Phospholipid and Plasmalogen Changes during Functional Differentiation of Adipose Tissue in the Newborn Rat*

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The experiments reported in this paper are concerned with (a) the changes in specific chemical constituents in presumptive adipose tissue undergoing functional differentiation, and (b) the possible role of plasmalogen and phospholipid in the anabolism and catabolism of neutral fat. In vertebrates, adipose tissue develops from spindle-shaped cells which appear to be primitive connective tissue elements (1, 2). As the cell undergoes functional differentiation, lipid droplets accumulate in the cytoplasm, and the cell withdraws its processes and rounds up. When differentiation is complete, the cell may be many times its original diameter, and the cytoplasm is reduced to little more than a peripheral film. This rapid accumulation of fat suggests marked changes in cell metabolism and cell permeability, particularly in regard to the metabolism and absorption of fatty acids. Since phospholipid in general (3), and plasmalogen in particular (4, 5), have been implicated in processes associated with the absorption and mobilization of fatty acids, it was decided to investigate the changes in plasmalogen relative to phospholipid and to total lipid in mesenchymal cells undergoing rapid accumulation of lipid. The subcutaneous connective tissue of the newborn rat was chosen for this investigation, since little is present at birth and rapid functional differentiation occurs during the first week of life.

Changes in the chemical composition of developing adipose tissue are expressed in terms both of tissue dry weight and of tissue DNA phosphorus content. During the period of lipid infiltration, there are marked changes in both cell size and cell number (via cell migration into the region), as well as changes in tissue water and lipid content. It is important, therefore, to distinguish between changes in phospholipid relative to other tissue components and the changes in phospholipid per cell. Since the amount of DNA per cell of somatic tissue shows no significant change with variations in the physiological or nutritional state of the animal (6, 7), it is possible to obtain an estimate of the average amount of each chemical constituent per cell in tissue undergoing physiological change. There are, however, two possible sources of error in the use of DNA as a reference substance. First, dividing cells have double the DNA content of connective tissue cells not undergoing functional differentiation. According to most authors (8), adipose tissue is loose connective tissue in which the fat cell has displaced most of the other elements. Compressed fibroblasts, lymphoid cells, and mast cells are scattered in the narrow spaces between the adipose cells. This possible source of variation will be considered later in connection with the observed net increase in phospholipid and plasmalogen in developing adipose tissue.

**EXPERIMENTAL PROCEDURE**

Newborn rats of the Wistar strain were killed by decapitation at birth (day 1), and at 24-, 48-, and 72-hour intervals as shown in Table I. The subcutaneous adipose tissue from the pelvic and pectoral regions was removed, freed from extraneous connective tissue, and frozen on glass at −72°. Variation among litters was minimized by taking one animal from each of five litters and pooling for each individual age group. The pooled tissue from each age group was extracted by brief homogenization in 20 volumes of a mixture of chloroform and methanol (2:1, v/v). The suspension was filtered on a Buchner funnel, and the residue was reextracted twice with several volumes of chloroform-methanol (2:1). The combined filtrates were washed with distilled water at 5° according to Folch et al. (9). The interfacial material was removed by freezing and filtration, and the residual chloroform was removed in a vacuum at 40°. Determinations were then made of abelhydogenetic lipids (plasmalogen), lipid phosphorus, and lipid weight. Plasmalogen was determined by the p-nitrophenylhydrazone method of Wittenberg, Korey, and Swenson (10) as modified by Rapport and Alonzo (11). Corrections were made for material absorbing at 390 μm in the acid-treated sample. Lipid phosphorus was determined by the procedure of Beveridge and Johnson (12). Total lipid was determined gravimetrically. Nucleic acids were extracted with hot trichloroacetic acid by the method of Schneider (13). DNA was estimated by the diphenylamine method of Dische (14). RNA was determined by the orcinol method of Meijbaum (15).

**RESULTS**

Total Lipid and Tissue Hydration—The amount of total lipid, lipid phosphorus, and plasmalogen (as p-nitrophenylhydrazone) found in subcutaneous adipose tissue from rats 1, 2, 3, 5, and 8 days old are shown in Table I. Each value represents the analysis of pooled tissue from five animals. For each tissue...
adult rat kidney or brain (16). However, the lipid content accumulate over successive days reaching a value of 80.7% of increases nearly 2-fold within the first 24 hours and continues to column lists the RNA:DNA ratios found for each age group. The last comparison with the tissue lipid phosphorus content. The last nucleic acids have been expressed in terms of micrograms of RNA phosphorus and DNA phosphorus per g of dry tissue for comparison with the tissue lipid phosphorus content. The last column lists the RNA:DNA ratios found for each age group.

The results show that at birth total lipid constitutes 31.6% of dry tissue weight, a value comparable to that reported for adult rat kidney or brain (16). However, the lipid content increases nearly 2-fold within the first 24 hours and continues to accumulate over successive days reaching a value of 80.7% of the dry weight on the 8th day of life. The average change per cell obtained by relating these changes to the DNA content is shown in Fig. 1. It is seen that the deposition of lipid per cell rises rapidly during the first 48 hours and the rate decreases during the subsequent 5-day period. The cell water content, on the other hand, does not decrease until the 3rd day of life and may even increase during the first 24 hours after birth. By the 8th day of life, a 10-fold increase in total lipid and a concomitant decrease of 34% in the water content of the cell are found.

**Phospholipid and Plasmalogen**—The results in Table I show that, at birth, the lipid phosphorus content of developing adipose tissue is 71.0 μmoles per g of dry tissue, a value comparable with published data for other rat tissues of predominantly mesodermal origin, i.e., skeletal muscle (10, 17) and spleen (17). It is interesting, however, that the aldehydogenic lipid content of developing adipose tissue is high compared to values reported for other rat tissues. At birth, adipose tissue contains 18.9 μmoles per g of dry tissue, a value similar to that found for rat lung (17) and exceeded only by rat brain (16, 17). If one assumes that the aldehydogenic chains are associated almost entirely with phospholipids, plasmalogen would account for over 26% of the total phospholipid content of the presumptive adipose cell in the newborn rat. Only in brain tissues of rat (10, 17) have plasmalogens been found to constitute as much as 28% of the total tissue phospholipids.

Initially high levels of plasmalogen and lipid phosphorus decrease rapidly as the adipose tissue fills with fat, reaching values of 6.1 and 28.6 μmoles per g of dry tissue, respectively after 8 days. This apparent inverse relationship between total phospholipid or plasmalogen and total lipid or dry tissue weight is not found, however, when lipid is correlated with DNA phosphorus. Fig. 2 shows the percentage of change in the total lipid phosphorus and higher fatty aldehyde (plasmalogen) content of the tissue. Each component is represented as the percentage increase over that present at birth. The results indicate that during the first 24 hours of life, as the total lipid of the cell increases nearly 4-fold (Fig. 1), the total phospholipid increases about 30%. This increase in lipid phosphorus per cell continues during successive 24-hour intervals and on the 8th day of life reaches a value 68% above the level at birth. On the other hand, plasmalogen accumulates much less rapidly than total phospholipid, indicating a differential accumulation of nonaldehydogenic phospholipids within the developing adipose cell. This shift in the phospholipid composition of developing adipose tissue confirms the histochemical studies of Mückel (4), and the more recent observations of Yarbro and Anderson (5). However, the plasmalogen concentrations (per g of fresh tissue)

### Table I

<table>
<thead>
<tr>
<th>Age</th>
<th>Water</th>
<th>Lipid</th>
<th>Lipid phosphorus</th>
<th>Plasmalogen</th>
<th>DNA phosphorus</th>
<th>RNA phosphorus</th>
<th>RNA DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>% dry weight</td>
<td>μg/g dry weight</td>
<td>μg/g dry weight</td>
<td>μg/g dry weight</td>
<td>μg/g dry weight</td>
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<tr>
<td>1</td>
<td>80.0</td>
<td>31.6</td>
<td>71.0</td>
<td>18.9</td>
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<td>2300</td>
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<tr>
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<td>30.5</td>
<td>49.3</td>
<td>11.7</td>
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<td>1320</td>
<td>5.74</td>
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<td>35.5</td>
<td>35.5</td>
<td>8.2</td>
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<tr>
<td>4</td>
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<td>40.0</td>
<td>30.2</td>
<td>6.6</td>
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<td>720</td>
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<tr>
<td>5</td>
<td>61.4</td>
<td>40.7</td>
<td>28.6</td>
<td>6.1</td>
<td>106</td>
<td>610</td>
<td>5.75</td>
</tr>
</tbody>
</table>

* Average absolute standard deviation based on three analyses of each of the pooled tissue samples.

**Fig. 1.** Changes in total lipid weight and tissue water content in developing subcutaneous adipose tissue of the newborn rat during the first 8 days postnatally. Each component is expressed as milligrams or grams per mg of tissue DNA phosphorus.

**Fig. 2.** Increase in total lipid phosphorus and p-nitrophenylhydrazine (NPH) plasmalogen in developing subcutaneous adipose tissue of the newborn rat during the first 8 days postnatally. Each component is expressed as milligrams per mg of tissue DNA phosphorus.
Lipids are predominantly phospholipids, it is apparent that the ratio of RNA to DNA does not change significantly during lipid deposition. Thus, the RNA content of the cell apparently remains unchanged during lipid infiltration even though the RNA-containing structures of the cell are progressively confined into the narrow rim of the cytoplasm that eventually constitutes only a very small fraction of the total cell volume. Finally, the ratio of RNA to DNA is much higher for developing adipose tissue than for other fetal or adult rat tissues. The average value of the ratio of RNA to DNA for the five age groups reported here is 5.62. In other rat tissues studied, only liver and pancreas have been reported (6) to have higher RNA:DNA ratio noted for developing adipose tissue may reflect the onset of specialization of tissue function in an incompletely differentiated cell.

**DISCUSSION**

The results of the present study show that the rate of deposition of total lipid with respect to the DNA content of developing subcutaneous adipose tissue is rapid in the first 48 hours postnatally, and decreases during the subsequent 5-day period. As lipid droplets accumulate within the cell, the concentration of both plasmalogen and total phospholipid increase relative to the DNA content of the tissue. These results suggest that there is a net synthesis of phospholipid per cell corresponding to the period of lipid deposition within the cytoplasm of the cell. By the 8th day, there was an increase of 68% in the phospholipid per cell. Since the tissue certainly contains a proportion of non-differentiating mesenchymal cells, this value is not characteristic of a single cell type but rather indicates a net increase of phospholipid per cell in those cells undergoing lipid accumulation.

However, plasmalogen increases much less rapidly than the total phospholipid. If one assumes that the aldehydogenic lipids are predominantly phospholipids, it is apparent that there must be a differential synthesis of specific phospholipids within the adipose cell, as has recently been reported for brain (18).

This study confirms one finding concerning phospholipid metabolism in adipose tissue (based on less satisfactory methodology) but contradicts another. The observation which is confirmed (4, 5) is that a specific type of phospholipid, plasmalogen, decreases relative to total phospholipid during rapid lipid infiltration of the developing adipose cell. However, the report that the total phospholipid decreases during the deposition of neutral lipid (5) is valid only for phospholipid change referred to weight of total lipid. In contrast, phospholipid increases during this process when the phospholipid change is related to the individual cell.

**SUMMARY**

The changes in the aldehydogenic lipid, phospholipid, total lipid, nucleic acid, and water content (expressed per mg of tissue DNA phosphorus) of developing adipose tissue of newborn rats have been followed during the first 8 days of life. The results indicate that the rate of deposition of total lipid referred to the DNA content of the tissue rises rapidly in the first 2 days postnatally, whereas the water content per cell does not decrease until the 3rd day. As the adipose tissue fills with lipid, the concentration of both plasmalogen and phospholipid increase with respect to DNA content, suggesting a net synthesis of phospholipid per cell during the period of accelerated lipid deposition. Plasmalogen, however, accumulates less rapidly than the total phospholipid, indicating a differential accumulation of nonaldehydogenic phospholipids. A characteristic of developing adipose tissue is the constant value for the RNA:DNA ratio.

**REFERENCES**

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