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Calorigenic Actions of Oleic Acid and Glucagon Infusions in Anesthetized Dogs[1] (38284)

PHILIP C. WEISER[2] AND FRANCISCO GRANDE

Jay Phillips Research Laboratory, Mount Sinai Hospital, Minneapolis, Minnesota 55404; and Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minnesota 55455

We have reported that glucagon has a greater calorigenic action than epinephrine in the dog (1). Such an effect cannot be ascribed to free fatty acid (FFA) mobilization because glucagon does not stimulate the *in vitro* lipolytic activity of adipose tissue of the dog (2, 3) and causes only a minimal elevation, followed by a marked decrease, of plasma FFA concentration when injected into this animal (4, 5).

It has been proposed (6) that the calorigenic effect of the catecholamines is due to increased fatty acid oxidation consecutive to the increase of plasma FFA concentration produced by these hormones. However, the fact that nicotinic acid completely suppresses the adipokinetic effect of norepinephrine but only partially inhibits its calorigenic effect (7) indicates that no more than a part of the calorigenic effect depends on FFA mobilization (8). On the other hand, elevation of the concentration of albumin-bound fatty acids in the perfusion fluid increased the oxygen consumption of the isolated rat heart (9). The increase in oxidation of fatty acids accounted for at least 73% of the increment in oxygen consumption.

It was therefore of interest to investigate whether an elevation of plasma FFA concentration produced by continuous infusion of a fatty acid in the dog has a calorigenic effect in the whole animal, and to determine whether the calorigenic effect of glucagon is enhanced when this hormone is administered together with the fatty acid infusion.

Methods. Four groups of 6 mongrel dogs (male and female), weighing between 16 and 23 kg (mean wt 19.3 kg), were studied. The dogs were housed in individual cages in an air-conditioned room (22–23°) and were fed a commercial low-fat dog food (Kibbies, Morton Dog Food Co., Minneapolis, MN), *ad libitum*. All experiments were done after 16–18 hr of fasting.

Anesthesia was induced by injection of sodium pentobarbital (Diabutal, Diamond Laboratories, Inc., Des Moines, IO; 26 mg/kg iv). After anesthesia, PE 60 catheters (Intramedic, Clay Adams, Inc., New York) were inserted into the femoral veins for the infusions and into the femoral artery for withdrawal of blood samples. Periodic injections of sodium pentobarbital were given iv to maintain anesthesia.

Oxygen uptake ws monitored with a recording metabolic apparatus connected through a two-way valve with a tracheal tube, as previously described (1).

A 0.4 *M* emulsion of oleic acid in sodium oleate (0.35 *M* oleic acid, 0.05 *M* oleate) containing 1% gelatin was prepared as described (10). Just prior to the infusion, the emulsion was diluted with saline according to the weight of the animal in order to deliver 1.0 mmole of oleic acid/kg/hr, at an infusion rate of 114.6 ml/hr.

Appropriate dilutions of crystalline glucagon (lot 258-234B-167-1, Eli Lilly Co., Indianapolis, IN) in glycine buffer (0.1 *M* pH 9.5) were prepared daily just before the beginning of the infusions.

Infusions were made with syringe infusion pumps (model 600-910, Harvard Apparatus Co., Dover, MA). The first group of dogs was infused with oleic acid (1.0 mmole/kg/hr) for 1 hr, and with a volume of glycine buffer equal to that used for the glucagon infusion, for the last

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[2] Present address: Cardiovascular Pulmonary Research Laboratory, University of Colorado Medical Center, Denver, Colorado 80220.

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30 min of the oleic acid infusion. The second group was infused for 1 hr with a solution of gelatin in saline containing the same amount of gelatin as the oleic acid emulsion, and with glucagon (2.0 μg/kg/min) for the last 30 min of the gelatin infusion. The third group of dogs received oleic acid (1.0 mmole/kg/hr) for 1 hr, and glucagon (2.0 μg/kg/min) for the last 30 min of the oleic acid infusion. Finally, the fourth group (control) received gelatin infusion for 1 hr and glycine buffer for the last 30 min of the gelatin infusion.

Arterial blood was collected in Corex centrifuge tubes with heparin powder and kept in an ice bath. After taking aliquots for blood sugar determination, the samples were centrifuged in a refrigerated centrifuge (−2.0°) and the plasma was separated.

Plasma FFA were measured by the method of Trout *et al.* (11) using 0.02 *N* NaOH in isopropanol for the titration, as described by Davis (12). Blood sugar was determined according to Nelson (13).

The data were compared by analysis of variance unless stated otherwise. Comparisons between treatment means were performed by using appropriate Newman–Keul procedures (14). Differences were considered significant when $P < 0.05$.

Results. Oxygen uptake. Figure 1 shows the changes of oxygen uptake for each group of dogs. The mean oxygen consumption values for the half an hour before the infusion for the four groups, reported in Table I, were not significantly different from each other. The oxygen consumption of the control group showed some increase during the infusion, but the mean value at the end of the period of observation was not significantly different from the preinfusion or infusion values for this group.

Infusion of oleic acid caused an elevation of oxygen consumption within 6 min of the beginning of the infusion in all the animals. Thereafter the oxygen consumption increased slowly with a peak after 48 min of infusion. Glucagon infusion caused an elevation of oxygen consumption with a peak 6 min after the end of the infusion. Infusion of glucagon during the infusion of oleic acid caused an elevation of oxygen consumption which corresponds roughly to the sum of those produced by each of these infusions given separately. The calorigenic effect was estimated by calculating the excess oxygen consumption above the mean preinfusion level, during the

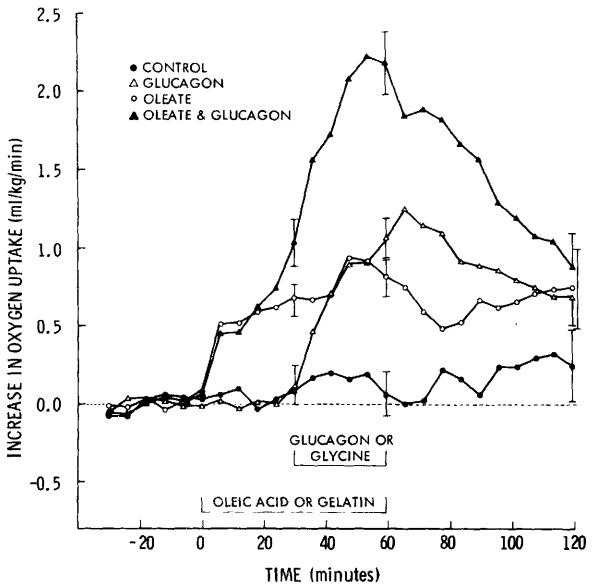

FIG. 1. Effect of oleate infusion (1.0 meq/kg/min for 1 hr) and/or glucagon infusion (2.0 μg/kg/min for 30 min) in anesthetized dogs. Points represent differences between the observed values and the mean of corresponding values obtained during the preinfusion period. Mean (±SE) preinfusion oxygen uptakes given in Table I. Control (•), glucagon only (Δ), oleate only (○), and oleate plus glucagon (▲). Ordinate: ΔVO_2, oxygen consumption differences (ml O_2/kg/min). Abscissa: Time in minutes. Means ± SE for 6 dogs in each group.

TABLE I. Oxygen Uptake Before Infusion and Excess Oxygen Uptake During and After Infusion for the Four Groups of Dogs. Excess Oxygen Uptake is Given as ml/kg Above the Mean Pre-infusion Value, for the Duration of the Corresponding Period.

	Infusion given			Excess oxygen uptake (ml/kg)			
Group	0–60 min	30–60 min	Pre-infusion oxygen uptake (ml/kg/min)	0–30 min	30–60 min	60–120 min	0–120 min
1	Oleic acid	Glycine	6.03[c] ± 0.43	14.5[a] ± 1.6	23.2[a] ± 1.5	38.3[a] ± 9.5	76.0[a] ± 10.8
2	Gelatin	Glucagon	5.73 ± 0.17	0.3 ± 1.5	26.0[a] ± 5.0	67.1[a] ± 9.8	93.4[a] ± 12.0
3	Oleic acid	Glucagon	6.23 ± 0.31	17.6[a] ± 3.5	55.4[a,b] ± 5.3	89.2[a,b] ± 14.8	162.2[a,b] ± 22.7
4 (control)	Gelatin	Glycine	6.43 ± 0.39	− 0.3 ± 0.4	3.8 ± 1.8	10.2 ± 12.1	13.7 ± 14.1

[a] Significantly greater ($P < 0.05$) than corresponding control value.
[b] Significantly greater ($P < 0.05$) than corresponding values for groups 1 (oleic acid) and 2 (glucagon).
[c] Means ± SE for 6 dogs in each group

infusion (0–30 min and 30–60 min) and postinfusion (60–120 min) periods.

Excess oxygen uptakes for the infusion and postinfusion periods are given in Table I. For the 30–60-min infusion period, both oleic acid and glucagon given separately elevated oxygen uptake. The elevations were significantly greater than that observed in the control group. The infusion of both oleic acid and glucagon caused significantly greater elevations of oxygen uptake than either of the infusions given separately, and somewhat greater than the sum of their separate effects. Analysis of variance, however, revealed no significant interaction between these effects, indicating that they are simply additive.

Analysis of variance for the total period of observation (0–120 min) showed significant effects of oleic acid (F (1, 20) = 17.79) and glucagon (F (1, 20) = 27.81), but again the interaction term was not significant (F (1, 20) = 0.03). The data indicate therefore that glucagon increases oxygen uptake to the same extent when infused with gelatin and when infused with oleic acid.

Plasma FFA. The changes in plasma FFA concentration are shown in Fig. 2. The mean FFA levels for the preinfusion period for the four groups of dogs were not statistically different from each other.

Infusion of oleic acid produced a rapid and marked elevation of plasma FFA concentration. The mean elevation for the 0–30-min infusion period was 167% above mean preinfusion level for the group infused with oleic acid alone and 190% for the group infused with oleic acid and glucagon. The difference between these elevations was statistically significant. The FFA level for the group infused with glucagon alone decreased slightly and was significantly lower than that of the control group at 30 min. No significant changes of FFA were observed in the control group.

Since the infusion of oleic acid was associated with an increase in oxygen uptake, the correlation between the increases in FFA (ΔFFA) and oxygen uptake (ΔVO_2) after 30 min of infusion was calculated using the data of the 12 dogs in groups 1 and 3 and those of 5 other dogs infused with higher doses of oleic acid (1.6 – 4.0 mmoles/kg/hr). The correlation was highly significant ($r = 0.91$, $N = 17$, $P < 0.01$). The relationship between ΔFFA and ΔVO_2 is described by the regression equation:

$$\Delta VO_2 \text{ (ml/kg/min)} = 1.56\ \Delta\text{FFA (meq/liter)} - 0.52$$

Blood sugar. The blood sugar changes are shown in Fig. 3. There were no significant changes during infusion in the control group. Infusion of oleate caused a significant decrease of blood sugar which was significant between 40 and 70 min. Glucagon produced marked elevation of blood sugar both when infused alone and when given during the infusion of oleic acid. The

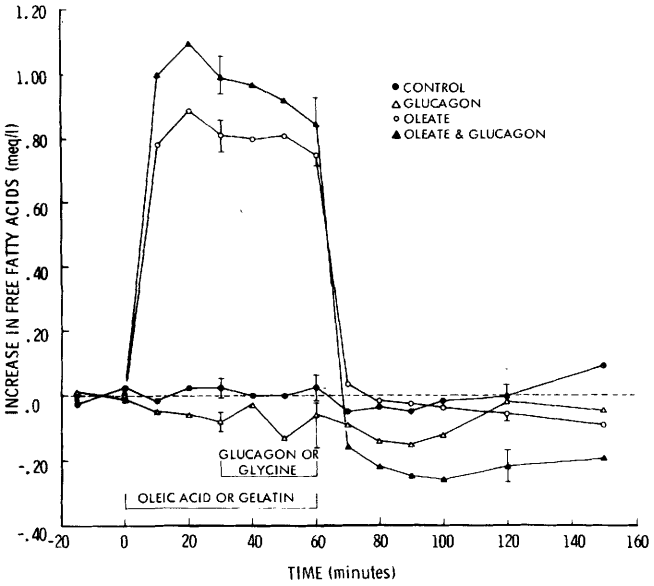

FIG. 2. Effect of oleate infusion and/or glucagon infusion on plasma free fatty acids (FFA) in anesthetized dogs. Symbols and points as in Fig. 1. Mean preinfusion FFA values were 0.53 ± 0.05, 0.52 ± 0.09, 0.48 ± 0.06, and 0.55 ± 0.06 meq/liter for the control, glucagon only, oleate only, and oleate plus glucagon groups, respectively. Ordinate: Increase in free fatty acids (meq/liter). Abscissa: Time in minutes. Means ± SE for 6 dogs in each group.

mean elevations at 60 min were 76 and 88% above the mean preinfusion levels for the group infused with glucagon and for the group infused with both oleic acid and glucagon, respectively.

Discussion. The infusion of oleic acid caused significant increases of oxygen uptake which were closely correlated with the corresponding elevations of plasma FFA. To our knowledge,

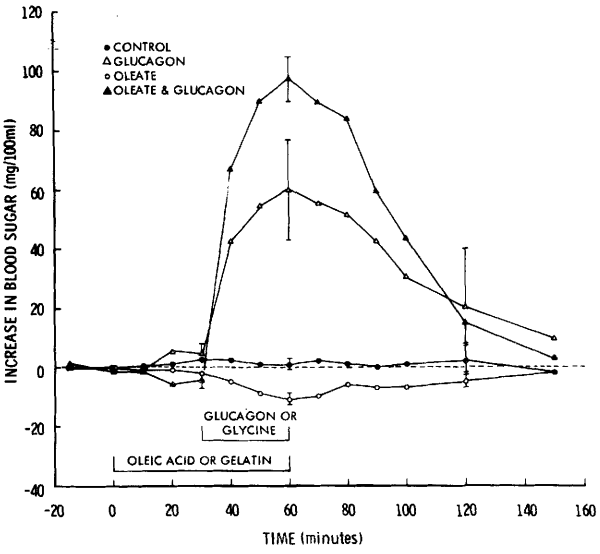

FIG. 3. Effect of oleate infusion and/or glucagon infusion on blood sugar in anesthetized dogs. Symbols and points as in Fig. 1. Mean preinfusion blood sugars were 76 ± 4, 79 ± 4, 67 ± 4, and 89 ± 3 mg/100 ml for the control, glucagon only, oleate only, and oleate plus glucagon groups, respectively. Ordinate: Increase in blood sugar (mg/100 ml). Abscissa: Time in minutes. Means ± SE for 6 dogs in each group.

the present data are the first to show that the infusion of a fatty acid has a calorigenic action in the anesthetized dog. These results, therefore, support the view that the calorigenic action of the adipokinetic hormones may be due in part to increased oxidation of fatty acids consecutive to the elevation of plasma FFA levels (6–9). However, the calorigenic action noted was small. The excess oxygen uptake for the 2 hr of observation corresponds to 10% of the oxygen uptake before infusion. It can be calculated that the total excess oxygen uptake for the 2 hr of observation can only account for the oxidation of some 14% of the oleic acid infused. The complete oxidation of the infused oleic acid, on the other hand, would require about 70% of the total oxygen consumed by the animals in the 2 hr period.

The present results confirm our previous observations regarding the calorigenic action of glucagon in the dog (1) and show that the calorigenic action of this hormone is independent of the concentration of plasma FFA. This suggests different mechanisms for the calorigenic effects of elevated plasma FFA and glucagon infusion.

In our previous report (1) we have suggested that the calorigenic action of glucagon may be due to the stimulation of the oxidation of long-chain fatty acids by the liver. The present findings are compatible with this suggestion because the effect of glucagon on the hepatic concentration of metabolites related to fatty acid oxidation seems to be independent of plasma FFA concentration (15, 16). The infusion of oleic acid produced a decrease of blood sugar concentration comparable to that observed by others (17, 18) using a different technique for the infusion of fatty acids in dogs.

Summary. Infusion of oleic acid at the rate of 1.0 mmole/kg/hr for 1 hr produced significant elevations of oxygen uptake in anesthetized dogs. The elevations of oxygen uptake (ml O_2/kg/min) after 30 min of infusion showed a high correlation with the corresponding elevations of plasma FFA ($r = 0.91$, $N = 17$, $P < 0.01$). Glucagon infusion produced elevation of oxygen uptake, as previously described. The increase in oxygen uptake produced by glucagon was the same when the hormone was infused alone and when it was infused during the infusion of oleic acid. Statistical analysis showed no interaction between the calorigenic effects of oleic acid and glucagon infusions. It is concluded that the calorigenic actions produced by infusing oleic acid and glucagon are additive and mediated by independent mechanisms.

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