Insulin Resistance in Cushing's Disease

Evaluation by Studies of Insulin Binding to Erythrocytes

BERNARDO L. WAJCHENBERG, CARLOS E. LEME, ANTONIO C. LERARIO, IRACELIA T. TOLEDO E SOUZA, HELENA W. RODBARD, AND DAVID RODBARD

SUMMARY

Studies of ^{125}I-insulin binding to erythrocytes (RBC) from 5 patients with Cushing's disease were performed in an attempt to evaluate the insulin resistance in this disease. Five obese, nondiabetic patients and six normal subjects served as controls. Insulin resistance was present in both the obese, nondiabetic subjects and in the patients with Cushing's disease. Patients with Cushing's disease showed insulin resistance out of proportion to obesity, and of greater severity than in the obese subjects. As in previous studies, the insulin resistance of the obese subjects could be at least partially ascribed to a reduced number of receptors. In contrast, in our patients with Cushing's disease, no physiologically significant changes in the parameters of insulin-receptor interaction could be demonstrated. This suggests that the RBC insulin receptor is not involved in this type of insulin resistance. DIABETES *33*:455–459, May 1984.

Abnormalities of carbohydrate metabolism induced by glucocorticoid hormones are well known,[1] and are consistent with an insulin-resistant state.[2] Since decreased binding of insulin to its specific receptors has been reported in other insulin-resistant states, evaluation of glucocorticoid effects on insulin binding to its target cells has been the subject of considerable interest. Two animal studies demonstrated that glucocorticoid excess induces a decrease in binding of insulin to its receptors.[3,4] However, more recent animal studies suggested that response may be variable: De Pirro et al.[5] showed that dexamethasone decreased while prednisolone increased insulin binding to isolated rat adipocytes in vivo, but no effects on binding were observed in vitro.

In humans, exogenous glucocorticoids also result in variable responses. Prednisone has been reported to increase specific binding of ^{125}I-insulin to monocytes from normal subjects.[6] Dexamethasone and cortisone administration to normal men induced a significant decrease of insulin binding to circulating monocytes.[7] Decreased insulin binding to erythrocytes from normal human subjects has been reported in response to dexamethasone and prednisone ingestion.[8]

Most reported studies designed to evaluate the effects of glucocorticoid excess on insulin binding have been performed in situation of short-term, experimentally induced hypercortisolism. The present report describes studies of the interaction of insulin with its receptors on erythrocytes from patients with Cushing's disease. Since the initial report by Gambhir et al.[9] and the subsequent demonstration that insulin binding to RBC and monocytes changes in parallel in many situations,[10–12] erythrocytes have gained general acceptance as important, easily obtainable, and representative target tissue for studies of insulin binding.

MATERIALS AND METHODS

Five female patients (ages 12–48 yr) with florid clinical presentations of Cushing's disease and without family history of diabetes mellitus were studied. Diagnosis of ACTH-dependent Cushing's syndrome was confirmed by the following laboratory studies (Table 1): (1) elevated 8 a.m. plasma cortisol; (2) increased urinary excretion of 17-hydroxycorticosteroids (17-OHCS); (3) lack of a normal circadian rhythm of plasma cortisol, with elevated levels throughout the day, and no suppression in response to 1 mg dexamethasone given at midnight; (4) responses to larger doses of dexamethasone in the suppression test were inconsistent (50% suppression of urinary 17-OHCS with 2 mg/day and further partial suppression with 8 mg/day were obtained in three subjects [MG, JS, ZBS], while the two remaining patients [FC, RA] showed approximately 50% suppression only with 8 mg/

From the Diabetes and Adrenal Unit, Hospital das Clinicas and Institute of Energetic and Nuclear Research, Sao Paulo, Brazil; and the Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland.
Address reprint requests to Bernardo L. Wajchenberg, M.D., Diabetes and Adrenal Unit, Hospital das Clinicas, Sao Paulo, Brazil.
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TABLE 1
Clinical and laboratory data for five female patients with Cushing's disease

Subject	Age (yr)	Known duration of disease (mo)	Body wt (kg)	Height (m)	Total RBC count ($\times 10^6/\mu l$)	Reticulocytes (%)*	Hb (g/dl)	Plasma cortisol 8 a.m. (μg/dl)†	Urinary 17-OHCS (mg/day)‡	Urinary 17KS (mg/day)§	Plasma cortisol diurnal rhythm (μg/dl)			Dexamethasone suppression test: urinary 17-OHCS (mg/day)			Metyrapone (SU-4885): urinary 17-OHCS (mg/day)		
											8 a.m.	4 p.m.	midnight‖	8 a.m.	Basal	2 mg/day (2nd day)	8 mg/day (2nd day)	Basal	SU-4885	Post-recovery
FC	48	8	78	1.72	4.76	0.9	13.9	33	77	22	29	21	26	32	68	49	43	71	87	117
MG	12	24	44	1.38	5.40	1.0	16.6	24	13	7	24	23	33	25	13	7	5	10	12	37
RA	25	18	92	1.65	4.93	1.1	15.6	29	30	13	34	35	32	33	35	37	17	48	70	100
JS	29	16	75	1.52	4.90	1.0	15.8	20	19	12	22	20	16	25	25	13	7	19	51	59
ZBS	30	24	77	1.58	4.76	1.2	13.7	24	17	13	25	20	18	22	21	9	7	18	19	39

Normal ranges: *0.2–2%; †5–18 μg/dl; ‡4–12 mg/day; and §4–14 mg/day.
‖Dexamethasone (1 mg) given p.o. at midnight.

day); (5) significant responses to metyrapone administration occurred in all patients, as evaluated by increased 24-h urinary 17-OHCS excretion; and (6) the sella turcica was radiologically normal in three patients (MG, RA, ZBS) and slightly enlarged in two (FC, JS).

After completion of these studies, the pituitary fossa was explored by the trans-sphenoidal route and the presence of an adenoma was confirmed in all patients. A group of six normal females (ages 25–35 yr) and another group of five obese, nondiabetic female subjects (ages 23–38 yr) were studied in a similar fashion as controls.

All subjects were maintained on a high carbohydrate diet for 3–5 days before testing. The diet consisted of 50% carbohydrate (300 g), 20% protein, and 30% fat, with a daily caloric intake of 2400 cal/day. An oral glucose tolerance test (OGTT) was performed in all patients, with blood collected at 0, 30, 60, 120, and 180 min after the glucose load (100 g as a 25% solution in water except in the youngest patient, who received 1.75 g of glucose/kg ideal body wt). Blood glucose (Technicon Autoanalyzer, Ferricyanide method) and plasma immunoreactive insulin (IRI)[13] were determined in these samples. A reticulocyte count in peripheral blood was performed for the Cushing's patients.

Insulin binding studies. Blood was collected after an overnight fast on the same day as the OGTT. We employed the method of Gambhir et al.[9] with minor modifications. Nonspecific binding was measured in the presence of 10^5 ng/ml of insulin and subtracted from total binding. Further details are described elsewhere (Wajchenberg, B. L., Lerario, A. C., El-Andere, W., Ohnuma, L. Y., and Toledo e Souza, I. T.: Human erythrocyte insulin receptors in normal male and female subjects. In press. Clin. Endocrinol. 1984). All studies in the control females (normal or obese) were done during the follicular phase of the menstrual cycle. The Cushing's patients were amenorrheic. Results of binding studies were analyzed by displacement curves and Scatchard plots. Conventional statistical methods[14] were used for the analysis of results, employing the BRIGHT statistics package at NIH. Logarithmic transformation of insulin values was used to achieve better normality (Gaussianness) and uniformity of variance. Student's *t* tests were based on equal or unequal variances as appropriate, as determined by objective (F-test) criteria. One-sided *t* tests were used when a priori information was available to predict the direction of an expected difference: this included analysis of glucose and insulin during the OGTT, and the comparison of insulin binding in normal and obese subjects.

RESULTS

Figure 1A shows the relationship between the ratio BW/H = (body weight, kg)/(height, cm) and basal plasma IRI. Pooling data from normal and obese subjects, a significantly positive, linear correlation between these parameters was observed: IRI = $-23.8 + 86.6$ (BW/H); $r = 0.92$, $P < 0.01$.

Figure 1A indicates that Cushing's patients have a disproportionately higher plasma IRI in relation to the ratio BW/H when compared with the data obtained from normal and nondiabetic, obese subjects. This suggests insulin resistance in excess of that expected on the basis of obesity per se.

Table 2 shows the results obtained in the OGTT. With the

exception of fasting values, patients with Cushing's disease showed significant elevations of blood glucose at all times, as compared with normal and obese, nondiabetic subjects ($P < 0.01$ at 120 and 180 min). Mean plasma insulin levels during OGTT were also higher at all times in Cushing's disease in relation to the normal subjects, with statistical significance at $P < 0.01$ for basal values and $P < 0.05$ at 120 and 180 min. Mean insulin values for the patients with Cushing's disease were higher than for the obese, nondiabetic subjects at all times, but they were not statistically significant by *t* tests at individual time points.

When mean serum IRI is plotted versus blood glucose at the corresponding time during the OGTT for all three groups (Figure 1B), it is evident that patients with Cushing's disease have higher blood glucose values than the other two groups, despite very high insulin levels. This again suggests the presence of significant insulin resistance in these subjects. Similarly, the obese subjects display insulin resistance relative to the normal subjects. The Cushing's patients showed a normal proportion of reticulocytes relative to the total RBC count (Table 1); the normal subjects and obese patients showed a reticulocyte count of 0.9 ± 0.3% (mean ± 1 SD).

Figure 2 shows the mean inhibition-competition curves for ^{125}I-insulin binding to erythrocytes for the three study groups. Normal subjects and Cushing's patients demonstrate binding curves that are statistically indistinguishable for any given insulin concentration. Obese subjects have uniformly lower binding curves, with statistical significance ($P < 0.05$) in comparison with Cushing's patients for concentrations below 5 ng/ml of unlabeled insulin. The ID_{50}, i.e., the concentration of unlabeled insulin required to displace half of the ^{125}I-insulin bound, was similar for all three groups (10–14 ng/ml). Note that the mean B/F was higher in the Cushing's patients than either the normals or the obese subjects at each of the nine concentration levels tested. This composite effect is significant at the $P < 0.01$ level.

Figure 2 (inset) shows the Scatchard analysis of the mean data for the three groups. The relationship is curvilinear, and the curves for the three groups are congruent in shape. Although the mean receptor concentration was lower for the

TABLE 2
Glucose tolerance tests and parameters of insulin-receptor interaction for patients with Cushing's disease (C); obese, nondiabetic subjects (O), and normal subjects (N)

	Oral glucose tolerance test (OGTT)												
	Blood glucose (mg/dl)					Plasma IRI (μU/ml)					^{125}I-insulin	Apparent receptors/	Apparent K_e
Time (min)	0	30	60	120	180	0	30	60	120	180	%$(B/F)_0$	cell	10^8 M^{-1}
Cushing's Disease (C)													
FC	93	267	255	183	108	25	120	150	180	87	4.5	62	1.0
MG	67	161	191	175	152	30	500	1000	1250	1500	4.8	32	2.1
RA	72	116	178	163	153	30	68	88	180	140	8.0	49	2.3
JS	76	160	140	178	168	35	120	120	260	390	8.0	36	3.3
ZBS	66	166	180	154	110	35	180	280	300	200	9.1	47	2.8
Mean	74.8	174	188.8	170.6	138.2	31.0	197.6	327.6	434.0	463.0	6.88	42.5	2.3
SEM	4.9	24.9	18.6	5.3	12.2	1.9	77.6	171.2	205.0	264.1	0.93	5.2	0.38
Statistics		*a,b*	*a**	*a**,b*	*a**,b*	*a**			*a*	*a*	b		
Obese, nondiabetic subjects (O)													
DIN	90	120	148	112	74	18	116	200	145	11	4.2	22	2.5
MMM	84	120	170	130	105	29	167	377	310	285	6.5	31	3.0
JAS	76	104	110	74	84	38	270	228	51	64	3.8	35	1.3
RS	89	118	159	129	107	19	108	260	320	148	3.6	18	2.8
CE	82	127	162	106	97	15	96	256	92	60	3.6	51	0.96
Mean	84.7	117.8	149.8	110.2	93.4	23.8	151.4	264.2	183.6	113.6	4.34	31.4	2.11
SEM	2.5	2.5	3.8	10.2	6.3	4.2	32	30.2	55.6	48.1	0.55	5.7	0.41
Statistics		b	*c*	*c*	*c*	c		c	c		c		
Normal subjects (N)													
MLC	78	122	98	82	76	2	90	110	60	40	7.2	37	2.9
JL	81	130	120	85	79	1	100	105	72	45	6.9	58	1.7
MJR	76	102	98	75	72	10	135	126	80	62	6.2	36	2.5
MN	80	112	102	78	76	10	145	148	120	72	4.8	20	4.3
CL	82	110	96	81	82	5	92	98	42	26	4.8	67	1.0
NR	77	105	100	76	70	0.5	106	116	80	142	5.5	31	2.5
Mean	79.0	113.5	102.3	79.5	75.8	5.5	111.3	117.2	75.6	47.8	5.9	41.5	2.48
SEM	1.0	4.3	3.6	1.5	2.0	2.0	9.5	7.3	10.6	6.7	0.42	7.2	0.45
Statistics	c	*a*	*a*,c**	a*,*c*	*a*,c*	*a*,c**		c*	*a*,c	*a*	c		

a, Cushing's patients statistically significantly different from normal subjects at $P < 0.05$ level.
b, Cushing's patients statistically significantly different from obese subjects at $P < 0.05$ level.
c, Normal subjects statistically significantly different from obese subjects at $P < 0.05$ level.
*Indicates significance at $P < 0.01$ level.
Italics denote Student's *t* test with unequal variance model.
All comparisons for OGTT data were one-sided. For insulin binding studies, the comparison of O versus N was one-sided; for C versus N, and C versus O, two-sided.

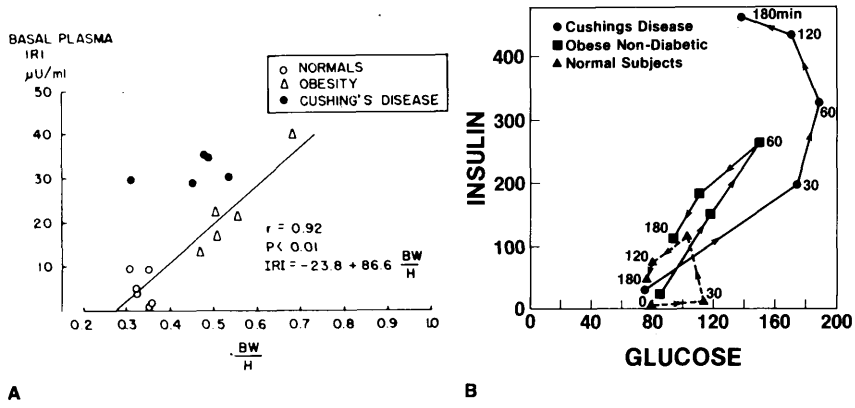

FIGURE 1. (A) Correlation between basal plasma immunoreactive insulin (IRI) and the ratio BW/H, where BW = body weight (kg), and H = height (cm). The regression line was constructed for the normal and obese, nondiabetic subjects. Patients with Cushing's disease have disproportionately higher plasma IRI levels than normal subjects and obese subjects with a corresponding index of obesity. (B) Relationship between mean plasma IRI (ordinate) and mean blood glucose (abscissa) at corresponding times during OGTT for the three groups. Note the insulin resistance of the obese subjects and Cushing's patients relative to controls.

obese subjects in relation to both normal and Cushing's patients, the differences were not statistically significant (Table 2). This is because maximal binding capacity (B_{max}) is an extrapolated value, subject to larger errors than individual measurements. As noted above, tracer binding for the obese subjects was statistically, significantly lower than in the normals or Cushing's patients (Table 2, $P < 0.05$).

Table 2 also shows K_e, the apparent effective affinity when receptors are empty, assuming the presence of a homogeneous class of receptors showing negative cooperativity as postulated by De Meyts. The results are not statistically significant, suggesting that the effective affinity was similar in all three groups.

DISCUSSION

The present results are consistent with the concept that subjects with hypercortisolism are in an insulin-resistant state. In the patients with Cushing's disease, this state is suggested by: (1) elevated levels of basal insulin in comparison with normal controls (Figure 1A, Table 2); and (2) clearly abnormal OGTT despite abnormally high levels of plasma insulin in response to the glucose load (Figure 1B, Table 2). These abnormalities of carbohydrate metabolism are indicative of insulin resistance.[2] The hyperinsulinemic state in our patients could not be entirely attributed to obesity, since they have disproportionally higher levels of plasma IRI relative to the ratio (BW/H) than the obese subjects (Figure 1A). This implies that chronic endogenous glucocorticoid excess can lead to insulin resistance independent of body weight alterations per se. This was confirmed by: (1) the finding of a somewhat reduced decrement in blood glucose during a standard insulin tolerance test (0.1 m/kg) in these five subjects; and (2) a significant reduction in blood glucose clearance (Cl_G) during an insulin clamp study in both of two patients studied in this manner (cases FC, ZBS).

Insulin resistance can result from defects located at a prereceptor level, at the receptor, or subsequent to the hormone-receptor interaction.[15] In obesity, defects have been postulated at both the receptor and postreceptor mechanisms.

In the present study, the obese, nondiabetic subjects and the patients with Cushing's disease have comparable degrees of obesity, and both demonstrate an insulin-resistant state. In the obese, nondiabetic subjects, the insulin resistance can be attributed at least in part to decreased insulin binding, i.e., a reduced $\%(B/F)_0$, and mean B/F for all insulin concentration levels, and a reduced number of receptors. In contrast, the Cushing's patients demonstrate normal binding and negligible alteration in the parameters of hormone-receptor interaction. This was somewhat surprising, since neither the presence of obesity nor the elevated plasma insulin had any apparent effect on the insulin-receptor interaction as evaluated in the RBC. This would imply that the insulin resistance associated with the long-term hypercortisolism of Cushing's disease might be independent from alterations at the receptor level, in contrast to the short-term experiments in which glucocorticoids are exogenously administered and distinct alterations at the receptor level on circulating cells are immediately evident.[6-8] Since tissue sensitivity to insulin is apparently heterogeneous in Cushing's syndrome,[16] it is possible that binding studies using other specific target cells (e.g., adipocyte, muscle cells) may per-

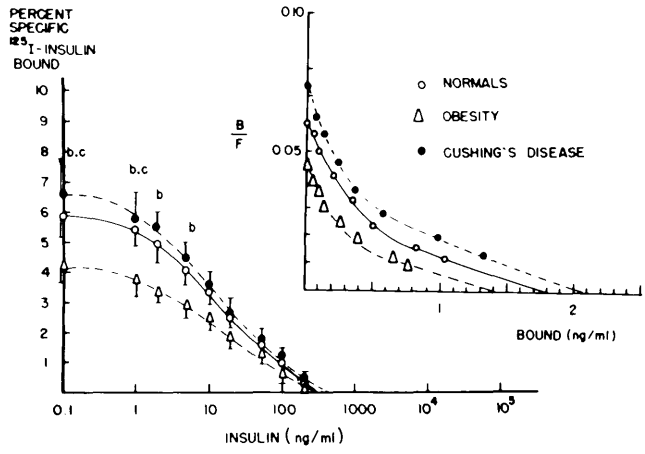

FIGURE 2. ^{125}I-Insulin binding inhibition-competition curves to erythrocytes from Cushing's patients, normals, and obese, nondiabetic subjects. Results expressed for a final cell concentration of 4.5×10^9 RBC/ml. The symbol (b) indicates that mean B/F for Cushing's patients was statistically, significantly different from that of obese subjects ($P < 0.05$). The symbol (c) indicates that normal subjects are statistically different from obese subjects ($P < 0.05$). Inset: Scatchard analysis using mean B/F data. Values for normal subjects and Cushing's patients are not statistically significantly different for individual dose levels, although a consistent effect was seen at all dose levels. Values for obese, nondiabetic subjects are statistically, significantly lower than for the other two groups ($P < 0.01$) when results at all dose levels are considered simultaneously.

mit detection of insulin-resistant tissues with possible receptor defects.

Another possible explanation for our results is suggested from the study by Fantus et al.[17] These authors demonstrated that glucocorticoids increase insulin binding to cultured lymphocytes in vitro, but that in vivo increases in prednisone-induced insulin concentration may counteract this influence. Thus, one possible explanation for the apparently normal binding observed in our patients with Cushing's disease is that hypercortisolism would have increased insulin binding to RBC, but the counteracting influences of obesity and hyperinsulinemia may neutralize this effect.

Finally, we note the recent report by Muggeo et al.[18] of a study similar to the present one. They also found no significant or systematic change in the competition curves, Scatchard plots, initial $\%(B/F)_o$, or ID_{50} for ^{125}I-insulin binding to erythrocytes in patients with long-standing endogenous hypercortisolism. They reported what appeared to be a subtle decrease in binding capacity, coupled with a compensatory increase in average or effective affinity. They observed a large increase in variability of results in patients with endogenous hypercortisolism, with no consistent shift. They also noted a disparity with results for lymphocytes, in which a significant increase in $\%(B/F)_o$ and apparent affinity was observed. Thus, the present studies support the concept that the interaction of two or more effects results in no net change in insulin binding to erythrocyte insulin receptors in patients with endogenous hypercortisolism.

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