Cell Size and Palmitate-1-14C Turnover of Rabbit Marrow Fat

By Sidney Trubowitz and Ambika Bathija

Adipose tissue is a major component of normal rabbit marrow. Morphological considerations suggest an active role for this tissue in hematopoiesis. This hypothesis was tested by injecting 50 µCi of palmitate-1-14C intravenously into fed, hematologically normal New Zealand rabbits. The animals were sacrificed 24 hr later and the femoral marrow removed. Samples of subcutaneous and perinephric fat were taken for comparison. The fat cells were isolated by the Rodbell method and the diameters measured. Incorporation of the 14C-palmitate into the triglyceride fraction was determined and the composition of the fatty acids was measured by gas chromatography. The mean diameter of the marrow fat cell was 46 µm (mean cell volume 55 pl); the mean diameter of the perinephric fat cell 70 µm (mean cell volume 200 pl). 14C-Palmitate turnover per gram triglyceride was some fivefold greater in the marrow fat; however, when expressed on a cell basis, the turnover for the marrow and perinephric fat cell was similar. The marrow fat contained a higher concentration of unsaturated fatty acids. These findings suggest that there is greater lipolysis and lesser storage in the marrow fat than in the perinephric.

Rabbit bone marrow, like human marrow, is a composite of rapidly proliferating and differentiating hematopoietic cells in a matrix of sinusoids, reticular cells, and adipose cells. Quantitively, the adipose tissue is a major component of the normal marrow matrix. Structurally, the adipose tissue in the marrow is a spherical pack of fat cells about which and between which the hematopoietic cells proliferate and the sinusoids course. The intimate relationship between the proliferating blood cell precursors and the fat cells, in a manner highly reminiscent of an embryo to its yolk sac, suggests a role for the fat cells in hematopoiesis other than purely structural.

The present study was therefore undertaken to determine and compare structural and functional parameters of the rabbit marrow fat cells and the fat cells from extramedullary sites, subcutaneous and perinephric, from the same animal.

MATERIALS AND METHODS

New Zealand white rabbits weighing 6-10 lb were used for the study. The animals appeared healthy and were determined to be hematologically normal by the usual blood studies, namely, hemoglobin, hematocrit determinations, and white blood cell and reticulocyte counts.

Fifty microcuries of palmitate-1-14C (New England Nuclear) containing 4-5 µmoles, bound to an equivalent concentration of serum albumin (fraction V) (Sigma Chemical) were injected intravenously into fed animals. The rabbits were sacrificed 24 hrs later under sodium barbital anesthesia. The entire marrow of both femora was removed, and samples of perinephric and subcutaneous fat were obtained for comparison.

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The fat cells of the marrow, subcutaneous, and perinephric adipose tissues were isolated by the Rodbell method using collagenase (Sigma Chemical). Plastic or siliconized glassware was used throughout for all preparations of fat cells.

On completion of the collagenase treatment the contents of the flask were centrifuged at 400 g for 1 min. The fat cells floated to the top of the flask, while the rest of the marrow cells (hematopoietic, reticular, and endothelial) sedimented to the bottom. The two layers were effectively separated by aspiration.

The total lipids were extracted from the fat cell layer by the method of Folch et al. The individual lipids were separated by thin-layer chromatography on silica gel G using petroleum ether, diethyl ether, and acetic acid (85:15:1) as the solvent. The component spots were visualized with iodine vapors, circumscribed, and upon disappearance of the iodine, scraped and transferred into scintillation vials. The spots were identified by comparison of the $R_f$ values with known standard mixtures.

Radioactivity was measured in a Packard Tri-carb liquid scintillation counter using 10 ml of the scintillation fluid containing 5.0 g 2,5-diphenyloxazole and 0.2 g $p$-bis[2-(5-phenyloxazolyl)] benzene/liter of toluene. Quenching was corrected by the use of the external standard method.

Fatty acid analysis was accomplished with the Hewlett-Packard 7610 gas chromatograph fitted with a flame ionization detector. Peak areas were determined with an automatic digital integrator model CRS-204 (Columbia Scientific). An aliquot of total lipids was used for the formation of methyl esters of fatty acids by transesterification with anhydrous methanol. The methyl esters of the fatty acids were separated at 185°C on 180 cm × 4 mm glass U columns packed with 10%, DEGS-PS on Supelcoport (80/100 mesh, Supelco) with nitrogen carrier gas flow of 40 cu cm/min. The fatty acids were identified by comparison of the relative retention times of unknown fatty acids with those of known fatty acids run under similar chromatographic conditions. The linearity of the response of the instrument was established by means of suitable NHI standards (Supelco). The percent composition of each fatty acid was determined by measurement of the peak area with the integrator.

The isolated fat cells were examined by interference microscopy and photographed for cell sizing. The diameters of approximately 100 isolated fat cells were determined by direct measurement of the projected photographed image. The results of such measurements compared favorably with the direct measurement of the cells with a filar micrometer. The mean cell diameter and

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Fig. 1. Adipose cells, femoral marrow of rabbit, interference microscopy. About × 490.
standard deviation were determined for each rabbit. Visual examination of the isolated fat cells revealed few adherent marrow cells. The bulk of the adipose cells were single and clean, but occasional small clumps of partially separated fat cells with adherent marrow cells were encountered. Crudely estimated, there were, on an average, about four to five marrow cells per fat cell in these preparations.

The mean cell volume was determined from the mean cell diameter and its standard deviation by the method of Goldrick utilizing the following formula:

$$\frac{4}{3} \pi (\bar{X}^2 + 3SD^2) \bar{X},$$

where $\bar{X}$ is the mean diameter of the cells and SD the standard deviation. The number of cells per gram of total lipid was calculated as $1.0 \, g \, total \, lipid/mean \, cell \, volume \times lipid \, density$. The density of the lipid was taken as 0.93.

Mean and standard deviation were calculated in the usual manner. Significance of differences between means was estimated by Student's $t$ test. Values of $p$ less than 0.05 were taken to indicate significance.

In the initial two animals of this study, the entire sedimented marrow cell layer was removed and treated in the same manner as outlined above for the fat cell layer. The total quantity of lipid recovered from this layer was 0.2 mg. The extracted lipid consisted wholly of phospholipid and free fatty acid, both of which contained the $^{14}C$ label. Triglyceride was virtually absent. It was clear, therefore, that the small contamination of marrow cells in the fat cell layer could make little contribution to the triglyceride extracted from that layer. It seemed reasonable to assume that the adipose cells were the sole source of the triglyceride obtained from the fat cell layer.

**RESULTS**

Except for size, the fat cells isolated from the adipose tissue of the marrow, perinephric, and subcutaneous sites appear identical. The cells are large spheres with an eccentrically placed nucleus. The Rodbell technique provides a fairly clean separation of the marrow fat cells, although some contamination by adherent hematopoietic cells is evident. Figures 1 and 2 demonstrate the isolated...
fat cells as seen by interference microscopy; the difference in size of marrow and perinephric fat cells is obvious. Since subcutaneous and perinephric fat cells are identical in size and metabolic behavior, the results are tabulated only for perinephric fat cells.

The mean marrow fat cell diameter was 0.65 of the mean diameter of the extramedullary fat cells. In all cases, the marrow fat cells were consistently and significantly smaller than the subcutaneous and perinephric fat cells. The diameter varied from animal to animal with a range in the marrow of 35-53 μm and a mean of 46 μm. The range of the diameters of the perinephric fat cells for the different animals was 54-81 μm with a mean of 70 μm (Table 1 and Fig. 3). The perinephric fat cell diameter was on an average about 1.6 times larger than the diameter of the marrow fat cell. Thus on a volume basis the perinephric fat cell was about four times larger than the marrow fat cell.

The rate of incorporation of labeled free fatty acid into triglyceride is a useful measure of triglyceride synthesis by adipose tissue. Although the technique does not measure the total esterification capacity of a given adipose tissue, it is a useful and valid method for the evaluation of triglyceride synthesis of different tissues. The palmitate turnover rate for bone marrow fat is about five times the palmitate turnover rate of the perinephric fat. The difference is consistent for all animals, although considerable individual variation is noted. Individual differences in turnover do not correlate with individual differences in fat cell size, but in every case the perinephric fat shows a significantly lower triglyceride turnover than the corresponding marrow fat (Fig. 4). However, when calculation of palmitate turnover is made on fat cell basis, there appears to be no significant difference (Table 2).

The fatty acid composition of bone marrow fat and perinephric or subcutaneous fat is similar. However, the total concentration of the unsaturated fatty acids is somewhat higher in the marrow fat. Figure 5 shows the ratios of

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**Table 1. Mean Cell Diameters of Rabbit Marrow and Perinephric Fat Cells**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Marrow (μm)</th>
<th>Perinephric (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35.0 ± 4.8</td>
<td>54.3 ± 6.3</td>
</tr>
<tr>
<td>2</td>
<td>41.2 ± 6.2</td>
<td>66.1 ± 8.8</td>
</tr>
<tr>
<td>3</td>
<td>52.4 ± 6.5</td>
<td>75.7 ± 12.7</td>
</tr>
<tr>
<td>4</td>
<td>48.7 ± 7.2</td>
<td>70.7 ± 10.9</td>
</tr>
<tr>
<td>5</td>
<td>50.8 ± 7.2</td>
<td>80.5 ± 11.2</td>
</tr>
<tr>
<td>6</td>
<td>44.7 ± 7.7</td>
<td>114.5 ± 21.7</td>
</tr>
</tbody>
</table>

Mean ± SD.

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**Table 2. Mean Fat Cell Volume and 14C-Palmitate Turnover**

<table>
<thead>
<tr>
<th>Fat Cell</th>
<th>Fat Cell Volume (pl)</th>
<th>No. of Cells/g TG (10⁶)</th>
<th>CPM/Cell (10⁻⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>54.58 ± 20.95</td>
<td>23.62 ± 12.25</td>
<td>5.02 ± 4.14</td>
</tr>
<tr>
<td>n = 6</td>
<td></td>
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</tr>
<tr>
<td>Perinephric</td>
<td>200.35 ± 75.18</td>
<td>6.42 ± 3.25</td>
<td>3.42 ± 3.34</td>
</tr>
<tr>
<td>n = 5</td>
<td></td>
<td></td>
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</tbody>
</table>

(p < 0.005) (p < 0.02) (p > 0.50)
saturated to unsaturated fatty acids of marrow and perinephric fat of the animals studied. Except for one animal where there is little difference between marrow and perinephric fat, the remaining animals show consistently higher unsaturation of the marrow. The mean value of the saturated to unsaturated fatty acid ratio for the marrow is 0.57 and for the perinephric fat 0.68. The difference is not large, but probably significant ($p < 0.05$).

DISCUSSION

Although adipose tissue is a major component of normal marrow, it has attracted little attention. Jolly$^9$ recognized the fat cell as a structural unit of the normal hemopoietically active marrow. Oberling et al.$^{10}$ concluded on the basis of ultrastructural studies of the rabbit marrow that the adipocyte not only played a fundamental role in the structural organization of the marrow matrix, but because of its high content of glycogen and cell organelles, an important metabolic or functional role as well. Tavassoli$^{11}$ and Tavassoli et al.$^{12}$ concluded from their studies on the effect of starvation and hemolysis on marrow fat that...
the development and the degradation of the marrow adipocyte was regulated by hemopoiesis rather than by extramedullary factors.

It is now a well-recognized fact that adipose tissue is metabolically active. The perfection of a technique for the isolation of individual cells by Rodbell and the development of sizing procedures by Hirsch and Gallian have been largely responsible for the knowledge explosion in the physiology and metabolism of the fat cell. Determination of the fat cell size has been a dominant theme in investigations on adipose tissue because of the established relationship between cell size, metabolic rate, and insulin sensitivity. In general, increasing fat cell volume correlates with decreasing lipolysis. These relationships are sharply modified by other variables such as age, nutrition, and animal species.

The marrow fat cells of the normal rabbit were shown to have a mean cell volume of about 55 pl; the perinephric fat cells, a mean cell volume of about 200 pl. This difference in cell size of the marrow and extramedullary adipose cells in the same animal suggested a difference in behavior, on a metabolic level, between the different adipose tissues. Thus greater metabolic rate and insulin sensitivity would be anticipated for the marrow adipocytes.

Palmitate turnover studies did demonstrate a fivefold greater uptake of the fatty acid in the marrow adipose tissue. On a cell basis, incorporation of palmitate into marrow and perinephric fat cells, however, appeared similar. One is, therefore, forced to conclude that the smaller marrow fat cell is lipolytically more active than the perinephric fat cell and stores less fat.

Studies of the fatty acid composition of the bone marrow fat have shown relatively small differences from the compositional characteristics of other adipose tissues. Zach and Shafrir demonstrated marked interspecies differences in fatty acid composition, but rather consistently similar composition of different adipose sites in the same animal. In the rabbit, only small differences were found between red and yellow marrow: the yellow marrow was somewhat richer in palmitoleic acid. In the present study, gas chromatography revealed a somewhat higher concentration of unsaturated fatty acids in the marrow. The ratios of saturated to unsaturated fatty acids obtained in this study were very similar to those found by Shore in the rabbit.

The findings of the present study suggest that fat storage is probably a secondary function of the marrow fat, and that metabolic activity in the marrow fat is regulated by marrow factors such as blood supply and hemopoiesis.

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