Decreased Response to Insulin in Adipose Tissue during Starvation

Effect of Hypophysectomy and Growth Hormone Administration

P. A. Trueheart, M.S. in Hygiene, and M. G. Herrera, M.D., Boston

SUMMARY

Fasting for twenty-four hours or longer reduced basal glucose oxidation and incorporation into lipid in rat adipose tissue. The "in vitro" effect of 50 and 100 μU./ml. insulin was also found to be significantly decreased. A ten-fold increase in insulin concentration increased glucose metabolism to levels comparable to those observed in tissues from fed rats. Hypophysectomy diminished markedly the difference in response to insulin between fed and fasted rats. Injection of 50 μgm. of growth hormone every twelve hours restored this difference. It is concluded that adipose tissue responsiveness to insulin is markedly reduced by fasting and that the hypophysis is implicated in this phenomenon. Growth hormone appears to be one of the factors that mediates this change in insulin response. DIABETES *20*:46-50, January, 1971.

The first descriptions of insulin resistance in starvation diabetes appeared in the 1920's.[1] Decreased sensitivity to insulin administration during fasting was shown in rabbits[1] and has since been shown in mice.[2] In humans there is evidence that glucose is spared by peripheral tissues during prolonged starvation at a time when glucose and insulin, although diminished in concentration, continue to be present in the extracellular fluid.[3] "In vitro" experiments have shown that glucose oxidation and glucose incorporation into lipid are markedly diminished in adipose tissue removed from fasted rats; responsiveness to insulin is also reduced.[4-6] Similar observations have been made in adipose tissue from rats fed a high fat, low carbohydrate diet.[5]

Himsworth and Scott[7] were the first to associate the hypophysis with the appearance of insulin resistance during starvation. More recently, elevation of circulating growth hormone levels during fasting have been documented in the human[3,8,9] while pituitary depletion of growth hormone has been shown in the fasting rat.[10] With these observations in mind, we have studied the effect of fasting on insulin sensitivity of adipose tissue from intact rats, hypophysectomized rats, and hypophysectomized rats treated with growth hormone.

METHODS

Sprague-Dawley male rats weighing between 120 and 180 gm. were used. Hypophysectomy and sham operations were performed by Charles River Laboratories at least ten days prior to use. Completeness of hypophysectomy was assessed by lack of growth and testicular atrophy. A high carbohydrate, low fat diet was fed ad libitum for seven days prior to the experiment. The animals were decapitated and their epididymal adipose tissue was severed at the stalk, weighed and immediately transferred to incubation flasks. The incubation medium consisted of 2 ml. of Krebs Ringer bicarbonate buffer containing per 100 ml., 5 mg. streptomycin, 5 mg. penicillin, 100 mg. gelatin, and 100 mg. glucose labeled with chromatographically pure glucose-U-C-14 (New England Nuclear). In all experiments the adipose tissue removed from one side of the rat was incubated in the absence of insulin while the contralateral tissue was incubated in the presence of various concentrations of glucagon-free insulin (Lilly).

Incubations were carried out for two hours in a Dubnoff Metabolic Shaking Incubator at 37° C. after the medium was equilibrated for five minutes with a gas mixture consisting of 95 per cent O_2 and 5 per cent CO_2. At the end of two hours, the tissue was removed

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From the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115.

TABLE 1

Glucose-U-C-14-carbon incorporation into CO_2 and total lipid by paired epididymal adipose tissues from fed and fasted rats in the presence and absence of 50 μU./ml. insulin

	μatoms glucose carbon per 100 mg. fat-free dry weight incorporated into:					
	Co_2			Total lipid		
Treatment group	Without insulin	With insulin	% increase due to insulin	Without insulin	With insulin	% increase due to insulin
Fed n = 7	12 ± 1.4	45 ± 2.9	310 ± 59	20 ± 2.2	85 ± 15	370 ± 61
Fasted:						
24 Hours n = 7, p	6.7 ± .42 < .005	27 ± 1.7 < .001	320 ± 50 n.s.	11 ± 0.4 < .005	51 ± 2.9 < .001	380 ± 44 n.s.
48 Hours n = 7, p	4.3 ± .33 < .001	10 ± 1.0 < .001	140 ± 18 < .02	8.0 ± 0.69 < .001	20 ± 1.9 < .001	160 ± 17 < .01

Incubation medium contained 100 mg./100 ml. glucose and 0.5 μC./ml. glucose-U-C-14.
Tissues from two animals were present in each flask.
n = number of pairs of animals per group.
p = significance of difference from fed in the same column.

and rinsed in buffer. Lipids were isolated by a modification of the method of Folch.[11] The fat-free remnant was dried to constant weight. C-14-O_2 was liberated from an aliquot of the medium by the addition of 5N H_2SO_4, and collected in hyamin hydroxide (Amersham/Searle). The hyamine and the purified lipid extracts were dissolved in toluene containing per liter 4 g. PPO and 50 mg. POPOP. Radioactivity was determined in a Nuclear-Chicago liquid scintillation spectrometer. In some of the experiments hypophysectomized rats were injected subcutaneously with 50 μgm. of NIH GH S9 ovine growth hormone* every twelve hours. The last injection was given three to four hours before the experiment. The data are expressed as the means ±

*Gift of the Endocrinology Study Section.

TABLE 2

Glucose-U-C-14-carbon incorporation into CO_2 and total lipid by paired epididymal adipose tissues from fed and seventy-two-hour fasted rats in the presence and absence of different doses of insulin

	μatoms of glucose carbon per 100 mg. fat-free dry weight incorporated into:					
	CO_2			Total lipid		
Treatment group and insulin dose	Without insulin	With insulin	% increase due to insulin	Without insulin	With insulin	% increase due to insulin
Fed, 100 μU./ml. n = 7	13 ± 2.0	38 ± 3.5	230 ± 40	33 ± 4.9	110 ± 11	250 ± 33
Fasted, 100 μU./ml. n = 7, p	3.8 ± .58 < .005	7.1 ± 1.81 < .001	85 ± 27 < .01	13 ± 2.1 < .005	25 ± 5.0 < .001	110 ± 29 < .01
Fasted, 250 μU./ml. n = 7, p	4.2 ± .79 < .005	15 ± 3.1 < .001	300 ± 74 n.s.	15 ± 2.6 < .01	51 ± 7.8 < .001	290 ± 64 n.s.
Fasted, 1 mU./ml. n = 7, p	3.6 ± .50 < .001	28 ± 5.3 n.s.	670 ± 61 < .001	12 ± 1.8 < .005	84 ± 17 n.s.	600 ± 64 < .001

Incubation media contained 100 mg./100 ml. glucose and 0.5 μC/ml. glucose-U-C-14.
Tissue from one animal was present in each flask.
n = number of animals per group.
p = significance of difference from fed in the same column.

S.E.M. The significance of differences was calculated between means using Student's t test and between variances using the F ratio.

RESULTS

Table 1 summarizes an experiment in which the glucose metabolism of adipose tissue from fed rats is compared with that of tissue obtained from rats fasted twenty-four and forty-eight hours. After a twenty-four-hour fast, significant decreases in the incorporation of glucose carbon into CO_2 and lipid in the presence and absence of insulin were observed. Further reduction was noted after forty-eight hours of fasting when both the absolute and the per cent increase in glucose metabolism due to insulin were significantly decreased.

Table 2 summarizes the data from an experiment in which tissues from fed and seventy-two-hour fasted rats were used. By increasing the insulin concentration in the medium, it was possible to stimulate glucose oxidation and lipogenesis in fasted adipose tissue to levels comparable to those observed in fed tissue. The per cent increase in glucose metabolism due to insulin observed in fasted tissue incubated in the presence of 250 μU./ml. insulin was not significantly different from that observed in fed tissue exposed to 100 μU./ml. insulin. The rate of glucose incorporation into CO_2 and lipid in fasted tissue at this insulin concentration approximates that of the unstimulated fed tissue. When ten times the insulin concentration utilized to incubate fed tissue is used to incubate tissue from fasted rats the activity of the fasted tissue is similar to that of the fed.

The results of an experiment in which the effect of hypophysectomy on glucose metabolism was studied are presented in figure 1. In both hypophysectomized and sham operated control rats there were significant decreases in baseline glucose metabolism in response to fasting. The levels of glucose oxidation and incorporation into lipid obtained in tissues from both groups after a seventy-two-hour fast were indistinguishable. On the other hand, the absolute or per cent increments in glucose oxidation or incorporation into lipid due to insulin were significantly greater in the tissues from fasted hypophysectomized animals than in those of the fasted controls.

The effect of growth hormone administration on the reduction in sensitivity to insulin during fasting was then studied. Three groups of hypophysectomized rats were used: fed injected with saline, fed injected with growth hormone, and seventy-two-hour fasted injected with growth hormone. Fifty micrograms of growth hormone in 0.2 ml. saline were injected subcutaneously

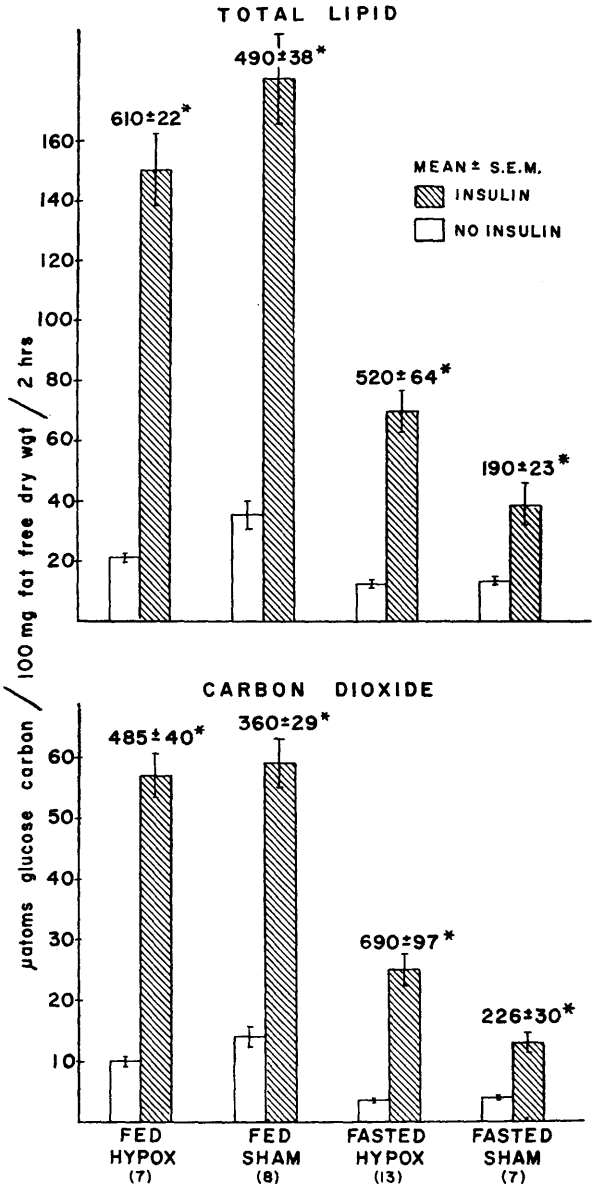

FIG. 1. Glucose-U-C-14-carbon incorporation into CO_2 and total lipid by paired epididymal adipose tissues from fed and seventy-two-hour fasted hypophysectomized and sham hypophysectomized rats in the absence and in the presence of 100 μU./ml. insulin. Numbers in parentheses equal number of pairs studied.
*Per cent increase above baseline due to insulin ± S.E.M.

every twelve hours from the beginning of the fasting period. The results are summarized in figure 2. There were no significant differences in any of the parameters measured between fed rats injected with growth hormone and those injected with saline. On the other hand,

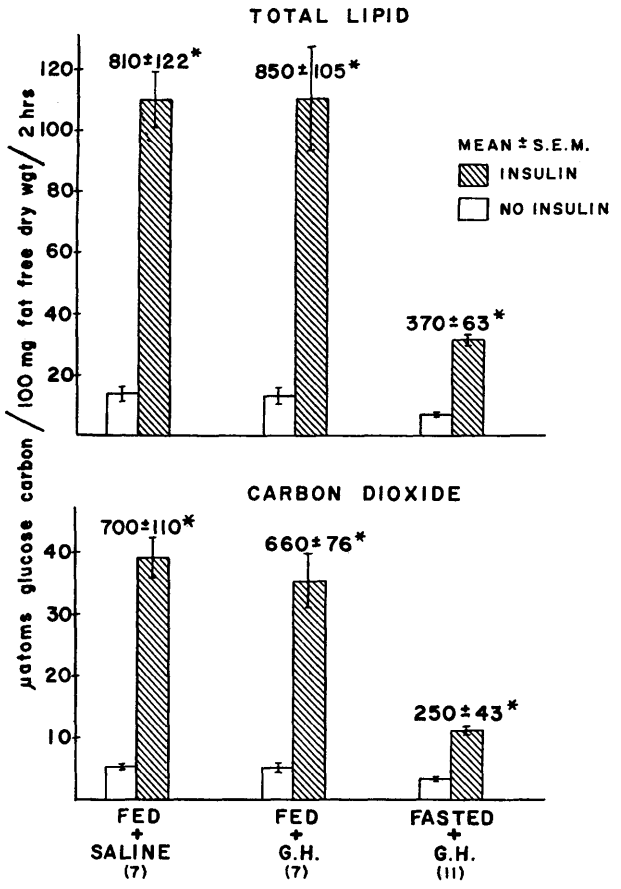

FIG. 2. Glucose-U-C-14-carbon incorporation into CO_2 and total lipid by paired epididymal adipose tissues from fed and fasted hypophysectomized rats injected with 50 μgm. growth hormone or saline, b.i.d., in the absence and presence of 100 μU./ml. insulin. Numbers in parentheses equal number of pairs studied.
*Per cent increase above baseline due to insulin ± S.E.M.

adipose tissue from fasted rats injected with growth hormone showed a significant depression in glucose metabolism and a significant reduction in its response to insulin. Comparison with figure 1 shows that the insulin effects on glucose oxidation and incorporation into lipid in tissues from hypophysectomized rats treated with growth hormone were similar to those observed in tissues from fasted sham operated controls.

DISCUSSION

The data presented in table 1 shows that glucose metabolism in adipose tissue diminishes over a period of forty-eight hours of fasting. The response of the tissue to insulin in vitro was also significantly reduced. Fasting for seventy-two hours did not decrease these parameters further (table 2). By increasing the insulin concentration in the medium used to incubate adipose tissue from fasted rats, it was possible to increase glucose metabolism to CO_2 and total lipid and the magnitude of the insulin response to levels comparable to those observed in tissue from fed rats (table 2). These findings are compatible with the appearance in adipose tissue of a competitive inhibitor of insulin during fasting. Such a possibility has been previously suggested by Masoro et al.[12]

Hypophysectomy did not prevent the reduction in basal glucose oxidation and lipogenesis but it did prevent to a large extent the reduction in the response to insulin as a result of fasting (figure 1). The consequent inability to spare glucose may explain in part the fact that hypophysectomized animals do not adapt well to starvation. This finding is in keeping with Himsworth's early "in vivo" observations.[7]

Long-term administration of growth hormone in doses comparable to that used in our experiments have been shown by Goodman[13,14] to decrease glucose oxidation and glucose incorporation into fatty acids in adipose tissue. On the other hand, this investigator did not find any decrement in adipose tissue sensitivity to insulin when fed animals were injected with a single 50-μgm. dose of growth hormone three hours before the incubation was carried out.[15]

Chattophadhyay et al.[16] have found that adipose tissue removed from twelve-hour fasted rats bearing growth hormone producing tumors exhibit a reduced rate of glucose metabolism to CO_2 and fatty acids as well as a marked reduction in the response to insulin, when compared to control rats. Serum growth hormone concentrations in these animals were fifteen-fold higher than in the controls while insulin levels were increased to three times the normal value. In our experiments administration of 50 μgm. growth hormone twice a day for three days did not reduce the response to insulin in tissue from fed hypophysectomized animals (figure 2). On the other hand, tissue from similarly treated fasted animals showed a distinct reduction in both parameters of glucose metabolism tested, as well as a marked reduction in insulin sensitivity. These various findings can perhaps be best explained by postulating that the sensitivity to insulin of excised adipose tissue is determined by the relative levels of growth hormone (or a factor dependent on its presence) and insulin to which it was exposed in the animal. A relative excess of insulin, as in the fed, would render the tissue more insulin sensitive while a relative excess of growth hormone, as in the fasting, would render it more resistant. This is in fact the type of insulin-modulated effect of growth hormone

on glucose utilization proposed by Rabinowitz et al.[9,17] and Luft and Cerasi,[18] based on studies of glucose metabolism in the human.

The findings are compatible with the concept that adipose tissue responsiveness to insulin is markedly reduced by fasting and that the hypophysis is implicated in this phenomenon. Growth hormone appears to be one of the factors that mediates this change in insulin response. It would be unwarranted, however, to conclude from these experiments that growth hormone is the only factor involved in the appearance of resistance to insulin during fasting. It is very likely that glucocorticoids play at least a permissive role in this phenomenon.[19] It has been shown that adrenal glucocorticoids are required for growth hormone to exert its normal lipolytic effect in adipose tissue from hypophysectomized rats.[20,21] This may also be true of growth hormone effects on insulin sensitivity. Glucocorticoids, in high but physiological concentrations, have been shown to inhibit glucose uptake, oxidation to CO_2 and incorporation into lipid.[22-25] Feldman and Lebovitz[2] have recently reported that insulin resistance does not develop in the adrenalectomized mouse in response to fasting even if cortisol is administered in small doses. The hypophysectomized rats utilized in our experiments may well have retained some adrenal function although fluctuations in the secretion rate of their glucocorticoids in response to fasting would be unlikely.

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REFERENCES

1 Tiitso, Max: Influence of nutritive condition on initial fall in blood sugar after insulin. Proc. Soc. Exp. Biol. Med. *23*: 40-43, 1923.

2 Feldman, J. M., and Lebovitz, H. E.: Effect of fasting on insulin secretion and action in mice. Endocrinology *86*:313-21, 1970.

3 Cahill, G. F., Jr., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. A., Jr., and Kipnis, D. M.: Hormone-fuel interrelationships during fasting. J. Clin. Invest. *45*:1751-69, 1966.

4 Herrera, M. G., Philipps, G. R., and Renold, A. E.: Stimulation of metabolic activity of adipose tissue from fasted rats by prolonged incubation in vitro I. Requirement for glucose and insulin. Biochim. Biophys. Acta *106*:221-33, 1965.

5 Hausberger, F. X., and Milstein, S. W.: Dietary effects on lipogenesis in adipose tissue. J. Biol. Chem. *214*:483-88, 1955.

6 Del Boca, J., and Flatt, J. P.: Fatty acid synthesis from glucose and acetate and the control of lipogenesis in adipose tissue. European J. Biochem. *11*:127-34, 1969.

7 Himsworth, H. P., and Scott, D. B. M.: The relation of the hypophysis to changes in sugar tolerance and insulin sensitivity induced by changes of diet. J. Physiol. *91*:447-58, 1938.

8 Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A.: Hypoglycemia—a potent stimulus to secretion of growth hormone. Science *140*:987-88, 1963.

9 Rabinowitz, D., and Zierler, K. L.: A metabolic regulating device based on the action of human growth hormone and of insulin, singly and together on the human forearm. Nature *199*:913-15, 1963.

10 Friedman, R., and Reichlin, S.: Growth hormone content of the pituitary gland of starved rats. Endocrinology *76*: 787-88, 1965.

11 Folch, J., Lees, M., and Sloane Stanley, G. H.: A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. *226*:497-509, 1957.

12 Masoro, E. J., Korchak, H. M., and Porter, E.: A study of the lipogenic inhibitory mechanisms induced by fasting. Biochim. Biophys. Acta *58*:407-18, 1962.

13 Goodman, H. M.: Early and late effects of growth hormone on the metabolism of glucose in adipose tissue. Endocrinology *76*:1134-40, 1965.

14 Goodman, H. M.: Effects of chronic growth hormone treatment on lipogenesis by rat adipose tissue. Endocrinology *72*:95-99, 1963.

15 Goodman, H. M.: Growth hormone and the metabolism of carbohydrate and lipid in adipose tissue. Annals N.Y. Acad. Sci. *148*:419-40, 1968.

16 Chattopadhyay, D. P., Akerblom, H. K., and Martin, J. M.: In vitro metabolism of glucose by adipose tissue from rats bearing a growth hormone secreting tumor (MtTW 15). Diabetes *18*:387-91, 1969.

17 Rabinowitz, D., Merimee, T. J., and Burgess, J. A.: Growth hormone-insulin interaction: fact and speculation. Diabetes *15*:905-10, 1966.

18 Luft, R., and Cerasi, E.: Human growth hormone as a regulator of blood glucose concentration and as a diabetogenic substance. Diabetologia *4*:1-9, 1968.

19 Ingle, D. J.: The role of the adrenal cortex in homeostasis. J. Endoc. *8*:xxiii-xxxvii, 1952.

20 Fain, J. N.: Studies on the role of RNA and protein synthesis in the lipolytic action of growth hormone in isolated fat cells. Adv. Enz. Reg. *5*:39-51, 1967.

21 Goodman, H. M.: Permissive effects of hormones on lipolysis, II. Growth hormone. Endocrinology *86*:1064-74, 1970.

22 Munck, A., and Koritz, S. B.: Studies on the mode of action of glucocorticoids in rats: I. Early effects of cortisol on blood glucose and glucose entry into muscle, liver and adipose tissue. Biochim. Biophys. Acta. *57*:310-17, 1962.

23 Munck, A.: Studies on the mode of action of glucocorticoids in rats: II. The effects in vivo and in vitro on net glucose uptake by isolated adipose tissue. Biochim. Biophys. Acta *57*:318-26, 1962.

24 Philipps, G. R., Herrera, M. G., and Renold, A. E.: Stimulation of metabolic activity of adipose tissue from fasted rats by prolonged incubation in vitro. II. Effects of physiologic levels of cortisol. Biochim. Biophys. Acta *106*:234-40, 1965.

25 Fain, J. N., Scow, R. O., and Chernick, S. S.: Effects of glucocorticoids on metabolism of adipose tissue in vitro. J. Biol. Chem. *238*:54-58, 1963.