Size of adipose cells in infancy

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Dauncey, M. J., and Gairdner, D. (1975). *Archives of Disease in Childhood*, **50**, 286. **Size of adipose cells in infancy.** Seventy-three samples of adipose tissue from 59 infants, aged from 25 weeks' gestation to 18 months of age, were obtained at necropsy, or at operation, or by needle biopsy. Adipose cell size was measured by microscopy.

During fetal life the mean cell diameter increases from about 40 μm at 25 weeks' gestation to 50–80 μm at term. Adipose cells from the buttocks are larger than those from the abdominal wall. After birth, adipose cell size continues to increase so that by 3 months the mean cell diameter is about 90 μm . Observations made on infants that had been born preterm showed that the growth of adipose cells proceeds at the same rate whether development is intrauterine or extrauterine.

Knowledge of how adipose tissue develops in early life is likely to be of importance if we are to establish whether an infant's diet influences the later development of disorders such as obesity or atheroma. This paper is concerned with the growth of fat cells in adipose tissue in fetal life and in infancy.

Material and methods

A series of 47 cases formed the main part of the study. 29 of these had died in the perinatal period; they were fetuses or infants delivered at gestational ages from 25–42 weeks, who were either stillborn or had died within 3 days of birth, usually from respiratory causes. Fat samples were obtained at necropsy; in 14 cases samples were taken from two sites. The remaining 18 cases were healthy term infants, samples being obtained at ages from 7 to 78 weeks, mainly from the abdominal wall at the time of operation, usually herniotomy. The total number of samples thus amounted to 61.

Additionally, samples were available from a series of 12 preterm infants born at gestational ages from 28 to 35 weeks, and from whom samples of buttocks fat were obtained by needle biopsy at from 4 to 14 weeks after birth. This formed part of a study of the effect of different milk formulae on the growth and on the chemical composition of the fat of premature and term infants (Widdowson *et al.*, 1975). This object was explained to the parents in gaining their permission.

Needle biopsy was carried out with local anaesthesia using a 12-gauge needle and a 10 ml syringe for aspiration. 10–20 mg adipose tissue was generally obtained,

enabling both chemical analysis and measurement of cell size to be performed. The fragments were transferred to a tube of isotonic saline, filtered through a nylon screen, washed, and examined either immediately or after storage at -20°C .

Examination of fat samples was carried out by a modification of the method described by Sjöström, Björntorp, and Vrána (1971). A fragment of adipose tissue was placed on a petri dish in a container of solid CO_2 for at least 10 minutes. Sections were then cut by hand with a scalpel kept in solid CO_2 . It was found that this was the most effective technique; with practise, sections of about 200 μm (as estimated by comparison with sections cut by freezing microtome) could be obtained. The section was transferred to a slide. A glass cylinder, diameter 7 mm, height 3 mm, was placed around it, held to the slide by vacuum grease. The cylinder was filled with isotonic saline and the section floated to the surface. The microscopical appearances are illustrated in Fig. 1 (a, b, c). Using an eyepiece micrometer, the diameters of 120 cells were measured in two horizontal axes. Large cells tend to be polyhedrons (Fig. 1c,) but Sjöström *et al.* found the error caused by assuming them to be of spherical shape was insignificant.

The mean cell diameter (MCD) of 120 cells, with the SE and coefficient of variation, was calculated. From the MCD (in μm) an approximate value for the mean cell volume could be calculated as $\pi/6 \times (\text{MCD})^3$ (see Appendix). The mean cell volume when multiplied by 0.015 (the density of triolein, Di Girolamo, Mendlinger, and Fertig, 1971), gave a value for the weight in μg of fat/cell. The precision of the method was established by duplicate analysis of 11 specimens (for details see Appendix); the MCD of these varied from 36 to 88 μm . The mean differences between duplicate estima-

FIG. 1.—Adipose cells (a) infant of 34 weeks' gestation, mean cell diameter (MCD) 45 μm ; (b) infant at term, MCD 60 μm ; (c) infant aged 11 months, MCD 132 μm .

tions of MCD was $0.18 \pm 0.83 \mu\text{m}$ (SE), this difference being nonsignificant ($P > 0.05$).

Results

The MCD in 61 samples of adipose tissue is shown in Fig. 2, buttock and abdominal wall sites being shown separately. If 14 of the cases (age range 27–42 weeks' gestation) paired samples were available from both buttock and abdominal wall sites. In 13 of the 14, the MCD of the buttock sample exceeded that from the abdominal wall; in the remaining case the values were the same. The difference between the MCD of the two sites was highly significant ($P < 0.001$). (In 7 of the 14, samples were in addition available from perirenal fat; the MCD of this did not differ significantly from that of abdominal wall fat.) Our data do not

indicate whether this site-to-site difference persists at later ages.

In samples from the youngest subjects, those of 25–30 weeks' gestation, the MCD from both the buttock and the abdominal wall sites was in the range 37–50 μm . After 30 weeks' gestation the MCD of the buttock samples increases steadily, so that at term the MCD of 12 infants born at 37–42 weeks' gestation was $68 \pm 2.6 \mu\text{m}$ (mean $\pm$ SE). The comparable figure for 8 abdominal wall samples was $50 \pm 1.6 \mu\text{m}$, the increase in cell size in the period 30–40 weeks' gestation being comparatively small.

After birth cell size continues to increase so that by 12 weeks the MCD is about 90 μm . At later ages samples were fewer, but the data in Fig. 2 show that between 6 and 12 months (5 samples)

FIG. 2.—Mean cell diameter of adipose cells, 61 samples from 47 fetuses and infants, aged from 25 weeks' gestation to 80 weeks after term. Samples from buttocks fat ○; from abdominal wall ●. Time scale logarithmic.

the MCD was between 110 and 132 μm , and that after 12 months (3 samples) MCD was lower, between 83 and 90 μm .

Preterm infants. In 12 cases samples were available from babies who had been born at from 28 to 35 weeks' gestation, and had thrived on a conventional feed derived from full-cream cow's milk. Samples were taken at from 4 to 14 weeks after birth, so that the time of sampling was often close to the expected date of delivery of the infant. Fig. 3 shows that the adipose cell size of infants who had been born prematurely, by up to 12 weeks, was similar to that of term babies of like gestational age.

Discussion

Novak, Monkus, and Pardo (1971) reported on fat cell size (buttock samples) in 16 term infants at birth; the MCD was 52 ± 11 μm (mean $\pm$ SE) with a range of 24–86 μm . This compares with our mean value of 68 μm for buttock samples and 50 μm for abdominal wall samples. The only other relevant data are from Hirsch and Knittle (1970) who derived values for the size of cells in terms of their lipid content, determined by enumerating the

cells in a known amount of adipose tissue. Their data are presented graphically but it appears that 4 of their cases were infants at about term, and that these had a mean lipid content per cell of between

FIG. 3.—Mean cell diameter of adipose cells of 12 infants born preterm at the gestational ages (in weeks) indicated; measurements made at from 4 to 14 weeks after birth. The curve is derived from the data in Fig. 2 and indicates average mean cell diameter at different ages. Note that the growth of adipose cells is similar whether development is intrauterine or extrauterine.

FIG. 4.—Frequency distribution of 11 duplicate analyses of adipose cell diameters; 120 cells measured in each instance. CV, coefficient of variation.

0.05 and 0.18 μg , corresponding approximately to an MCD of 50–70 μm , which is in satisfactory agreement with our values. For the months after birth, their data give no indication about changes in cell size, but between 4 and 12 years cell size appeared to remain at about 0.4 μg lipid per cell, corresponding to an MCD of 95 μm ; this is similar to the value of about 90 μm found by us for 3 cases aged between 1 and 2 years. Comparable values are given by Brook (1971) who quotes a figure of 0.3 μg for the mean cell lipid content of children (age range 0.8–11.4 years) corresponding to a MCD of 85 μm .

Present evidence therefore indicates that fat cell size increases steadily during the period from 28 weeks' gestational age (i.e. 3 months preterm), up to 3 months postnatal age, MCD during this 6-month period increasing from about 40 to 90 μm . There is a suggestion from our data (see Fig. 2) that cell size then continues to increase further with MCD values of 110–130 μm between 3 and 6 months, before returning to about 90 μm after 12 months. According to the observations of Hirsch and Knittle (1970) cell size remains at this value of about 90 μm throughout childhood (their value for the average MCD of adults is 110 μm). Further observations will be needed, however, before it can be accepted that in childhood adipose cell size is actually larger in the second 6 months of life than subsequently.

Up to 28 weeks' gestation, the fetus has little adipose tissue, this it amasses rapidly in the course of the last 12 weeks of intrauterine life. Of interest, therefore, is the observation made on infants born preterm that the adipose cells grow at the same rate during this period, whether the infant remains *in utero* and is nourished placentally, or is delivered and then milk fed.

Regarding the differences between the size of fat cells in adipose tissue at different sites of the body, our own observations provide adequate data only for the period up to 40 weeks' gestation, when we have found that cells from buttock adipose tissue are larger than those from the abdominal wall (or than those from perirenal fat). Similar conclusions were reached by Duckerts and Bonnet (1973), who studied children aged between 17 days and 16 years: adipose cells from the buttocks were larger than those from the subscapular area and abdominal wall; differences were at a maximum at 5 years. Brook (1971) found in children aged 0.8–11.4 years adipose cells from superficial sites to be larger than from deep sites. Site-to-site cell size differences were found also in adults by Salans, Cushman, and Weismann (1973).

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Appendix

Calculation of the mean cell diameter (MCD) from individual diameters in a sample of adipose tissue depends on the assumption that the distribution of cell diameters is gaussian. Fig. 4. shows representative frequency distributions of cell diameters from 11 duplicate analyses of adipose tissue. In most cases the distribution is gaussian (as Di Girolamo *et al.*, 1971, found), though there is a slight tendency for the distribution to be skewed to the left. The figure also illustrates the similarity of frequency distributions between duplicate measurements.

The matter is of practical importance, since when the distribution is nongaussian the mean cell diameter can give a misleading idea of the size of cells in a sample of adipose tissue. For instance, Ashwell and Garrow (1973) found that there might be important differences between mean and modal adipose cell diameters. e.g. after a weight gain of 6 kg, a subject showed a paradoxical decrease in mean cell diameter caused by an increase in the number of small adipose cells. Calculation of the mean cell volume from mean cell diameter, as we have done by the simple formula $\pi/6 \times (\text{MCD})^3$ will, with a gaussian distribution, give an underestimate, the error being greater when the coefficient of variation is large. Sjöstrom *et al.* (1971), Di Girolamo *et al.* (1971), and Lemonnier (1972) have given more correct formulae for converting cell diameters to cell volume.

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