Effect of Weight Reduction on in Vitro Adipose Tissue Lipolysis and Cellularity in Obese Adolescents and Adults

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SUMMARY

Adipose tissue cellularity, in vitro glycerol release, and in vivo glucose metabolism were examined in ten markedly obese subjects, ages thirteen to fifty-four, before and after weight reduction.

Prior to reduction adipose cells were larger and exhibited higher rates of lipolysis than smaller postreduction cells. Incubations containing epinephrine, epinephrine plus insulin and insulin alone also revealed significant decreases in absolute rates of glycerol release in the postreduction state. However, when the data were expressed as per cent change, in vitro hormonal activity did not appear to be influenced by changes in cell size. Weight reduction was also accompanied by improvement in oral glucose tolerance tests, lower fasting levels of plasma free fatty acids and decreased values of circulating immunoreactive insulin, fasting and following oral glucose. Studies of adipose cell number revealed that obese adolescents display hypercellularity comparable to that observed in obese adults. As in the adult, weight loss resulted in improved glucose tolerance and a decrease in adipose cell size without a significant change in total adipose cell number.

Thus decreased rates of lipolysis of smaller adipocytes were associated with the improved lipid and glucose metabolism found in obese subjects after weight reduction. DIABETES *21*:754-61, June, 1972.

In the adult, obesity is characterized by hypercellularity of the adipose depot, and to a lesser degree by an increase in cell lipid content (cell size).[1] In addition abnormalities in glucose metabolism and hyperinsulinemia are commonly associated findings.[2-6] Weight loss results in improved glucose tolerance and is achieved almost exclusively by a decrease in adipose cell size while cell number remains unchanged.[1,7] Although abnormalities in lipid metabolism have also been found in obese patients,[8-10] the effect of weight loss and decreased adipose cell size on in vitro adipose cell lipolysis has not been reported. Furthermore, previous studies of the effect of weight loss on adipose cell size and number have been limited to adult subjects.

In the present investigation ten obese subjects (five adults and five adolescents) were examined before and after weight reduction. Body composition, adipose tissue cellularity and in vitro glycerol release were determined. The in vitro studies included the evaluation of the action of epinephrine, epinephrine plus insulin and insulin alone on rates of lipolysis pre- and postreduction. The effect of weight loss on plasma free fatty acids, immunoreactive insulin and oral glucose tolerance was also determined.

MATERIALS AND METHODS

Subjects

Ten markedly obese patients ages thirteen to fifty-four were hospitalized for an average of eight to twelve months on a metabolic ward. Pertinent clinical data are given in table 1. All patients were placed on a liquid formula diet consisting of 15 per cent of calories as protein, 45 per cent as carbohydrate and 40 per cent as fat, with daily supplements of iodized salt, iron and vitamins during each study period. During an initial four- to six-week period of hospitalization sufficient calories were provided to maintain constant body weight (Period I). This was followed by a six- to twelve-month period of caloric restriction of 600/day. During this time, the protein content of the formula was increased to 20 per cent and the carbohydrate reduced to 40 per cent. After weight reduction another four- to six-week

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TABLE 1

Total body weight, total fat, adipose cell size and number in obese patients before (Period I) and after (Period II) weight reduction

Patient	Age	Sex	Height	Total weight		Total fat*		Cell size		Cell number	
				I	II	I	II	I	II	I	II
	(yr.)		(cm.)	(kg.)		(kg.)		(μg. lipid/cell)		(x 10^9)	
C.J.	13	F	169	131	121	66	58	0.6737	0.6025	97.9	96.2
B.R.	14	M	168	137	102	55	45	0.6260	0.4670	81.6	96.3
H.P.	15	F	165	121	106	49	34	0.5721	0.4191	85.6	81.1
O.S.	16	F	172	174	83	86	32	0.6600	0.2524	130.0	126.0
C.V.	17	F	180	134	115	48	42	0.6746	0.5207	71.1	80.6
G.R.	22	F	162	148	110	72	47	0.8619	0.6640	83.5	70.7
S.R.	25	M	194	169	130	71	42	0.7218	0.5083	98.3	82.6
U.L.	35	F	169	170	79	77	16	0.8730	0.1778	88.2	89.9
G.L.	50	M	177	194	133	104	54	0.9148	0.4915	113.0	109.8
G.D.	54	F	157	112	81	56	36	0.8158	0.5224	68.6	68.0

*Calculated from total body potassium in adolescents and tritiated water space in adults.

Liquid formula diets were fed to obese subjects as described in the text. In both periods sufficient calories were provided to maintain constant body weight.

period followed during which sufficient calories were provided to maintain a constant weight at the new lower level (Period II) and studies were then repeated. All studies were performed after an overnight fast during both Periods I and II.

Adipose tissue sampling

Adipose tissue samples were obtained in all patients from the subcutaneous tissue of the buttocks by the method of needle aspiration.[11] No studies were performed during Period II until one week of maintenance diet had been instituted. Sufficient quantities of tissue were obtained for both metabolic studies and determination of the number and size of adipose cells. The tissue fragments were immediately placed in Krebs-Ringer bicarbonate buffer kept at 37° C. under 95 per cent O_2:5 per cent CO_2 in a thermos flask and subsequently washed with large amounts of warm buffer to remove adherent oil droplets and blood prior to cell sizing and incubations.

Determination of adipose cell size and number

Fragments of adipose tissue were processed according to the method described by Hirsch and Gallian.[12] With this method the tissue is placed in a flask containing 25 ml. of a 2 per cent solution of osmium tetroxide in collidine buffer and incubated at 37° C. for forty-eight hours. Individual intact osmium-fixed cells separate from the tissue matrix during this time interval and are separated from supporting tissue and debris by washing with saline through a 250 μ Nitex filter.* The free cells are collected on a 25 μ sieve and then suspended in a 400 ml. beaker containing a known amount of saline. The cells are then counted in a Coulter counter and the total number of cells in the sample determined.

Another sample of tissue was placed on a tared filter and its wet weight determined. The filter and sample were then placed in 30 ml. of a chloroform:methanol (2:1) solution and kept overnight. Ten milliliters of water were added and a two-phase system obtained. The upper phase was removed and anhydrous Na_2SO_4 was added to the remaining chloroform phase. Aliquots were then taken for determination of carboxyl ester bonds.[13] Cell size (average lipid content per cell) was calculated by the following equation:

$$\text{Lipid content/cell} = \frac{\text{per cent of lipid} \times \text{wet weight of sample}}{\text{total number of cells in sample.}}$$

Using the average content of lipid per cell, it is possible to make an estimate of the total adipose cell number in an individual. This is done by dividing the average cell lipid content into the total amount of lipid present in all adipose tissues.

Total adipose lipid was derived from a measurement of tritiated body water in adults. Approximately 100 μc. of tritiated water were injected intravenously into each subject. After a minimum of four hours for equilibration of the labeled water a sample of plasma was obtained and assayed for isotope by lipid scintillation counting with an internal standard to correct for quenching. All of the injected label was assumed to be diluted into a single water space and the calculated volume of this space is considered to be total body water. Lean body mass (LBM) was then calculated as total body water

*Cut from Nitex nylon screen, mesh size 202 μ, obtained from Tobler, Ernst and Traber, Inc., N. Y.

divided by 0.74.[14] The difference between body weight and lean body mass was considered to be equal to the total mass of adipose tissue lipid. Tritiated water was not administered to the adolescents. LBM was calculated by measurement of total body potassium in a total body counter.[15,16]

Incubation of tissue

Individual fragments of adipose tissue were placed in 25 ml. siliconized Erlenmeyer flasks containing 2 ml. Krebs-Ringer bicarbonate buffer, pH 7.4, at 37° C. Glucose (0.5 mg./ml.) and defatted fat free albumin* (50 mg./ml.) were also added.[17] Three additional flasks were prepared with added epinephrine† (3 μg./ml.) and epinephrine plus insulin‡ (3 μg./ml. and 1,000 μU./ml. respectively) and insulin (1,000 μU./ml.) alone. Blanks containing buffer alone, added epinephrine and epinephrine plus insulin and insulin alone were also prepared without the addition of tissue.

Each flask was capped with a rubber stopper and the tissue and/or buffer equilibrated with 95 per cent O_2:5 per cent CO_2 for five minutes. Incubations were continued for four hours at 37° C. with shaking at 72 cycles/min.

At the end of the incubation period adipose tissue was collected on a Nitex filter and thoroughly washed with saline and transferred to 20 ml. of isopropanol:heptane: 1 N sulfuric acid (4:1:0.1) and total lipid extracted overnight.[18] After the addition of water and heptane, aliquots were taken from the upper heptane phase for lipid determination by measurement of carboxyl esters. Incubation media were collected from the experimental and blank flasks and glycerol was determined enzymatically by a modification of the method of Wieland.[19] The rate of glycerol release is expressed as μmoles glycerol x 10^{-6}/cell/4 hr. and per cent change was calculated as follows:

1) $\frac{\text{epinephrine—basal}}{\text{basal}} \times 100 =$ per cent increase epinephrine

2) $\frac{\text{epinephrine—(epinephrine + insulin)}}{\text{epinephrine}} \times 100 =$ per cent decrease epinephrine + insulin

3) $\frac{\text{basal—insulin}}{\text{basal}} \times 100 =$ per cent decrease insulin.

*Armour Pharmaceutical Co., Chicago, Ill.

†Parke-Davis & Co., Detroit, Mich.

‡Glucagon-free beef zinc insulin, courtesy of Dr. W. R. Kirtley, Eli Lilly Laboratories, Indianapolis, Ind.

Thus "per cent increase epinephrine" refers to the increase of glycerol release over basal values produced by epinephrine alone, "per cent decrease epinephrine + insulin" to the effect of epinephrine plus insulin compared to epinephrine alone, and "per cent decrease insulin" to the effect of insulin on basal lipolysis.

Blood glucose and immunoreactive insulin determinations

Oral glucose tolerance tests were performed before and after weight reduction. All subjects were tested with 100-gm. doses of glucose during each period. Blood glucose was determined by the glucose oxidase method.

Plasma immunoreactive insulin was measured by a modification of the method of Herbert et al.[20] Insulin values are the means of triplicate analyses. Measurements were made during periods of weight maintenance before and after weight reduction, after a twelve-hour fast and after the ingestion of glucose.

RESULTS

A. *Determination of adipose cell size and number*

Pertinent clinical data for each subject before (Period I) and after (Period II) weight reduction are given in table 1. Ages ranged from thirteen to seventeen for adolescent subjects and twenty-two to fifty-four for adults. Prior to reduction total body fat in all subjects comprised 40 per cent or more of total body weight as measured by tritiated water space in the adults and total body potassium in the children. The net weight loss ranged from 10 to 91 kg. and was accompanied by a significant reduction in adipose cell lipid content. The values for cell size in Period I ranged from 0.5721 to 0.9148 μg. of lipid per cell with a mean and SEM of 0.7393 ± 0.0360 compared to a range of 0.1778 to 0.6640 with a mean and SEM of 0.4625 ± 0.0458 for Period II. The differences in cell size in Periods I and II were statistically significant, $P < 0.01$ by the paired "t" test. Prior to weight reduction values for adipose cell number ranged from 71.1 to 130 x 10^9 cells in the adolescent group and 68.6 to 113.0 x 10^9 for the adults. However, no significant change was noted for either group after weight reduction. Thus weight loss was accomplished almost exclusively by a decrease in cell lipid content in all subjects studied.

B. *Oral glucose tolerance tests and immunoreactive insulin*

Mean blood glucose levels during oral glucose tolerance tests are shown in figure 1. The fasting levels and those at one-half, one and two hr. were significantly higher prior to weight loss ($P < 0.05$). After reduction the glucose tolerance curves were flatter, and peak values

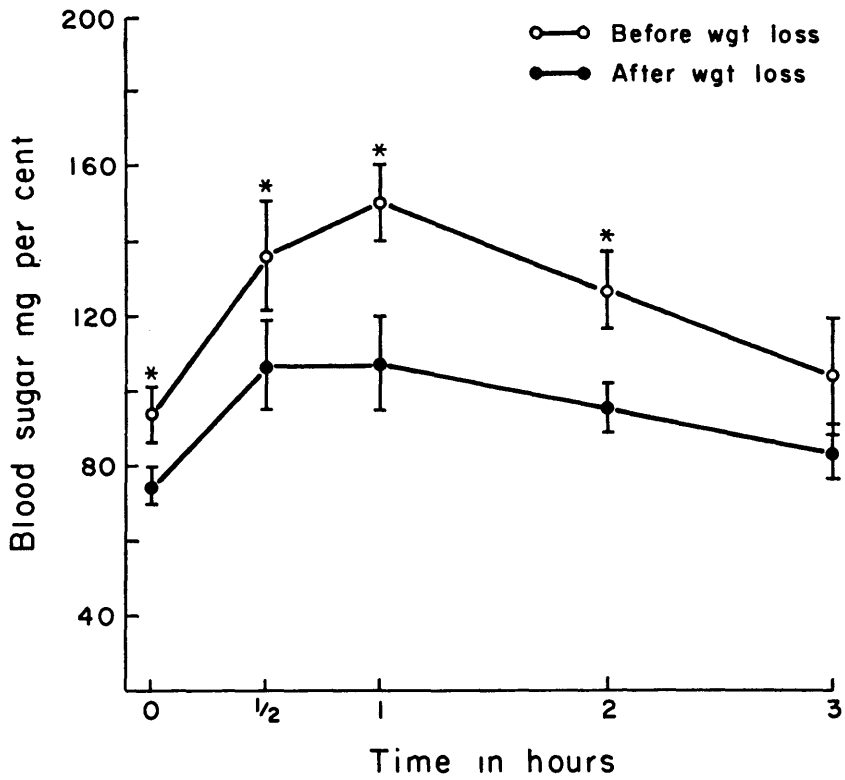

FIGURE 1

Plasma glucose responses after 100 gm. of oral glucose. Values represent means and SEM. (*) indicates significant differences between treatment periods as determined by paired "t" test ($P < 0.05$).

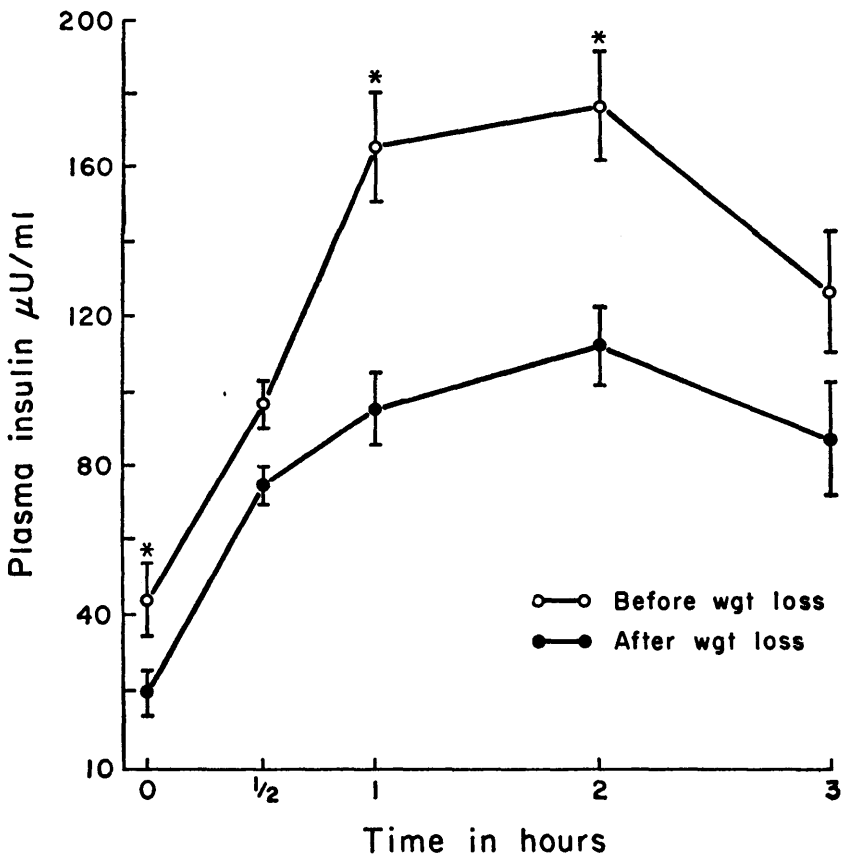

FIGURE 2

Plasma insulin responses for 100 gm. of oral glucose. Values represent the mean and SEM of triplicate determinations. (*) indicates significant differences between treatment periods as determined by paired "t" test ($P < 0.05$).

TABLE 2

In vitro glycerol release and fasting FFA in obese subjects pre- and postreduction

	Cell size	Basal release	Epinephrine (3 μg./ml.)	Epinephrine (3 μg./ml.) + insulin (1,000 μU./ml.)	Insulin (1,000 μU./ml.)	FFA
	(μg. lipid/cell)		μM glycerol x 10^{-6}/cell/4 hr.			(μEq./L.)
Prereduction	0.7393 ± 0.0360 (10)*	3.49 ± 0.35 (10)	4.53 ± 0.36 (10)	2.35 ± 0.25 (10)	2.05 ± 0.28 (10)	592 ± 20 (5)
Postreduction	0.4625 ± 0.0458 (10)	1.95 ± 0.47 (10)	2.91 ± 0.26 (10)	1.50 ± 0.36 (10)	1.33 ± 0.22 (10)	436 ± 38 (5)
P values Post vs. Pre	< 0.01	< 0.01	< 0.05	< 0.05	< 0.05	< 0.05)

* ( ) = number of subjects.
All values represent the mean and SEM of duplicate determinations for each subject. Significance levels calculated by paired "t" test.

were achieved at an earlier time than that prior to reduction (one-half hr. vs. 1 hr.). Fasting plasma insulin values and those obtained one and two hours after a glucose load were also significantly lower after weight loss ($P < 0.05$) (figure 2). As with glucose the curves were flatter after reduction. However, peak values were reached at two hours during both study periods. Thus after weight loss, less insulin was secreted during the three-hour test and levels of glucose were lower, indicating more efficient utilization.

C. *In vitro glycerol release and serum free fatty acids*

The mean values for adipose cell size, in vitro glycerol release, and serum free fatty acids are shown in table 2. Values for basal glycerol release were significantly higher prior to reduction: 3.49 vs. 1.95 μmoles x 10^{-6}/cell/4 hr. ($P < 0.01$). This was also true for the rates of in vitro release during incubations containing epinephrine and insulin. The absolute value of glycerol release with added epinephrine (3 μg./ml.) during Period I was 4.35 compared to 2.91 μmoles x 10^{-6}/cell/4 hr. in Period II ($P < 0.05$). When insulin (1,000 μU./ml.) was added to the epinephrine the inhibitory effect was quite striking and values before reduction were 2.35 compared to 1.50 after ($P < 0.05$). These values were similar to those for insulin alone 2.05 vs. 1.33 ($P < 0.05$; Period I vs. Period II) indicating a profound effect of insulin on glycerol release both prior to and after weight reduction. Indeed, rates of release during incubation in which insulin was present with or without epinephrine were lower than basal rates. Thus all values for glycerol release were significantly lower after weight reduction and these changes were also reflected in levels of fasting free fatty acids shown in table 2. Only five of the ten subjects (B.R., S.R., G.L., G.D., and G.R.) were studied but the differences were statistically significant: 592 vs. 486 μEq./L. ($P < 0.05$; Period I vs. Period II).

D. *Effect of in vitro hormonal activity*

Although absolute rates of glycerol release were decreased after weight loss, when measured as per cent change no significant differences were observed. The data expressed as per cent change are shown in table 3. Prior to reduction the stimulatory effect of epinephrine compared to basal values was 30 per cent. After reduction the increase over basal activity was 49 per cent. These differences were not statistically significant by paired data analysis or unpaired analysis.

When the effect of insulin plus epinephrine was compared to epinephrine alone, the per cent decrease was 39 per cent in both periods indicating no significant difference. Insulin alone also displayed comparable per cent changes when compared to basal values during both periods, 41 vs. 32 per cent. Thus no correlation could be discerned between hormonal activity and cell size, and prior to reduction adipose cells were as responsive to hormonal action as the smaller postreduction cells even though initial lipolytic rates were different.

TABLE 3

Per cent change of in vitro glycerol release in obese subjects pre- and postreduction

	Basal vs. epinephrine $\frac{(E-B}{B}$ x 100)	Epinephrine vs. epinephrine + insulin $\frac{(E-EI}{E}$ x 100)	Basal vs. insulin $\frac{(B-I}{B}$ x 100)
Prereduction	30±5 (10)*	39±5 (10)	41±12 (10)
Postreduction	49±8 (10)	39±10 (10)	32±9 (10)
P values Pre vs. Post	NS	NS	NS

* ( ) = number of subjects
All values represent the mean and SEM of duplicate determinations for each subject. Significance levels calculated by paired "t" test.

DISCUSSION

Impaired glucose tolerance and elevated plasma insulin levels are common findings in obesity, and the improvement found in our subjects following weight reduction is consistent with previous reports.[2-6] A number of authors have speculated on the role of adipose cell size in the improved glucose metabolism following weight reduction. Salans et al.[2] have suggested that decreased responsiveness to insulin of enlarged adipocytes may play a role in decreased peripheral glucose utilization by the adipose depot prior to reduction. After weight loss, and reduction in adipose cell size, normal insulin sensitivity and glucose utilization are restored with a resultant decrease in insulin production. It should be noted, however, that the insulin resistance observed in these in vitro studies was limited to the enhancement of glucose oxidation. Lipolysis was not examined and no effect of cell size on insulin responsivity was noted when the conversion of glucose to triglyceride was measured.

In vivo studies by Sims et al.[21] and Bjorntorp et al.[22] have also shown correlations between elevated plasma insulin levels, blood sugar and increased fat cell size in normal males. More recently Kalkhoff and Ferrou have demonstrated exaggerated insulin responses in obese subjects when compared to more muscular subjects of the same weight, indicating a relationship between metabolic changes in glucose metabolism and the degree of body fat accumulation.[23] It has been argued, however, that in vivo glucose uptake by adipose tissue is relatively small and therefore could not fully explain the impairment of glucose utilization found in obesity.[22]

Randle and co-workers[24] have suggested that an accumulation of excess fatty acid could impair glucose uptake of skeletal muscle and result in a compensatory increase in insulin production. The findings of the present study are consistent with but do not bear directly on this postulate. Thus in the obese state the enlarged adipose cells by virtue of their increased lipolysis, could interfere with peripheral glucose utilization; after weight reduction the decrease in lipolysis would result in more efficient glucose utilization. This role of increased lipolytic activity of enlarged adipose cells is further supported by the findings of Goldrick and McLoughlin.[25] These authors found higher rates of in vitro basal lipolysis in subcutaneous fat cells when compared to smaller omental cells derived from the same subject. On the other hand, Bjorntorp and co-workers[22] failed to show any correlation between fat cell size and plasma glycerol levels in normal weight men, but plasma levels of glycerol and/or fatty acids may not accurately reflect tissue levels as demonstrated by Schonfeld and Kipnis.[26]

Our data for epinephrine stimulation do not agree with those reported by Goldrick and McLoughlin who found increased per cent epinephrine change in smaller adipose cells. Variations in hormone concentration and cell preparation may account for these discrepancies, but the data could reflect real differences between the cells of obese and non-obese subjects and are consistent with our previous findings in obese and non-obese children.[27] It is of further interest that Goldrick and McLoughlin found no effect of cell size on the inhibitory action of insulin compared to epinephrine-stimulated lipolysis utilizing as little as 5 μU./ml. of insulin. This is in agreement with our present findings and in sharp contrast to the reported effect of insulin on the enhancement of glucose oxidation.[2]

The reasons for these variations in insulin responsiveness on different parameters of adipose tissue metabolism are not readily apparent. However, the fact remains that in studies of human adipose tissue, decreased insulin responses in enlarged adipocytes are only found when glucose oxidation is measured. No influence of cell size on insulin sensitivity has been demonstrated for glyceride, synthesis or fatty acid release.[25] Thus at present a precise evaluation of the influence of adipose cell enlargement on in vivo metabolic function is not possible. All one can say is that increased lipid content of adipose cells is associated with a decrease in insulin enhancement of glucose oxidation and higher rates of glycerol release. The determination of the extent to which either of these factors contributes to, or is secondary to, the development of obesity and/or abnormalities in lipid or glucose metabolism must await more definitive studies. Indeed, it has been shown that many obese subjects display normal adipose cell size.[1,22]

Whatever the final determination of the metabolic role of adipose cell enlargement, the present studies clearly indicate that altered rates of in vitro basal lipolysis result after weight reduction. Since rates of lipolysis appear to be regulated by levels of 3′5′ cyclic AMP,[28] it is tempting to speculate that the differences in basal lipolysis pre- and postreduction may be due to either altered hormonal sensitivity or to varying levels of adenyl cyclase activity. These postulates are consistent with our finding of decreased absolute values for epinephrine-stimulated lipolysis which is mediated through the action of adenyl cyclase and 3′5′ cyclic AMP.

Another objective of the present study was to evaluate the cellular nature of the obese state in adolescent sub-

jects and the effect of weight loss on their adipose cell number. Previous studies of human and rat adipose tissue have indicated that in the adult, cell number is unaltered by weight reduction.[1,29] Only by early caloric restriction prior to weaning have significant changes in adipose tissue cellularity been achieved in the rat.[30] In man, the age at which an individual reaches adult cellular values is not known, nor has it been determined at what age alterations in total cell number can still be accomplished. In the present report only subjects in whom body fat comprised 40 per cent or more of total body weight were studied. Thus the results do not necessarily apply to normal adipose tissue development nor to other degrees of overweight in adolescents or adults. Indeed Forbes[31] has described two types of adolescent obesity based on measurement of total body potassium. One group displays increased lean body mass (LBM) and is taller than average while the other is of average height with normal LBM. Although the latter group was not encountered in the present study, it is important to be aware of its existence and to include measurements of body composition in all studies of obese subjects. The numerous conflicting reports of metabolic function found in the literature may be due to the lack of a precise definition of obesity. Many authors rely solely on body weight without any reference to body composition and/or adipose tissue cellularity, thus making assessment of metabolic data difficult because of the absence of comparable specific activities.

Within the limits of our definition of the obese state it is apparent that adolescents can achieve a significant degree of adipose tissue hypercellularity, comparable to that observed in obese adults. As in the obese adult, caloric restriction results in a decrease in cell size without any significant change in adipose cell number. Furthermore, the metabolic changes found in vitro and in vivo after weight loss are qualitatively similar to those found in the adults. Thus the hypercellularity encountered in the obese state can occur early in the adolescent period and does not appear to be influenced by dietary manipulation.

Whether or not these findings indicate a predisposition to a more rapid rate of adipose cell growth in obese as compared to non-obese subjects or is the result of overnutrition early in life, cannot be determined at present. However, the data do lend further support to the hypothesis that if it is to be effective, treatment of obesity by dietary intervention should be instituted early in life prior to the development of hypercellularity of adipose tissue.

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