Insulin Receptors on Monocytes from Patients with Ketosis-prone Diabetes Mellitus

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SUMMARY

I-125-Insulin binding to its receptor on monocytes was measured in 30 young, insulin-treated diabetic outpatients and in 13 newly diagnosed, ketotic diabetic patients before and after insulin treatment.

In insulin-treated diabetic outpatients, insulin binding was similar on average to that of 25 age-matched normal subjects. A slight but statistically significant negative relationship was found between the insulin concentration required to displace specific binding of I-125-insulin by 50 per cent and the plasma concentration of ketone bodies ($R = -0.46$, $p < 0.02$), indicating a positive correlation between apparent receptor affinity and the plasma concentration of ketone bodies. No correlation was found between insulin binding and total plasma insulin concentration.

Insulin binding to monocytes from newly diagnosed diabetic patients exhibited large variability. Based on the initial insulin-binding values and the receptor response to insulin treatment, the patients could be divided into two groups. Group 1 comprised eight diabetic subjects who had had specific cell-binding fractions at an insulin tracer concentration above the median of normal subjects before therapy; after insulin treatment for 10 days, insulin binding was restored to normal. Group 2 consisted of five diabetic patients who had had specific cell-binding fractions at a tracer level below the median of normal subjects when admitted; insulin therapy caused no significant change in insulin binding. In both groups of newly diagnosed, diabetic patients, no correlation could be found between the cellular insulin binding and the plasma concentrations of insulin and ketone bodies.

We conclude that (1) insulin-treated diabetic outpatients have normal insulin binding to monocytes; (2) among newly diagnosed, ketotic diabetics with identical metabolic presentation, some may have a raised but reversible insulin binding initially, while others seem to have defective cellular insulin binding in addition to insulin deficiency; and (3) no consistent relationships exist between the plasma levels of insulin and ketone bodies and the insulin binding on monocytes from insulin-dependent diabetics. DIABETES *27*:1098-1104, November, 1978.

Decreased cellular insulin binding has been described repeatedly in nonketotic diabetic states of man.[1-6] Moreover, both normal and reduced insulin binding to placental membranes from insulin-dependent diabetic mothers have been reported.[7,8] In contrast, an increase in insulin receptor binding by liver cell membranes was found in studies of animals rendered diabetic with streptozotocin.[9-11] Studies in vitro show that insulin[12] and beta-hydroxybutyrate[13] affect insulin binding to cultured lymphocytes. In this study we examined 30 insulin-treated, juvenile diabetic outpatients and 13 newly discovered, ketotic diabetic patients before and after insulin treatment in order to estimate insulin binding to monocytes and the receptor-regulatory influence of the plasma concentrations of insulin and ketone bodies.

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MATERIALS AND METHODS

Insulin-treated diabetic outpatients. The study comprised 30 randomly selected, juvenile diabetic patients who attended our outpatient clinic. All had onset of diabetes before the age of 30 years. None of them had symptoms of long-term vascular complications, and all had normal serum creatinine values. They were fed a standard diabetes diet with a daily

TABLE 1

Clinical data of 30 insulin-treated diabetic outpatients and of 25 normal subjects

Subjects	n	Age (yr.)	Body weight (kg.)	Duration of diabetes (yr.)	Insulin dose† (U./day)	Urine glucose‡ (mmol/ 24 hr.)
Normal	15 F 10 M	25* 21-31*	63 45-90	—	—	—
Diabetic	8 F 22 M	26 20-35	64 52-76	9 1-23	40 16-72	165 0-850

*Median and range, respectively.

†Insulin dose was the requirement at the time of investigation.

‡Urine glucose excretion was measured for three days before the day of the receptor study, and the average values are given.

energy supply from 2000 to 2600 kcal. (40 per cent carbohydrate, 41 per cent fat, and 19 per cent protein). Diabetics received no insulin on the morning of study, and all measurements were undertaken at 0800 hours after an overnight fast. Clinical and experimental data are given in tables 1 and 2.

Newly diagnosed diabetic patients. Thirteen consecutively admitted patients with newly discovered, insulin-requiring diabetes mellitus were studied before insulin treatment and for the first few days while inpatients. Clinical and metabolic data are presented in table 3. The patients had body weights within plus-minus 10 per cent of their ideal weight.[14] None of them suffered from other diseases. Four patients (LB, HSN, AJ, and NP) had slightly depressed consciousness and acetone odor and were clinically dehydrated (diabetic precoma), whereas the remaining nine diabetics showed no signs of metabolic decompensation at physical examination when admitted.

Normal subjects. The reference group included 25 apparently healthy volunteers (table 1). All were within plus-minus 15 per cent of their ideal weight,[14] and all had normal fasting plasma concentrations of glucose and insulin.

Insulin. Plasma insulin concentration in persons who had never received exogenous insulin was measured by the radioimmunoassay of Heding.[15] Total plasma insulin (IRI) in insulin-treated diabetic patients was measured after acid-ethanol extraction in order to separate insulin from insulin-binding antibodies.[16]

Insulin-binding IgG (insulin antibodies). The insulin-binding capacity of IgG was determined after incubation of the plasma with ^{125}I-labeled bovine insulin followed by separation of the free and antibody-bound ^{125}I-bovine insulin by immunoelectrophoresis.[17]

Glucose. Glucose concentration in plasma and urine was measured using an ortho-toluidine method.[18]

Ketone bodies. Plasma acetoacetate concentration and plasma 3-hydroxybutyrate concentration were measured separately by an enzymatic micromethod.[19] Plasma ketone bodies are the total of 3-hydroxybutyrate and acetoacetate.

Free fatty acids (FFA). Plasma FFAs (aliphatic carboxylate, C_8-C_{18} nonesterified) were analyzed by the colorimetric method of Duncombe[20] as modified by Itaya.[21] Both palmitic acid in chloroform and an aqueous solution of oleic acid were used as standards. Analysis of FFA was always performed the next day after storage of plasma at −20° C.

Cell-binding studies. These experiments were performed at 0800 hours after an overnight fast, with the exception of newly diagnosed diabetics, who were examined at the time of admission. Whole blood (175 ml.) was drawn from an antecubital vein and transferred to tubes containing EDTA (dipotassium salt). Mononuclear leukocytes were isolated by gradient centrifugation ad modum Boyum.[22] The cells were washed twice and were incubated in tris-HCl buffer (25 mmol per liter, pH 8.0, at 15°) at a cell-number concentration of about 70 × 10^6 per milliliter for 100 minutes at 15° C. with ^{125}I-insulin (Novo Research

TABLE 2

Experimental data of 25 normal subjects and of 30 insulin-treated diabetic outpatients

Subjects	Fasting plasma glucose (mmol/L.)	Fasting plasma insulin (μU./ml.)	Fasting plasma insulin binding IgG (mU./ml.)	Fasting plasma ketone bodies (mmol/L.)	Fasting plasma FFA (mmol/L.)	Specific cell-binding fraction × 10^{-2}
Normal	5.0* 4.0-5.8*	5 0-15	—	0.068 0.02-0.32	0.25 0.09-0.62	2.1 1.2-4.4
Diabetic	13.0 4.4-23.7	141 0-2880	0.430 0.014-8.919	0.888 0.12-2.60	0.52 0.12-1.53	2.3 1.2-4.1

*Median and range, respectively.

TABLE 3

Individual characteristics of newly diagnosed diabetic patients*

Patient	Sex	Age (yr.)	Specific cell-binding fraction × 10^{-2}	Plasma glucose (mmol/L.)	Plasma insulin (μU./ml.)	Plasma ketone bodies (mmol/L.)	Plasma FFA (mmol/L.)	Insulin requirement (U./24 hr.)
LB	F	17	5.2-3.3	16.4-10.6	0	16.250-0.060	1.14-0.18	100-48
HSN	F	27	3.5-1.3	16.7-13.2	0	4.820-0.580	0.72-0.42	28-28
ALG	F	27	2.5-1.9	21.9-9.9	0	12.900-0.810	0.41-0.40	120-44
EH	M	68	2.4-1.7	26.7-9.1	3	6.351-1.056	-0.23†	36-36
AJ	F	68	4.3-3.6	46.0-18.0	0	3.410-1.377	0.54-0.17	60-40
NP	M	13	3.7-2.1	12.0-7.0	0	6.420-0.091	0.60-0.16	24-20
EKH	M	30	2.3-1.3	12.1-9.6	4	2.039-0.153	0.73-0.22	20-16
IDA	M	12	4.4-2.1	17.9-8.4	0	2.590-0.125	0.98-0.09	24-20
PBH	M	20	1.6-1.9	17.0-18.0	4	2.433-0.287	0.50-0.40	20-36
JW	M	23	1.7-1.6	18.0-10.0	0	7.570-1.179	0.93-0.47	16-36
BJ	M	21	1.0-1.0	17.9-12.4	6	6.067-0.583	0.61-0.70	36-28
HJ	M	31	0.8-1.7	18.9-8.8	6	5.750-1.380	0.46-0.36	16-18
PSN	M	20	1.6-1.9	17.9-18.0	4	2.433-0.287	0.50-0.40	20-36

*Experimental data are given from the time of admission and after 10 days' treatment, at which time all measurements were undertaken at about 0800 hours after an overnight fast. Plasma insulin concentration was determined only at the time of admission. Specific cell-binding fraction was measured at a tracer concentration of 172 pmol per liter.

†Sample lost for measurement.

Institute, Copenhagen) at a concentration of 172 pmol per liter.[23] The specific activity of the tracer was 25 to 30 μCi. per microgram, and there were 0.1 to 0.2 iodine atoms per insulin molecule.[24] At this low degree of iodination, more than 95 per cent of the iodoinsulin is monoiodinated.[25]

For competition studies, native insulin was added to the incubation medium in increasing concentrations. 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 fraction is defined as total binding fraction minus nonspecific binding fraction. Radioactivity that remained bound in the presence of an excess of native insulin at 13 μmol per liter was considered nonspecific. In all three groups this fraction averaged about 25 per cent of the total binding fraction. The monocytes were identified by morphologic criteria in cytocentrifuged smears stained with alpha naphthyl acetate esterase.[26] There was no significant difference between the number of monocytes isolated from diabetic and from normal subjects. On an average, monocytes comprised 14.7 per cent of the isolated mononuclear leukocytes, and the specific cell-binding fraction was adjusted to a cell-number concentration of 10^7 monocytes per milliliter.[27] All binding studies were done in duplicate.

Binding analysis. The results of the binding studies are presented in four ways: (1) the specific cell-binding fraction (bound/total insulin) at the insulin tracer concentration, 172 pmol per liter, (2) the specific cell-binding fraction plotted as a function of total insulin concentration (competition curve), (3) specific cell-bound insulin/free insulin plotted as a function of specific bound insulin (Scatchard plot);[28] the intercept on the x-axis indicates the maximum amount of insulin bound (an approximation of the insulin receptor concentration = R_0); the validity of this plot was discussed previously,[23] and (4) the concentration of native insulin necessary to inhibit the specific cell-binding fraction of ^{125}I-insulin by 50 per cent is taken as an estimate of apparent receptor affinity (a low, 50 per cent inhibition indicates a high apparent receptor affinity and vice versa) in comparative studies.

Statistical methods. Wilcoxon's two-sample test and test for paired differences were employed for comparison of values among groups and in groups before and after treatment, respectively. Spearman's coefficient of rank was applied in correlation studies.

RESULTS

Insulin-treated diabetic outpatients. Specific cell-binding fraction at each insulin concentration tested was statistically the same in normals and diabetics (table 2 and figure 1). A negative correlation was found between the concentration of insulin required to reduce specific binding of ^{125}I-insulin by 50 per cent and the plasma concentration of ketone bodies (figure 2, $R = -0.46$, $p < 0.02$). Specific cell-binding fraction at insulin tracer concentration was slightly correlated negatively with the total plasma insulin concen-

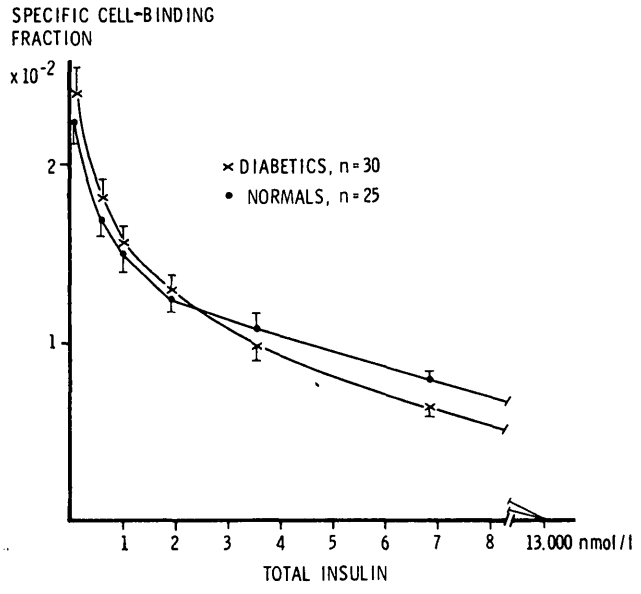

FIG. 1. The inhibiting effect of native insulin on ^{125}I-insulin binding to monocytes from 25 normal subjects and 30 insulin-treated diabetic outpatients. Mononuclear leukocytes, in a cell-number concentration of 70 × 10^6 per milliliter, were incubated in a tris-HCl buffer (pH = 8) at 15° C. for 100 minutes with ^{125}I-insulin in a concentration of 172 pmol per liter and native insulin in increasing concentration. The specific cell-binding fraction was corrected to a monocyte concentration of 10^7 per milliliter. Each point on the curve represents mean ± S.E.M.

tration (figure 3, R = −0.17, p > 0.10) and with the plasma level of insulin-binding IgG (R = −0.12, p > 0.10).

Newly diagnosed diabetic patients. Individual values of specific cell binding, plasma glucose, plasma insulin, plasma ketone bodies, plasma free fatty acids, and daily insulin requirement at hospitalization and at five days and 10 days, respectively, after treatment with insulin and diet are given in table 3. When admitted, all patients were ketotic (range of plasma ketone bodies was 2.04 to 16.25 mmol per liter). Insulin binding to monocytes varied greatly. Based on the initial values and the course of cellular insulin binding during treatment, the patients could be divided into two groups (figure 4).

Group 1 comprised eight diabetics who had specific cell-binding fractions at insulin tracer concentration above the median of the reference group when admitted (p < 0.01). After treatment for 10 days, specific cell-binding fraction decreased in all patients (figure 5, left, p < 0.05 at the three lowest insulin concentrations and p > 0.1 at the four highest insulin concentrations). It can be seen from the corresponding Scatchard plots (figure 5, right) that the approximated number of receptors per monocyte remained fairly constant during the first 10 days of treatment (the extrapolated plots have grossly the same intercept on the x-axis). The insulin concentration giving 50 per cent inhibition of specific ^{125}I-insulin binding, in contrast, was significantly raised (figure 5, left, p < 0.05) after treatment for 10 days, suggesting that insulin administration was associated with a down-regulation of apparent receptor affinity.

Group 2 consisted of five diabetics who had specific cell-binding fractions at a tracer concentration below the median of the reference group initially (p < 0.01). Insulin administration caused no significant alterations (figure 6, p > 0.1) in receptor concentration or apparent receptor affinity.

There was no significant difference between the two groups in the median plasma concentrations of insulin, glucose, ketone bodies, or free fatty acids either at the time of admission or after 10 days of treatment. No correlation could be shown between values of cellular insulin binding and the plasma level of ketone bodies.

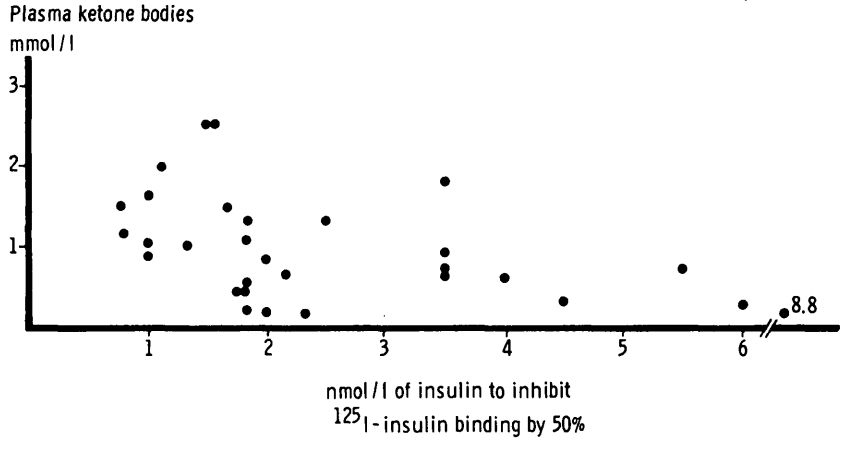

FIGURE 2

Correlation between fasting plasma concentration of ketone bodies and the amount of insulin that caused a 50 per cent reduction in specific cell binding of ^{125}I-insulin in 30 insulin-treated juvenile diabetics.

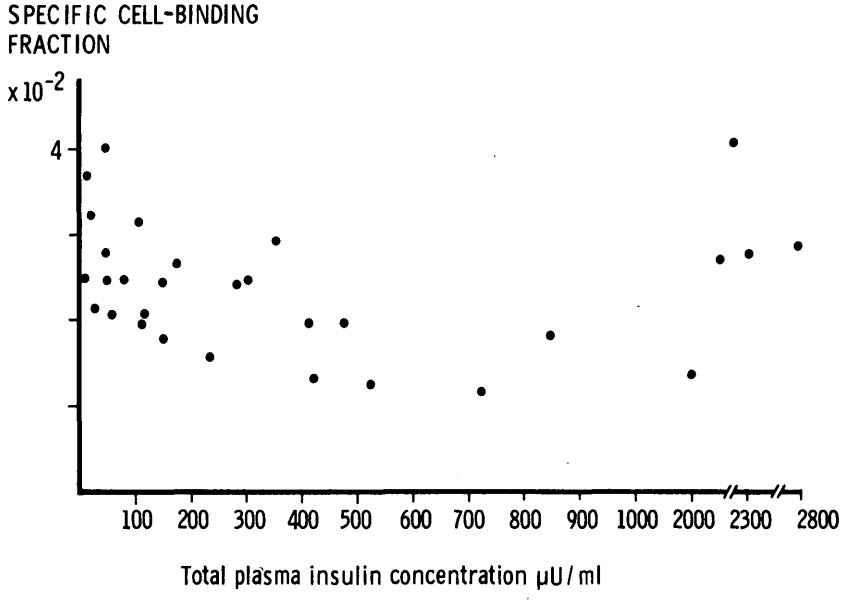

FIGURE 3

Correlation between the specific cell-binding fraction at a tracer concentration and the total fasting plasma insulin concentration in 30 insulin-treated diabetics.

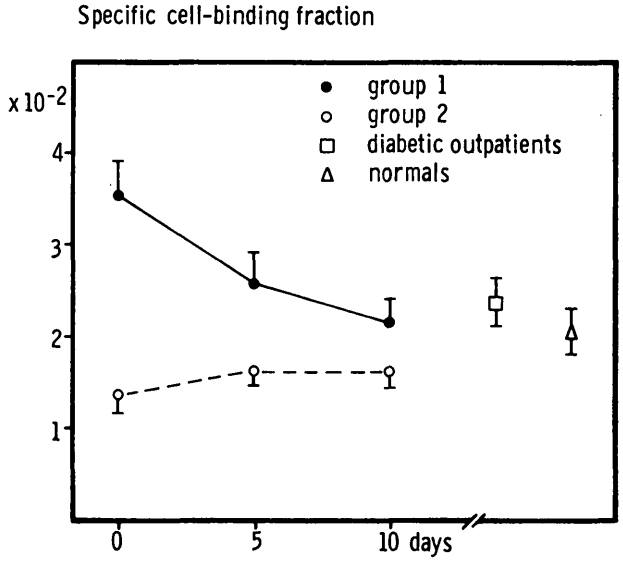

FIG. 4. Average values (mean ± S.E.M.) of specific cell-binding fraction at tracer concentration in group 1 and group 2, respectively, of newly diagnosed diabetics at the time of admission and for the first few days under treatment with insulin and a standard diabetes diet. These binding values are compared to the average specific cell-binding fractions obtained in 30 insulin-treated diabetic outpatients and in 25 normals.

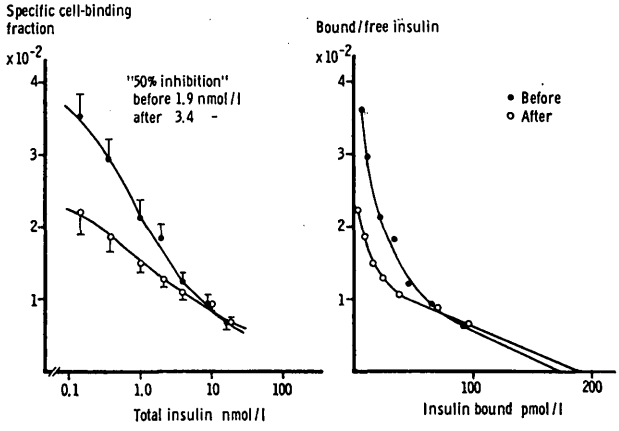

FIG. 5. *(Left)* The inhibiting effect of native insulin on ^{125}I-insulin binding to monocytes from eight newly diagnosed insulin-dependent diabetics on admission before insulin administration and again 10 days after the start of treatment (mean ± S.E.M.). Experimental conditions were as described in legend to figure 1. The concentration of insulin required to reduce specific binding of ^{125}I-insulin by 50 per cent before and after insulin therapy for 10 days is given in the inset; *before* and *after* treatment for 10 days are as symbolized in the other panel. *(Right)* Scatchard analysis of the binding data derived from the competition curves (figure 5, left).

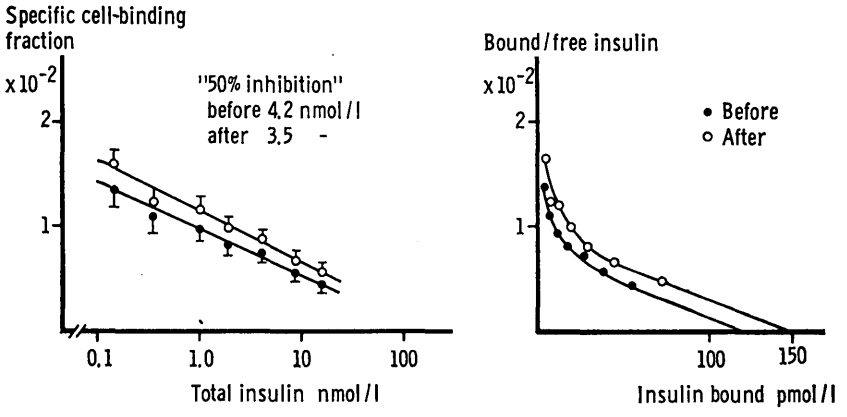

FIGURE 6

(Left) The inhibiting effect of native insulin on ^{125}I-insulin binding to monocytes from five newly diagnosed insulin-dependent diabetics on admission before insulin administration and again 10 days after the start of treatment (mean ± S.E.M.). Experimental conditions were as described in legend to figure 1. The concentration of insulin required to reduce specific binding of ^{125}I-insulin by 50 per cent before and after insulin therapy for 10 days is given in the inset; *before* and *after* treatment for 10 days are as indicated in the second panel. *(Right)* Scatchard analysis of binding data derived from competition curves (figure 6, left).

DISCUSSION

We found normal cellular insulin binding in well controlled, insulin-treated diabetic subjects. The observation is consistent with the finding of Ginsberg[29] that well treated subjects with ketosis-prone diabetes mellitus have normal sensitivity to insulin.

In a preliminary study[30] of a few, untreated ketotic diabetic patients, we reported increased insulin binding to monocytes. However, as the group of examined patients with untreated juvenile-type diabetes grew, we found considerable variation in their cellular insulin binding. In an attempt to uncover differences between the newly diagnosed diabetics, several clinical and metabolic characteristics were compared. The differences in insulin binding could not be related to differences in the metabolic variables. It was noted, however, that the four patients who had diabetic precoma when first admitted also exhibited the greatest rise in cellular insulin binding.

Based on the cellular insulin binding at the time of admission and the receptor response to insulin treatment the patients could be referred arbitrarily to two groups. Group 1 comprised eight persons who had elevated baseline values of insulin binding. Insulin treatment returned insulin binding to normal. These findings accord with studies of hepatic plasma membranes from insulin-deficient Chinese hamsters and rats and mice, respectively.[9-11] Group 2 comprised five diabetics who were characterized by low cellular insulin binding with no response to insulin treatment, suggesting that some ketotic diabetics may have a defective cellular insulin binding like that of nonketotic diabetics.[1]

Receptor-regulatory mechanisms. Subsequent to the original hypothesis of Gavin et al.[12] that insulin receptors may be subject to negative feedback regulation by the height of the ambient insulin concentration, significant negative correlations between insulin binding and fasting plasma insulin levels have been found in hyperinsulinemic states.[1,31-35] In the study of insulin-treated outpatients, no linear relationship could be shown between insulin binding to monocytes and total plasma insulin. Measurement of free insulin may have shown a better relationship, however. No evidence for a receptor-regulatory influence of insulin was found in the total group of newly diagnosed diabetics.

Merimee et al.[13] have reported increased insulin binding to cultured lymphocytes induced by beta-hydroxybutyrate. In insulin-treated outpatients we found a positive correlation between apparent receptor affinity and the plasma concentration of ketone bodies. In contrast, no relationship could be shown between the same two variables in newly diagnosed diabetics either before or after insulin treatment. Elevated plasma levels of ketone bodies in diabetics reflect decreased cellular action of insulin, and a correlation between apparent receptor affinity and plasma ketone bodies might as well be a mere concomitant of the diabetic disorder.

Both receptor concentration and receptor affinity are probably subject to numerous regulatory influences. Our findings should serve to emphasize that the ambient levels of insulin and ketone bodies under certain biologic conditions might be but two of several factors to affect the insulin receptor.

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