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DEVELOPMENT OF ADIPOSE TISSUE IN THE HUMAN FOETUS

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Summary

Studies on foetal and infant adipose tissue have shown that in addition to maturing fat-containing adipocytes there are small cells that do not contain fat. Ultrastructural studies showed that these small cells were metabolically active, and many showed early accumulation of triglyceride as vacuoles within the cell. These cells possessed hormone sensitive lipase activity with an active adenyl cyclase system, a feature thought to be unique to fat cells. The cells were also shown to be capable of fat synthesis.

During foetal development there was a rise in activity of an enzyme concerned with fat synthesis, acetyl CoA carboxylase from 10 weeks gestation, and this activity preceded the appearance of triglyceride within the cell.

These studies appear to have characterised pre-adipocytes in foetal tissue at a time when active growth may set the stage for future fat stores.

There is increasing evidence that obesity that starts in early childhood tends to persist into childhood and adult life (Lloyd and Wolffe 1961; Asher 1966; Eid 1970; Heald and Hollander 1965). These studies tend to confirm that many fat babies remain fat throughout childhood. The reasons for this are not known but it has been suggested that the degree of obesity is largely dependent on the number of fat cells present in the body (Brook 1972a; Hirsch and Knittle 1970). There is experimental evidence from both animals and humans that the final fat cell number is influenced by early nutritional factors. However, it is not known whether these factors operate in foetal life. In the rat the final number of adipocytes is reached by 15 weeks of age, and is profoundly influenced by the variations in the energy intake produced by alterations in the litter size, particularly in the first six weeks of life before weaning (Knittle and Hirsch 1968). These observations on rats cannot be directly applied to humans, but this stage of development of the young rat probably corresponds to the last trimester of pregnancy in the human, and it has been suggested that the sensitive period for fat cell replication includes these months, and extends to the end of the first year. Brook found that children who became obese in the first year had more fat cells than those becoming obese after the first year, and children who were growth retarded in the foetal stage had fewer fat cells (Brook 1972b). Despite the lively interest in the cellular basis of fat stores and the relationship to the development of obesity, there is little known about the normal development of fat tissue in the foetus.

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In a study reported earlier (Boulton et al. 1974), we studied the size of cells from adipose tissue in the foetuses and infants, after separating the cells by collagenase, and fixing with osmium tetroxide. All cell particles in each sample were measured using a particle sizer which was used to count and size the cells, and which presented data as curves of cell size distribution. Subcutaneous tissue from the anterior abdominal wall of 13 foetuses of approximately 11-15 weeks gestation obtained at therapeutic abortion for medico-social reasons, and two preterm infants of 28 weeks gestation who died soon after birth were studied. These studies suggested the presence of cells smaller than the mature fat cell.

In a further study of 10 foetuses of gestational age 10 to 24 weeks, the particle distribution profile consisted of a single peak in which all cells were less than 17.5 microns in diameter. Microscopic examination showed that the peak was comprised of particles of connective tissue debris and cells in which osmium fixing was only apparent in the membrane. No centrally osmium fixed cells were present. Thus these cells apparently contained no fat.

In a group of five foetuses and infants of 20 weeks gestational age to two months postnatal age, the particle distribution profile showed an increasing number of larger cells extending the peak seen in the younger foetuses. Microscopic examination showed that in addition to the connective tissue debris and membrane fixed cells seen in the first group, a number of larger cells with central osmium deposition characteristic of fat cells prepared by this method were seen.

These studies showed that in foetal life and early infancy there are two types of cells in adipose tissue. The smaller cells, particularly found in tissue before birth and the first months of post-natal life, did not contain fat and this observation led to the proposition that these cells might represent preadipocytes.

Foetal tissues containing these cells were then examined by electron microscopy. Subcutaneous adipose tissue was taken from the anterior abdominal wall of four children aged 2 months and 22 months. Sub-cutaneous tissue was also obtained from a para-umbilical site in four foetuses of gestational age 16 weeks. These areas were chosen because they are the sites for future white fat development and avoids areas containing brown fat. Electron microscopic ultrastructural studies show that the tissue was composed of a relatively uniform population of cells within sparsely collagenous connective tissue. The cells showed very uniform nuclear characteristics and the cytoplasm was rich in mitochondria and polyribosomes. Some of the cells showed no intra-cytoplasmic aggregates of lipid material but others showed variable amounts of lipid material which could occupy a very large part of the cell volume. These studies suggested that in a relatively uniform population of cells within human foetal subcutaneous tissue there is a variable expression of lipid content.

There seemed little doubt morphologically that these cells could be regarded as preadipocytes, as they demonstrated a metabolically active cytoplasm and a progressive accumulation of intracellular lipid material. If it could be proved that these cells were in fact pre-adipocytes, and were metabolically active, it might pave the way for a study of factors that influence the growth of fat tissue. It is

presumed that preadipocytes can undergo replication, whereas mature fat cells are no longer capable of this.

A metabolic feature of mature fat cells, and one that is thought to be unique to fat cells, is hormone sensitive lipase activity. This activity was then studied in foetal tissue composed of the small cells we regarded as preadipocytes. As these cells contained little or no intracellular triglyceride, glycerol trioleate was added to the incubations to provide a substrate for lipolytic activity. Additions of epinephrine were made at timed intervals. Levels of cyclic AMP were also determined in homogenates of tissue following epinephrine stimulation without added triglyceride. After the addition of epinephrine (10^{-4} M) there was substantial release of F.F.A. 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 cyclic AMP. A dose relationship for cyclic AMP production, after epinephrine, was present at each age studied. Changing levels in cyclic AMP in foetal tissue with epinephrine suggested 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 a functional ability of the cell.

The findings appear to confirm that the small foetal cells do have this metabolic functional ability characteristic of the mature fat cell. The next stage in characterization of the foetal tissue we have regarded as containing preadipocytes was to demonstrate the ability of such cells to synthesise fatty acid, and to study factors that might influence the synthesis of fatty acid. Although the energy conditions present in foetal life favour the storage of nutritional substrates, it is often stated that adipose tissue is undeveloped in early foetal life with little accumulation of tissue triglyceride. The correlations made of body fat to body weight which show less than 1% body weight as fat at 20-25 weeks gestation rising to 15% at term would support this (Widdowson and Spray 1951). However the presence in the foetus, at early stages of gestation, of cells with the potential to store triglyceride and thus form mature fat cells, draws attention to the need to study the factors influencing initial triglyceride deposition.

There have been few observations in the human foetus of the source of triglyceride fatty acid for lipid accumulation. Prior to 28 weeks gestation, maternal transfer of fatty acids has been regarded as the major source of lipid with lipogenesis from substrates such as glucose occurring only after this time (Harding 1971). Our next study was an investigation on the contribution of de novo fatty acid synthesis to the establishment and maintenance of adipose cell lipid in human foetal subcutaneous tissue.

Incorporation of 1-C^{14} acetate into neutral lipid and long chain fatty acyl CoA derivatives was determined in the presence of citrate (30 mM). Acetyl CoA dependent incorporation of 1-C^{14} bicarbonate into acid stable malonic acid was also determined as a measure of the level of activity of one of the rate limiting enzymes in fatty acid synthesis, acetyl CoA carboxylase (EC 6.4.1.2). Activity was expressed as nmol C^{14} bicarbonate incorporated.

In foetal subcutaneous tissue, lipogenesis from acetate could be demonstrated and the rate of de novo synthesis of neutral lipid shown to be dependent on the level of insulin present. Although acetyl CoA carboxylase activity, in adipose tissue taken from children was low (30 nmol/min/g), measurement of acetyl CoA carboxylase activity in foetal tissue showed it to be highest at ages when initial lipid deposits are found in the cell (85 nmol/min/g). At this time it was also possible to demonstrate the presence of insulin in those tissue homogenates. Thus in developing adipose tissue a contribution to lipid content is possible from de novo fatty acid synthesis. Regulation of the rate of lipid deposition is affected by insulin suggesting that nutrient and hormonal balances are factors which begin to affect the fat store in foetal life.

Thus these studies have demonstrated the development of small cells from foetal tissue that appear destined to form mature white fat tissue. These small cells are metabolically active, have the capacity of de novo fatty acid synthesis and show hormone sensitive lipase activity. Ultrastructural studies show features consistent with a metabolically active cell that is starting to accumulate intracellular lipid.

It is possible that these cells may provide the key for the study of the development of mature adipose tissue. It is also possible that a definition of the factors that influence the growth - both by replication and by fat accumulation - of these cells may help in an understanding of the development of obesity.

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