Influence of Glucocorticoids on Glucagon Secretion and Plasma Amino Acid Concentrations in Man

JONATHAN K. WISE, ROSA HENDLER, and PHILIP FELIG

From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

ABSTRACT Plasma concentrations of glucagon, insulin, glucose, and individual plasma amino acids were measured in normal nonobese and obese subjects before and after 3 days of dexamethasone treatment (2 mg/day) and in patients with Cushing's syndrome. The subjects were studied in the basal postabsorptive state and following the infusion of alanine (0.15 g/kg) or ingestion of a protein meal.

In nonobese subjects dexamethasone treatment resulted in a 55% increment in basal glucagon levels and in a 60–100% increase in the maximal glucagon response to alanine infusion or protein ingestion. In obese subjects, basal glucagon rose by 110% following dexamethasone, while the response to alanine increased fourfold. In patients with Cushing's syndrome basal glucagon levels were 100% higher and the glucagon response to alanine infusion was 170% greater than in normal controls.

Dexamethasone treatment in normal subjects resulted in a 40% rise in plasma alanine concentration which was directly proportional to the rise in basal glucagon. The remaining 14 amino acids were unchanged. In the patients with Cushing's syndrome alanine levels were 40% higher than in normal controls and were directly proportional to basal glucagon concentrations. No other plasma amino acids were significantly altered in the group with Cushing's syndrome.

It is concluded that (*a*) glucocorticoids increase plasma glucagon concentration in the basal state and in response to protein ingestion or aminogenic stimulation; (*b*) this effect of glucocorticoids occurs in the face of obesity and persists in chronic hypercorticism; (*c*) hyperalaninemia is a characteristic of acute and chronic glucocorticoid excess, and may in turn contribute to steroid-induced hyperglucagonemia; and (*d*) increased alpha cell secretion may be a contributory factor in the gluconeogenic and diabetogenic effects of glucocorticoids.

This work was presented in part at the 65th Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., April 1973 (1973. *J. Clin. Invest.* **52**: 90a [Abstr.]).

Dr. Felig is recipient of a Research Career Development Award (AM 70219) from the National Institutes of Health.

Received for publication 24 May 1973 and in revised form 29 June 1973.

INTRODUCTION

It is well recognized that glucocorticoids increase the production of glucose by the liver (1). This gluconeogenic effect has generally been ascribed to accelerated protein catabolism (2), resulting in augmented availability of precursor amino acids (3). Changes in hepatic gluconeogenic processes (4) and resistance to the action of insulin (5) have also been demonstrated following administration of corticosteroids. Despite the evidence indicating enhancement of gluconeogenesis, information concerning the effects of glucocorticoids on the secretion of glucagon has only recently become available (6). This is of particular interest since a variety of in vitro (7) and in vivo studies (8) indicate that the relative availability of glucagon and insulin determines the net balance of glucose exchange across the liver.

The present study was consequently undertaken to determine the influence of glucocorticoids on alpha cell secretion. This was done by examining the effects of short term (3 days) administration of dexamethasone on plasma glucagon concentration in the basal state and following stimulation with known alpha cell secretogogues: infusion of alanine (9), and ingestion of protein (8). The studies were carried out in normal weight subjects as well as in obese individuals. The latter group was investigated because obesity has recently been demonstrated to have a suppressive influence on alpha cell function (9, 10). The effect of

chronic hypercorticism was also examined by studying glucagon secretion in patients with Cushing's syndrome. Finally, individual plasma amino acid levels were measured to evaluate the effect of corticosteroids on endogenous substrate availability and to determine if hyperaminoacidemia is a contributory factor in glucocorticoid-induced changes in alpha cell secretion.

METHODS

Subjects. Three groups of subjects were studied. The first group consisted of 14 healthy, nonobese volunteers within 10% of ideal body weight (based on Metropolitan Life Insurance Table, 1959), with no history or evidence of diabetes. The subjects ranged in age from 18–36 yr. The second group consisted of five obese subjects with normal glucose tolerance (11), 20–44 yr of age, who were 25–103% (mean±SE 59±16%) above ideal weight (based on Metropolitan Life Insurance Tables, 1959). The third group was composed of four patients (21, 26, 38, and 48 yr of age) with clinical and laboratory evidence of Cushing's syndrome of six months' duration or longer. Three of the subjects subsequently underwent surgical exploration and were demonstrated to have bilateral adrenal hyperplasia. In the fourth subject Cushing's syndrome was iatrogenic in origin, a consequence of long term administration of pharmacological doses of glucocorticoids (prednisone, 40 mg/day) for control of asthma. This subject had not experienced an acute asthmatic exacerbation for one month prior to study. In three of the Cushingoid subjects, glucose tolerance was within normal limits by oral testing (11). The fourth subject (D. G.) was mildly diabetic (fasting blood glucose 110–120 mg/100 ml) and was controlled by dietary measures. The nature, purpose, and possible risks of the study were explained to all of the subjects prior to obtaining their voluntary consent to participate.

Procedures. All subjects received a weight-maintaining diet containing 2,200–3,000 cal, 40% of which was in the form of carbohydrate, for at least 1 wk prior to study, and while receiving dexamethasone. Dexamethasone (Decadron, Merck Sharp & Dohme, West Point, Pa.) was administered to the healthy nonobese and obese subjects in a dose of 0.5 mg every 6 h (2 mg/day) for 3 days. The three subjects with endogenous hypercorticism received no dexamethasone. The patient with iatrogenic Cushing's syndrome continued to take prednisone in a dose of 40 mg/day until the morning of the study.

Alpha cell responsiveness was determined by infusion of alanine or ingestion of a protein meal. The studies were performed in the morning after an overnight (12–14 h) fast prior to institution of dexamethasone treatment, and the same study (either alanine infusion or protein ingestion) was repeated after completion of three days of dexamethasone administration. The subjects with Cushing's syndrome received a single infusion of alanine after an overnight fast. L-alanine (ICN Nutritional Biochemical Div., Cleveland, Ohio) was prepared as a 10% sterile, pyrogen-free solution as described previously (9), and was infused intravenously over 3–4 min in a dose of 0.15 g/kg. Protein in-

TABLE I

*Plasma Glucagon Concentration in the Basal State before and after 3 Days of Dexamethasone (Dex) Treatment in Nonobese and Obese Subjects and in Patients with Cushing's Syndrome**

Nonobese Subjects			Obese Subjects			Cushing's syndrome‡	
Subject	Pre-Dex	Post-Dex	Subject	Pre-Dex	Post-Dex	Subject	Conc.
	pg/ml	*pg/ml*		*pg/ml*	*pg/ml*		*pg/ml*
R. K.	50	100	S. B.	20	65	D. G.	140
J. W.	25	75	H. G.	150	170	M. D.	110
P. D.	50	150	F. K.	60	150	L. M.	185
A. R.	210	420	A. P.	20	200	G. G.	240
M. F.	75	105	D. N.	115	150		
M. T.	90	90					
M. V.	160	250					
J. W.	35	45					
J. H.	80	95					
D. C.	115	135					
R. K.	30	140					
F. L.	90	45					
R. A.	120	120					
L. M.	115	140					
Mean	89	136		73	147		169
±SE	±14	±26		±26	±22		±28
P	<0.01§			<0.05§		<0.025‖	

* Each value represents the mean of two to three determinations in the basal, postabsorptive state.

‡ The patients with Cushing's syndrome did not receive dexamethasone.

§ Significance of difference between pre- and post-dexamethasone values (paired *t* test).

‖ Significance of difference between mean values in Cushing's syndrome and in healthy (nonobese, pre-dexamethasone) subjects (unpaired *t* test).

gestion consisted of a meal of boiled lean beef (3 g/kg of body weight) consumed in its entirety over 30 min. Blood samples were obtained from an indwelling needle in an antecubital vein at 10-min intervals for 20–30 min before the administration of alanine or protein, and at intervals of 10–30 min thereafter for 2 h (alanine infusion) or 4 h (protein ingestion).

Analyses. Plasma glucose was determined by the glucose oxidase technique (12). For the measurement of glucagon, 3 ml of whole blood was added to tubes containing 9 mg EDTA to which 3,000 U of a kallikrein-trypsin inhibitor (Trasylol, FBA Pharmaceuticals, New York) was added in a volume of 0.3 ml. Glucagon was determined in plasma by radioimmunoassay using an antibody (Lot 30 K, supplied by Dr. Roger Unger) which cross reacts minimally with extracts of gastrointestinal tissue (13). The coefficient of variation with this assay system on replicate analysis of quality control samples over a 6 month period was 8%. All plasma samples from a given patient (pre- and post-dexamethasone treatment) were analyzed for glucagon in the same assay. Insulin was measured by radioimmunoassay employing talc to separate bound from free insulin (14). Total alpha amino nitrogen in serum was determined colorimetrically using ninhydrin (15). Individual neutral and acidic amino acids were measured by the automated ion exchange chromatographic method (16) on plasma samples deproteinized with sulfosalicyclic acid (17). Since the plasma samples had been frozen prior to deproteinization, cystine was not measurable (18). Samples for amino acid analysis were available from eight of the nonobese subjects and from the patients with Cushing's syndrome.

For the statistical analyses, the paired *t* test (19) was employed in comparing observations pre- and post-dexamethasone treatment in the healthy nonobese and obese groups, each subject thus serving as his own control. In comparing the group with Cushing's syndrome to the healthy nonobese group, the unpaired *t* test was used (19). Data are presented as the mean±SE.

TABLE II

*Plasma Glucose and Insulin in the Basal State and Maximal Increments (Max Δ) after a Protein Meal or Infusion of Alanine before and after 3 Days of Dexamethasone (Dex) Treatment in Nonobese and Obese Subjects and in Patients with Cushing's Syndrome**

	N‡	Pre-Dex	Post-Dex	P
Nonobese Subjects				
Glucose (mg/100 ml)				
Basal	14	76±2	89±3	<0.005§
Max Δ (protein-meal)	8	2±2	8±3	<0.005§
Max Δ (alanine-infusion)	6	4±1	15±2	<0.002§
Insulin (μU/ml)				
Basal	14	8±1	18±3	<0.002§
Max Δ (protein-meal)	8	18±2	32±4	<0.001§
Max Δ (alanine-infusion)	6	12±2	56±17	<0.02§
Obese Subjects				
Glucose (mg/100 ml)				
Basal	5	76±2	92±5	<0.05§
Max Δ (alanine infusion)	5	4±1	13±2	<0.01§
Insulin (μU/ml)				
Basal	5	23±7	61±20	<0.05§
Max Δ (alanine infusion)	5	40±13	101±14	<0.005§
Cushing's Syndrome				
Glucose (mg/100 ml)				
Basal	4	83±10	—	NS‖
Max Δ (alanine infusion)	4	18±4	—	<0.01‖
Insulin (μU/ml)				
Basal	4	31±10	—	<0.05‖
Max Δ (alanine infusion)	4	95±33	—	<0.05‖

* Values are expressed as mean±SE.
‡ Number of subjects.
§ Significance of difference between pre- and post-dexamethasone values (paired *t* test).
‖ Significance of difference between mean values in Cushing's Syndrome and in healthy subjects (nonobese, pre-dexamethasone).

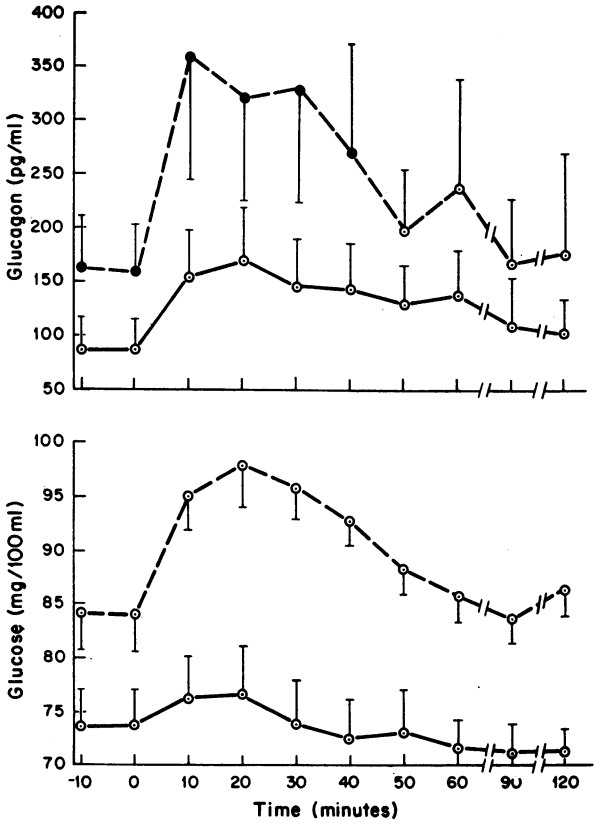

FIGURE 1 Plasma glucagon and glucose concentrations (mean±SE) following the infusion of alanine in six healthy nonobese subjects before (solid lines), and after (broken lines) 3 days of dexamethasone treatment. The solid circles in the upper panel represent those points at which glucagon levels rose to a significantly greater extent after dexamethasone treatment than before ($P < 0.05$, paired *t* test). The specific increments in the glucagon response to alanine after dexamethasone were 202±76 pg/ml at 10 min, 152±70 pg/ml at 20 min, 182±70 pg/ml at 30 min, and 137±57 pg/ml at 40 min.

RESULTS

Basal concentrations of glucagon, glucose, and insulin. In Table I individual plasma glucagon concentrations in the basal state are shown for the nonobese and

TABLE III

Maximal Increments in Plasma Glucagon in Response to a Protein Meal or Infusion of Alanine before and after 3 Days of Dexamethasone (Dex) Treatment in Nonobese and Obese Subjects and in Patients with Cushing's Syndrome

Nonobese						Obese			Cushing's syndrome*	
Protein meal			Alanine infusion			Alanine infusion			Alanine infusion	
Subject	Pre-Dex	Post-Dex	Subject	Pre-Dex	Post-Dex	Subject	Pre-Dex	Post-Dex	Subject	Max Δ
	pg/ml			*pg/ml*			*pg/ml*			*pg/ml*
M. V.	150	180	R. K.	60	120	S. B.	40	110	D. G.	340
J. W.	180	320	J. W.	50	120	H. G.	60	275	M. D.	160
J. H.	110	125	P. D.	115	300	F. K.	75	375	L. M.	420
D. C.	30	140	A. R.	200	400	A. P.	50	400	G. G.	250
R. K.	115	280	M. F.	75	115	D. N.	60	130		
F. L.	20	75	M. T.	110	150					
R. A.	100	85								
L M	200	240								
Mean	113	181		102	201		57	258		293
±SE	±23	±32		±22	±49		±6	±60		±56
P	<0.02‡			<0.02‡			<0.02‡		<0.02‖	

* The patients with Cushing's syndrome received no dexamethasone.

‡ Significance of difference between pre- and post-dexamethasone values (paired *t* test).

‖ Significance of difference between mean values in Cushing's syndrome and in healthy (nonobese, pre-dexamethasone) subjects infused with alanine (unpaired *t* test).

obese subjects before and after dexamethasone administration and for the subjects with Cushing's syndrome. The mean basal levels of glucose and insulin for each of the groups are shown in Table II. Plasma glucagon concentration was higher in 11 of 14 nonobese subjects and in all five obese subjects following dexamethasone treatment (Table I). The mean increment in plasma glucagon was 55% in the nonobese group and 113% for the obese subjects. Mean basal glucagon concentration in the patients with Cushing's syndrome was double the value observed in the untreated controls ($P < 0.025$), and was comparable to the levels demonstrated following dexamethasone administration (Table I). As anticipated (20), a small (10–20 mg/100 ml), but significant increase in plasma glucose, and a two- to threefold increment in basal insulin were demonstrable following dexamethasone treatment (Table II). Basal hyperinsulinemia was also observed in the group with Cushing's syndrome (Table II).

Response to alanine infusion and protein ingestion. The individual maximal increments in plasma glucagon in response to alanine or protein administration are shown in Table III for each of the subject groups. The absolute glucagon and glucose concentrations following the infusion of alanine are shown in Fig. 1 for the nonobese group, in Fig. 2 for the obese subjects, and in Fig. 3 for the group with Cushing's syndrome. In the nonobese group, dexamethasone resulted in a doubling of the mean maximal increment in plasma glucagon following alanine administration and in a 60% increment in the response to protein ingestion (Table III). That the augmented glucagon response was not short-lived is indicated by the fact that after dexamethasone administration, a significantly greater increment in plasma glucagon was observed for 40 min during the postinfusion period (Fig. 1).

In the obese group the effect of dexamethasone treatment on the response to alanine was even more striking. The mean maximal increment in glucagon rose fourfold (Table III), while mean glucagon levels were significantly higher throughout the two hour period of observation (Fig. 2). In the group with Cushing's syndrome, the maximal glucagon increment in response to alanine was 170% higher than in untreated controls and was comparable to the dexamethasone-treated subjects (Table III). Furthermore, the absolute concentration of plasma glucagon after the infusion of alanine remained significantly higher throughout the 2 h period of observation in the group with Cushing's syndrome as compared with untreated controls (Fig. 3).

In each of the subject groups the augmented glucagon response to alanine or protein associated with dexamethasone administration or chronic hypercorticism was accompanied by a greater increment in plasma glucose (Table II, Figs. 1–3). For the entire population of studies performed, a highly significant direct linear correlation was observed between the maximal incre-

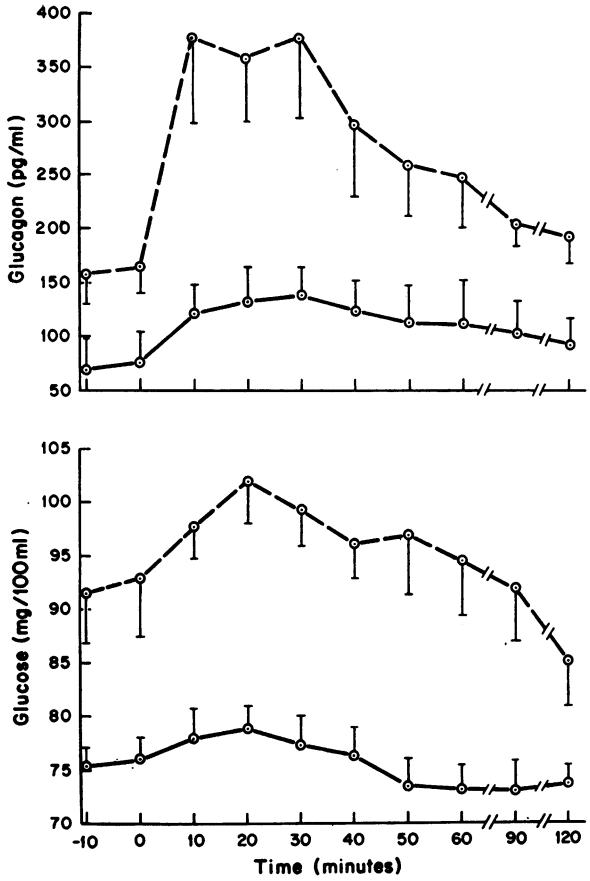

FIGURE 2 Plasma glucagon and glucose concentrations (mean±SE) following the infusion of alanine in five obese subjects before (solid lines), and after (broken lines) 3 days of dexamethasone treatment. The glucagon levels were significantly higher after dexamethasone at all points shown ($P < 0.02$ at -10, 0, 10, 20, 30, and 90 min; $P < 0.05$ at 40, 50, 60, and 120 min; paired t test).

ment in glucose and glucagon following alanine infusion ($r = 0.76$, $P < 0.01$).

The plasma insulin response to alanine and protein was also two- to fourfold greater following dexamethasone treatment and was elevated in the group with Cushing's syndrome (Table II).

Plasma amino acid concentrations. In Table IV, the basal concentrations of 15 individual neutral and acidic amino acids in the nonobese group are shown before and after dexamethasone treatment. A 40% increment was observed in plasma alanine after 3 days of dexamethasone administration ($P < 0.01$). In marked contrast, no significant changes were observed in the remaining 14 amino acids measured. To evaluate the possible role of endogenous alanine as a glucagon secretogogue, the relation between the changes induced by dexamethasone in plasma alanine and plasma glucagon in the basal state were examined. As shown in Fig. 4, a significant direct linear correlation was observed ($r = 0.75$, $P < 0.05$).

Plasma amino acid concentrations in the patients with Cushing's syndrome are also shown in Table IV. As indicated, plasma alanine concentration in patients with chronic hypercorticism was 40% higher than in healthy subjects studied before dexamethasone treatment ($P < 0.01$), and was comparable to the value observed in the normal subjects after dexamethasone administration. In contrast, no significant differences were observed between the Cushing group and normal subjects with respect to any of the remaining 14 amino acids measured. Despite the small number of subjects in the group with Cushing's syndrome, a striking relationship was observed between endogenous alanine and glucagon concentrations. As shown in Fig. 5, a highly significant direct linear correlation was observed be-

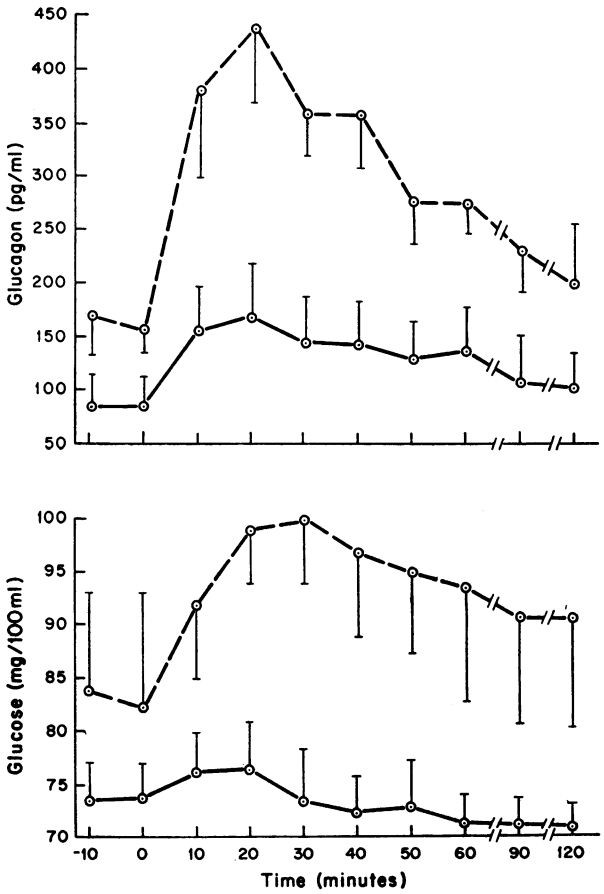

FIGURE 3 Plasma glucagon and glucose concentrations (mean±SE) following the infusion of alanine in four patients with Cushing's syndrome (broken lines) and in six healthy nonobese controls (solid lines). The glucagon levels were significantly higher in the group with Cushing's syndrome at all points except 90 and 120 min ($P < 0.05$ at -10, 0, 10, 50, and 60 min; $P < 0.02$ at 20, 30, 40 min; unpaired t test).

TABLE IV

*Basal Plasma Amino Acid Concentrations in Normal Subjects before and after Dexamethasone (Dex) Administration and in Patients with Cushing's Syndrome**

	Normal subjects‡			Cushing's syndrome	
	Pre-Dex	Post-Dex	P§	Conc.	P‖
	μmol/liter	*μmol/liter*		*μmol/liter*	
Taurine	41±3	36±3	NS	48±16	NS
Aspartic acid	19±4	23±4	NS	20±3	NS
Threonine	164±13	172±11	NS	134±23	NS
Serine	163±9	154±5	NS	137±10	NS
Proline	213±19	288±29	NS	230±54	NS
Citrulline	34±2	33±3	NS	30±2	NS
Glycine	235±18	236±19	NS	213±34	NS
Alanine	347±26	482±41	<0.01	488±31	<0.01
α-aminobutyric acid	22±4	30±5	NS	23±2	NS
Valine	206±8	210±11	NS	331±85	NS
Methionine	28±2	36±4	NS	27±4	NS
Isoleucine	67±3	68±5	NS	68±4	NS
Leucine	121±7	126±8	NS	147±15	NS
Tyrosine	56±5	64±6	NS	62±5	NS
Phenylalanine	60±3	67±6	NS	76±7	NS

* Data are presented as the mean±SE.

‡ The normal subjects were eight nonobese individuals.

§ Significance of difference between pre- and post-dexamethasone values (paired *t* test).

‖ Significance of difference between mean values in patients with Cushing's syndrome and in untreated (pre-dexamethasone) normal subjects (unpaired *t* test).

tween basal concentration of plasma alanine and plasma glucagon ($r = 0.97$, $P < 0.01$).

In Table V, plasma alanine concentrations following the infusion of this amino acid are shown for the nonobese subjects before and after dexamethasone treatment. No significant differences were noted at any of the times examined. Thus the augmented glucagon response to alanine infusion induced by dexamethasone could not be attributed to higher ambient alanine concentrations during the postinfusion period. Similarly, the mean maximal increment in total alpha amino nitrogen concentration following protein ingestion was not affected by dexamethasone treatment (2.4±0.2 μmol/liter, pre-dexamethasone; 2.3±0.2 μmol/liter post-dexamethasone.

DISCUSSION

The present data provide evidence that glucocorticoids increase glucagon secretion in man. An elevation in plasma glucagon concentration was demonstrable following short-term (3 day) administration of dexamethasone as well as in association with chronic endogenous or iatrogenic hypercorticism. In a recent study, Marco et al. reported an increased alpha cell response to infusion of arginine in patients treated with prednisolone, but noted only a minimal rise in basal glucagon levels (6). The current findings indicate that glucocorticoids increase plasma glucagon concentration

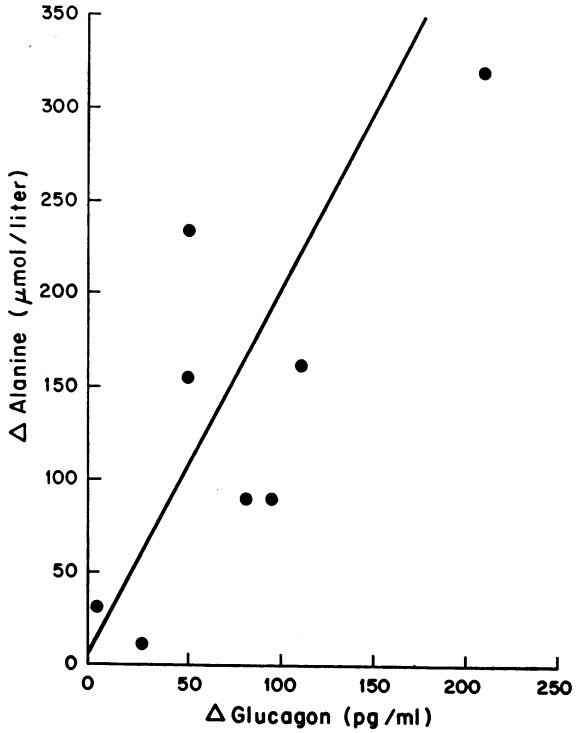

FIGURE 4 Relation between changes in the basal concentrations of alanine and glucagon following dexamethasone administration in healthy, nonobese subjects; $r = 0.75$, $P < 0.05$.

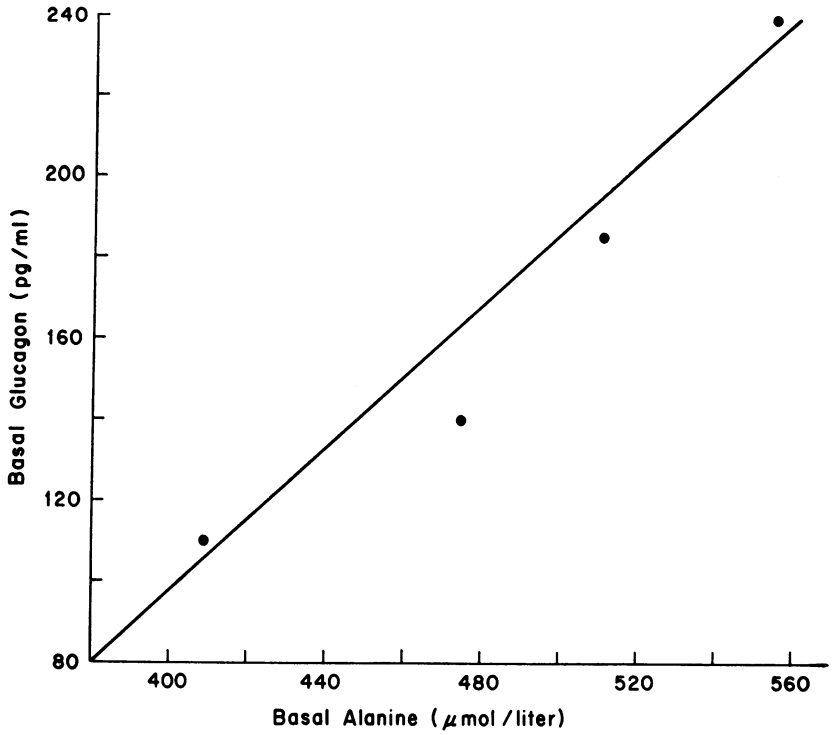

FIGURE 5 Relation between basal concentrations of plasma alanine and glucagon in patients with Cushing's syndrome; $r = 0.97$, $P < 0.01$.

in the basal state as well as in response to aminogenic stimulation by infusion of alanine. Furthermore, the results also demonstrate augmented alpha cell secretion in response to ingestion of a protein meal (Table III). The significance of the latter observation is underscored by the recent suggestion that protein ingestion may constitute the most important physiologic stimulus for glucagon secretion (21, 22).

The response to glucocorticoids in obese subjects was of particular interest since truncal, and often generalized obesity is a characteristic finding in chronic hypercorticism (23). In addition, obesity per se has recently been demonstrated to have a suppressive effect on glucagon secretion in response to starvation (10, 24) or infusion of alanine (9, 10). Furthermore, even in the face of overt diabetes, a blunted alpha cell response has been noted in obese subjects (9, 25). The present data indicate that obesity does not interfere with the enhancement of glucagon secretion induced by glucocorticoids. In fact, the rise in basal as well as alanine-stimulated glucagon levels following dexamethasone administration was even more marked in obese than in nonobese subjects (Tables I and III).

TABLE V

*Plasma Alanine Concentrations after the Infusion of Alanine in Nonobese Subjects**

Time	Plasma alanine	
	Pre-dexamethasone	Post-dexamethasone
min	*μmol/liter*	
10	4,275±190	4,520±369
20	2,470±110	2,787±292
30	1,980±120	1,856±231
60	1,066±81	986±52

* Data are presented as the mean±SE.

The observations on the effects of short term administration of dexamethasone and chronic hypercorticism on plasma amino acid levels are of interest both with respect to the mechanism of hyperglucagonemia as well as in regard to the changes in gluconeogenic substrate availability induced by glucocorticoids. Although the protein catabolic effect of corticosteroids has been well documented (2), and an augmented rise in total alpha amino nitrogen has been demonstrated in eviscerated animals (3), little information is available on the effect of glucocorticoids on individual plasma amino acid concentrations in man. Considering the important role of alanine as the key endogenous gluconeogenic substrate (26, 27), one might anticipate that adrenal steroids, in increasing availability of glu-

cose precursors, would induce an elevation in plasma alanine levels. However, Zinneman, Johnson, and Seal reported no significant changes in the concentrations of 19 plasma amino acids measured in four subjects given 100 mg of cortisol daily for 5 days (28). Nevertheless, examination of their data reveals higher mean plasma levels during cortisol treatment only in the case of alanine, at each of three time periods examined (24 h, 1–3 days, and 3–5 days). In acute experiments, a single dose of glucocorticoid has been observed to increase plasma alanine concentration in children (29) and in rats (30). The present data demonstrate a specific elevation in plasma alanine after 3 days of dexamethasone treatment. Furthermore, hyperalaninemia of comparable magnitude was demonstrable in association with chronic hypercorticism. These data thus further underscore the unique role of alanine as an endogenous gluconeogenic substrate (26, 27) and provide direct evidence of glucocorticoid-mediated changes in circulating glucose precursors in intact man. The mechanism whereby glucocorticoids bring about an elevation in alanine levels may be related to their ability to raise pyruvate concentration (31). A similar explanation has been advanced to account for exercise-induced hyperalaninemia (32). Such a formulation is supported by the direct linear relationship between alanine and pyruvate levels in intact man (32).

With respect to the mechanism of glucocorticoid-induced hyperglucagonemia, alanine has been demonstrated to be a potent stimulus of glucagon secretion (9, 33). On that basis, hyperalaninemia has been suggested as the possible mediator of augmented glucagon secretion in exercise (34). In addition, in dogs increased glucagon secretion has been demonstrated to accompany a 30–50% increment in plasma alanine (33), an elevation comparable to that observed in the present study. Furthermore, in the current investigation a direct linear correlation was observed between the rise in alanine and the increase in plasma glucagon in dexamethasone-treated subjects (Fig. 4), and between basal alanine and glucagon levels in patients with chronic hypercorticism (Fig. 5). These data thus raise the possibility that hyperalaninemia may be a contributory factor in glucocorticoid-induced hyperglucagonemia. While this explanation may account for the elevation in basal glucagon levels, it should be noted that the rise in alanine following the infusion of this amino acid was no higher after dexamethasone treatment than before (Table V). Thus an increase in the responsiveness of the alpha cell to marked elevations in plasma alanine appears to be a contributing factor to the hypersecretory state brought about by dexamethasone. Interference by glucocorticoids with the uptake of glucose by the alpha cell, an effect analogous to the insulin antagonism observed in other tissues (5), has been suggested as a possible mechanism for the increase in reactivity to aminogenic stimulation (6).

With regard to the implications of hyperglucagonemia, an increased concentration of glucagon would be expected to enhance hepatic glucose output. Moreover, the interaction of corticosteroids and glucagon is such that adrenal steroids markedly enhance the gluconeogenic response to glucagon (35). Thus in addition to augmented availability of aminogenic substrate and possible direct effects on hepatic gluconeogenic processes (4), hyperglucagonemia constitutes an additional mechanism whereby glucocorticoids augment glucose production and exert a diabetogenic effect. This mechanism appears to be of importance in acute as well as chronic hypercorticism.

Finally, it has been stressed that glucocorticoids are far more effective in increasing gluconeogenesis in the intact organism as compared to their in vitro action when added to the isolated liver (1). In fact, addition of dexamethasone to perfused liver obtained from adrenalectomized animals fails to increase basal glucose production (35). This discrepancy between in vivo and in vitro effectiveness in enhancing glucose production has been ascribed to accelerated amino acid mobilization induced by glucocorticoids in the intact organism (1). An additional explanation however, may be provided by the augmentation in glucagon secretion, an effect which would not apply to the isolated liver.

ACKNOWLEDGMENTS

We thank Marion Holmes, Kathy Chu, and Richard Korszun for their expert technical assistance and the nurses and staff of the Clinical Research Center for their efforts in caring for our patients.

This work was supported in part by U. S. Public Health Service Grants AM 13526 and RR 00125.

REFERENCES

1. Cahill, G. F., Jr. 1971. Action of adrenal cortical steroids on carbohydrate metabolism. *In* The Human Adrenal Cortex. N. P. Christy, editor. Harper & Row, Publishers, New York. 205.
2. Long, C. N. H., B. Katzin, and E. G. Fry. 1940. Adrenal cortex and carbohydrate metabolism. *Endocrinology.* **26:** 309.
3. Smith, O. K., and C. N. H. Long. 1967. Effect of cortisol on the plasma amino nitrogen of eviscerated adrenalectomized-diabetic rats. *Endocrinology.* **80:** 561.
4. Eisenstein, A. B., S. Spencer, S. Flatness, and A. Brodsky. 1966. Carbohydrate synthesis in the isolated perfused rat liver: role of the adrenal cortex. *Endocrinology.* **79:** 182.
5. Munck, A. 1971. Glucocorticoid inhibition of glucose uptake by peripheral tissues: old and new evidence, molecular mechanism, and physiological significance. *Perspect. Biol. Med.* **14:** 265.
6. Marco, J., C. Calle, D. Roman, M. Diaz-Fierros, M. Villanueva, and I. Valverde. 1973. Hyperglucagonism

induced by glucocorticoid treatment in man. *N. Engl. J. Med.* **288**: 128.
7. Mackrell, D. J., and J. E. Sokal. 1969. Antagonism between the effects of insulin and glucagon upon the isolated liver. *Diabetes.* **18**: 724.
8. Unger, R. H. 1971. Glucagon physiology and pathophysiology. *N. Engl. J. Med.* **285**: 443.
9. Wise, J. K., R. Hendler, and P. Felig. 1973. Evaluation of alpha cell function by infusion of alanine in normal, diabetic and obese subjects. *N. Engl. J. Med.* **288**: 487.
10. Wise, J. K., R. Hendler, and P. Felig. 1972. Obesity: evidence of decreased secretion of glucagon. *Sci. (Wash. D. C.).* **178**: 513.
11. Seltzer, H. S. 1971. Oral glucose tolerance tests. *In* Diabetes Mellitus: Diagnosis and Treatment. S. S. Fajans and K. E. Sussman, editors. American Diabetes Association, New York. **3**: 101.
12. Huggett, A. S. G., and D. A. Nixon. 1957. Use of glucose oxidase, peroxidase, and o-dianisidine in determination of blood and urinary glucose. *Lancet.* **2**: 368.
13. Aguilar-Parada, E., A. M. Eisentraut, and R. H. Unger. 1969. Pancreatic glucagon secretion in normal and diabetic subjects. *Am. J. Med. Sci.* **257**: 415.
14. Rosselin, G., R. Assan, R. S. Yalow, and S. Berson. 1966. Separation of antibody-bound and unbound peptide hormone labelled with iodine-131 by talcum powder and precipitated silica. *Nature (Lond.).* **212**: 355.
15. Moore, S., and W. H. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.* **211**: 907.
16. Spackman, D. H., W. H. Stein, and S. Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **30**: 1190.
17. Block, W. D., M. E. Markovs, and B. F. Steele. 1966. Comparison between free amino acid levels in plasma deproteinated with picric acid and with sulfosalycylic acid. *Proc. Soc. Exp. Biol. Med.* **122**: 1089.
18. Stein, W. H., and S. Moore. 1954. The free amino acids of human blood plasma. *J. Biol. Chem.* **21**: 915.
19. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State University Press, Ames. 6th edition.
20. Perley, M., and D. M. Kipnis. 1966. Effect of glucocorticoids on plasma insulin. *N. Engl. J. Med.* **274**: 1237.
21. Cahill, G. F., Jr. 1973. Glucagon. *N. Engl. J. Med.* **288**: 157. (Editorial)
22. Unger, R. H., A. Ohneda, E. Aguilar-Parada, and A. M. Eisentraut. 1969. The role of aminogenic glucagon secretion in blood glucose homeostasis. *J. Clin. Invest.* **48**: 810.
23. Hollifield, G. 1968. Glucocorticoid-induced obesity—a model and a challenge. *Am. J. Clin. Nutr.* **21**: 1471.
24. Floyd, J. C., Jr., S. Pek, S. S. Fajans, D. E. Schteingart, and J. W. Conn. 1972. Effect upon plasma glucagon of severe and prolonged restriction of food intake in obese and non-obese subjects. *Diabetes.* **21**: 331. (Abstr.)
25. Gerich, J. E., M. Langlois, V. S. Schneider, S. Dippe, G. M. Gustafson, J. H. Karam, and P. H. Forsham. 1973. Abnormalities of glucagon secretion in diabetic syndromes: influence of obesity and response to oral glucose tolerance test. *Clin. Res.* **21**: 201. (Abstr.)
26. Felig, P., T. Pozefsky, E. Marliss, and G. F. Cahill, Jr. 1970. Alanine: key role in gluconeogenesis. *Science (Wash. D. C.).* **167**: 1003.
27. Felig, P. 1973. The glucose-alanine cycle. *Metab. (Clin. Exp.).* **22**: 179.
28. Zinneman, H. H., J. J. Johnson, and U. S. Seal. 1963. Effect of short-term therapy with cortisol on the urinary excretion of free amino acids. *J. Clin. Endocrinol.* **23**: 996.
29. Pagliara, A. S., I. E. Karl, D. C. DeVivo, R. D. Feigin, and D. M. Kipnis. 1972. Hypoalaninemia: a concomitant of ketotic hypoglycemia. *J. Clin. Invest.* **51**: 1440.
30. Ryan, W. L., and M. J. Carver. 1963. Immediate and prolonged effects of hydrocortisone on the free amino acids of rat skeletal muscle. *Proc. Soc. Exp. Biol. Med.* **114**: 816.
31. Hennes, A. R., B. L. Wajchenberg, S. S. Fajans, and J. W. Conn. 1957. The effect of adrenal steroids on blood levels of pyruvic and alpha-ketoglutaric acids in normal subjects. *Metab. (Clin. Exp.).* **6**: 339.
32. Felig, P., and J. Wahren. 1971. Amino acid metabolism in exercising man. *J. Clin. Invest.* **50**: 2703.
33. Muller, W. A., G. R. Faloona, and R. H. Unger. 1971. The effect of alanine on glucagon secretion. *J. Clin. Invest.* **50**: 2215.
34. Felig, P., J. Wahren, R. Hendler, and G. Ahlborg. 1972. Plasma glucagon levels in exercising man. *N. Engl. J. Med.* **287**: 184.
35. Exton, J. H., N. Friedmann, E. H. Wong, J. P. Brineaux, J. D. Corbin, and C. R. Park. 1972. Interaction of glucocorticoids with glucagon and epinephrine in the control of gluconeogenesis and glycogenolysis in liver and lipolysis in adipose tissue. *J. Biol. Chem.* **247**: 3579.