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Kinetics of glucagon in man: Effects of starvation

(fasting/gluconeogenesis/carbohydrate/hormone turnover/glucose)

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ABSTRACT **The kinetics of glucagon in man was evaluated by continuously infusing crystalline glucagon in normal postabsorptive subjects at rates of 1.5, 3.0, and 6.0 ng/kg per min, and in obese subjects at a rate of 120 ng/m^2 per min. The obese group was studied in the postabsorptive state, and after 3 days and 3–4 weeks of starvation. In the postabsorptive state, the increment in plasma glucagon was directly proportional to the infusion rate, indicating that glucagon turnover is linear over the range of plasma concentrations studied (200–600 pg/ml). The metabolic clearance rate of glucagon (MCR_G) was 537 ± 27 (SE) ml/m^2 per min, which is comparable to that reported for insulin. The basal systemic delivery rate ($BSDR_G$) was 51 ± 5 (SE) ng/m^2 per min, which extrapolates to a 24-hr glucagon delivery rate of 139 ± 14 (SE) μg/day. MCR_G and $BSDR_G$ in the postabsorptive state were comparable in obese and nonobese subjects. Hyperglucagonemia induced by a 3-day fast was associated with a 20% reduction in MCR_G, but no change in $BSDR_G$. After 3–4 weeks of fasting, MCR_G declined further to 35% below baseline. Plasma glucagon, however, fell, returning to postabsorptive levels, as $BSDR_G$ decreased 37% below postabsorptive values.**

It is concluded that (*a*) in postabsorptive man, glucagon removal is proportional to the plasma concentration; (*b*) during early starvation, hyperglucagonemia results primarily from decreased glucagon removal rather than hypersecretion; and (*c*) in prolonged fasting, plasma glucagon returns to baseline levels as a consequence of a reduction in secretion in association with a progressive decline in metabolic clearance.

The secretion of glucagon in man has generally been evaluated on the basis of measurements of peripheral venous concentrations of immunoreactive hormone (1). Changes in plasma hormone concentration may be a consequence of altered secretion, altered hormonal clearance, or both. Although determinations of portal venous glucagon levels provide a more direct means of evaluating alpha cell secretion (2), such measurements in man are not feasible in most circumstances. The current study was undertaken to evaluate the kinetics of glucagon in normal man, and to determine the effect of starvation, a circumstance characterized by hyperglucagonemia (3) on glucagon turnover. In addition to providing normative data on the metabolic clearance and systemic delivery of glucagon, our findings indicate that the hyperglucagonemia of starvation is due to decreased hormonal removal rather than to hypersecretion.

METHODS

Subjects. Two groups of subjects were studied. The first group consisted of 17 healthy volunteers (14 men and 3 women) aged 22–31 years, within 15% of ideal body weight (based on Metropolitan Life Insurance Co. Tables, 1959). They consumed weight-maintaining diets containing at least 200 g of carbohydrate and were taking no drugs. All had negative primary family histories for diabetes mellitus and none had an elevated fasting plasma glucose. Thirteen subjects received an oral glucose tolerance test (100 g) and were considered to have a normal response (4).

The second group consisted of seven healthy obese subjects (4 men and 3 women) who were hospitalized at the Clinical Research Center of the Yale-New Haven Hospital. Each had volunteered to undergo prolonged starvation after failure to lose weight with diet therapy. They ranged in age from 21 to 50 years and were 72–171% (102 ± 12%, mean ±SE) above ideal weight (based on Metropolitan Life Insurance Co. Tables, 1959). All had normal fasting glucose levels; however, three of the seven obese subjects had mild glucose intolerance (2-hr plasma glucose >150 mg/100 ml). They had normal thyroxine levels, and normal renal and hepatic function. All obese subjects consumed a 3000 kcal (13,000 megajoule) diet containing 300 g of carbohydrate and 125 g of protein prior to study. None was receiving any drugs.

Subjects were informed of the nature, purpose, and possible risks of the study before we obtained their written consent to participate. Daily intake during fasting was restricted to 2000 ml of water, one multivitamin tablet (Theragran, E. R. Squibb & Sons, New York), 1 mg of folic acid (Volvite, American Cyanamid Co., Lederle Laboratories Div., Pearl River, N.Y.) and intermittently, 650 mg of sodium bicarbonate (sugar-free) and 20 mEq of potassium gluconate [Kaon, Warren-Teed Products (Pharmaceuticals), Columbus, Ohio]. Three of the obese subjects received 300 mg/day of allopurinol (Zyloprim, Burroughs Wellcome and Co., Tuckahoe, N.Y.). No potassium gluconate or allopurinol was given for 3–4 days prior to the glucagon infusion studies.

Procedures. The nonobese subjects were studied after a 12- to 15-hr overnight fast (postabsorptive state). The obese subjects were studied in the postabsorptive state, and after 3 days and 3–4 weeks of starvation. Beef and pork crystalline glucagon (obtained from Eli Lilly & Co., Indianapolis, Ind.) was diluted in sterile, pyrogen-free saline containing 300 mg/100 ml of human serum albumin (Armour Pharmaceutical Co., Phoenix, Ariz.). The glucagon-albumin solution was further diluted with physiologic saline and an aliquot of the subject's whole blood (4 ml/100 ml of infusate, to prevent adsorption of glucagon to glassware and tubing) so as to achieve a final concentration of glucagon in the infusate of 500 ng/ml.

The glucagon solution was administered as a continuous infusion over a 3-hr period via peristaltic infusion pump (Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.). The infusion doses of glucagon in nonobese subjects were 1.5 ng/kg per min (n = 3), 3.0 ng/kg per min (n = 21; includes five subjects studied twice), and 6.0 ng/kg per min (n = 8). In obese subjects the infusion dose was 120 ng/m^2 of body surface area per min; this dose was chosen so as to achieve increments in plasma glucagon that were comparable to the 3.0 ng/kg per min dose in the nonobese group. Venous blood samples were drawn at 10- to 15-min intervals prior to the infusion (control values represent the mean of three preinfusion measurements),

Abbreviations: MCR_G, metabolic clearance rate of glucagon; $BSDR_G$, basal systemic delivery rate of glucagon.

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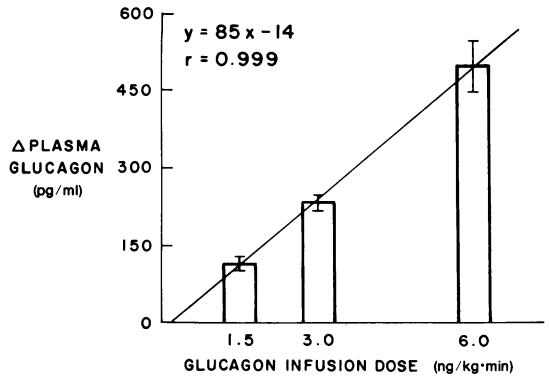

FIG. 1. Changes in plasma glucagon concentration in relation to the glucagon infusion rate in normal, nonobese subjects. In all of the infusion studies, plasma glucagon reached a stable plateau at 30 min. The height of the bars represents the mean increment in plasma glucagon ±SE.

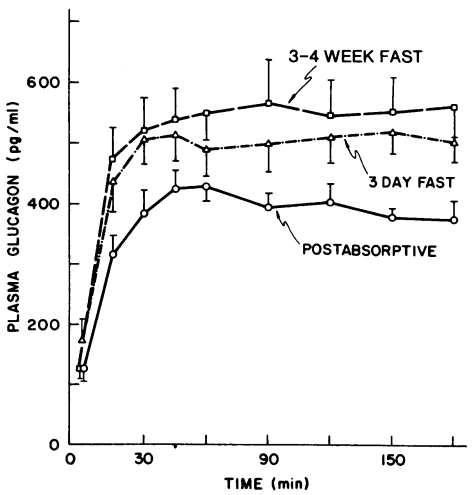

FIG. 2. The effect of starvation on the plasma glucagon response to exogenous glucagon infusion (120 ng/m^2 per min) in obese subjects. Plasma glucagon concentration during the infusion progressively increased above postabsorptive values after 3 days ($P < 0.01$) and 3–4 weeks ($P < 0.01$) of starvation.

and thereafter at 15- to 30-min intervals until completion of the infusion at 180 min.

Analyses. The methods used for the determination of plasma glucose, plasma immunoreactive insulin, and plasma immunoreactive glucagon (using Unger antibody 30K) have been described previously (5). Statistical analyses were performed with the Student's *t* test (using the paired *t* test when applicable) and linear regression analysis (6). Data in the *text* are presented as the mean ±SE.

Calculations. The metabolic clearance rate of glucagon (MCR_G) (the volume of plasma completely and irreversibly cleared of glucagon per min) was calculated according to the formula (7):

$$MCR_G = (\text{glucagon infusion rate}) \div (\text{glucagon concentration at equilibrium} - \text{basal glucagon concentration})$$

It was assumed that exogenous glucagon metabolism is indistinguishable from that of endogenous glucagon, and that basal, endogenous glucagon secretion does not change during the infusion. The latter assumption is supported by the recent demonstration of glucose-induced suppression of plasma glucagon levels during infusion of exogenous hormone (8).

The basal systemic delivery rate of glucagon ($BSDR_G$) was calculated as the product of the basal glucagon concentration and MCR_G. The term $BSDR_G$ was used since the techniques employed exclude that portion of total endogenous glucagon secretion removed by the liver before entering the systemic circulation.

RESULTS

Glucagon kinetics in normal, nonobese subjects

Fig. 1 demonstrates the changes in plasma glucagon concentration in normal nonobese subjects who received continuous infusion of glucagon at rates of 1.5, 3, and 6 ng/kg per min. The basal plasma glucagon concentrations prior to each of the three infusion rates were 70 ± 5, 93 ± 8, and 101 ± 14 pg/ml, respectively. In each of the studies, plasma glucagon rose rapidly during the infusion and reached a stable plateau by 30 min. The coefficient of variation at 30–180 min was 11 ± 1%. The increments in plasma glucagon were 118 ± 15 pg/ml for the 1.5 ng/kg per min dose, 233 ± 13 pg/ml for the 3 ng/kg per min dose, and 497 ± 49 pg/ml for the 6 ng/kg per min infusion dose. There was a direct linear correlation between the glucagon infusion rate and the mean change in plasma glucagon concentration ($r = 0.999$, $P < 0.05$) (Fig. 1). The calculated metabolic clearance rate of glucagon (MCR_G) was the same regardless of the glucagon infusion dose: 1.5 ng dose, MCR_G = 13.1 ± 1.5 ml/kg per min; 3 ng dose, MCR_G = 13.8 ± 0.9 ml/kg per min; 6 ng dose, MCR_G = 12.9 ± 1.3 ml/kg per min. These results support the conclusion that the glucagon removal system is linear over the range of concentrations studied.

The mean MCR_G for all 32 studies (combining the data from the three infusion rates) was 13.5 ± 0.6 ml/kg per min or 537 ± 27 ml/m^2 per min. The mean $BSDR_G$ was 1.3 ± 0.1 ng/kg per min or 50.5 ± 4.7 ng/m^2 per min. These values correspond to a daily (24 hr) basal systemic delivery rate of glucagon in these subjects of 139 ± 14 μg/day.

Glucagon kinetics in obese subjects before and after starvation

In the obese subjects, plasma glucagon was 128 ± 20 pg/ml in the basal postabsorptive state, and rose to 393 ± 16 pg/ml during the glucagon infusion (Fig. 2). The MCR_G in the postabsorptive state (468 ± 22 ml/m^2 per min) was slightly, but not significantly, lower than in the nonobese group (537 ± 27, $P > 0.05$). The $BSDR_G$ in the obese subjects in the postabsorptive state (61.4 ± 11.3 ng/m^2 per min) was also comparable to that of nonobese subjects (50.4 ± 4.7, $P > 0.1$).

During prolonged fasting, plasma glucagon initially rose to 178 ± 35 pg/ml at 3 days of starvation ($P < 0.05$). As fasting continued for 3–4 weeks, plasma glucagon, as expected (3), returned to levels comparable to the postabsorptive state (125 ± 18 pg/ml). In contrast, plasma glucagon concentration during exogenous glucagon infusion progressively increased above postabsorptive values (393 ± 16 pg/ml), after 3 days (506 ± 39 pg/ml, $P < 0.01$) and 3–4 weeks (538 ± 50 pg/ml, $P < 0.01$) of starvation (Fig. 2). The effects of starvation on MCR_G and $BSDR_G$ are shown in Fig. 3. MCR_G was reduced by 20% after 3 days of starvation (375 ± 25 ml/m^2 per min, $P < 0.005$) and fell to 35% below postabsorptive levels (309 ± 27 ml/m^2 per min, $P < 0.001$) after 3–4 weeks of fasting (Fig. 3). In contrast, $BSDR_G$ after 3 days of fasting (67.4 ± 16.4 ng/m^2 per min) showed no significant change from the postabsorptive state (61.4 ± 11.3). However, as fasting continued for 3–4 weeks, $BSDR_G$ fell 37% below postabsorptive levels to 38.8 ± 7.6 ng/m^2 per min ($P < 0.02$) (Fig. 2).

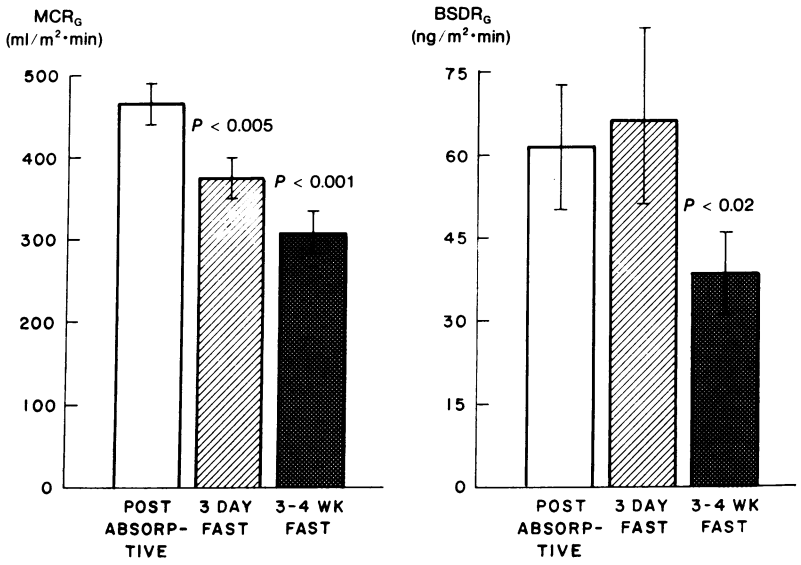

FIG. 3. The effects of short-term (3 day) and prolonged (3–4 weeks) fasting on the metabolic clearance rate (MCR_G) and basal systemic delivery rate ($BSDR_G$) of glucagon in obese subjects. *P* values represent the significance of differences in MCR_G and $BSDR_G$ as compared to the postabsorptive state.

Plasma glucose and insulin response to glucagon administration during starvation

In the postabsorptive state, the mean maximal increment in plasma glucose in response to comparable elevations in plasma glucagon was the same in obese (11 ± 3 mg/100 ml) and nonobese subjects (10 ± 1 mg/100 ml; 3 ng/kg per min glucagon infusion dose). After a 3-day fast, glucagon failed to elicit a consistent rise in plasma glucose (Fig. 4). However, after a 3 to 4 week fast, the glycemic response was restored; the mean maximal glucose increment was 7 ± 2 mg/100 ml. The peak plasma glucose rise, which occurred at 30–60 min in the postabsorptive state, was, however, delayed to 90–150 min after 3–4 weeks of starvation (Fig. 4).

The increment in plasma insulin in response to the glucagon infusion tended to be greater in the obese subjects (19 ± 5 μU/ml) than in nonobese subjects (9 ± 1, $0.05 < P < 0.1$) in the postabsorptive state. After 3 days and 3–4 weeks of fasting, the glucagon infusion failed to consistently alter plasma insulin levels.

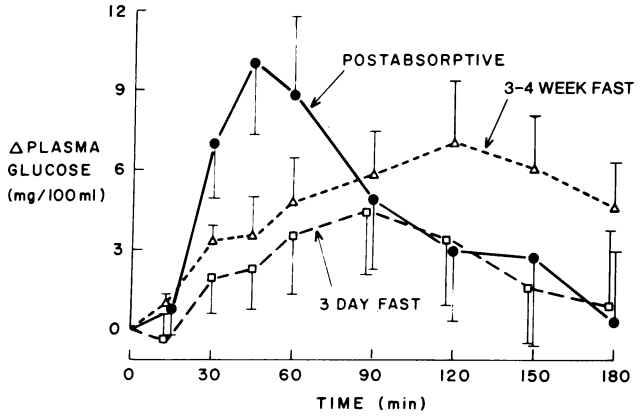

FIG. 4. The effect of starvation on the plasma glucose response to glucagon in obese subjects. The response in the obese group in the postabsorptive state was comparable to that of nonobese subjects (not shown in figure; see *text*). After a 3-day fast the glucose response to glucagon was not significantly different from zero. With more prolonged fasting (3–4 weeks) the glucose response returned but the peak increment, which occurred at 30–60 min in postabsorptive subjects, was delayed to 90–150 min.

DISCUSSION

In the current study, infusion of unlabeled hormone was used to evaluate glucagon kinetics in man. Labeled hormone was not used in view of previous studies with insulin indicating that the metabolism of ^{131}I-labeled insulin differs from that of unlabeled insulin (9, 10). The present results reveal a direct correlation between the rate of glucagon infusion and the increment in plasma concentration (Fig. 1). These findings indicate that glucagon removal is linear over the range of concentrations studied. In a previous report in which a glucagon infusion rate of 50 ng/kg per min was used, plasma glucagon increments of 4200–4300 pg/ml were observed (11). The latter value fits precisely with that predicted from the data in Fig. 1 (4235 pg/ml), suggesting that glucagon disposal is linear over a range of concentrations well in excess of those observed in physiological or pathophysiological circumstances.

The MCR_G demonstrated in normal postabsorptive subjects (537 ± 27 ml/m^2 per min) is comparable to that reported for insulin (450–500 ml/m^2 per min) (9, 10). The basal systemic delivery rate of glucagon in the postabsorptive state was 50.5 ± 4.7 ng/m^2 per min or 1.3 ± 0.1 ng/kg per min. This value extrapolates to a 24-hr delivery rate in a normal 70-kg man of 130 μg/day. In view of the small portal:peripheral gradient for glucagon in the basal state (1.3:1) (2, 12), the $BSDR_G$ is likely to represent no more than a 30% underestimate of the true pancreatic secretory rate of glucagon. The validity of the MCR_G and $BSDR_G$ as determined in the present study may be tested by calculating, on the basis of these values, the predicted concentration of glucagon in the hepatic vein (the site of glucagon delivery into the systemic circulation). Assuming an hepatic venous plasma flow of 800 ml/min (13), and a $BSDR_G$ of 91 ng/min (in a 70-kg man), the predicted concentration of plasma glucagon in the hepatic vein (by the Fick principle) is 114 pg/ml. This value fits closely with the hepatic venous glucagon concentration of 101 ± 6 pg/ml observed in 19 normal postabsorptive subjects†.

† P. Felig and J. Wahren, unpublished data.

Of particular interest was the effect of starvation on glucagon kinetics. Hyperglucagonemia induced by 3 days of starvation was associated with a 20% reduction in MCR_G in the absence of an increase in $BSDR_G$. Furthermore, as starvation continued for 3–4 weeks, MCR_G continued to decline while glucagon levels returned to baseline. The fall in plasma glucagon as starvation was extended for 3–4 weeks was a consequence of a 35–40% fall in $BSDR_G$. Inasmuch as starvation is known to be associated with a diuresis, particularly during the first week (14), the decline in MCR_G and rise in plasma levels may reflect, in part, a diminution in the volume of hormonal distribution and a decrease in flow to catabolic tissues. However, extracellular volume generally falls by no more than 5% during a 3-day fast (14) and is thus unlikely to be a major factor in the 20% reduction in MCR_G observed in early starvation or in the 35% reduction observed in more prolonged fasting. Regardless of the mechanism involved, these findings indicate that a rise in plasma glucagon need not reflect increased secretion but may be accounted for by a decrease in hormone clearance. These results raise the possibility that the hyperglucagonemia associated with such diverse influences as infection (15), trauma (16), and diabetic ketoacidosis (17) may be due in part to reduced hormonal turnover. Direct evidence of the latter mechanism has recently been reported in uremic subjects (18). In uremia, hyperglucagonemia is associated with a 60% reduction in metabolic clearance rate and an unchanged basal systemic delivery rate (18).

The evidence of a decline in glucagon secretion in prolonged starvation may at first glance appear to contradict the notion that glucagon is a hormone of "glucose need" (1). It should be noted, however, that in prolonged (3–6 weeks) fasted subjects, liver glycogen is depleted (19), hepatic gluconeogenic rates are reduced (20), and protein breakdown is markedly reduced from the levels observed at 3 days of fasting (20). In this circumstance of protein conservation and glycogen depletion a decline in the secretion of a glycogenolytic and gluconeogenic hormone is not unexpected.

It should be noted that the calculations used in determining MCR_G assume that endogenous glucagon secretion is not suppressed during the infusion of exogenous hormone. The greater glycemic and insulin response to the glucagon infusion in the postabsorptive as compared to the fasted state does raise the possibility of such suppression in the postabsorptive condition. However, evidence against such suppression derives from a variety of studies. In nonobese and obese subjects, glucose-induced increments in plasma insulin of 15–50 μU/ml and in plasma glucose of 10–15 mg/100 ml (comparable to or greater than those observed with the glucagon infusion), fail to lower plasma glucagon levels (13). Second, administration of oral glucose during a continuous infusion of glucagon results in a 60–70 pg/ml decline in plasma glucagon levels, suggesting ongoing endogenous glucagon secretion prior to glucose administration (8). Finally, in insulin-dependent diabetic subjects (whose insulin response to exogenous glucagon is negligible), the rise in plasma glucagon induced by infusion of exogenous hormone is identical to that of normal controls (8). Thus the available data suggest that secretion of endogenous hormone is not suppressed during the glucagon infusion.

The loss of the glycemic response to glucagon after a 3-day fast is consistent with liver biopsy data demonstrating depletion of hepatic glycogen stores after a 3-day fast (19). Noteworthy was the restoration of the blood glucose rise after a 3- to 4-week fast (Fig. 4). Previous studies have failed to demonstrate glycogen repletion in humans fasted beyond 3 days (19). On the other hand, the delayed rise in blood glucose suggests the possibility of a gluconeogenic rather than glycogenolytic effect of glucagon. The time course of the rise in plasma glucose in the subjects who fasted 3–4 weeks is in fact comparable to the curve of alanine incorporation into blood glucose (21). It is noteworthy in this regard that marked sensitivity to the hypoaminoacidemic effects of glucagon has been reported in prolonged fasted subjects (3).

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