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The effect of insulin on concentration of plasma glycerol*

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SUMMARY

Intravenous infusion of insulin into anesthetized rabbits causes a decrease in the concentration of plasma glycerol. Insulin does not alter the *in vitro* release of glycerol from mesenteric adipose tissue of fasting rabbits either in the presence or absence of glucose. Insulin increases the rate of disappearance of infused glycerol *in vivo*. Possible sites of insulin action to produce these effects are discussed.

In a previous paper (1), it was reported that the intravenous infusion of noradrenaline into rabbits caused an increase in the concentration of plasma glycerol. This increase was probably due to a stimulation by the noradrenaline of lipolysis in the adipose tissue, with the release of glycerol and free fatty acids (FFA) (2). The present paper describes studies of the action of insulin on concentration of plasma glycerol in rabbits. Strack et al. (3) have reported that insulin increases the concentration of glycerol in the plasma of rabbits receiving a glycerol infusion. However, the concentration of plasma glycerol in their experiments was elevated by the glycerol infusion either to about 500 μmoles/100 ml or to about 2,200 μmoles/100 ml, which is very much greater than the normal level of 8–20 μmoles/100 ml. In the present experiments, it was found that insulin caused a decrease in the amount of glycerol normally present in plasma. Since the major source of plasma glycerol is probably the adipose tissue, the effect of insulin on glycerol release by rabbit adipose tissue *in vitro* was also studied.

METHODS

Rabbits were anesthetized by the intraperitoneal injection of 7.5% sodium phenobarbital solution, 2.5 ml/kg. Polyethylene cannulae (PE 50) were passed into the femoral artery and one or both ear veins and were tied in place. The collection of blood samples was started not less than 2 hr after tying in the cannulae, during which time the animals remained undisturbed. Arterial blood was collected in heparinized tubes on ice and was centrifuged, and samples of the plasma were then removed. Plasma (0.5 ml) was acidified with 0.25 ml 2 N perchloric acid, the precipitated protein was removed by centrifugation, and an aliquot of the supernatant fluid was neutralized with 1 N potassium hydroxide. The precipitated potassium perchlorate was removed by centrifugation after the samples had stood overnight in the cold room at 2°.

A dual infusion pump (Harvard Apparatus Co.) was used for the infusions. Glycerol in 0.154 M sodium chloride solution was infused intravenously at a rate of 10 μmoles kg/min and 0.0764 ml/min. Insulin[1] in 0.154 M sodium chloride solution was infused at a rate of 0.1 unit/kg/min and either 0.0388 or 0.0764 ml/min. Insulin stock solution (40 units/ml in 0.001 N hydrochloric acid) and glycerol stock solution (1.0 M) were diluted in 0.154 M sodium chloride solution just before use.

Mesenteric adipose tissue was obtained from small (1.1–1.3 kg) rabbits that had been fasting for 2 days. The tissue was cut into small (20–30 mg) fragments with scissors and the fragments distributed among the incubation vessels so that each received approximately 150 mg of a representative sample of the whole tissue.

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[1] I am grateful to Dr. O. K. Behrens of Eli Lilly and Company for a gift of crystalline zinc insulin (Lot No. 466368).

The incubation vessels contained 3.5 ml of Krebs' bicarbonate buffer solution (4) containing bovine serum albumin (Nutritional Biochemicals Corp.) at a concentration of 3 g/100 ml. This albumin solution had previously been dialyzed for 18 hr against the Krebs' bicarbonate buffer solution. In addition, the vessels contained either 0.1 ml insulin solution in Krebs' bicarbonate buffer–albumin solution or a further 0.1 ml of Krebs' bicarbonate buffer–albumin solution and either 0.4 ml 0.154 M sodium chloride or 0.4 ml 0.1 M glucose in 0.154 M sodium chloride. Thus, tissues were incubated in the presence and absence of insulin both with and without glucose. The final concentration of insulin was 0.1 unit/ml, and of glucose, 0.01 M. Ten vessels (five pairs of duplicates) were used in each experiment. Control vessels containing all solutions but lacking tissue were included in each experiment. The vessels were incubated in a Dubnoff shaking incubator at 37° in an atmosphere of 95% oxygen and 5% carbon dioxide for 1 hr. At the end of the incubation, the media were centrifuged to remove tissue fragments and samples removed for estimations of glycerol (2 ml medium plus 0.5 ml 2 N perchloric acid) and FFA (1 ml medium plus 5 ml acid extraction mixture [5]). Glycerol was estimated with the glycerol dehydrogenase method described previously (1), FFA were measured by the method of Dole (5), glucose was measured by the glucose oxidase method (6).

RESULTS

The Effect of Insulin on Concentration of Plasma Glycerol in vivo. Intravenous infusion of insulin, 0.01 unit/kg/min for 1 hr usually caused the concentration of plasma glycerol to fall to less than 60% of its initial value within 30 min. The results of five experiments are shown in Table 1; two representative experiments are illustrated in Fig. 1. The glycerol concentration started to rise again after about 30 min despite the continued infusion of insulin and before the concentration of plasma glucose had reached its lowest level. After the insulin infusion was stopped, the glycerol concentration rose rapidly to the initial level.

Infusion of glycerol (10 μmoles/kg/min for 1 hr) resulted in a very rapid rise in concentration of plasma glycerol in the first 3–10 min of the infusion (Table 2); during the subsequent 50 min of the infusion, the glycerol concentration increased much more slowly. When the infusion was stopped, the glycerol concentration returned to normal levels quite rapidly. Attempts to demonstrate an effect of insulin on plasma glycerol by infusing insulin intravenously during a glycerol infusion were not successful (Table 2). After a glycerol-plus-insulin infusion, however, the elevated concentration of blood glycerol returned to the initial level more rapidly than after an infusion of glycerol alone. In three experiments in which glycerol was infused together with insulin (Table 2), the glycerol concentration had fallen to or below the initial level within 3 min in one experiment and within 10 min in the other two experiments. In contrast, in the three control experiments in which glycerol alone was infused, the glycerol concentration had fallen to normal levels in 10 min in one experiment and within 60 min in the other two experiments. Thus, insulin infusion does appear to increase the rate of removal of the infused glycerol but this can be demonstrated only when the infusion has ended. A single injection of insulin during the glycerol infusion decreased the glycerol concentration briefly (Table 2).

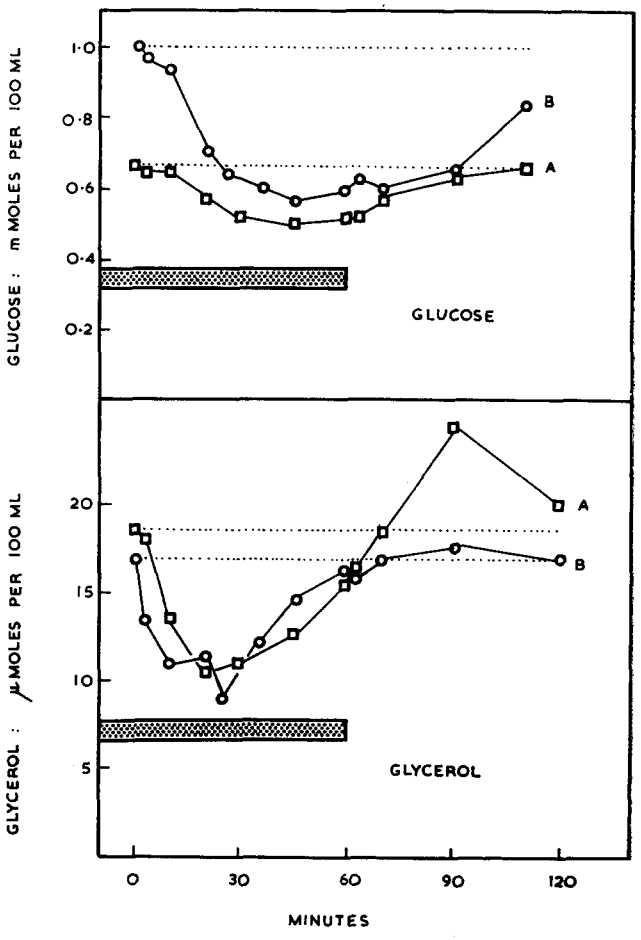

FIG. 1. The effect of an intravenous insulin infusion on plasma glycerol and glucose concentrations. Two experiments with two rabbits (A. ♀, 4.2 kg; B. ♀, 2.8 kg) are shown. Insulin dissolved in 0.154 M sodium chloride was infused at the rate of 0.0764 ml/min and 0.01 unit/kg/min as indicated by the horizontal stippled bars.

TABLE 1. EFFECT OF AN INTRAVENOUS INSULIN* INFUSION

Rabbit		Glycerol Concentration in Plasma (μmoles/100 ml)†												
		Time (min)												
Wt	Sex	−5	3	10	19	25	30	35	45	59	63	70	90	120
kg														
4.2	F	18.5	18.0	13.5	10.5		11.0		12.5	15.5	16.5	18.5	24.5	20.0
			(−0.5)	(−5.0)	(−8.0)		(−6.5)		(−6.0)	(−3.0)	(−2.0)	(0)	(+6.0)	(−1.5)
4.2	F	5.0	2.0	0	0		0		0.4	0.7		1.0	1.4	3.5
			(−3.0)	(−5.0)	(−5.0)		(−5.0)		(−4.6)	(−4.3)		(−4.0)	(−3.6)	(−1.5)
2.8	F	16.9	13.3	10.9	11.4	8.9		12.2	14.6	16.2	15.7	16.9	17.5	16.9
			(−3.6)	(−6.0)	(−5.5)	(−8.0)		(−4.7)	(−2.3)	(−0.7)	(−1.2)	(0)	(+0.6)	(0)
2.9	F	25.6	25.0	18.2	21.6	13.7		21.8	20.0	24.7		21.9	24.2	
			(−0.6)	(−7.4)	(−4.0)	(−11.9)		(−3.8)	(−5.6)	(−0.9)		(−3.7)	(−1.4)	
3.4	F‡	25.0	21.2	19.4	20.0	20.0		17.9	19.2	23.7	28.0	25.0	27.5	28.8
			(−3.8)	(−6.6)	(−5.0)	(−5.0)		(−7.1)	(−5.8)	(−1.3)	(+3)	(0)	(+2.5)	(+3.8)

* Insulin dissolved in 0.154 M sodium chloride was infused at the rate of 0.0764 ml/min and 0.01 unit/kg/min for 1 hr (0–60 min).

Since the decrease in plasma glycerol caused by insulin might be due to an action of insulin either on glycerol production or on glycerol utilization, the effect of insulin on the release of glycerol by adipose tissue was studied *in vitro* (Fig. 2). As might be expected, insulin did not alter glycerol release by adipose tissue either in the presence or absence of glucose, although the known effect of insulin to decrease FFA release in the presence of glucose (7) was observed. The lack of effect of glucose itself to decrease FFA release by rabbit adipose tissue (Fig. 2), in contrast to the known effect of glucose to decrease FFA release from rat adipose tissue *in vitro* (e.g., 8) might be due to a difference between rabbit adipose tissue and rat adipose tissue (9). No FFA release by mesenteric adipose tissue from fed rabbits could be demonstrated in the present experiments although a small release could be demonstrated after 48 hr of fasting (Fig. 2).

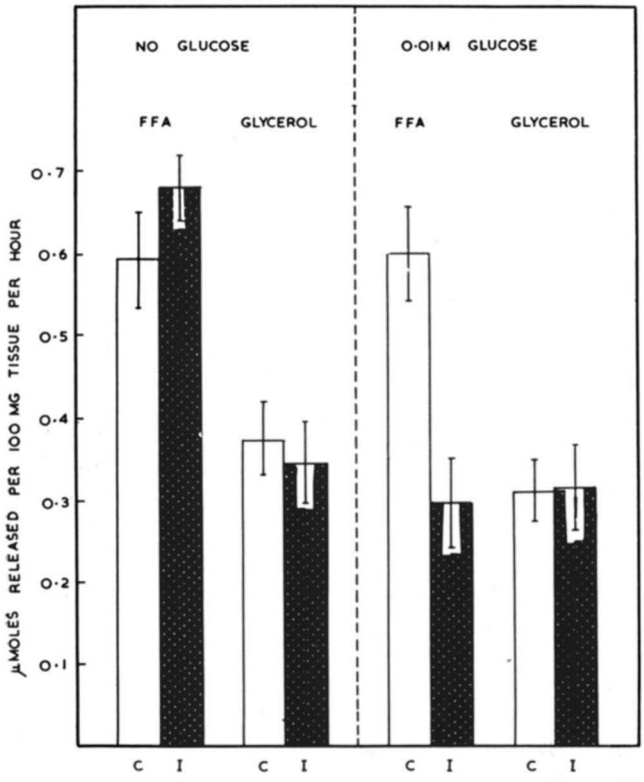

FIG. 2. The effect of insulin on glycerol and FFA release by adipose tissue *in vitro*. The bars represent the means of four estimations; the lines represent the standard errors of the means. The release of glycerol or FFA (μmoles/100 mg of tissue/hr) without (C) and with (I) insulin is shown. Mesenteric adipose tissue (about 150 mg) from rabbits weighing 1.1–1.3 kg and fasting 48 hr was used.

DISCUSSION

The decrease in the concentration of glycerol in plasma caused by insulin could be due either to a decrease in the rate of entry of glycerol into the plasma or to an increase in the rate of removal of glycerol from the plasma.

There are four known possible sources of plasma glycerol: (1) lipolysis of triglyceride in adipose tissue, (2) lipolysis of plasma lipids, (3) lipolysis of dietary lipids in the intestine, and (4) synthesis from glucose by the intestinal mucosa (10).

Of these possible sources, the first is probably the major one. It is known that adipose tissue *in vitro* can release both glycerol and FFA (2) and that this release is increased by adrenaline (2), ACTH (11), and glucagon (12). In rabbits (1, 13) and in man (14), the injection of noradrenaline causes an increase in the concentration of glycerol in the

ON PLASMA GLYCEROL AND GLUCOSE CONCENTRATIONS

Glucose Concentration in Plasma (mg/100 ml)†												
Time (min)												
−5	3	10	19	25	25	30	35	59	63	70	90	120
119	116	116	102		94		90	93	93	102	116	119
	(−3)	(−3)	(−17)		(−25)		(−29)	(−26)	(−26)	(−17)	(−3)	(0)
256	252	224	180		172		112	104		112	156	144
	(−4)	(−32)	(−76)		(−84)		(−144)	(−152)		(−144)	(−100)	(−122)
184	173	168	127	114		108	103	108	114	108	114	151
	(−11)	(−16)	(−57)	(−70)		(−74)	(−81)	(−76)	(−70)	(−76)	(−70)	−33)
150	150	140	115	100		82.5	82.5	95		110	120	
	(0)	(−10)	(−35)	(−50)		(−67.5)	(−67.5)	(−55)		(−40)	(−30)	
140	145	147.5	127.5	120		112.5	110	120	127.5	127.5	135	145
	(+5)	(+7.5)	(−12.5)	(−20)		(−27.5)	(−30)	(−20)	(−12.5)	(−12.5)	(−5)	(+5)

† The numbers in parentheses are the changes, in μmoles/100 ml of plasma (glycerol) or in mg/100 ml of plasma (glucose), from the initial values.
‡ Pregnant rabbit.

plasma; this effect is interpreted as most probably due to stimulation by the noradrenaline of lipolysis in adipose tissue (1). An action of insulin to decrease the release of glycerol by adipose tissue seems unlikely in view of the present findings that insulin does not alter spontaneous glycerol release from the adipose tissue of a fasting rabbit either in the presence or absence of glucose.

Plasma glycerol might also be derived from the lipolysis of plasma lipids, whether this occurred intravascularly or within the tissue after uptake of the lipids and prior to storage (15). The known action of insulin to increase liproprotein lipase activity of adipose tissue both *in vivo* (16) and *in vitro* (16, 17) would be expected to increase uptake and lipolysis of plasma lipids, an action that could not explain the present

TABLE 2. THE EFFECT OF GLYCEROL INFUSION ON CONCENTRATION OF PLASMA GLYCEROL AND THE EFFECT OF INSULIN ON CONCENTRATION OF PLASMA GLYCEROL DURING A GLYCEROL INFUSION

Expt.	Rabbit		Glycerol Concentration in Plasma (μmoles per 100 ml)*												
			Time (min)												
	Wt.	Sex	−5	3	10	19	25	30	35	45	59	63	70	90	120
	kg														
Glycerol Infusion†	3.8	F	7.5	21.7	21.7	23.0		23.6		23.6	24.5	14.5	10.0	9.0	6.5
				(+14.2)	(+14.2)	(+15.5)		(+16.1)		(+16.1)	(+17.0)	(+7.0)	(+2.5)	(+1.5)	(−1.0)
	2.9	F	24.3	39.6	44.6	45.2	49.5		47.3	48.8	44.6	30.6	24.3	18.0	18.0
				(+15.1)	(+20.3)	(+20.9)	(+25.2)		(+23.0)	(+24.5)	(+20.3)	(+6.3)	(0)	(−6.3)	(−6.3)
	2.4	F	18.9	27.0	31.8	31.8	34.4		37.8	39.8	41.8	28.4	22.2	20.2	19.5
				(+8.1)	(+12.9)	(+12.9)	(+15.5)		(+18.9)	(+20.9)	(+22.9)	(+9.5)	(+3.3)	(+1.3)	(+0.6)
Insulin infusion during a glycerol infusion†‡	3.3	F	21.1	31.2	34.2	34.8	39.0		37.7	34.2	32.4	18.5	17.8	16.3	19.6
				(+10.1)	(+13.1)	(+13.7)	(+17.9)		(+16.6)	(+13.2)	(+11.3)	(−2.6)	(−3.3)	(−4.8)	(−1.5)
	2.5	F	13.2	20.8	25.8	27.0	25.8		31.8	27.6	24.6	15.0	9.1	9.1	12.1
				(+7.6)	(+12.6)	(+13.8)	(+12.6)		(+18.6)	(+14.4)	(+11.4)	(+1.8)	(−4.1)	(−4.1)	(−1.1)
	2.3	M	18.7	34.4	37.5	39.1	41.8		34.0	36.5	39.8	20.2	18.7	16.8	16.2
				(+15.7)	(+18.8)	(+20.4)	(+23.1)		(+15.3)	(+17.8)	(+21.1)	(+1.5)	(0)	(−1.9)	(−2.5)
Insulin injection during a glycerol infusion†§	4.5	M	38.0	60.0	70.0	69.5	60.0		77.0	77.0	81.0	51.5	53.0	60.0	43.0
				(+22.0)	(+32.0)	(+31.5)	(+22.0)		(+39.0)	(+39.0)	(+43.0)	(+13.5)	(+15.0)	(+22.0)	(+5.0)
	3.6	M	12.5	27.5	31.0	35.0	29.5		34.0	35.0	35.0	19.0	13.8	12.5	9.5
				(+15.0)	(+18.5)	(+22.5)	(+17.0)		(+21.5)	(+22.5)	(+22.5)	(+6.5)	(+1.3)	(0)	(−3.0)
	2.4	M	15.7	27.5	34.0	41.8	31.3		32.7	35.4	31.8	20.2	13.6	12.8	14.2
				(+11.8)	(+18.3)	(+26.1)	(+15.6)		(+17.0)	(+19.7)	(+16.1)	(+4.5)	(−2.1)	(−2.9)	(−1.5)

*The numbers in parentheses are the changes, in μmoles/100 ml of plasma, from the initial values.
† Glycerol was infused for 1 hr (0–60 min) at the rate of 0.0764 ml/min and 10 μmoles/kg/min.
‡ Insulin was infused for 40 min at the rate of 0.0388 ml/min and 0.01 unit/kg/min from the 20th to 60th min of the glycerol infusion.
§ Insulin was injected in a volume of 2 ml during 1 min at a concentration of 0.5 unit/kg at the 20th min of the glycerol infusion.

finding of a decrease in plasma glycerol concentration caused by insulin.

The contribution of lipolysis of dietary lipids to plasma glycerol is unknown. Recent evidence that such lipolysis might proceed only to the monoglyceride stage and be followed by reacylation to triglyceride (18) would exclude this as a major source of glycerol. Although some complete hydrolysis might occur, it seems unlikely that intermittent absorption from the intestine could maintain the observed constancy of the concentration of plasma glycerol. Whether insulin has any action on this source of glycerol or on the synthesis of glycerol by intestinal mucosa (10) is unknown.

The major fate of plasma glycerol is conversion to glucose and glycogen in the liver (19); glycerol can also be metabolized in kidney (20), in reticulocytes (21), and in mammary gland (22, 23). Glycerol is metabolized very rapidly *in vivo* (19), *in vitro* (24, 25), and in the perfused liver (26); most of the radioactivity of injected glycerol-C^{14} appears in carbon dioxide within 6 hr (19, 27). Phosphorylation to form α-glycerophosphate appears to be the initial reaction of glycerol metabolism (28); oxidation by glycerol dehydrogenase (29) does not appear to be a significant route of glycerol metabolism (30). An action of insulin to alter glycerol metabolism is uncertain. Although the distribution pattern of the products of glycerol metabolism is changed in livers from diabetic rats, the uptake of glycerol is not altered (25). The same incorporation of deuterio-glycerol into liver glycogen of normal and diabetic rats re-fed after a period of fasting has also been observed (31). In view of these results, an effect of insulin on the liver to increase glycerol utilization seems unlikely.

The experiments of Strack et al. (3) deserve mention because, under the conditions of their experiments, insulin caused an increase in concentration of plasma glycerol. They infused glycerol (intraduodenally or intravenously) for up to 32 hr so that plasma levels of glycerol were elevated to about 500 μmoles/100 ml or to about 2200 μmoles/100 ml. Injection of insulin (Novo) towards the end of such an infusion caused an increase in concentration of plasma glycerol. Also, the equilibrium glycerol concentration in the plasma reached during such an infusion in diabetic rabbits was lower than in normal rabbits. These authors concluded that insulin inhibits glycerol utilization by the liver. Other interpretations of these results are also possible. If the rate of removal of glycerol from plasma when high concentrations of glycerol are present is determined by the capacity of the liver to produce glucose, then an increased removal in diabetics and a decreased removal after insulin might be expected. Also, increased excretion of glycerol in the urine could partly account for the lower levels in the plasma of the diabetic rabbits. In the present experiments, the effect of insulin on the very low levels of glycerol normally present in plasma was studied. A limit to the capacity of the liver to handle glycerol is almost certainly not reached under these conditions and an action of insulin on glucose output would therefore be unlikely to influence the normal concentration of plasma glycerol.

In view of the many possible sources and fates of glycerol and the lack of evidence of an action of insulin on many of these sources or fates, it is not possible at the present time to come to any conclusion about the mechanism by which insulin lowers the concentration of glycerol in plasma.

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