Lack of Insulin Effect on Free Fatty Acid Mobilization Produced by Glucagon in Birds* (33639)

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Insulin suppresses the release of free fatty acids, (FFA) in rat adipose tissue *in vitro* and antagonizes the stimulation of lipolysis caused by epinephrine and ACTH in this preparation (1). Jungas and Ball (2) reported that insulin exerts an inhibitory influence upon the lipolytic effect of glucagon on the adipose tissue of the rat, but Fain *et al.* (3) found that small doses of insulin do not reduce the lipolytic action of glucagon on the isolated fat cells of the rat. The observation that injection of glucagon produces a marked elevation of plasma FFA in various avian species (4) gave an opportunity to study whether insulin injected *in vivo* influences the changes of FFA concentration produced by glucagon in birds.

Methods. Experimental animals and analytical methods used were as described in a previous report (4). Crystalline glucagon,[1] dissolved in 0.1 *M* glycine buffer (pH 9.5), was injected into a wing vein followed immediately by the injection of crystalline insulin,[2] dissolved in glycine buffer. Blood samples were taken before and at various intervals after injection for the determination of plasma FFA and blood sugar. All the animals had been fasting for 16–18 hr at the time of injection.

Results. The effects on plasma FFA concentration of glucagon alone (20.0 μg/kg) and of glucagon plus insulin are shown in Fig. 1. It is apparent that the changes in FFA concentration produced by the intravenous injection of glucagon were not modified by the administration of insulin at the doses of 0.5 or 2.0 U/kg.

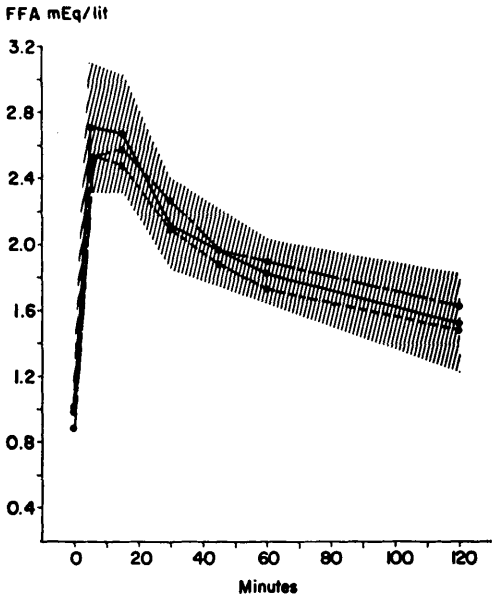

FIG. 1. Effect of glucagon alone and of glucagon and insulin on plasma free fatty acids in geese. The shaded area includes ± 3SE of the mean value for 17 geese injected with glucagon alone; abscissa: time after injection. (●—●) 17 geese, 20 μg glucagon/kg; (●- - - -●) 6 geese 20 μg glucagon + 2.0 U insulin/kg; (●— - —●) 6 geese 20 μg glucagon + 0.5 U insulin/kg.

The effect of insulin on the blood sugar changes produced by injection of glucagon is presented in Fig. 2. In contrast with the lack of effect of insulin on the changes of FFA caused by the injection of glucagon, Fig. 2 shows that insulin counteracted the hyperglycemic effect of glucagon. When 2.0 U of insulin/kg were injected, the hyperglycemic effect of glucagon was practically abolished and marked hypoglycemia was produced.

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[1] Crystalline beef–pork glucagon, lot 258-234 B-167-1, Eli Lilly and Co., Indianapolis, Indiana; kindly supplied by Drs. W. W. Bromer and W. N. Shaw.

[2] Crystalline beef insulin, lot P-4609, 23.8 U/mg and less than 0.0003% glucagon, Eli Lilly and Co., Indianapolis, Indiana; kindly supplied by Dr. W. N. Shaw.

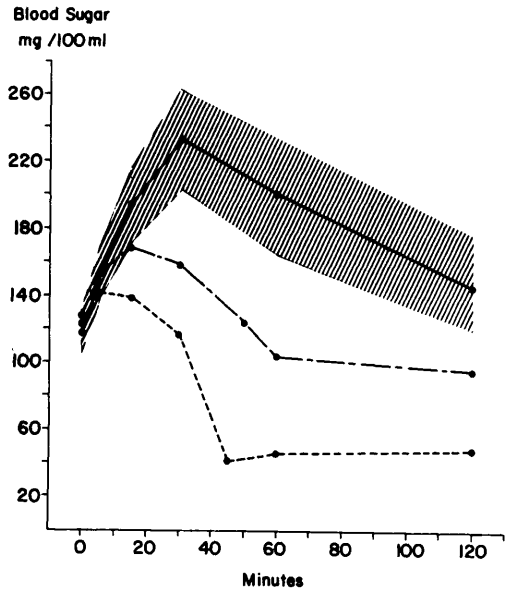

FIG. 2. Effect of glucagon alone and glucagon plus insulin on blood sugar in geese. The shaded area includes ± 3 SE of the mean values for 17 geese injected with glucagon alone; abscissa: time after injection. (●—●) 17 geese 20 μg glucagon/kg; (●- - - -●) 6 geese 20 μg glucagon + 2.0 U insulin /kg ●— - —● 6 geese 20 μg glucagon + 0.5 U insulin/kg.

Similar experiments were performed with 2 Peking ducks and 2 leghorn roosters injecting 20.0 μg of glucagon and 2.0 U of insulin/kg, with identical results as observed in geese.

In view of the failure of insulin to prevent the elevation of plasma FFA produced by glucagon injection, the effect of insulin alone was tested in geese using doses of 0.1, 0.5, and 2.0 U/kg. The results obtained in 8 geese with the dose of 2.0 U/kg are presented in Table I. The data indicate that insulin alone caused the expected decrease of blood sugar, but no significant decrease of plasma FFA concentration. A slight increase of FFA concentration was noted at 30, 45, and 120 min after the injection.

Discussion. The results reported indicate that injection of insulin at doses of 0.5 and 2.0 U/kg does not prevent the lipolytic effect of glucagon in birds. This finding is in agreement with the observations on the isolated fat cells of the rat reported by Fain *et al.* (3), but at variance with the results reported for the adipose tissue of the rat *in vitro* (1, 2, 5). Administration of insulin is known to cause a rapid and marked decrease of FFA concentration in several mammalian species, and this effect can be obtained with small doses of insulin not having any appreciable effect on blood sugar concentration (6). In contrast, our observations indicate that a dose of insulin which produced a marked decrease of blood sugar failed to lower the concentration of plasma FFA in the goose. Bierman *et al.* (7) showed that the decrease of plasma FFA concentration produced by insulin in the dog and the rabbit, is due to decreased release of fatty acids from tissue stores, and not to an increased removal from the blood. Spitzer and Hohenleitner (8) showed that insulin inhibits the release of

TABLE I. Effect of Intravenous Injection of Crystalline Beef Insulin (2.0 U/kg) on Plasma FFA and Blood Sugar in Geese.[a]

		Control		Time after injection (min)					
		1[b]	2[c]	5	15	30	45	60	120
FFA (meq/liter)	Mean	0.97	0.98	0.89	0.90	1.10	1.09	0.98	1.24
	SE	0.073	0.074	0.101	0.110	0.089	0.072	0.065	0.093
	p			0.15	0.12	0.02	0.025	—	0.03
Blood sugar	Mean	123	125	115	74	52	47	47	51
(mg/100 ml)	SE	3.3	3.5	3.6	3.7	3.5	3.6	3.5	3.6
	p			0.025	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] Means, SE, and probability of chance occurrence (*p*) of differences with mean control value, for 8 birds.

[b] Control sample taken 30 min before injection.

[c] Control sample taken just before injection.

FFA by the adipose tissue of the dog *in vivo*. Accordingly, our results may be interpreted as indicating that insulin has no inhibitory effect on the release of fatty acids by the avian adipose tissue. Such view is supported by the observation of Goodridge and Ball (9) that insulin has no effect on the spontaneous or epinephrine-stimulated rate of lipolysis in pigeon adipose tissue *in vitro*, regardless of the presence or absence of glucose. It appears therefore that insulin does not inhibit the release of fatty acids from the adipose tissue of certain avian species, *in vitro* or *in vivo*. Injection of insulin produced a very small, but significant, elevation of plasma FFA concentration which was noticed first 30 min after the injection. Heald *et al.* (11) reported an elevation of the plasma FFA level after injection of insulin in the domestic fowl and suggested that such an elevation may be related to release of glucagon from the pancreas, induced by the injection of insulin. Our results, as well as those reported by Goodridge and Ball (9) and by Heald *et al.* (11) indicate that the adipose tissue of various avian species is insensitive to the antilipolytic effect of insulin. However, since these results were obtained with insulins of mammalian origin (beef and pork) the possibility that avian insulin may have a different effect shall be considered.

Summary. Intravenous injection of insulin (2.0 U/kg) did not have any effect on the elevation of plasma FFA concentration produced by the injection of 20.0 μg/kg of glucagon in geese, ducks, and roosters. Intravenous injection of insulin (2.0 U/kg) in geese caused marked hypoglycemia, but no decrease of plasma FFA concentration.

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