Lysosomal acid hydrolases of normal adipose tissues of man, monkey and rat

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Summary. The activities of 3 lysosomal acid hydrolases were examined in the normal adipose tissues of 3 species, man, monkey and rat and very low levels of these hydrolases were found.

Biochemical and morphological evidence suggests that the adipose cell is a highly specialized cell[2-4] derived from a stromal precursor present in the adipose tissue[5,6]. The biochemical mechanisms involved in the differentiation of the putative preadipose cell are not understood, although it appears that the ability to synthesize lipid is present in the preadipose cell before it differentiates morphologically into an adipocyte. The cultured human preadipocyte has been found to incorporate more labelled glucose into lipids[5], accumulate triglycerides more efficiently[5], contain much more triglyceride synthetase, fatty acid synthetase and lipoprotein lipase activities than cultured skin fibroblasts[6]. The cultured adipose cell also appeared able to acquire some of the characteristics of its precursor enzymatically[4] and morphologically[7].

The importance of lysosomal enzymes in normal cellular metabolism is suggested by the very high level of activities of these enzymes in many tissues[8-10]. Little is presently known about the activities of lysosomal enzymes in adipose tissue. The low level of lysosomal acid lipase activity which has been found in the adipose cells increased significantly when the cells were maintained in culture for weeks and after the cells had mobilized their stored lipids[11], suggesting that a relationship may exist between the lysosomal acid lipase activity in the adipose cell and its lipid storing and lipid free states.

The significantly reduced levels of lysosomal acid lipase reported in human adipose tissue[11], and the lack of information on other lysosomal enzymes in this tissue, led us to examine the activities of other lysosomal acid hydrolases, acid B-galactosidase, N-acetyl-B-D-glucosaminidase as well as acid-lipase in the normal adipose tissues of 3 species, man, monkey and rat.

Methods. Adipose tissues obtained from perirenal masses of 2 fetuses aged 16 and 20 weeks, the anterior abdominal wall of 19 lean children aged 2–10 years, fasted overnight and undergoing routine operative procedures; 5 normal adult monkeys (*Macaca mulatta*), also fasted overnight and 5 normal adult Charles River rats fasted overnight, were immediately transferred to the laboratory and processed as described.

The tissues were freed of as much vascular elements as possible, washed thoroughly in 0.2 M phosphate buffer, pH 7.0, and homogenized in a volume of distilled water equal to the volume of the tissue in a Teflon grinder for 1–2 min. The homogenates were centrifuged at 20,000 × g for 10 min at 4 °C and the pellet and top fat cake discarded. Earlier studies (unpublished) had demonstrated that lysosomal enzymes were absent from the pellet. The middle soluble aqueous fraction was kept on ice and immediately assayed. Lysosomal acid lipase (LAL) was assayed by the fluorometric method of Cortner et al.[9] using 4-methylumbelliferyl oleate (4MUO) as substrate. Acid-B-galactosidase (B-Gal) and N-acetyl B-D-glucosaminidase, otherwise called hexosaminidase (Hex) were assayed by the methods of Kolodny and Mumford[12] using the appropriate 4-methyl-umbelliferyl glycosides as substrates. The specific activity was expressed as 1 nmole of substrate hydrolyzed per min per mg protein. Protein was assayed by the method of Lowry et al.[13].

Results. The table shows that the activity of B-Gal in the rat adipose tissue was 7 times that in the adipose tissues of man ($p < 0.005$) and of the monkeys ($p < 0.005$), but similar in the adipose tissues of the monkey and man.

The specific activity of Hex in the rat adipose tissue was twice that in the adipose tissue of man ($p < 0.025$), and not significantly different in the adipose tissues of monkey and rat or of man and monkey. The specific activities of LAL in all samples were very low, although significantly higher in rat adipose tissue than in the monkey adipose tissue ($p < 0.025$), higher in the monkey adipose tissue than in man's adipose tissue ($p < 0.005$) and higher in rat adipose tissue than in the adipose tissue of man ($p < 0.005$). The specific activities of all 3 lysosomal enzymes, LAL, B-Gal and Hex were significantly and markedly higher in the human fetal adipose tissue than in the adipose tissues of children and monkeys. Compared with the activities of these enzymes in the rat tissue, human fetal LAL and Hex activities were significantly higher but the B-Gal activity was higher in the rat tissue than in the human fetal tissue (table).

Discussion. This study shows that the activities of all 3 lysosomal acid hydrolases are higher in the adipose tissue of the rat than in the adipose tissue of man or monkey. These levels of activities are very low in the adipose tissues of all 3 species. The activities of the same enzymes in other tissues, e.g. liver, muscles, and lymphocytes of the same species[8-10] are some 10–100 times those in the adipose tissues. Human fetal adipose tissues, however, reveal lysosomal enzyme activities comparable to those seen in the skin fibroblasts and other tissues.

Specific lysosomal enzymes have been found to be deficient in various forms of diseases in which the substrates of the enzymes accumulate and are intralysosomally stored in the cell[8,10]. In the adipose tissue, however, the activities of these lysosomal enzymes are very low, probably because of the specialized function, which adipocytes have differentiated to perform. But at which stage in adipocyte differentiation the marked reduction in lysosomal enzymes occurs

The activities (nmole substrate hydrolyzed per min per mg protein) of 3 lysosomal acid hydrolases, acid-B-galactosidase (B-Gal), hexosaminidase (Hex) and lysosomal acid lipase (LAL) in normal adipose tissues of fetal human, man, monkey and rat. Activities are expressed as mean ± SEM.

	N	B-Gal	Hex	LAL
Fetal human	2	0.68 ± 0.15	11.93 ± 0.71	2.53 ± 0.33
Man	19	0.17 ± 0.02[a]	1.78 ± 0.27[c]	0.23 ± 0.02[e,f]
Monkey	5	0.16 ± 0.01[b]	1.93 ± 0.45[d]	0.39 ± 0.05[e,f]
Rat	5	1.11 ± 0.17[a,b]	3.16 ± 0.83[c,d]	0.68 ± 0.10[f,g]

Student 't'-test

	t	Degree freedom	p-value
a	10.280	22	< 0.005
b	5.296	8	< 0.005
c	2.150	22	< 0.025
d	1.302	8	> 0.10
e	3.063	22	< 0.005
f	6.870	22	< 0.005
g	2.588	8	< 0.025

remains unknown, although our present preliminary data would suggest that it occurs after 20 weeks of gestation. We are studying more fetal adipose tissues at different gestational ages to determine when the reduction in the activities of the lysosomal enzymes occur in the differentiating adipose cell. If it occurs after the cell had fully differentiated, it may be possible to determine if the reduction in the lysosomal hydrolases is primary or secondary to the process of lipid accumulation in the cell.

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Purification and partial characterization of a human plasma α_1-heteroglycan

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Summary. A heteroglycan was purified from human plasma and partially characterized in terms of its major properties. It is noteworthy that the carbohydrate content of this blood constituent is unusually high (75%).

During our studies on the very soluble macromolecular constituents of human plasma, we have recently discovered a nondialyzable heteroglycan which distinguishes itself by an unusual chemical composition. The present paper describes the isolation, purification and some of the major chemical and physicochemical properties of this blood polysaccharide. *Isolation of the heteroglycan.* Pooled normal plasma (250 l) was fractionated according to Cohn's method 6[2]. After removal of the 5 major protein fractions[2] a supernatant solution[3] (400 l) was obtained which contained, in addition to low mol.wt acidic[4], neutral[5] and basic[6] proteins, the hitherto unknown heteroglycan. For the isolation of this heteropolysaccharide, 200 g of CM-cellulose previously equilibrated against pH 5.5, $\Gamma/2$ 0.05 sodium acetate buffer was mixed with the mentioned supernatant solution, stirred overnight and allowed to settle. Because of the presence of the high concentration of ethanol in this solution (40%), the isolation procedure was carried out at -5 °C. Subsequently, the CM-cellulose with the adsorbed protein was suspended at 4 °C in the above mentioned buffer and centrifuged. This technique which was repeated once, led to the removal of the bulk of ethanol and protein. The washed CM-cellulose was then transferred in the cold into an appropriate column and eluted with the same buffer but employing a NaCl gradient (figure). The first fraction contained primarily albumin and the second, in addition to the latter protein, α_1- and β-globulins. Fraction 3, however, contained essentially a heteroglycan (0.25 g) which on paper electrophoresis stained intensely with PAS and weakly with amidoblack. Fractions 4 and 5 contained albumin and α_1-globulins, respectively.

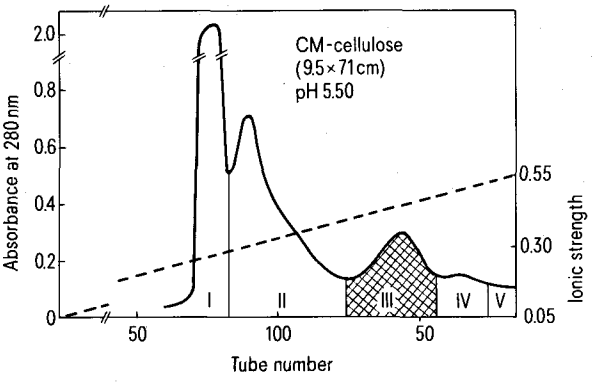
Purification of the plasma heteroglycan. Rechromatography of fraction 3 under the same conditions as described above afforded the removal of a considerable portion of the contaminating proteins at low ionic strength while the carbohydrate was displaced at higher ionic strenght. Other proteins (β_1- and γ-globulins and basic proteins) were eluted at even higher salt concentrations.

Partial characterization of the plasma heteroglycan. Homogeneity of this plasma heteroglycan was established by paper (pH 8.6, $\Gamma/2$ 0.1 citrate-barbiturate buffer) and disc

Composition of human plasma α_1-heteroglycan

Physicochemical properties	
Molecular weight	70,000
Sedimentation constant (S)	3.6
Electrophoretic mobility[a] ($cm^2 \cdot volt^{-1} \cdot sec^{-1}$)	-4.5×10^{-5}
Chemical properties	
Sialic acid (%)	25
Neutral hexoses (%)	25
Hexosamines (%)	25
Total carbohydrate content (%)	75
Peptide moiety (%)	30

[a] At pH 8.6 $\Gamma/2$ 0.1 citrate-barbiturate buffer.



Chromatography on CM-cellulose of a crude heteroglycan preparation isolated from normal human plasma. A flow rate of 200 ml/h was employed and 18-ml fractions were collected. The proteins and the heteropolysaccharide were eluted at a constant pH of 5.5 using an ionic strength gradient (initial $\Gamma/2$ 0.05 as NaAc and final $\Gamma/2$ 0.55 as 0.05 NaAc plus 0.50 M NaCl). Appropriate fractions were pooled as indicated by the roman numerals. Fraction III (cross-hatched) contained the heteroglycan.