Modulation of Fatty Acid Metabolism by Glucagon in Man

IV. Effects of a Physiologic Hormone Infusion in Normal Man

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SUMMARY

This study was done to explore the role of physiologic elevations of glucagon concentration in plasma ketone body concentration in normal man. During the period of hormone elevation, plasma free fatty acids were pharmacologically elevated to ensure adequate free fatty acid substrate delivery to the liver to support hepatic ketogenesis.

Eighty-minute infusions of glucagon resulted in a plasma hormone concentration of approximately 300 pg./ml. During the infusion, ketone bodies declined from their basal concentration and remained below basal for the duration of the infusion. An acute heparin-induced pharmacologic elevation of plasma free fatty acid concentration resulted in a transient rise in plasma ketone body concentration, but at no time did it attain the concentration observed during the control saline infusion. Plasma glucose concentration was not altered by glucagon infusion, but plasma insulin concentration rose by approximately 2.5 μU./ml.

These results suggest that glucagon is not ketogenic in normal man as has been previously reported in insulin-deficient diabetics. The glucagon-induced rise in plasma insulin concentration may participate in the observed reduction in plasma ketone body concentration.

DIABETES *25*:978-83, October, 1976.

Previous studies from this laboratory have demonstrated that bolus injection of pharmacologic concentrations of glucagon in normal man results in a positive dose-response increase in plasma ketone body concentration.[1] At the lowest pharmacologic dosage administered (0.1 μg./kg.), plasma ketone body concentration rose to a maximum of only 78 μmol/L. This observation suggests that lower concentrations of glucagon may not result in augmentation of hepatic ketogenesis. This postulate is supported by the report that infusions of low pharmacologic concentrations of glucagon result in a decrease in plasma ketone body concentration.[2] In order to determine the role of this hormone in stimulating hepatic ketogenesis at concentrations physiologically obtained in normal subjects, we infused physiologic concentrations of glucagon into nondiabetic subjects. To assess the contribution of free fatty acid substrate to this ketogenic response, the change in plasma ketone bodies was examined in both the basal state and during heparin-induced augmentation of free fatty acid substrate availability.

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Accepted for publication May 28, 1976.

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METHODS

Six healthy subjects (three males and three females) were studied between 7:00 a.m. and 9:00 a.m. fol-

lowing a 12-hour overnight fast. All normal subjects were nondiabetic by U.S.P.H.S. criteria following a standard 100-gm. oral glucose tolerance test.[3] They were instructed to consume at least 300 gm. of carbohydrate for one week prior to testing. Ages ranged from 21 to 26 years, and all subjects were within 5 per cent of their ideal body weight by the Metropolitan Life Insurance Tables.[4]

All subjects assumed the supine position for 30 minutes prior to testing and maintained this position throughout the duration of the test. Each antecubital vein was catheterized with a no. 19 scalp-vein needle, and patency was maintained by constant infusion of normal saline (1 ml./min.) from a Harvard infusion pump. Blood samples were withdrawn from one arm while infusion of saline or of glucagon in saline was administered into the contralateral arm.

Two studies were performed in each subject one week apart. The "control study" consisted of an 80-minute saline infusion (1 ml./min.) plus an intravenous sodium heparin injection (5,000 U.) after a 30-minute basal period to acutely raise plasma FFA concentration.[5] The "glucagon infusion study" consisted of a glucagon infusion (3.0 ng./kg./min.) administered at the rate of 1.0 ml. per minute instead of the saline infusion and prepared so as to deliver a hormone concentration of 3.0 ng./kg./min. as previously described.[6] Assay of this glucagon preparation for insulin indicated that each milliliter of the glucagon infusate contained less than 10 μU. of insulin. Blood samples were obtained at 10-minute intervals over the initial 30-minute baseline period and following bolus heparin injection at +2, +5, +10, +15, +20, +30, +40, and +50 minutes.

All blood samples were assayed in duplicate for betahydroxybutyrate, acetoacetate, free fatty acids, and glucose, as previously described.[1] Insulin concentration was assayed as previously reported by a double antibody precipitation method.[1] Glucagon concentration was assayed in plasma as described[7] with 30K antibody obtained from Dr. Roger Unger, V. A. Hospital, Dallas, Texas. Growth hormone was assayed by double-antibody precipitation.[8]

For each individual subject, the control and glucagon infusion tests were performed one week apart according to a random schedule. Plasma ketone bodies were assayed on the day of testing. All other substrates and the hormones insulin, glucagon, and growth hormone were assayed from individually frozen plasma samples within three weeks of testing. Significance of the difference of sample means was calculated by the two-tailed Student's *t* test for paired data.[9] Variance of the mean is expressed as the standard error of the mean (S.E.M.).

RESULTS (MEAN ± S.E.M.)

Plasma Glucagon Concentration

The mean plasma concentration of glucagon in the normal subjects in both the control saline infusion and glucagon infusion studies are given in tables 1 and 2. When the basal plasma glucagon concentration of the control study (28 ± 4 pg./ml.) was compared with the basal hormone concentration in the glucagon infusion study (37 ± 10 pg./ml.), no statistical difference was observed ($p > 0.1$). Following the initiation of the

TABLE 1

Concentration of hormones and substrates during control saline infusion

	Basal ← Duration of saline infusion (minutes) →											
	0	10	20	29	32	35	40	45	50	60	70	80
Hormones/substrates				Heparin inj. ↓								
Glucagon (pg./ml.)	28 ±4	34 ±5	30 ±13	37 ±4	26 ±4	25 ±4	25 ±4	33 ±6	23 ±11	29 ±6	37 ±7	32 ±7
Insulin (μU./ml.)	15 ±2.2	14 ±2.1	14 ±1.6	14 ±1.8	12 ±1.9	13 ±1.6	12 ±1.1	13 ±1.3	12 ±1.1	13 ±.96	11 ±8.2	12 ±1.2
Growth hormone (ng./ml.)	2.7 ±0.9	2.4 ±0.8	2.8 ±1.2	2.0 ±0.7	2.7 ±1.0	2.3 ±1.3	2.0 ±1.0	3.0 ±1.5	4.0 ±1.7	4.0 ±1.3	4.0 ±1.3	2.9 ±1.0
Ketone bodies (μmol/L.)	146 ±15	172 ±18	196 ±32	187 ±44	196 ±34	182 ±35	204 ±33	232 ±48	285 ±52	263 ±50	261 ±59	258 ±61
Free fatty acid (μmol/L.)	759 ±43	753 ±43	759 ±45	759 ±46	1,514 ±241	1,638 ±252	1,825 ±213	1,807 ±158	1,551 ±167	1,381 ±148	1,118 ±105	1,282 ±106
Glucose (mg./dl.)	88 ±3.6	86 ±3.4	87 ±2.0	88 ±2.3	88 ±1.9	84 ±2.6	82 ±3.6	83 ±2.9	85 ±2.5	87 ±2.6	83 ±5.5	91 ±3.6

All concentrations are expressed as the mean ± standard error of the mean.

TABLE 2

Concentration of hormones and substrates during glucagon infusion

	Basal	Duration of glucagon infusion (minutes)										
	0	10	20	29	32	35	40	45	50	60	70	80
					Heparin inj. ↓							
Hormones/substrates												
Glucagon	37	280*	331*	280*	304*	284*	275*	285*	275*	382*	490*	410*
(pg./ml.)	±10	±68	±94	±85	±66	±38	±43	±52	±64	±125	±197	±140
Insulin	13	16	17	17	16*	17*	15	15	14	15*	15*	15*
(μU./ml.)	±2.0	±1.7	±1.9	±1.7	±2.0	±1.8	±1.8	±2.4	±2.3	±1.7	±1.6	±1.6
Growth hormone	3.5	3.7	4.5	5.4	5.2	5.0	4.6	3.8	4.2	2.8	3.1	2.8
(ng./ml.)	±1.2	±1.3	±1.4	±2.0	±2.2	±2.5	±1.6	±1.4	±1.8	±1.0	±1.0	±1.2
Ketone bodies	201	198	185	154	147	137	157	183	204	200	179	150
(μmol/L.)	±34	±31	±23	±17	±18	±19	±19	±24	±37	±31	±27	±21
Free fatty acids	708	792*	1,107*	857	1,582	1,749	1,671	1,603	1,435	1,225	1,128	1,089
(μmol/L.)	±68	±79	±151	±159	±284	±285	±252	±237	±219	±196	±253	±221
Glucose	90	91	94	94	92	92	88	91*	99	94	88	93
(mg./dl.)	±5.8	±4.8	±4.6	±4.9	±4.6	±5.7	±6.1	±3.0	±8.5	±7.5	±4.9	±7.0

All concentrations are expressed as the mean ± standard error of the mean.
*indicates that the concentration is statistically different ($p < 0.05$) than the corresponding concentration given in table 1.

glucagon infusion a steady increase in plasma glucagon was observed in all normal subjects and by 20 minutes had attained a steady state concentration of approximately 330 pg./ml. No significant change in glucagon concentration occurred during the control saline infusion study.

Plasma Insulin Concentration

As shown in table 2, infusion of glucagon resulted in a small but statistically significant increase in circulating plasma insulin at five observation points over that of the saline control study ($p > 0.05$). This glucagon-induced rise of peripheral plasma insulin concentration contrasted with insulin levels in the saline control study. During this control study no significant change in circulating insulin concentration was observed ($p > 0.05$) (table 1).

Plasma Growth Hormone Concentration

No significant difference in growth hormone concentration was observed between the control saline infusion and the glucagon infusion studies ($p > 0.05$). No change in growth hormone was observed following heparin-induced lipolysis (table 1).

Plasma Free Fatty Acid Concentration (FFA)

Except for a transient elevation occurring after 10 and 20 minutes of glucagon infusion, the plasma concentration of FFA was statistically indistinguishable in the saline control and glucagon infusion studies (tables 1 and 2, figure 1). In response to heparin, a rise to a maximum FFA concentration of 1,825 ± 251 μmol/L. in the control study was observed. This lipolytic response was indistinguishable from the FFA rise to a maximum of 1,749 ± 285 μmol/L. following heparin administration in the glucagon infusion study ($p > 0.1$). Thus, the doubling in FFA concentration during both control and glucagon infusion studies assured adequate substrate availability for ketogenesis.

Plasma Ketone Body Concentration

Total ketone bodies are expressed as the sum of acetoacetate (AcAc) and betahydroxybutyrate (BOH). Tables 1 and 2 give the total ketone bodies for both the saline control and glucagon infusion studies, respectively. Figure 2 depicts the mean changes in the individual ketone bodies in addition to the change in total ketone bodies. In the saline infusion study in normal subjects, heparin administration resulted in a progressive increase in plasma ketone bodies in parallel with the simultaneous rise in FFA concentration. The maximal concentration of total ketone bodies (285 ± 52 μmol/L.) was attained at 20 minutes postheparin-injection. As shown in table 2, glucagon infusion resulted in a progressive decline in ketone body concentration from basal concentration to a nadir of 137 ± 19 μmol/L. by five minutes postheparin-injection. The mean ketone body concentration then rose transiently but remained below the concentration observed following heparin administration in the saline control study at all observed times. Thus, as plotted in figure 2, the net effect of glucagon on ketone body concentration was negative, even during the majority of the period of increased FFA substrate availability generated by heparin.

This reduction in total plasma KB concentration in the normal subjects was reflected by a similar response in the change in betahydroxybutyrate and acetoacetate

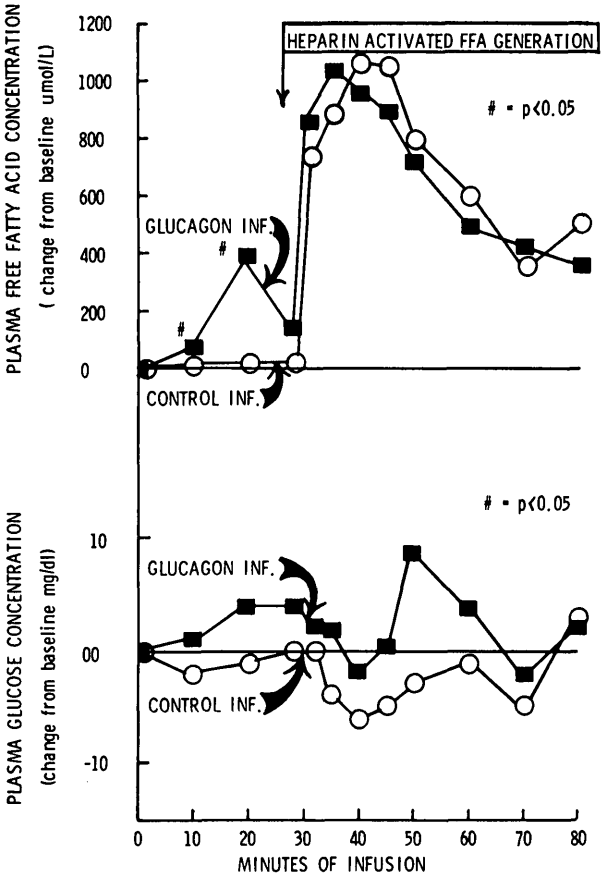

FIG. 1. Mean substrate change from basal concentration in the glucagon infusion study and the saline control study. Plasma free fatty acid concentration (top) was different in the two studies at only two time points—10 and 20 minutes postinitiation of glucagon infusion ($p < 0.05$). The change in plasma glucose concentration was not different in the two studies ($p > 0.05$). Five thousand units of heparin was administered as an intravenous bolus at the arrow.

(figure 2). For both of these individual ketone bodies, glucagon infusion resulted in a significant decline below basal concentration at the majority of observation times ($p < 0.05$). Thus, glucagon infusion into normal subjects resulted in a significant decline in the concentration of both major ketone bodies in spite of adequate availability of free fatty acid substrate to support ketogenesis.

Plasma Glucose Concentration

Basal plasma glucose concentration (figure 1, tables 1 and 2) did not differ in the control or glucagon infusion study ($p < 0.1$). The infusion of glucagon did not alter the plasma concentration of glucose in the normal subjects (table 1) when compared with the saline control study (figure 1, tables 1 and 2). This was true whether the glucose was calculated as the change from baseline or as the true plasma glucose concentration.

DISCUSSION

The present study examined the normal ketone response to physiologic elevations in plasma glucagon both during a basal period and during increased plasma free fatty acid concentration. It was observed that glucagon infusion resulted in a progressive decline in plasma ketone body concentration whether expressed as total ketone bodies or expressed as the individual ketones, acetoacetate and betahydroxybutyrate. This negative ketone response differs markedly from that observed previously in insulin-dependent diabetic subjects.[6] In this latter population, glucagon infusion resulted in a progressive increase in plasma ketone body concentration.[6] These

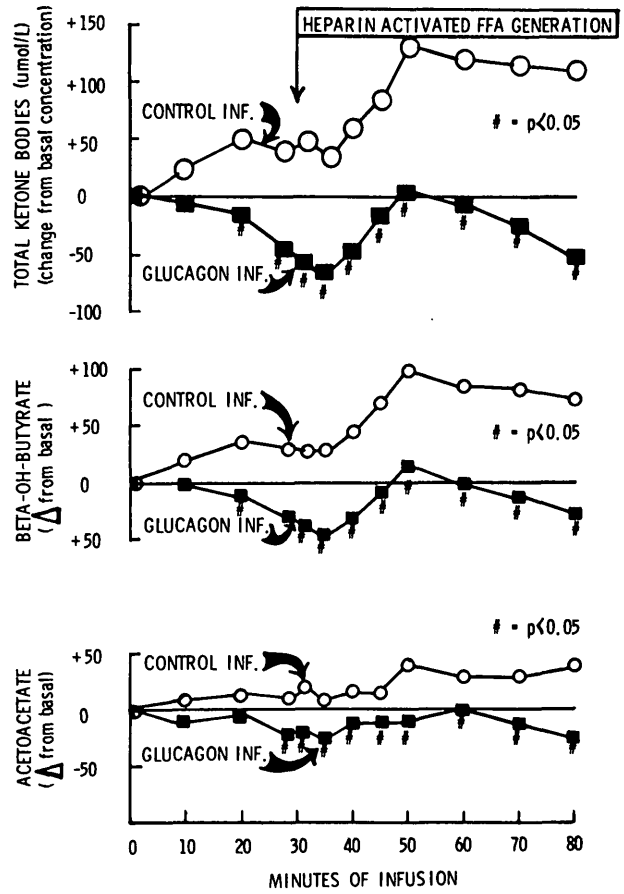

FIG. 2. Mean change from basal concentration of both total ketone bodies and the individual ketone bodies— betahydroxybutyrate and acetoacetate. Both prior to and following heparin-induced lipolysis, plasma ketone bodies declined below basal concentrations of the saline control study ($p < 0.05$). Five thousand units of heparin was administered as an intravenous bolus at the arrow.

observations suggest that glucagon's ketogenic activity is modulated by the simultaneous concentration of other hormones or substrates.

Although the net ketone body response during glucagon infusion was negative, a transient rise in plasma ketone body concentration was observed following the heparin-induced rise in plasma free fatty acid concentration (figure 2). This transient elevation occurred in the presence of the simultaneous rise in plasma glucagon and insulin concentration (table 2). These data suggest that under our experimental conditions, the substrate-product relationship of free fatty acids to ketone bodies remained in effect in spite of concurrent alterations in circulating hormones. This observation is consistent with the in-vitro liver perfusion experiments of Heimberg et al.[10] who demonstrated that hepatic ketone body production was directly proportional to perfusate fatty acid concentrations. Addition of glucagon to the perfusate did not qualitatively alter this relationship.

In addition to the "negative" ketone body response, plasma glucose concentration was unchanged during hormone infusion. This contrasts with the positive response to glucagon in insulin-dependent diabetics. In these latter subjects, a 26-mg./dl. rise in blood glucose concentration was observed by 20 minutes postinitiation of glucagon infusion.[6]

In agreement with our observations, a similar lack of positive glycemic and ketonemic response to physiologic elevations of plasma glucagon in nondiabetic man has been reported. Thus, Marliss and associates[11] demonstrated no alteration in plasma glucose or ketone body concentration in fasting man until plasma glucagon attained a concentration of 752 ± 166 pg./ml. More recently, Sherwin et al. demonstrated only a very transient rise in glucose during glucagon infusion.[12] Although the "counterregulatory" mechanisms at work in the "nonresponse" are not resolved, the concurrent elevation of plasma insulin has been suggested.[13] In the perfused liver, exposure to insulin inhibits ketone body production.[14] Similarly, in human ketoacidosis, the administration of insulin is rapidly associated with a reduction in plasma ketone and glucose concentration.[15] In man, glucagon is an insulin secretagogue.[1] In our studies a rise in plasma insulin was demonstrated in the normal subjects during physiologic glucagon infusion, which confirms previous observations by Sherwin et al.[12] However, the magnitude of this rise in our study averaged only 2.5 μU./ml. Since only the peripheral concentration of insulin was measured, the hormonal concentration in the portal vein can only be estimated. Considering the report of W. G. Blackard and N. C. Nelson,[16] portal vein hormone concentration may be greater than 10 times that assayed in peripheral venous samples following stimulation of endogenous insulin secretion. Thus, in our normal subjects, portal vein insulin concentration may have attained concentrations above basal values by 25 μU./ml.

This differential response to glucagon in normal versus diabetic subjects has previously been demonstrated with pharmacologic dosages of glucagon.[2,13] In this latter study,[13] the ketogenic response to exogenous glucagon was inversely related to the magnitude of the rise in endogenous plasma insulin concentration. Thus, the greatest response in plasma ketone bodies was present in insulin-deficient diabetic subjects and contrasted to the negative response in hyperinsulinemic obese subjects. Normal subjects demonstrated a ketonemic and insulinemic response to glucagon administration intermediate between those of the diabetic and obese groups.

It is attractive to suggest from our data that the differing glycemic and ketotic response to glucagon in normal and diabetic subjects relates to the glucagon-induced secretion of insulin. However, our experimental design does not permit exclusion of another possible mechanism, which may include an altered preëxisting metabolic "set" within the hepatocyte, a differential rate of peripheral metabolite utilization, or a glucagon-induced change in other hormones. Since these potential mechanisms were not assessed in the present study, conclusions concerning the relative importance of insulin secretion cannot be made.

In summary, this study demonstrates that physiologic elevations of glucagon concentrations do not result in ketonemia and hyperglycemia in nondiabetic man. It supports the recent suggestion that elevations of this hormone may not result in hyperglycemia if counterregulatory events occur.[12] In addition, our study extends this concept to plasma ketone body concentration, which actually declines when physiologic concentrations of glucagon are infused in normal man.

ACKNOWLEDGMENTS

This investigation was supported by grants from the American Diabetes Association, the Kroc Foundation, and Eli Lilly and Company.

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