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Fluorometric Measurements of Lipase Activities in the Adipose Tissue of Infants and Children

FO ADEBONOJO*

Summary

Adebonojo FO. Fluorometric Measurements of Lipase Activities in the Adipose Tissue of Infants and Children. *Nigerian Journal of Paediatrics* **1982; 9: 51.** Using a fluorometric assay method, the activities of acid lipase and neutral lipase were measured in isolated adipose cells obtained from 41 infants and children. The values of the activities of both lipases were directly correlated and both increased with age and cell size, suggesting that the control of lipid mobilization in the adipose cell may be partially related to these lipases. Since body weight ultimately depends on the size of the adipose depot and since the activities of these lipases reflect the size of that depot, determination of these enzymes may be useful in the assessment of the nutritional state in severe dysnutrition such as in grade IV malnutrition where they may help to focus attention on the affected children with the greatest need for expert hospital-based care.

Introduction

RECENT interest in adipose tissue lipolytic enzymes recognizes the significance of this tissue in energy metabolism and the clinical importance of errors of lipid metabolism in the different dysnutritional states.[1-6] The size of the adipocyte directly reflects the size of its lipid content.[7 8] It has been suggested that the lipid depot in an adipocyte consists of two pools. The first of these is the large, slow, central, relatively stable globule making up the largest bulk (80–90%) of the cell. This pool consists principally of long chain triglycerides. The second pool is smaller, faster, more labile and cytosolic in location. It consists primarily of microdroplets of short chain triglycerides.[9] These two pools appear to be in equilibrium with each other.

Earlier, we have demonstrated that the mean adipose cell size of normal children progressively increases with age up to five years.[10] Other workers have shown that the adipose cell size of children peaks at about eight years of age, but increases again in early adulthood.[11] Culture studies on adipose cells of at least, two species appear to suggest that the levels of activities of some of the lipolytic enzymes, especially the neutral lipase, may directly correlate with the size of the intracellular lipid pool.[1] It appears, therefore, of interest to study the levels of activities of some of the lipases in the adipose tissue of children of different ages to see if there is any

Faculty of Health Sciences, University of Ife, Ile-Ife

Department of Paediatrics and Child Health
*Professor

correlation in the enzyme activities with age. In the present study, we have examined the activities of acid and neutral lipases in the adipose tissue of children of different ages from six months to 18 years.

Materials and Methods

At the beginning of elective surgery, adipose tissue specimens were excised from the anterior abdominal wall of 41 children (25 males and 16 females), aged six months to 18 years. The children were divided into 5 age groups (Table). The specimens were taken in McCoy's 5A modified medium directly to the laboratory, where they were thoroughly washed several times in saline. The samples were then collagenased to isolate the individual adipose cells from their stromal support as previously described.[12] The isolated cells were then washed thrice in 0.2 M phosphate buffer pH 7.0 to eliminate any residual transfer medium still present. The cells were homogenized in a Teflon grinder in an equal volume of the buffer for two minutes. The homogenates were centrifuged at 20,000 x g for 10 min at 4°C. The soluble aqueous fraction below the top fat layer was removed and stored on ice and assayed generally within one hour. However, this fraction was sometimes stored at —70°C as the enzymes under study had been found to be stable for periods up to one month if thawed only once (Adebonojo, unpublished observation).

Acid lipase (AL) was assayed by a fluorometric method[13] in an Aminco-Bowman spectrophotofluorometer using 4-methylumbelliferryl oleate (MUO) as substrate 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.[13] One unit of enzyme activity was defined as one nanomole of 4-MUO hydrolysed per min per mg of protein. Protein was assayed by the method of Lowry *et al.*[14]

The data was analysed in two ways. The first employed the paired Student t test, assigning statistical significance only when the p value was less than 0.05 between groups. The values of the enzymes activities were stated as the Mean ± SEM. The second plotted the log of NL (ordinate) against the log of AL (abscissa) in a scattergram and using linear regression analysis, the correlation coefficient (r) and slope (m) and y intercept (b) were calculated where $y = mx + b$ is the least squares regression equation of y on x.

Results

Table 1 depicts the activities of AL and NL in the adipose tissue of the different groups of children and the number of children in each age group. The activities of AL increased progressively from the youngest groups, Group I (0.18 ± 0.07) and Group II (0.34 ± 0.10), reached a peak in Group III (0.84 ± 0.36), then declined in the older children, Group IV (0.39 ± 0.09) and Group V (0.36 ± 0.17). However, the differences between any two groups did not reach statistical significance.

TABLE

Specific Activities (nmole of 4 MU released/min/mg/prot) of Acid Lipase (AL) and Neutral Lipase (NL) in the Adipose Tissue of 41 Infants and Children According to Age

Age Group (Yrs)	*No. of Children*	*AL*	*NL*
< 1	6	0.18 ± 0.07	0.58 ± 0.28[a]
1–3	6	0.34 ± 0.10	0.65 ± 0.20[b]
4–7	9	0.84 ± 0.36	2.82 ± 0.79 [abcd]
8–13	10	0.39 ± 0.09	1.35 ± 0.39[c]
14–18	10	0.36 ± 0.17	1.24 ± 0.26[d]

Student t test

	t	*df*	*p*
a	2.217	13	<0.025
b	2.180	13	<0.025
c	1.717	17	NS
d	1.985	17	<0.05

NS = Not significant

The activities of NL showed a group trend similar to that seen in the AL activities. The Group I value (0.58 ± 0.28) was similar to that in Group II (0.65 ± 0.20) and both were significantly lower than Group III value (2.82 ± 0.79) with $p < 0.025$ in each case. The NL value in Group IV (1.35 ± 0.39) was also similar to that in Group V (1.24 ± 0.26), but only the Group V value was significantly lower than Group III value with $p < 0.05$. The NL value in Group IV was not statistically different from the value in Group III ($p < 0.05$).

The values of each pair of 2 + log NL on the ordinate and 2 + log AL on the abscissa were plotted for the 41 samples. Linear regression analysis of the values of log NL + 2 and log AL + 2 yielded a correlation coefficient (r) of 0.473, linear regression (lr) of 0.496 ($p < 0.005$) and the equation $y = 0.39x + 1.43$ where $y = \log NL + 2$ and $x = \log AL + 2$. From this equation, it is possible to predict a probable range of values for neutral lipase when the value for acid lipase is known or vice versa.

Discussion

Using a fluorometric assay method, it has been possible to estimate the levels of activities of acid lipase, an enzyme believed to be lysosomally located and that of neutral lipase, probably cytosolic in location, although it may be tightly bound to the surface of the large central lipid globule. The findings suggest a correlation in the levels of these enzymes in the same sample and a progressive increase in the activities of both enzymes with age, peaking at 4 to 7 years. This agrees with our earlier findings[10] and those of Knitlle[11] who demonstrated a peak in the mean adipose cell size at 8 years of age. In recent culture studies, we speculated that there may be a relationship between the triglyceride pool in the adipocyte and its level of neutral lipase activity.[1] The findings of this study appear to lend support to that speculation, since with increasing adipose cell size, the neutral lipase activity also increases

We have also suggested, from studies on human adipocytes, that the acid lipase activities may correlate with the ability of the cell to clear cytosolic lipid microdroplets[1] but rat studies (Adebonojo, unpublished observations) have not confirmed this suggestion.

At present, it is difficult to classify the different grades of malnutrition encountered in Nigeria. Some employ the ratio of actual weight to expected weight for age and sex as an indication of the degree of malnutrition. Others use skinfold thickness and clinical assessments plus the ratio mentioned above. In the outpatient situation where the patient population is rather large, simple mid-arm circumference measurements plus clinical assessment are used. However, in the admitted very ill child, there is no reliable single measure which is predictive of the grade of malnutrition and which may portend the prognosis appropriate for the grade, and perhaps the level of expert care required for the particular child.

It would be of great interest to extend this study to infants and children in different stages of malnutrition in which the amount of lipid deposit in the adipose cell ranges from slightly less than normal (Grade I) to depleted (Grade IV). It may turn out to be a more accurate measure of the degree of dysnutrition for children of given ages.

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