Decreased Insulin Binding of Human Erythrocytes After Dexamethasone or Prednisone Ingestion

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SUMMARY

We have investigated changes in insulin binding in erythrocytes in response to overnight ingestion of 1 mg dexamethasone or 10 mg of prednisone in two groups of eight lean, healthy subjects. Dexamethasone administration reduced insulin binding from 9.6 to 6.8% ($P < 0.001$) with concomitant increase in basal plasma insulin from 10.5 to 14.1 μU/ml ($P < 0.05$). Prednisone ingestion reduced insulin binding from 9.9 to 7.9% ($P < 0.01$), but the increase in basal insulin from 16.9 to 20.6 μU/ml was not significantly different. The decrease in insulin binding with both dexamethasone and prednisone was associated with decreased affinity of erythrocyte for insulin at low occupancy and the increase in the dose of unlabeled insulin resulted in 50% inhibition of specific binding without changes in the number of receptors. The earliest decrease in insulin binding was noted within 2 h after ingestion of 1 mg of dexamethasone. These data suggest that acute alteration of insulin receptor function could occur in erythrocytes by small amounts of dexamethasone or prednisone through a mechanism consistent with a decrease in receptor affinity rather than a decrease in the number of receptors. **DIABETES 29:811–814, October 1980.**

Hypercortisolism is clinically associated with the state of insulin resistance, but the mechanism of this phenomenon has not been fully elucidated. Although in vitro studies of insulin binding in insulin-sensitive cells of experimental animals with hypercortisolism have provided equivocable results,[1–3] which partially depend on the type of steroid used to induce hypercortisolism, very few studies have been conducted with human subjects. After administration of prednisone to man, an increase rather than a decrease in specific binding of ^{125}I-insulin to monocytes has been observed.[4] On the other hand, the infusion of cortisol for 4 h in man had no effect on insulin binding to monocytes.[5] Recently, a report has appeared in the literature on the specific insulin binding receptor in human erythrocytes,[6] which has binding characteristics comparable to insulin receptors in other tissues.[6–9] In the present study, therefore, we have investigated in detail the acute effects of small doses of dexamethasone and prednisone on insulin receptors of erythrocytes in man.

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MATERIALS AND METHODS

Subjects and experimental protocol. Two groups each of eight healthy adults (six males, two females, age range 28–50 yr, 39.3 ± 2.7 yr in dexamethasone group; eight males, age range 25–47 yr, 37.5 ± 3.8 yr in prednisone group) were studied. All subjects were within 10% of their ideal weight (Metropolitan Life Insurance Tables, 1959) and were consuming a weight-maintaining diet. None of the subjects were taking any medications and none had a family history of diabetes. The purpose of this study was explained to all subjects before obtaining their oral consent to participate and subsequent admission to the outpatient department of the Clinical Research Center. Our studies were performed at 8 a.m. after 12 h of fasting before and after an oral dose of 1 mg of dexamethasone or 10 mg of prednisone at 11 p.m. on the preceding night. In the study of the time course of the effect of dexamethasone, the drug was administered at 8 a.m. in two of the subjects after overnight fasting, and blood was then drawn every 2 h for the subsequent 8 h in the state of fast.

Methods. Insulin binding using erythrocytes was measured by a modification of the method of Gambhir.[6] Erythrocytes were separated using Ficoll-Hypaque solution by the method of Boyum[10] and resuspended in buffer containing 50 mM Hepes, 50 mM Tris, 50 mM NaCl, 10 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM glucose, 5 mM KCl, 2 mM EDTA, and 0.1% bovine serum albumin, pH 8.0, in a final red cell concentration of 5×10^9 cells/ml. ^{125}I-insulin was purchased from Cambridge Nuclear Corp., Billerica, Massachusetts

(specific activity 150–180 μCi/μg) and was purified by the method reported previously.[11]

Erythrocytes (2×10^9 cells) were incubated with labeled insulin (80–100 pg) plus various amounts of unlabeled porcine monocomponent insulin (gift of Eli Lilly and Co.) at 15°C for 4 h in a total volume of 0.5 ml. After the incubation, duplicate 200-μl aliquots were transferred into prechilled microfuge tubes containing 200 μl of buffer and 200 μl of dibutylphthalate and centrifuged. Nonspecific binding, defined as the radioactivity associated with erythrocyte pellets in the presence of 1.0×10^5 ng/ml of unlabeled insulin, was subtracted from the total binding to yield specific binding. For the study of dissociation kinetics, 9×10^9 erythrocytes and 400 pg of ^{125}I-insulin were incubated with or without 1.0×10^5 ng of unlabeled insulin in a total volume of 2.0 ml at 15°C for 4 h. After the incubation, erythrocytes were washed twice with the buffer and resuspended to a final volume of 2.0 ml. Duplicate 50-μl aliquots were diluted with 3.0 ml of buffer. These were reincubated at 15°C for 5, 10, 20, 30, 60, and 120 min for measurements of the rate at which the previously bound ^{125}I-insulin dissociates from the cells. The cells were then centrifuged after the addition of 400 μl of dibutylphthalate. After the separation of cell pellet, residual-bound radioactivities were counted. The amount bound at time 0 was estimated as 100%. To investigate degradation of ^{125}I-insulin by erythrocytes, cells were incubated with labeled insulin at time 0 and for 4 h at 15°C. Insulin degradation was calculated by the ratio of radioactivity of the TCA-solubilized material over the total ^{125}I-radioactivity as previously described.[11]

Analyses. Plasma glucose was measured by the glucose oxidase method. Plasma insulin[13] and cortisol[14] were determined by radioimmunoassay. Insulin binding data were analyzed by Scatchard analysis, in which the highest insulin concentration used was 10^2 ng/ml,[15] and the average affinity profile was evaluated by the method of DeMeyts and Roth.[16] Statistical analyses were done by the paired Student's *t* test. All data are expressed as mean ± SEM.

RESULTS

Figure 1 depicts individual values for insulin binding to erythrocytes before and after dexamethasone or prednisone administration. The data show that with dexamethasone administration, specific insulin binding was reduced in all subjects from 9.6 ± 0.6% to 6.8 ± 0.3% ($P < 0.001$), and prednisone reduced insulin binding from 9.9 ± 0.3% to 7.9 ± 0.6% ($P < 0.001$). The percent decrease in specific insulin binding ranged from 18 to 36% (mean, 29%) after dexamethasone and from 7 to 42% (mean, 20%) after prednisone. Although the mean percent decrease in the dexamethasone group was larger than in the prednisone group, there were no significant differences between the two groups of steroid-treated subjects.

Figure 2 presents a competition curve of ^{125}I-insulin binding to erythrocyte by unlabeled insulin (panel A), Scatchard analysis (panel B), and the average affinity profile (panel C) before and after dexamethasone or prednisone administration. Significant changes were noted in the competition curve and average affinity.

As seen in Figure 2B, the abscissa intercept in the Scatchard plot (Ro) has not significantly changed (5.3 ± 0.5 ng before and 5.7 ± 0.8 ng after dexamethasone; 4.9 ± 0.3

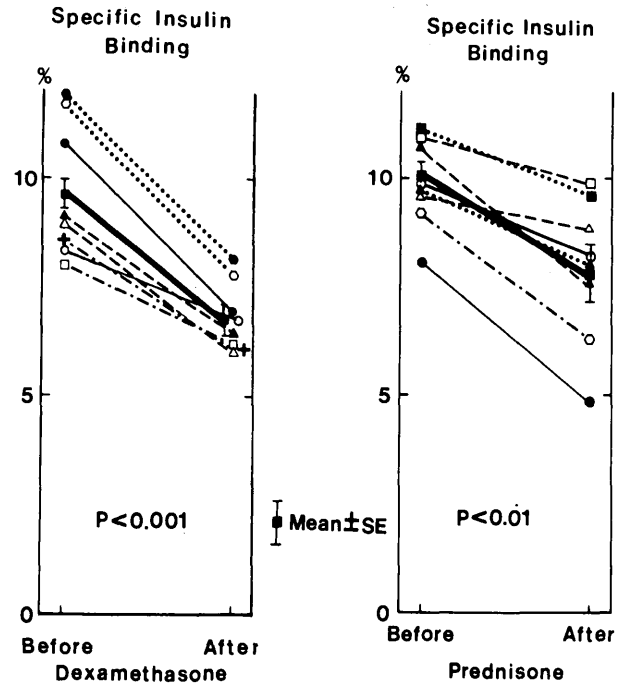

FIGURE 1. Individual values of specific insulin binding to human erythrocytes before and after administration of 1 mg of dexamethasone or 10 mg of prednisone.

before prednisone, 5.2 ± 0.5 after prednisone), but the average affinities both at the low occupancy ($\bar{K}e$) and high occupancy ($\bar{K}f$) have decreased significantly (Table 1). The concentration of insulin necessary to inhibit 50% of the specific bindings was significantly increased after dexamethasone (11.7 ± 1.2 ng/ml versus 27.6 ± 3.3 ng/ml) or prednisone (13.0 ± 1.0 ng/ml versus 21.1 ± 3.4 ng/ml) (Figure 2A and Table 1).

No significant change was noted in the dissociation curve by dexamethasone administration (Figure 3) and, furthermore, no significant differences were noted either in degradation or nonspecific binding between the two studies by erythrocytes. The total amount of insulin degradation at 15°C for 4 h in any condition did not exceed 1%.

The results of the time-course effect of dexamethasone in two subjects and prednisone in one subject are presented in Table 2, where maximum insulin binding decrement was noted at 4 h but was still present after 8 h. These results also indicate that changes in receptor affinity could occur as early as 2 h after glucocorticoid administration. Additional studies have shown no in vitro effects of dexamethasone in concentrations of 1–100 ng/ml or of prednisone in concentrations of 10–1000 ng/ml on insulin binding to erythrocytes (data not shown).

In eight subjects investigated, the reticulocytes, which are known to affect insulin binding to red blood cells,[17] were not changed by dexamethasone or prednisone administration (data not shown).

DISCUSSION

Our results on the effect of dexamethasone or prednisone on insulin binding are consistent with the data obtained from animal experiments,[1,2] although our data show the difference to be noted with smaller doses of steroid. In our study,

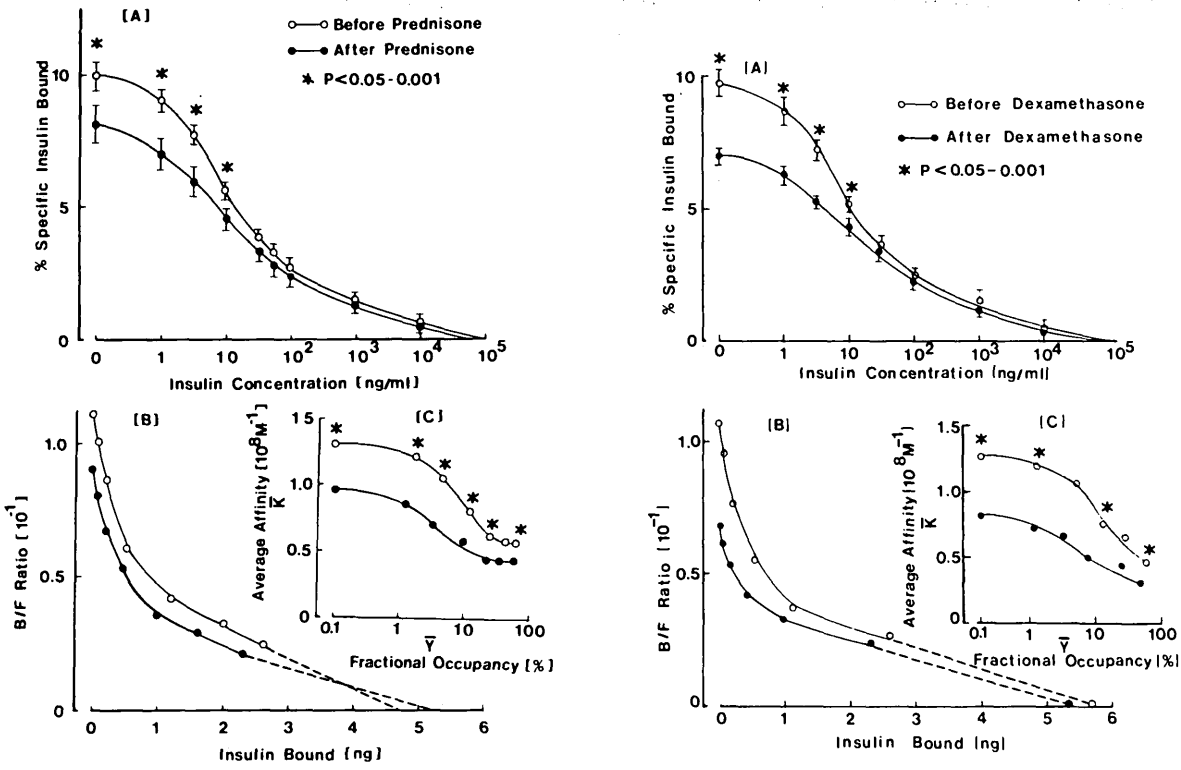

FIGURE 2. Analytic figures of binding studies before (open circle) and after (solid circle) dexamethasone (right) or prednisone (left). The data represent the mean of eight normal subjects. (A) Comparison of the ability of unlabeled insulin to compete with the binding of ^{125}I-insulin to erythrocytes. (B) Scatchard plot of mean values from the above study. Bound to free ratio (B/F ratio) was plotted as a function of the insulin bound. Interception of the curve to the abscissa (Ro, broken line), which is used for calculation of receptor number/cell, was not significantly changed. (C) The average affinity profile obtained by the method of DeMeyts and Roth.[16] Average affinity of receptor ($\bar{K}$) was plotted as a function of receptor occupancy ($\bar{Y}$). Asterisks show significant difference in average affinity.

the effect of dexamethasone appears as early as 2 h after administration and become maximal 4 h after the dose. This might be closely related to the absorption of dexamethasone and the peak level of plasma dexamethasone when orally administered.[18]

Our results are different from the recent report[5] in which no influence of acute infusion of cortisol on insulin binding to monocytes was observed. The cause of this difference remains unknown. Our results also indicate that overnight administration of prednisone decreases insulin binding in erythrocytes. Recent studies by Beck-Nielsen et al.[4] on the effect of prednisone on monocytes of normal subjects indicated increased binding; however, these studies differ from ours in many respects. First, the studies were not conducted on the overnight administration of prednisone, as all patients received the dose at 8 a.m. and were evaluated 3, 7, 24, 48, and 72 h later. It is of interest that the monocyte binding studies in the first 3 h of prednisone were quite variable and only three out of six showed clear increases in insulin binding.[4] Second, we have not investigated the effect of chronic therapy of glucocorticoid on either monocyte or erythrocyte, and, finally, our studies have been limited to erythrocytes.

TABLE 1
Insulin binding to erythrocytes in two groups of eight normal, lean subjects before and after 1 mg of dexamethasone or 10 mg of prednisone (mean ± SEM)

	Dexamethasone		Prednisone	
Number of subjects	8 (2 females and 6 males)		8 (males)	
Parameters measured	Before	After	Before	After
Plasma glucose (mg/dl)	106 ± 4.4	103 ± 4.3	101 ± 0.4	109 ± 4.4
Plasma insulin (μU/ml)	10.5 ± 0.8	14.1 ± 1.6*	16.9 ± 1.7	20.6 ± 2.4
Plasma cortisol (μg%)	14.7 ± 1.1	1.9 ± 0.2‡	17.2 ± 1.6	3.9 ± 0.3‡
Specific insulin binding (%)	9.6 ± 0.6	6.8 ± 0.3‡	9.9 ± 0.3	7.9 ± 0.6†
Receptor number (per cell)	132 ± 12	142 ± 21	127 ± 6	133 ± 12
Average affinity (10^8 M^{-1})				
at low occupancy ($\bar{K}e$)	1.33 ± 0.15	0.82 ± 0.12*	1.32 ± 0.07	0.90 ± 0.09†
at high occupancy ($\bar{K}f$)	0.52 ± 0.08	0.32 ± 0.02*	0.67 ± 0.06	0.44 ± 0.04†
½ inhibiting conc. (ng/ml)	12.3 ± 1.3	28.5 ± 3.7†	12.3 ± 1.0	21.1 ± 3.4*

* $P < 0.05$, † $P < 0.01$, and ‡ $P < 0.001$ when compared before and after treatment in the same group. All other comparative values are not significantly different before and after treatment.

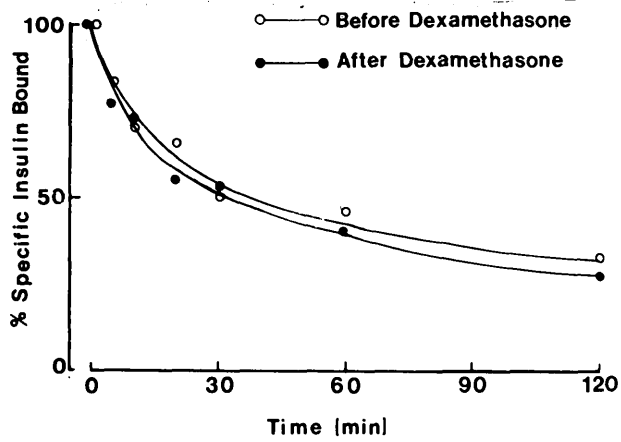

FIGURE 3. Dissociation curve, obtained as described under METHODS, before (open circle) and after (solid circle) dexamethasone.

Although recent studies suggest that monocyte binding parallels insulin binding in erythrocytes,[6-9] the differential effect of various glucocorticoids on insulin binding in various tissues needs to be further investigated.

Lack of alterations in receptor number, the decreased average affinity at low occupancy, and the increased insulin concentration of 50% inhibition of insulin binding in our studies suggest that a decrease in insulin binding could be explained by a decrease in receptor affinity rather than a change in the number of receptors. The decrease in receptor affinity can be due to either a decrease in association rate constant, an increase in dissociation rate constant, or both.[19] The lack of difference on the dissociation curve in our study (Figure 3) supports the possibility that a decrease in affinity by dexamethasone may be due to a decrease in association rate constant. That these effects could not be directly related to the action of dexamethasone or prednisone is indicated by our in vitro studies.

These results suggest that a process of acute regulation of insulin binding to erythrocytes could be a reflection of acute changes in receptor binding affinity. Similar short-term binding alteration by change in affinity in response to changes in insulin level by glucose clamp, in glucose levels by insulin clamp,[20] and alteration with exercise[9,20] have been reported recently. These data, taken together with the present studies, suggest that the erythrocyte may be an appropriate model to study acute changes in insulin receptor function.

It is tempting to postulate that alteration of insulin affinity may be an important means by which the state of insulin resistance could be acutely altered in an in vivo condition, such as increases noted with a prolonged fast of obese individuals or the decrease in binding noted with hyperinsulinemia of glucose challenge or hypercortisolism reported here. The mechanism of this alteration in receptor affinity is not known, but presence of a regulator appears plausible.

TABLE 2
The time course of effect of dexamethasone (subjects 1 and 2) and prednisone (subject 3) on specific insulin binding to human erythrocytes after ingestion of 1 mg of dexamethasone or 10 mg of prednisone*

	Hours after the administration of glucocorticoids				
	0	2	4	6	8
Subject 1	8.8	6.8	6.5	7.1	7.6
Subject 2	8.0	5.8	5.0	7.2	6.6
Subject 3	9.9	8.5	8.2	9.2	7.7

* Values are reported as percent binding.

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