Adipose Tissue Fatty Acids and Size and Number of Fat Cells From Birth to 9 Years of Age—A Cross-Sectional Study in 96 Boys

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We studied interrelations between the size and number of fat cells and the composition of fatty acids in 96 boys from birth to 9 years of age. The size of fat cells increased during the first months of life and decreased over the second 6 months, in parallel with the tissue fat concentration and the skinfold thickness of subcutaneous fat. From the end of the first year of life, the size of the fat cell increased slowly. The number of fat cells did not begin to increase until the end of the first year of life, maintaining a continuous increase until 9 years of age. During the first years of life, important changes occurred in the concentration of adipose tissue fatty acids. Palmitic acid had the greatest concentration in adipose tissue at the moment of birth and then decreased, becoming stabilized from the age of 2 or 3 years. The concentrations of lauric, myristic, and myristoleic acids followed a course similar to that of adipocyte growth, probably reflecting changes in the accumulation of fat by the adipocyte. At the moment of birth, the concentrations of linoleic acid (C18:2), an essential fatty acid not synthesized by the organism, were low, increasing from the very first months of life. This increase correlated with the increase in fat cell size and number. These interrelations between the size and number of fat cells and the composition of adipose tissue fatty acids suggest the important role dietary fat can play in the childhood endowment of adipocytes.

DURING THE FIRST years of life, concomitant with the increase in weight, important modifications take place in body composition. Adipose tissue grows rapidly during the first months of life and then decreases, continuing to increase slowly until adulthood.[1,2] Some investigators[3,4] have proposed that the increase in the number of adipocytes in some adult obesities takes place right from infancy, suggesting the hypothesis that during development there are periods during which adipose tissue would be especially sensitive to different stimuli, thereby provoking an irreversible cellular response, as demonstrated by Knittle and Hirsch[5] in rats submitted to a hypercaloric diet. Later transverse[6] and longitudinal[7] studies supported this hypothesis. The irreversible acquisition of new fat cells during both development and adulthood has recently received wide experimental attention in both humans and rodents.[8-10]

This capacity for replication and differentiation may be modulated by numerous endogenous and exogenous factors.[11] Some polyunsaturated fatty acids (n-6) such as arachidonic acid are able to amplify the differentiation of adipocyte precursor cells in vitro.[12] Adipose tissue fatty acids are derived from both endogenous and alimentary sources, requiring approximately 20 weeks in the adult for a change in the composition of the type of dietary fat to modify the composition of adipose tissue fatty acids. In infancy and in situations of rapid weight gain, this time may be shorter.[13]

Increasing evidence suggests that dietary fatty acid composition modulates the utilization of body fuel and alters body composition.[14] If changes in caloric intake induce modifications in the endowment of adipocytes, it can be hypothesized that there is an association between changes in the composition of adipose tissue fatty acids, whose use as nutritional markers is well known,[15] and changes in the size and number of fat cells. We therefore examined from birth to the age of 9 years (prepuberty period) the adipose tissue cellularity and fatty acid composition simultaneously.

SUBJECTS AND METHODS

Subjects

The study included a total of 96 boys ranging in age from birth to 9 years. All boys whose weight was less than or equal to the 10th percentile or greater than or equal to the 90th percentile were excluded from the study.[16] The distribution by age was as follows: birth to 6 months, n = 18; 7 to 12 months, n = 26; 13 to 24 months, n = 17; 25 to 47 months, n = 12; 48 to 83 months, n = 15; and 84 to 98 months, n = 8. All subjects were studied during admission for inguinal hernia surgery, except the term newborn boys dying in hospital of various causes, who were studied at autopsy.

Variables

The following variables were studied in each boy.

Tricipital, subscapular, bicipital, and suprailiac skinfold thicknesses were measured with a Holtain-type constant-pressure lipocaliper.[17-18]

Total adipose tissue was calculated according to the following equations: in newborns, $TBF = W - (k \times TBW)$,[18] and $TBW = 0.843 \times W^{0.89119}$; in the other boys, $TBF = 4.95\ D - 4.5$,[20] and $D = 1.169 - 0.0788\ (\log S4Sf)$[21] (where k is 1/0.82, assuming the concentration of water in the fat-free body to be 82%; TBF is total body fat; TBW is total body water; D is body density; log S4Sf is the logarithm of the sum of four skinfold thicknesses; and W is weight).

In each case, a sample of subcutaneous adipose tissue was taken from the area of the incision during the course of inguinal hernia surgery, and the following were studied in each sample of adipose tissue.

The diameter of 200 adipose cells[22] was determined after separation of the adipocytes by Rodbell's method.[23] Cell volume

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was calculated using Goldrick's formula,[24] assuming the adipose cells to be spherical. Fat cell size was expressed as a mass of lipid per cell by assuming that the mass of a cell is the same as the mass of lipid contained within it and having a density of 0.915 g/mL. The number of body fat cells was calculated by dividing the total mass of body fat by the mean mass of lipid per cell.

The concentration of fat per gram of wet adipose tissue was measured by gravimetry after extraction of the fat with Folch's method.[25]

The composition of adipose tissue fatty acids was studied by gas chromatography. Hydrolysis-methylation was performed on the fat extract.[26]

Statistical Analysis

The data are presented as the mean ± SD. The intervariable tendency was measured by Pearson's correlation coefficient (r).

Although not used in the statistical analysis, we also present results of the adipose cellularity in 39 adults (13 with normal weight and 26 with body mass index > 35) and the composition of adipose tissue fatty acids in 89 healthy adults from the same population. In the adults, samples were taken by aspiration puncture with a large-caliber trocar.

The study was approved by the Ethics and Clinical Investigation Committee of Carlos Haya Regional Hospital.

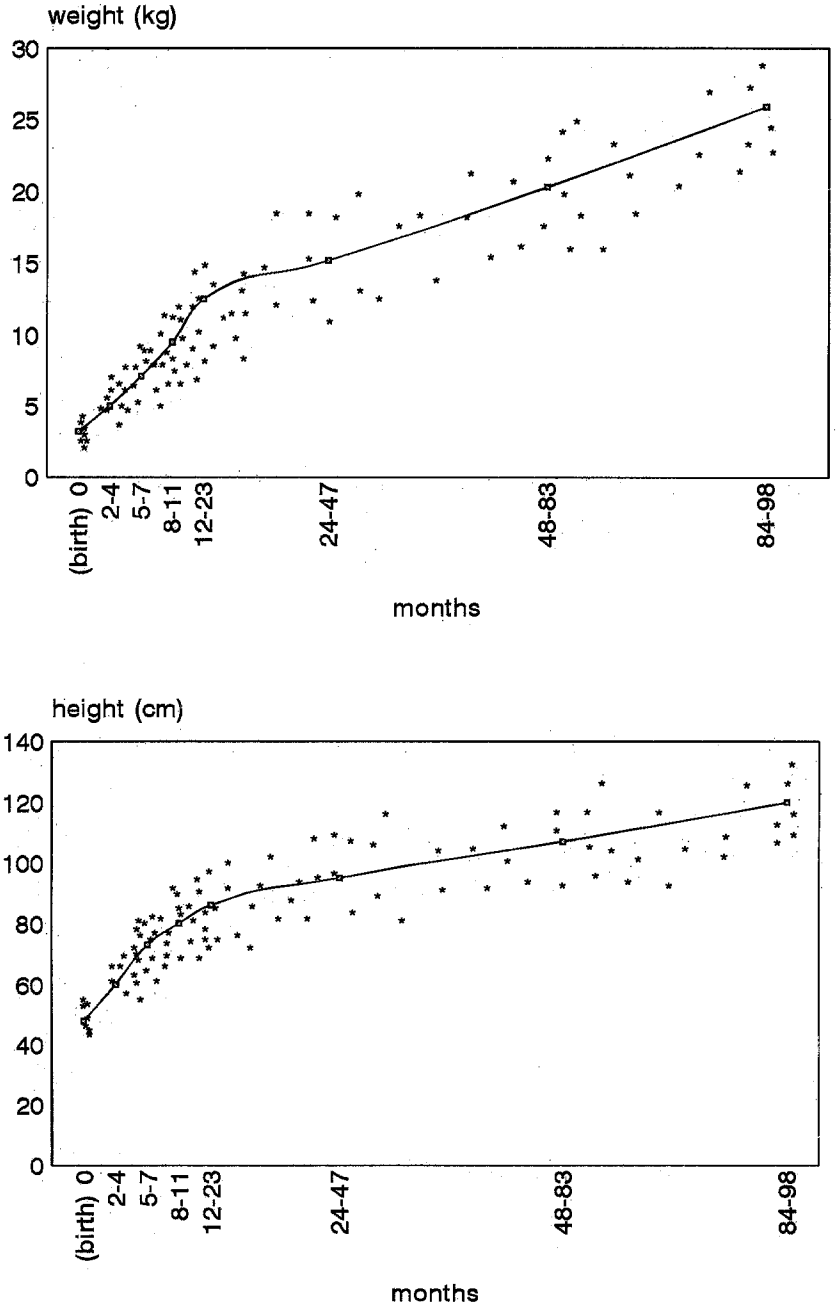

Fig 1. Weight and height of 96 boys. All boys whose weight was $\leq P_{10}$ or $\geq P_{90}$ of the weight distribution curve for Spanish boys were excluded from the study.[15]

RESULTS

The weight, height, and sum of four skinfold thicknesses in the study children followed a distribution parallel to the standards for weight, height, and skinfold thicknesses in the Spanish population[16] (Figs 1 and 2).

Subcutaneous adipose tissue (sum of four skinfold thicknesses) grew rapidly from birth to 7 months of age, and was correlated during this time with body weight ($r = .66$, $P = .05$; Fig 2). During this time, the fat concentration in adipose tissue increased rapidly and progressively, and was closely correlated with age ($r = .71$, $P = .01$). At the same time, the adipose cells rapidly increased in size (from 0.037 ± 0.023 μg fat per cell at the moment of birth to 0.261 ± 0.098 μg fat per cell at 7 months of age). The number of adipose cells remained stable during these first months of life (Fig 2).

In the second 6 months of life, the thickness of the layer of subcutaneous fat decreased, whereas body weight increased (Fig 2). At the same time, cell size decreased from the eighth month on, in parallel with the reduction in subcutaneous adipose tissue ($r = .60, P = .05$). The number of adipose cells remained stable during the first year of life, and began to increase between months 12 and 18, correlating with weight ($r = .54, P = .01$). In the second 6 months of life, the concentration of fat in adipose tissue continued to increase, decreasing in the second year of life and becoming stabilized thereafter (Fig 2).

From 2 years of life, adipose tissue continued growing slowly both in size and in number of fat cells, with both correlating with age and weight (size *v* age, $r = .54, P = .01$; size *v* weight, $r = .48$, $P = .05$; number *v* age, $r = .36$, $P = .05$; and number *v* weight, $r = .53, P = .01$; Fig 2).

At the moment of birth, adipose tissue concentrations of lauric (C12:0), myristic (C14:0), and myristoleic (C14:1) acids were very low, increasing rapidly over the first 7 months (Fig 3), and correlated with age (C12:0, $r = .59$, $P = .01$; C14:0, $r = .71$, $P = .01$), weight (C12:0, $r = .68$, $P = .01$; C14:0, $r = .73, P = .01$), and height (C12:0, $r = .73$, $P = .01$; C14:0, $r = .78$, $P = .01$). In the second 6 months, the concentration decreased and correlated negatively with age (C12:0, $r = -.63$, $P = .01$; C14:0, $r = -.60$, $P = .01$), height (C12:0, $r = -.69, P = .01$; C14:0, $r = -.65, P = .01$), and the number of fat cells (C12:0, $r = -.50$, $P = .05$; C14:0, $r = -.55$, $P = .001$) and positively with the sum of

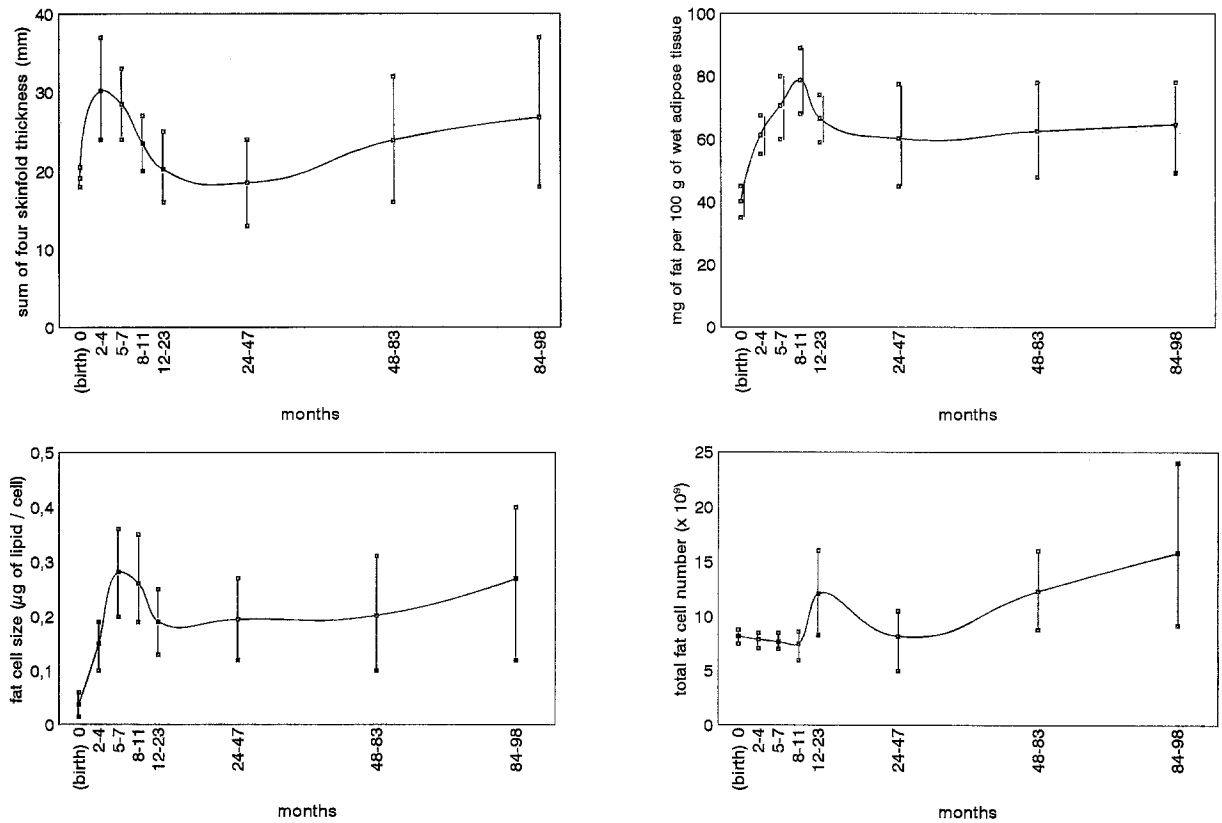

Fig 2. Sum of 4 skinfold thicknesses (bicipital, tricipital, subscapular, and suprailiac) measured with a Holtain-type constant-pressure lipocaliper, fat concentration per unit of weight of wet adipose tissue, size of the fat cell (volume calculated from Goldrick's formula, expressed in μg lipid per cell), and estimated total number of adipose cells (×10⁹). Data on size and number of adipose cells in 13 adults with normal weight and 24 with body mass index > 35 are included for reference purposes.

the four skinfold thicknesses (C12:0, $r = .58$, $P = .01$; C14:0, $r = .57$, $P = .01$) and the size of the fat cells (C12:0, $r = .50$, $P = .05$; C14:0, $r = .55$, $P = .05$).

Palmitic acid (C16:0) was the fatty acid with the greatest adipose tissue concentration at the moment of birth, decreasing rapidly and progressively over the first 7 months of life, correlating (Fig 3) in this first period with age ($r = -.78$, $P = .01$), weight ($r = -.77$, $P = .01$), height ($r = -.76$, $P = .01$), fat cell size ($r = -.76$, $P = .01$), and adipose tissue fat concentration ($r = -.62$, $P = .01$). The concentration of C16:0 continued to decrease until the second to third year of life. Palmitoleic acid (C16:1) followed a similar course.

Stearic acid (C18:0) increased during the first 6 months of life, correlating negatively over this time with the number of fat cells ($r = -.69$, $P = .05$), continuously increasing until the third year of life (Fig 4).

Oleic acid (C18:1) also increased from birth, becoming the fatty acid with the highest adipose tissue concentration in the children studied. In the first 6 months, C18:1 correlated positively with the size of the fat cells ($r = .65$, $P = .01$) and negatively with the number of fat cells ($r = -.48$, $P = .01$) (Fig 4).

Linoleic acid (C18:2) deserves special attention, since it is an essential fatty acid that the organism is unable to synthesize. At the moment of birth, its concentration was low (3.45% ± 1.5%). In the first 6 months of life, its concentration increased and correlated significantly with age ($r = .53$, $P = .01$), weight ($r = .51$, $P = .01$), height ($r = .66$, $P = .05$), and fat cell size ($r = .51$, $P = .05$). This increase stabilized during the second 6 months and decreased during months 12 to 18 of life, correlating positively with fat cell size ($r = .64$, $P = .01$), which over this period was reduced in volume and correlated negatively with the total number of fat cells ($r = -.58$, $P = .01$), which began to increase from the sixth month of life (Fig 4).

DISCUSSION

The increase in fat mass during the first months of life, as well as during adolescence,[27,28] probably represents a physiologic response to energy demand to ensure survival and reproduction.[29] However, in these periods of rapid growth, the fatty tissue has to compete for energy with the increased lean body mass and physical activity. The total amount of fat at any one time is the result of the size and number of fat cells, and following the pioneering studies by Lemmonier,[30] the hyperplastic development of adipose tissue in aging animals fed a high-carbohydrate or high-fat diet has been thoroughly studied.[8]

Both our results and those of other investigators show that the gain in fat mass during infancy is accompanied by an increase in the size of adipose cells.[2,6,7,9,29,31] However,

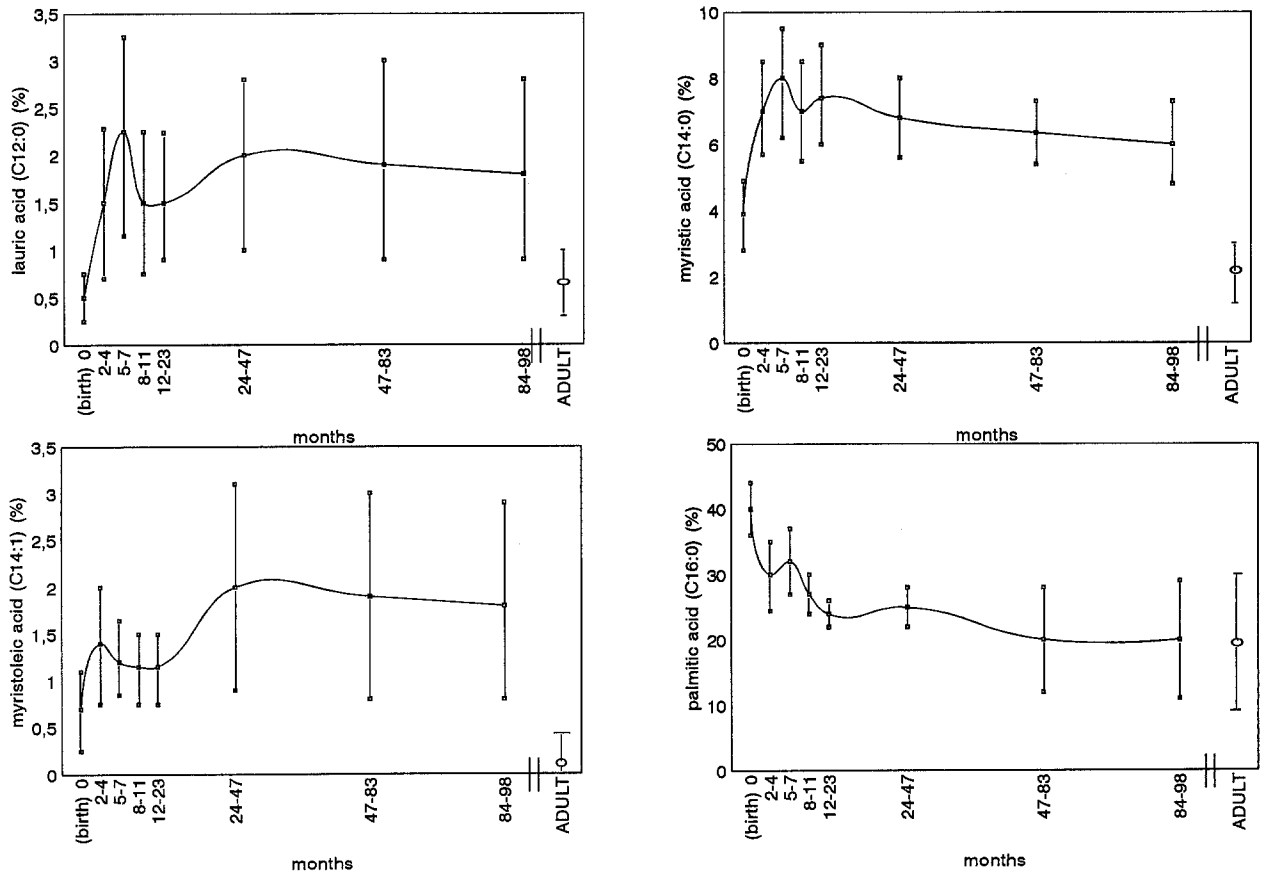

Fig 3. Concentration (%) of lauric acid (C12:0), myristic acid (C14:0), and myristoleic acid (C14:1) in adipose tissue of 96 boys with ages ranging from birth to 9 years. Data from the adults correspond to 89 healthy adults from the same community.

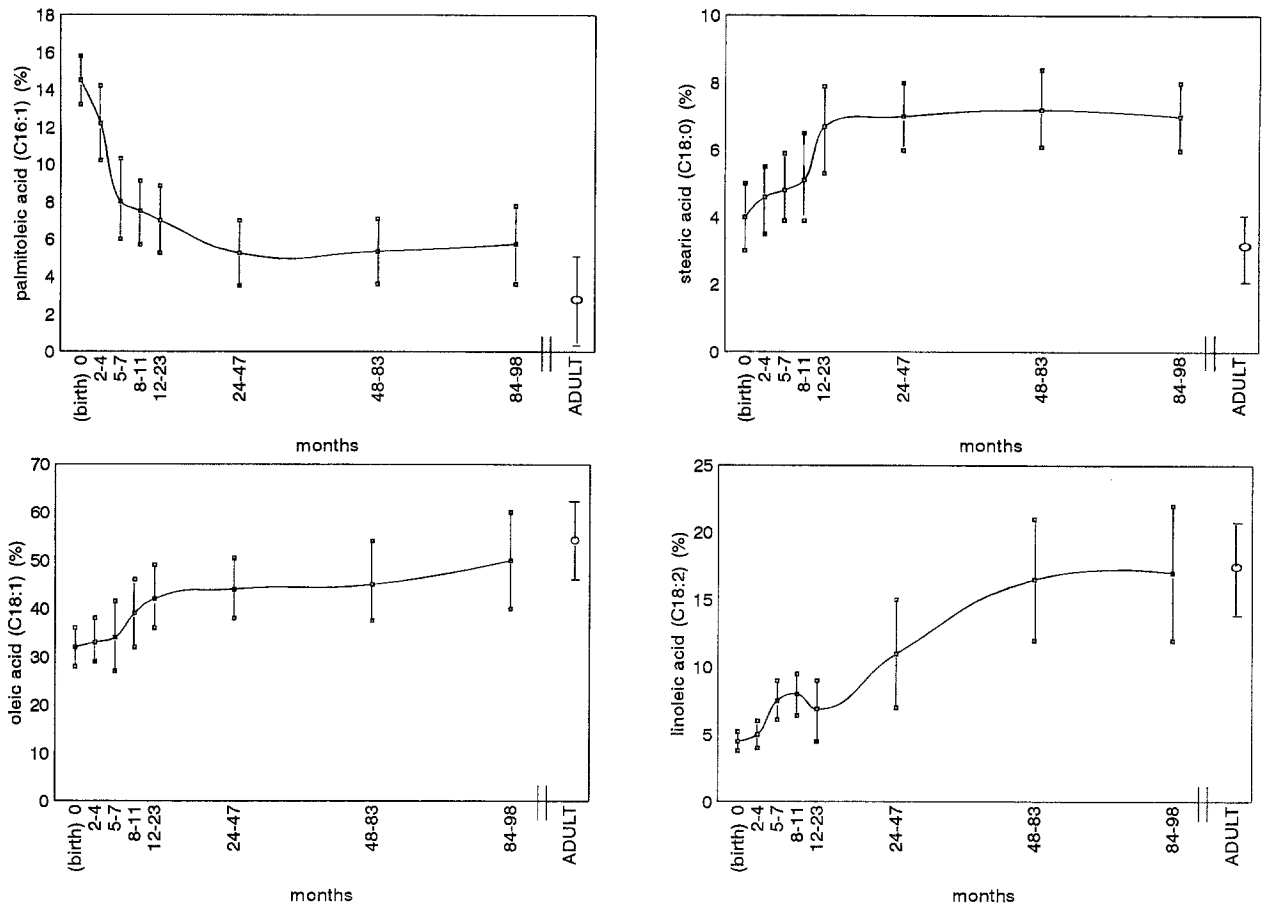

Fig 4. Concentration (%) of stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) in adipose tissue of 96 boys with ages ranging from birth to 9 years. Data from the adults correspond to 89 healthy adults from the same community.

the increase in size does not appear to be sufficient to respond to the increasing demand of fat deposition, so an increase in the number of adipose cells is necessary. This recruitment of new cells may in fact represent up to 50% of the total fat accumulated over this period.[29]

Our results suggest that modifications in dietary fat may influence the endowment of adipocytes during the first years of life. Although the weight increase was relatively constant, the thickness of the panniculus adiposus increased rapidly over the first 6 months of life and then decreased until the age of 12 to 18 months, when it again started to increase. These changes were basically due to the size of the fat cell. The concentration of fat per gram of wet tissue also changed in parallel with the changes in cell volume. These results are similar to those reported for the size and estimated number of fat cells in human subcutaneous[3,6,7,9] and primate epiploic[29] adipose tissue.

The changes in fat cell size were accompanied by important modifications in the type of fatty acid of the lipids in the fat cells. The composition of adipose tissue fatty acids is the result of endogenous synthesis and exogenous origin. The adipose tissue fatty acid pattern of infants is different from that of adults, with palmitic acid being the most important fatty acid.[13] At birth, the concentration of linoleic acid, an essential fatty acid, must come from the mother, since it is known that this and other fatty acids can cross the placental barrier,[32] with important population differences in the composition of wet tissue fatty acids right from birth.[33]

Hager et al[7] proposed that the increase in fat cells could begin when the cells reach a certain size. The recruitment of new cells might take place when the size of the fat cell is unable to maintain the physiologic requirements of a calorie reserve. This moment might correspond in the first year of life to the decrease in the size of the fat cell seen from the sixth month. The increasing incorporation from birth of fatty acids from the diet may be one of the factors leading to a reduction in fat cell size and a later increase in fat cell number. Moreover, this hypothesis is supported by others.

Firstly, dietary fatty acids are catabolized via different pathways, including oxidation for energy, acylation into tissue lipids for membrane remodeling or storage, and desaturation or elongation of other fatty acids.[34] Recent studies appear to show that dietary fatty acid composition alters the efficiency of energy substrate accretion in rats.[35] Herzberg[36] found that triglyceride synthesis was inversely related to the content of dietary unsaturated fat. On the other hand, both saturated and monounsaturated fatty

acids are more easily acylated into triglycerides.[37] A study by Clarke et al[38] showed that palmitate and stearate failed to cause inhibition of fatty acid synthesis, whereas oleate produced a slight inhibition and linoleate and linolenate a marked reduction. Consequently, a reduction in the lipogenic capacity of the adipose cell secondary to the presence of a greater amount of polyunsaturated fatty acids and a reduction in adipose tissue could be produced.

Secondly, the simultaneous incorporation of exogenous fatty acids into the fat cell membrane leads to changes in its permeability and ability to receive different hormonal messages,[39,40] which could make the preadipocytes more sensitive to the effect of numerous cell growth–stimulatory substances.[11] Thirdly, the possibility that the levels of fat unsaturation may affect cell multiplication in adipose tissue was already proposed nearly 25 years ago.[41]

We therefore conclude that changes in adipose tissue cellularity throughout infancy are no more than an expression of the balance between nature and nurture. The observation in humans of the interrelation between C18:2 and cell changes, found in our study, supports the hypothesis of Knittle and Hirsch[5] that early dietary changes in rats can modify the endowment of adipocytes.

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