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Research Article

The effect of glucagon (50 ng/kg/min) on arterial glycerol concentration and net splanchnic production of total ketones and glucose was studied after an overnight fast in four normal and five insulin-dependent diabetic men. Brachial artery and hepatic vein catheters were inserted and splanchnic blood flow determined using indocyanine green. The glucagon infusion resulted in a mean circulating plasma level of 4,420 pg/ml.

In the normal subjects, the glucagon infusion resulted in stimulation of insulin secretion indicated by rising levels of immunoreactive insulin and C-peptide immunoreactivity. Arterial glycerol concentration (an index of lipolysis) declined markedly and net splanchnic total ketone production was virtually abolished. In contrast, the diabetic subjects secreted no insulin (no rise in C-peptide immunoreactivity) in response to glucagon. Arterial glycerol and net splanchnic total ketone production in these subjects rose significantly (P=<0.05) when compared with the results in four diabetics who received a saline infusion after undergoing the same catheterization procedure.

Net splanchnic glucose production rose markedly during glucagon stimulation in the normals and diabetics despite the marked rise in insulin in the normals. Thus, the same level of circulating insulin which markedly suppressed lipolysis and ketogenesis in the normals failed to inhibit the glucagon-mediated increase in net splanchnic glucose production.

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ABSTRACT The effect of glucagon (50 ng/kg/min) on arterial glycerol concentration and net splanchnic production of total ketones and glucose was studied after an overnight fast in four normal and five insulin-dependent diabetic men. Brachial artery and hepatic vein catheters were inserted and splanchnic blood flow determined using indocyanine green. The glucagon infusion resulted in a mean circulating plasma level of 4,420 pg/ml.

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Net splanchnic glucose production rose markedly during glucagon stimulation in the normals and diabetics despite the marked rise in insulin in the normals. Thus, the same level of circulating insulin which markedly suppressed lipolysis and ketogenesis in the normals failed to inhibit the glucagon-mediated increase in net splanchnic glucose production.

It is concluded (*a*) that glucagon at high concentration is capable of stimulating lipolysis and ketogenesis in insulin-deficient diabetic man; (*b*) that insulin, mole for mole, has more antilipolytic activity in man than glucagon has lipolytic activity; and (*c*) that glucagon, on a molar basis, has greater stimulatory activity than insulin has inhibitory activity on hepatic glucose release.

INTRODUCTION

Glucagon has been shown to stimulate lipolysis and ketogenesis in many animal species both in vitro (1–4) and in vivo (2, 5, 6). In human adipose tissue studied in vitro, however, the lipolytic activity of glucagon has been difficult to detect (7–10), and in intact man the ketogenic activity of glucagon has never been conclusively established (11–13). Hence, the ability of glucagon to stimulate these two processes in man has been questioned (8, 9). The assessment of the lipolytic and ketogenic activity of glucagon in man has been complicated by the inherent capability of glucagon to stimulate insulin secretion (14) and the fact that insulin inhibits both lipolysis and ketogenesis (2, 15–17). Indeed, early studies in vivo in various species, including man, showed a marked decline in circulating free fatty acids (FFA) after glucagon administration (18–22), attributed to the observed rise in plasma immunoreactive insulin (IRI)[1] (23, 24).

This study, utilizing normal and juvenile-onset diabetic subjects, was undertaken in order that in vivo studies could be performed with glucagon in the presence and absence of glucagon-induced insulin release. In this experimental setting and using high levels of glucagon, it

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[1] *Abbreviations used in this paper:* EHPF, estimated hepatic plasma flow; IRI, immunoreactive insulin.

was felt that the lipolytic and ketogenic activity of glucagon in man could be established.

METHODS

The studies reported in this paper were performed concomitantly and in the same subjects as the cyclic AMP studies reported in an accompanying paper (25). Except for the following details, the methods employed in the two studies were the same.

Materials. All enzymes and cofactors used in the determinations of glycerol, acetoacetate, and β-hydroxybutyrate were obtained from Boehringer Mannheim Corp., New York.

Subjects and procedure. In brief, after an overnight fast, four normal and five insulin-dependent diabetic men underwent hepatic vein and brachial artery catheterization. A 2 h 50 ng/kg/min intravenous glucagon infusion was then administered. Four diabetic men serving as controls also underwent the catheterization procedure and received saline infusions. Splanchnic blood flow was measured using a continuous infusion of indocyanine green. Blood samples were obtained simultaneously from the brachial artery and hepatic vein throughout the study. The nature, purpose, and possible risks of the catheterization procedure were fully explained to the subjects before obtaining their voluntary consent.

Analytical methods. Immediately after being drawn, blood samples were deproteinized with barium hydroxide and zinc sulfate (26) for determination of ketone bodies and with 0.6 M perchloric acid for the glycerol determination. Acetoacetate and β-hydroxybutyrate were measured enzymatically (27, 28) in the Aminco fluoromicrophotometer (American Instrument Co., Inc., Silver Spring, Md.). Glycerol was measured by the microfluorometric enzymatic assay (29). Acetoacetate was assayed on the day of the catheterization study, and β-hydroxybutyrate and glycerol within 3 days thereafter. Student's *t* test and analysis of covariance were employed in the statistical analyses (30).

RESULTS

Glucagon, insulin, C-peptide immunoreactivity, and estimated splanchnic plasma flow. The results of these determinations are presented in detail in an accompanying paper (25). The 50 ng/kg/min glucagon infusion resulted in an arterial plasma glucagon concentration of 4,420 pg/ml (1.3×10^{-9} M) in the diabetic subjects (assayed by Dr. Roger Unger, Dallas, Tex.) (31). This concentration was achieved in 15 min and maintained thereafter for the duration of the infusion. Glucagon was not measured in the normal subjects. The mean plasma IRI concentration rose progressively throughout the glucagon infusion in the normal subjects reaching a peak of 225 μU/ml (1.6×10^{-9} M) at 90 min. As endogenous anti-insulin antibodies interfered with the IRI measurements in the diabetic subjects, C-peptide immunoreactivity (assayed by Doctors Marshall Block and Arthur

TABLE I

Effect of Glucagon and Saline on Arterial Glycerol Concentration in Normal and Diabetic Men

	Arterial concentration											
Subjects Time, *min*...	−30	−20	−10	0	5	15	25	35	45	60	90	120
	μmol/liter											
Normals + glucagon												
P. F.	—	48	—	60	60	40	36	28	—	24	16	28
W. E.	—	44	—	64	64	60	48	40	—	36	24	20
G. H.	—	44	—	36	44	44	20	20	—	28	12	12
D. R.	—	24	—	40	44	—	16	36	—	8	4	0
Mean	—	40	—	50	53	48	30	31	—	24	14	15
Diabetics + glucagon												
D. M.	—	56	—	56	88	96	92	72	—	60	60	32
K. C.	—	48	—	68	88	88	88	100	—	96	60	68
D. W.	—	40	—	32	52	72	48	48	—	80	42	56
C. A.	—	12	—	12	16	20	20	16	—	20	24	16
R. S.	—	40	—	44	42	80	68	88	—	92	56	52
Mean	—	39	—	42	57	71	63	65	—	70	48	45
Diabetics + saline												
M. P.	—	40	—	56	44	48	52	44	—	48	45	44
C. A.	40	60	35	20	20	32	40	32	24	—	—	—
D. W.	16	20	28	28	32	24	36	28	44	—	—	—
J. T.	56	60	64	80	84	88	92	92	76	—	—	—
Mean	37	45	43	46	45	48	55	49	48	—	—	—

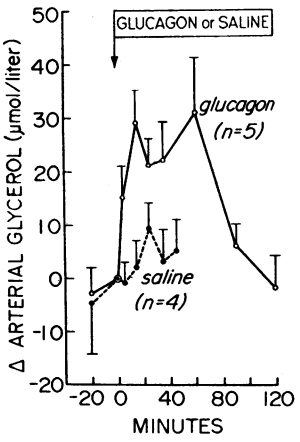

FIGURE 1 Effect of glucagon and saline on the change in arterial glycerol concentration in diabetic men. Mean values ± SE are shown.

Rubenstein, Chicago, Ill.) (32) was measured instead, as an index of secretion of endogenous insulin. C-peptide was found to rise in parallel with the IRI in the normal subjects but did not rise in the diabetics. Estimated hepatic plasma flow (EHPF) was measured in all subjects using indocyanine green (33). Glucagon did not increase EHPF in any of the subjects. EHPF was 20% lower in the diabetics than the normals, a significant difference at the $P = < 0.01$ level.

Glycerol. Glucagon infusion in the normal subjects resulted in a marked decline in arterial glycerol concentration (Table I). Net splanchnic glycerol extraction (Table II) rose initially (at 5 min); thereafter, it fell sharply, paralleling the steep decline in arterial glycerol concentration.

In the diabetics subjects, glucagon infusion resulted in a significant rise in arterial glycerol concentration (Table I and Fig. 1). The difference between the glucagon and saline-treated diabetics was significant at the $P = < 0.05$ level. At 90 min of the glucagon infusion, arterial glycerol concentration fell to basal levels despite continuing glucagon infusion. A significant increase in net splanchnic glycerol extraction also occurred in those diabetic subjects receiving glucagon (Table II).

Total ketones. In the normal subjects, glucagon infusion resulted in an immediate and profound decline in net splanchnic total ketone production (Table III) which contrasted with a lack of decline in ketone production in a saline-treated normal subject.

In the diabetics, glucagon infusion resulted in a rapid and marked increase in net splanchnic total ketone production which was sustained throughout the 2 h infusion (Table III and Fig. 2). The mean increase was

TABLE II

Effect of Glucagon and Saline on Net Splanchnic Glycerol Extraction in Normal and Diabetic Men

	Net splanchnic glycerol extraction											
Subjects Time, *min*...	−30	−20	−10	0	5	15	25	35	45	60	90	120
						μmol/min						
Normals + glucagon												
P. F.	—	69	—	76	96	62	55	28	—	14	21	34
W. E.	—	57	—	41	74	66	57	16	—	33	25	8
G. H.	—	56	—	35	56	56	7	21	—	28	7	7
D. R.	—	30	—	60	66	—	18	54	—	12	6	0
Mean	—	53	—	53	73	61	34	30	—	22	14	12
Diabetics + glucagon												
D. M.	—	79	—	73	118	129	129	73	—	79	79	28
K. C.	—	44	—	58	75	75	44	84	—	75	49	58
D. W.	—	53	—	35	61	99	53	29	—	94	44	76
C. A.	—	17	—	17	23	29	29	23	—	29	35	6
R. S.	—	38	—	44	41	87	60	114	—	114	57	51
Mean	—	46	—	46	64	84	63	65	—	78	53	44
Diabetics + saline												
M. P.	—	32	—	40	44	40	44	44	—	48	45	44
C. A.	31	44	36	18	22	4	40	27	13	—	—	—
D. W.	26	20	45	45	39	39	52	32	58	—	—	—
J. T.	53	63	58	53	73	78	73	87	73	—	—	—
Mean	37	40	46	39	44	40	52	48	48	—	—	—

TABLE III

Effect of Glucagon and Saline on Net Splanchnic Total Ketone Production in Normal and Diabetic Men

	Net splanchnic total ketone production											
Subjects Time, *min*...	−30	−20	−10	0	5	15	25	35	45	60	90	120
						μmol/min						
Normals + glucagon												
P. F.	—	467	—	584	478	371	206	192	—	39	21	45
W. E.	—	62	—	29	90	29	86	57	—	29	41	33
G. H.	—	229	—	115	118	97	104	42	—	17	21	101
D. R.	—	21	—	60	48	63	48	60	—	18	70	12
Mean	—	195	—	197	184	140	111	88	—	26	38	48
Diabetics + glucagon												
D. M.	—	146	—	197	208	242	430	453	—	456	498	146
K. C.	—	364	—	352	597	646	564	644	—	658	494	545
D. W.	—	486	—	548	545	655	—	589	—	698	961	1011
C. A.	—	278	—	279	218	291	382	356	—	341	421	501
R. S.	—	313	—	322	389	493	525	711	—	768	697	920
Mean	—	317	—	340	391	465	475	551	—	584	614	625
Diabetics + saline												
M. P.	—	265	—	387	345	323	303	343	—	411	263	377
C. A.	339	—	304	285	230	264	289	242	279	—	—	—
D. W.	667	—	581	589	445	540	432	433	555	—	—	—
J. T.	321	256	235	264	307	367	396	404	424	—	—	—
Mean	442	260	373	381	332	374	355	356	419	—	—	—

significant at the $P = < 0.05$ level when compared with the four saline-control diabetics (Fig. 2) which showed no increase.

Glucose. Glucagon administration resulted in a prompt rise in net splanchnic glucose production, followed by a decline to a plateau in both groups (Fig. 3). During the 2 h glucagon infusion, net splanchnic glucose production remained markedly elevated in the normal as well as in the diabetic group despite marked endogenous insulin secretion in the former.

DISCUSSION

Arterial glycerol concentration was used as an index of lipolysis in this study (34). The sharp decline in arterial glycerol in the normal subjects during glucagon administration contrasted with its prompt rise in the diabetics. Lipolysis was, thus, suppressed during the glucagon infusion in the normals but stimulated in the diabetics. Rising levels of IRI and C-peptide immunoreactivity in the normals and the lack of rise of C-peptide immunoreactivity in the diabetics indicated that the normal subjects responded to glucagon by secreting insulin but the diabetics subjects did not. It appears, therefore, that glucagon has the capability of stimulating lipolysis in man. However, this effect was seen only in the diabetic subjects, those who failed to secrete insulin in response to glucagon, and was seen with glucagon levels well above the physiologic range. In the normal subjects, who secreted insulin in response to glucagon, lipolysis was inhibited, resulting in a profound fall in arterial glycerol similar to that reported after insulin adminis-

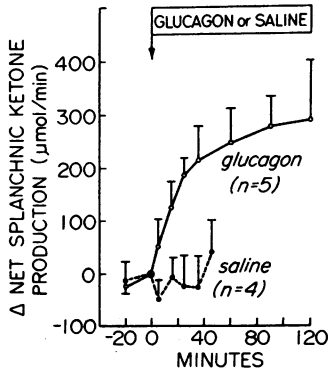

FIGURE 2 Effect of glucagon and saline on the change in net splanchnic total ketone production in diabetic men. Mean values ± SE are shown.

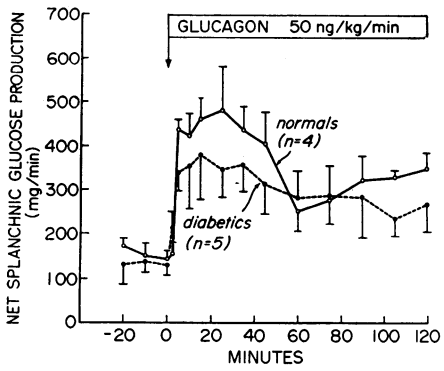

FIGURE 3 Effect of glucagon on net splanchnic glucose production in normal and diabetic men. Mean values ± SE are shown.

tration (35, 36). These findings are consistent with data from in vitro studies using rat epididymal adipose tissue in which a physiologic concentration of insulin (25 μU/ml) completely inhibited glucagon-induced lipolysis until the molar concentration of glucagon was raised to levels 8–12 times higher than that of insulin (24). In the normal subjects, endogenous insulin secretion occurred so rapidly in response to the glucagon infusion and the subsequent hyperglycemia that the peripheral glucagon: insulin molar ratio probably never exceeded 5 (based on the glucagon levels measured in the diabetics during the 50 ng/kg/min glucagon infusion). Thus, it is not surprising that no detectable stimulation of lipolysis occurred with glucagon in the normal subjects.

Net splanchnic glycerol extraction increased and decreased as the arterial glycerol level increased and decreased. That hepatic glycerol extraction is directly proportional to the arterial concentration has previously been reported in many species including man (37, 38). In the normal subjects, however, splanchnic glycerol extraction rose immediately after glucagon administration despite only a minimal rise in arterial glycerol, suggesting that hepatic glycerol extraction may have been directly stimulated by glucagon.

Hepatic ketogenesis was quantified in this study by measuring net splanchnic total ketone production. As the diabetics' last dose of insulin had been given only 24 h previously, the arterial ketone levels in these subjects were not grossly elevated at the time of study when compared with those levels seen in diabetic ketoacidosis (39). Despite these low arterial ketone levels (0.42 mM) glucagon administration resulted in a marked rise in net splanchnic total ketone production. This rise was in marked contrast to a profound decline in net splanchnic total ketone production observed in the normal subjects. The question as to whether this stimulation of ketogenesis was a direct hepatic effect of glucagon or not cannot be answered by this study. It has long been known that simply increasing FFA supply to the liver resulted in enhanced ketone body formation (40). The acceleration of ketogenesis in this study, therefore, may well have been due to glucagon-stimulated lipolysis resulting in an elevation of arterial FFA concentration and increased hepatic FFA uptake, the latter having been shown to be directly proportional to the arterial FFA concentration (41–43). A direct hepatic effect of glucagon in stimulating ketogenesis has also been described (4, 44), in which glucagon acts to shunt intrahepatic FFA away from synthetic pathways (i.e., triglyceride synthesis) towards oxidative pathways (i.e., ketogenesis and Krebs cycle oxidation). This effect can be reversed by insulin (44). Thus, the marked inhibition of ketogenesis in the normal group can be attributed to insulin acting either centrally on the liver or peripherally on the fat cell.

The present study provides no evidence for a physiologic role of glucagon in stimulating lipolysis and ketogenesis in man. The arterial glucagon levels of 4,420 pg/ml (1.3×10^{-9} M) are greatly in excess of normal values. However, peripheral levels as high as 2,000 pg/ml have been reported in a patient in severe ketoacidosis accompanied by pancreatitis and one may assume that the portal vein glucagon levels would have been higher in such a patient (45). Unger has also noted that the highest glucagon levels occur in the most severe cases of diabetic ketoacidosis (45), despite the fact that elevated levels of FFA and ketone bodies have been shown to suppress glucagon secretion (46–48). These data coupled with the demonstration in this study of a lipolytic and ketogenic effect of glucagon in diabetic men suggest that glucagon could possibly play a pathophysiologic role in accelerating these two processes in insulin-lacking man. That lipolytic hormones can play a role in the development of ketosis in diabetic animals has recently been demonstrated (49).

Glucagon in pharmacological doses, has long been known to stimulate adrenal cathecholamine release (50–52) and both epinephrine and norepinephrine are known lipolytic agents in man (9). Indeed, the initial and transient rise in circulating FFA noted after large bolus injections of glucagon has been attributed to glucagon-induced mobilization of catecholamines (23, 53, 54). The doses of glucagon used in these studies, however, were far in excess of the dose used in the present study. In an attempt to determine whether low-dose glucagon infusion stimulates adrenal catecholamine release, Broadus and co-workers infused glucagon at 100 ng/kg/min, twice the level of the present study, yet noted no change in blood pressure, pulse, nor half-hourly urinary catecholamines (55).

Trauma and anxiety are also known to increase sympathetic nervous system activity and stimulate catecholamine release. To determine whether the catheterization procedure itself played a major role in stimulating lipolysis and ketogenesis, four saline infusion studies were performed in diabetic men who had been similarly catheterized. No significant change in their rates of lipolysis and ketogenesis was observed.

We conclude, therefore, that the lipolytic and ketogenic activity attributed to glucagon in this study is most likely a primary effect of glucagon. This conclusion is strengthened by the recent demonstration that glucagon administered into the brachial artery in man caused immediate lipolysis which was not mediated by catecholamine release or systemic sympathetic nervous system discharge (56).

The present study also demonstrates a difference in sensitivity of various metabolic processes to control by insulin and glucagon. In the normal subjects, the glucagon-induced insulin secretion raised intraportal and peripheral arterial insulin concentrations high enough to suppress ketogenesis and lipolysis and, obviously, to override any glucagon stimulation of these two processes. The peripheral glucagon: insulin molar ratio during the 2nd h of the glucagon infusion was probably about 1.0 (based on the glucagon levels measured in the diabetics during the 50 ng/kg/min glucagon infusion). These same levels of circulating insulin, however, were unable to inhibit net splanchnic glucose production which had been stimulated by glucagon. This observation is consistent with studies in perfused rat liver in which insulin, even when used in extremely high concentration, failed to suppress hepatic glucose release induced by supraphysiologic levels of glucagon (57). Similar observations have been made by Mackrell and Sokal who demonstrated differences in sensitivity of various hepatic processes to control by insulin and glucagon (58). It is also consistent with the observation that net glucose uptake by the canine liver does not begin until the glucagon: insulin ratio is less than 1/10 (59). Thus, in intact man, glucagon, mole for mole, has more activity in stimulating hepatic glucose release than insulin has in opposing this process. On the other hand, insulin, on a molar basis, has more antilipolytic activity than glucagon has lipolytic activity.

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