (each 1 ml) were counted in a gamma counter and a main peak of three separate peaks identified from these fractions was collected, divided to several tubes and stored at −65°C until used (Fig. 1).

Preparation of erythrocytes.

Isolation of erythrocytes was made according to the modified method of Gambhir et al[13]. Ten milliliters of blood was drawn from the cubital vein of normal subjects and NIDDM patients

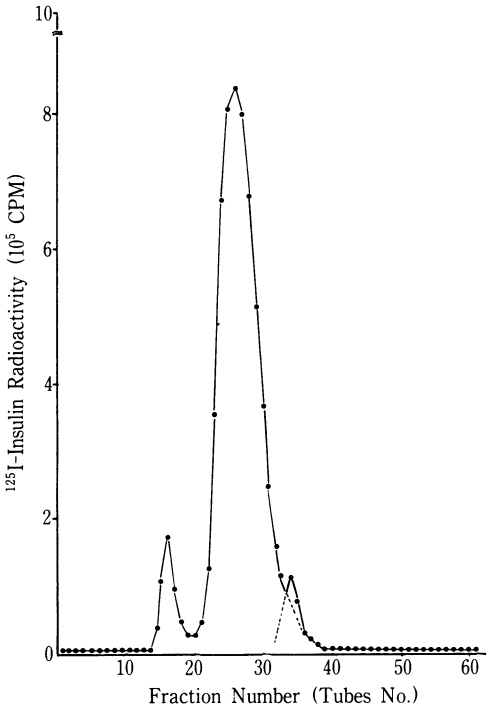

Fig. 1. Purification of ^{125}I-monoiodinated porcine insulin. Monoiodinated porcine insulin solution was applied to a Sephadex G-50 column (50 × 2.5 cm) eluted in Buffer G at pH 7.9. All fractions (each 1 ml) were counted with a gamma counter. The main peak of the three separate peaks was collected and stored in −65°C until use.

after overnight fast, collected in heparinized tube and then centrifuged at 1500 × *g* for 15 min. Plasma was collected for IRI determination and stored at −65°C until use. The erythrocytes pellet was suspended with three volumes of saline and 3 ml of Ficoll-Conray (SG 1.074) was placed at the bottom of the tube. Erythrocytes were isolated from other cells by centrifugation at 400 × *g* for 30 min at 18°C. The supernatant, buffy coat and upper layer of the erythrocytes pellet (granulocytes) were aspirated and the remaining pellet was resuspended with two volumes of saline, and centrifuged. The supernatant was aspirated and then pellet was added with Buffer G to make the volume contain 2.5 × 10^6 cells/ml.

Binding studies.

Binding of ^{125}I-insulin to erythrocytes was made by incubating 0.4 ml of cell suspension with 50 μl (500 pg) of ^{125}I-insulin and 50 μl of each concentration of native porcine insulin ranging from 0 to 10^4 ng/ml in a plastic tube to a final volume of 0.5 ml. After incubating for 24 hr at 4°C, 0.5 ml of cold Buffer G and 0.3 ml of di-n-butylphthalate were added, mixed and centrifuged 2.5 min using Beckman Microfuge in a cold room. Buffer G and the upper layer of di-n-butylphthalate were aspirated. To avoid free ^{125}I-insulin in the tube wall, centrifugation was repeated for 30 seconds and reaspiration of the remaining buffer and the tubes were counted using an auto gamma counter. To obtain a similar condition during the experiments, all tubes were placed on ice using an icebox. Determination of IRI concentrations was made at the same time to eliminate interassay variation. Blood glucose was meassured using glucose analyzer (YCI), while total cholesterol and triglyceride were determined by enzyme methods.

Evaluation of binding data.

The percentage of specific ^{125}I-insulin bound was determined by subtracting the percentage of ^{125}I-insulin bound in the presence of 10^4ng/ml native porcine insulin from the total percentage of ^{125}I-insulin bound. Although the existence of two independent populations[20] or single population of receptors[9] remains undecided, in order to facilitate a formal description for quantitative purposes, the Scatchard plot[54], which appear curvilinear in this present study, was interpreted as the sum of two linear Scatchard plots with one of high affinity and low capacity for insulin binding and the other having low affinity and high capacity. In this present study as the receptor of low affinity sites determined by two points only, gave difficulty in interpretation, therefore the author was emphasized to the receptors of high affinity site. Differences in the values between normal subjects and NIDDM were analyzed using non paired t-test and paired t-test for values of NIDDM before and after treatment.

RESULTS

Insulin binding, affinity and receptor number in normal subjects and NIDDM before treatment.

Specific ^{125}I-insulin binding to erythrocytes from 98 normal subjects and 40 NIDDM patients before treatment are shown in Fig. 2. The mean value of NIDDM before treatment was 5.64 ± 0.30% (mean ± SEM), and significantly decreased compared with that of normal subjects

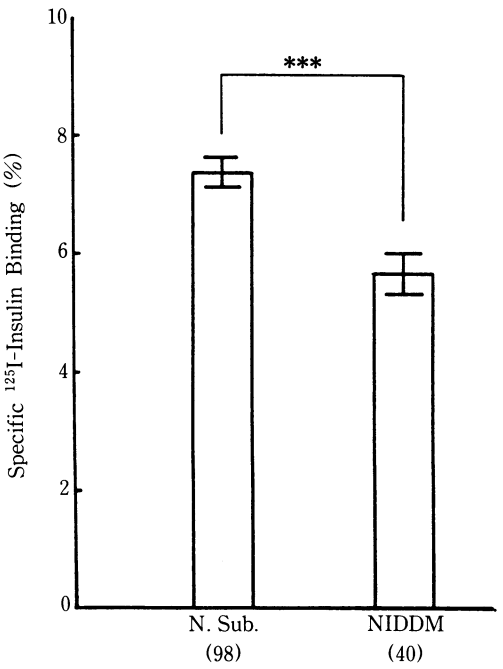

Fig. 2. Comparison of specific ^{125}I-insulin binding to erythrocytes between normal subjects (N. Sub.) and NIDDM patients before treatment. The values are expressed as mean ± SEM. *** $p<0.001$.

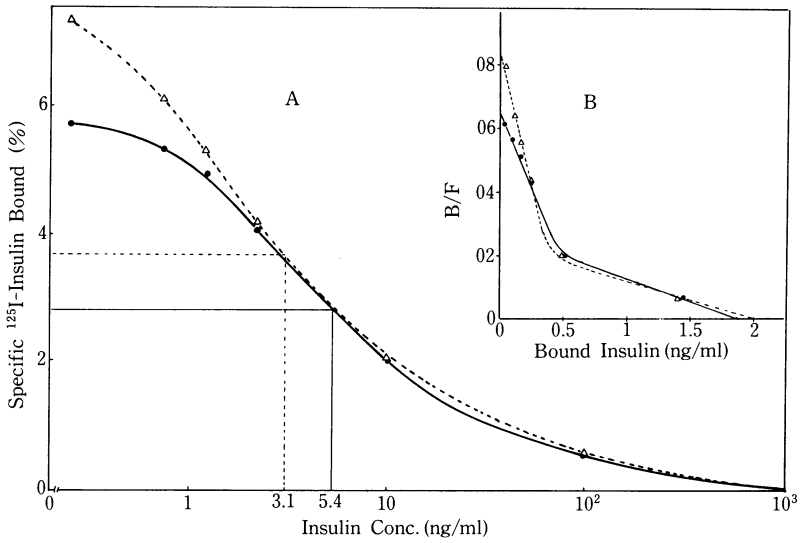

Fig. 3. Displacement curves and Scatchard plots of normal subject's erythrocytes and NIDDM patient's erythrocytes before treatment. Panel A: 0.4 ml of cell suspension (about 2.5×10^6 cells/ml) was incubated with 50 μl (250 pg/ml) of ^{125}I-insulin and 50 μl of each concentration of native porcine insulin ranging from 0 to 10^4 ng/ml. Non-specific binding was subtracted as described in procedure. The intercepts of the vertical lines to abscissa are the native insulin concentrations required for HMB. Each point represents the mean value. Panel B is Scatchard plots derived from panel A.
△ ··· △ normal subjects. ●——● NIDDM patients before treatment.

(7.35 ± 0.20%, $p<0.001$). Displacement curves in panel A of Fig. 3 showing the mean concentration of native insulin required for inhibition of 50% maximum specific ^{125}I-insulin binding or half maximum binding (HMB) of normal subjects and NIDDM patients before treatment were 3.1 and 5.4 ng/ml, respectively. These results indicate that affinity of insulin receptors is reduced in untreated NIDDM patients. Scatchard plot in panel B of Fig. 3 shows that reduction in the insulin binding is mainly due to reduction in affinity of receptors of high affinity site. The average affinity constants of the receptor of high affinity site ($\overline{K}e$) calculated from Scatchard plots of normal subjects and NIDDM patients were 1.49 ± 0.05 and 0.75 ± 0.05 × $10^9 M^{-1}$ $p<0.001$, respectively (Table 2), whereas receptor concentration of this site was found to be higher in NIDDM (33 ± 1.2 for NIDDM and 22 ± 0.9 sites/cell for normal subjects, $p<0.001$).

Insulin binding, affinity and receptor number in NIDDM after treatment.

Of 40 NIDDM patients, 13 were treated with insulin, 16 with sulfonylurea and 11 with diet

Table 2. The average affinity constant and receptor number of high affinity erythrocytes insulin receptor site on normal subjects and NIDDM patients before treatment

Groups	$\overline{K}e \times 10^9 M^{-1}$	Receptor number/cell
Normal subjects	1.49 ± 0.05	22 ± 0.9
NIDDM patients	0.75 ± 0.05***	33 ± 1.2***

The values are expressed as mean ± SEM.
*** $p<0.001$.

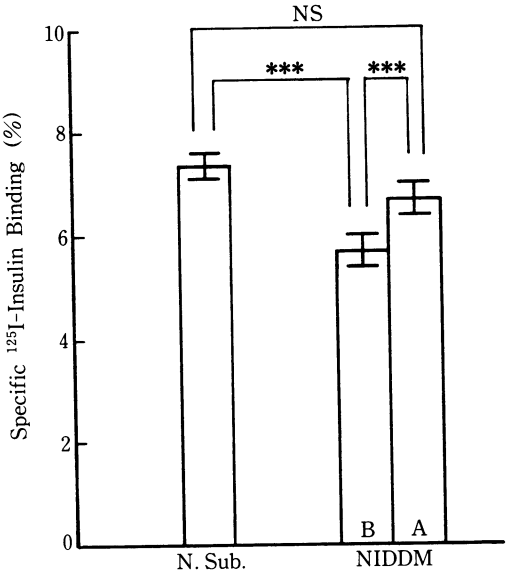

Fig. 4. Comparison of specific ^{125}I-insulin binding to erythrocytes between NIDDM patients before and after treatment. The values are expressed as mean ± SEM. B = before treatment. A = after treatment. *** $p<0.001$, NS: not significant.

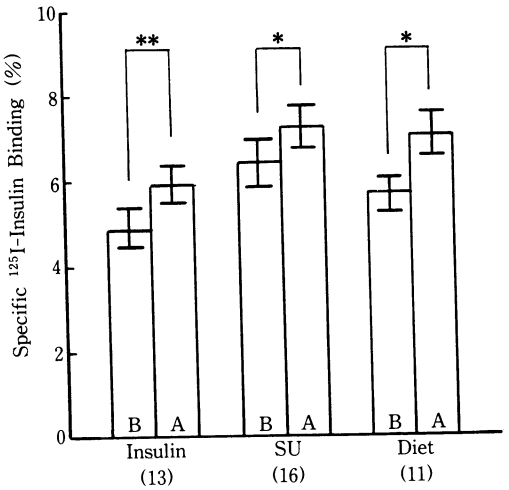

Fig. 6. Specific ^{125}I-insulin binding to erythrocytes of NIDDM patients treated with insulin, sulfonylurea and diet alone. The values are expressed as mean ± SEM. B = before treatment. A = after treatment. * $p<0.05$, ** $p<0.02$.

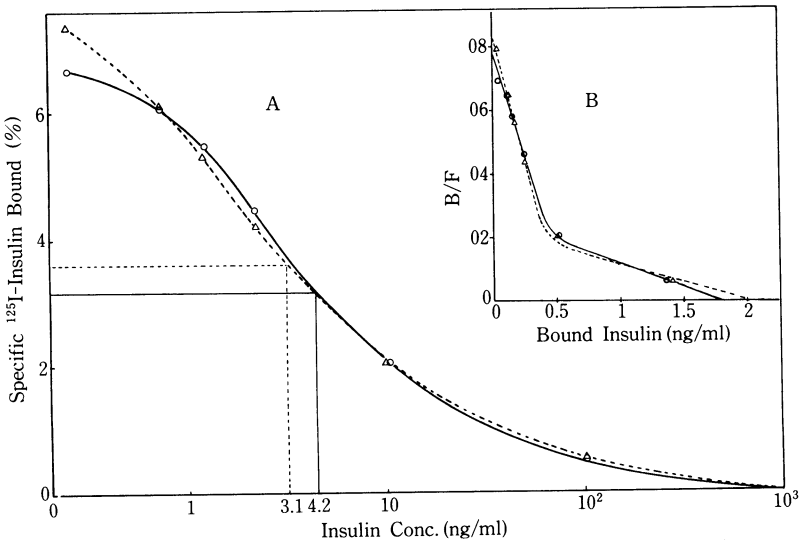

Fig. 5. Displacement curves and Scatchard plots of normal subject's erythrocytes and NIDDM patient's erythrocytes after treatment.
△ ··· △ normal subjects. ○———○ NIDDM patients after treatment.

Table 3. The average affinity constant and receptor number of high affinity erythrocyte insulin receptor site on NIDDM patients before and after treatment

Groups	$\overline{K}e \times 10^9 M^{-1}$	Receptor number/cell
NIDDM before treat.	0.75 ± 0.05	33 ± 1.2
NIDDM after treat.	1.12 ± 0.07***	28 ± 1.5**

The values are expressed as mean ± SEM
** $p<0.01$, *** $p<0.001$

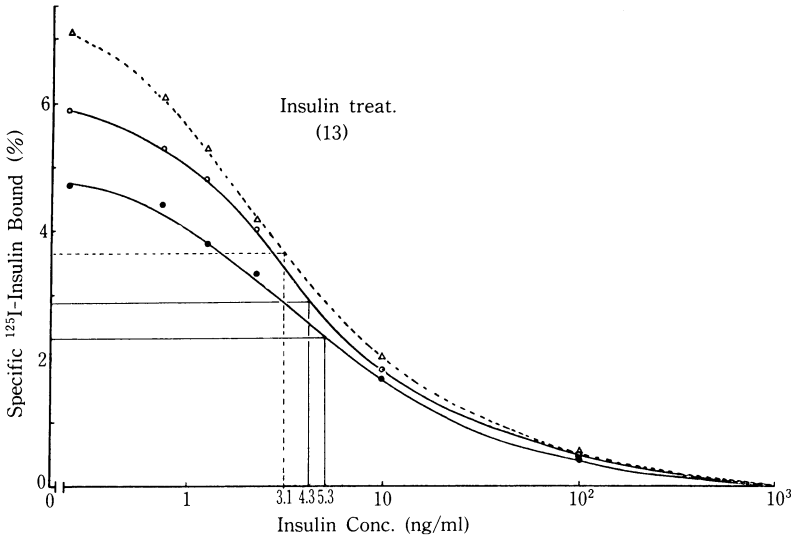

Fig. 7. Displacement curves of NIDDM patients treated with insulin. Procedure as described in Fig. 3. Δ ··· Δ normal subjects. ●———● NIDDM before insulin treatment. ○———○ NIDDM after insulin treatment.

alone. After treatment, the mean specific ^{125}I-insulin binding was significantly increased from 5.64 ± 0.30 to 6.68 ± 0.29%, $p<0.001$ (Fig. 4), but this value remains lower compared with the mean value of normal subjects. The native insulin concentration required for HMB decreased from 5.4 to 4.2 ng/ml (Fig. 3A, 5A), and the mean $\overline{K}e$ was increased from 0.75 ± 0.05 to 1.12 ± 0.07 × $10^9 M^{-1}$, $p<0.001$, but the receptor numbers of high affinity site decreased from 33 ± 1.2 sites/cell before treatment to 28 ± 1.5 sites/cell after treatment, $p<0.01$ (Table 3).

To investigate whether the changes of insulin binding, affinity and receptor number were similar or different with respect to therapy, displacement curves and Scatchard analyses were made for each group. Before treatment, the mean specific ^{125}I-insulin binding of NIDDM treated with insulin, sulfonylurea and diet alone were 4.78 ± 0.44, 6.35 ± 0.60 and 5.64 ± 0.30%, respectively (Fig. 6), but after treatment, these values increased to 5.68 ± 0.39 ($p<0.02$), 7.15 ± 0.49 ($p<0.05$) and 6.96 ± 0.50% ($p<0.05$), respectively. Displacement curve of each group revealed a similar decrease in the native insulin concentrations for HMB (Fig. 7, 8 and 9), indicate that similar increase of receptors affinity in response to insulin, sulfonylurea and diet therapy. Before treatment, the native insulin concentration required for HMB of insulin, sulfonylurea and diet groups were 5.3, 5.8 and 5.1 ng/ml, respectively. After treatment, these concentrations decreased to 4.3, 4.0 and 4.2 ng/ml, respectively. Scatchard analyses of each group before and after treatment are given in

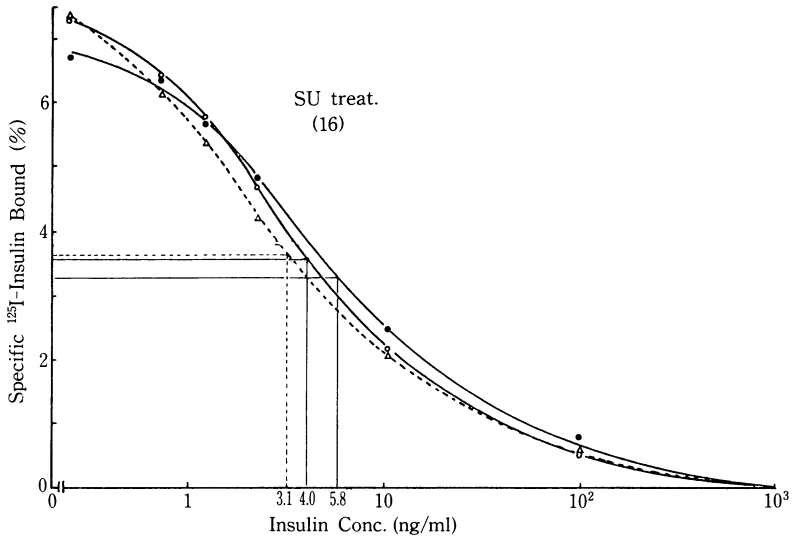

Fig. 8. Displacement curves of NIDDM patients treated with sulfonylurea. Procedure as described in Fig. 3. Δ ··· Δ normal subjects. ●———● NIDDM before sulfonylurea treatment. ○———○ NIDDM after sulfonylurea treatment.

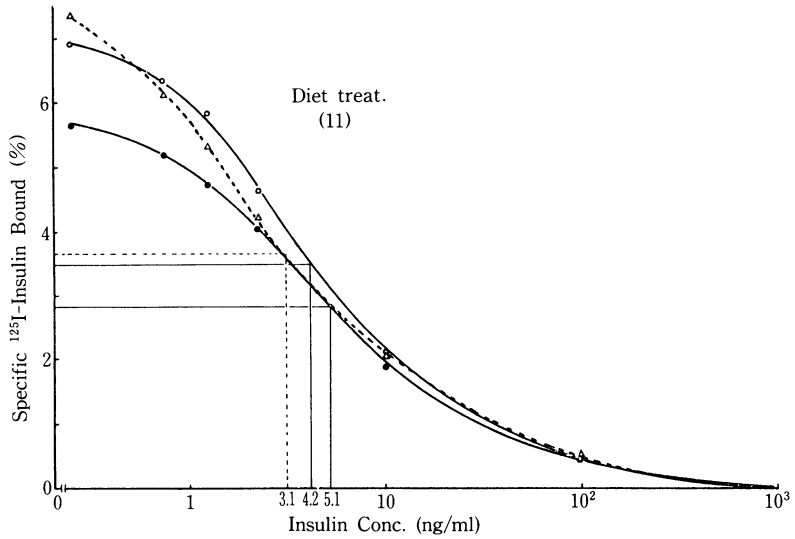

Fig. 9. Displacement curves of NIDDM patients treated with diet alone. Procedure as described in Fig. 3. Δ ··· Δ normal subjects. ●———● NIDDM before diet treatment. ○———○ NIDDM after diet treatment.

Fig. 10. $\overline{Ke}$ of insulin, sulfonylurea and diet groups before treatment were 0.72 ± 0.08, 0.79 ± 0.09 and 0.75 ± 0.08 × 10^9M^{-1}, respectively. After treatment these values increased to 1.02 ± 0.10 ($p<0.001$), 1.19 ± 0.15 ($p<0.001$) and 1.14 ± 0.09 × 10^9M^{-1} ($p<0.001$), respectively, as shown in Table 4. However, the post treatment values of each group all remained at a lower level compared with those of normal subjects. The insulin receptor concentrations of high affinity site were found to be decreased in all groups after treatment, but significant change was only observed in NIDDM patients treated with sulfonylurea. Before treatment,

Table 4. The average affinity constant and receptor number of high affinity erythrocyte insulin receptor site on NIDDM patients treated with insulin, sulfonylurea and diet alone

Groups	$\overline{K}e \times 10^9 M^{-1}$		Receptor number/cell	
	Before	After	Before	After
Insulin	0.72 ± 0.08	1.02 ± 0.10***	30 ± 3.0	26 ± 2.2
Sulfonylurea	0.79 ± 0.09	1.19 ± 0.15***	37 ± 2.8	28 ± 2.0*
Diet	0.75 ± 0.08	1.14 ± 0.09***	33 ± 4.4	29 ± 3.6

The values are expressed as mean ± SEM.
* $p<0.05$, *** $p<0.001$.

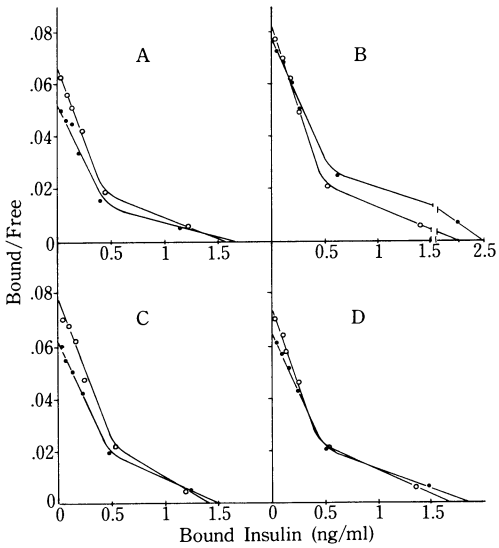

Fig. 10. Scatchard plots of NIDDM patients treated with insulin, sulfonylurea and diet alone. Panel. A, B and C are Scatchard plots derived from Fig. 7, 8 and 9, respectively. Each point represents the mean value of each group. Panel D is the Scatchard plot of total NIDDM. ●———● before treatment. ○———○ after treatment.

receptor concentrations of high affinity sites of insulin, sulfonylurea and diet groups were 30 ± 3.0, 37 ± 2.8 and 33 ± 4.4 sites/cell, respectively. After treatment decreased to 26 ± 2.2 (NS), 28 ± 2.0 ($p<0.05$) and 29 ± 3.6 sites/cell (NS), respectively. Results shown in Fig. 7, 8, 9 and 10 and Table 4 revealed that the effects of insulin, sulfonylurea and diet therapy on receptor affinity and receptor concentrations of high affinity site were found to be similar.

Clinical and metabolic changes after treatment.

Clinical and metabolic characteristics of the study groups are presented in Table 1, while changes in clinical and metabolic findings following treatment are given in Table 5. Significant decrease was observed in fasting blood sugar (FBS) and total cholesterol in NIDDM patients treated with insulin (FBS 215.78 ± 16.94 to 123.78 ± 5.78 mg/dl, $p<0.001$ and total cholesterol 239.50 ± 17.20 to 213.00 ± 19.06 mg/dl, $p<0.05$) despite the increase in mean body weight. Immunoreactive insulin concentration was also slightly increased from 9.80 ± 2.21 to 12.60 ± 1.90 μU/ml (NS). Treatment of NIDDM with sulfonylurea led to a decrease of FBS (187.13 ± 10.33 to 106.60 ± 4.47 mg/dl $p<0.001$) without any change in the fasting plasma IRI concentrations. Total cholesterol and triglyceride were also decreased but statistically not significant. The mean body weight of NIDDM patients treated with diet alone decreased from 58.15 ± 2.40 to 57.05 ± 2.30 kg, $p<0.01$. Reduction of body weight was associated with decrease in FBS from 130.45 ± 13.96 to 104.00 ± 4.73 mg/dl (NS). Total cholesterol and triglyceride were also decreased ($p<0.02$ for triglyceride).

Relationship between ^{125}I-insulin binding and other parameters ($\overline{K}e$, receptor concentration, IRI) and between $\overline{K}e$ and metabolic factors.

Relationship between ^{125}I-insulin binding and $\overline{K}e$, and between ^{125}I-insulin binding and receptor concentration of high affinity site is given in Fig. 11. The coefficient of correlation (r) between ^{125}I-insulin binding and $\overline{K}e$ before treatment was 0.363, and after treatment it increased to 0.417, whereas the coefficient of correlation of ^{125}I-insulin binding and receptor concentration of high affinity site decreased from 0.387 before to 0.344 after treatment.

A number of evidence from other studies sug-

Table 5. Changes in body weight and metabolic findings following therapy with insulin, sulfonylurea and diet alone

	Insulin treatment		Sulfonylurea treatment		Diet treatment	
	Before	After	Before	After	Before	After
Body weight (kg)	51.83 ± 2.74	52.03 ± 2.44	54.04 ± 3.42	53.04 ± 3.88 ***	58.15 ± 2.40	57.05 ± 2.30 ***
FBS (mg/dl)	215.78 ± 16.94	123.78 ± 5.78 ****	187.13 ± 10.33	106.60 ± 4.47 ****	130.45 ± 13.96	104.00 ± 4.73
T. Cholest. (mg/dl)	239.50 ± 17.20	213.00 ± 19.06 *	223.73 ± 14.56	186.06 ± 17.73	224.55 ± 19.95	202.55 ± 11.73
Triglyceride (mg/dl)	198.71 ± 40.35	119.14 ± 24.39 *	169.42 ± 19.80	155.59 ± 18.31	165.33 ± 17.91	127.66 ± 10.88 **
F-IRI (μU/ml)	9.80 ± 2.21	12.60 ± 1.90	11.40 ± 2.14	9.90 ± 0.64	8.83 ± 1.00	9.66 ± 1.07

The values are expressed as mean ± SEM.
* $p<0.05$, ** $p<0.02$, *** $p<0.01$, **** $p<0.001$.

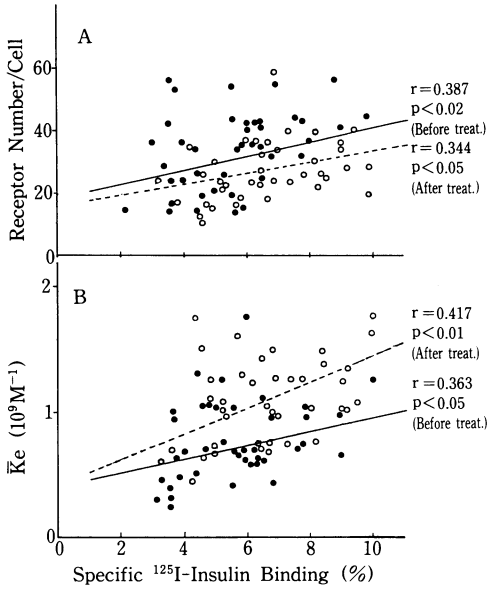

Fig. 11. Relationship between ^{125}I-insulin binding and receptor number of high affinity site (panel A) and between ^{125}I-insulin binding and receptor affinity of high affinity site (panel B) in NIDDM patients. (●) before treatment. (○) after treatment.

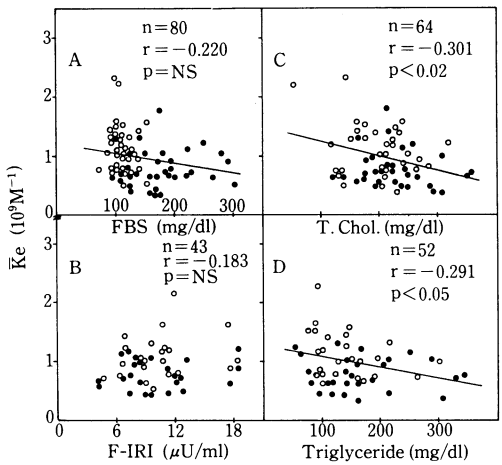

Fig. 12. Relationship between affinity of the receptor of high affinity site and plasma factors (FBS, fasting IRI, total cholesterol and triglyceride). (●) before treatment. (○) after treatment.

gest that plasma factors may play a role in the regulation of insulin binding by affecting the affinity of insulin receptors[3,24,35,38,44]. Therefore, the relationship between $\bar{K}e$ and plasma factors such as blood glucose, total cholesterol, triglyceride and IRI were evaluated in this study (Fig. 12). An inverse relationship existed between $\bar{K}e$ and total cholesterol and between $\bar{K}e$ and triglyceride with the coefficient of correlation being $r = -0.301$ and $r = -0.291$, respectively. Inverse relationship was also observed between $\bar{K}e$ and FBS, but it was not statistically significant. No relationship was found between $\bar{K}e$ and fasting plasma IRI concentration.

Down regulation is known as a key of homeostatic mechanism which there is a decrease in the number of specific hormone

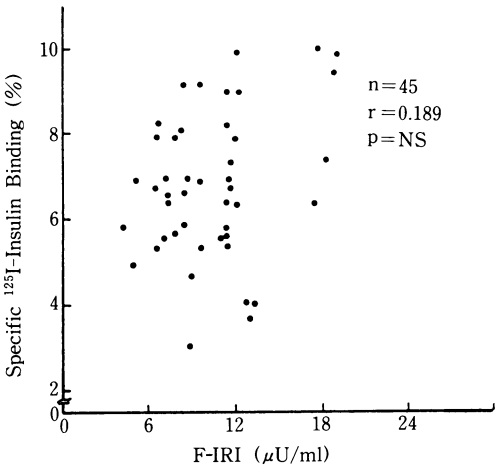

Fig. 13. Relationship between ^{125}I-insulin binding and fasting plasma IRI concentration. The values are derived from NIDDM patients, before and after treatment.

receptors in response to elevated level of that hormone. Studies using nucleated cells such as monocytes[2], and adipocytes[31,32,40] have shown that insulin can inversely regulate its receptor concentration in these cells, but no relationship was observed between ^{125}I-insulin binding and fasting plasma IRI concentration in this present study (Fig. 13).

DISCUSSION

It is generally recognized that impaired glucose metabolism in NIDDM patients is partly due to decrease of insulin binding to its target cell receptor. Studies on several tissues such as adipocytes[41] monocytes[2,5] and erythrocytes[10,31,39] have revealed that decrease of insulin binding to these cells in NIDDM is due to reduction in the concentration of insulin receptors. Obesity is frequently associated with hyperinsulinemia. Since the majority of NIDDM patients are obese, it has been suggested that a chronic elevation of plasma insulin is responsible for reduction of receptor concentration in these patients. Although in the present study the determination of insulin receptor concentration of low affinity site seems to be less valuable, at least the Scatchard plot in Fig. 3B has presented an impression that decrease of insulin binding is not due to reduction in insulin receptor concentration. Scatchard analysis shows that decrease of insulin binding is mainly due to decrease in affinity of insulin receptor. The native insulin concentration required for HMB was higher (Fig. 3), and $\overline{K}e$ was significantly lower in NIDDM patients compared with those of normal subjects (Table 2), indicating a decrease in affinity of insulin receptors of high affinity site. No reduction in insulin receptor concentration observed on NIDDM patients in this present study may be explained because these patients did not exhibit hyperinsulinemia. The mean fasting plasma IRI concentration and the mean body mass index did not differ from those of normal subjects (Table 1). In addition, sex ratio and age were also comparable. These data indicate that even in the absence of hyperinsulinemia in uncontrolled NIDDM, there is a decrease of insulin binding to erythrocytes due to reduction in affinity of insulin receptors.

NIDDM after treatment.

Amelioration of blood glucose in NIDDM patients after treatment is associated with a significant increase in insulin binding to erythrocytes (Fig. 4). Scatchard analysis shows that increase of insulin binding after treatment is due to increase in affinity of insulin receptors as indicated by a decrease in native insulin concentrations needed for HMB and by an increase in $\overline{K}e$ (Table 3). The mean receptor concentration of high affinity sites was found to have decreased. However, Scatchard analysis (Fig. 5B) does not suggest any increase in total receptor concentration. These data indicate that an increase in insulin binding to erythrocytes after a short term therapy is mediated through changes in affinity of insulin receptors and reduction of receptor affinity in untreated NIDDM patient is reversible.

To investigate whether changes in insulin binding were similar or differed with respect to therapy, Scatchard analyses of each group were further evaluated.

Insulin treatment.

Studies of insulin binding in diabetics undergoing insulin treatment have rarely been reported. To avoid the possible effect by insulin antibody which develops during therapy, patients having considerably high concentration of insulin antibody were excluded from this studies. Results from studies on several cells such as

lymphocytes[14], erythroid cells[15] and adipocytes[16,27] have indicated that insulin can inversely regulate its receptor concentration in these cells. Soman et al[56] have reported that physiologic hyperinsulinemia by insulin infusion in man reduces insulin concentration on monocytes. Insel et al[22] have also reported that short term insulin infusion leads to decrease in insulin receptor affinity on erythrocytes. These results indicate that elevation of plasma insulin concentration can reduce both affinity and concentration of insulin receptors on these cells. Contrary the present results, there was a significant increase of insulin binding to erythrocytes in NIDDM patients after 20 days of therapy with conventional insulin injection (Fig. 6). Scatchard analysis in Fig. 10A shows an increase in affinity of insulin receptors, in particular the receptor of high affinity site (Table 4), whereas receptor concentration of this site is decreased. Reduced receptor concentration of high affinity site after therapy with insulin cannot be attributed to a down-regulatory effect of insulin, because this phenomenon was also observed in NIDDM patients treated with either sulfonylurea or diet alone (Table 4). The author speculated that decrease receptor affinity of the receptor of high affinity site in untreated NIDDM is probably compensated by increase receptor concentration of this site by spare receptor in erythrocytes. No significant increase in fasting plasma IRI concentration could be observed, but the insulin effect could be seen as improvement in metabolic factors.

Since insulin can reduce both affinity[22] and concentration[56] of its receptors, increase in insulin binding to erythrocytes in NIDDM patients after 20 days of conventional insulin therapy cannot be attributed to the direct effects of insulin, but other underlying mechanisms such as secondary increase by improve in the metabolic factors in response to insulin therapy must be considered.

Sulfonylurea treatment.

The mechanism of hypoglycemic action of sulfonylurea remains unclear. Earlier studies have emphasized stimulation of endogenous insulin secretion, but subsequent reports have shown that long term administration frequently results in return of insulin to or below the pretreatment levels[12,51] and leads to the concept of extra-pancreatic effects of these drugs. This present study showed that treatment of NIDDM patients with sulfonylurea led to decrease in FBS and concomitant increase in insulin binding to erythrocytes. These results are consistent with previous *in vivo* studies on monocytes[42], adipocytes[29], mice plasma membranes[11] and erythrocytes[37]. Whether increase in insulin binding is related specifically or not to sulfonylurea remains unclear. An *in vitro* study[50] has shown that sulfonylurea increases insulin receptors in human fibroblasts, whereas an *in vitro* study by Nowak et al on liver plasma membranes[36] has suggested that sulfonylurea acts predominantly on processes beyond the binding portion of the insulin receptor. Although the possible direct effect of sulfonylurea on affinity of insulin receptors cannot be ruled out in this present study, similar changes in affinity of insulin receptors with those of NIDDM treated with either insulin or diet therapy alone suggest the increase not as the direct effect of these drugs. Decrease in the total insulin receptor concentrations in NIDDM treated with sulfonylurea can be related to the increase in endogenous insulin secretion. Although no difference was observed in the fasting plasma IRI concentration, the mean sum IRI concentration on 50 g oral glucose tolerance test in this group after treatment was significantly increased (data not shown). Thus there was a possibility that down-regulatory effect caused by increment of endogenous insulin secretion which was induced by drugs.

Diet treatment.

Total calory restriction on NIDDM patients leads to decrease of FBS, total cholesterol and triglyceride. The mean body weight was significantly decreased, but no significant change was observed in fasting plasma IRI concentrations (Table 5). The finding of improved metabolic control without alteration of IRI concentrations may suggest an enhancement of insulin effectiveness. In fact, there was a significantly increase of insulin binding to erythrocytes after total calory restriction, and this was related to change in receptor affinity (Fig. 9 and 10, Table 4). Other previous studies on several cells such as monocytes[2] and adipocytes[28] have shown that increase of insulin binding to these cells following diet restrictions is related to increase in

insulin receptor concentrations. Scatchard analysis in Fig. 10C suggests no changes in the total receptor concentration. The difference in the mechanism of increased binding between erythrocytes and cells such as monocytes, lymphocytes, and adipocytes can be explained in part because mature erythrocytes have lost the capacity for de novo protein synthesis, therefore unable for receptors up-regulation in responses to diet restriction. However, it has been suggested that prolonged diet restriction can increase insulin receptor concentration such as in cases of anorexia nervosa[53], since the concentration of insulin receptors on erythrocytes can be modulated at a premature stage[58]. A difference in the mechanism of binding increase between monocytes and erythrocytes after 14 days of diet restriction has also been reported by Spanheimer et al[57].

It seems that the effect of fat differs from carbohydrates in the metabolic control of diabetics. Studies in rats have shown that high fat feeding induces a state of insulin resistance as a results of impairment of both insulin binding and glucose processing at post receptor levels[17,23,55], while other studies have demonstrated that increasing the intake of indigestible carbohydrates without changing digestible carbohydrates or fat have beneficial effect on the control of diabetics[25,33]. In this present study, it could not be determined whether improved metabolic control in NIDDM patients is related only to fat restriction or also with other diet components.

The inverse relationship existing between $\overline{K}e$ and plasma factors (total cholesterol, triglyceride and blood glucose), suggests that plasma factors may play a role in the regulation of receptor affinity, where plasma lipids seem to be more dominant. However, changes in receptor affinity cannot be related directly to either cholesterol, triglyceride or blood glucose since such relationship could not be observed in pre or post-treatment. Moreover, *in vitro* studies are not available to support this. It suggested that either insulin or sulfonylurea can improve metabolic control through their action on the processes beyond the binding portion of the insulin receptor, and normalization of the metabolic factors will restore receptor affinity.

Several investigators have already demonstrated that ambient insulin can regulate the receptor concentration of many cells *in vitro*[7,14,30,35] and in obesity the concentration of insulin receptors on monocytes and adipocytes is inversely proportional to plasma insulin[2,41]. No inverse relationship between fasting plasma IRI and insulin binding to erythrocytes was observed in this present study (Fig. 13). This finding is consistent with those of other investigators[15,59]. Several investigators have reported that both up and down regulation of insulin receptors require de novo protein synthesis[7,14,15,30,35]. Since mature erythrocytes have no nuclei, limit to protein synthesis, thus these cells will not down-regulate their receptor concentrations. It has been reported that concentration of insulin receptors can be modulated at a premature stage[58]. In this present study, a short term therapy (mean of 20 days) was likely to cause minimal changes in the population of erythrocytes over the duration of the study. So the exact reason why the erythrocytes insulin receptor concentration of high affinity site was changed in the short term therapy is not clear. Further examination should be requested for understanding to this problem.

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