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Release of free fatty acids by adipose tissue *in vivo**

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SUMMARY

A technique is described to determine changes in plasma FFA on passage through adipose tissue *in vivo*. The subcutaneous adipose tissue of the abdominal region was shown to be constantly releasing FFA. After the injection of insulin, mobilization of FFA was depressed in normal as well as in diabetic dogs, but seldom was there any evidence of FFA uptake. Lymph infusion from fat-absorbing donors did not seem to influence the release of FFA by adipose tissue in the recipient animals.

In the past few years considerable effort has been directed to the study of metabolic behavior of adipose tissue. It has been shown that adipose tissue is capable of releasing or taking up fatty acids, depending on the experimental conditions, and that its metabolic activity is influenced by certain hormones *in vitro* (1, 2, 3). While there is little difficulty in obtaining isolated adipose tissue for studies *in vitro*, experiments *in vivo* have been far more limited, and have been restricted largely to sampling of veins which drain, along with adipose tissue, large amounts of muscle or visceral organs (4, 5). The present study is an attempt to provide information concerning the nature of plasma FFA transport through adipose tissue *in vivo* under various conditions. It concerns FFA mobilization in fasting and fat-absorbing animals, as well as the influence of insulin in normal and diabetic animals.

MATERIALS AND METHODS

Mongrel dogs weighing 11 to 24 kgs, and fasted 18 to 20 hours, were used under pentobarbital anesthesia. Arterial and venous samples were obtained through cannulae placed in a side branch of the femoral artery (sample A) and femoral vein (sample V). To obtain samples of blood passing through the subcutaneous adipose tissue of the abdominal region, the inferior epigastric vein was prepared by ligating the vein draining the rectus abdominis muscle, and passing a cannula through the latter vein to its junction with the inferior epigastric vein (sample E). Thus, sample E represented blood coming from the adipose tissue with some contribution from the skin. No muscular drainage could be visibly detected in this sample. Blood flow from the adipose tissue was not interfered with, except during sampling. It was necessary to recannulate this vessel for almost each sampling, to avoid intravascular clotting, since no anticoagulant was administered to the animals in order to preserve physiological conditions as much as possible. Backflow of blood from the femoral vein into the sampled vein was prevented by the valves of the latter vessel, or, if required, by means of a temporary ligature passed around the inferior epigastric vein at its juncture with the femoral vein. In animals in which cannulation of the vein was impossible, samples were obtained by direct venipuncture with a #21 needle. Simultaneous blood samples of an artery, femoral vein, and inferior epigastric vein were withdrawn into syringes at intervals, and immediately transferred to cold, oxalated test tubes and refrigerated at 3°. On each sample, duplicate determinations of FFA were performed by Dole's method (6).

Insulin (0.5 to 1.2 units/kg) was administered intravenously, and arterial glucose changes were followed, using Nelson's colorimetric adaptation of Somogyi's method (7). Seven dogs were made diabetic by an intravenous injection of alloxan (0.75 mg/kg). The dogs were used 48 hours later, after showing marked glycosuria.

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The effect of fat absorption was studied by employing two dogs for each of three experiments. The donor was fed 20 ml triolein, and the recipient was simultaneously anesthetized. Control samples from the recipient animal were taken several hours later, and shortly before infusion of chyle. The donor dog was anesthetized 3 hours after feeding, and its thoracic duct was cannulated. The distal end of this cannula was inserted into the jugular vein of the recipient dog, and chyle was permitted to flow in this manner for 90 minutes or more, with blood samples being drawn from the recipient dog at intervals. FFA, glucose, and esterified fatty acids were determined in the plasma samples.

TABLE 1. STUDIES ON NORMAL DOGS

Dog and Sample No.	Time	Sample A	Sample V	Sample E	E − A
	min				
M 2—1	0	0.673	0.635	0.726	0.053
2	20	0.715	0.811	0.781	0.066
M 3—1	0	0.536	0.963	0.899	0.363
2	10	0.564	1.065	0.959	0.395
3	20	0.565	0.788	0.886	0.321
4	35	0.556		0.802	0.246
5	45	0.504	0.848	0.788	0.284
M 4—1	0	0.538	0.610	0.574	0.036
2	15	0.558	0.611	0.482	−0.076
3	45	0.445	0.542	0.438	−0.007
F 5—1	0	0.636	1.170	0.896	0.260
2	20	0.860	1.300	1.200	0.340
3	50	0.706	1.480	0.966	0.260
F 6—1	0	0.720	0.966	1.277	0.557
M 7—1	0	0.377	0.556	0.691	0.314
M 8—1	0	0.676	0.720	0.919	0.243
M 9—1	0	1.266	1.369	1.556	0.290
M 10—1	0	1.380	1.625	1.690	0.310
F 11—1	0	0.331	0.429	0.430	0.099
2	15	0.340	0.425	0.462	0.112
M 12—1	0	0.230	0.258	0.328	0.098
2	12	0.223	0.213	0.297	0.074
M 13—1	0	0.763	0.830	0.950	0.187
2	10	0.798	0.888	0.910	0.112
3	32	0.936	0.975	1.044	0.108
M 14—1	0	0.316	0.350	0.386	0.070
2	10	0.282	0.319	0.365	0.083
F 20—1	0	0.379	0.493	0.937	0.558
2	5	0.365	0.538	1.029	0.664
3	100	0.341	0.378	0.974	0.633
F 24—1	0	0.756	0.900	0.896	0.140
2	40	0.589	0.695	0.718	0.129
3	82	0.579	0.694	0.696	0.118
M 25—1	0	0.264	0.332	0.596	0.332
2	3	0.279	0.332	0.550	0.271
F 27—1	0	0.281	0.232	0.293	0.012
2	105	0.179	0.254	0.244	0.065

All FFA values in mEq/liter.

It should be noted that dog #20 had milk-laden *post partum* mammary glands, and these tissues were drained by the inferior epigastric vein.

RESULTS

Table 1 shows a quite consistent release of FFA by the adipose tissue, i.e., higher concentrations in the epigastric vein than in the artery. The magnitude of this release equals about 43% of the arterial concentration (calculation based on the first sample of each animal).

After administration of insulin, the arterial FFA level often decreased within 10 minutes after injection, and frequently rather markedly. The average decrease in epigastric vein−arterial (E−A) FFA difference after insulin was 0.179 mEq/liter, with a standard error of 0.05. In six out of the eight dogs the E−A difference decreased within 25 minutes after injection. In the two remaining dogs there was no apparent cessation or near cessation of FFA release from the subcutaneous adipose tissue at some time within 25 minutes after insulin, and in one of these, a negative E−A difference was observed for the same tissue. In about half of the experiments an uptake of glucose by the adipose tissue was found after the administration of insulin. One typical experiment is shown in Figure 1.

As expected (8), the diabetic dogs had, statistically, significantly higher arterial FFA concentrations (mean = 1.116) than the normal dogs used in this study (mean = 0.566). In all samples but one the epigastric vein

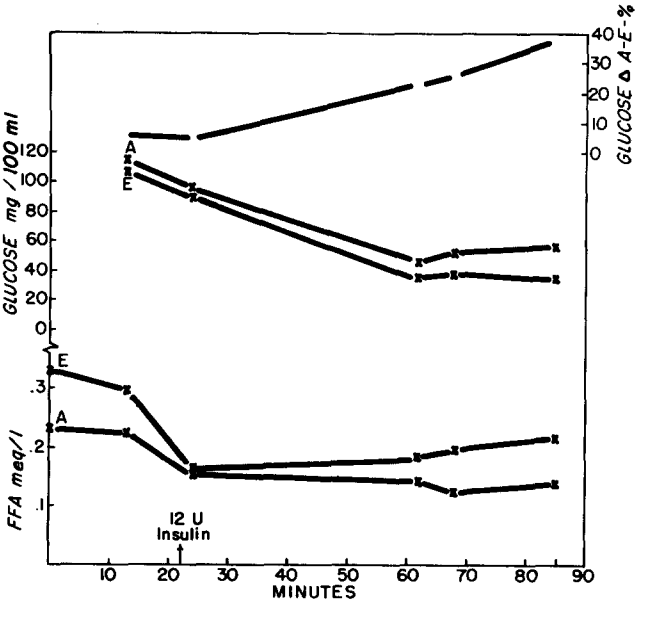

FIG. 1. Effect of insulin injection on FFA release in a normal dog.

had higher FFA concentration than the artery, the differences being similar to those found in normal animals.

In all cases E−A differences were decreased after insulin. One typical experiment is shown in Figure 2. The average decrease in E−A difference was found to be 0.121 meq/liter, with a standard error of 0.028. In two dogs marked uptake of FFA by adipose tissue was observed after insulin. In one of them the uptake was present even before insulin.

E−A differences did not differ markedly during lymph infusion, although a slight increase was noticed. One example of these studies is given in Figure 3.

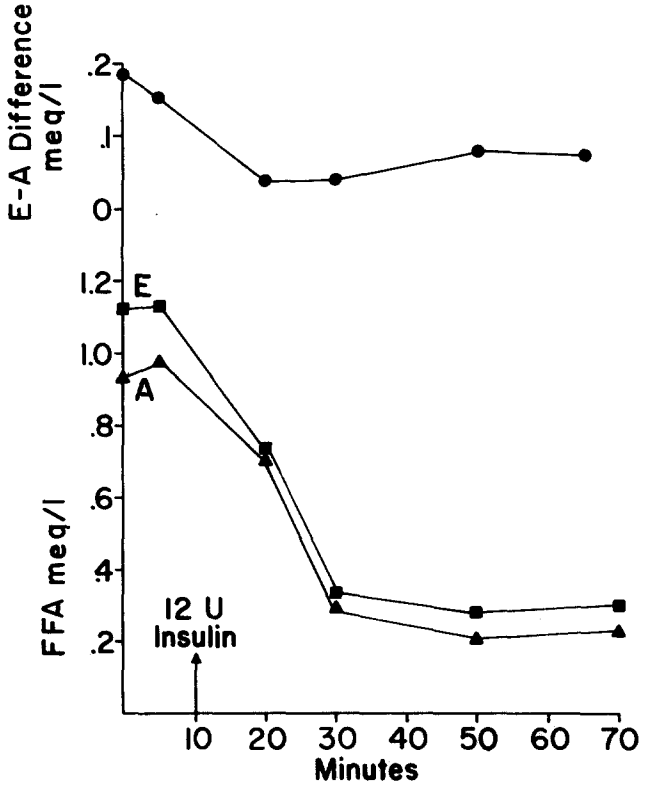

FIG. 2. Effect of insulin injection on FFA release in a diabetic dog.

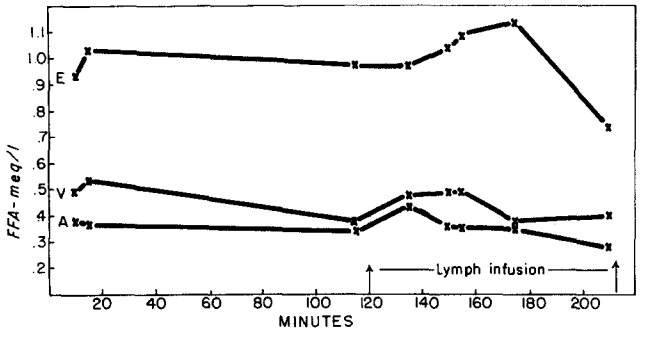

FIG. 3. Effect of lymph infusion on FFA release.

DISCUSSION

Simultaneous determmations of E−A FFA differences yielded valuable information on the behavior of adipose tissue, but this information cannot be translated into strict quantitative terms, unless the amount of blood flow through the adipose tissue in question is known. Our attempts to determine this have been unsuccessful so far. Therefore the reported changes can represent quantitative information only if one assumes that no major change in blood flow through adipose tissue occurred in the course of the experiments. Such assumption is justified under the present experimental conditions since neither diabetes nor the same amount of insulin seems to influence blood flow through coronary arteries, liver, or thigh muscles.[1] Even excessive doses of insulin (40 to 65 units/kg) caused only a small (10% to 20%) increase in femoral venous flow after about 10 minutes in dogs (9). In man (10), massive doses of from 40 to 280 units of insulin increased blood flow in the hand and forearm in 60 to 145 minutes after injection. The relatively small volume of infused lymph was also unlikely to alter blood flow through adipose tissue. In the normal animal a continuous release of FFA could be observed from the adipose tissue. Hormonal influences undoubtedly play a role in influencing this release. Insulin decreased it; epinephrine seemed to increase it. The latter hormone was tested only in one experiment, because any change caused by epinephrine is hard to interpret, depending on the simultaneous blood flow changes that occur. Although the arterial FFA level has an influence on the uptake of FFA by both the liver (11, 12) and the myocardium (13), it does not seem to correlate with the release of FFA by adipose tissue.

Using the Fick principle, rapidly falling arterial concentration of the measured metabolite may introduce an error. Since an E−A FFA gradient existed in the present experiments, such error would have increased rather than decreased the E−A difference. Thus the decreased E−A difference across the adipose tissue after insulin administration cannot be explained on this basis.

It is difficult either to prove or disprove a cause-effect relationship from the present data between the diminished E−A difference and the lowering of arterial FFA after insulin. Seldom was there any evidence for FFA uptake by adipose tissue caused by insulin.

The effect of insulin on FFA release from the adipose tissue *in vivo* is similar to its effect in the presence of glucose *in vitro* (14). The uptake of glucose by the adipose tissue after insulin that occurred in half of the

[1] Unpublished observation.

experiments is also reminiscent of the effect of insulin on adipose tissue *in vitro* (2).

Adipose tissue continued to release FFA during lymph infusion, in spite of the greater influx of triglycerides and of FFA (15) from the lymph to the blood.

It can be observed from Table 1 that the FFA level in the femoral vein is frequently equal to or higher than that in the epigastric vein. Since only a portion of the femoral venous blood comes from adipose tissue, one possibility is that deep-lying adipose tissue is perhaps more active, metabolically, than the subcutaneous tissue. Difference in temperature may be one of the possible explanations. Similar assumptions could be made from experiments involving the region (predominantly muscles) drained by the profunda femoris vein in the dog; this vein frequently contains higher concentration of FFA than the femoral vein (16). Therefore more information is needed before the findings of the present study can be safely applied to all adipose tissue regardless of location.

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