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# Lipoprotein lipase content and triglyceride fatty acid uptake in adipose tissue of rats of differing body weights

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ABSTRACT Increasing body weight appears to alter lipid metabolism in adipose tissue. We have measured the content of lipoprotein lipase and the uptake of chylomicron triglyceride fatty acids in epididymal fat pads of rats of different weights. In order that the results might be expressed in terms of cell numbers, the relationship between the weights of fat pads and the numbers and volumes of fat cells isolated from them was determined.

Highly significant correlations were found between fat pad weight and both the number and the volume of the individual adipocytes. In rats weighing from 140 to 350 g, the increase in the size of fat pads was attributable almost equally to increases in cell size and in cell number.

Lipoprotein lipase activity was measured in acetone powders of whole fat pads and of isolated fat cell preparations. With both, lipoprotein lipase activity per cell diminished significantly as the weight of fat tissue increased, i.e., larger fat cells contained less enzyme per cell than smaller cells.

The uptake of triglyceride fatty acid radioactivity was measured after incubation of fat pads with radiolabeled rat lymph chylomicrons in flasks containing either buffer alone or with added glucose or glucose plus insulin. The addition of glucose and insulin led to a mean increase of 70% in the uptake of radioactivity, but larger adipocytes were stimulated less than smaller cells. This resulted in a significant negative correlation between the weights of fat pads and the uptake of radioactivity. Enlargement of fat cells also led to a diminution in their capacity to esterify fatty acids.

SUPPLEMENTARY KEY WORDS adipocyte number · adipocyte size

INCREASING WEIGHT and age have been reported to be associated with changes in the metabolism of lipids and glucose in rat adipose tissue. These include decreased lipogenesis (1, 2), reduced esterification of fatty acids (1, 2), diminished responsiveness to insulin (2–4), and lower activity of lipoprotein lipase (5), an enzyme responsible for the uptake of triglyceride by tissues such as fat.

This paper reports further studies on the behavior of adipose tissue obtained from rats of differing weights. We have measured the uptake of triglyceride fatty acids and the activity of lipoprotein lipase in epididymal fat pads and related these to the weights of the fat pads. The volume and number of adipocytes in fat pads were determined in isolated fat cell preparations and the metabolic activity of the tissue was expressed in terms of cell numbers.

## METHODS

### *Lipoprotein Lipase Activity*

Lipoprotein lipase activity was measured in acetone powder extracts of whole epididymal fat pads obtained from 35 male rats. The rats were of the white, John Curtin School strain. Their weights were between 130 and 345 g and their ages between 6 and 18 wk.

They had access to food until 9 or 10 a.m., when they were killed by a blow on the head. The fat pads were excised, weighed, immediately homogenized with acetone, and washed several times with acetone and ether. The acetone–ether residue was dried and then homogenized in 0.025 N $NH_4OH$, left for 1 hr at 0°C, and centrifuged. An aliquot of the supernatant solution was incubated at 37°C for 1 hr, together with 0.75 ml of

Abbreviations: FFA, free fatty acids; TGFA, triglyceride fatty acids.



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16% (w/v) bovine serum albumin in 0.1 M $(NH_4)_2SO_4$ (pH 8.2), 0.15 ml of 1 M Tris buffer (pH 8.2), 50 μg of sodium heparin, and 0.1 ml of a preincubated mixture (1:1, v/v) of 16 mg of triglyceride as Ediol (Schenlabs, New York) and serum from a fasted human subject. The free fatty acids (FFA) released during the incubation were estimated by the method of Dole (6). Lipoprotein lipase activity was expressed in the units: micromoles of FFA released per hr.

Lipoprotein lipase activity was also measured in acetone–ether residues of isolated fat cells obtained from the epididymal fat pads of 13 male rats weighing between 150 and 310 g. The fat cells and the acetone–ether residues were prepared as described by Rodbell (7) and modified by Goldrick (8). The fat pads were incubated with collagenase, albumin, and glucose for about 1.5 hr. The fat cells were then homogenized in acetone, filtered, and washed with acetone and ether, and the lipoprotein lipase activity was eluted with $NH_4OH$ from the residue on the filter paper.

### *Uptake of Triglyceride Fatty Acids*

The uptake of triglyceride fatty acids (TGFA) was measured in a series of experiments on a total of 62 fat pads. The pads were obtained from fed male rats whose weights were between 140 and 345 g.

Palmitic acid-9,10-$^3$H was obtained from the Radiochemical Centre, Amersham, England and its purity, checked by thin-layer and gas–liquid chromatography, was 98%. Labeled TGFA were obtained by the feeding of the labeled palmitic acid in corn oil to rats in which the cisterna chyli had been cannulated. The chyle was layered under 0.9% NaCl, 4% bovine albumin solution, and centrifuged at 20,000 rpm for 30 min in the 40.3 rotor of a Spinco ultracentrifuge. The supernatant chylomicrons were resuspended in saline. More than 90% of the chylomicron radioactivity was in TGFA. Distal portions of fat pads, between 500 and 700 mg in weight, were incubated in 5 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 0.1 ml of chylomicrons (0.5–0.7 mg of triglyceride/ml). The left and right fat pads from each of 31 rats were randomly allocated to flasks containing either buffer alone (28 studies) or buffer plus glucose, 0.5 mg/ml (15 studies), or buffer plus glucose and glucagon-free porcine insulin (Eli Lilly, Indianapolis, Ind.), 2 mU/ml (19 studies).

Incubation was at 37°C with gentle shaking. After 3 hr the pads were washed three times in buffer and the lipids were extracted by the Folch procedure (9). The TGFA and FFA fractions were separated by the method of Borgström (10) and the radioactivity was counted in a Packard liquid scintillation counter. We determined adsorbed radioactivity by dipping control fat pads into the incubation mixture for 30 sec, but since the amount of $^3$H in TGFA and FFA never exceeded 2% of the total found as "incorporated," no correction was made.

The variation in TGFA uptake between pieces of fat pads taken from the left and right sides was studied in 15 additional rats, using buffer alone. Variation was computed from the formula $\Sigma\Delta^2/2N$ (where $\Delta$ = difference between left and right pads expressed as a percentage of the mean of the pair and $N$ = number of pairs), and was found to be 10.9%.

### *Determination of Fat Cell Volume and Number*

The volume of individual fat cells and the total number in a fat pad were determined in an additional 21 rats weighing between 140 and 350 g. Isolated fat cells were prepared (8) and the mean diameter of the cells was calculated after measurement of at least 300 cells under a microscope fitted with a calibrated grid. The volume of the cells was calculated on the assumption that the cells were spherical and using the equation: $(\Pi/6)(3\sigma^2 + x^2)x$, where $\sigma^2$ is the variance of the diameter and $x$ is the mean diameter (11). The weight of each cell was then calculated by assuming that its density was that of triolein (12). The total lipid in the fat pad was obtained by measuring the carboxyl ester content (13) and expressing it as micrograms of triolein. The number of fat cells in each fat pad could then be calculated.

## RESULTS

### *Volume and Number of Adipocytes in Fat Pads*

Fig. 1 shows that increasing fat pad weight (which was correlated very closely with the weight of the whole rat) was associated with highly significant increases in fat cell volume ($r = +0.87$, $P < 0.001$) and numbers of fat cells ($r = +0.86$, $P < 0.001$). Fat pads of 800 mg, obtained from rats weighing about 150 g, were calculated to contain $4.6 \times 10^6$ adipocytes having a mean cell volume of 110,000 μm$^3$ each. A fat pad of 3600 mg from a rat weighing 350 g was calculated to contain $9.7 \times 10^6$ cells with a mean volume of 260,000 μm$^3$. The increase in the size and weight of the fat pads in this population of rats was therefore attributable to an increase in both size and number of adipocytes in approximately equal proportions.

In the following sections, lipoprotein lipase activity and triglyceride fatty acid uptake will be expressed in terms of cell numbers which have been derived from the calculated regression relating weight and number of cells in the 21 fat pads (Fig. 1).

### *Lipoprotein Lipase Activity*

The lipoprotein lipase content of whole fat pads varied from 10.8 to 25.7 units (μeq FFA per hr) per pad with a mean of 19.5 ± 3.7 (SD). Mean lipase content, ex-



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pressed per $10^6$ cells, was 3.4 ± 0.97 units and declined with increasing weight ($r = -0.82$, $P < 0.001$) (Fig. 2).

The lipoprotein lipase content of isolated fat cells was 4.7–18.6 units per fat pad (mean ± SD = 12.6 ± 5.3) or 2.3 ± 1.3 units per $10^6$ cells. However, these data cannot be compared with those for the whole pads since the preparation of the fat cells involved 1.5-hr incubations in the presence of albumin and glucose. We have previously found that lipoprotein lipase activity in vitro declines with a half-time of 1–2 hr but that this is reduced if albumin and glucose are added (14). Nevertheless, assuming a decline in activity of about 25% during the incubation and a recovery of only 75% of fat cells following treatment with collagenase, it is clear that the total amount of enzyme found in the fat cells from most rats could account for much of the enzyme content of the whole pad. With the isolated fat cells, there was also a significant inverse relationship between lipase activity per $10^6$ cells and the weight of the fat pads ($P < 0.01$).

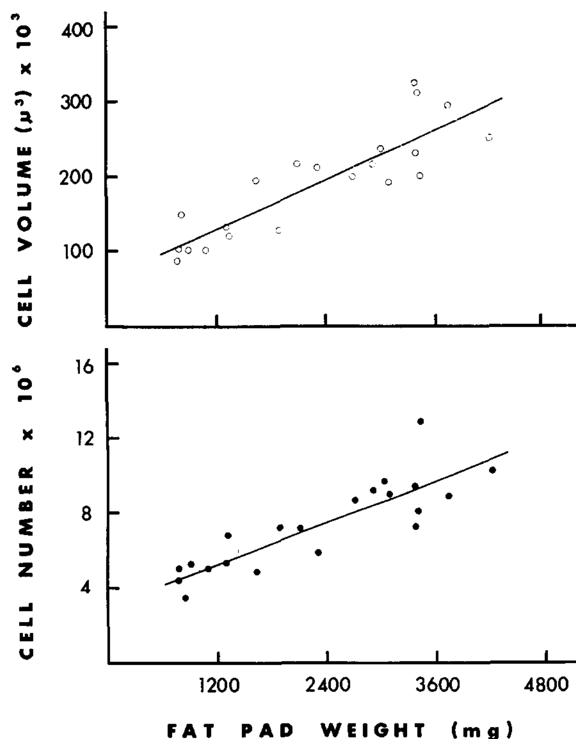


Fig. 1. Relationship between weights of rat epididymal fat pads and the number and the volume of the fat cells isolated from the pads.

### Triglyceride Fatty Acid Uptake

The results shown in Table 1 have been expressed in terms of the correlation coefficients that relate the weights of the fat pads with the uptake of radioactivity (cpm/$10^6$ cells) and with the proportions of radioactivity found in glyceride and FFA fractions (TGFA/FFA).

In the 28 incubations in buffer alone, there was no clear relationship between TGFA uptake per cell and the size of the fat pads (Fig. 3). That is, TGFA uptake was neither enhanced nor diminished with increasing cell size. The presence of glucose in the medium increased the total uptake of radioactivity by 32% but did not reveal a significant relationship between the weight of fat pads and the uptake of TGFA. However, whereas the relationship was positive in the buffer studies ($r = +0.12$), it became negative when glucose was added ($r = -0.12$). Since the total uptake of radioactivity was stimulated by glucose, the negative correlation suggested that larger cells were more resistant to stimulation by glucose then smaller cells. This trend was seen more clearly when insulin was also present in the incubation

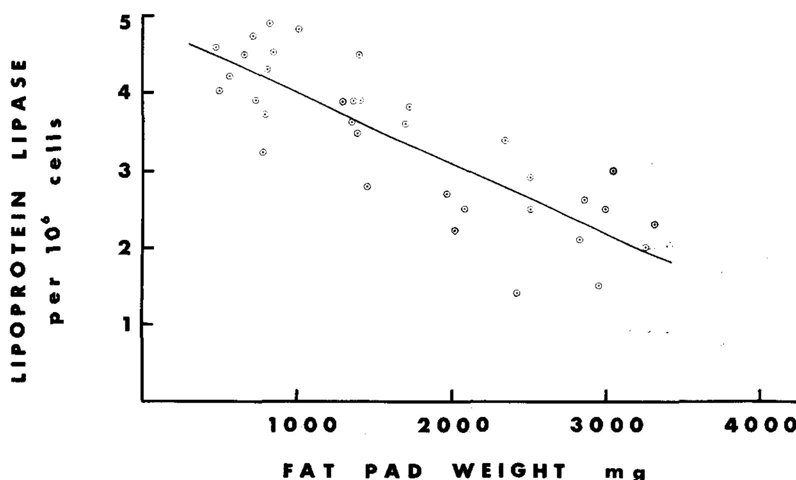


Fig. 2. Lipoprotein lipase content of rat epididymal fat pads (units = μmoles FFA released per hr per $10^6$ cells) related to the weights of the pads. Fat pads were homogenized, the residue was washed with acetone and ether, and the enzyme was extracted with $NH_4OH$.



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TABLE 1 CORRELATION COEFFICIENTS EXPRESSING THE RELATIONSHIPS BETWEEN THE WEIGHTS OF RAT EPIDIDYMAL FAT PADS AND THE UPTAKE OF TOTAL RADIOACTIVITY (cpm/$10^6$ cells) AND THE PROPORTIONS OF RADIOACTIVITY IN GLYCERIDE FATTY ACIDS AND FREE FATTY ACIDS (TGFA/FFA)

| Incubation Medium | Number of Studies | Range of Weights | | Radioactivity in fat pads | |
|---|---|---|---|---|---|
| | | Carcass | Fat Pads | cpm/$10^6$ cells | TGFA/FFA |
| | | *g* | *mg* | *r* | *r* |
| Buffer | 28 | 140–325 | 783–3838 | +0.12 | −0.54* |
| Glucose–Insulin | 19 | 140–325 | 822–3111 | −0.46† | −0.54† |
| Glucose | 15 | 145–345 | 508–4472 | −0.12 | −0.59† |

Adipose tissue was incubated with labeled chylomicron TGFA in Krebs' bicarbonate buffer to which glucose, 0.5 mg/ml, and insulin, 2 mU/ml, were added where shown.
* $P < 0.01$.
† $P < 0.05$.

mixture. The addition of glucose and insulin stimulated the uptake of radioactivity by an average of 70% compared to the uptake in buffer and resulted in a significant negative correlation between TGFA uptake and weight of fat pads ($r = -0.46$) (Fig. 3). Larger fat cells appeared therefore to respond less readily to glucose and insulin than smaller cells.

The relationships between the weights of the fat pads and the esterification of labeled fatty acids in the pads (TGFA/FFA) are also shown in Table 1. A significant inverse relationship was found in the three sets of experiments. Fatty acid esterification was therefore diminished in the larger fat cells; this was true in both the presence and the absence of glucose and insulin.

## DISCUSSION

These results show that epididymal fat pads of actively growing male rats increase in size and weight by roughly equal increments in cell numbers and cell volume (Fig. 1). Both Goldrick (11) and Zinder, Arad, and Shapiro (15) had previously shown that increasing adiposity and body weight were associated with an increase in the size of adipocytes. Goldrick found that rats, which were heavier than those we studied, increased the size of their fat pads predominantly by enlargement of cells (11). Hirsch and Han (16) have also suggested that an increase in cellularity and cell size are responsible for adipose tissue growth in younger animals, but that cellular enlargement accounts for most of the increase in the fat tissue of older animals.

When the activity of lipoprotein lipase was expressed in terms of cell numbers, it was clear that the enlargement of cells was associated with a decrease in enzyme content (Fig. 2). Since the total number of cells was greater in larger than smaller fat pads, the total enzyme contents of pads differing in weight were rather similar. Chlouverakis had previously reported reduced lipopro-

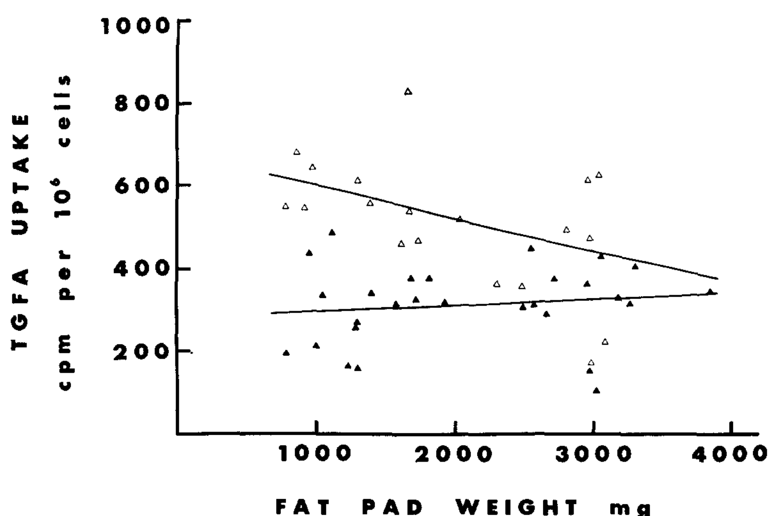


FIG. 3. Radioactivity (per $10^6$ cells) recovered from rat epididymal fat pads that had been incubated with labeled chylomicron TGFA in Krebs–Ringer buffer alone (▲) or in the presence of glucose plus insulin (△).



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tein lipase activity with increasing rat weight but had expressed the results per unit of wet weight (5).

Although the uptake of TGFA radioactivity was not influenced by the size of adipocytes when fat pads were incubated in buffer solution, the addition of glucose and insulin demonstrated that the enlargement of adipocytes blunted their responsiveness (Fig. 3). Furthermore, diminished esterification of fatty acids was demonstrable in the three series of experiments (Table 1). Our studies therefore support the findings of Benjamin, Gellhorn, Wagner, and Kundel (1) and of Di Girolamo and Rudman (2) who reported a reduction in esterification (or an increase in lipolysis) in adipose tissue of fat rats. We have previously reported that in fat pads of rats, glucose and insulin enhance both the activity of lipoproten lipase and the capacity of the tissue to take up TGFA (17). It seems that increasing adiposity interferes with this process.

Our studies confirm Rodbell's finding of lipoprotein lipase in isolated fat cells (7). The amount of enzyme recovered in the fat cells of the smaller pads was of the order generally found in whole pads that had been incubated for 1.5 hr (14).

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