Adrenergic Regulation of Insulin Secretion During Fasting in Normal Subjects

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SUMMARY

It is well known that a prolonged period of fasting produces, in normal subjects, a "diabetic-like" condition manifested by glucose intolerance and diminished insulin secretion. Administration of the alpha adrenergic blocking agent, phentolamine (Regitine), to healthy fasting subjects caused hyperinsulinemia in response to an intravenous glucose load without amelioration of glucose intolerance. These observations indicate that (1) reversal of hypoinsulinemia does not acutely alter those factors which cause fasting glucose intolerance, and (2) the increase in sympathetic tone observed during starvation is responsible for the suppression of insulin release. The latter suggests that the autonomic nervous system plays an important physiological role in the regulation of the glucose-insulin axis. DIABETES *19*:688-93, October, 1970.

Glucose is generally held to be the prime stimulant of insulin secretion in man. An infusion of glucose to normal subjects causes a prompt rise in the serum insulin concentration followed by a gradual decline as plasma glucose concentration falls. After a prolonged period of fasting by healthy subjects, the normal glucose-insulin relation is altered. The rapid rise in serum insulin levels following glucose administration is significantly diminished, and the concentration of insulin remains low despite persistent hyperglycemia.[1] Decreased insulin synthesis is not a likely explanation for this phenomenon, since recent animal studies have shown that pancreatic insulin stores are not depleted during a similar period of starvation.[2,3]

Porte et al.[4] have shown that catecholamines inhibit insulin secretion and that this inhibition is prevented by alpha adrenergic blocking agents. It is well known that acute hypoglycemia causes the release of epinephrine from the adrenal medulla.[5] It is not generally appreciated however, that starvation may be associated with a rise in urinary excretion of catecholamines.[6] In view of this finding, it appears likely that adrenergic suppression is responsible for the hypoinsulinemia of starvation.

The object of the present experiment was to study the role of catecholamines in insulin secretion in fasting subjects by the use of the alpha adrenergic blocking agent phentolamine.

METHODS

Fourteen nonobese (within 10 per cent of ideal body weight according to Metropolitan Life Insurance tables) male volunteers, between the ages of twenty-one and thirty-one were selected from the Schools of Medicine and Public Health of The Johns Hopkins University. All subjects were in excellent health and had no family history of diabetes mellitus or other endocrinopathy. Subjects were hospitalized for study in the Clinical Research Unit of The Johns Hopkins Hospital. Each subject received an intravenous glucose tolerance test (0.5 gm./kg. in 3 min.) following an overnight fast of 12 hrs. A soft catheter was placed percutaneously into a forearm vein. At least two basal blood samples were drawn and samples were drawn intermittently for three hours following administration of glucose. Blood samples were quickly chilled and allowed to clot. Serum was collected and stored at —20° C. Following the glucose tolerance test each subject received a standard breakfast and for the subsequent sixty-eight hours intake was limited to water and decaffeinated coffee. Normal physical activity was not restricted. Twenty-four-hour urine samples, collected from nine subjects throughout the course of the experiment, were acidified and stored in brown glass bottles for catecholamine determina-

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TABLE 1

Metabolic alterations observed in seventeen normal subjects during starvation

Serum:	Days of fasting 0	1	2	3
Glucose, mg./100 ml.	98 ±3	83 ±3	76 ± 3	70 ± 2
Free fatty acids, mmoles/L.	0.98± .08	1.19± .08	1.58± .10	1.75± .11
Uric acid, mg./100 ml.	4.9 ± .3	6.1 ± .4	7.7 ± .5	9.2 ± 1.8
Growth hormone, mμg./ml.	1.0 ± .1	1.9 ± .8	9.1 ± 2.3	4.3 ± 1.3
Insulin, μU./ml.	17 ±3	15 ±2	15 ± 2	14 ± 2
Urinary catecholamines, μg./24 hr.*	72.8 ±6.8	92.8 ±9.0	134.9 ±14.8	178.8 ±16.4

*Nine subjects.
Values are means ± S.E.M.

tions. Basal blood samples were obtained each morning at 7 a.m. throughout the course of the fast.

At the conclusion of the fast each subject was randomly assigned to either an experimental or control group. Three additional nonobese apparently normal subjects who followed an identical protocol were subsequently added to the control group. Intravenous glucose tolerance tests were performed as described above except that the nine subjects chosen as experimentals received an intravenous infusion of phentolamine (5 mg. stat + 0.5 mg./min. for 210 min.), while the eight subjects chosen as controls received half normal saline. Infusions of phentolamine or half normal saline were begun thirty minutes before the initiation of the glucose tolerance test. Serum glucose was measured by the AutoAnalyzer ferricyanide method;[7] several determinations using the glucose oxidase method[8] were in close agreement. Immunoreactive insulin was measured by a modification of the technic of Yalow and Berson[9] and serum growth hormone by the method of Glick et al.[10]

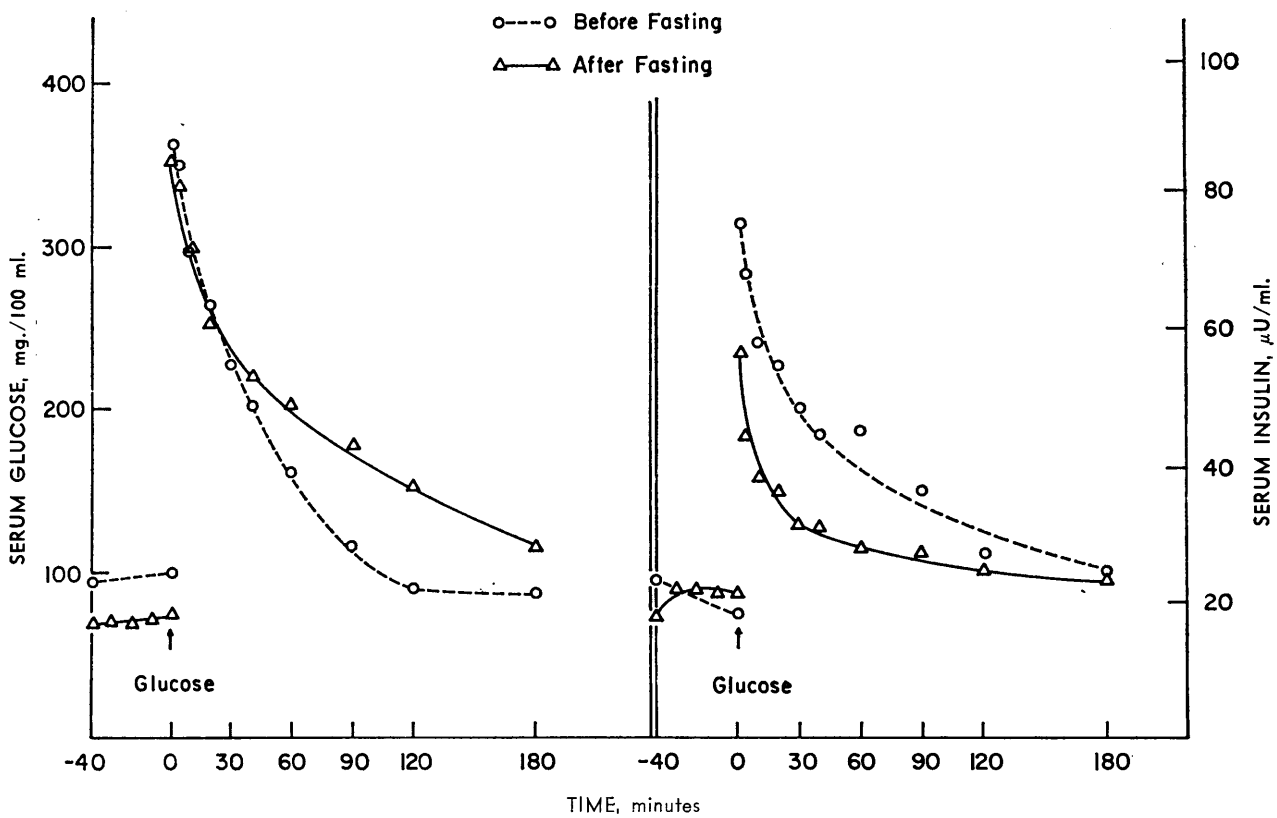

FIG. 1. Intravenous glucose tolerance tests in control subjects before and after starvation. For statistical analysis see table 2.

Free fatty acid was measured by extraction and titration,[11] and uric acid was determined by the Cupric-Neocuproine method.[12] Twenty-four-hour total urinary catecholamines were measured fluorimetrically by the method of Sobel and Henry.[13]

RESULTS

The mean weight loss after three days of starvation was 3.9 kg. (S.E.M. ± 0.2). Most subjects had small amounts of urinary ketones after the first day and large ketonuria after the third day. Serum sodium, potassium and chloride levels were unchanged.

Characteristic metabolic changes of starvation were observed in all subjects (table 1). The serum glucose concentration declined throughout the course of the fast, while the concentrations of growth hormone, free fatty acids, and uric acid increased. The urinary excretion of catecholamines rose significantly by the second day in the nine subjects in whom it was measured. Eight-hour (4:00 a.m. to 12:00 noon) urine collections were obtained on Day 0 and Day 3 from the remaining eight subjects and revealed a mean rise from 3.2 to 7.1 μg./hr. Although not reflected by the mean values (table 1), twelve out of seventeen subjects had a decline in the concentration of serum insulin over the course of the fast. This decline of serum insulin was not statistically significant ($p = .10$).

Figure 1 compares the serum concentrations of glucose and insulin after intravenous administration of a glucose load to the control group. Starvation resulted in a "diabetic-like" glucose tolerance curve in these subjects and a substantial decrease in the insulin response. Delta glucose, the total area under the glucose curve minus the area contributed by the basal glucose concentration, was significantly increased ($p < .001$). As shown in table 2, the disappearance rate of glucose (k) decreased from 1.23 to 0.54 per cent/min. The lower limit of k in normal individuals is generally accepted to be between 0.9 and 1.1 per cent/min.[14] Seltzer et al.[15] have used delta insulin, expressed as μU./ml. × min., as a quantitative measure of beta cell secretion. Following starvation, the control subjects had a 45 per cent mean decline in delta insulin ($p < .03$).

Figure 2 shows the concentrations of serum glucose and insulin after administration of glucose to the experimental subjects. This group also had a mean "diabetic-like" glucose tolerance curve after starvation. The mean changes of delta glucose and glucose disappearance rate (table 2) did not differ significantly from those of the control group. However, delta insulin increased after starvation ($p < .03$). In eight out of nine of the experimental subjects there was an increase in insulin secretion. In the remaining subject insulin secretion was diminished by 20 per cent following the three-day fast. The difference in the mean value of delta insulin between the experimental and control group before the fast reflects the variability of insulin secretion among individuals. This variability is not of consequence in this study since each subject served as his own control, and analysis of the data in table 2 utilizes only the change in insulin area observed in each individual after fasting. When the mean change of delta insulin in the experimental group after fasting (2,149 ± 802 μU./ml. × min.) is compared to that of the control group (—1,325 ± 458), it is clear that phentolamine increases insulin secretion in response to glucose infusion after starvation ($p < .01$).

To evaluate the possibility that phentolamine affects

TABLE 2

Analysis of the intravenous glucose tolerance tests

Control group	Before starvation	After starvation	Mean change	p*
Delta glucose,† mg./100 ml.× min.	7,646±1,022	20,058±6,142	12,412±1,900	<.001
k (glucose disappearance), %/min.	1.23± .10	0.54± .02	− .69± .09	<.001
Delta insulin,† μU./ml. × min.	2,951± 544	1,626± 595	−1,325± 458	<.03
Experimental group (phentolamine)				
Delta glucose	5,502±1,069	18,169±2,869	12,669±2,631	<.001
k	1.95± .34	0.61± .03	− 1.34± .27	<.001
Delta insulin	1,807± 352	3,956± 829	2,149± 802	<.03

Values are mean ± S.E.M.

*Statistical analysis was performed by considering each subject as his own control and computing the mean change in each response variable observed after fasting. p is the significance limit for rejecting the hypothesis by a two sided *t* test, that there is no mean change after starvation.

†Area under the glucose or insulin curve evaluated over 180 min. minus the basal level.

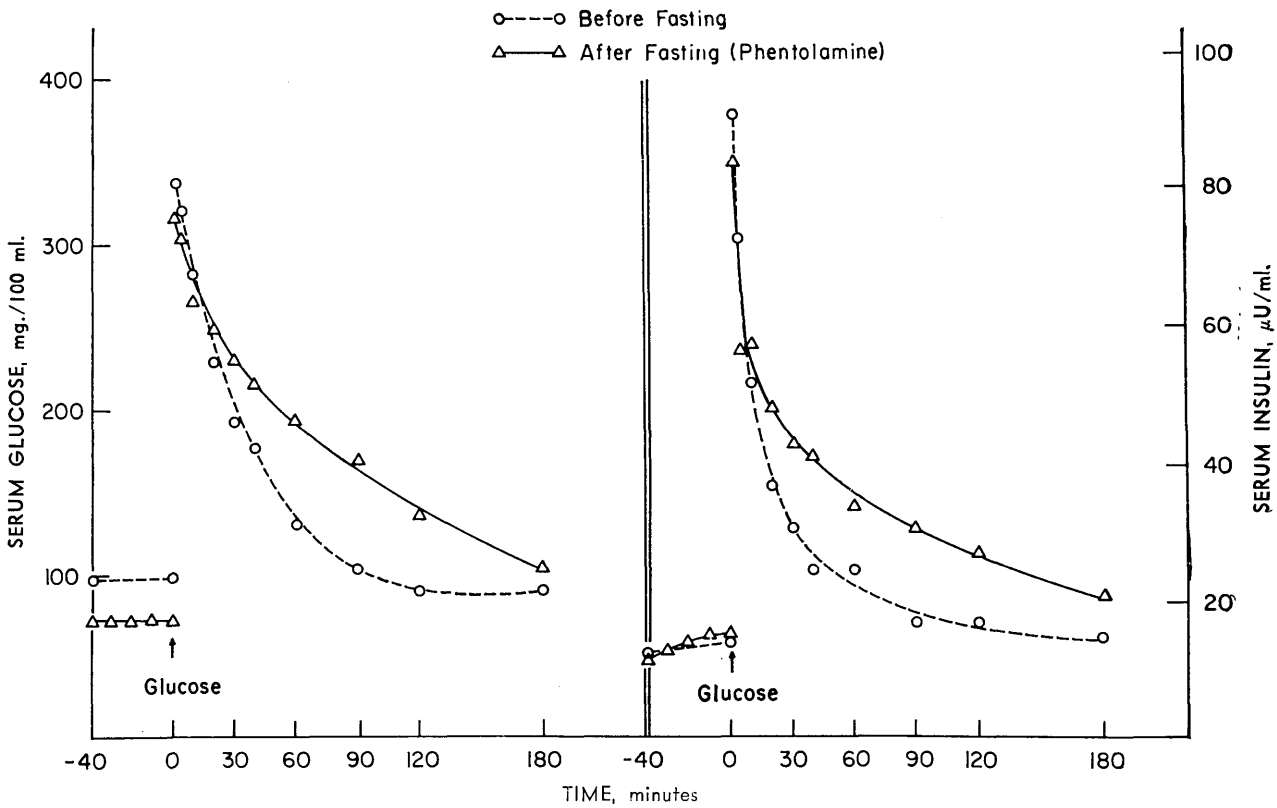

FIG. 2. Intravenous glucose tolerance tests in experimental subjects before and after starvation. For statistical analysis see table 2.

glucose clearance and insulin secretion in the unfasted state, two subjects were restudied while on a normal diet. They were given glucose tolerance tests under the influence of phentolamine, and no consistent change from the prefast values obtained in the previous tests could be demonstrated. This finding corroborates a recently published study by Colwell.[16]

DISCUSSION

The urinary excretion of catecholamines rose after three days of fasting in association with a blunted insulin response to a glucose infusion. Our finding that alpha adrenergic blockade with phentolamine increased glucose-induced insulin secretion above its prefasting level suggests that adrenergic suppression of insulin release is the cause of this hypoinsulinemia. Control subjects had relatively low insulin levels until two hours after glucose administration, suggesting that the chronic hypoglycemia of starvation causes an increase in adrenergic activity that is not readily suppressed by an instantaneous rise in blood glucose.

In recent years there has been increasing evidence that the autonomic nervous system plays a direct role in the modulation of the insulin-glucose axis. Electrical stimulation of the components of the autonomic nervous system, as well as the use of cholinergic and adrenergic drugs in vivo and in vitro, have indicated that parasympathetic activity augments insulin secretion and sympathetic activity suppresses it.[3,17-21] The recent demonstration of cholinergic and adrenergic nerve endings in the islets of Langerhans of the cat[22] has given an anatomical basis for these observations. From our data it is not possible to say whether suppression of insulin secretion during starvation is due to an increase in the level of circulating catecholamines or to local sympathetic activity in the pancreas. The minimal concentration of epinephrine and norepinephrine required to chronically suppress insulin secretion has not been determined in vivo. However, the urinary excretion of catecholamines in patients with diabetes secondary to pheochromocytoma[16] greatly exceeded that observed in our fasting subjects. Furthermore, according to Januszewicz et al.,[6] 90 per cent of the total catecholamine excreted during starvation is norepinephrine. This suggests that local adrenergic activity in the islets of Langerhans may be responsible for suppression of

insulin secretion during starvation in man.

Subjects who did not receive phentolamine had a "diabetic-like" glucose tolerance test after fasting in association with diminished levels of serum insulin. These results agree with those reported recently by Cahill et al.[1] Although subjects who received phentolamine had an increased insulin response to a glucose load, they also remained glucose intolerant. It has been known since the work of Zierler and Rabinowitz[23] that exogenous insulin is less effective in promoting glucose uptake in the forearm of subjects who have been fasted. There are several possible explanations for this apparent insensitivity to insulin:

(1) Proinsulin, the metabolically inactive insulin precursor, would be measured as active insulin in the immunoassay employed in this study. The possibility that phentolamine stimulated the secretion of proinsulin must be considered. However, the finding that substantial amounts of proinsulin do not appear in the peripheral circulation until two hours after administration of glucose to fasting subjects makes this explanation unlikely.[24]

(2) Human growth hormone antagonizes the action of insulin on adipose tissue, and our subjects showed a rise in the serum concentration of growth hormone after two days of fasting. However, other investigators have found an inconsistency in growth hormone response during fasting.[1] Indeed, several of our subjects had a prompt decline of serum growth hormone to normal values during the fasting glucose tolerance test but remained glucose intolerant.

(3) Gluconeogenesis has been emphasized as the main determinant of carbohydrate metabolism during starvation.[1] The enzymes of hepatic gluconeogenesis are elevated in starvation whereas those of glucose utilization are depressed. Because several hours are required after refeeding to change the profile of these hepatic enzymes in fasted rats,[25] it is possible that the liver contributes to glucose intolerance after starvation.

(4) Free fatty acids in serum concentrations, comparable to that which we have observed during fasting, have been shown to inhibit glucose uptake in man.[26]

(5) Epinephrine antagonizes the actions of insulin on liver and adipose tissue. Since this activity is not retarded by alpha-blockade, it appears likely that catecholamines contribute significantly to glucose intolerance. This explanation is supported by the report that fasted rats are relatively resistant to exogenous insulin, and insulin sensitivity is restored by adrenalectomy with glucocorticoid replacement.[27]

The physiological role of the autonomic nervous system as a modulator of the insulin-glucose axis, in the pancreas, liver and peripheral tissues, has not been well investigated in man. The importance of the autonomic nervous system is suggested by a number of situations in which the rate of insulin secretion does not consistently reflect changes in the concentration of glucose in the blood. Schalch[28] has shown that during exercise the plasma concentration of free fatty acids and glucose are elevated while the insulin concentration is depressed, and has suggested that these metabolic changes are caused by epinephrine. It is well known that glucose absorbed from the jejunum is much more effective in stimulating insulin release than an equivalent amount administered intravenously.[29] The explanation has been offered that humoral factors of the intestinal tract promote insulin secretion; however, another possible cause is direct vagal stimulation. Clinical studies have demonstrated that emotional stress can precipitate ketosis in the diabetic patient.[30] Since there is a close anatomical and physiological relationship between emotional stress and the sympathetic nervous system, adrenergic regulation of insulin secretion provides a basis for this observation. Finally, our finding that phentolamine corrects the blunted response of serum insulin to a glucose infusion during starvation is evidence in support of the physiological importance of adrenergic regulation of insulin secretion in man.

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