

Studies on Developing Adipose Tissue: Lipolytic Activity in Human Fetal Subcutaneous Tissue as an Indication of Adipose Potential

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Summary

In a previous study of children in the first 2 years of life in which all cells in adipose tissue were examined, we found numbers of small cells which do not contain the extensive intracellular triglyceride deposits characteristic of the mature fat cell. Similar cells have also been detected in fetal subcutaneous tissue. It seems important to establish whether the cells of fetal subcutaneous origin are adipocyte precursors. Isolated adipose cells and homogenates of isolated cells can be expected to show hormone-sensitive lipase activity. This paper reports studies on the lipolytic activity of fetal subcutaneous tissue cells. Fetal subcutaneous tissue, obtained after therapeutic abortion, was dissected from the abdominal wall. To provide a substrate for lipolytic activity in fetal tissue where little intracellular triglyceride is present, triolein as glycerol trioleate was added to the incubations. Additions of either heparin or epinephrine were made at timed intervals. Levels of cAMP were determined in homogenates of tissue after epinephrine stimulation without added triglyceride. Accumulation of tissue triglyceride starts at 16-17 weeks and increases through the ages studied. After the addition of epinephrine (10^{-4} M) there is substantial release of free fatty acid (FFA) with all tissue homogenates. The addition of epinephrine to the preparation in which no exogenous triglyceride substrate had been added, was associated with a rise in cAMP. Glycerol release was also detected at this time. A dose relationship for cAMP production after epinephrine is present at each age studied. Changing levels in cAMP in fetal tissue with epinephrine suggests an activity similar to the mature adipose cell membrane. These findings do not necessarily indicate an *in vivo* process occurring at this period of development but rather show a functional ability of the cell. When considering this potential in a cell taken from a site where adult subcutaneous adipose deposition occurs, the definition of an adipose cell should not rely entirely on the presence of accumulated triglyceride.

Speculation

The recognition of fetal tissue with the lipolytic characteristics of mature fat tissue suggests that in fetal life, this tissue may be responsive to changes in the fetal environment. The nutritional and hormonal environment of the fetus may thus influence the development of adipose stores.

There has been considerable importance placed on the significance of numbers of cells in developing adipose tissue. Obese children have increased numbers of fat cells in their adipose tissue and there have been several studies defining the age when fat cell replication can occur. Brook *et al.* (2) have reported that fat cells increase in number in the fetus from 30 weeks of gestation. Hirsch and Knittle (14) have shown that hyperplasia in adipose tissue may occur to 13 years of age. In these studies

only mature adipocytes were studied, as identification of cells has depended on the presence of accumulated triglyceride within the cell. Adipocytes may only undergo replication before they accumulate triglyceride as no further division is thought to occur once the cell contains fat (12). Thus the reports of critical times for fat cells replication are not based on observations made on dividing fat cells and may not reflect the actual time of adipose cell replication.

In a previous study of children in the first 2 years of life, in which all cells in adipose tissue were examined, we have found numbers of small cells which do not contain the extensive intracellular triglyceride deposits characteristic of the mature fat cell (1). Similar cells have also been detected in fetal subcutaneous tissue from gestational age 16 weeks. It was not possible in that study to establish morphologically whether these cells were precursors to fat cells, although their presence in abundance in fetal subcutaneous and in infant adipose tissue has led us to believe that they may be so. It would seem important to establish whether the cells of fetal subcutaneous origin are in fact adipocyte precursors, and this has been attempted by investigating one of their metabolic characteristics, lipolytic ability.

Lipolytic activity in adipose tissue may be attributed to the action of intracellular triglyceride lipases and lipoprotein lipase (17, 19, 27). Of the intracellular triglyceride lipase activities, hormone-sensitive triglyceride lipase has been demonstrated in rat and human adipose tissue (11, 21). The initial activation in the lipolytic response to hormones is that of adenylyl cyclase at the cell membrane. This results in the activation of cAMP dependent protein kinase with subsequent activation of triglyceride lipase within the adipose cell (5, 16).

The extracellular lipoprotein lipase is found in human adipose tissue and is located in the capillary endothelium of the tissue (8, 24). This enzyme hydrolyses protein bound triglyceride and activation by heparin is a distinguishing feature of its activity (7, 15).

Isolated adipose cells and homogenates of isolated cells can be expected to show hormone sensitive lipase activity, whereas total adipose tissue and its homogenates may in addition show heparin-activated lipase activity. This paper reports studies on the lipolytic activity of fetal subcutaneous tissue cells to establish their potential as adipocyte precursors.

MATERIALS AND METHODS

Fetal subcutaneous tissue, obtained after therapeutic abortion, was dissected from the abdominal wall in a region immediately above the entry of the umbilicus. This area is known to be one of adipose tissue deposition in the adult and was chosen to exclude any known area of brown fat accumulation in the fetus. Gestational age was from 10-22 weeks judged from maternal history and crown-rump measurements. Tissue was transported at room temperature and used immediately wher-

ever possible. In studying fetal tissue in which triglyceride content is not constant and isolation of cells achieved only with difficulty, finely ground tissue was used throughout. Results were expressed per g tissue wet wt. Homogenates of fetal tissue were prepared at 4° in Krebs-Ringer phosphate buffer at pH 7.4 containing half the reported calcium concentration (22) by using a glass hand-held homogenizer.

TRIGLYCERIDE CONTENT

Triglyceride content was estimated as glyceride-glycerol (26) after Folch extraction (10). Triglyceride content was expressed as milligrams of triglyceride per tissue wet wt.

HOMOGENATE LIPOLYTIC ABILITY

To provide a substrate for lipolytic activity in fetal tissue where little intracellular triglyceride is present, triolein as glycerol trioleate (BDH Chemicals Ltd.) was added to the incubations. Triolein was purified by chromatography on florisil (6) and homogenized in 0.05 M phosphate buffer. Human serum albumin obtained from the Commonwealth Serum Laboratory, Melbourne, as Cohn fraction V (recrystallized), extracted with *n*-heptane acidified to pH 3.2 with glacial acetic acid to remove preformed fatty acids, was added before homogenization. Final concentration of triolein was 25 μ mol/incubation with albumin concentration at 4%. In the initial experiments no glucose, insulin, or cofactor lipoprotein was added. Triolein substrate was incubated with tissue homogenate at pH 7.4 at a temperature of 37° in an atmosphere of 95% O₂-5% CO₂. Additions of either heparin (heparin injection BP 1000 μ /ml; Boots Co. Ltd., Nottingham, England) or epinephrine (adrenalin injection 1:1000, Abbott Laboratories, Sydney, Australia) were made at timed intervals. In addition, tissue homogenate was incubated with phosphate buffer as a measure of release from endogenous triglyceride. The reaction was terminated by the addition of copper nitrate-triethanolamine reagent (27) followed by 2.0 ml chloroform for the determination of free fatty acids as copper soaps by the method of Duncombe (9). Results were expressed as micromoles of FFA released from substrate triglyceride per g tissue over 20 min.

Levels of cAMP were determined in homogenates of tissue after epinephrine stimulation without added triglyceride. Aliquots of the incubation medium were precipitated with 6% trichloroacetic acid and the supernatant washed with water-saturated ethyl ether. The washed supernatant was dried under N₂ and reconstituted in Tris-EDTA buffer. cAMP levels were measured using a competitive binding radioimmunoassay technique (Amersham Radiochemicals, The Radiochemical Centre, Amersham, Bucks, England). Results were expressed as picomoles of cAMP released per g tissue.

Although in some tissue the release of glycerol to the incubation medium could be measured by an enzymic micromethod (18), the low endogenous triglyceride content of fetal tissue gave resulting glycerol levels at the limit of detection. To confirm lipolytic activity in the incubation, 1 μ mol [2(n)-H³]glycerol trioleate (Amersham Radiochemicals) was added in a parallel experiment. The use of [8-H³]cAMP in the immunoassay of cAMP prevented the simultaneous use of [2(n)-H³]glycerol trioleate. After extraction of the incubation medium with heptane, tritiated glycerol levels were determined in the aqueous phase.

RESULTS

Accumulation of triglyceride in the tissue with gestational age of the fetus is shown in Figure 1. Although the triglyceride content of the tissue is below that found in adipose tissue containing mature fat cells (780-900 mg/g), a substantial accumulation of tissue triglyceride starts at 16-17 weeks and increases through the ages studied.

Table 1 tabulates the increase in lipolytic activity of whole homogenates of fetal tissue induced by addition of either heparin or epinephrine. Release of FFA after addition of heparin was high in early gestation which is consistent with the higher proportion of capillary endothelium at this stage. After the addition of epinephrine (10⁻⁴ M) there is substantial release of FFA with all tissue homogenates.

The addition of epinephrine to the preparation in which no exogenous triglyceride substrate had been added, was associated with a rise in cAMP. Glycerol release was also detected at this time (Table 2). When radiolabeled triglyceride was added to the preparation, epinephrine again produced glycerol release, confirming lipolytic activity (Fig. 2). A dose relationship for cAMP production after epinephrine is present at each age studied (Fig. 3) but is more easily demonstrated in tissue taken at later gestation. Increases in basal cAMP production of 39, 106, and 170 pmol/g/10 min for gestational ages 16, 20, and 22 weeks, respectively, seen when epinephrine is omitted, may be a function of tissue maturity.

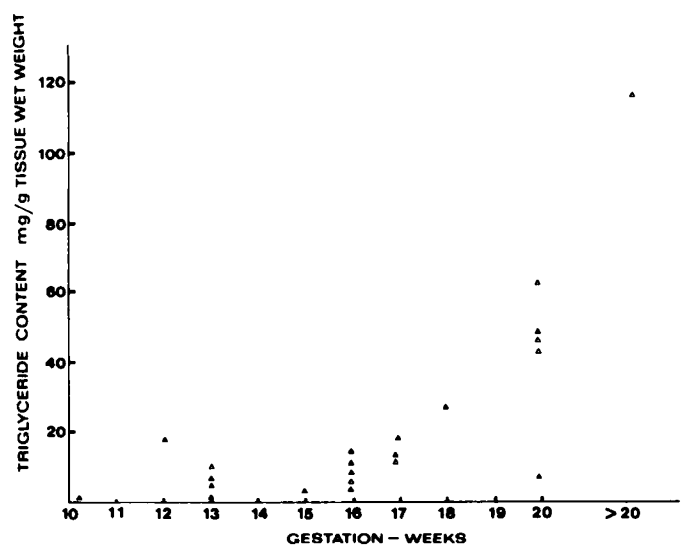


Fig. 1. Age-related changes in triglyceride content of fetal subcutaneous tissue.

Table 1. Release of FFA from 25 μ mol added triolein substrate by fetal tissue homogenates

Gestational age of tissue	FFA release, μ mol/g tissue/20 min ¹	
	13-16 weeks	16-18 weeks
Basal	0.8 (.04)	0.28 (0.14)
Heparin added (25 U)	18.6 (2.7)	13.5 (2.4)
Epinephrine added (10 ⁻⁴ M)	14.4 (4.4)	27.2 (5.5)

¹ Mean and SD of four duplicate measurements at each point shown.

Table 2. Glycerol and cAMP levels after epinephrine

Time after epinephrine (10 ⁻⁴ M) ¹	Glycerol, nmol/g tissue wet wt	cAMP, pmol/g tissue wet wt
0	5.3	170
2 min	11	445
5 min	14	430
10 min	24	350
20 min	32	225

¹ Measurement of glycerol and cAMP was made after epinephrine stimulation (10⁻⁴ M) in fetal tissue homogenate (40-60 mg) of 22 weeks of gestation. Triglyceride level of this tissue was estimated as 120 mg/g wet wt. Values are the mean of four observations at each time.

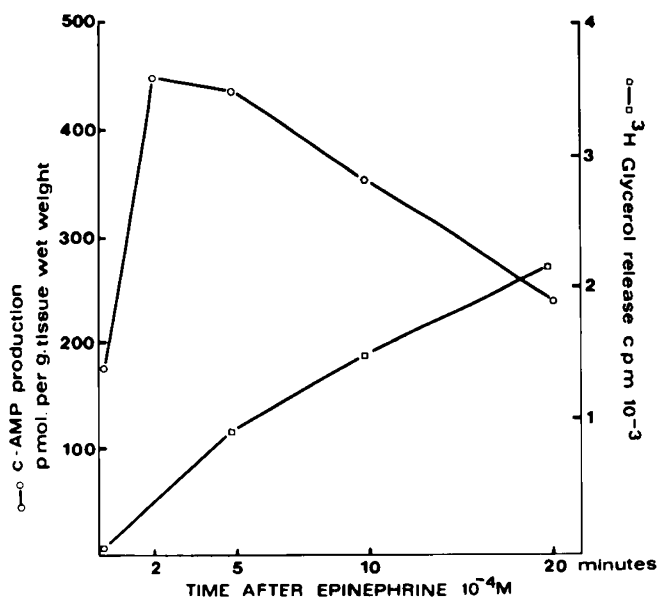


Fig. 2. cAMP production after epinephrine stimulation in fetal subcutaneous tissue homogenate (40–60 mg wet wt) taken at 22 weeks of gestation. Glycerol measurements obtained from separate incubations. Points shown are the means of four observations on a single tissue sample.

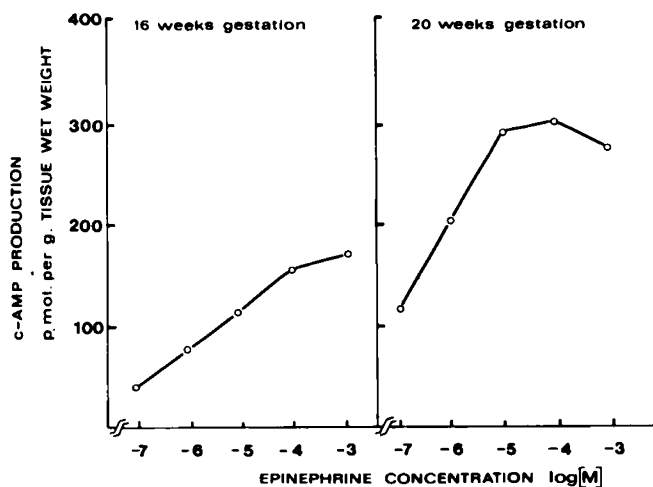


Fig. 3. Dose-response curve of epinephrine in fetal subcutaneous tissue. cAMP measured after incubation of fetal subcutaneous tissue (15 mg wet wt) with epinephrine 10^{-3} – 10^{-7} M at 37° for 10 min. Points shown are the mean for four observations on a single sample at each gestational age.

DISCUSSION

Most studies on the metabolism of mature adipose tissue have been made on isolated cell preparations in which cells are released from their connective tissue matrix after incubation with collagenase and separated by flotation (22). These cells have the metabolic features of intact tissue characterized by synthesis of triglyceride and by lipolytic activity. Lipogenesis is not unique to adipose tissue and may be demonstrated in a number of tissues including liver and intestinal mucosa (20). We believe, however, that demonstration of intracellular hormone-dependent lipase activity is a specific characteristic of adipose cells and therefore may be used to identify them. cAMP acts as a common mediator for various hormones in mammalian tissues, and differentiation within the target cell determines the physiologic response. For an adipose cell the characteristic action of lipolytic hormones is the release of FFA and glycerol from

stored triglyceride through activated triglyceride lipase (4). Activation of this lipase through the adenylyl cyclase system has been demonstrated in mature adipose tissues of many mammalian species (21, 27).

It has been reported for rat adipose tissue that the ability of heparin to increase lipolytic activity in fat pad homogenates is due to increased activity in the stromal cells only (15). It was not possible to separate lipolytic activity of fetal cells from that of other components of fetal tissue in this homogenate, but the differential lipolytic responses to heparin and epinephrine implies that the tissue possesses the function of mature adipose tissue. Although in the adult, triglyceride lipase is most active during fasting, the activity of lipoprotein lipase is maximal in the fed state (15). When investigating fetal tissue, although the maternal nutritional status is assessed as fasting, the same metabolic condition cannot be assumed for the fetus. From this study, demonstration of triglyceride hydrolysis after the addition of heparin to fetal tissue suggests that lipoprotein triglyceride may be made available to the fetal cell.

Of greater importance is the demonstration of increase in medium cAMP levels after epinephrine stimulation. Epinephrine was used as an hormonal stimulus, for although both epinephrine and norepinephrine stimulate activity of adenylyl cyclase (25), norepinephrine is specifically implicated in the response to cold exposure in developing animals (23). This specificity may introduce a limitation in studying the undefined tissue used in these studies. Similarly, isoproterenol, a β -receptor stimulus, was not used. Glucagon has transient sensitivity in rat subcutaneous adipose tissue (13), but human adipose tissue has been reported to be unresponsive to glucagon (3). Changing levels in cAMP in fetal tissue with epinephrine suggests an activity similar to the mature adipose cell membrane.

These findings demonstrate a potential for this tissue. They do not necessarily indicate an *in vivo* process occurring at this period of development but rather show a functional ability of the cell. When considering this potential in a cell taken from a site where adult subcutaneous adipose deposition occurs, the definition of an adipose cell should not rely entirely on the presence of accumulated triglyceride. A study of the factors regulating triglyceride appearance in fetal subcutaneous tissue may determine critical times for hypercellularity in adipose tissue which precede those currently accepted.

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