Acid and Neutral Lipases of Cultured Adipocytes of Infants and Children

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Summary

In order to determine whether the mobilization of intracellular triglycerides observed in cultured human adipocytes is associated with changes in the activities of acid and neutral lipases, the activities of both enzymes were measured weekly on cultured adipocytes for several months. The activity of acid lipase was initially very low, but rose to levels 40 times the original activity within 3 months. The activity of neutral lipase decreased rapidly within the first 2-4 weeks and remained at approximately 25% of original levels thereafter.

Speculation

There is increasing evidence that the human adipocyte is highly specialized and that it is derived from a stromal precursor present in the adipose tissue. The cell has the capacity to acquire in culture the properties of the precursor, as evidenced by the appearance of acid lipase and disappearance of neutral lipase. We submit that the low level of acid lipase in the adipocyte is related to its lipid storage function. It remains to be proven that it is not primary to the differentiation of the adipocyte from its precursor. Furthermore, we speculate that a direct biochemical relationship exists between the neutral lipase and intracellular triglyceride stores in the adipocyte.

Although lipid mobilization from adipose tissue appears to be under the control of a number of endogenous and exogenous influences, the final step in mobilization is hydrolysis of triglycerides by lipases. Neutral lipases have been characterized in the adipose and other tissues of a number of species (4-7, 11-17, 20, 21, 23, 27, 29-33). They are active at neutral pH, have affinity for long chain triglyceride esters, and sensitive to hormones such as epinephrine and insulin. Acid lipases are lysosomal in subcellular location (13, 20, 32). First described in the liver (13, 28, 32), acid lipases are active around pH 4.0 and appear to have a somewhat broader chain length preference. In adipose tissue, an acid lipase, which could be stimulated by lipolytic hormones (18, 28) has been described as sedimenting with mitochondria (18, 28). This is the class of lipases deficient in the tissues of patients with lipidoses such as Wolman's disease and cholesteryl ester storage disease (8).

Earlier, we showed that human adipose cells can be sustained in culture for long periods of time (1, 2) and have described some of the morphologic and enzymatic properties of these cells in culture (2). Other investigators (9, 24, 34) have also established presumptive "preadipose" or adipose cells in culture. In one study (24), cultured "preadipose cells" accumulated triglycerides and incorporated glucose into lipids, thus resembling adipose cells rather than skin fibroblasts. In a recent study, Van *et al.* (34) found measurable levels of lipoprotein lipase activity in cultured "adipocyte precursors" of human adipose tissue. These putative "preadipose cells" were also found to possess 15 times

as much triglyceride synthetase activity as skin fibroblast from the same patients and twice the fatty acid synthetase activity. Dixon-Shanies *et al.* (9) have found that cells cultured from adipose tissue of human adults have some metabolic characteristics which distinguish them from skin fibroblasts. We showed recently that the cultured human adipocyte is capable of mobilizing its own stored lipids over a period of weeks in culture (3). In the present study, we have attempted to determine the changes in the activities of acid and neutral lipases which accompany the mobilization of triglycerides from cultured adipocytes.

MATERIALS AND METHODS

Two to 3 g adipose tissue were obtained from the anterior abdominal wall of each of nine infants and children, aged 6 days to 16 years. Eight children were over 2 years of age. These children were undergoing routine operative procedures and the samples were excised at the onset of the operation. The samples were transferred to the laboratory for culture and for assay immediately after excision.

Tissues were divided into three portions of similar weight. Two portions were incubated with collagenase to isolate adipocytes, as previously described (1). The isolated adipocytes were cultured, as described previously (1, 2), in 25-ml plastic flasks with 3 ml McCoy's 5A (modified) medium (35) supplemented with 20% fetal calf serum (35). Cultures were kept at 37° in 5% CO₂/95% balanced air. The initial cultures were left undisturbed for 4-7 days and the cells were fed three times weekly thereafter. The cells in each flask were transferred to a new flask weekly (25) and split every 2-3 weeks depending on when confluence occurred. Duplicate flasks from each cell line were harvested at the times indicated in Figure 1 by methods previously described (1, 3); the homogenate was centrifuged at 20,000 × g for 10 min at 4° and the soluble aqueous fraction was kept on ice and assayed within 1 hr.

One of the portions of the whole tissue and a portion of the initial fresh adipocytes were each washed three times in 0.1 M phosphate buffer, pH 7.0, and homogenized in an equal volume of the buffer in a Teflon grinder for 1-2 min. The homogenates were centrifuged at 20,000 × g for 10 min at 4°. The pellets were discarded; the middle portion, called the soluble aqueous fractions, was stored on ice and assayed generally within 1 hr. However, this could be stored at -70° because both enzyme activities were stable for periods up to 1 month if thawed only once.

Acid lipase (AL) was assayed fluorometrically (8) in an Aminco-Bowman spectrophluorometer (36) using 4-methylumbelliferyl oleate (MUO) as substrate (37) in 0.2 M acetate buffer, pH 4.0, in the presence of sodium taurocholate and lecithin. Neutral lipase (NL) was assayed by a similar method, except that the buffer was 0.2 M phosphate, pH 7.0. One unit of enzyme activity was defined as 1 nmol MUO hydrolyzed/

min/mg protein. Protein was determined by the method of Lowry *et al.* (19). The data was analyzed by the paired Student *t*-test with statistical significance assigned only when the *P* value was less than 0.05. The values of protein and enzyme activities were stated as the mean $\pm$ SEM.

RESULTS

The dispersed adipocytes had a mean protein content of 4.02 ± 0.85 mg/g wet packed cells. The specific activity of NL in the soluble fraction of the adipocytes was 2.19 ± 0.45 , which was not significantly different from that in the soluble fraction of the whole adipose tissue, 2.29 ± 0.64 ($P > 0.1$). The specific activity of AL in freshly dissociated adipose cells was 0.67 ± 0.19 , comparable to the value in whole adipose tissue, 0.46 ± 0.13 ($P > 0.1$).

The adipocytes in culture gradually changed shape and assumed a stellate fibroblast-like appearance. At the same time, they gradually lost their stored lipids. As shown in Figure 1, serial determinations of NL and AL in the cultured adipocytes revealed striking changes with time. The initial NL activity in the adipocytes 2.19 ± 0.45 decreased progressively in about 6 weeks to a level between 0.46 and 0.65, at which it stabilized. The difference in the NL values between day 0 and day 32 is highly significant ($P < 0.001$).

By contrast, a slow but steady increase of AL activity in adipocyte was found during the first 4–7 weeks of culture, after which it increased more rapidly to a peak 40 times the original levels ($P < 0.001$) by 10 weeks (Fig. 1).

DISCUSSION

Cultured human adipocytes are capable of mobilizing their stored triglycerides in the absence of specific stimulator of lipolysis (3). This is reflected in the release of considerable amounts of glycerol by cultured adipocytes into the culture medium (22). We (3) have found, as did Nyberg *et al.* (22), that intracellular triglycerides are not reduced much during the first week of culture; they are reduced considerably thereafter (3). In the current study, NL activity was seen to decline as early as 8 days of culture.

The process by which cultured adipocytes mobilize their triglycerides appears to involve a significant and simultaneous alteration in the activity of NL. The similarity in the pattern of decrease in the activity of NL in cultured adipocytes, reported

here, and the decrease in the intracellular triglyceride content of cultured adipocytes reported previously (3) raises the possibility that the two events are related biochemically.

The activity of AL in adipocytes is extremely low, and considerably lower than that found in normal fibroblast (8) and other nonadipose tissues (13, 20, 32). It is even lower than that found in tissues of patients with Wolman's disease and cholesteryl ester storage disease (8), in which the deficiency of AL in various nonadipose tissues has been associated with the inability of cells to clear triglycerides resulting in intralysosomal lipid accumulation.

The large increase in AL activity of adipose cell after it has been in culture for a long time suggests an adaptation which may reflect the loss of the "lipid storage" function of the cell under the culture conditions employed in this study. It is possible but unlikely that the increase in AL activity played a significant role in the observed lipid mobilization since most of the intracellular triglycerides had been mobilized by the time the large increase in AL activity occurred. On the other hand, as noted earlier, the decrease in NL activity of the cell occurred nearly simultaneously with the observed lipid mobilization. The fate of AL and NL under lipogenic conditions may reveal more about their importance in lipid storage and mobilization in cultured adipocytes.

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Fig. 1. Specific activities (nanomoles of 4MU(4-MU)/min/mg protein) of acid and neutral lipases (AL and NL) in the soluble fractions of cultured adipocytes of children.

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Lead
metabolic balance studies

Absorption and Retention of Lead by Infants

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Summary

Eighty-nine metabolic balance studies were performed with 12 normal infants ranging in age from 14-746 days. Intake and fecal and urinary excretions of lead were determined and net absorption and net retention were calculated. Subjects were fed milk or formula and commercially prepared strained foods. Intakes of lead ranged from 0.83-22.61 $\mu\text{g/kg/day}$ with a mean of 9.43 $\mu\text{g/kg/day}$. Urinary excretion averaged 1.02 (SD 0.68) $\mu\text{g/kg/day}$ and was positively correlated with lead intake (Fig. 1). Fecal excretion was highly correlated with intake of lead (Fig. 2); fecal excretion exceeded intake in 10 studies. In 61 balance studies with lead intakes greater than 5 $\mu\text{g/kg/day}$, net absorption averaged 41.5% of lead intake and net retention averaged 31.7% of intake.

Retention of lead was highly correlated with lead intake (Fig. 3). Urinary plus fecal excretion of lead exceeded intake in 19 of 28 balances in which lead intakes were less than 5 $\mu\text{g/kg/day}$. Absorption and retention of lead were inversely correlated with intake of calcium (Fig. 4). Absorption and retention of lead accounted for greater percentages of intake of lead in this study of infants and young children than have been reported in studies of older subjects.

Speculation

Highly efficient absorption and retention of ingested lead by young children may be partly responsible for the high prevalence of lead intoxication in this age group.

In 1971 an *ad hoc* committee of the Bureau of Community Environmental Management, PHS (9) suggested 300 $\mu\text{g/day}$ for children as the daily permissible intake of lead from all sources. Children 1-3 years of age were specifically included. The daily permissible intake was based, in part, on the assumption that 90% of ingested lead would be excreted—an assumption based on extrapolation from data concerning adults.

However, it is known that suckling rats absorb a greater percentage of ingested lead than do older rats (6, 10). In addition, Alexander *et al.* (1) have reported data from 11 metabolic balance studies with subjects less than 8 years of age, including six studies with three subjects less than 2 years of age. As estimated from the figures included in the publication, these studies indicated that considerably less than 90% of ingested lead was excreted.