# Metabolism of adipose tissue in the fat tail of the sheep in vivo

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ABSTRACT The metabolism of the large mass of adipose tissue constituting the fat tail of the Syrian sheep has been investigated by measuring arteriovenous concentration (A–V) differences. The tail in situ in the intact anesthetized animal, as well as the isolated tail perfused with blood through a constant flow pump oxygenator, was used.

In fed animals, the adipose tissue took up glucose and ketone bodies and released lactate and free fatty acids (FFA), although in some animals uptake of FFA also occurred. After 48–144 hr of fasting, uptake of glucose and ketone bodies continued and the FFA release increased. Total lipid esters and phospholipids were not released even after food had been withheld for 6 days. Insulin increased the A–V difference and the uptake of glucose, and reduced the FFA release.

Adrenaline increased the A–V difference and uptake of glucose; the simultaneous increase in serum FFA was not accompanied by an increase in A–V difference for FFA in most experiments, which suggests that this adipose tissue is relatively insensitive to the lipolytic effect of the hormone. The effect of noradrenaline was similar to that of adrenaline. Glucagon hyperglycemia was not accompanied by increase in glucose uptake in most experiments.

KEY WORDS metabolism · adipose tissue · fat tail sheep · in vivo · perfusion · arteriovenous differences · free fatty acids · glucose · insulin · adrenaline · glucagon · fatty acid composition

THE METABOLISM of adipose tissue has been extensively studied in recent years and a considerable understanding has been gained concerning the metabolic pathways and their hormonal regulation in this tissue (1–3). Most of these studies have been carried out in vitro, mainly on the rat epididymal fat pad. Information about the physiological pathways existing in the intact animal and in adipose tissue in various sites is scanty. The Syrian fat tail sheep, *Ovis aries var. crassicundus* (found primarily in the Middle East), which has a large tail composed entirely of white adipose tissue covered with skin, provides a large mass of such tissue for study (4). The tail weighs several kilograms in the adult animal and serves as a fat store. The veins draining the tail can be reached by a minor dissection and large samples of blood can be drawn. This advantageous anatomic arrangement prompted us to study the metabolism of this adipose tissue in vivo by determining the arteriovenous concentration (A–V) differences for various metabolites in the intact anesthetized animal as well as in the isolated tail perfused with blood through a constant-flow pump oxygenator.

Abbreviations: A–V, arteriovenous concentration; A, arterial evel s; FFA, free fatty acid; GLC, gas–liquid chromatography.

## MATERIALS AND METHODS

### *Animals*

The mass of fat comprising the tail of the Syrian sheep is equal to about 10–12% of the total body weight and appears as a large fold of the gluteal subcutaneous fat (Fig. 1). It is uniform in consistency and similar in appearance to subcutaneous fat seen in other species of the sheep. The arteries and the veins supplying and draining the tail are the presacral artery and vein, located in the midline, and the terminal branches of the two internal iliac arteries and veins, located laterally. No visible anastomoses exist between the blood supply of the adipose tissue and that of the overlying skin.

Adult male sheep weighing 26–42 kg were bought from the local market and kept on a diet of barley and bran for at least 10 days before experiments. In experiments on "fed" animals food was withheld for 16–20 hr before the experiment to prevent intratracheal aspiration

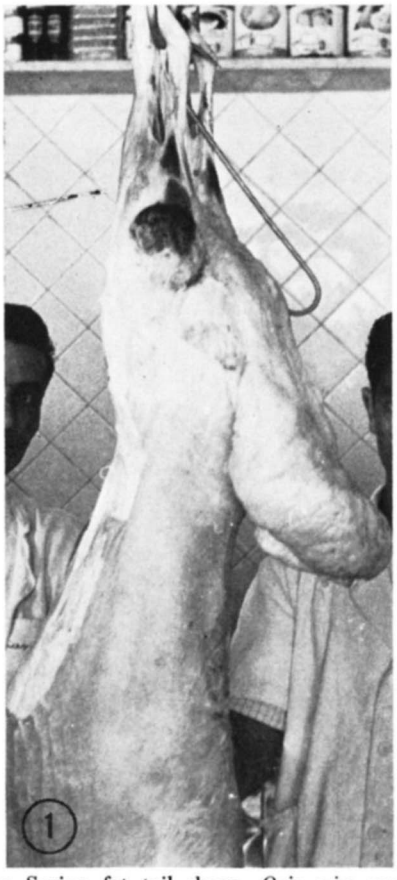

FIG. 1. The Syrian fat tail sheep, *Ovis aries var. crassicundus.*

of food particles during anesthesia. Since the sheep has a large rumen where food is stored for long periods, it can be considered nonfasting.

Anesthesia was induced with intravenous pentobarbital and the ventral aspect of the tail was dissected to locate a large vein. This was best achieved by starting the incision 5 cm from the midline at a point midway between the base and the tip of the tail. A polyethylene catheter was introduced into the vein via a tributary when possible. This catheter was kept patent during the experiment by injecting small amounts of EDTA solution into it periodically. The femoral artery was then dissected and a Cournand needle inserted. A peripheral vein was also cannulated and used for the injection of anesthetic or hormones and glucose.

For the perfusion experiments the animals were anesthetized, a tracheotomy was performed through a midline cervical incision, and a cuffed, endotracheal tube was inserted into the trachea. The animal was then placed on an automatic respirator. The abdomen was opened through a midline incision extending from the xiphoid to the pubis. The gastrointestinal tract, from the esophagogastric junction to the rectum, was resected, along with the spleen and pancreas, in order to increase available space in the abdomen and facilitate dissection of the pelvic vessels. The circulation to the tail was isolated by ligating the external iliac arteries and veins in that order. Arterial and venous branches supplying the iliopsoas muscles were also divided. Initially an effort was made to divide the branches of the internal iliac supplying the gluteal muscles, but frequently this resulted in trauma to the main vessels. Instead, tight tourniquets were placed on both thighs to cut off their circulation.

A catheter was introduced into the lower aorta through one of the external iliac arteries proximal to the previously placed tie. A second catheter was introduced into the lower end of the inferior vena cava in a similar fashion through the contralateral external iliac vein. Both catheters were connected to an extracorporeal circuit as outlined in Fig. 2. The circuit was primed with 2–5 liters of sheep blood obtained from the slaughter house on the morning of the experiment and anticoagulated with 20 mg of heparin per liter. Glucose was added to the blood initially and infused at a constant rate into the reservoir during the experiment. The lower aorta and vena cava were then ligated and the extracorporeal circulation was started.

Cross-circulation between the extracorporeal circuit and the rest of the body was minimized by disconnecting the upper body of the animal from the pelvis at a point just above the ligatures on the aorta and the inferior vena cava. Bleeders on the cut edge of the experimental specimen were secured with ligatures. Venous blood returning from the tail was drained by gravity through the catheter in the inferior vena cava. A side arm was provided at this point for the collection of venous samples.

The blood was passed through a 13 inch rotating disc oxygenator and a filter, and then pumped back into the tail through the catheter in the aorta. A second side arm was provided at this point for the collection of arterial samples. A reservoir was interposed between the oxygenator and the filter to increase the priming volume. The system was kept at 37°C by means of a resistance coil wrapped around the oxygenator. A uniform blood flow was maintained throughout each experiment by a constant-flow digital pump, the rates in different experiments varying between 30 and 73 ml/min. The extent of lipolysis in the perfusate was measured by removing blood samples from the reservoir at various periods during the experiment and incubating them at 37°C. The average increase in FFA during the incubation was 1.4 μeq/liter per min.

Blood samples were collected at various intervals from the arterial and venous sides; clotting was prevented with EDTA. The venous sample was collected at the same rate as the arterial sample, with a delay of 15 sec to allow for the passage of blood from the arterial to the venous bed. Collection of each sample lasted about 1 min and the total volume removed did not exceed 200 ml per

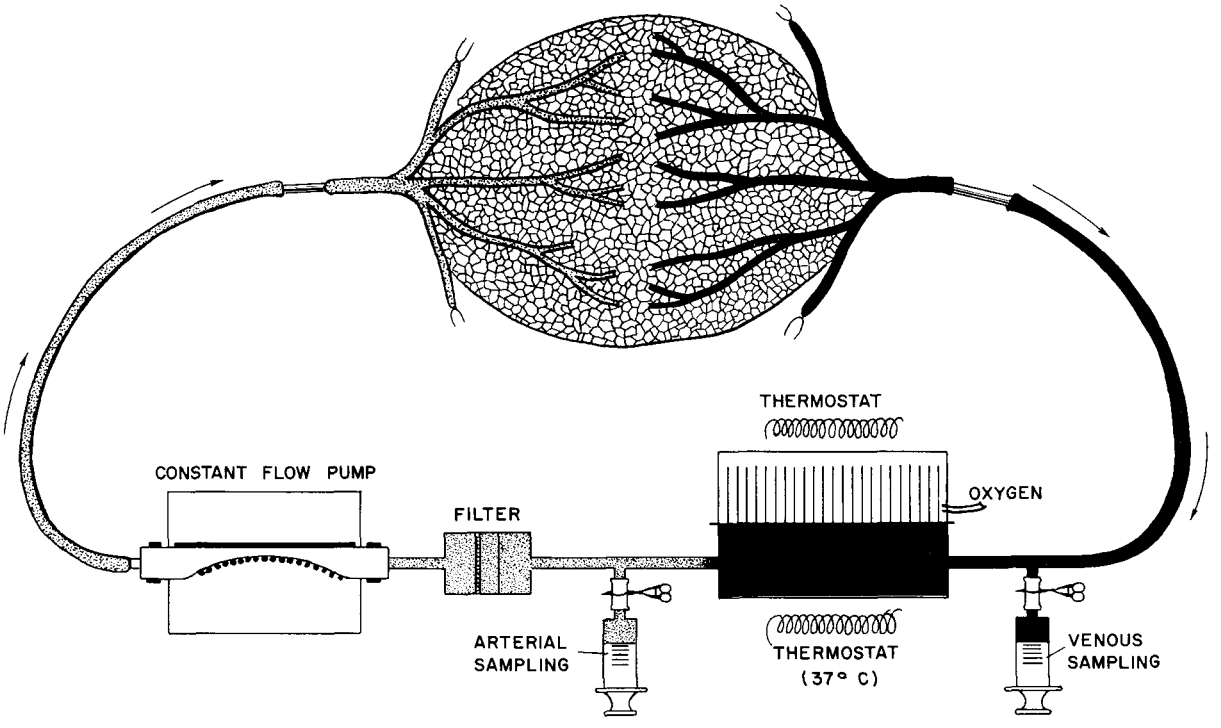


FIG. 2. Perfusion of sheep tail. Diagrammatic representation of perfusion assembly.

animal. The blood was centrifuged immediately in a refrigerated centrifuge and duplicate analyses were carried out within 30 min.

### *Analytical Procedures*

Glucose was determined by Nelson's photometric adaptation of Somogyi's method (5) or with the aid of glucose oxidase reagent obtained from Worthington Biochemical Corporation, Freehold, N.J. Other analytical methods were: lactate (6); ketone bodies (7); cholesterol (8); phospholipid phosphorus (9); total lipid esters (10, 11); free fatty acids (FFA) (11); tissue total lipids (12); DNA (13).

The tissue fatty acids were extracted with chloroform–methanol 2:1 (v/v) (14). They were methanolyzed by refluxing in 0.5 N anhydrous methanolic HCl for 2 hr on a water bath.

The fatty acids in the sera were extracted by Dole's extraction mixture (11). The FFA were then extracted into 0.02 N NaOH. This fraction was washed several times with petroleum ether to remove nonsaponifiable substances. The FFA were liberated by the addition of 1 N $H_2SO_4$, then extracted in petroleum ether, dried over anhydrous $Na_2SO_4$, and methanolyzed as described above. Plasma triglycerides were not appreciably hydrolyzed during the extraction procedure, for when tripalmitin-$^{14}$C (fatty acid-labeled) was added to plasma and the radioactivity counted in the FFA fraction, a maximum of 0.37% of the radioactivity added was recovered.

Margaric acid (17:0) was used as an internal standard for gas chromatographic analyses. Appropriate amounts of a standard solution of this acid in heptane were added to the extraction mixture simultaneously with the serum. Gas–liquid chromatography was carried out on Apiezon L (20% on Celite) at 198°C argon flow 50–75 ml/min, $^{90}$Sr detector.

Separations of NIH standard fatty acid mixture F (15) gave an average error of 3.9%, and a maximum error of 8.8% (for 20:0). Triplicate analyses of the same blood sample extracted separately also gave satisfactory results, with an average variation of 2.2 μeq/liter for each fatty acid and a maximum difference of 4.4 μeq/liter.

Statistical analysis included an evaluation of the significance of differences between means of small samples (16).

## RESULTS

### Composition of the Tail Adipose Tissue

Table 1 shows the composition of the tail adipose tissue in the fed animal. Table 2 compares the relative composition of tissue triglyceride and free fatty acids with that of plasma FFA. The adipose tissue contains primarily oleic, palmitic, and stearic acids. The plasma FFA contain relatively more stearic and less palmitic acid.

TABLE 1 Composition of Sheep Tail Adipose Tissue in Control Animals

| | % wet weight |
|---|---|
| Water | 13 |
| Total lipids | 82 |
| Total nitrogen | 0.45 |
| Total ash | 1.4 |
| DNA | 0.017 |
| Cholesterol | 0.4 |
| Phospholipids | 0.08 |

TABLE 2 Fatty Acid Composition of Tissue Triglycerides, Tissue Free Fatty Acids, and Arterial Free Fatty Acids in the Fed Animal

| | Tissue Fatty Acids | | | |
|---|---|---|---|---|
| | Glyceride (x) | Free | Arterial FFA (y) | $\frac{y}{x}$ |
| | *moles per cent* | | | |
| 18:0 | 12.62 | 16.86 | 27.46 | 2.18 |
| 18:1 | 45.87 | 44.68 | 42.25 | 0.92 |
| 18:2, 18:3 | 4.60 | 4.87 | 5.71 | 1.24 |
| 17:0 | 4.87 | 4.45 | 2.69 | 0.55 |
| 16:0 | 29.08 | 26.73 | 19.63 | 0.68 |
| 16:1 | 2.96 | 2.41 | 2.25 | 0.76 |

## Experiments on Intact Animals

### *A–V Differences in the Fed Animal*

Table 3 shows the A–V differences for the various metabolites in the fed animals. In all of the experiments the tail took up glucose and ketone bodies and released lactic acid. In five experiments there was an appreciable release of total FFA (A–V differences >90 μeq/liter), in nine the A–V difference was small (0–50 μeq/liter), and in one a small uptake of FFA was demonstrated. There was no correlation between the A–V differences and arterial levels of FFA.

The A–V difference for FFA was studied further by measuring the individual long-chain FFA by gas–liquid chromatography in seven fed animals. According to this method, uptake of FFA occurred in three experiments (Table 4). Since these A–V differences are greater than the error of the method in our laboratory, an uptake of FFA by adipose tissue is demonstrated in these animals. The A–V differences for phospholipids and total lipid esters suggest a very small release and uptake respectively for these lipids from adipose tissue (Table 3). However, the values are not statistically significant.

### *A–V Differences in Fasted Animals*

The effect of fasting for 48–144 hours was examined in 10 animals. Table 5 summarizes the results of these experiments, and Table 6 compares the results with those obtained in the fed animals. Glucose uptake continued to occur in all these animals. In one (experiment 16) a definite uptake of glucose occurred in spite of an arterial glucose level of 35 mg/100 ml. Similarly, ketone bodies were taken up in all animals and lactate was released in all except one. There was a definite increase in the arterial level and the A–V differences for FFA. The A–V differences for phospholipid and total lipid esters were not significant.

Table 7 shows the FFA composition of plasma in fasted animals. Comparison with values found in fed animals reveals a relative decrease in stearic and a relative increase in oleic acids.

### *Effect of Insulin*

The effect of glucagon-free insulin, 0.4 units/kg of body weight, injected intravenously over 3 min was studied in eight animals. Fig. 3 shows the results obtained in four typical experiments. The arterial glucose level decreased, as expected, in all experiments and the A–V difference for glucose increased in all except one that was terminated 45 min after the injection of insulin. This increase in A–V difference was seen in the 30 min sample at the

TABLE 3 Arterial Levels (A) and Arteriovenous Concentration Differences (A–V) for Various Metabolites in the Fed Animals

Values represent average of 1–4 pairs of determinations for each experiment.

| Metabolite | No. of Expts. | Arterial Levels | | A–V | | |
|---|---|---|---|---|---|---|
| | | Mean ±SD | Range | Mean ±SD | Range | *P* |
| | | | *mg/100 ml* | | | |
| Glucose | 15 | 80.7 ± 17.4 | 62–124 | 7.7 ± 4.8 | 1 to 19 | <0.01 |
| Ketone bodies | 6 | 3.6 ± 0.9 | 2.6–5.2 | 0.65 ± 0.41 | 0.07 to 1.3 | <0.02 |
| Lactic acid | 12 | 14.9 ± 8.3 | 3–31 | −3.8 ± 2.8 | −0.2 to −7.5 | <0.02 |
| | | | *μeq/liter* | | | |
| FFA | 15 | 310 ± 186 | 88–800 | −92 ± 155 | −560 to 55 | <0.01 |
| Total lipid esters | 7 | 2800 ± 530 | 2200–3500 | 140 ± 230 | −100 to 500 | <0.2 |
| | | | *μmoles/liter* | | | |
| Phospholipid P | 7 | 790 ± 280 | 400–1160 | −86 ± 127 | −360 to 4 | <0.2 |

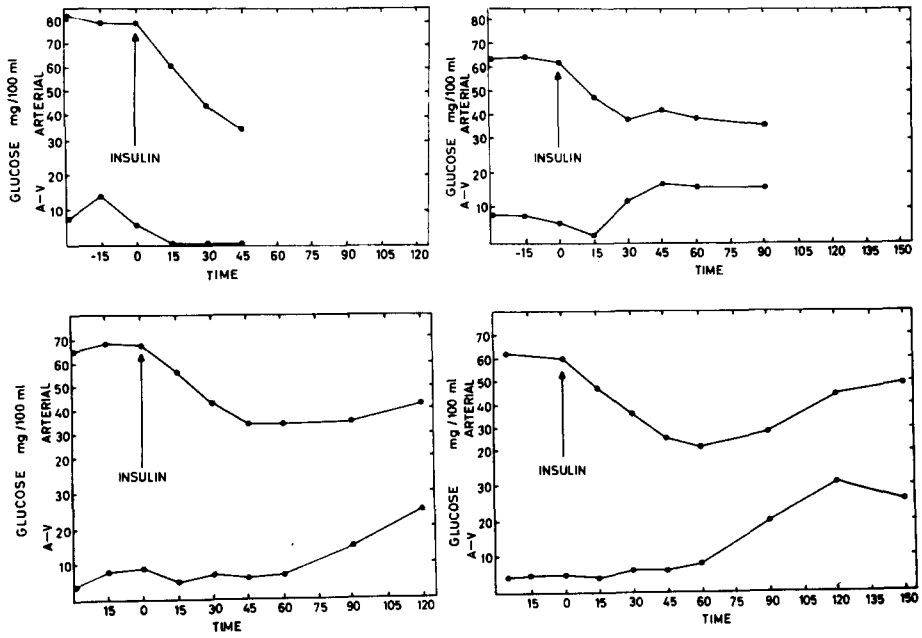


FIG. 3. Effect of intravenous insulin, 0.4 units/kg body weight, on the arterial level (upper curves) and A–V difference of glucose (lower curves). Each pair of graphs represents a separate animal.

TABLE 4 UPTAKE OF LONG-CHAIN FREE FATTY ACIDS BY THE ADIPOSE TISSUE IN THE FED ANIMAL

| Fatty Acids | Expt. 2 | | Expt. 6 | | Expt. 8 | |
|---|---|---|---|---|---|---|
| | A | A–V | A | A–V | A | A–V |
| | | | *μeq/liter* | | | |
| $C_{16-18}$ | 78.5 | 51.3 | 110.6 | 36.7 | 481.7 | 148.0 |

earliest and in one experiment did not occur until 120 min after the injection.

The mean A–V difference measured 15–150 min after the injection of insulin for ketone bodies decreased from 0.60 to 0.34 mg/100 ml, while the mean negative A–V difference for lactate rose from 4.5 to 8.1 mg/100 ml. These changes were not statistically significant.

The arterial level and the A–V difference for FFA showed wide fluctuations during the experiments. A decline in the arterial level and a decrease in the negative A–V difference for FFA were observed in five out of eight experiments after the injection of insulin. In three experiments the positive A–V differences for FFA rose significantly. A secondary rise in the arterial FFA levels without an increase in the negative A–V difference occurred in two experiments (Table 8.)

TABLE 5 ARTERIAL LEVELS AND ARTERIOVENOUS CONCENTRATION DIFFERENCES IN FASTED ANIMALS
Values represent averages of 1–4 pairs of determinations for each experiment.

| Expt. No. | Fast | Glucose | | Ketone Bodies | | Lactate | | FFA | | PLP | | TLE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A–V | A | A–V | A | A–V | A | A–V | A | A–V | A | A–V |
| | *hr* | *mg/100 ml* | | *mg/100 ml* | | *mg/100 ml* | | *μeq/liter* | | *μmoles/liter* | | *μeq/liter* | |
| 6 | 48 | 66 | 7.3 | 1.5 | 0.10 | 13.6 | −9.1 | 363 | −144 | — | — | — | — |
| 18 | 48 | 73 | 3.7 | — | — | 15.8 | −5.2 | 462 | −136 | — | — | — | — |
| 21 | 48 | 88 | 9.4 | 5.4 | 0.8 | 18.3 | −3.5 | 340 | −200 | 830 | 50 | 2600 | 0 |
| 7 | 72 | 61 | 4.6 | 5.6 | 1.4 | 23.5 | 0 | 906 | −764 | 656 | 6 | — | — |
| 8 | 72 | 64 | 4.5 | 5.3 | 0.3 | 12.5 | −1.5 | 533 | −170 | 500 | −15 | 3100 | 0 |
| 16 | 72 | 35 | 6.7 | 5.2 | 0.3 | 10.6 | −0.5 | 781 | −469 | 890 | 100 | 2700 | 20 |
| 17 | 72 | 64 | 10.3 | — | — | 18 | −7.7 | 625 | −317 | 930 | 5 | 2900 | 70 |
| 50 | 72 | 80 | 6.0 | — | — | 18.9 | −3.7 | 981 | −630 | — | — | 2900 | −300 |
| 47 | 120 | 66 | 8.0 | 3.7 | 1.0 | 21.0 | −2.0 | 210 | −150 | 975 | −10 | 3100 | −100 |
| 22 | 144 | 86 | 1.2 | 7.0 | 0 | — | — | 800 | −100 | 950 | −10 | 3500 | 100 |
| Mean | | 68.3 | 6.2 | 4.8 | .56 | 16.9 | −3.7 | 600 | −308 | 820 | 18 | 3000 | 30 |
| ±SD | | ±15.7 | ±2.7 | ±1.8 | ±.5 | ±4.2 | ±3.1 | ±261 | ±234 | ±310 | ±42 | ±310 | ±150 |
| *P* | | — | <0.01 | — | <0.05 | — | <0.01 | — | <0.01 | — | NS | — | NS |

TABLE 6 MEAN ARTERIAL LEVELS AND ARTERIOVENOUS CONCENTRATION DIFFERENCES (A–V) OF VARIOUS METABOLITES EXPRESSED AS RATIOS OF FED TO FASTED ANIMALS

| Metabolite | Arterial Levels Fed/Fasted | P | A–V Fed/Fasted | P |
|---|---|---|---|---|
| Glucose | 1.18 | <0.1 | 1.26 | <0.4 |
| Ketone bodies | 0.77 | <0.2 | 1.17 | <0.9 |
| Lactic acid | 0.89 | <0.5 | 1.03 | <0.9 |
| FFA | 0.52 | <0.01 | 0.30 | <0.02 |
| Phospholipid | 0.95 | <0.1 | — | — |
| Total lipid esters | 0.94 | <0.6 | 4.7 | <0.3 |

TABLE 7 FREE FATTY ACID COMPOSITION OF ARTERIAL PLASMA IN VARIOUS EXPERIMENTAL CONDITIONS

Number of experiments in each series indicated by figures in parentheses.

| Fatty Acids | Normal (6) | 90–150 Hr Fast (4) | After Adrenaline (3) | After Noradrenaline (2) | After Glucose (2) |
|---|---|---|---|---|---|
| | *moles per cent* | | | | |
| 18:0 | 27.5 | 25.6 | 22.9 | 21.5 | 28.4 |
| 18:1 | 42.2 | 48.2 | 42.6 | 41.4 | 38.3 |
| 18:2, 18:3 | 5.7 | 7.9 | 7.4 | 8.1 | 6.3 |
| 17:0 | 2.7 | 2.4 | 3.6 | 2.3 | 2.2 |
| 16:0 | 19.6 | 14.3 | 21.1 | 24.5 | 22.7 |
| 16:1 | 2.2 | 1.6 | 2.4 | 2.2 | 2.2 |

### *Effect of Adrenaline and Noradrenaline*

The effect of intravenous adrenaline, 0.25–0.5 mg injected over 5 min was tested in five experiments. In each, there was a sharp rise in plasma glucose concentration together with an increase in A–V difference. The arterial lactate rose and the negative A–V difference for lactate increased. There was a moderate increase in the arterial level of FFA in four experiments. A definite increase in the negative A–V difference for FFA could be demonstrated only in one experiment (Table 9). In two additional experiments measurement of individual FFA showed a slight decrease in the A–V difference following the injection of adrenaline. There was an increase of 10–25% in the arterial levels of total lipid esters 15–45 min after the injection, but no effect on the A–V difference. The effect of noradrenaline in two experiments was similar to that of adrenaline (Table 10). Table 7 shows the relative concentrations of various FFA in the arterial plasma of animals injected with adrenaline and noradrenaline. Comparison with control values reveals that the relative concentration of stearic acid decreased.

The hyperglycemic effect of the hormones, which alters the FFA response, was minimized by fasting one animal for 6 days and then infusing 0.9 mg of noradrenaline over 30 min by means of a constant-flow pump. The results given in Table 11 show a slight increase in the arterial level of glucose and its A–V difference, a marked increase in the arterial FFA but no change in the A–V difference for total FFA, and a decrease in the A–V difference for the fatty acids measured by GLC.

### *Effect of Glucagon*

The effect of glucagon, 2 mg given intravenously, was tested in four animals fasted for 16–144 hours. In all animals there was a substantial rise in the arterial glucose level. A definite increase in the A–V difference for glucose occurred in only one of the four experiments. There was a decline in the arterial level and in the negative A–V difference for FFA (Table 12).

### *Effect of Intravenous Glucose*

The effect of the infusion of 10% glucose on the arterial level and the A–V differences of FFA was studied in two animals (Table 13). Measurements were done before and at the end of the infusion. The results indicate a reversal of the direction of flow for FFA in the animal that had

TABLE 8 EFFECT OF INSULIN ON A AND A–V OF GLUCOSE AND FFA

Animal in experiment 5 fasted for 18 hr, animal in experiment 7 fasted for 72 hr before the experiment.

| Time from Insulin Injection | Experiment 5 Glucose A | Experiment 5 FFA A | Experiment 5 FFA A–V | Experiment 7 Glucose A | Experiment 7 FFA A | Experiment 7 FFA A–V |
|---|---|---|---|---|---|---|
| *min* | *mg/100 ml* | *μeq/liter* | | *mg/100 ml* | *μeq/liter* | |
| −20 | 63 | 500 | −70 | 62 | 772 | −790 |
| −10 | 64 | 525 | −50 | 61 | 976 | −817 |
| 0 | 62 | 525 | 25 | 61 | 1248 | −680 |
| 15 | 47 | 325 | 0 | 47 | 749 | −635 |
| 30 | 38 | 325 | −25 | 36 | 476 | −273 |
| 45 | 42 | 350 | −25 | 26 | 499 | −250 |
| 60 | 39 | 375 | 50 | 22 | 1067 | −250 |
| 90 | 36 | 475 | 75 | 28 | 1498 | −68 |
| 120 | — | — | — | 44 | 1248 | −68 |
| 150 | — | — | — | 48 | 749 | 113 |

TABLE 9 EFFECT OF ADRENALINE, 0.25–0.5 mg INTRAVENOUSLY ON A AND A–V OF GLUCOSE AND FFA

Values represent average of 2–4 pairs of measurements done before and after injection.

| | Glucose | | | | FFA | | | |
|---|---|---|---|---|---|---|---|---|
| | A | | A–V | | A | | A–V | |
| Expt. No. | Before | After | Before | After | Before | After | Before | After |
| | *mg/100 ml* | | | | *μeq/liter* | | | |
| 8 | 64 | 108 | 5 | 15 | 560 | 703 | −182 | −189 |
| 10 | 79 | 146 | 6 | 22 | 371 | 462 | −42 | −95 |
| 45 | 91 | 166 | 4 | 51 | 88 | 176 | −88 | −89 |
| 46 | 68 | 126 | 16 | 31 | 265 | 206 | −30 | −147 |
| B11 | 91 | 134 | 23 | 34 | 144 | 256 | 19 | 1 |
| Mean | 79 | 136 | 11 | 31 | 286 | 361 | −65 | −104 |
| ±SD | ±8.9 | ±19.3 | ±8 | ±10.3 | ±188 | ±221 | ±75 | ±71 |
| *P* | | <0.01 | | <0.02 | | <0.8 | | <0.4 |

TABLE 10 EFFECT OF NORADRENALINE, 0.25–0.5 mg INTRAVENOUSLY, ON A AND A–V OF GLUCOSE AND FFA

| Expt. No. | Time from Injection | Glucose | | FFA | |
|---|---|---|---|---|---|
| | | A | A–V | A | A–V |
| | *min* | *mg/100 ml* | | *μeq/liter* | |
| B9 | 0 | 65 | 6 | 173 | −30 |
| | 30 | 123 | 11 | 311 | −27 |
| 55 | 0 | 71 | 7 | 217 | −17 |
| | 5 | 141 | 31 | 334 | −16 |
| | 15 | 123 | 15 | 267 | +33 |
| | 30 | 114 | 15 | 350 | −34 |

TABLE 11 EFFECT OF INTRAVENOUS NORADRENALINE ON A AND A–V FOR FFA AND GLUCOSE IN THE TAIL OF THE SHEEP

The animal was fasted for 6 days. Samples were collected before and at the end of the infusion of 900 mg of noradrenaline over 40 min.

| | Control | | After Noradrenaline | |
|---|---|---|---|---|
| | A | A–V | A | A–V |
| | | | *μeq/liter* | |
| Total FFA by titration | 395 | −447 | 1000 | −420 |
| Total FFA by GLC | 381 | −476 | 667 | −266 |
| | | | *mg/100 ml* | |
| Glucose | 82 | 9 | 103 | 12 |

fasted for 60 hr and a suppression of release in the animal fasted for 144 hr.

### EXPERIMENTS WITH THE ISOLATED PERFUSED TAIL

Four complete experiments were carried out, two with insulin and two with adrenaline.

The A–V measurements in the control period showed that the perfused tail was taking up glucose and total lipid esters and releasing lactate. The A–V differences for FFA, phospholipid, and ketone bodies were small and variable.

Insulin, 20 units added to the reservoir, caused a significant increase in glucose uptake in both experiments (Table 14). The effect was noted in both experiments at the third measurement, 22.5 min after the addition of insulin. The average A–V difference for ketone bodies changed from −0.2 to 0.8 mg/100 ml. The effect on the other metabolites was very small and variable.

Adrenaline, 1 mg added to the reservoir, caused a significant increase in glucose uptake and a slight increase in lactate release in both experiments (Table 15). In one experiment there was a small increase in the uptake of FFA by the tail while in the other no effect was noted. The uptake of total lipid esters decreased in one experiment and was not affected in the other. No appreciable effect on the other metabolites could be demonstrated.

## DISCUSSION

The anatomical structure of the tail in the Syrian sheep is particularly suited for the study of adipose tissue metabolism in vivo because blood which is draining exclusively a large mass of white adipose tissue can be repeatedly sampled. However, since measurements of blood flow could not be done in the intact animal, changes in uptake and release caused by the various experimental procedures can be predicted only if the changes in the A–V differences are large enough to outbalance possible changes in blood flow resulting from these procedures. Constant-flow perfusion experiments were done to eliminate this uncertainty but were limited in number because of the technical difficulties in obtaining successful preparations.

The A–V difference for glucose was positive in all control animals, indicating uptake of glucose by adipose tissue. Fasting caused a small decline in the average arterial glucose concentration but one animal that had fasted for 144 hr still had a level of 86 mg/100 ml, evidence of the active gluconeogenic mechanism in rumi-

TABLE 12 EFFECT OF INTRAVENOUS GLUCAGON UPON A AND A–V OF GLUCOSE AND FFA

| No. of Expt. | Glucose | | | | FFA | | | |
|---|---|---|---|---|---|---|---|---|
| | Before | | After | | Before | | After | |
| | A | A–V | A | A–V | A | A–V | A | A–V |
| | *mg/100 ml* | | | | *μeq/liter* | | | |
| 49 | 108 | 6 | 189 | 2.4 | 305 | 55 | 236 | 4 |
| 50 | 80 | 6 | 170 | 24 | 981 | −630 | 734 | −111 |
| 51 | 125 | 7 | 206 | 8 | 640 | −375 | 735 | −92 |
| 53 | 124 | 9 | 262 | 15 | 178 | −54 | 214 | 18 |
| Average | 107 | 7 | 208 | 12 | 526 | −251 | 479 | −45 |

TABLE 13 EFFECT OF INTRAVENOUS GLUCOSE ON A AND A–V OF LONG-CHAIN FATTY ACIDS MEASURED BY GLC

The animal in experiment 50 was fasted for 60 hr and received 50 g of glucose over 90 min. The animal in experiment B20 was fasted for 144 hr and received 30 g of glucose over 60 min.

| | Experiment 50 | | | | Experiment B20 | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | After Glucose | | Control | | After Glucose | |
| | A | A–V | A | A–V | A | A–V | A | A–V |
| | | | | *μeq/liter* | | | | |
| Total FFA by GLC | 492 | −246 | 252 | 103 | 589 | −489 | 297 | −8 |
| | | | | *mg/100 ml* | | | | |
| Glucose | 80 | 6 | 250 | 17 | 70 | 2 | 369 | 5 |

nants, which normally absorb only small amounts of glucose from the gastrointestinal tract (17). Bergman (17) has shown that hypoglycemia due to fasting occurs readily in pregnant ewes. In our experiments on male animals hypoglycemia was seen in only one animal (experiment 16, Table 5). A significant uptake of glucose by adipose tissue in this animal at an arterial glucose concentration of 35 mg/100 ml is noteworthy. Uptake of glucose by isolated tail was also noted at blood glucose levels below 35 mg/100 ml in two perfusion experiments.

The stimulating action of insulin on uptake of glucose by adipose tissue has been repeatedly demonstrated. This effect appears to be immediate in the rat epididymal fat pad in vitro since an appreciable effect was noted at measurements done after a 15 min incubation (18). However, in vivo, the increase in glucose uptake after intravenous injection of insulin is not immediate. Thus in the experiments of Bell and Burns (19) the femoral A–V difference increased 45 min after the injection of intravenous insulin. With intraarterial insulin injection, a lag period of 18–26 min was noted before increases were observed in A–V differences across the arm of obese individuals, although the insulin effect appeared to be immediate in normal controls (20). In both series of experiments the changes in arterial blood glucose were very small and the lack of immediate response to insulin could not be explained by the error (predicted from the Fick principle) created by the declining arterial glucose levels. In our experiments on the intact animals measurements were done at 15 min intervals; the earliest rise was seen 30 min after injection, but was delayed until 120 min in another experiment. The decline in arterial glucose level after insulin was 1–1.5 mg/100 ml per min. This change in arterial glucose level makes the interpretation of A–V differences difficult. In two experiments with the isolated tail, in which the arterial concentration of glucose and the blood flow were kept constant, the increase in uptake was first noted at the third sample, taken 22.5 min after addition of insulin. Although these findings suggest a delay in the action of insulin in increasing glucose uptake, further experiments are needed to establish this finding.

The hyperglycemia resulting from the injection of adrenaline and noradrenaline to the intact fed animal was accompanied by a significant increase in the A–V difference for glucose. This observation suggests that adrenaline stimulates glucose uptake by adipose tissue in vivo as it does in vitro (3). This possibility is supported by the results of the perfusion experiments, in which the stimulating effect of adrenaline is well defined. In the experiments reported here, the degree of hyperglycemia caused by noradrenaline was comparable to that after adrenaline. This disagrees with findings in other species, in which the hyperglycemia resulting from noradrenaline is very slight (21). The reason for this difference is unknown.

Studies on the effect of glucagon on peripheral uptake of glucose have yielded contradictory results. Thus, Van Itallie, Morgan, and Dotti (22) and Bondy and Cardillo (23) reported increases in A–V difference across the arm in humans, while Kibler, Taylor, and Myers (24) could

find no increase in uptake across the leg or the brain in the human, and Drury, Wick, and Sherrill (25) found a decrease in glucose uptake by the eviscerated rabbit during a constant glucose infusion. The results reported here suggest that glucagon hyperglycemia is not accompanied by a significant increase in glucose uptake by adipose tissue.

The release of FFA from adipose tissue in vivo and into suitable media in vitro is well established (3, 26). A release of FFA by adipose tissue was seen in all of our experiments with fasted sheep as well as in most experiments on control animals. The lack of significant release from the isolated perfused tail could possibly be due to the denervation of the tail in this preparation (27) as well as to the relatively high concentration of glucose in the perfusing blood.

Net uptake of FFA by adipose tissue in vitro occurs at high concentrations of FFA in the medium (28). In the intact animal, uptake of FFA from blood by the liver, myocardium, and peripheral tissue has been repeatedly demonstrated by measuring A–V differences. However, Spitzer and Hohenleitner (29) and Miller, Gold, and Spitzer (30), measuring A–V differences across the abdominal adipose tissue of the dog fasted for 16 hr, found a constant release of FFA by this adipose tissue and could not obtain definite evidence for FFA uptake after insulin injection. A definite uptake of FFA by adipose tissue could be demonstrated in some of our experiments on control animals. A possible explanation for this discrepancy could be that, unlike the dog, the sheep is still absorbing food maximally from the gastrointestinal tract 16–20 hr after the animal is deprived of food. The uptake was more evident when the measurements were done by GLC, which measures only long-chain fatty acids in contrast to the less specific titration method.

Adrenaline and noradrenaline are known to increase the serum level of FFA and their release from adipose tissue in vitro. Our failure to demonstrate an increase in the A–V difference for FFA across the tail despite elevations in serum levels is contrary to expectation. Possible explanations for this discrepancy are a relative insensitivity of tail adipose tissue to the hormones or the suppression of the lipolytic effect of the hormones by their hyperglycemic effect. The findings on a fasted sheep reported

TABLE 14 Effect of Insulin on A and A–V of Various Metabolites in Perfusion Experiments (Expts. 38 and 43)

Figures in parentheses represent number of determinations.

| | A | | A–V | | |
|---|---|---|---|---|---|
| | Before | After | Before ±SD | After ±SD | *P* |
| | | | *mg/100 ml* | | |
| Glucose | 117 (6) | 118 (9) | 29 ± 45 | 41 ± 3.4 | <0.01 |
| | 152 (6) | 137 (9) | 48 ± 2.8 | 60 ± 4.5 | <0.01 |
| Ketone bodies | 6.3 (4) | 7.0 (2) | −0.4 ± 0.68 | 0.8 ± 1.2 | <0.3 |
| | 4.2 (3) | 4.3 (3) | 0.03 ± 0.67 | 0.73 ± 0.71 | <0.3 |
| Lactate | 53 (3) | 71 (3) | −32 ± 5.3 | −43 ± 8.5 | <0.2 |
| | | | *μeq/liter* | | |
| FFA | 339 (5) | 435 (3) | 31 ± 43 | 20 ± 58 | <0.8 |
| | 519 (6) | 521 (3) | −6 ± 67 | 0 | <0.9 |
| Total lipid esters | 6200 (1) | 6200 (2) | 200 ± 140 | 400 ± 320 | <0.7 |
| | 3800 (2) | 3800 (3) | 200 ± 0 | 200 ± 280 | — |

TABLE 15 Effect of Adrenaline on A and A–V of Various Metabolites in Two Perfusion Experiments

Figures in parentheses represent number of determinations.

| | A | | A–V | | |
|---|---|---|---|---|---|
| Metabolites | Before | After | Before ±SD | After ±SD | *P* |
| | | | *mg/100 ml* | | |
| Glucose | 173 (7) | 191 (12) | 38 ± 4.7 | 53 ± 7 | <0.01 |
| | 170 (9) | 151 (8) | 46 ± 3.4 | 67 ± 7 | <0.01 |
| Lactate | 59 (3) | 89 (3) | −39 ± 5.15 | −52 ± 11 | <0.3 |
| | 56 (2) | 106 (4) | −42 ± 1 | −51 ± 5 | <0.1 |
| | | | *μeq/liter* | | |
| FFA | 445 (5) | 422 (4) | 4.6 ± 10 | 80 ± 28 | <0.01 |
| | 478 (4) | 619 (7) | −31 ± 56 | −34 ± 38 | >0.9 |
| Total lipid esters | 3900 (2) | 3600 (2) | 400 ± 140 | 400 ± 0 | — |
| | 4300 (2) | 4100 (3) | 800 ± 70 | 200 ± 170 | <0.02 |

in Table 11, as well as the results in the perfusion experiments, favor the first possibility. Studies now in progress (Khachadurian, A. K., and B. Adrouni) on minced adipose tissue incubated in plasma or buffered albumin with and without glucose reveal again a lack of lipolytic effect of both adrenaline and noradrenaline on FFA release into the medium as measured by titration or GLC. These hormones caused a marked increase in FFA release from the rat epididymal fat pad in parallel experiments. These findings represent another example of the metabolic differences existing in adipose tissues from different sites or species. The lack of lipolytic response to adrenaline and noradrenaline, as well as the ability of the tail adipose tissue to extract glucose from blood at low arterial concentrations, may be an important factor favoring the accumulation of the large mass of lipid in the tail of the sheep.

Mobilization of lipids from adipose tissue in the form of triglycerides could not be demonstrated in our experiments even in animals that had fasted for 6 days. These results support the presently held view that the FFA are the main form under which the lipids are mobilized from adipose tissue. A small positive A–V difference for total lipid esters was seen in most of the intact animal experiments and in all perfusion experiments. Bragdon has demonstrated uptake of chylomicron palmitate by adipose tissue (31). Efforts to study lipid uptake by adipose tissue in hyperlipemia induced by infusion of fat (Lipomul) in two animals failed since both animals developed convulsions and died shortly after the start of the infusion. Extensive thromboses were found at necropsy.

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