

## Effects of Glucagon and Insulin on Plasma Free Fatty Acids and Blood Sugar in Owls\* (34514)

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Crystalline glucagon of mammalian origin produces marked elevations of plasma free fatty acids (FFA) and blood sugar (BS) when injected intravenously into a number of avian species (1, 2, 3) and stimulates the release of FFA when added *in vitro* to the adipose tissue of the sparrow, the pigeon, and the domestic fowl (4, 5, 6). Birds appear to be less sensitive to the hypoglycemic effect of mammalian insulin than to that of insulin of avian origin (7), but mammalian insulin, at doses which produced a marked decrease of BS, did not cause a decrease of plasma FFA in chickens and geese (1, 8, 9), in contrast with its effect in man, dog and rabbit (10, 11, 12). Moreover, mammalian insulin does not prevent the elevation of plasma FFA caused in birds by the injection of glucagon (9) and it has no effect on the spontaneous or epinephrine-induced rate of lipolysis of pigeon's adipose tissue *in vitro* (5). Since these studies of the effects of glucagon and insulin on plasma FFA in birds have been made with grain-eating species, and it is known that grain-eating and carnivorous birds have some metabolic differences, it was considered of interest to examine the effects of glucagon and insulin on the plasma FFA and BS of a carnivorous bird.

The owl was selected for the studies reported here. This carnivorous bird develops marked hyperglycemia and ketosis upon removal of the pancreas (13), in contrast with a number of grain-eating birds that do not develop full manifestations of chronic diabetes when their pancreas is removed (14).

**Methods.** Eighteen horned owls (males and

females) weighing 1.2 to 1.9 kg were used in the experiments. They were kept in individual cages in an air-conditioned room (22-23°), lighted for 10 hr every day and in the evening they were fed *ad libitum* daily with fresh meat. The animals were weighed periodically and were used after it was established that their body weight was constant for at least 3 weeks. No food was given the evening before the experiments.

Injections of crystalline glucagon,<sup>1</sup> or crystalline insulin,<sup>2</sup> both dissolved in 0.02 M glycine buffer, pH 9.5 in saline, were made into a wing vein. Blood samples were taken from the wing veins at various periods of time before and after the injections. The blood was collected into Corex centrifuge tubes containing heparin powder and kept in an ice water bath. After removing an aliquot for BS determination, the samples were centrifuged in a refrigerated centrifuge (-2°) and the plasma was separated for FFA determination. BS was determined by Nelson's modification of Somogyi's method (15).

Plasma FFA were determined by the method of Trout *et al.* (16) as modified by Davis (17).

**Results.** The effect of injecting 50.0 µg of glucagon/kg intravenously is presented in Table I. Glucagon caused a prompt and significant elevation of plasma FFA that reached a maximum 15 min after injection. At that time, the mean plasma FFA level was 335% of the mean control value. There was

<sup>1</sup> 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.

<sup>2</sup> Crystalline beef insulin, lot P-4609, 23.8 U/mg and < 0.0003 % glucagon, Eli Lilly and Co., Indianapolis, Indiana. Kindly supplied by Dr. W. N. Shaw.

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TABLE I. Effect of Glucagon (50.0  $\mu\text{g}/\text{kg}$  iv) on Plasma Free Fatty Acids and Blood Sugar in Owls (Means and SE for 8 Owls).

Control samples		Minutes after injection			
C1 <sup>a</sup>	C2 <sup>b</sup>	5	15	30	60
Free fatty acids (meq/liter)					
0.42 $\pm$ 0.06	0.44 $\pm$ 0.06	1.08 $\pm$ 0.09	1.44 $\pm$ 0.10	1.38 $\pm$ 0.13	1.01 $\pm$ 0.13
		0.65 $\pm$ 0.08 <sup>c</sup>	1.01 $\pm$ 0.09 <sup>c</sup>	0.95 $\pm$ 0.12 <sup>c</sup>	0.58 $\pm$ 0.12 <sup>c</sup>
Blood sugar (mg/100 ml)					
236 $\pm$ 10	238 $\pm$ 10	256 $\pm$ 9	301 $\pm$ 8	335 $\pm$ 8	355 $\pm$ 12
		19 $\pm$ 3 <sup>c</sup>	64 $\pm$ 5 <sup>c</sup>	98 $\pm$ 6 <sup>c</sup>	118 $\pm$ 8 <sup>c</sup>

<sup>a</sup> Control sample taken 30 minutes before glucagon injection.

<sup>b</sup> Control sample taken just before glucagon injection.

<sup>c</sup> Difference: sample minus mean control;  $p < 0.01$ .

also a significant elevation of BS. The maximum value was 150% of the mean control level, 60 min after the injection.

Injection of insulin (2.0 U/kg iv) caused no significant change of plasma FFA concentration but produced a significant decrease of BS 30 and 60 min after injection, as shown in Table II.

*Discussion.* The results presented in Table I show that the owl, a carnivorous bird, responds to the intravenous injection of crystalline glucagon of mammalian origin with elevations of plasma FFA and BS comparable to those observed in various species of grain-eating birds (1, 2, 3). The relative elevation of plasma FFA in the owl was similar to that observed in this Laboratory in geese receiving the same amount of glucagon. In absolute

terms, however, the elevation of plasma FFA produced in the owl by 50.0  $\mu\text{g}$  of glucagon/kg was similar to that observed in turkeys with 10.0  $\mu\text{g}/\text{kg}$  of glucagon (2). It is of interest to note that the fasting (pre-injection) levels of FFA and BS of turkeys and owls are practically identical. Fasting geese and ducks have higher FFA and lower BS than owls and turkeys (2), whereas mature chickens have high fasting BS, like owls and turkeys, and high fasting FFA, like geese and ducks (1, 8). As observed in other avian species, the hyperglycemic response in the owl to the administration of glucagon was, in relative terms, smaller and slower in developing than was the elevation of plasma FFA (2). Injection of insulin (2.0 U/kg) produced significant hypoglycemia, but failed

TABLE II. Effect of Insulin (2.0 U/kg iv) on Plasma Free Fatty Acids and Blood Sugar in Owls (Means and SE for 10 Owls).

Control samples		Minutes after injection			
C1 <sup>a</sup>	C2 <sup>b</sup>	5	15	30	60
Free fatty acids (meq/liter)					
0.42 $\pm$ 0.05	0.43 $\pm$ 0.05	0.35 $\pm$ 0.05	0.40 $\pm$ 0.06	0.40 $\pm$ 0.06	0.34 $\pm$ 0.05
		-0.07 $\pm$ 0.06 <sup>c</sup>	-0.02 $\pm$ 0.06 <sup>c</sup>	-0.02 $\pm$ 0.06 <sup>c</sup>	-0.08 $\pm$ 0.05 <sup>c</sup>
Blood sugar (mg/100 ml)					
235 $\pm$ 13	234 $\pm$ 15	244 $\pm$ 16	227 $\pm$ 15	190 $\pm$ 14	152 $\pm$ 17
		10 $\pm$ 9 <sup>c</sup>	-7 $\pm$ 9 <sup>c</sup>	-44 $\pm$ 9 <sup>d</sup>	-82 $\pm$ 10 <sup>d</sup>

<sup>a</sup> Control sample taken 30 minutes before glucagon injection.

<sup>b</sup> Control sample taken just before glucagon injection.

<sup>c</sup> Difference: sample minus mean control;  $p > 0.05$ .

<sup>d</sup> Difference: sample minus mean control;  $p < 0.01$ .

to cause any significant change of plasma FFA concentration in the owl, as shown in Table II. The same dose of insulin in geese caused only a very small elevation of plasma FFA at 30, 45 and 120 min after injection (9). The results obtained in the owl are in variance with observations in chicken reported by Heald *et al.* and by Lepkovsky *et al.* Heald *et al.* (1) noted a marked elevation of plasma FFA in both mature and immature chickens treated with 1.0 to 1.5 U/kg of crystalline, glucagon-free insulin *iv.* The maximum FFA elevation was observed 1 hr after the injection. Lepkovsky *et al.* (8) noted a significant elevation of plasma FFA in adult chickens 1 hr after the intramuscular injection of 5.0 U of insulin/kg. Since the elevation of plasma FFA was also observed after the injection of glucagon-free insulin and when insulin was injected into pancrectomized chicken, Lepkovsky *et al.* concluded that the elevation of plasma FFA was caused by insulin *per se.* It appears, therefore, that insulin elevates plasma FFA in some birds, like the chicken, but not in others, like the owl. There is, however, no indication that in birds insulin causes a decrease of plasma FFA similar to that well-documented in various mammalian species, in which the reduction in plasma FFA occurs with doses of insulin having no appreciable hypoglycemic effect (10, 11). The decrease of plasma FFA produced by insulin in mammals is considered to be mainly due to a decreased release of fatty acids from tissue stores (12, 18, 19); consequently, these observations would indicate that insulin has no inhibitory effect on the release of FFA by the avian adipose tissue *in vivo.* This is in agreement with the lack of effect of insulin on the rate of lipolysis by the pigeon's adipose tissue *in vitro,* reported by Goodridge and Ball (5).

The BS decrease observed in the owl 1 hr after the intravenous injection of 2.0 U of insulin/kg was comparable, in absolute terms, to that observed in geese receiving the same dose of insulin. In relative terms, however, the blood sugar level 1 hr after the injection was 65% of control in the owl and 38% in the goose (9).

Because all these studies were made with insulin of mammalian origin, it is pertinent to ask whether the lack of inhibition of FFA release reflects differences in activity between avian and mammalian insulins, rather than metabolic differences between the adipose tissues of these two classes of vertebrates. Hazelwood *et al.* (7) have recently demonstrated in chickens that 0.02 U/kg of chicken insulin produces a hypoglycemic response equal to that obtained with a 10-fold greater dose of beef insulin, but there is no information regarding the relative effects of avian and mammalian insulins on the plasma FFA of birds and mammals.

*Summary.* Intravenous injection of crystalline glucagon (50.0  $\mu\text{g}/\text{kg}$ ) of mammalian origin in owls caused significant elevations of plasma FFA and BS, results similar to those observed in other avian species. Intravenous injection of crystalline beef insulin (2.0 U/kg) in owls caused marked hypoglycemia, but no significant change of plasma FFA concentration.

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