

# Experimental Obesity in Man: Cellular Character of the Adipose Tissue

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**ABSTRACT** Studies of adipose tissue cellularity were carried out in a group of nonobese adult male volunteers who gained 15–25% of their body weight as the result of prolonged high caloric intake. Adipose cell size (lipid content per cell) was determined in tissue obtained from three subcutaneous sites (gluteal, anterior abdominal wall, and triceps) and total adipose cell number estimated from measurement of total body fat.

Five experimental subjects gained an average of 16.2 kg of body weight, of which 10.4 kg was determined to be fat. Expansion of the adipose mass was accompanied by a significant and relatively uniform increase in fat cell size in each subcutaneous site tested. Total adipose cell number did not change as a result of weight gain and expansion of the adipose depot in adult life. Subsequent loss of weight and restoration of original body fat was associated with a reduction in adipose cell size at each subcutaneous site, but no change in total number. In two control subjects who neither gained nor lost weight there were no changes in total adipose cell number or cell size. These observations suggest that expansion and retraction of the adipose depot in adult life is accompanied by changes in adipose cell size only.

Significant differences in both the size and total number of adipose cells were observed between subjects in both the experimental and control groups. In addition, within individuals of both groups there were significant differences in cell size when adipose cells from the three subcutaneous sites were compared. These findings indicate that wide variations in adipose cell size and number exist in nonobese individuals having similar adipose depot sizes.

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## INTRODUCTION

Obesity is characterized by an increased adipose tissue mass. The enlargement of the adipose depot may be the result of an increase in the number or size (lipid content per cell) of its constituent fat cells. It has been suggested that definition of the cellular character of this expanded tissue may provide a means for categorizing different patterns of human obesity and may lead to a more rational therapeutic approach (1). Consideration of the cellular character of the adipose tissue may be of more than just morphologic interest since it has recently been demonstrated that some aspects of glucose tolerance, insulin secretion and sensitivity, and adipose tissue metabolism are influenced by adipose cell size and number (2, 3).

Human obesity of early onset is accompanied by a marked increase in adipose cell number and to a lesser extent in adipose cell size (1). Weight loss by dietary restriction is achieved solely by reduction in adipose cell size and the hypercellularity persists. Glucose intolerance and hyperinsulinemia in these individuals is associated with the presence of enlarged, insulin-resistant fat cells in the adipose depot, abnormalities which disappear upon weight loss and reduction in fat cell size (2). Similar abnormalities of impaired glucose tolerance and hyperinsulinemia are seen in human obesity of adult onset, but little is known of the cellular and metabolic character of their expanded adipose depot and of its relationship to the metabolic disorders.

Earlier studies of experimentally induced obesity in adult man indicate that expansion of the adipose depot is accompanied by an increase in the size of fat cells in the subcutaneous tissue of the gluteal region (4). It was postulated that the adipose depot enlarged as a result of a generalized increase in fat cell size but that there was no change in total adipose cell number. The current studies were undertaken to more clearly define the cellular char-

acter of the adipose depot when it is expanded and contracted by experimental means in adult human volunteers.

## METHODS

**Subjects.** All subjects were inmates of the Vermont State Prison who volunteered for the study. They were selected so as to exclude those with a history or family history of diabetes mellitus, obesity, or other metabolic and nutritional disorders. The seven volunteers ranged in age from 20 to 30 yr and in normal body weight from 61 to 84 kg, as indicated in Table I.

All subjects followed normal prison routine during the entire period of the study, except that they ate meals together as a group in a dining room set aside for the purpose and during the period of weight gain they reduced their physical activity. The caloric content and composition of their diet was estimated from standard dietary tables. The quantity of food ingested by each individual at each meal was carefully recorded. During an initial 6 wk study period sufficient calories were provided to maintain constant body weight (base line weight). In five of the seven subjects this initial period was followed by a 3-4 month period of high caloric intake to produce weight gain. After desired or maximum obtainable weight was reached, each of these five subjects ingested sufficient numbers of calories to maintain constant weight during the second study period (peak weight), which was of 10 wk duration. The final phase of the study began after a period in which caloric restriction and increased activity induced loss of weight to original levels. During the final study period sufficient calories were provided to maintain constant normal body weight (reduced base line). The body weight of the two control subjects was maintained at a constant level throughout all three study periods. Determination of total body fat, adipose cell size, and adipose cell number was made on each patient during each of these study periods.

TABLE I  
Age and Body Weight of Volunteer Subjects

Subject	Age	Height	Body weight		
			Base line	Peak	Reduced base line
	yr	cm	kg	kg	kg
<b>Experimentals</b>					
A. C.	25	182	73	90	76
N. H.	26	177	75	94	75
B. H.	30	176	84	102	84
R. P.	29	181	80	96	82
P. W.	20	163	61	72	59
Mean			74.6	90.8	75.2
			Base line 1	Base line 2	
<b>Controls</b>					
S. L.	20	170	62	62	
L. M.	24	164	61	60	
Mean			62	61	

TABLE II  
Body Fat and Per Cent of Body Weight as Fat in Volunteer Subjects

Subject	Base line		Peak		Reduced base line	
	Fat	Fat	Fat	Fat	Fat	Fat
	kg	%	kg	%	kg	%
<b>Experimentals</b>						
A. C.	13.1	(18.1)	25.0	(27.2)	15.2	(20.0)
N. H.	18.9	(25.0)	30.1	(32.1)	17.9	(23.9)
B. H.	19.7	(23.5)	33.0	(31.7)	17.7	(21.9)
R. P.	10.7	(13.2)	21.3	(21.2)	9.1	(11.2)
P. W.	8.6	(13.9)	13.7	(18.9)	6.9	(11.7)
Mean	14.2	(18.7)	24.6	(26.2)	13.4	(17.7)
			Base line 1	Base line 2		
<b>Controls</b>						
S. L.	7.8	(12.6)	6.7	(10.8)		
L. M.	10.4	(17.2)	10.0	(16.7)		
Mean	9.1	(14.9)	8.4	(13.8)		

Body fat was determined from measurement of body density and correction for residual respiratory volume.

**Adipose tissue sampling.** Adipose tissue was obtained from all subjects from the subcutaneous tissue of the buttock, anterior abdominal wall, and triceps area by needle aspiration (5). One aspirate containing many tissue fragments was obtained from each of the three subcutaneous sites and from this aspirate, adipose cell size and number were determined either in duplicate or triplicate. The identical procedure was repeated at weekly intervals so that tissue was obtained from each of the three sites at least twice during each study period. No studies were performed during the periods of active weight gain or loss.

The adipose tissue fragments were immediately placed in bicarbonate buffer kept at 37°C under 95% oxygen; 5% CO<sub>2</sub> in a thermos flask.

**Determination of adipose cell size and number.** The tissue fragments were carefully and repeatedly washed with warm buffer to remove blood and adherent oil droplets. Then they were processed according to the method of Hirsch and Gallian (6). One aliquot of adipose tissue of known weight was extracted overnight in chloroform:methanol (2:1) to determine per cent of wet weight which is fat. Aliquots of adipose tissue of known wet weight were incubated in duplicate or triplicate for 48 hr in a solution of osmium tetroxide in collidine buffer at 37°C. The resultant osmium-fixed free adipose cells were counted in a Coulter Electronic Counter (Coulter Electronics, Hialeah, Fla.) Mean adipose cell size (lipid content per cell) for each sample was calculated by dividing the total lipid in the tissue fragments by the number of fat cells in that amount of lipid. The same methods were used to determine mean adipose cell size at weekly intervals during each study period. Adipose cell size for each individual as shown in Table III represents the mean of all determinations during the study period.

The total number of adipose cells in the body was estimated by dividing total body fat by the average fat per cell at each site (mean cell size, Table III). Body fat was

calculated from under-water weighing as described by Goldman and Buskirk (7) except that body density was measured by immersion in the supine position (8). The residual lung volume used in the calculations was measured by a closed-circuit helium dilution technique (9) with subjects in the same position in air as when being weighed under water. There was no difference in residual lung volume between base line and peak body weight.

## RESULTS

### Body weight

Table I indicates the body weight of each individual and the mean body weight of the group at each study

period. Prolonged high caloric intake resulted in a mean weight gain of 16.2 kg in the group, with an individual range of from 9 to 19 kg. This represents a 20.9% increase in body weight for the group as a whole with individual gains ranging from 14.8 to 25.3%. Caloric restriction restored body weight to normal. There was no change in the body weight of the two control subjects from base line 1 to base line 2.

### Body fat

These changes in body weight were largely due to changes in body fat as is shown in Table II. Prolonged

TABLE III  
Adipose Cell Size ( $\mu\text{g TG/cell}$ )—Individual Subjects

Subject	Study period	Gluteal	Abdomen	Triceps
Experimental				
A. C.	Base line	0.46 $\pm$ 0.02*	0.31 $\pm$ 0.01‡	0.45 $\pm$ 0.02
	Peak	0.99 $\pm$ 0.16*	0.76 $\pm$ 0.01‡	0.85 $\pm$ 0.04
	Reduced base line	0.50 $\pm$ 0.02	0.49 $\pm$ 0.08	0.52 $\pm$ 0.01
	P§	<0.001	<0.001	<0.001
N. H.	Base line	0.61 $\pm$ 0.03*	0.51 $\pm$ 0.03	0.56 $\pm$ 0.02
	Peak	0.88 $\pm$ 0.02	0.86 $\pm$ 0.02	0.86 $\pm$ 0.07
	Reduced base line	0.44 $\pm$ 0.01*	0.52 $\pm$ 0.11	0.39 $\pm$ 0.08
	P§	<0.01	<0.001	<0.001
B. H.	Base line	0.65 $\pm$ 0.01*	0.54 $\pm$ 0.01‡	0.60 $\pm$ 0.02
	Peak	1.02 $\pm$ 0.02	0.85 $\pm$ 0.05‡	1.04 $\pm$ 0.04
	Reduced base line	0.52 $\pm$ 0.03	0.39 $\pm$ 0.02‡	0.46 $\pm$ 0.03
	P§	<0.001	<0.001	<0.001
R. P.	Base line	0.37 $\pm$ 0.03*	0.32 $\pm$ 0.01‡	0.25 $\pm$ 0.03
	Peak	0.72 $\pm$ 0.01*	0.65 $\pm$ 0.14‡	0.50 $\pm$ 0.03
	Reduced base line	0.36 $\pm$ 0.02*	0.23 $\pm$ 0.02‡	0.25 $\pm$ 0.01
	P§	<0.001	<0.001	<0.001
P. W.	Base line	0.41 $\pm$ 0.02*	0.31 $\pm$ 0.04	0.28 $\pm$ 0.01
	Peak	0.71 $\pm$ 0.03*	0.43 $\pm$ 0.08	0.54 $\pm$ 0.04
	Reduced base line	0.31 $\pm$ 0.02*	0.28 $\pm$ 0.02	0.23 $\pm$ 0.01
	P§	<0.001	<0.001	<0.001
Control				
S. L.	Base line 1	0.34 $\pm$ 0.01*	0.24 $\pm$ 0.03	0.28 $\pm$ 0.02
	Base line 2	0.35 $\pm$ 0.02*	0.22 $\pm$ 0.01	0.29 $\pm$ 0.01
	P§	NS¶	NS	NS
L. M.	Base line 1	0.40 $\pm$ 0.02*	0.25 $\pm$ 0.02	0.27 $\pm$ 0.03
	Base line 2	0.34 $\pm$ 0.03	0.24 $\pm$ 0.03	0.25 $\pm$ 0.03
	P§	NS	NS	NS

Values represent the mean  $\pm$ SD of all determinations of adipose cell size in each individual at each subcutaneous site during each study period. TG = triglyceride. Significance levels were determined from F ratios calculated by two-way analysis of variance.

\*  $P < 0.05$  gluteal cell size vs. abdomen cell size within a study period.

‡  $P < 0.05$  abdomen cell size vs. triceps cell size within a study period.

§  $P$  for comparisons of adipose cell size between base line and peak, between peak and reduced base line, and between base line 1 and base line 2.

||  $P < 0.05$  triceps cell size vs. gluteal cell size within a study period.

¶ NS = nonsignificant ( $P > 0.05$ ).

TABLE IV  
Group Adipose Cell Size ( $\mu\text{g TG/cell}$ )

Group (n)	Study period	Gluteal*	Abdomen*	Triceps*
Experimental (5)	Base line	0.50 $\pm$ 0.12	0.41 $\pm$ 0.11	0.43 $\pm$ 0.16
	Peak	0.87 $\pm$ 0.14	0.71 $\pm$ 0.18	0.76 $\pm$ 0.23
	Reduced base line	0.43 $\pm$ 0.09	0.38 $\pm$ 0.04	0.37 $\pm$ 0.13
	<i>P</i> †	<0.001	<0.001	<0.001
Group (n)	Study period	Gluteal§	Abdomen§	Triceps§
Control (2)	Base line 1	0.37 $\pm$ 0.03	0.22 $\pm$ 0.08	0.27 $\pm$ 0.03
	Base line 2	0.34 $\pm$ 0.02	0.23 $\pm$ 0.03	0.27 $\pm$ 0.03
	<i>P</i> †	NS	NS	NS

Values represent the mean  $\pm$ SD of all determinations of adipose cell size for each subcutaneous site during that study period in (n) individuals. TG = triglyceride. Significance levels were determined from F ratios calculated by two-way analysis of variance.

\* No significant differences ( $P > 0.05$ ) from site-to-site in each study period: gluteal vs. abdomen, abdomen vs. triceps, triceps vs. gluteal, in experimental subjects.

† *P* for comparisons of adipose cell size between peak values with base line or reduced base line values in experimental subjects, and between base line 1 and base line 2 in control subjects. No difference in adipose cell size between base line and reduced base line is present. (NS =  $P > 0.05$ .)

§  $P < 0.05$  for comparison of adipose cell size between gluteal and abdomen, and gluteal and triceps during base line 1 and base line 2 in control subjects. No significant difference between abdomen and triceps during either base line period is present.

high caloric intake resulted in a mean gain of 10.4 kg of fat. The per cent of body weight which was fat increased by 41% in the group, with an individual rise of from 28 to 60%. Weight loss was accompanied by an 11.2 kg reduction in body fat in the group, restoring body fat content and its per cent of body weight to previous base line levels. There was no change in the two control subjects.

### Adipose cell size

*Variability.* Samples of adipose tissue were obtained from three subcutaneous sites (gluteal, anterior abdominal wall, and triceps) and comparisons of mean cell size were made within and between subjects. The results of an analysis of the sources of variance (10) in these determinations of adipose cell size indicate: (a) Replicate variability within subjects was small (same site, within subjects triplicates or duplicates) and in every case is less than the variance between subjects at each site (same site, between subjects); (b) The major source of intraindividual variance is week-to-week differences, which however were quite small; (c) The variability of adipose cell size from one subcutaneous site to another within individuals (site-to-site, gluteal vs. abdomen vs. triceps) was greater than same site variability within individuals (same site, within subject replicates) and same site variability between subjects (gluteal vs. gluteal, etc.); (d) In some instances the variability in cell size from site-to-site within individuals was greater than

site-to-site variance between subjects. These observations of adipose cell size variability were true for each study period, although variance increased at peak weight and returned to base line levels after reduction.

*Mean adipose cell size.* Table III summarizes the data on mean adipose cell size of each experimental and control subject during each study period. Within each individual there were significant differences ( $P < 0.05$ ) in cell size when adipose cells obtained from three subcutaneous sites were compared. In some subjects all three sites were different, while in others only two sites differed. At each subcutaneous site the degree of change in adipose cell size resulting from weight gain varies from subject to subject and, therefore, site-to-site differences within subjects were altered accordingly. In general, site-to-site differences which existed at base line weight were restored after weight reduction. Significant differences ( $P < 0.05$ ) in adipose cell size were also found when cells from one subcutaneous site or from different sites were compared between some, but not all individuals. As seen in Table IV, however, when adipose cell size at each subcutaneous site is averaged for the experimental group there are no site-to-site differences in any study period. When individual data for the two control subjects are averaged these site-to-site differences do not completely disappear.

*Effect of weight gain and weight loss upon adipose cell size.* In every individual, weight gain and expansion of the adipose depot was accompanied by a signifi-

cant increase in the size of the fat cells at each site tested (Table III,  $P < 0.01$ ). As a group the increase in mean adipose cell size was relatively uniform for all sites (Table IV, 74–77%); however, as shown in Table III, individually there was considerable variation from site to site (41–144%). Weight loss in every case, was associated with a significant reduction of fat cells size ( $P < 0.01$ ); again, as a group, relatively uniform for each site tested (Table IV, 87–107%), but, individually variable from site to site (Table III, 55–182%). Although as a group adipose cell size at each subcutaneous site was smaller after weight reduction compared to the original base line, the differences were not statistically significant. This was due to individual and site-to-site variability: in some subjects cell size at one or more sites was smaller after weight loss (N. H., B. H., P. W.), in one cell size was larger (A. C.), and in many sites there was no change.

The two control subjects in whom body weight remained constant had no change in adipose cell size at any of the three sites.

### Adipose cell number

*Variability.* Since adipose cell number is calculated by dividing the total body fat by the average fat cell content this value will vary depending upon whether the smallest or largest cell sizes are used in the calculation. The total number of adipose cells estimated in the body of each individual is shown in Table V, as the range of estimates of adipose cell number (calculated from the smallest and largest cell sizes) and as the mean cell number calculated from the mean of the three sites. Marked differences in total adipose cell number within and between patients can be seen by examining the ranges. At normal base line body weight the estimate of total adipose cell number in the experimental group ranged from  $21.2 \times 10^9$  to  $43.0 \times 10^9$ , and in the control group from  $22.9 \times 10^9$  to  $41.6 \times 10^9$ . The widest range estimated within an individual was noted in control subject L. M. ( $26.3 \times 10^9$  to  $41.16 \times 10^9$ ). At peak body weight the estimates of the total number of adipose cells in the experimental group ranged from  $19.2 \times 10^9$  to  $42.6 \times 10^9$  cells, and this was similar to that at base line weight. The widest range estimated was noted in subject R. P. ( $29.5 \times 10^9$  to  $42.6 \times 10^9$ ). Upon reduction to previous base line weight, estimates of adipose cell number ranged from  $22.4 \times 10^9$  to  $45.9 \times 10^9$  with the widest range noted in patient P. W. ( $22.4 \times 10^9$  to  $37.3 \times 10^9$ ). The estimated total adipose cell number at this time in the control group was similar ranging from  $19.4 \times 10^9$  to  $40.9 \times 10^9$ . The interindividual differences largely disappear when total adipose cell number is calculated as the mean of the three sites, except that experimental subject P. W. had fewer cells.

TABLE V  
Total Adipose Cell Number ( $\times 10^9$ )—Individual Subjects

Subject	Study period	Range	Mean $\pm$ SEM	P*
Experimental				
A. C.	Base line	28.9–42.3†	33.3 $\pm$ 4.5	NS
	Peak	25.3–33.0†	29.2 $\pm$ 2.2	NS
	Reduced base line	29.2–31.0	30.2 $\pm$ 0.5	NS
N. H.	Base line	30.9–37.4†	34.1 $\pm$ 1.9	NS
	Peak	34.0–35.1	34.6 $\pm$ 0.5	NS
	Reduced base line	32.5–44.4†	38.6 $\pm$ 5.8	NS
B. H.	Base line	30.5–36.5†	33.3 $\pm$ 2.8	NS
	Peak	31.6–38.9†	34.2 $\pm$ 2.4	NS
	Reduced base line	34.2–45.9†	39.7 $\pm$ 4.4	NS
R. P.	Base line	29.1–43.0†	35.3 $\pm$ 4.1	NS
	Peak	29.5–42.6†	35.0 $\pm$ 3.9	NS
	Reduced base line	25.1–39.7†	33.6 $\pm$ 4.4	NS
P. W.	Base line	21.2–30.6†	25.1 $\pm$ 2.8	NS
	Peak	19.2–31.6†	25.4 $\pm$ 3.6	NS
	Reduced base line	22.4–37.3†	29.8 $\pm$ 4.4	NS
Control				
S. L.	Base line 1	22.9–32.2†	27.7 $\pm$ 3.8	NS
	Base line 2	19.4–31.2†	30.2 $\pm$ 2.7	NS
L. M.	Base line 1	26.3–41.6†	35.6 $\pm$ 4.1	NS
	Base line 2	29.7–40.9†	36.9 $\pm$ 3.4	NS

Values represent the range and mean  $\pm$  SEM of total adipose cell number for each individual as calculated from fat cell size from the three subcutaneous sites during that study period. Significance levels were determined from F ratios calculated from two-way analysis of variance.

\*  $P$  for base line vs. peak, peak vs. reduced base line, reduced base line vs. base line, base line 1 vs. base line for controls. (NS =  $P > 0.05$ .)

† Significant differences ( $P < 0.05$ ) in estimated adipose cell number within individuals when cell number was calculated from the smallest and largest cells from the three subcutaneous sites.

*Effect of weight gain and loss upon total adipose cell number.* The estimated total number of adipose cells did not change in any subject as the result of weight gain ( $P > 0.05$ ). This was true for both the range (Table V, base line vs. peak, each individual) and the mean (Table V, base line vs. peak, each individual). The same was observed when total adipose cell number was considered as a group (Table VI). There were no differences ( $P > 0.05$ ) in total adipose cell number between the control and the experimental groups (Table VI).

### DISCUSSION

The development of a relatively simple and reliable technique for sizing and counting fat cells in small fragments of adipose tissue in man and experimental animals has made possible a detailed study of the cellular character of this tissue in normal and abnormal conditions (1–3, 6, 11, 12). The study reported here has made use of these techniques to examine the adipose tissue of individuals who have been made obese as adults by experimental means.

These studies indicate that induction of mild degree of obesity and expansion of the adipose depot in adult

TABLE VI  
Group Total Adipose Cell Number ( $\times 10^6$ )

Group (n)	Study period	Range	Mean $\pm$ SEM	P*
Experimental (5)	Base line	21.2-43.0†	32.2 $\pm$ 1.6	NS
	Peak	19.2-42.6†	31.7 $\pm$ 1.5	NS
	Reduced base line	22.4-45.9	34.9 $\pm$ 1.8	NS
Control (2)	Base line 1	22.9-41.6†	31.6 $\pm$ 3.0	NS
	Base line 2	19.0-40.9†	30.7 $\pm$ 3.5	NS

Values represent the range and the mean  $\pm$  SEM of total adipose cell number for all subjects during each study period as calculated from fat cell size from the three subcutaneous sites during that period. Significance levels were determined from F ratios calculated from two-way analysis of variance. \* P for base line vs. peak, peak vs. reduced base line, reduced base line vs. base line, and base line 1 vs. base line 2. (NS =  $P > 0.05$ .) † Significant difference ( $P < 0.05$ ) in estimated adipose cell number within individuals when cell number was calculated from the smallest and largest cells from the three subcutaneous sites.

humans by prolonged excessive caloric intake is accomplished by hypertrophy of existing fat cells, and is not accompanied by a detectable change in the total number of fat cells. Moreover, loss of weight and reduction in adipose tissue mass in these same individuals was accomplished without a change in cell number, but solely by a reduction in fat cell size. The current findings in adult humans support those previously made in experimental animals in which weight gain or loss in the adult animal was shown to be associated with changes in adipose cell size only (11). These observations in experimentally induced obesity lend further credence to the concept that adipose cell number may be altered only early in life (1, 12), and provide additional evidence for believing that human obesity may be categorized according to the cellular pattern of the adipose depot: early onset obesity characterized by a hypercellular adipose mass and adult onset a normocellular, hypertrophic tissue.

These conclusions should, however, be drawn with great care, as the following considerations suggest. The changes in the current study were experimentally and acutely induced, and the degree of obesity which resulted was relatively mild. In spontaneous, lifelong human obesity the abnormality is a more chronic one and is usually more severe. It is possible that years of excessive caloric intake in adult man leading to severe expansion of the adipose depot could lead to changes in cell number. Bray and Gallagher have reported a marked increase in adipose cell number in an individual who became obese as an adult as a result of a hypothalamic tumore (13). Although Hirsch and Knittle report that increased cell number is characteristic of the adipose depots of patients with early onset obesity, there is some increase in cell number in individuals whose obesity began after age 20 (1). Thus, the question of when and, if cell number becomes fixed remains unsettled. Additionally, the current data indicate that there may be sig-

nificant differences in the size of adipose cells from one subcutaneous site to another in nonobese individuals. In this respect, the data differ from those of Hirsch and Knittle but are consistent with the recent observations of Goldrick and McLaughlin (14). The significance of these findings lies in the fact that total adipose cell number of the body is calculated on the basis of mean fat cell size. Thus, until the size of adipose cells in all major fat depots of the body as well as the relative contribution of each depot to the total adipose tissue mass can be defined, conclusions about differences in total adipose cell number between individuals when calculated on the basis of one, two, or even three subcutaneous sites should be drawn with caution. This is particularly true in view of the observation that in some individuals omental and mesenteric fat cell size may differ considerably from those in the subcutaneous depots.<sup>1</sup> The possibility that intra-abdominal and subcutaneous fat depots are influenced differently in obesity (spontaneous or experimentally induced) has not been excluded by these studies.

An additional factor must be considered in interpreting the present data as well as those of Hirsch: the sensitivity of the method used for cell counting to detect very small fat cells. It should be noted that according to Hirsch and Knittle (1), in a few obese subjects who had undergone extreme degrees of weight loss there was an apparent reduction in total adipose cell number. The authors believe this apparent reduction in cell number to be artifactual since the method used for cell counting depends on the lipid content per cell. Thus, cells containing less than 0.01  $\mu$ g of lipid might not be counted, leading to an erroneous overestimation of mean cell size and underestimation of total cell number. The apparent reduction in cell number was found only in those individuals with extremely small cells. Similarly, it is possible that precursors of adipose cells containing little lipid may exist in the adipose depot and thus not be measured by the present techniques (15). If forced feeding induced the formation of substantial numbers of these precursor cells, an increase in cell number would go undetected. Thus, although the current data taken together with those of Hirsch and coworkers seem to support the concept of a fixed number of adipose cells determined early in life and the categorization of obesity into two cellular types, for the above reasons some modification may be necessary.

In spite of site-to-site variability in adipose cell size the current data indicate that in these individuals the lipid content per cell increased relatively uniformly over the three sites examined. This is contrary to the gross impression of earlier studies in experimentally induced

<sup>1</sup> Salans, L. B., and R. Weismann. Adipose cell size and number in obese patients undergoing jejunoileal bypass. Manuscript in preparation.

human obesity in which it appeared that the excess subcutaneous fat was preferentially deposited in central rather than peripheral depots (4). Such differences may reflect differences in the total number of fat cells in a given subcutaneous depot.

The data in the current study do not indicate that those subjects who were fatter initially and who gained more weight (A. C., N. H., B. H.) had either more cells or a tendency towards a change in cell number when compared to their leaner colleagues (R. P., P. W., S. L., L. M.).

The mean values for adipose cell size in the seven patients of this study are below those reported by Hirsch and Knittle using the same technique (1). One possible explanation may lie in differences in body weight between the two groups: individuals in the current study weighing less. Hirsch and Knittle do not provide information on the body weights of their nonobese group. Other differences in technical procedures between the two laboratories may play a role in these differences.

The present studies indicate that experimentally induced obesity in adult humans is achieved primarily by an increase in adipose cell size without a change in adipose cell number. It is well recognized that weight gain and increased adiposity under these conditions are associated with the development of abnormalities of carbohydrate and lipid metabolism. The mechanism(s) by which this occurs is unknown. Studies currently in progress in these laboratories are examining the role of factors such as dietary intake and physical activity as well as adipose cell size and insulin sensitivity in the development of these metabolic abnormalities of obesity (3, 16).

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