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Research Article

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The Growth of Adipose Tissue in Children and Adolescents

CROSS-SECTIONAL AND LONGITUDINAL STUDIES OF ADIPOSE CELL NUMBER AND SIZE

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ABSTRACT Adipocyte size and number were determined in 288 subjects ranging in age from 4 mo to 19 yr. The study was performed in 110 obese and 178 nonobese subjects. 4-yr, longitudinal, follow-up studies were also performed in 132 subjects. The results demonstrate that the contribution of cell number and size to the growth of the fat depot in nonobese children varies with age. Deviations from this normal development were observed in obese children shortly after 1 yr of age. By 11 yr of age obese children exceeded the mean cell number found in nonobese adults. Indeed, obese subjects displayed more rapid and earlier elevations in both cell number and size, which were maintained throughout the study. Thus obese children display both quantitative and qualitative differences in fat tissue development when compared to nonobese children. The data indicate that the rate and type of adipose tissue cellular development one encounters in children may play a role in the development of the enlarged fat depots found in obese subjects.

INTRODUCTION

The most apparent and universally accepted fact about the obese state is the enlargement of the fat depots found in obese individuals. However, immediate disagreement arises when one attempts to delineate to what degree, and at what time interval, increments in adipose tissue are a result of either increases in adipose cell number and(or) cell lipid content (cell size). Previous studies of human adipose tissue cellularity have reported varying results (1–13). Although some authors have stressed the importance of cellular proliferation during the earliest periods of development in nonobese subjects, others have maintained that cellular enlargement may play a more important role. Cellular studies of obese subjects have also generated conflicting data. Some investigators have reported that those subjects with the earliest onset of obesity tend to display a more marked degree of hyperplasia than those with adult-onset obesity, whereas others have not found this association.

Differences from one investigator to another have been attributed to possible variations in either methodology or populations studied. However, interpretation of the results are further complicated by the fact that most of the studies are based on cross-sectional and retrospective data from adult subjects in whom the actual onset of obesity was at best conjectural, whereas cross-sectional and longitudinal studies in children are not common. The few prospective studies reported in children have been performed on small numbers of subjects over short periods of time (up to 2 yr). Thus our knowledge of either normal or abnormal fat depot development early in life is rather meager. It was therefore of interest to study prospectively larger numbers of subjects over longer periods of time to determine: (*a*) normal adipose tissue development, (*b*) how and at what age obese children deviate from normal development, and (*c*) at what age obese children exceeded nonobese adult values. The answers to these questions are of importance if one is to understand the pathogenesis of obesity in man and make meaningful judgments related to the time of dietary intervention in childhood. In the present report we have summarized our longitudinal findings in obese and nonobese children of varying age followed for a period of 4 yr.

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TABLE I
Body Composition of Obese and Nonobese Subjects

Age		n	LBM	Total body fat	Weight	Height	Fat
yr			*kg*	*kg*	*cm*	*cm*	*%*
2–4	Obese	17	18.3±2.8	8.6±2.3	27±5	102±7	31±2.9
	Nonobese	31	11.5±2.7	2.8±1.4	14.3±4	92±9.7	19±4.1
5–7	Obese	23	26.1±48	13.7±5.1	39±9	120±6.9	33±4.3
	Nonobese	29	16.4±4.6	4.5±2.6	21±7	113±11.7	20±4.4
8–10	Obese	15	31.9±9.1	17.7±8.6	49±1.7	133±1.4	34±5.1
	Nonobese	29	21.1±4.7	6.5±3.4	27±7	128±10.3	22±6.5
11–13	Obese	27	45.8±1.0	30±1.3	76±2.3	153±1.0	38±4.2
	Nonobese	22	29.7±6.6	11.1±5.3	45±1.1	147±9.6	24±5.3
14–16	Obese	19	46.8±1.2	33±4.0	80±3.5	157±1.1	40±5.7
	Nonobese	16	36.7±1.2	12.0±1.7	63±2.1	159±1.9	19±7.5
17–19	Obese	9	54.8±1.6	40.4±1.9	95±3.1	161±1.1	40±5.2
	Nonobese	6	36.8±1.2	12.0±1.9	65±2.2	169±12.3	18±1.6

METHODS

Initial cross-sectional studies were performed on 288 subjects ranging in age from 4 mo to 19 yr. Of these, 132 have now been followed longitudinally for a period of 4 yr. Subjects who attended the General Pediatric and Pediatric Nutrition Clinics of The Mount Sinai Hospital in New York participated in this study after detailed explanation of the procedures to be performed was given to their mothers and informed consent obtained.

The children were classified as either obese or nonobese on the basis of ideal weight-for-height tables at the time of entry into the program. Those subjects who were 130% or greater than ideal weight were designated obese. Nonobese were those <130% of ideal. Under the age of 2 yr no obese subjects were encountered. Individuals who partook in successful weight-reduction programs were dealt with separately, and their data were not included in the present report. Subjects with endocrinopathies or nutritional disorders were also excluded.

Fragments of adipose tissue were obtained from the upper outer gluteal quadrant of the buttock by means of a percutaneous needle aspiration (14). 1% Xylocaine (lidocaine hydrochloride; The Vitarine Co., Inc., New York) was used for local anesthesia. The tissue fragments were immediately placed in Krebs-Ringer bicarbonate buffer and kept at 37°C under 95% oxygen and 5% carbon dioxide in a thermos flask until processed. Lipid content per cell (cell size) was measured in triplicate by the method of Hirsch and Gallian (15). The total number of adipose cells in the body was estimated by dividing total body fat by mean adipose cell size.

Total body fat was calculated as body weight minus lean body mass (LBM).[1] Lean body mass was determined by either height-weight relationships or measurement of total body potassium (TBK). One cannot accurately measure TBK in the under age two group because of limitations of the total body counter used. We therefore relied exclusively on height and weight measurements for estimates of total body fat in this group.

TBK was measured at the whole-body counter located at Brookhaven National Laboratories, Upton, Long Island, N. Y. Brookhaven has an on-line computer facility with a relatively invariant response to radionuclide distribution and body size with an accuracy and precision for measuring TBK of ±3.3%. A complete description of the 54-detector Brookhaven counter has been published (16). Routine counting procedures involved a 15-min count of the subject who donned hospital pajamas and slippers before the counting procedure. Calibration of the counter was first performed. The whole-body count of the Alderson random phantom, containing a known amount of potassium homogeneously distributed, was obtained in the same geometry as that for the subjects. The count was corrected for geometry and absorption effects.

In adolescents, LBM in kilograms was determined by dividing TBK (milliequivalents obtained from the whole-body count) by 68.1 meq assuming 1 kg LBM contains 68.1 meq of potassium (17–19). In determining LBM in prepubescent children we have used a factor of 89 meq/kg based on other studies (20–22). This value is close to calculations by Hager et al. who used a value of 90 meq/kg (2). Children were classified as prepubescent or pubescent on the basis of secondary sexual characteristics (23). All statistical analyses and X, Y plottings of the data were performed on the City University of New York time-sharing computer system.

RESULTS

Studies related to body composition. Obese subjects displayed significantly greater values for LBM, total body fat, weight, height, and percent body fat than nonobese subjects of the same age. Table I summarizes the data. No sex differences were encountered in the obese group; however, after age 16 nonobese girls tended to have slightly higher values for percent fat than the nonobese boys.

Intercorrelation matrix analyses were performed for cell size and cell number with total body weight, total body fat, percent body fat, LBM, and percent ideal

[1] *Abbreviations used in this paper*: LBM, lean body mass; TBK, total body potassium.

TABLE II

Correlation Analysis of Cell Size and Cell Number with Body Weight, Body Fat, Percent Body Fat, Percent Ideal Weight, and LBM

	Cell number	Cell size
Total body weight	0.80*	0.258
Total body fat	0.90*	0.47
Body fat, %	0.77*	0.58‡
Ideal weight, %	0.28	0.30
LBM	0.71*	0.23

* $P < 0.01$.
‡ $P < 0.05$.

weight. The data are summarized in Table II. Significant correlations were found between cell number and total body weight ($r = 0.80$, $P < 0.01$), total body fat ($r = 0.90$, $P < 0.01$), LBM ($r = 0.71$, $P < 0.01$), and percent body fat ($r = 0.77$, $P < 0.01$). No correlation between cell number and percent body weight, total body fat, or percent ideal weight was found. However, a significant correlation between cell size and percent body fat ($r = 0.58$, $P < 0.05$) was seen. In Fig. 1, cell number is plotted against total body fat. A similar plotting for cell size is seen in Fig. 2. The plottings indicate that cell number is a major contributor to the absolute enlargement of fat depots (total body fat) regardless of age. On the other hand, cell size, though not a particularly useful indicator of absolute body fat, does indicate the degree of fatness. It is of interest that neither parameter correlated with percent ideal weight.

Cross-sectional studies of adipose cell size and number. These studies were performed in 110 obese and 133 nonobese children from ages 2 through 19 and in 45 infants at ages 6 mo and 1 yr. Table III shows the breakdown by sex and age of each group in this study. The data represent the first determination of adipose cell size and number upon entry into our program. Analyses of variance revealed no significant differences in cellularity between the sexes, so the data was pooled and plotted against age (Fig. 3).

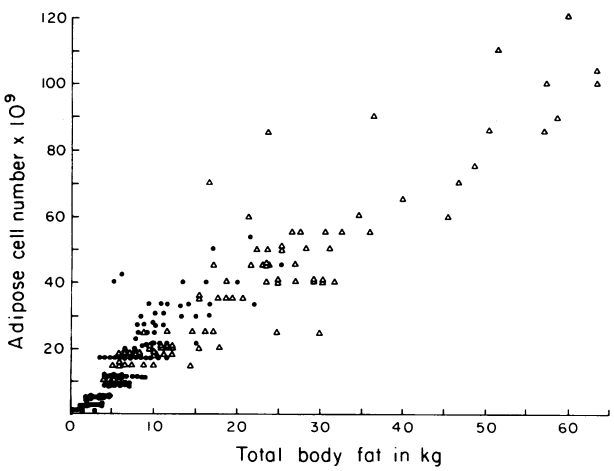

FIGURE 1 Adipose cell number as a function of total body fat. Obese subjects are represented as triangles and nonobese as black dots.

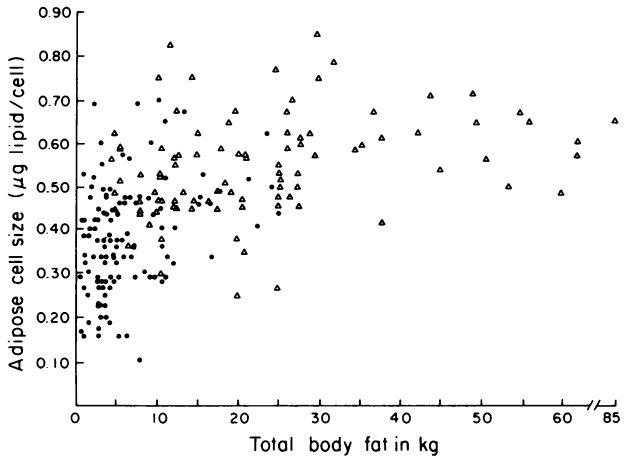

FIGURE 2 Adipose cell size as a function of total body fat. Obese subjects are represented as triangles and nonobese as black dots.

Under age two, all subjects were nonobese and therefore were plotted accordingly. From 6 mo of age to 1 yr, cell size appeared to increase to adult levels (0.45 μg lipid/cell) and then decreased between 1 and 2 yr of age to previous levels (0.35 μg lipid/cell). By age two, 17 obese subjects were identified on the basis of elevated weight-for-height and were compared to 31 nonobese children. The cells from the obese subjects were significantly larger when compared to the nonobese group (0.52 ± 0.03 vs. 0.35 ± 0.03, $P < 0.001$). One can see that no significant changes in cell size occurred in obese children from ages 2 through 16. However, there did appear to be an increase after the age of 17 especially

TABLE III

Age and Sex of Obese and Nonobese Subjects in Cross-Sectional Studies

	Obese			Nonobese		
Groups	Male	Female	Total	Male	Female	Total
<2	—	—	—	22	23	45
2–4	6	11	17	11	20	31
5–7	13	10	23	11	18	29
8–10	7	8	15	17	12	29
11–13	13	14	27	13	9	22
14–16	6	13	19	10	6	16
17–19	4	5	9	3	3	6
Total	49	61	110	87	91	178

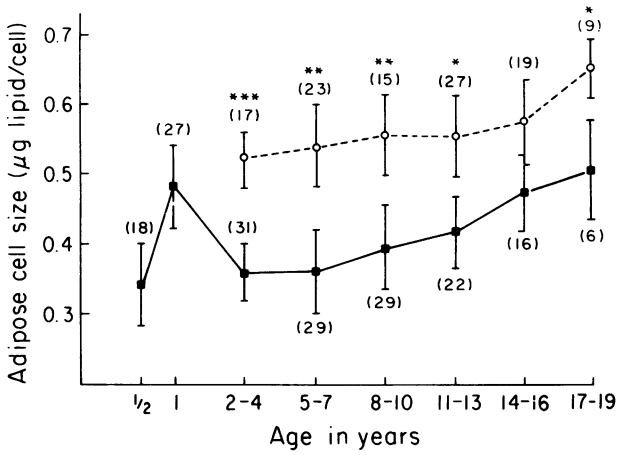

FIGURE 3 Cross-sectional studies of adipose cell size as a function of age in years. Obese subjects are represented as open circles and nonobese as black squares. (n) = number of subjects in each age group. Each point represents mean values±SEM; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; obese vs. nonobese; Student's t test.

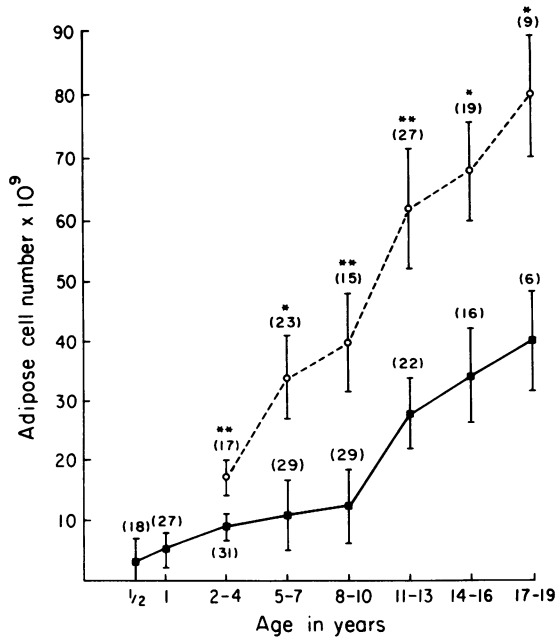

FIGURE 4 Cross-sectional studies of adipose cell number as a function of age in years. Obese subjects are represented as open circles and nonobese as black squares. (n) = number of subjects in each age group. Each point represents mean values±SEM; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; obese vs. nonobese; Student's t test.

when compared to age 2–4 ($P < 0.01$). Although slight increments in cell size seemed to be occurring in nonobese subjects, the differences were not statistically significant until one compared ages 14–19 with those from ages 2–7 ($P < 0.05$). Thus in nonobese children after 2 yr of age, cell size does not alter significantly until the early adolescent period. Differences in cell size were statistically significant when obese and nonobese subjects were compared in all age groups except at ages 14–16.

By age 2, obese children had achieved adult nonobese values for cell size (0.4–0.7 μg lipid/cell), whereas adult levels were not reached in the nonobese until ages 11–13. Although nonobese subjects showed a very slow increase in cell size with age in the intervals studied, obese cell sizes remained relatively constant. Only after 14 yr of age were increases noted, and the values at ages 17–19 (0.65±0.03) were significantly greater than those at age 2–4 (0.52±0.03, $P < 0.05$).

Cross-sectional studies of adipose cell number are shown in Fig. 4. Here one can see once again that significant differences between obese and nonobese subjects can be observed as early as age two and persist throughout all ages studied. It is of interest to note that no significant alteration in cell number occurred in the nonobese children from 2 to 10 yr of age, and only after age 10 were significant elevations observed ($P < 0.05$). Conversely, in obese children significant increases in cell number occurred throughout all age groups.

A regression analysis of cell number against age from 2–10 yr of age revealed an r value of 0.70 ($P < 0.01$) for the obese and 0.25 (NS) for the nonobese. A similar analysis for cell size and age revealed no differences between the two groups. Thus the cross-sectional studies indicate that, from ages 6 mo to 1 yr, increments in fat depots are primarily because of increases in cell size with only minor contributions by cell number. After age one, nonobese children appear to decrease cell size and display small increments in cell number until age two. Thereafter fat depots remain quiescent until age 10, when both cell number and size increase. Obese children, on the other hand, attain adult fat-cell sizes by age two without much change thereafter until age 17. However, they continue to show increases in fat depots and cell number at all ages studied.

Longitudinal cellular studies. 4-yr follow-up studies have currently been completed in 60 obese and 53 nonobese subjects over age 2 and in 19 infants under age 2 born to obese mothers starting at 4 mo of age. The age and sex for each group are given in Table IV. The mean cell sizes and cell numbers±SEM are shown in Figs. 5 and 6, respectively. Obese subjects who had been involved in successful weight reduction programs were not included in this study in order to determine "natural development patterns" of cellularity. The same subjects were studied over a 4-yr interval.

In the over two group, a pattern similar to that observed in the cross-sectional data emerges in both cell size and number. Although differences in cell size were

TABLE IV
Age and Sex of Obese and Nonobese Subjects in Longitudinal Studies Over Age Two

Groups	Obese			Nonobese		
	Male	Female	Total	Male	Female	Total
2–4	5	7	12	5	7	12
6–8	6	8	14	5	6	11
10–12	6	7	13	6	6	12
14–16	5	8	13	7	6	13
18–20	4	4	8	2	3	5
Total	26	34	60	25	28	53

observed in the obese groups over time, they were not statistically significant, and size was fairly constant until age 14. After this age, cell size showed an increase, and values after this age were significantly greater than those under age 14 ($P < 0.05$). In nonobese subjects significant changes in cell size were found when children 10–12 yr of age were examined 4 yr later ($P < 0.05$), but not in the other groups. All values after age 14 were significantly greater than those found in the younger children ($P < 0.01$). These findings are similar to but not identical to our cross-sectional data. The longitudinal data suggests a more abrupt increase in cell size after age 10.

The longitudinal studies of cell number in children over age two are almost identical to our cross-sectional results. One can see that, at all ages studied, obese children displayed significant increments in number over a 4-yr time interval. Before age 10, no significant changes in cell number were observed in nonobese children. However, after this time significant increments could be observed through age 18. After age 18, cell number appears to plateau in the nonobese, indicating that they have leveled off at their final adult number. However, this remains to be proven by further follow-up.

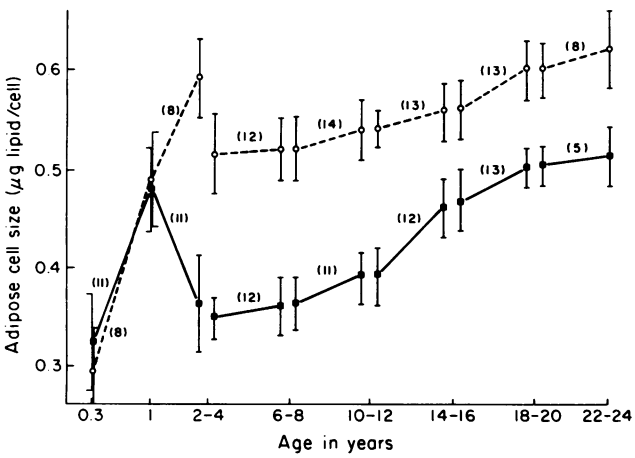

FIGURE 5 Longitudinal studies of adipose cell size as a function of age in years. Obese subjects are represented as open circles and nonobese as black squares. (n) = number of subjects studied at each 4-yr interval. Each point represents mean values±SEM.

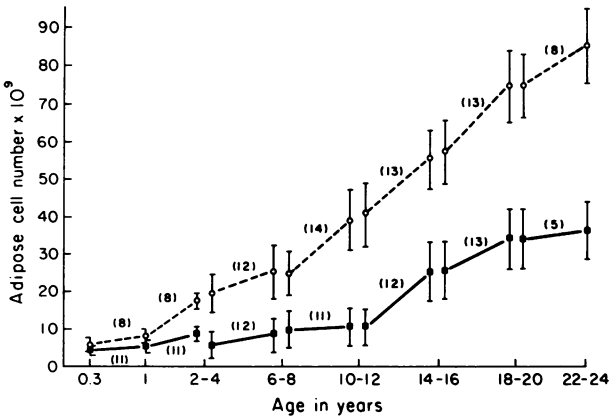

FIGURE 6 Longitudinal studies of adipose cell number as a function of age in years. Obese subjects are represented as open circles and nonobese as black squares. (n) = number of subjects at 4-yr interval. Each point represents mean values ±SEM.

Studies of cell size in children under age two, born to obese mothers, were performed starting as early as 4 mo of age. Eight of the subjects studied have now developed clinically overt obesity (i.e., over 130% of ideal weight). The data obtained from these individuals during the course of our observations were matched with 11 subjects of comparable age who did not become obese. In both Figs. 5 (cell size) and 6 (cell number) data are depicted up to the age of two; however, the differences found at this age persisted until age four. No significant differences in body weight were observed before and up to 12 mo of age. Indeed, although not statistically significant, the mean weight of the subsequently obese children was less than that observed in the nonobese at birth and 4 mo of age. However, by 2 yr of age the obese group was significantly heavier with a mean weight of 19 kg compared to 13.0 kg in the nonobese, and they remained heavier until 4 yr of age ($P < 0.01$). Under the age of two, estimates of cell number did not show significant differences when obese and nonobese groups were compared, nor did either group show significant increases with age. By 2 yr obese subjects did display a significantly greater cell number ($P < 0.05$) than the nonobese, and these differences continued until four years of age ($P < 0.01$). In both groups, significant increments were observed in cell number from 1–2 yr ($P < 0.01$ and $P < 0.005$, obese and nonobese, respectively). However, after 2 yr the nonobese no longer displayed significant increases whereas obese subjects did. Thus, one could not clearly distinguish between obese and nonobese children on

the basis of either total cell number or rate of cellular development at any time before 1 yr of age.

From 4 mo to 1 yr of age both groups showed significant increases in cell size ($P < 0.05$), but one could not distinguish between groups at either of these ages. However, from 1–2 yr of age, though obese children continued to display significant increments in lipid content ($P < 0.05$), nonobese subjects displayed a marked decrease, and by 2 yr had a mean cell size that was significantly less than the obese child ($P < 0.05$). Thus rates and patterns of cellular development varied markedly when nonobese and obesity-prone subjects were compared. However, the differences noted could not be detected before 1 yr of age.

DISCUSSION

The development of techniques for the measurement of adipose tissue cellularity has made it feasible for one to perform meaningful studies of the growth of fat depots in man (15). Such studies are important for the understanding of the pathogenesis of the obese state. One must be cautious, however, in the interpretation of adipocyte cellular data because site-to-site variation in cell size has been shown to occur within a subject, and the methods available for the measurement of body fat are far from ideal (3, 24). Although it would have been preferable to obtain tissue samples from a variety of subcutaneous areas, this was not feasible in the present study of children and infants. However, it has been shown that correlations exist between fat cell sizes of different regions of the body. Thus, samples obtained at a single site from a large group of subjects will provide a reasonable estimate of the variation among individuals in fat cell sizes in all depots (11). In the present report only the buttock area was used for sampling.

The measurement of total body fat poses a more perplexing problem because all methods are based upon a number of quantitative relationships between different body components that vary within a given population. Thus any estimate of body fat for a given individual has an unknown degree of uncertainty. In the use of TBK one is uncertain about the possible variations in concentration of potassium in the compartment being measured and the degree of hydration of the patient. The use of height-weight relationships are no better and yield varying results depending upon the methods used (25–27). However, despite these shortcomings, the techniques used in the present study have proven useful in defining and comparing cellular characteristics of obese and nonobese subjects and in studying the same individual over time. Indeed, the differences found in body fat between obese and nonobese subjects at any given age over 2 yr was large enough to offset small errors as a result of methodology. Thus although one cannot state with certainty what the absolute size of the fat depot is in a given subject, comparisons of extreme sizes (as were found in our study) and of the same subject over time can yield useful data. Furthermore, the two techniques used for determining body fat in this study had a correlation coefficient of 0.95 in children over the age of two. Estimates of the fat depot size and(or) cell number in children under age two are less reliable; however, one can measure cell size directly. Indeed, our results indicate that changes in adipose cell size appear to be a more reliable index of future obesity.

Although disagreement may exist as to which method of body fat determination should be used, most investigators agree that basically two forms of obesity can be identified in man on the basis of cellular studies (1, 3, 5, 8). One type is hyperplastic and is associated with either normal or enlarged adipose cell size; the other is primarily hypertrophic with only a moderate contribution of cell number. In general, investigations of adults and children have demonstrated marked increments of both parameters early in life (1, 3–5, 7). This is true despite variations in cell size from site to site, because the majority of obese children can be classified as hypercellular when compared to nonobese, independent of the site chosen (3, 6). Thus it is fairly well accepted that obese children and adults with childhood-onset obesity display the greatest degree of hypercellularity. More recently, Hager et al. failed to find a correlation between age of onset and cellularity in 18 obese girls studied at 8 yr of age (6). The obese girls did, however, display increased cell numbers and size when compared to nonobese, age-matched controls. In the nonobese group no alterations in cell number were observed over a 1.9-yr period, whereas the obese group did show some increase. They also found that those obese girls who were most successfully treated had the lowest increases in cell number. In another longitudinal study of infants, Hager et al. followed 16 subjects from 1–18 mo of age and suggested that fat depots during this age period grew by virtue of cell size alone; increases in cell number were only detected from 12–18 mo of age (2). This is in contrast to earlier cross-sectional data reported by Hirsch and Knittle in infants and children that indicated that fat-cell number and size both played a role in early development with cell number a more prominent contributor (1). However, in that study no follow-up data were available as to the subsequent weight of these children. It is of interest that, as in the present report, Hager et al. (2) showed increases in cell size from 1–12 mo with a fall in size in the 2nd yr of life. They did not, however, comment on this phenomenon. On the other hand, Bonnet et al. have reported in their study of children a slow increase in cell number with no change in cell size (12).

Some of these inconsistencies in findings may be a

result of methodologic problems or differences in the populations studied. However, they may also be a result of differences in the growth of fat depots in obese and nonobese subjects. Thus studies that do not follow their subjects longitudinally for sufficient periods of time may yield varying results depending upon the number of obesity-prone subjects involved in their initial investigation. Our studies indicate that the development of fat depots in obese children differ both quantitatively and qualitatively when compared to nonobese children of the same age and that these differences have important consequences for the ultimate size that the depot attains. In obese children significant increases in cell size to nonobese adult values occur early in life at a time when nonobese subjects display marked reductions or no change in lipid content. During this time while cell number continued to increase in the obese no significant change was noted in the nonobese child. However, after age 10 when cell size in the nonobese reached adult values there were also significant increments in cell number. Hager et al. have also suggested that there may be an important coupling between fat-cell size and adipocyte multiplication in man (2). A further indication of the possible interaction of cell size and cell number was suggested by the correlation of these parameters with percent body fat. In the analysis of cell number vs. percent fat it was found that there were two separate slopes for subjects with <25% vs. >25%. After percent body fat exceeded 25% of total body weight, cell number rose dramatically. The fact that percent fat was the only factor in body composition that correlated with cell size ($r = 0.58$, $P < 0.05$) is suggestive of a "triggering" of fat-cell proliferation at a set level of body fatness and(or) cell lipid content.

What the actual triggering mechanism might be is at present unknown; however, it appears to occur at different ages for obese and nonobese subjects. The variations observed in adipocyte size and development could be a result of either inherent differences in the fat cells of obese children resulting in abnormal responses to normal environmental factors (hormones, etc.) or to an altered hormonal milieu causing an increase in fat deposition or decreased lipolytic activity.

At present all one can say is that two time intervals appear to be of importance in adipose tissue cellular proliferation. One is before age two and the other during the adolescent growth spurt. If new cells are actually being produced at these time intervals the possibility of future hypercellular obesity exists. Although our studies do not support a limited early critical period or set point for determination of cellularity, they do suggest that certain time intervals may have more important consequences for ultimate cellularity and size of fat depots that one attains in adult life.

It has been argued that the aforementioned results, using the osmium tetroxide technique for fat-cell counting, are limited in that only cells with sufficient lipid content can be identified. Smaller cells that contain little or no lipid cannot be counted; hence, precursor cells, if present, are not included. Thus statements that cell number increased with age may not reflect multiplication of new cells but merely the "filling up" of preformed precursor cells. However, if the increases in cell number after birth that we have described are actually a result of the filling up of preformed adipocytes one must explain why nonobese children, who continue to increase in height, weight, and body potassium from ages 2–10, do not also display significant increases in adipose cell number in either cross-sectional or 4 yr longitudinal studies. If pre-adipocytes are present by age two, why do they remain unfilled until age 10? and why don't obese children display a similar inhibition?

One must also explain findings by us and those of Hager et al. who found that although one cannot decrease cell number in the obese by dietary intervention, one can decrease or abolish cellular proliferation (2, 28). Ideally, of course, one would like to measure adipose cellular division in man directly, but techniques for doing so are presently unavailable.

At the present time, the existence of an adipocyte precursor cell in man has not been clearly established, and a marker of the pre-obese or obesity-prone subject still eludes investigators. However, in vitro studies using tissue culture techniques currently in progress in our laboratory and reported by others may provide the answers to this question (29–33). With these techniques one may also determine to what extent variations in the development of fat depots are a result of either innate differences in the adipocyte or to alterations in environmental factors that control adipose cell size and number.

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