Influence of Fasting on Changes in Glucose Metabolism Induced by Growth Hormone Injection in the Normal Dog

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SUMMARY

Normal dogs were fasted four to eight days, then subjected to a regimen of bovine growth hormone (1 mg./kg. per day, intramuscularly) for an additional four to six days with fasting continued. Plasma glucose concentration, and the rate of release and uptake of circulating glucose (as measured with C-14-glucose), were not increased significantly by growth hormone. This contrasts with the increases seen in the normally-fed dog given growth hormone. Nevertheless, the growth hormone regimen produced very high plasma insulin levels (as measured by radioimmunoassay) in the long-fasted animals, and produced the usual resistance in these animals to the effects of administered insulin to enhance glucose uptake and diminish glucose release. Thus neither increased glucose turnover nor increased plasma glucose concentration is a required factor in the development of hyperinsulinemia and insulin resistance in the growth hormone-treated intact dog. DIABETES *19*:487-91, July, 1970.

Experiments carried out in this laboratory a number of years ago led to the surprising finding that a growth hormone regimen increases glucose production and uptake in normal dogs in the postabsorptive state.[1] Because resistance to the blood sugar lowering action of insulin was evident in these dogs, it was conjectured that the rate of insulin secretion in such animals was high.

In order to gain further insight into the interrelationships between the changes in glucose production, glucose uptake, plasma glucose concentration and plasma insulin concentration which are seen during growth hormone administration, conditions were sought which might allow some of these changes to occur in the absence of others. Since the fasted dog, unlike the fed dog, fails to develop diabetes during a regimen in which growth hormone is injected in large doses,[2] fasted dogs were used in the present experiments. In these experiments glucose production and uptake were measured using labeled glucose; plasma insulin levels were measured by radioimmunoassay.

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METHODS

Dogs were used which were trained to lie quietly during the experimental procedures without anesthesia. Prior to fasting the animals were maintained on a standard mixed diet previously described.[3] Uniformly tagged C-14-glucose was given as a priming dose and as a continuous infusion (in trace amount, by way of a peripheral vein) to measure the rates of glucose release to the blood by the liver and glucose uptake from the blood by the tissues. Serial samples of blood were drawn into heparinized syringes through an indwelling polyethylene catheter in the jugular vein. Plasma filtrates were prepared for determination of plasma glucose concentration and for the isolation of glucose as the glucosotriazole derivative for liquid scintillation counting of C-14. These procedures have been fully described elsewhere.[4] The C-14 counting results were used to calculate plasma glucose specific activity. The values of plasma glucose concentration and specific activity were used to calculate glucose release by liver and uptake by tissues by methods already described.[5-7] The rates were expressed as grams of glucose per square meter of body surface per hour, using the formula,[8] $A = 0.2864 \times W^{0.367} \times L$, where A is square meters, W is dog body weight in kilograms and L is dog length, in meters, from tip of snout to root of tail. No corrections were made for the minor errors introduced by the recycling of C-14 back into the glucose output of the liver, a process which results in

5 to 8 per cent of the total plasma glucose C-14 being recycled C-14 at 120 to 360 minutes of continuous infusion.[9] It was ascertained in separate experiments (unpublished) that such errors in the growth hormone-treated dog are no more serious than they are in the untreated normal dog.

The resting rates of glucose turnover were measured during three to four-hour periods of continuous infusion of C-14-glucose; where insulin (trypsin-treated bovine insulin, low in glucagon content, gift from Eli Lilly and Co.) was infused to measure its effect on glucose uptake, the insulin infusion was begun after such a three to four-hour basal period. Plasma insulin concentrations were measured by radioimmunoassay by a modification of the Yalow and Berson technic.[10] When bovine growth hormone (NIH—GH—B8, gift of the Endocrine Study Section, National Institutes of Health) was administered, it was given once a day (intramuscularly, at 1 mg./kg. body weight) at about 4 p.m. Glucose turnover experiments were done beginning at about 11 a.m.; samples for insulin assay were collected prior to 4 p.m. Glucose turnover experiments "in the postabsorptive state" were done beginning about eighteen hours after the last meal.

RESULTS

In table 1 is shown the influence of fasting on some changes produced in normal dogs by a growth hormone regimen. The changes in glucose metabolism produced by growth hormone in the fed dog are fully developed prior to the third day of such a regimen, and remain the same over the ensuing five days of the regimen. It will be noted that the moderate increase in plasma glucose concentration and the large increase in glucose turnover which the regimen produces in the fed animal (observed in the postabsorptive state) are not seen when the growth hormone is given during a period of continued fasting. Fasting alone reduces glucose turnover as shown in table 1; this change was reported earlier by others.[11] The most rapid change toward lower values occurred during the first four days of the fast. There was a slight tendency (last column of table 1, not of statistical significance) for the fasted dog receiving growth hormone to have a higher glucose turnover than the dog which is fasted but not treated with growth hormone. The important point is the absence of a large increase in turnover as is seen during a growth hormone regimen in the postabsorptive state in the fed dog.

In table 1 are shown also the changes in immunoassayable plasma insulin concentration brought about by growth hormone. In the normally-fed dog, growth hormone causes a seven-fold increase when insulin concentration was measured in the postabsorptive state; in the fasted dog growth hormone still brought about a three-fold increase.

Figure 1 shows the time course of the changes which occurred when a growth hormone regimen was instituted on the fourth day of a long fasting period. Prior to growth hormone, glucose turnover fell, in four days of fasting, from about 3.5 to 3.0 gm./m^2 per hour. During the growth hormone regimen with continuation of fasting, there was no further significant change in glucose turnover. Plasma glucose concentration was not significantly affected by the preliminary four-day fast, and changed only slightly during the growth hor-

TABLE 1

Effect of growth hormone (GH)* on plasma glucose and insulin concentrations and on glucose turnover in normal and fasted dogs

	Normal (20)†	Normal+GH‡ (24)	Fasted§ (13)	Fasted+GH‖ (14)
Plasma glucose concentration (mg./100 ml.)	104±1¶	123±3	113±2	108±3
Plasma insulin concentration (μU./ml.)	18±1	138±29	18±1	52±9
Glucose turnover (gm./m^2/hr.)	3.72±0.09	6.87±0.32	2.66±0.15	2.93±0.15

*Bovine growth hormone, 1 mg./kg./day (intramuscularly).
†Number of animals.
‡Bovine GH for three to eight days.
§Fasted for four to eight days.
‖Growth hormone administered daily for four to six days with continued fasting, to dogs previously fasted four to eight days.
¶Mean and standard error of mean.

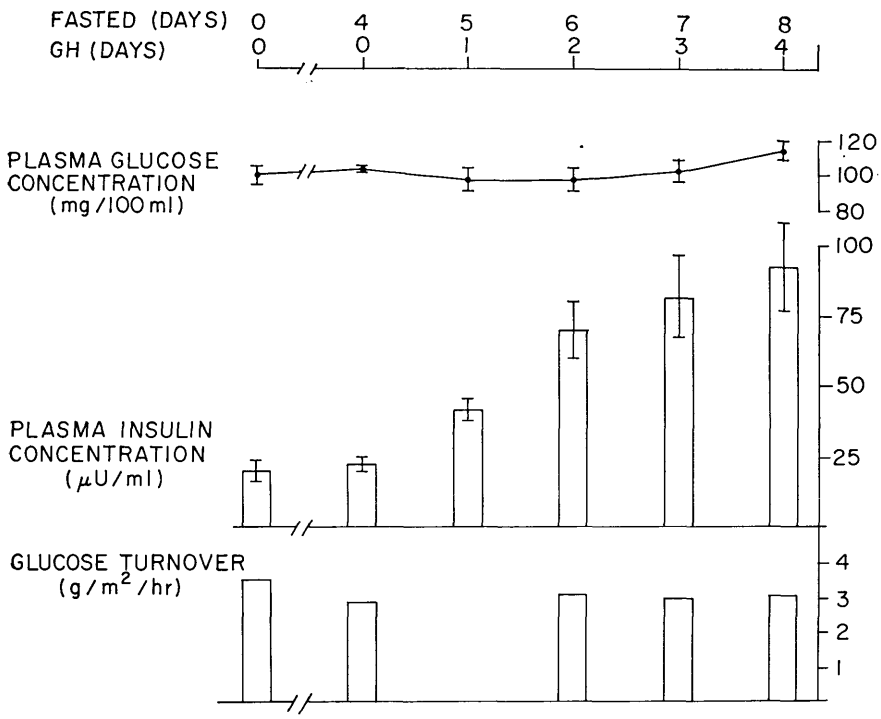

FIG. 1.
The effects of fasting and a growth hormone (GH) regimen (1 mg./kg./day) during fasting, on plasma glucose and insulin concentrations and on glucose turnover in six normal dogs. The legends Fasted (Days) and GH (Days) refer to the number of days the dog has been fasted and on growth hormone respectively at the time the observations were made. Vertical line (I) represents 2 × standard error of mean. The glucose turnover on Days 2, 3 and 4 of the GH regimen represents mean values for two different dogs for each day; calculation of standard error was not attempted, but all values are quite similar.

mone regimen. Plasma insulin concentration, unaffected by the preliminary four-day fast, increased progressively during the first four days when the growth hormone regimen was superimposed on the continued fasting period.

Figures 2 and 3 demonstrate that fasting had no influence on the resistance to insulin-induced glucose uptake brought about by a growth hormone regimen. In figure 2 are compared the response to intravenous insulin infusion of a dog fasted eight days with the same dog fasted a total of fourteen days and with a growth hormone regimen superimposed during the last five days. Glucose uptake increased markedly in response to insulin in the eight-day fasted dog, and the release of unlabeled glucose by the liver increased in response to the induced hypoglycemia. In contrast, very little increase in glucose uptake was induced by the same amount of insulin infused in the fasted dog during the growth hormone regimen. Glucose release in response to hypoglycemia was not challenged sufficiently in this animal because this same dose of insulin was ineffective in producing hypoglycemia in the growth hormone-treated dog.

The responses to infused insulin of normally fed, and growth hormone-treated normally-fed dogs, when the responses were measured in the postabsorptive state, are shown in figure 3. The responses are quite similar to those in the fasted animal in that growth hormone administration resulted in a similar resistance to the insulin-induced increase in glucose uptake.

DISCUSSION

A finding of central importance in the present study was that a growth hormone regimen caused a very large increase in the rate of insulin secretion even when the administration of growth hormone took place during a prolonged period of fasting. Just as in the normally-fed animal, this increased rate of insulin secretion was associated with insensitivity of the animal to the blood sugar-lowering effect of injected insulin. In large part this insensitivity to insulin was due to deficient increase in glucose uptake by the tissues.

A second item of central importance in the present findings was that the increased insulin secretion and the insulin resistance which are caused by growth hormone were not mediated entirely by increased glucose release by the liver and consequent elevation of plasma glucose concentration. In the normally-fed dog these latter changes coincided with increased insulin secretion, but in the long-fasted dog they did not; hence the increased insulin secretion and the insulin resistance appear not to be due exclusively to an effect of growth hormone to increase glucose release by the liver, whereas previously this was not clear.

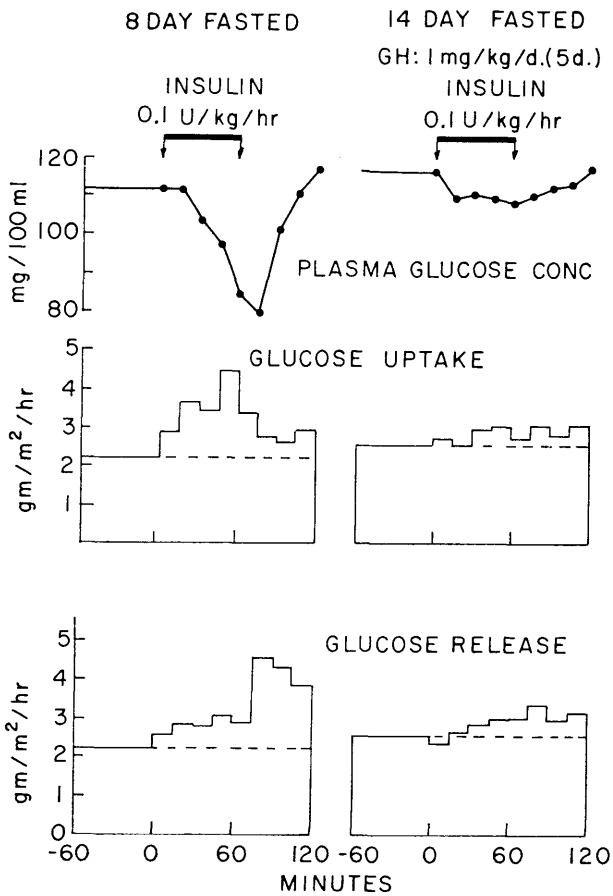

FIG. 2. Changes in sensitivity to infused insulin (0.1 U./kg./hr. for 60 min.) brought about by growth hormone (GH) in a long-fasted dog: Effects on plasma glucose concentration, plasma glucose uptake by the tissues and glucose release by the liver into the plasma.

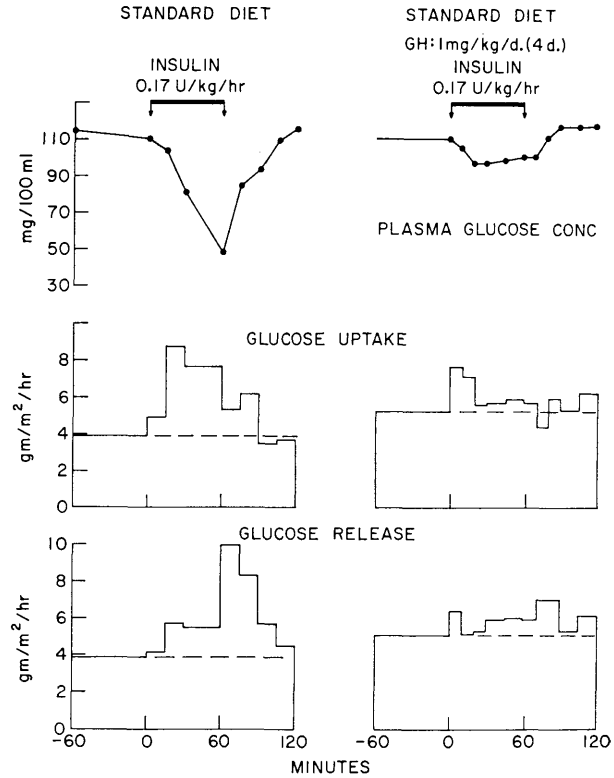

FIG. 3. Changes in sensitivity to infused insulin (0.17 U./kg./hr. for 60 min.) brought about by growth hormone (GH) in a normally-fed dog in the postabsorptive state: Effects on plasma glucose concentration, plasma glucose uptake by the tissues and glucose release by the liver into the plasma.

Measurements of plasma free fatty acid concentration in the growth hormone-treated long-fasted dog have shown that growth hormone is still active in mobilizing fatty acids under these conditions.[12] These findings are in agreement with recent observations[13] that growth hormone, in fasted humans, elevates blood acetoacetate and β-hydroxybutyrate concentrations.

The conclusion drawn in the present work, that the increase in plasma insulin concentration caused by growth hormone is due to an increase in secretion rate rather than a decrease in the rate of extraction of insulin from the blood, is based on the findings of Campbell and Rastogi.[14] These workers found an increased basal secretion rate of insulin in the normally-fed dog during a growth hormone regimen. They also observed increased increments in insulin secretion in response to glucose administration in such animals. They suggested that the increased blood ketone body levels brought about by growth hormone might stimulate insulin secretion; also that there might be an increase in the sensitivity of the pancreatic beta cells to any given level of blood glucose concentration.

Either or both of these mechanisms may well be operating to increase the plasma insulin concentration in the long-fasted dog treated with growth hormone. Other possibilities are increased uptake by beta cells of amino acids capable of stimulating insulin secretion, increased glucose uptake by beta cells in response to an "insulin-like" action of growth hormone as seen in certain other tissues, and an increase in the absolute number of beta cells.

The glycogen content of the liver of the long-fasted growth hormone-treated dog is not elevated (unpublished findings), whereas in the normally-fed growth hormone-treated dog in the postabsorptive state liver glycogen is extremely high.[15] It is believed that the elevated liver glycogen content may be responsible in

some way for the elevated rate of glucose release seen eighteen hours after the last meal in the normally-fed growth hormone-treated dog. This increased rate of glucose release may be a temporary phenomenon reflecting the preferential utilization of the fatty acid portion (and the consequent sparing of the carbohydrate portion) of the last meal over the eighteen-hour period following its ingestion.

Insensitivity to the effect of insulin to lower the rate of glucose release by the liver is also evident in the long-fasted dog kept on growth hormone, just as it is in the normally-fed dog on a growth hormone regimen.[15] Without exogenous insulin being infused, both the fed and fasted growth hormone-treated dogs satisfy the conditions required in the normal dog to demonstrate depression by insulin of glucose release, namely elevated plasma insulin concentration in the absence of hypoglycemia. The concentration of insulin in the plasma in the growth hormone-treated dog is sufficiently high to depress glucose release by the liver, as judged from the amount of insulin which is necessary to infuse into the normal dog to produce this effect. This is not to say that the high plasma insulin level in the growth hormone-treated dog has no restraining effect at all on glucose release. What is evident is the insensitvity of the liver of such an animal to the usually effective amount of insulin, so that glucose release goes on at the normal fasting rate even after growth hormone has brought about a very large increase in plasma insulin concentration.

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