A Nonsuppressible Increase of Glucagon Secretion by Isolated Islets of High-protein-fed Rats

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SUMMARY

We recently demonstrated increased plasma glucagon but normal insulin in rats fed a high-protein, carbohydrate-free (HP) diet; however, other investigators have reported that both plasma glucagon and insulin are increased after protein feeding. For this reason, we have investigated the effects of an HP diet on pancreatic secretion of insulin and glucagon. Male rats were fed an HP or control diet for one, three, or five days, and, at the end of the feeding period, blood was taken for glucose, insulin, and glucagon determinations. Additional animals fed the HP and control diets for up to 10 days were sacrificed, the pancreases removed, and islets of Langerhans isolated. Islets were incubated for 30 minutes in media with glucose concentration of 1.7, 8.3, 16.7, or 33.4 mM. Insulin and glucagon secreted into the media were determined by radioimmunoassay.

Plasma insulin was markedly reduced after one day of HP feeding but gradually returned to normal by the fifth day. Plasma glucagon was not altered on day 1 but was significantly increased after three days of HP feeding. The I/G molar ratio, which declined precipitously on day 1, increased thereafter but, as shown previously, remained at a level that promotes gluconeogenesis for up to 10 days.

Insulin secretion by isolated islets of control and HP rats increased more than 10-fold as medium glucose was raised from 1.7 to 16.7 mM. There was no difference in insulin release by the two groups of islets. Glucagon secretion by HP islets at low medium glucose remained normal during the first five days; however, beginning on day 3 there was gradual loss of the suppressive effect of high medium glucose on glucagon secretion. After one week of HP feeding, glucagon secretion at low medium glucose was doubled and there was complete lack of suppression of the elevated hormone production by high medium glucose. The alterations of alpha-cell function induced by HP feeding are similar to those found in human and experimental diabetes. DIABETES *25*:51-55, **January, 1976.**

It was recently demonstrated that in rats fed a high-protein, carbohydrate-free (HP) diet for one week, plasma glucagon was elevated by more than 50 per cent while the insulin level was unchanged.[1] These findings were not readily explained, since other investigators have reported that protein ingestion stimulates secretion of both pancreatic hormones.[2-6] Insulin and glucagon are secreted into the portal vein and are partially degraded by the liver; thus, it was apparent that more precise information regarding the effects of diet on hormone production could be obtained by direct determination of pancreatic hormone secretion. For this reason, the effect of an HP diet on insulin and glucagon secretion by isolated pancreatic islets has been determined. The results reveal that glucagon secretion by isolated islets of rats fed an HP diet for several days is greatly elevated and cannot be suppressed by high concentrations of glucose. In contrast, insulin secretion was unaffected by increased protein intake.

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MATERIALS AND METHODS

Male Holtzman rats weighing 225-275 gm. were kept in individual cages and allowed to acclimate to their surroundings for several days. During this time, they were fed a nutritionally complete, semisynthetic food that during the experiment was used as the control diet.[7] At the onset of experiments, rats were randomly divided into two groups, one of which was fed a high-protein, carbohydrate-free (HP) diet[7] while the other consumed the control regimen. Animals were fed the diets ad libitum for one, three, or five days. Food intake of each rat was determined daily, and animals were weighed at the start of the feeding period and prior to being sacrificed. At the end of the experiment, rats were anesthetized with intraperitoneal sodium pentobarbital (4.5 mg./100 gm. body weight) and blood was drawn from the inferior

vena cava for glucose, insulin, and glucagon assays with the use of previously described technics.[1,8]

Isolated Islet Incubation Experiments

Rats fed HP or control diets ad libitum for 1, 3, 5, or 8-10 days were sacrificed, and the pancreases were removed and digested in collagenase solution for 10-12 minutes. Islets of Langerhans were then isolated according to the procedure described by Lacy.[9,10] Sixty islets, approximately equal in size, were obtained from each pancreas and preincubated for 30 minutes. The medium used for preincubation and incubation was a modified Krebs bicarbonate solution[11] supplemented with sodium salts of pyruvic acid, glutamic acid, and fumaric acid at concentrations of 5 mM and containing 0.2 per cent bovine serum albumin. Glucose concentration of the preincubation media was 5.5 mM. On completion of preincubation, islets were washed and resuspended in media with glucose concentration of 1.7, 8.3, 16.7, or 33.4 mM. Two beakers, each containing 10 islets, were incubated for 30 minutes at the glucose concentrations used in the experiment being conducted. In all experiments, islets were incubated in media with low (1.7 mM) and high glucose concentrations (16.7 or 33.4 mM), and, in most studies, an intermediate glucose level (8.3 mM) was also used. Trasylol (1,000 KIU.) was added to all beakers to prevent degradation of glucagon. After incubation, media were frozen and stored at $-20°$ C. until insulin and glucagon could be determined by radioimmunoassay.

RESULTS

The HP diet fed to experimental animals in these studies has been used in our laboratory for several years.[1,7,8] Despite the lack of carbohydrate, the food is readily consumed so that there is no impairment of growth even when it is fed for as long as two months. In the experiments reported here, food intake and weight gain of experimental groups were the same as those of controls.

Plasma glucose was slightly but significantly elevated on the third and fifth days of HP feeding (table 1). In previous studies we found normal glucose levels in animals fed the HP diet for one week.[7] The ability of rats to maintain normal blood sugar when carbohydrate intake is totally restricted indicates the potential for gluconeogenesis in these animals.

Plasma insulin showed a precipitous decline after one day of HP feeding (C = 48 ± 4.2 μU./ml.; HP = 17 ± 0.8, P = < 0.001) although food intake of these animals was not reduced (table 1). After three days on the HP diet, the insulin level rose but was still significantly less than that of controls (table 1). Plasma insulin returned to the control level after five days and, as shown previously, remained normal for at least five more days.[1,7] Glucagon was not significantly altered in rats fed the HP diet for one day; however, there was an abrupt decline of the insulin-glucagon molar ratio (C= 27.5 ± 2.9; HP 8.0 ± 1.5) because of the low insulin value at that time. After three days, glucagon was significantly elevated (C= 42 ± 3.1 pg./ml.; HP = 62 ± 6.6, P = < 0.01), and the plasma level of this hormone remained high on the final day of the study (table 1). Earlier investigation revealed that plasma glucagon was increased by more than 50 per cent in animals fed the HP diet for 8-10

TABLE 1

Plasma glucose, insulin, glucagon, and the insulin-glucagon ratio in high-protein-fed rats

	Body weight (gm.)	Glucose (mg./100 ml.)	Insulin (μU./ml.)	Glucagon (pg./ml.)	I/G ratio
Control diet (8)*	227.6 ± 3.5	173 ± 3.3	48 ± 4.2	42 ± 3.1	27.5 ± 2.9
High-protein diet—1 day (6)	221.5 ± 3.6	164 ± 3.2	17 ± 0.8†	51 ± 3.3	8.0 ± 1.5†
High-protein diet—3 days (8)	219.3 ± 5.0	188 ± 3.4‡	38 ± 2.8¶	62 ± 6.6§	13.6 ± 1.7†
High-protein diet—5 days (8)	228.4 ± 2.0	191 ± 5.1§	53 ± 3.8	59 ± 3.4†	21.4 ± 2.3

Mean values ± S.E.M.
*Number of animals.
†Significantly different from control value, P= < 0.001
‡Significantly different from control value, P= < 0.005
§Significantly different from control value, P= < 0.01
¶Significantly different from control value, P= < 0.05

days.[1] The I/G ratio in HP rats gradually rose from a nadir of 8.0 on day 1 to 21.4 ± 2.3 on day 5 (table 1). The latter value is not significantly different from that of control animals.

Insulin secretion by isolated islets of control and HP-fed rats increased more than 10-fold as medium glucose was raised from 1.7 to 16.7 mM (table 2). The responsiveness of isolated islets to changes in medium glucose was not affected by the HP diet even when it was fed for as long as 10 days (table 2).

Glucagon secretion by islets of control and one-day-HP rats was almost identical (table 3). In both groups of islets, hormone release declined by 50 per cent as medium glucose concentration was increased from 1.7 to 16.7 mM. After three days, glucagon production by HP and control islets at 1.7 and 8.3 mM glucose was the same; however, at 16.7 mM glucose, hormone secretion was not suppressed as effectively in HP islets as in controls (C = 478 ± 31 pg./10 islets/30 minutes; HP = 601 ± 35, P = < 0.025). After five days, glucagon release by HP islets at low and intermediate medium glucose did not vary significantly from control levels (table 3), but at 16.7 mM glucose, there was much less suppression of hormone production in HP islets than in controls (C = 478 ± 36; HP = 903 ± 78, P = < 0.001). In islets of rats fed the experimental diet for 8-10 days, glucagon secretion at 1.7 mM glucose was twice that

TABLE 2

Insulin secretion by islets of control and high-protein-fed rats

	Experiment†	Days on diet	Body weight (gm.)	Insulin secretion μU./10 islets/30 minutes 1.7 mM*	8.3 mM*	16.7 mM*
Control (6)	1	1	284.4 ± 7.1	151 ± 13	328 ± 25	1,750 ± 94
High-protein (6)	1	1	263.5 ± 7.4	150 ± 15	260 ± 29	1,807 ± 104
Control (6)	2	3	259.3 ± 10.8	84 ± 6	273 ± 22	1,011 ± 54
High-protein (6)	2	3	254.3 ± 9.0	75 ± 7	227 ± 12	1,105 ± 76
Control (6)	2	5	248.2 ± 5.8	101 ± 12	239 ± 17	1,105 ± 74
High-protein (7)	2	5	249.2 ± 9.6	110 ± 7	240 ± 14	1,025 ± 55
Control (5)	3	8-10	279.2 ± 8.4	139 ± 14	—	1,374 ± 93
High-protein (5)	3	8-10	270.2 ± 5.8	113 ± 10	—	1,444 ± 101

Mean values ± S.E.M.
*Medium glucose concentration.
( ) = Number of animals.
†Results of three separate experiments are presented in this table.

TABLE 3

Glucagon secretion by islets of control and high-protein-fed rats

	Experiment†	Days on diet	Glucagon secretion pg./10 islets/30 minutes 1.7 mM*	8.3 mM*	16.7 mM*
Control (6)	1	1	1,525 ± 104	910 ± 77	740 ± 42
High-protein (6)	1	1	1,450 ± 115	960 ± 104	720 ± 67
Control (6)	2	3	1,122 ± 42	635 ± 60	478 ± 31
High-protein (6)	2	3	1,212 ± 63	762 ± 46	601 ± 35‡
Control (6)	2	5	1,138 ± 72	660 ± 60	478 ± 36
High-protein (7)	2	5	1,314 ± 67	831 ± 59	903 ± 78§
Control (5)	3	8-10	1,700 ± 114	—	1,055 ± 110
High-protein (5)	3	8-10	3,364 ± 230§	—	3,275 ± 275§

Mean values ± S.E.M.
*Medium glucose concentration.
( ) = Number of animals
†Results of three separate experiments are presented in this table.
‡Statistically different from Control, P= < 0.025
§Statistically different from Control, P= < 0.001

of controls (C = 1,700 ± 114; HP = 3,364 ± 230, P = < 0.001). At 16.7 mM glucose, glucagon release of control islets declined by 38 per cent, whereas hormone secretion by HP islets was not suppressed (table 3). Loss of the inhibitory effect of high medium glucose on hormone release resulted in glucagon secretion being three times greater in HP islets than in controls (C = 1,055 ± 110; HP = 3,275 ± 275, P = < 0.001).

To determine whether pancreatic alpha cells of HP-fed rats might respond to the suppressive action of a higher glucose concentration, islets of 8-10-day-HP-fed and control rats were incubated in medium containing 33.4 mM glucose. Glucagon secretion by HP islets was markedly elevated at 1.7 mM glucose and did not decline despite a 20-fold increase in medium glucose (table 4). In contrast, insulin release by HP islets was the same as that of controls.

DISCUSSION

Rats fed a high-protein, carbohydrate-free diet show remarkable ability to maintain a normal blood glucose. In these studies and those reported previously, plasma glucose of fed HP animals was normal or slightly elevated, and in fasted HP rats the decline of blood sugar and mobilization of liver glycogen were less than in controls.[7] A major reason why these animals are capable of maintaining carbohydrate stores despite restricted intake is that hepatic gluconeogenesis is enhanced.[7] It also appears likely that carbohydrate utilization is diminished by HP feeding.[7]

The marked fall of plasma insulin in one-day-HP rats was not expected in view of their undiminished food intake and maintenance of a normal blood glucose. It is clear that the low plasma insulin does not reflect reduced capability of the pancreatic beta cell for hormone secretion, since insulin release by islets of HP rats was normal at low, intermediate, and high glucose levels regardless of the duration of HP feeding. Perhaps the sudden withdrawal of carbohydrate from the diet resulted in reduced formation of certain enteric factors, which ordinarily enhance the beta-cell response to oral carbohydrate.[12,13] It might be expected that the HP diet would augment insulin secretion since there are several reports showing that serum insulin rises after protein ingestion.[2,3] However, our experiments reveal that insulin release by isolated islets was not elevated despite a very high protein intake. It is probable that lack of dietary carbohydrate inhibited the beta-cell response to protein, since Unger has shown a reduced insulin response to protein in human subjects consuming a carbohydrate-restricted diet.[4] The gradual return of plasma insulin from the low value found after one day of HP feeding to normal after five days is probably due in part to the 16 per cent increase of plasma glucose that occurred during that interval. It is also possible that the beta-cell response to the blood sugar may be greater when enteric hormone secretion has been diminished for several days.

The decrease of plasma insulin that occurred shortly after the onset of HP feeding was reflected in a similar decline of the insulin-glucagon molar ratio. As Unger has stated,[4] a low I/G ratio favors increased hepatic glucose production, an adaptation that is vitally important for animals whose carbohydrate intake has been abruptly discontinued. Although blood insulin gradually increased, the I/G ratio remained low on day 3 because of a rise of plasma glucagon. Despite a further increase of the I/G ratio on day 5, it is evident that plasma levels of the two hormones favor accelerated gluconeogenesis after prolonged HP feeding, because we previously observed a reduced I/G ratio and elevated liver cyclic AMP in rats fed the experimental diet for 8-10 days.[1]

In earlier studies we found elevated plasma

TABLE 4

Effects of high-glucose concentration on insulin and glucagon secretion by isolated islets of control and high-protein-fed rats*

	Body weight (gm.)	Insulin μU./10 islets/30 minutes		Glucagon pg./10 islets/30 minutes	
		1.7 mM†	33.4 mM†	1.7 mM†	33.4 mM†
Control (3)	282.3 ± 5.0	95 ± 10	1,124 ± 75	1,000 ± 85	640 ± 61
High-protein (3)	279.0 ± 4.5	89 ± 10	1,127 ± 67	2,238 ± 78‡	2,588 ± 357‡

Mean values ± S.E.M.
*Animals fed the respective diets for 8-10 days.
†Medium glucose concentration.
‡Statistically different from control, P= < 0.001.

glucagon in HP-fed rats and demonstrated that the increased hormone level was due to protein ingestion and not to restricted intake of carbohydrate.[8] The investigations reported here demonstrate that a high protein intake stimulates glucagon secretion by isolated pancreatic islets. The first evidence of altered glucagon production was partial inhibition of the suppressive effect of high-medium glucose concentrations on hormone release, which appeared on day 3. Loss of the suppressive action of high glucose levels gradually became more marked, so that after one week glucagon secretion was not at all influenced by the high medium glucose (33.4 mM). Increased protein consumption also resulted in hypersecretion of glucagon by islets incubated in media with low glucose concentration, although this effect did not appear until rats had been fed the HP diet for one week. The mechanism by which protein ingestion stimulates alpha-cell function has not been elucidated by our investigations; however, it is known that certain amino acids are potent glucagon secretagogues.[14] The elevation of plasma amino acids that results from protein ingestion[15] is the most likely explanation for enhanced alpha-cell function, but it is not clear why the maximal increase of glucagon secretion develops slowly over a period of one week. Perhaps enzymes operative in glucagon biosynthesis are induced by amino acids and this induction requires several days because of slow enzyme turnover.

Increased glucagon secretion and failure of high glucose levels to inhibit glucagon release that occur in response to protein feeding are similar to the alterations of alpha-cell function that occur in human and experimental diabetes. It is interesting that plasma concentrations of branched-chain amino acids are reported to be elevated in both HP-fed and diabetic animals.[15,16] This raises the question of whether the hyperglucagonemia of diabetes is a primary abnormality of alpha-cell function or is secondary to the hyperaminoacidemia of diabetes.

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