

# Studies of Human Adipose Tissue

## ADIPOSE CELL SIZE AND NUMBER IN NONOBESE AND OBESE PATIENTS

LESTER B. SALANS, SAMUEL W. CUSHMAN, and RODGER E. WEISMANN

*From the Departments of Medicine and Surgery, Dartmouth-Hitchcock Medical Center, Hanover, New Hampshire 03755*

**ABSTRACT** The cellular character of the adipose tissue of 21 nonobese and 78 obese patients has been examined. Adipose cell size (lipid per cell) was determined in three different subcutaneous and deep fat depots in each patient and the total number of adipose cells in the body estimated by division of total body fat by various combinations of the adipose cell sizes at six different sites. Cell number has also been estimated on the basis of various assumed distribution of total fat between the subcutaneous and deep fat depots.

Obese patients, as a group, have larger adipose cells than do nonobese patients; cell size, however, varies considerably among the fat depots of individuals of either group. The variation in cell size exists not only between, but also within subcutaneous and deep sites. Estimates of total adipose cell number for a given individual based upon cell size can, therefore, vary by as much as 85%. On the basis of these studies it is suggested that the total adipose number of an individual is best and most practically estimated, at this time, by division of total body fat by the mean of the adipose cell sizes of at least three subcutaneous sites.

Irrespective of the method by which total adipose cell number is estimated, two patterns of obesity emerge with respect to the cellular character of the adipose tissue mass of these patients: hyperplastic, with increased adipose cell number and normal or increased size, and hypertrophic, with increased cell size alone. These two cellular patterns of obesity are independent of a variety of assumed distributions of fat among the

subcutaneous and deep depots. When these different cellular patterns are examined in terms of various aspects of body size, body composition, and the degree, duration, and age of onset of obesity, only the latter uniquely distinguishes the hyperplastic from the hypertrophic: hyperplastic obesity is characterized by an early age of onset, hypertrophic, by a late age of onset. These studies indicate that there are two distinct periods early in life during which hypercellularity of the adipose tissue are most likely to occur: very early within the first few years, and again from age 9 to 13 yr.

### INTRODUCTION

Obesity is characterized anatomically by an excessive adipose tissue mass. The development of new and reliable methods for counting adipose cells and measuring their size in isolated fragments of adipose tissue has permitted a detailed examination of the cellular character of the expanded adipose depot of obese humans (1-3). Two apparently distinct cellular patterns have been described: in one, the expanded adipose depot contains increased numbers of somewhat enlarged adipose cells; in the second, the adipose cells are enlarged but are present in normal numbers (4-6).

In all of the above studies total adipose cell number has been estimated by dividing total body fat by adipose cell size (lipid content per cell). To date only subcutaneous fat cell size has been used in this calculation; in some instances, a single subcutaneous site has been used (2, 6); in others, three sites (4, 5, 8). The size of the subcutaneous cells measured had been assumed to represent the average adipose cell size of the body and the number of fat cells calculated from this average to be a meaningful estimate of the total for that individual. Adipose cell size, however, differs significantly

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Dr. Salans is the recipient of a Research Career Development Award from the Institute of Arthritis and Metabolism of the National Institutes of Health. Dr. Cushman is a recipient of an American Diabetes Association Research and Development Award.

*Received for publication 21 September 1972 and in revised form 13 December 1972.*

not only from one subcutaneous site to another (5), but also from subcutaneous to intra-abdominal sites (9). Thus, a cell derived from one or even multiple subcutaneous sites may yield an erroneous estimate of total adipose cell number. Furthermore, differences in cell number between nonobese and obese individuals based on these estimates may be misleading.

The present study was undertaken to more fully define the cellular character of the adipose depots of nonobese and obese individuals. Adipose cell size has been determined in several deep, as well as subcutaneous fat depots and a comparison of these sizes made. Several methods for estimating total cell number on the basis of these subcutaneous and deep cell sizes have been evaluated. Finally, the relationships among various parameters of body size and composition, age, and adipose tissue cellularity have been examined.

## METHODS

78 obese and 21 nonobese patients were studied. Individuals were classified as obese or nonobese on the basis of standard height, weight tables: obese patients ranged from 15% to greater than 100% overweight; nonobese patients were never more than 5% above their ideal body weight. 10 of the nonobese and 52 of the obese were female. Details of the anthropometric traits, body composition, and general characteristics of each of the two groups are shown in Table I. Individuals with recent weight loss or a history of nutritional or endocrine dysfunction, and nonobese patients with a past or family history of obesity were excluded from this study. A careful history was obtained from each patient in order to establish the age of onset of obesity; where possible, photographic and medical record documentation was obtained. Patients were hospitalized at the Mary Hitchcock Memorial Hospital and all studies were performed during periods of relatively constant body weight. Most of the obese patients underwent jejunio-ileal bypass operations after completion of this study. The nonobese patients were individuals undergoing other intra-abdominal surgical procedures.

Fragments of adipose tissue were obtained from three subcutaneous sites (upper outer *gluteal* quadrant, *abdominal* wall just lateral to the umbilicus, and midarm *triceps* region) by the method of needle aspiration (10). Tissue from three deep sites (*preperitoneal* just lateral to the umbilicus, *mesenteric* at the ligament of Treitz, and midline *omentum*)

were obtained at the time of surgery. Tissues were kept in Krebs-Ringer bicarbonate buffer, pH 7.4 at 37°C until processing. Lipid per cell (adipose cell size) at each site was measured in duplicate by the method described by Hirsch and Gallian (1). The total number of adipose cells in the body was estimated by dividing total body fat, calculated from measurements of body water and lean body mass (7, 11), by various combinations of the adipose cell sizes at the six different tissue sites.

Statistical analyses of the experimental results were performed as described by Sokal and Rohlf (12), on the Dartmouth College GE 635 Time-Sharing Computer System. A nested analysis of variance was employed to define the variability of cell size and cell number within and between patients and sites. In order to evaluate the various alternatives for calculating the average adipose cell size and estimating the total cell number, a simple linear regression analysis, stepwise multiple linear regression analysis, and a complete second order polynomial regression analysis were employed. The degree of association between the cellular character of the adipose tissue and the general patient characteristics was examined by correlation analysis and calculation of the coefficient of determination. Significance testing was performed by analysis of variance and significant values reported at  $P < 0.05$ .

## RESULTS

*Body size, body composition, and general characteristics of obese and nonobese patient groups.* The obese patients weigh more and have a significantly greater body fat, percent body fat, lean body mass, body fat/lean body mass, surface area, and ponderal index ( $\text{height}/\sqrt[3]{\text{weight}}$ ) than the nonobese patients (Table I). The nonobese females have increased percent body fat and fat:lean ratio compared with the men but these sex differences are not present in the obese. Fig. 1 shows the relationship between each of these indices and body weight. When all of the patients are considered together, each of the anthropometric and body composition parameters correlates highly with body weight, but these relationships may differ within the nonobese and obese subgroups.

The mean age of onset of obesity for the 78 patients is just under 10 ( $\pm 1\text{SEM}$ ) yr of age, the individual ages ranging from birth to 45 yr. Age of onset corre-

TABLE I  
General Characteristics of Nonobese and Obese Patient Groups

Patient group	Age	Weight*	Height	Surface area*	Body fat*	Percent body fat*	LBM*	Body fat/LBM*	Ponderal index*
	yr	kg	cm	m <sup>2</sup>	kg		kg		
Nonobese (21)	41 ± 3	66 ± 2	168 ± 2	1.75 ± 0.04	14 ± 1	21 ± 1	51 ± 2	0.27 ± 0.02	42 ± 0.2
Obese (78)	39 ± 1	130 ± 3	164 ± 1	2.28 ± 0.03	60 ± 3	45 ± 1	69 ± 1	0.87 ± 0.03	33 ± 0.3

Values, representing the mean  $\pm$ SEM for (n) patients, were calculated as follows: surface area =  $(\text{weight}^{0.425} \times \text{height}^{0.725}) \times 71.84$ , lean body mass (LBM) = total body water/0.73, body fat = weight - LBM, percent body fat = body fat/weight, and ponderal index =  $\text{height}/\sqrt[3]{\text{weight}}$ . Significance of differences between obese and nonobese groups was tested by analysis of variance; differences significant at the  $P < 0.05$  are indicated by an asterisk.

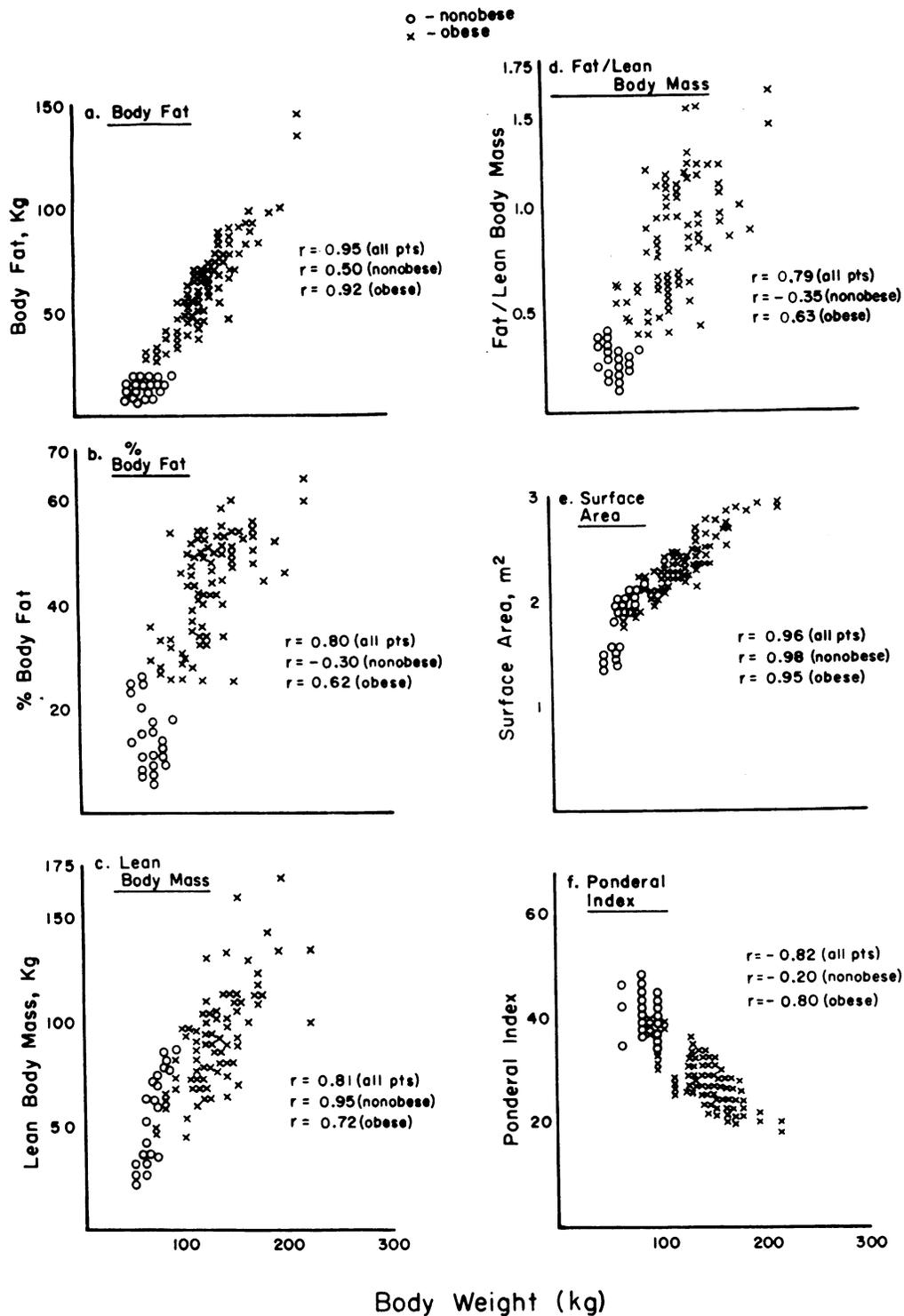


FIGURE 1 The relationship of the various parameters of body size and composition to the body weight of the nonobese and obese individuals. The correlation coefficient ( $r$ ) is shown for all 99 patients, as well as for the 21 nonobese and 78 obese patients, separately.

lates negatively with body weight ( $r = -0.80$ ) indicating that, in general, the earlier that obesity begins, the more severe the obesity.

*Adipose cell size in obese and nonobese patient groups.* Adipose cell size was determined at six individual subcutaneous and deep sites and the mean calculated for the three subcutaneous, the three deep and all of the six sites. Cell size was highly variable in these 99 patients.

The sources of variance of adipose cell size were examined by means of a nested analysis of variance. 70% of the total variance is accounted for by the differences between obese and nonobese patients, obese patients having larger cells. Fig. 2a illustrates the bimodal distribution of the mean of the cell sizes at all six sites among the 99 patients. A similar pattern was observed for each of the individual cell sizes and the mean of the three subcutaneous and three deep sizes. Fig. 2b, in which the percent frequency distribution of cell size within the nonobese or obese patient groups has been calculated, demonstrates that this bimodal distribution

is entirely attributable to the differences in cell size between the nonobese and obese patients. Table II summarizes the mean cell size of the individual sites and the means of the three subcutaneous, the three deep, and all six sites. The adipose cells of the obese patient group are significantly larger than those of the nonobese group for each of the nine determinations.

18% of the total variance of adipose cell size in the 99 patients is due to differences among the six sites sampled within each patient. The site to site variability of cell size was similar in the obese and nonobese patients (expected means squares = 0.0052 vs. 0.0054). The extent to which cell size may vary between fat depots within an individual is, in large part, masked when the mean cell sizes of the group are considered (Table II), and can only be appreciated by examining the cell sizes from different sites in individual patients as shown in Table III. The 12 patients, for whom the individual cell sizes have been listed, are representative of the wide range of body weights and cell sizes of those who comprise the nonobese and obese groups. Cell

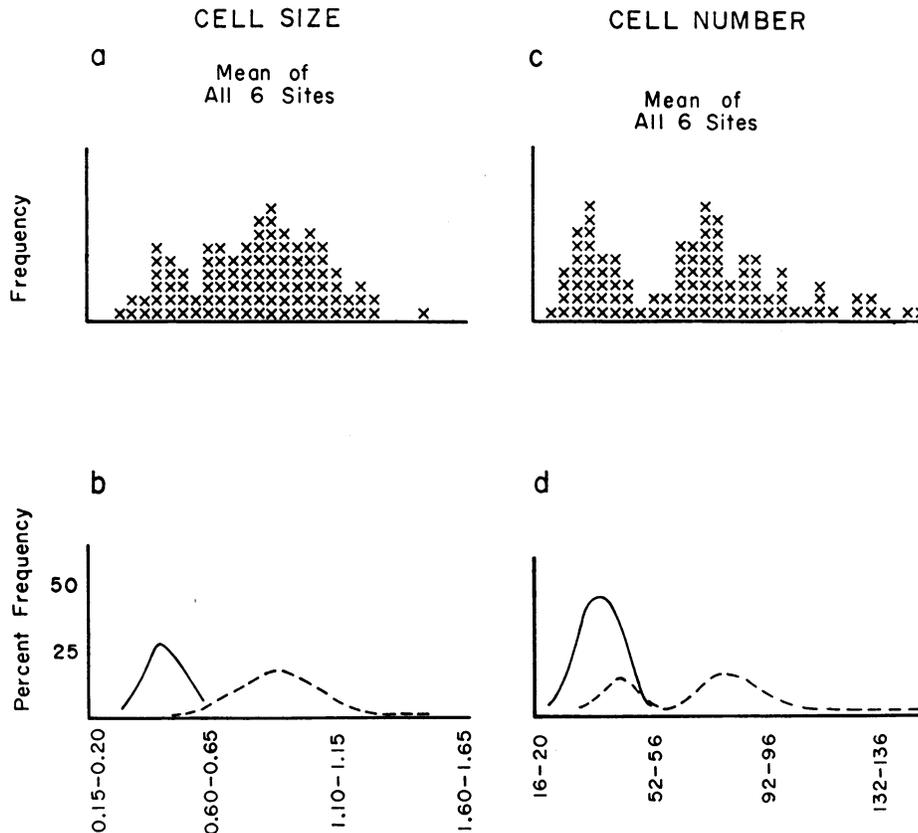


FIGURE 2 Distribution of adipose cell size and total cell number. Adipose cell size is expressed as the mean of the six individual cell sizes and total cell number is estimated by division of total body fat by this mean cell size. *a,c*): Frequency distribution of adipose cell size and number over the 99 patients ( $\times$ ). *b,d*): Percent frequency distribution of adipose cell size and number in the nonobese (—) and 78 obese (---) patients.

TABLE II  
Mean Values for Adipose Cell Size

Patient group	Subcutaneous			Deep			Mean		
	Gluteal	Abdomen	Triceps	Preperitoneal	Mesentery	Omentum	Subcutaneous	Deep	All
	$\mu\text{g lipid/cell}$								
Nonobese (21)	0.49	0.45	0.37	0.48	0.40	0.30	0.44	0.39	0.41
	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.03$	$\pm 0.03$	$\pm 0.03$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$
Obese (78)	0.93	0.89	0.83	0.90	0.80	0.73	0.88	0.81	0.84
	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$

Values represent the mean  $\pm$ SEM for (n) patients in each group. Significance of differences between obese and nonobese groups was tested by analysis of variance; all differences were significant at the  $P < 0.05$ .

size within a single patient may differ by more than 100% (T. B., M. R.), or only slightly (R. G., R. B.). The three subcutaneous cell sizes may be quite similar in a single patient while the three deep sizes differ not only from the subcutaneous sizes but from each other (E. J., E. D.); the reverse is also observed (H. J., N. Y.). Furthermore, although Table II indicates that the site to site order of cell size within each patient group is gluteal  $\geq$  preperitoneal  $\geq$  abdomen  $>$  mesentery  $\geq$  triceps  $>$  omentum, Table III demonstrates that the site to site order of cell size may differ considerably within the individual patients in each group. Less than 1% of the total variance of cell size represents the interaction between sites and patients. Neither the site to site order of cell size nor the magnitude of site to site variability appears, therefore, to be a function of the degree of adiposity.

The variability between duplicate determinations of cell size from a given site in an individual patient was quite small and within the previously defined precision of the method (1, 5).

The variability of cell size among the female and male patients is similar within each group, and the sources of variance are similar to those described above.

*Adipose cell number in obese and nonobese patient groups.* Adipose cell number is estimated by dividing total body fat by adipose cell size. The variability of total cell number, although quantitatively greater, is, therefore, qualitatively similar to that observed for adipose cell size. The largest source of variance in cell number is due to the difference between the obese and nonobese groups. As with cell size, this source of variance was considerably greater than that due to patient to patient and site to site variability within each group.

An attempt was then made to examine the extent to which the variability in the size of adipose cells obtained from different fat depots influences the estimation of total cell number and the comparison of obese and nonobese patients. Seven methods for estimating cell number from body fat and adipose cell size have been tested.

TABLE III  
Adipose Cell Size in Selected Nonobese and Obese Patients

Patient	Body weight	Subcutaneous			Deep			Mean		
		Gluteal	Abdominal	Triceps	Preperitoneal	Mesentery	Omental	Subcutaneous	Deep	All
		$\mu\text{g lipid/cell}$								
H. J.	58	0.51	0.36	0.31	0.34	0.33	0.30	0.39	0.32	0.35
E. J.	65	0.31	0.32	0.30	0.36	0.19	0.14	0.31	0.23	0.27
T. B.	70	0.61	0.52	0.37	0.52	0.30	0.26	0.50	0.36	0.43
H. M.	74	0.40	0.56	0.50	0.64	0.54	0.35	0.48	0.51	0.50
C. S.	77	0.59	0.51	0.50	0.51	0.59	0.64	0.53	0.58	0.56
R. G.	81	0.55	0.54	0.51	0.57	0.61	0.60	0.53	0.59	0.56
C. M.	105	0.94	0.80	0.70	0.75	0.80	0.76	0.81	0.77	0.79
E. D.	137	0.90	0.90	0.92	0.92	0.72	0.58	0.90	0.74	0.82
R. B.	143	0.95	0.92	0.90	0.91	0.94	0.89	0.92	0.91	0.91
N. Y.	144	1.18	0.89	0.71	0.95	0.68	0.70	0.93	0.78	0.85
M. R.	145	0.93	0.85	0.69	0.73	0.57	0.40	0.82	0.57	0.69
N. G.	205	0.91	0.99	1.11	0.95	1.20	1.23	1.01	1.13	1.07

Method I estimates total cell number from total body fat and the size of adipose cells at the six individual sites. The cell size at each site is assumed to represent the "average" cell size of a given patient. Methods II, III, and IV estimate total cell number from total body fat and the mean size of the three subcutaneous, three deep and all six sites, respectively.

In Methods V, VI, and VII, total cell number is estimated from various assumed distributions of total body fat between the subcutaneous and deep depots. The following five distributions of body fat have been assumed: (a) all of the body fat is in the subcutaneous depot and cell number is estimated from subcutaneous cell size only; (b) all of the fat is in the deep depots and cell number is estimated from deep cell size only; (c) 60% of the fat is in the subcutaneous, 40% in the deep depot and cell number is estimated from subcutaneous and deep cell size; (d) 40% of the fat is in the subcutaneous and 60% in the deep depots, and cell number is estimated from subcutaneous and deep cell size; (e) total body fat is distributed evenly between subcutaneous and deep depots and cell number is estimated using both subcutaneous and deep cell size. In Method V the mean subcutaneous and the mean deep cell sizes are assumed to represent the "average" cell size of their respective depots. In Method VI the largest of the three subcutaneous and three deep cell sizes are assumed to be the most representative cells of each depot; and in Method VII, the smallest.

The total number of adipose cells per individual varies considerably and significantly depending upon the method used: in general, cell number is greatest with omental cell size (range over 21 nonobese patients,  $23\text{--}65 \times 10^6$ ; over 78 obese patients,  $37\text{--}237 \times 10^6$ ) and smallest with gluteal (range over 21 nonobese patients,  $20\text{--}41 \times 10^6$ ; over 78 obese patients,  $28\text{--}128 \times 10^6$ ). Nevertheless, mean adipose cell number for the obese group is always significantly greater than that for the nonobese, irrespective of the cell size used and of the assumed distribution of total body fat (nonobese,  $28\text{--}49 \times 10^6$ ; obese  $65\text{--}88 \times 10^6$ ). Individual nonobese and obese patients do, however, overlap considerably in cell number; the overlap is greatest with omental cell size and least with the mean size of all six sites.

*Factors responsible for the difference in total cell number between obese and nonobese patient groups.* Fig. 2c illustrates the frequency distribution over all patients of total cell number calculated from total body fat and the mean of cell sizes determined at all six sites. A bimodal distribution of cell number is observed, but in contrast to cell size, the two populations are not simply a reflection of the obese and nonobese patient groups (Fig. 2d); the population with the lower cell number contains not only the 21 nonobese, but in addi-

tion, 17 of the obese patients. All 61 of the individuals within the population of higher cell number are obese. An evaluation of the factors which might be responsible for the two patterns of cellularity in the obese patients has been undertaken.

The hypercellular obese group weighs significantly more ( $138 \pm 4$  vs.  $101 \pm 4$ ), and has greater body fat ( $67 \pm 3$  vs.  $32 \pm 2$ ), percent body fat ( $48 \pm 1$  vs.  $36 \pm 1$ ), lean body mass ( $71 \pm 2$  vs.  $65 \pm 2$ ), surface area ( $2.35 \pm 0.02$  vs.  $2.05 \pm 0.04$ ), and fat:lean ratio ( $0.97 \pm 0.03$  vs.  $0.57 \pm 0.03$ ) than does the normocellular group of obese patients. The hypercellular obese patients have, in addition, a much earlier age of onset of their obesity ( $7 \pm 0.05$  vs.  $27 \pm 2$ ). Any of these factors, therefore, could be responsible for the increased number of adipose cells in the fat depots of the hypercellular obese patients. Fig. 3 indicates that two populations of obese patients can be differentiated on the basis of cell number, even within groups of obese patients matched for each of several parameters of body size and composition. In contrast, Fig. 4 demonstrates the relationship between total cell number and age of onset of obesity in these same matched patients. Regardless of the parameter on which these patients are matched, the hypercellular obese patients are those with an earlier onset of obesity than those with a normal cell number. Although all of the massively obese patients with an early age of onset of their obesity have increased cell number, Figs. 3 and 4 indicate that hypercellularity also occurs in patients with a moderate degree of obesity (body weight 91–123 kg) and early age of onset. Furthermore, cell number in the 78 obese patients is poorly correlated with the duration of obesity ( $r = 0.37$ ). Thus, as Fig. 5 indicates, the one factor which is most closely related to the differences in cell number between the two groups of obese patients is the age of onset of their obesity; obesity of onset early in life is associated with increased adipose cell number while adult onset obesity is associated with normal cell number. Although the age of onset of obesity in the hypercellular obese patients covers a wide range, the obesity of most patients began either within the first 5 yr of life, or between ages 9–13 yr. Those who became obese earliest in life have, as a group, the greatest degree of hypercellularity.

Since the age of onset of obesity appears to most clearly delineate the hypercellular from the normocellular obese patients, the age of onset of the two patient groups has been examined in detail. Of the 78 obese patients, none with onset of obesity after 20 yr of age are hypercellular regardless of the method by which total cell number is estimated. In several obese patients, however, classification into the normocellular or hypercellular category does depend upon which cell size is used to estimate cell number. Nine obese patients classi-

fied as having hypercellular adipose depots when the individual subcutaneous cell sizes are used to estimate total cell number have normal numbers of adipose cells when omental or mesenteric cell size is used (age of onset of obesity, 7-19 yr). Three obese patients with normal cell number estimated from the individual subcutaneous cell sizes have hypercellularity on the basis of omental or mesenteric size (age of onset of obesity, 18-19 yr). When the mean size of the three subcutaneous sites is employed to estimate cell number, these dif-

ferences in classification are observed in eight obese patients. When cell number is estimated from the mean of all six cell sizes the differences in classification are greatly reduced: two obese patients (age of onset of obesity, 18 and 19 yr) classified as normocellular on the basis of mean subcutaneous cell size are hypercellular, and one obese patient (age of onset of obesity, 18 yr) classified as hypercellular on the basis of mean subcutaneous cell size is normocellular. Estimation of total cell number on the basis of the various assumed dis-

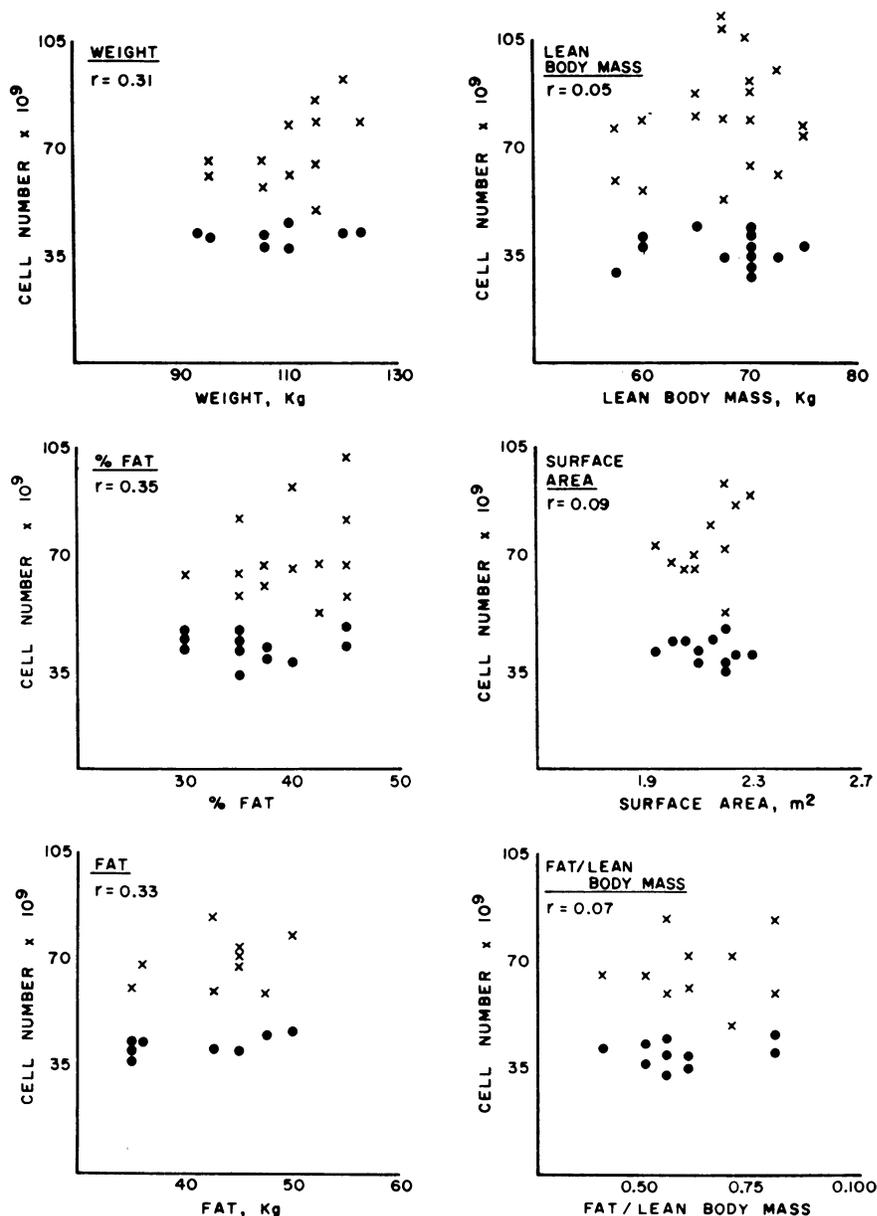


FIGURE 3 Total adipose cell number in obese patients matched for the various parameters of body size and composition. Cell number is estimated by dividing total body fat by the mean of the six cell sizes.

tributions of subcutaneous and deep body fat does not alter these observations. Thus, when cell number is estimated from the mean of all six cell sizes, 61 obese patients exhibit adipose hypercellularity and 17, normal cell number compared with the 21 nonobese patients.

Table IV summarizes the cell number data of these three patient groups. In the early onset obese patients cell number is greater than that of either the nonobese

or the late onset obese groups. Cell number in the late onset obese patient group is similar to that of the non-obese except when calculated from the gluteal, preperitoneal, or the superficial abdominal cell sizes, in which cases the differences are small.

Fig. 6 demonstrates that the relationship between mean cell number and mean cell size in the 99 patients studied falls into three patterns which correspond to

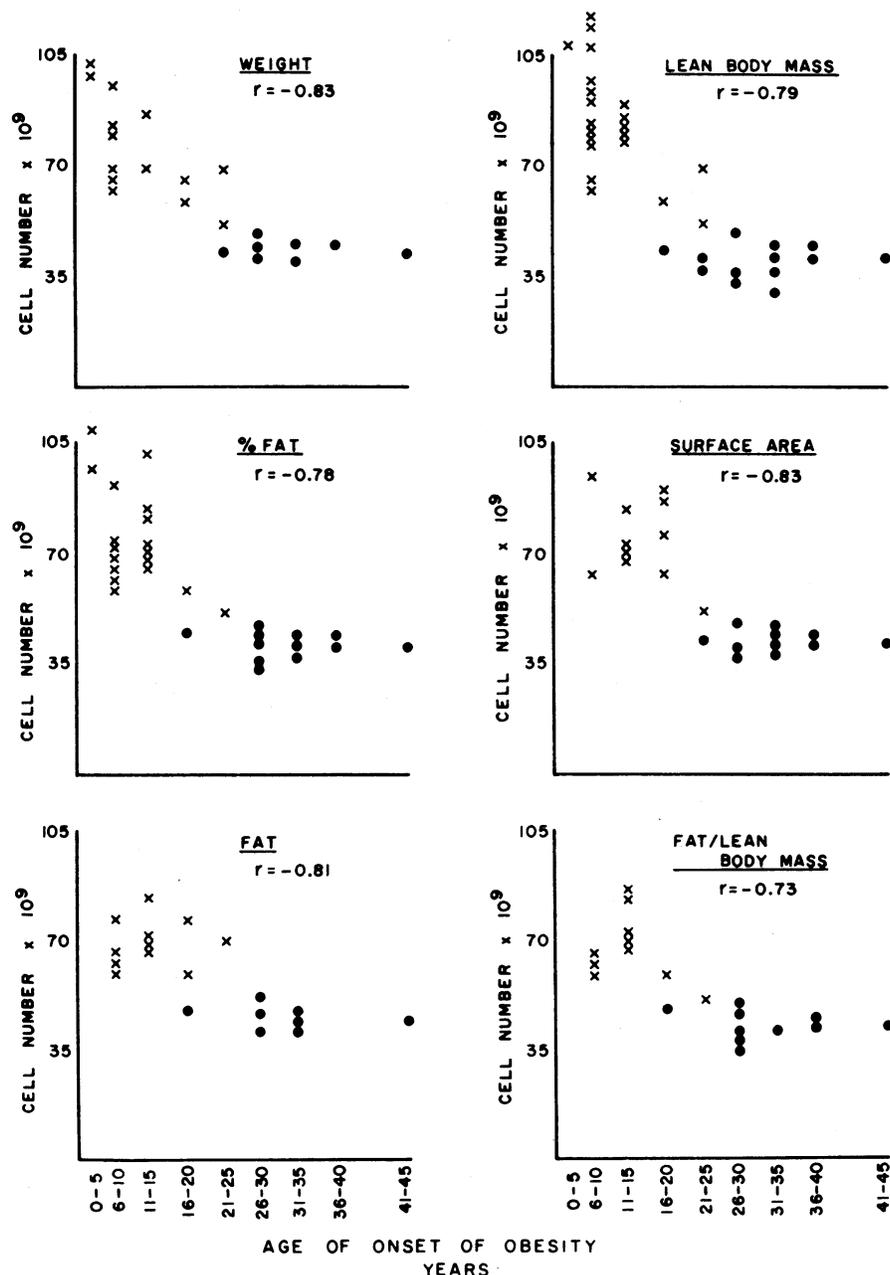


FIGURE 4 The relationship of total cell number and the age of onset of obesity in the obese patients matched for the various parameters of body size and composition. Cell number is estimated by dividing total body fat by the mean of the six cell sizes.

the three patient groups. Nonobese patients have a relatively small number of relatively small adipose cells. The normocellular obese have normal numbers of enlarged fat cells. The obesity of this group of individuals began relatively late in life compared with the third group. In the third group, obesity began early in life and the adipose depots of these patients contain increased numbers of normal or enlarged fat cells. The adipose cell size of this group is significantly smaller than that of the normocellular, late onset obese patients for each of the six individual cell sizes as well as for each of the three means ( $P < 0.05$ ). Some of the hypercellular obese patients even display normal adipose cell size. In neither of the obese patient groups are cell size and number highly correlated ( $r = -0.22$  and  $0.42$ , respectively).

*Relationship of adipose cell size and total cell number to body size and composition.* The relationships between the individual general characteristics of the 99 patients and the cellular character of their adipose tissues have been examined by measuring the correlation of adipose cell size and number with various parameters of body size (weight, surface area, ponderal index), composition (fat, percent fat, fat/lean mass), and age. Table V summarizes some of these correlation coefficients for each of the five patient groups. Although the data in this table pertain only to the correlation of the mean of the cell sizes at all six sites and the total cell number derived from this mean cell size, they are illustrative of those obtained from the other expressions of cell size and number. An estimate of the proportion of the variation of cell size or number within a patient group that can be explained by each of the various parameters is obtained by calculating  $r^2$ , where  $r$  is the simple correlation coefficient, shown in Table V.

To a large extent the nature of these relationships depends upon the patient group being examined. Thus, cell size correlates highly with body fat and the other

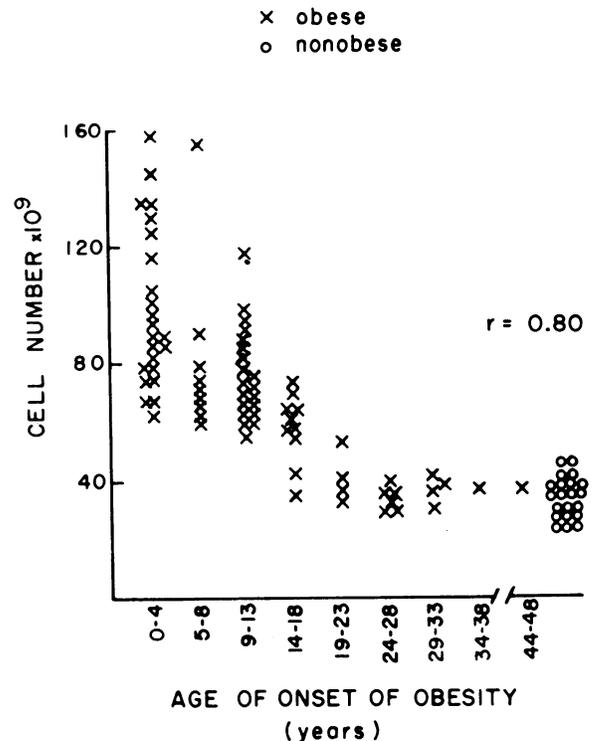


FIGURE 5 The relationship of total cell number and age of onset of obesity. Cell number is estimated by dividing total body fat by the mean of the six cell sizes.  $r$  represents the correlation coefficient for the obese patients only.

parameters of adiposity in normocellular obese patients, but only poorly in the hypercellular individuals. In contrast, adipose cell number correlates well with the parameters of adiposity in hypercellular obese but not in the normocellular obese group.

In all patient groups, adipose cell size correlates more highly with the parameters of adiposity than with those of body size, somatotype, or age. Adipose cell number is

TABLE IV  
*Adipose Cell Number in Normocellular and Hypercellular Obese Patients*

Patient group	Subcutaneous			Deep			Mean		
	Gluteal	Abdominal	Triceps	Preperitoneal	Mesentery	Omental	Subcutaneous	Deep	All
$\times 10^9$									
Normocellular									
Nonobese (21)	28 $\pm$ 1	31 $\pm$ 2	38 $\pm$ 2	29 $\pm$ 1	36 $\pm$ 2	49 $\pm$ 3	33 $\pm$ 2	39 $\pm$ 2	37 $\pm$ 1
Obese (17)	36 $\pm$ 2*	37 $\pm$ 1*	39 $\pm$ 1*	38 $\pm$ 1*	39 $\pm$ 1*	45 $\pm$ 2	37 $\pm$ 1	41 $\pm$ 1	39 $\pm$ 1
Hypercellular									
Obese (61)	73 $\pm$ 3‡	78 $\pm$ 3‡	84 $\pm$ 3‡	78 $\pm$ 3‡	90 $\pm$ 4‡	100 $\pm$ 5‡	78 $\pm$ 2‡	89 $\pm$ 4‡	84 $\pm$ 3‡

Values represent the mean  $\pm$ SEM for (n) patients in each group. Significance of differences was tested by analysis of variance:

\*  $P < 0.05$ , normocellular obese vs. nonobese.

‡  $P < 0.05$ , hypercellular obese vs. normocellular obese.

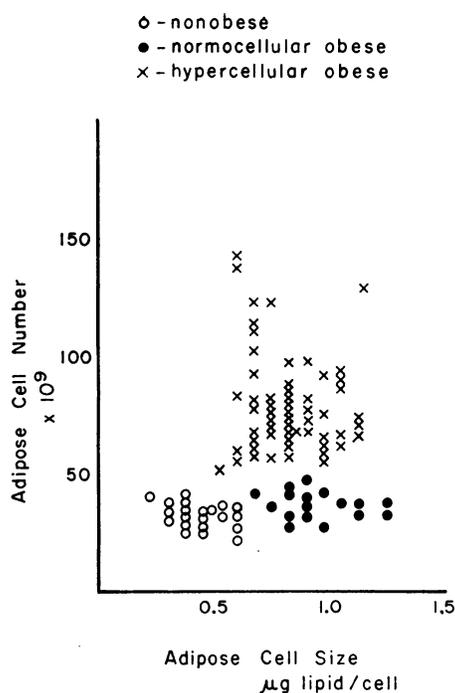


FIGURE 6 The relationship of adipose cell size and total adipose cell number. Adipose cell size is expressed as the mean of the six individual cell sizes and cell number estimated by division of total body fat by this mean cell size.

also most highly correlated with the parameters of adiposity in those groups which contain all of the patients, all of the obese patients, or only the hypercellular obese patients. In the remaining two groups of patients, cell number correlates best with the ponderal index but the relationship is poor ( $r = < 0.30$ ). Cell number is more highly correlated with the various parameters of adi-

posity than is cell size in all but the nonobese and normocellular obese groups, in which the association of this cellular character and all parameters of body size and composition is poor ( $r = < 0.30$ ). Both cell size and number correlate more highly with body fat than with percent body fat or the fat:lean ratio.

An estimate of the proportion of the variation of cell size or number within each patient group that can be explained by combinations of parameters of body size (weight, surface area, and height) or adiposity (fat, percent fat, and fat:lean ratio) is obtained by calculating  $r^2$ , where  $r$  is the multiple correlation coefficient (12). Such combinations of parameters increase the proportion of explained variance only very slightly over that explained by the most highly correlated individual parameter and do so without substantially altering the relationships described above. In all of the patient groups, the combination of body fat, percent fat, and fat:lean ratio accounts for more of the variation in cell number and cell size than do the combined parameters of body size. Within the nonobese group, the ponderal index accounts for more of the variation in cell number (9%) than do combinations of either body size or adiposity parameters.

*Predicting cell size and cell number from parameters of body size, body composition, and age.* The ability to predict cell size and cell number in an individual patient from the various anthropometric, body composition and age parameters has been tested. For this purpose the general characteristics of the 99 patients were first regressed, alone and in combination, against their adipose cell size and number. Both linear and curvilinear (complete second order polynomial regression) relationships were examined by means of a multiple regression analysis. The value of each of the parameters for predicting

TABLE V  
Correlations of Adipose Cell Size and Adipose Cell Number with Body Weight and Adiposity

Patient group		Body weight	Body fat	% Body fat	
				Body fat	Body fat/LBM
Adipose cell size					
All	(99)	0.70	0.70	0.68	0.68
Nonobese	(21)	0.52	0.77	0.41	0.34
All obese	(78)	0.38	0.38	0.19	0.33
Hypercellular obese	(61)	0.52	0.54	0.32	0.32
Normal cellular obese	(17)	0.65	0.81	0.69	0.65
Adipose cell number					
All	(99)	0.80	0.86	0.72	0.82
Nonobese	(21)	0.08	0.15	0.18	0.24
All obese	(78)	0.73	0.81	0.27	0.74
Hypercellular obese	(61)	0.61	0.71	0.56	0.61
Normal cellular obese	(17)	0.10	-0.05	-0.05	-0.06

cell size and number was then evaluated from the regression analyses and their associated residual variances (measures of the extent of scatter of observed points about the line of best fit). Finally, prediction equations were generated from these regression models and 95% confidence limits set for the predicted response. The data presented relate only to regressions against the mean of the cell sizes of all six sites and the total cell number estimated from this mean cell size. These relationships have been examined, however, for each of the other cell size and cell number measurements and the general pattern found to be similar.

General patient characteristics are of more value in predicting cell size than cell number for each patient group, but in all cases the value of the prediction models is poor. Cell size can be predicted within  $\pm 0.17 \mu\text{g}$  lipid in 95% of the nonobese and  $\pm 0.26 \mu\text{g}$  lipid in 95% of the obese patients, and cell number within  $\pm 15 \times 10^9$  in 95% of the nonobese and  $\pm 33 \times 10^9$  in 95% of the obese patients from the best linear and second order equations.

## DISCUSSION

Two distinct types of human obesity have been identified on the basis of the cellular character of the mass of adipose tissue: hyperplastic, with a small to moderate increase in adipose cell size, and hypertrophic, with a large increase in cell size alone. Of the various general patient characteristics examined, only the age of onset of obesity is uniquely characteristic of these two types: hypercellular obesity is associated with an early onset, hypertrophic with a later onset, of obesity. Neither body size nor degree or duration of obesity are particularly characteristic of one or the other type of obesity. These studies also establish two distinct periods during early life in which hypercellularity of the adipose tissue are most likely to develop: very early, within the first few years and again from age 9 to 13 yr. Those individuals who became obese very early in life are the ones who have most nearly normal cell size, but have the greatest increase in cell number, whereas those with onset of obesity between ages 9–13 yr have more change in cell size than cell number.

The size of adipose cells within an individual can vary considerably from fat depot to fat depot. Such variability in cell size has been observed not only among the subcutaneous depots, as has previously been reported (5), but also among the deep, as well as between the subcutaneous and deep, depots. The factors responsible for this variability could not, however, be defined in these investigations. Neither obesity, sex, age, age of onset of obesity, nor any of the various parameters of body size, somatotype, or composition could explain the variation of adipose cell size among depots. Additional factors such as adipomuscular ratio remain unexplored (13).

Variation in adipose cell size among fat depots may be of more than just morphologic significance since the metabolic activity of adipose tissue may be related, in part, to the size of its constituent adipose cells (14, 15). Extrapolation of metabolic information from adipose cells of a single depot to the entire adipose organ may be misleading. Several studies already indicate differences in the metabolic activity and hormone responsiveness of adipose cells obtained from different fat depots of the body (9, 16). Furthermore, since estimates of an individual's total adipose cell number are based upon cell size, these estimates will vary with varying cell size. Among the 21 nonobese patients examined in this study, estimated cell number within individuals varied by as much as 60% ( $41 - 65 \times 10^9$ ) over the six individual and three mean cell sizes measured. Among the obese patients, estimated cell number varied by as much as 85% ( $128 - 237 \times 10^9$ ).

In spite of the variation in the estimate of cell number within patients, however, classification of the great majority of obese patients (66 of 78) into the hypercellular or normocellular category is independent of the cell size employed. In some of the obese patients, categorization does depend upon which cell size is used to estimate cell number. When the mean size of the three subcutaneous, three deep, and all six sites are used, classification of patients is more uniform than when individual cell sizes are employed. Thus, although the absolute number of adipose cells within an individual cannot be determined by currently available methods, an approximation can be obtained and comparisons of the cellularity of this tissue among patients made through estimates of cell number based upon the simple division of total body fat by the adipose cell size at several sites.

Since no method presently exists through which the true number of adipose cells in an individual can be determined, no one of the several calculations described in this study can be selected as the best method for estimating cell number. These investigations have assumed, then, that the best estimate of cell number is obtained by dividing total body fat by the "average" adipose cell size and that the best estimate of the "average" adipose cell size of the body is the mean of the sizes determined at all of the six sites sampled in this study. This estimator of cell number has been denoted Method IV above. This assumption is based on the observed variability of cell size within an individual and the fact that no a priori information exists as to which, if any, of the cell sizes is most representative of the "average" adipose cell size. Since the determination of cell size in the deep fat depots is not practical in most patients, estimators of cell size and cell number based upon the subcutaneous cell size alone have been evaluated relative to

those based upon the mean of the cell sizes at all six sites.

The mean of the cell sizes at all three of the subcutaneous sites correlates more highly with the mean cell size of the six sites than does the size at any individual subcutaneous site, in both nonobese ( $r = 0.97$ ) and obese ( $r = 0.93$ ) patient groups. The variation of the mean subcutaneous size from the mean of all six sizes is  $\pm 0.05 \mu\text{g}$  lipid/cell in 95% of the nonobese patients and  $\pm 0.12 \mu\text{g}$  lipid/cell in 95% of the obese patients. Similarly, cell size estimated from the mean subcutaneous size correlates more highly with that estimated from the mean of all six sizes in both the nonobese ( $r = 0.83$ , error =  $\pm 5 \times 10^9$  in 95% of the patients) and obese ( $r = 0.97$ , error =  $12 \times 10^9$ ) patients, than does cell number estimated from the individual subcutaneous sizes. Triceps cell size in the nonobese and superficial abdominal cell size in the obese yield the best estimates of total cell number of the individual subcutaneous sizes ( $r = 0.93$ , error =  $\pm 8 \times 10^9$ ;  $r = 0.90$ , error =  $\pm 18 \times 10^9$ , in 95% of the patients, respectively). Classification of individuals as normo- or hypercellular is more difficult, as discussed above, when total cell number is estimated from individual cells sizes than from mean cell size. Thus, until a direct measure of total cell number becomes available, or until the contribution of each fat depot to total body fat is known, division of total body fat by the mean of the cell sizes of at least the three subcutaneous depots examined in this study appears to provide the best estimate of total adipose cell number.

The inability to differentiate cell lipid from structural fat and the insensitivity to extremely small cells which contain less than  $0.01 \mu\text{g}$  lipid/cell, remain limitations of the osmic acid method by which adipose cells in this study have been counted and sized. The former would lead to an underestimation, and the latter, an overestimation of adipose cell number. The recent observations of Sjostrom, Bjorntorp, and Vrana (3), however, suggest that the error of the latter type is likely to be quite small. Other limitations of potential significance in the calculation of total cell number by this method relate to the error in the measurement of total body fat from body water. Although not specifically examined in this study, others have found that the technique of isotope dilution provides an estimate of total body fat in non-obese and obese patients that is similar to that obtained by other methods, is reproducible, and is associated with a relatively small measurement error (2, 7, 17).

In the present study adipose cell size and total cell number were found to be more highly correlated with body fat than any parameter of adiposity including percent body fat and the fat:lean ratio. These observations conflict with a recent preliminary report which suggests that adipose cell size correlates more highly with

percent body fat than with total fat mass and that adipose cell hypertrophy is the major characteristic of the expanded adipose depot in obesity (18). The present study clearly demonstrates two different mechanisms for the expansion of the adipose depot in obese patients. The difference between these conflicting studies may lie, in part, in the type of obese patients included in the latter, since the present study indicates that the relationships between these general patient characteristics and the cellular character of the adipose tissue depends upon the group of patients being examined.

The present study also suggests that the different cellular patterns of the expanded adipose tissue mass of obese patients is not a simple function of the degree of obesity (8); several of the moderately obese patients exhibit a hypercellular tissue mass. All of the grossly obese patients, however, are hypercellular. These studies have not included massively obese individuals who became obese as adults, the greatest weight in the normocellular obese being 123 kg. It remains possible that more severely obese individuals, with onset of obesity in adult life, might show mild degrees of hypercellularity.

The one factor most closely associated with, and perhaps which best accounts for, the two different mechanisms by which expansion of the adipose tissue mass occurs in obesity is the time of life at which obesity begins; the earlier the onset the more likely that cell number will be increased. These studies do not indicate, however, that this phenomenon is related to the duration of the obesity. These observations support those previously reported by Hirsch and Knittle (4) and Brook, Lloyd, and Wolf (19). The age of onset of obesity could not be documented with assurance in most patients; too often this depended upon the reliability of historical information. Thus, the age at which adipose cell number can no longer be influenced could not be established with certainty. Although the latest age of onset of obesity for which hypercellularity has been observed in this study was 19 yr, small increases in cell number (estimated from subcutaneous cell size alone) in patients with onset of obesity later in life have been reported by Bray and Gallagher (20) and Hirsch and Knittle (4). This inability to reliably document the age of onset of obesity also limits the interpretation of the observation that age of onset of obesity in the hypercellular patients is most prevalent within the first 5 yr of life and then again from ages 9–13 yr. This finding is, however, compatible with the observation of Tanner that the adipose tissue mass grows rapidly during the first 2 yr of life, then plateaus until about age 7 yr when its rate of growth increases once again (21).

Finally, the value of general patient characteristics towards a prediction of adipose cell size and number has been examined through the construction of linear and

complete second order multiple regression models. The error of prediction from even the best of these models is, however, of such magnitude that the separation of non-obese and obese patients and the classification of obese patients into normo- and hypercellular groups are unreliable. The estimation of adipose cell size or number without direct measurements is, therefore, not recommended at least until additional parameters are evaluated and/or better models constructed.

#### ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent technical assistance of Mary Jane Zarnowski and Ruth Segal and the aid of Professor Harrison D. Weed, Department of Mathematics, Dartmouth College, in the statistical analysis of these data. We wish to express special gratitude to Dr. Jules Hirsch, The Rockefeller University, for his help and guidance in establishing the methods by which adipose cells were sized and counted during this study.

This study was supported by grant no. AM 13321 from the National Institutes of Health, and a grant from the Hitchcock Foundation.

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