Lipolysis and Lipogenesis from Glucose in Human Fat Cells of Different Sizes

EFFECTS OF INSULIN, EPINEPHRINE, AND THEOPHYLLINE

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ABSTRACT Lipogenesis from glucose and lipolysis in human omental and subcutaneous fat cells were studied as functions of adipose cell size and number in adult females. Since subcutaneous fat cells were larger than those prepared from the greater omentum, a comparison could be made of the metabolism of different sizes of cells within individual subjects. Rates per cell of glyceride-glycerol and glyceride–fatty acid synthesis from glucose were similar in omental and subcutaneous fat cells incubated in the presence or absence of insulin. However, subcutaneous fat cells exhibited higher rates of basal lipolysis than omental fat cells and these differences were maintained when lipolysis was stimulated with theophylline. Different rates of lipolysis were not demonstrable after incubations with epinephrine, indicating that subcutaneous fat cells were less responsive to this hormone than smaller omental fat cells. Correlation and partial correlation analysis showed that differences in basal and theophylline-stimulated lipolysis between fat cells prepared from different subjects and between omental and subcutaneous fat cells could be accounted for by differences in adipose cell volume. In subcutaneous fat cells highly significant intercorrelations were demonstrated between cell volume, basal lipolysis, and the basal conversion of glucose to glyceride-glycerol. There was no correlation between fat cell volume, age, or relative obesity and the effects of theophylline or insulin on lipolysis or lipogenesis from glucose in vitro when the data were expressed as percentage changes above basal values.

INTRODUCTION

Obese humans exhibit a number of metabolic abnormalities including high concentrations of plasma insulin (1) and elevated rates of turnover of plasma free fatty acids and triglycerides (2). Such individuals are also resistant to the effects of exogenous insulin in vivo (3) and their adipose tissue is relatively unresponsive to this hormone in vitro (4). Randle, Garland, Hales, and Newsholme (5) have proposed that the primary defect which causes insulin resistance in obesity is located in adipose tissue and muscle. This concept has gained support from the observations of Salans, Knittle, and Hirsch (4) that the ability of human adipose tissue and rat epididymal fat cells to oxidize glucose in response to insulin is an inverse function of cell lipid content. Similarly, an inverse relationship between adipose cell volume and the effects of insulin on the uptake and esterification of chylomicron triglyceride fatty acids has been demonstrated in rat epididymal fat pads (6). However, an interpretation of these findings as cause and effect is complicated by the fact that enlarged fat cells are obtained from obese humans (4) and obese rats (7, 8). It is therefore possible that relationships between the volume or lipid content of adipose cells and insulin resistance in vitro are merely coincidental and reflect an alteration in the general in vivo hormonal environment in overweight humans and rats.

A precise evaluation of the influence of adipose cell enlargement on its function requires that metabolic studies be performed on fat cells of different sizes prepared from the same fat depot. In the present investigation we have used an indirect approach to the problem, taking advantage of the fact that human subcutaneous fat cells are generally larger than those prepared from the greater omentum. It was thus possible to carry out paired comparisons in vitro of the metabolism of differently sized fat cells derived from a common in vivo internal environment. The data from different subjects were also examined to determine whether there was a correlation between the volume and metabolism of a fat

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cell independent of factors such as age and relative body weight. We reasoned that if both sets of analyses showed that adipose cell volume was the common factor in accounting for differences in cellular function within and between subjects, then this would be strong supportive evidence for an effect of cellular enlargement on metabolism.

METHODS

Source of adipose tissue. Samples of fat (8–12 g) were obtained from the subcutaneous tissues of the anterior abdominal wall and from the greater omentum of 31 subjects who were undergoing elective abdominal surgery. Operations were performed in the mornings after an overnight fast. Morphine and atropine or their derivatives were used for premedication. Anesthesia was induced with thiopentone sodium and maintained with nitrous oxide and halothane. Tissues were removed between 60 and 90 min after induction of anesthesia. On the basis of previous reports (9, 10) it has been assumed that variations in the duration and depth of anesthesia did not significantly affect the metabolism of subcutaneous and omental fat cells in vitro.

Relevant clinical data as well and individual values for adipose cell volume are shown in Table I. The subjects, most of whom were females, showed no evidence of endocrine or metabolic disease and none had experienced recent changes in body weight or an acute illness such as cholecystitis within a period of 6 wk before surgery. Their body weights

TABLE I
Clinical Data

Subject	Age	Sex	Diagnosis	Height	Weight	μg triolein per cell	
						Subcutaneous fat cells	Omental fat cells
	yr			*inches*	*lbs.*		
1	34	F	Functional uterine bleeding	60	140	0.215	0.094
2	45	F	"	64	161	0.445	0.510
3	40	F	Chronic cholecystitis and cholelithiasis	64	199	0.862	0.510
4	48	F	"	62	127	0.476	0.298
5	40	F	Uterine fibroids	65	196	0.587	0.255
6	41	F	Chronic cholecystitis and cholelithiasis	63	145	0.648	0.288
7	74	F	"	65	175	0.463	0.423
8	33	F	"	64	123	0.699	0.272
9	68	F	Colonic polyp	65	128	0.607	0.400
10	44	F	Chronic cholecystitis and cholelithiasis	65	142	0.329	0.170
11	46	F	"	62	106	0.194	0.077
12	72	F	"	60	174	0.522	0.261
13	46	F	Chronic cholecystitis	57	126	0.657	0.400
14	59	F	Chronic cholecystitis and cholelithiasis	60	137	0.624	0.573
15	44	F	Uterine fibroids	62	132	0.445	0.150
16	28	F	Focal adenomatous hyperplasia uteri	62	120	0.349	0.084
17	38	M	Chronic cholecystitis and cholelithiasis	66	175	0.583	0.436
18	60	F	Chronic duodenal ulcer	62	132	0.545	0.310
19	76	F	Chronic cholecystitis and cholelithiasis	64	148	0.299	0.196
20	56	F	"	59	121	0.459	0.287
21	58	M	"	70	147	0.442	0.445
22	33	F	Varicosities of the broad ligament	63	142	0.307	0.114
23	29	F	Chronic cholecystitis and cholelithiasis	65	134	0.305	0.153
24	43	M	"	70	187	0.424	0.346
25	51	M	"	70	178	0.561	0.353
26	26	F	"	62	132	0.579	0.317
27	65	F	Ventral hernia	64	187	0.449	—
28	33	F	Chronic cholecystitis and cholelithiasis	65	134	0.313	0.158
29	64	F	Ventral hernia	65	139	—	0.270
30	42	M	Chronic cholecystitis and cholelithiasis	66	196	0.500	0.815
31	50	F	Uterine fibroids	64	145	0.454	0.257
1–21	48*					0.498 ±0.036‡	0.285 ±0.034‡
1–31	46*					0.478 ±0.027‡	0.307 ±0.030‡

* Antilogs of the logarithmic means.
‡ Means ±SEM.

standardized for age, height, and sex (11) averaged 6.7% in excess of ideal (range −19% to +40%). Paired comparisons of adipose cell volume (expressed here as micrograms triolein per cell) carried out on 29 of the 31 subjects showed a highly significant difference ($P < 0.001$) between the volumes of subcutaneous and omental fat cells, the volume of the former being 57% in excess of the volume of omental fat cells. Metabolic studies were performed on fat cells prepared from subjects 1–21. In this subgroup the mean difference in volume between omental and subcutaneous fat cells was 75%. Methods for estimating adipose cell volume are described below. The data in Table I show a considerable range of variation in the absolute differences in volume between omental and subcutaneous fat cells. Such differences bore no clear relationship to the type of operative procedure and were independent of the volume of omental fat cells and the indices of relative obesity used in this study. However, they were positively correlated with the volume of subcutaneous fat cells ($r = 0.540$; $P < 0.01$) and inversely related to the age of the subjects ($r = -0.358$; $P < 0.05$), suggesting that absolute differences in volume between these two types of fat cell will vary according to the method of selecting subjects for study. Under the circumstances, the finding of a signifiacnt, but rather poor correlation ($r = 0.591$; $P < 0.001$) between the volumes of subcutaneous and omental fat cells was not unexpected; as shown in Fig. 1, the scatter of points was most apparent with the larger fat cells.

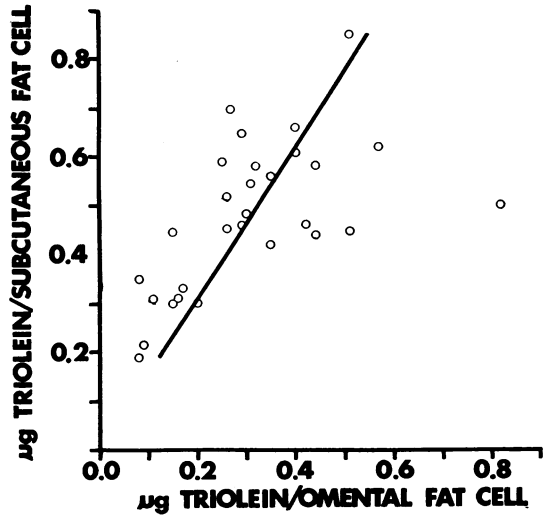

FIGURE 1 Relationship between the volumes of omental and subcutaneous fat cells ($r = 0.591$; $P < 0.001$).

General incubation conditions. Incubations were performed in siliconized flasks (12) at 37°C under an atmosphere of 95% O_2–5% CO_2 using a shaker water bath set at 60 cycles/min. Two buffer systems were employed: (*a*) Krebs-Ringer bicarbonate buffer containing half the suggested concentration of calcium ion (13) and 1 mg of glucose per ml; and (*b*) Krebs-Ringer bicarbonate buffer as above, containing 1 mg glucose and 40 mg bovine serum albumin[1] per ml and adjusted to pH 7.4 with saturated sodium bicarbonate under an atmosphere of 95% O_2–5% CO_2. The latter system will be referred to as albumin-bicarbonate buffer.

Preparation of fat cells. Immediately after excision, samples of adipose tissue were placed in warm (37°C) isotonic saline and processed within 15 min. Fat cells were prepared as described previously (12), divided into 5-ml lots, washed three times in 35-ml volumes of warm (37°C) Krebs-Ringer bicarbonate buffer, concentrated into a solid mass, and any free floating fat was aspirated from the upper layer of cells. In most preparations, the volume of free floating fat was too small to be accurately assessed. However, significant amounts of free floating fat (up to 1/15 of the volume of the final cell suspension) were produced when tissues containing large fat cells (> 0.65 μg triolein) were cut into small pieces before digestion with collagenase. Comparisons of cells dispersed from samples of tissue previously fixed in osmium tetroxide (14) with free fat cells fixed in osmium tetroxide after the digestion with collagenase of vigorously dissected samples of the tissue indicated that cellular breakdown did not selectively involve large or small fat cells.[2]

Determination of glycerol production by fat cells. Concentrated fat cells were diluted 1:1 (v/v) with albumin-bicarbonate buffer, dispensed in 0.5 ml aliquots into 7 ml of albumin bicarbonate buffer, and incubated for 2 hr. Additions to the incubation flasks were as follows: epinephrine bitartrate[3] equivalent to 0.1, 0.5, and 1.0 μg of epinephrine base per ml of incubation medium; 0.5 μg epinephrine base plus 5 μU of insulin[4] per ml of incubation medium; and theophylline[3] 9, 18, and 90 μg/ml of incubation medium (0.05, 0.1, and 0.5 mmole/liter respectively). Glycerol was estimated enzymatically (15) on deproteinized extracts of mixed cells and incubation medium. Determinations were performed in duplicate and were corrected for the glycerol contents of nonincubated mixtures of fat cells and buffer. Incubations of albumin-bicarbonate buffer with additions of epinephrine, theophylline, or insulin plus epinephrine as listed above yielded no measurable glycerol in the absence of fat cells.[2] The concentrations of epinephrine and theophylline used here were arbitrarily selected to produce submaximal rates of glycerol production. Maximal lipolytic effects were achieved with 5 μg/ml of epinephrine, in agreement with the findings of Burns and Langley (16), and with 360 μg/ml of theophylline. The concentration of insulin was chosen in a like fashion to produce submaximal inhibitory effects on lipolysis when incubated in the presence of 0.5 μg/ml of epinephrine. The production of glycerol by omental and subcutaneous fat cells was linear during 2 hr of incubation and was directly proportional to the number of fat cells in the system.[2] Examination of cell suspensions before and after incubation showed no significant change in mean cell volumes indicating that preferential loss of large or small fat cells did not occur under these conditions.[2]

Incorporation of glucose into glyceride-glycerol and glyceride-fatty acids. 0.5 ml aliquots of concentrated fat cell suspensions were dispensed into 0.5 ml Krebs-Ringer bicarbonate buffer containing 0.5 μCi glucose-U-^{14}C and incubated in triplicate flasks for 4 hr in the presence or absence of 1 mU insulin. Methods employed for extracting and saponifying adipose cell lipids and determining the in-

[1] Fraction V containing approximately 10 moles FFA per g; Armour and Company, Ltd., Eastbourne, England.

[2] Unpublished observations.

[3] British Drug Houses Ltd., Poole, England.

[4] Glucagon-free porcine insulin, Eli Lilly & Co., Indianapolis, Ind.

TABLE II

Glucose Metabolism in Omental and Subcutaneous Fat Cells

	Total glycerides		Glyceride-glycerol		Glyceride–fatty acids		Glyceride-glycerol		Glyceride–fatty acids	
	Basal	+1 mU insulin	Basal	+1 mU insulin	Basal	+1 mU insulin	Basal	+1 mU insulin	Basal	+1 mU insulin
	*μ moles glucose incorporated/10^6 cells per 4 hr**						*% total glyceride radioactivity**			
Omentum	0.152 ±0.020	0.229‡ ±0.025	0.102 ±0.010	0.140‡ ±0.014	0.050 ±0.015	0.089‡ ±0.018	72 ±4	65 ±4§	28 ±4	35 ±4§
Subcutaneous	0.147 ±0.025	0.202‡ ±0.032	0.102 ±0.130	0.131‡ ±0.017	0.045 ±0.017	0.071‡ ±0.021	77 ±5	73 ±5§	23 ±5	27 ±5§
	insulin-stimulated minus basal / *basal* × 100									
Omentum		59 ±13		39 ±6		62‖				
Subcutaneous		39 ±6		30 ±5		49‖				

* Mean values ±SEM; n = 20.

‡,§ Indicate P values <0.001 and <0.01 respectively (control vs. insulin) obtained by paired comparisons using the analysis of variance. There were no significant differences (P >0.05) when omental and subcutaneous fat cells were compared.

‖ Antilogs of the means of logarithmic transformations of the data; n = 20.

corporation of isotope[5] into glycerol and fatty acids have been described previously (12, 17). The properties of the present in vitro system have also been detailed in another report (17) wherein attention was drawn to the fact that significant rates of incorporation of glucose-U-^{14}C into glyceride-fatty acids by human fat cells required the use of concentrated cell preparations. Such preparations have been employed routinely in the present investigation. However, differences in the total numbers of fat cells added to the incubation flasks in the various comparisons were unavoidable, even though the ratio of the volume of packed cells to that of the incubation medium was constant throughout. The significance of variations in the number of fat cells per flask (range 0.5×10^6 to 4×10^6 cells) was assessed retrospectively by determining whether this variable was related to the rate of glyceride–fatty acid synthesis from glucose expressed per 10^6 cells. As no such correlation was demonstrable with either omental ($r = 0.290$) or subcutaneous ($r = 0.049$) fat cells, it seems unlikely that variations in cell number significantly affected measurements of fatty acid synthesis in these concentrated preparations.

Determination of adipose cell size and number. Aliquots of concentrated fat cell suspension were diluted on a siliconized glass slide at room temperature with Krebs-Ringer bicarbonate buffer and examined under a cover glass supported on a thick layer of silicone grease. The diameters of at least 300 cells from each sample of adipose tissue were measured at random by means of an eyepiece micrometer calibrated against a standard scale. Fat cell suspensions were maintained in Krebs-Ringer bicarbonate buffer at 37°C for up to 3 hr before examination after preliminary studies had failed to show any systematic changes in cell size during this period. Furthermore, serial observations as well as comparisons[2] with glutaraldehyde-fixed cells (14) indicated that the diameters of human fat cells were stable under the conditions employed for determining cell size.

Cell volume was calculated from the mean and the variance of the diameters (8) and was expressed as micrograms of triolein. The number of fat cells was derived from the total lipid content of the incubation mixture (18) divided by the average lipid content per cell. This calculation is based on the assumption that the external dimensions of a fat cell accurately reflect the volume of storage triglyceride. Its validity may be judged from the observations of Hirsch and Gallian (14) who showed that comparable values for adipose cell lipid are obtained from direct estimates of cell diameter as well as from the total lipid content of the sample divided by the number of fat cells. The precision of the present estimates of cell number may be gauged from the coefficients of variation[6] of duplicate determinations of cell volume (3.9%; n = 10) and total lipid content of the sample (2.5%; n = 20). All metabolic parameters measured here have been corrected for the number of fat cells in the sample and expressed per 10^6 cells.

Estimation of relative obesity. Relative obesity was derived from two parameters, weight in excess of ideal body weight at age 20–24 yr (11), and from body weight ÷ (height)^2 (W/H^2). Each of these parameters appears to be a satisfactory simple estimate of relative obesity in this population wherein variations of body weight per unit of height or bulk largely reflect variations in body fat content (19). We emphasize that in determining ideal body weight no correction has been applied for an increase in weight with age, which represents progressive deposition of fat in this community (20).

[5] The Radiochemical Centre, Amersham, England. Ascending paper chromatography of the glucose-U-^{14}C in isopropyl alcohol–n-butanol–water 70:10:20 v/v revealed a radiopurity of 97%.

[6] $\sqrt{\frac{\Sigma D^2}{2n}}$

where D = difference between duplicates × 100 ÷ mean and n = number of duplicate pairs.

Σ refers to the "sum of D^2."

Statistical methods. Comparisons were performed by the analysis of variance (21) using paired data from each subject. Complete matrices of correlation coefficient were calculated by an IBM 360/50 computer and partial correlation coefficients were calculated (21) from selected variables. The frequency distributions of the following data were skewed and so were normalized by logarithmic transformation: age, W/H^2, and all the data on glycerol production with the exception of the effects of insulin plus epinephrine and theophylline on lipolysis. Similar transformations were applied to the effects of insulin on glyceride fatty acid synthesis from glucose. When logarithmic transformations were

TABLE III

Glycerol Production by Omental and Subcutaneous Fat Cells

		+ Theophylline		
	Basal	0.05 mM	0.1 mM	0.5 mM
	*glycerol production μmoles/10^6 cells per 2 hr**			
Omentum	0.112	0.225‡	0.302§	0.785§
Subcutaneous	0.209	0.601§	0.805§	2.012§
Omentum vs. subcutaneous	*P* <0.01	<0.01	<0.01	<0.001
	Theophylline-stimulated minus basal glycerol production × 100‖			
	basal glycerol production			
Omentum		160 ±45	277 ±67	975 ±210
Subcutaneous		259 ±47	406 ±79	1038 ±135
	P	<0.05	NS	NS

* Antilogs of the means of logarithmic transformations of the data n = 20 for basal glycerol production and n = 16 for theophylline-stimulated glycerol production.
‡,§ Indicate *P* values <0.02 and <0.001 respectively. These were obtained by comparing glycerol production in the presence of theophylline with the corresponding value for basal glycerol production.
‖ Mean values ±SEM.

TABLE IV

Glycerol Production by Omental and Subcutaneous Fat Cells

		+ Epinephrine (μg/ml)			
	Basal	0.1	0.5	1.0	0.5 + insulin 5 μU/ml
	*glycerol production μmoles/10^6 cells per 2 hr**				
Omentum	0.112 (20)	0.444‡ (16)	0.911‡ (20)	1.184‡ (20)	0.661§ (17)
Subcutaneous	0.209 (20)	0.517§ (16)	0.931‡ (20)	1.413‡ (20)	0.613§ (17)
Omentum vs. subcutaneous	*P* <0.01	NS	NS	NS	NS
	Epinephrine-stimulated minus basal glycerol production × 100*				*% inhibition of epinephrine-stimulated glycerol production*‖
	basal glycerol production				
Omentum		245	684	929	26 ±5
Subcutaneous		108	327	568	35 ±5
Omentum vs. subcutaneous	*P*	NS	<0.01	<0.05	NS

* Antilogs of the means of logarithmic transformations of the data. Numbers of observations are shown in parenthesis.
‡,§ Indicate *P* values <0.001 and <0.05 respectively. These were obtained by comparing (*a*) glycerol production in the presence of epinephrine with the corresponding data for basal glycerol production, or (*b*) glycerol production in the presence of epinephrine plus insulin with the corresponding data obtained with 0.5 μg/ml epinephrine. Paired comparisons were carried out by the analysis of variance.
‖ Mean values ±SEM.

performed, the mean values shown in the tables are the antilogs of the logarithmic means. Under these circumstances no attempt has been made to indicate the degree of dispersion of the data. Small residual deviations from normality of distribution will be apparent in the text as discrepancies between the calculated values for the effects of hormones and theophylline and the absolute values for lipogenesis and lipolysis.

RESULTS

Comparisons of glucose metabolism and glycerol production in omental and subcutaneous fat cells

Glucose metabolism (*Table II*). Omental and subcutaneous fat cells incorporated glucose-U-^{14}C at virtually identical rates into total glycerides, glyceride-glycerol, and glyceride–fatty acids under basal conditions and when incubations were performed in the presence of 1 mU of insulin. There were also no significant differences between the two types of fat cell in regard to their responsiveness [7] to insulin or the distribution of glucose label between glyceride-glycerol and glyceride–fatty acids.

Glycerol production. The rate of basal lipolysis expressed in terms of 10^6 cells was almost twice as great in subcutaneous as in omental fat cells (Table III). Subcutaneous fat cells also exhibited consistently higher absolute rates of glycerol production than omental fat cells when lipolysis was stimulated submaximally by 0.05, 0.1, and 0.5 mM theophylline (Table III). The stimulation of glycerol production over basal values was greater with subcutaneous fat cells when theophylline was present at a concentration of 0.05 mmole/liter; but no significant difference in responsiveness between the two types of fat cell was observed when lipolysis was further stimulated by higher concentrations of this compound. On the other hand, in the presence of epinephrine (Table IV) there was no statistically significant difference in the absolute rates of glycerol production between subcutaneous and omental fat cells. This indicates that both types of cell were capable of approximately equal rates of lipolysis when exposed to submaximal stimulating concentrations of epinephrine. As might be expected from these findings, the relative stimulation with epinephrine was greater in omental fat cells and attained statistical significance at concentrations of 0.5 and 1.0 μg/ml. 5 uU/ml of insulin inhibited the lipolytic effects of 0.5 μg/ml of epinephrine to an equivalent extent in both types of fat cell (Table IV).

[7] Percentage increase over the rate of basal lipogenesis from glucose or the percentage increase over basal glycerol production.

TABLE V

Correlations between Glycerol Production in Subcutaneous Fat Cells and Age, Relative Obesity, and Adipose Cell Volume

					μmoles glycerol produced per 10^6 subcutaneous fat cells per 2 hr							
						+ Epinephrine					+ Theophylline	
		Excess weight	Weight / Height²	μg triolein per cell	Basal	0.1 μg/ml	0.5 μg/ml	1 μg/ml	0.5 μg/ml + insulin 5 μU/ml	0.05 mM	0.1 mM	0.5 mM
	Age	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Excess weight		0.841*	0.445‡	NS	NS	NS	NS	NS	NS	NS	NS
	Weight/height²			0.421‡	NS	NS	NS	NS	NS	NS	NS	NS
	μg triolein per cell				0.711*	0.644§	0.563§	0.555§	0.658§	0.849*	0.902*	0.835*
+ Epinephrine	Basal					0.815*	0.638§	0.521‡	0.790*	0.751*	0.671§	0.656§
+ Epinephrine	0.1 μg/ml						0.877*	0.737*	0.812*	0.851*	0.794*	0.787*
+ Epinephrine	0.5 μg/ml							0.931*	0.867*	0.789*	0.769*	0.767*
+ Epinephrine	1.0 μg/ml								0.735*	0.755*	0.742*	0.749*
+ Epinephrine	0.5 μg/ml + insulin, 5 μU/ml									0.867*	0.894*	0.853*
+ Theophylline	0.5 mM										0.966*	0.942*
+ Theophylline	0.1 mM											0.968*
+ Theophylline	0.5 mM											

μmoles glycerol produced per 10^6, subcutaneous fat cells per 2 hr

* P <0.001.
‡ P <0.02.
§ P <0.01.

TABLE VI

*Partial Correlations between Glycerol Production in Subcutaneous and Omental Fat Cells and Adipose Cell Volume**

Dependent variable, μmoles glycerol/10^6 cells per 2 hr	Variable held constant	Partial correlation coefficient, r		No. of observations	Level of significance of r	
		Subcutaneous	Omental		Subcutaneous	Omental
Basal	Excess body wt	0.719	0.502		P <0.001	P <0.02
	W/H²	0.682	0.512	20	P <0.001	P <0.02
	Age	0.763	0.627		P <0.001	P <0.01
+0.1 μg/ml epinephrine	Excess body wt	0.646	0.283		P <0.01	NS
	W/H²	0.670	0.327	16	P <0.01	NS
	Age	0.652	0.366		P <0.01	NS
+0.5 μg/ml epinephrine	Excess body wt	0.516	0.608		P <0.02	P <0.01
	W/H²	0.529	0.609	20	P <0.02	P <0.01
	Age	0.599	0.672		P <0.01	P <0.001
+1.0 μg/ml epinephrine	Excess body wt	0.510	0.636		P <0.02	P <0.01
	W/H²	0.534	0.675	20	P <0.02	P <0.001
	Age	0.598	0.699		P <0.01	P <0.001
+0.5 μg/ml epinephrine +5 μU/ml insulin	Excess body wt	0.638	0.481		P <0.01	P <0.05
	W/H²	0.648	0.487	17	P <0.01	P <0.05
	Age	0.707	0.596		P <0.001	P <0.01
+0.05 mM theophylline	Excess body wt	0.823	0.717		P <0.001	P <0.001
	W/H²	0.847	0.885	16	P <0.001	P <0.001
	Age	0.875	0.712		P <0.001	P <0.001
+0.1 mM theophylline	Excess body wt	0.884	0.485		P <0.001	P <0.05
	W/H²	0.844	0.481	16	P <0.001	P <0.05
	Age	0.928	0.577		P <0.001	P <0.02
+0.5 mM theophylline	Excess body wt	0.814	0.492		P <0.001	P <0.05
	W/H²	0.841	0.494	16	P <0.001	P <0.05
	Age	0.886	0.581		P <0.001	P <0.02

* Independent variable.

Relationships between adipose cell volume, metabolism, age, and indices of relative obesity

Interrelationships between Adipose Cell Volume, Age, and Indices of Relative Obesity (Table V)

Age bore no relationship to the indices of relative obesity or adipose cell volume. Excess weight and W/H² were strongly associated, and both parameters showed positive but relatively low order relationships with the volume of subcutaneous and omental fat cells.[8] These findings indicated that although the size of fat cells paralleled the degree of adiposity, wide variations in cell volume occurred among individuals possessing approximately equal amounts of body fat.

[8] When body weight was standardized for age as well as for height and sex there was no relationship between body weight in excess of ideal body weight and the volume of subcutaneous or omental fat cells.

Relationships between Glycerol Production, Age, Cell Volume, and Indices of Relative Obesity

Subcutaneous fat cells (Table V). The absolute rates of glycerol production under basal conditions and in the presence of epinephrine, epinephrine plus insulin, or theophylline were strongly interrelated. None of these parameters was related to variations in age, excess body weight, or W/H², nor was the responsiveness of fat cells to hormones or theophylline related to age, cell volume, or relative obesity (data not shown). On the other hand, highly significant positive correlations were found between the volume of subcutaneous fat cells and their absolute rates of glycerol production under basal conditions and when epinephrine, epinephrine plus insulin, or theophylline were present. Partial correlation analysis showed that the relationships between the volume of subcutaneous fat cells and their rates of glycerol

TABLE VII

Correlations between Glycerol Production in Omental Fat Cells and Age, Relative Obesity, and Adipose Cell Volume

					μmoles glycerol produced per 10^6 fat cells per 2 hr							
						+ Epinephrine				+ Theophylline		
		Excess weight	Weight / Height²	μg triolein per cell	Basal	0.1 μg/ml	0.5 μg/ml	1 μg/ml	0.5 μg/ml + insulin 5 μU/ml	0.05 mM	0.1 mM	0.5 mM
	Age	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Excess weight		0.841*	0.419‡	0.499‡	0.496§	0.594*	0.624*	0.469§	0.795‖	0.725‖	0.589*
	Weight/ height²			0.428‡	0.440§	NS	0.522‡	0.553*	NS	0.725‖	0.629*	0.548*
	μg triolein per cell				0.604*	NS	0.693‖	0.713‖	0.582‡	0.728‖	0.607*	0.608*
+ Epinephrine	Basal					0.509§	0.688‖	0.657‖	0.763‖	0.746‖	0.593*	NS
+ Epinephrine	0.1 μg/ml						0.798‖	0.798‖	0.743‖	0.748‖	0.771‖	0.767‖
+ Epinephrine	0.5 μg/ml							0.982‖	0.889‖	0.857‖	0.819‖	0.810‖
+ Epinephrine	1 μg/ml								0.802‖	0.861‖	0.807‖	0.802‖
+ Epinephrine	0.5 μg/ml + insulin, 5 μU/ml									0.714‖	0.776‖	0.657*
+ Theophylline	0.05 mM										0.906‖	0.833‖
+ Theophylline	0.1 mM											0.930‖
+ Theophylline	0.5 mM											

μmoles glycerol produced per 10^6, omental fat cells per 2 hr

* $P < 0.01$.
‡ $P < 0.02$.
§ $P < 0.05$.
‖ $P < 0.001$.

production were independent of variations in age, excess body weight, and W/H² (Table VI).

Omental fat cells (Table VII). Here also there were highly significant interrelationships between the absolute rates of glycerol production under the various conditions of incubation employed. The responsiveness of omental fat cells to epinephrine, epinephrine plus insulin, or theophylline was unrelated to age, relative obesity, or cell volume (data not shown); variations in age bore no relationship to the absolute rates of glycerol production. However, cell volume, excess body weight, and W/H² each exhibited highly significant positive relationships to the absolute rates of glycerol production under basal conditions and when epinephrine, epinephrine plus insulin, or theophylline were present. Partial correlation analysis showed that the relationships between adipose cell volume and rates of glycerol production were independent of variations in age, excess body weight, and W/H² (Table VI).

It was apparent from the correlation analysis that variations in the absolute rates of glycerol production in fat cells prepared from different subjects could be largely accounted for by variations in adipose cell volume. Therefore, it remained to be decided whether the differences in rates of glycerol production between subcutaneous and omental fat cells could be accounted for on the same basis. In other words it was necessary to show whether the data for subcutaneous and omental fat cells constituted a single regression line y (log glycerol production per 10^6 cells) $= a + b\,x$ (μg triolein per cell). Because the correlation analyses and paired comparisons both showed that large fat cells exhibited relatively high rates of basal and theophylline-stimulated glycerol production, attention was focused on these two parameters. With respect to basal glycerol production, the linear regression equations were almost identical in subcutaneous and omental fat cells (Fig. 2). Likewise, there were no significant differences in the regression lines for glycerol production in the presence of theophylline in subcutaneous and omental fat cells (Fig. 3), although the parallel nature of the lines was not as impressive as in Fig. 2. Thus, adipose cell volume appeared to be a common factor in accounting for differences in basal and theophylline-stimulated glycerol production between fat cells obtained from different subjects and between omental and subcutaneous fat cells.

Relationship of Glucose Metabolism to Age, Relative Obesity, Cell Volume, and Basal Glycerol Production

The volume of subcutaneous fat cells was closely related to the rate of incorporation of glucose into glyceride-glycerol under basal conditions ($r = 0.670$; $P < 0.001$) independently of variations in age or relative obesity. There was a close relationship between the

basal rates of glyceride-glycerol synthesis and glycerol production in subcutaneous fat cells ($r = 0.853$; $P < 0.001$). When these findings were considered in the light of the close correlation between cell volume and basal glycerol production (Table V) it was evident that the turnover of glyceride-glycerol was increased in enlarged subcutaneous fat cells. In omental fat cells, the basal rates of glyceride-glycerol synthesis and glycerol production were also related ($r = 0.460$; $P < 0.05$), but variations in cell volume were not associated with parallel changes in the rate of glyceride-glycerol synthesis from glucose. Aside from these findings, there was no evidence to indicate that factors such as age, relative obesity, or adipose cell volume affected the absolute rates of glucose metabolism in omental and subcutaneous fat cells. Furthermore, the capacity of insulin to stimulate the incorporation of glucose into glyceride-glycerol or glyceride–fatty acids by omental and subcutaneous fat cells was unrelated to age, relative obesity, or adipose cell volume.

DISCUSSION

The present investigation has shown the rate of basal lipolysis per cell to be almost twice as great in human subcutaneous fat cells as in omental fat cells. This difference was exaggerated in the presence of 0.05 mM theophylline and was maintained during incubations with higher submaximally stimulating concentrations of theophylline. However, subcutaneous and omental fat cells exhibited equivalent rates of glycerol production

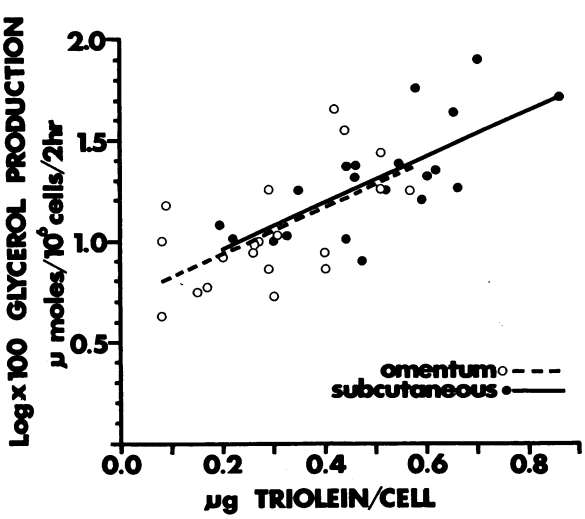

FIGURE 2 Relationship between basal glycerol production and cell volume in subcutaneous (●) and omental (○) fat cells. Regression lines y (log glycerol production) $= a + b\,x$ (μg triolein per cell) were $y = 0.71 + 1.14\,x$ for subcutaneous (——) and $y = 0.74 + 1.14\,x$ for omental (- - -) fat cells.

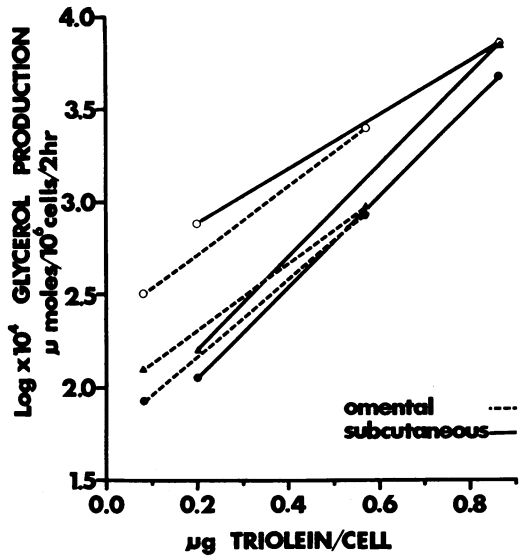

FIGURE 3 Regression lines y (log glycerol production $\times 10^4$) $= a + b\,x$ (μg triolein per cell) in the presence of 0.05 mM (●), 0.1 mM (▲), and 0.5 mM (○) theophylline for omental (- - -) and subcutaneous (——) fat cells. Regression equations for omental fat cells in the presence of 0.05, 0.1, and 0.5 mM theophylline were $y = 1.76 + 2.06\,x$, $y = 1.97 + 1.77\,x$, and $y = 2.37 + 1.84\,x$ respectively. Corresponding regression equations for subcutaneous fat cells were respectively $y = 1.56 + 2.45\,x$, $y = 1.59 + 2.64\,x$, and $y = 2.58 + 1.46\,x$.

when lipolysis was stimulated submaximally with several concentrations of epinephrine. Increases above basal values were thus significantly greater in subcutaneous fat cells at low concentrations of theophylline and were similar to those of omental fat cells at higher concentrations of this compound. On the other hand, increases above basal values were generally higher in omental fat cells during incubations with epinephrine. Both types of cell appeared to be equally responsive to the inhibitory effects of insulin on epinephrine-stimulated lipolysis. Previous comparisons of basal lipolysis in pieces of human subcutaneous and omental adipose tissue have yielded conflicting results (22, 23), presumably due to failure to correct for differences in the numbers of fat cells in the tissue preparations. However, there appears to be general agreement that human subcutaneous fat is less responsive than omental adipose tissue to the lipolytic effects of epinephrine and norepinephrine (22–24). The present findings of equivalent rates of glycerol production in subcutaneous and omental fat cells during stimulation with epinephrine suggest that the lesser responsiveness of subcutaneous fat cells to this hormone simply reflects their higher rates of basal lipolysis. The latter in turn may be due to a higher basal activity of adenyl cyclase in subcutaneous fat cells; this would be consistent with the

data obtained during incubations with theophylline or epinephrine and insulin. In this connection, it should be noted that lipolysis in human fat cells appears to be regulated by the level of cyclic 3′,5′-AMP as is the case with rat adipocytes (25). Under the circumstances it was interesting to find that the differences in rates of basal and theophylline-stimulated glycerol production observed between omental and subcutaneous fat cells could be accounted for by differences in their respective volumes. This suggests that the anatomical site of the fat depot from which the cells were prepared was not an important determinant of lipolytic activity.

The virtually identical patterns of basal and insulin-stimulated lipogenesis from glucose in omental and subcutaneous fat cells provide further evidence which indicates that the metabolism of omental adipose tissue is basically similar to that of subcutaneous fat. The discrepancy between the present observations and those of earlier comparisons which showed higher rates of glucose oxidation, esterification, and neutral lipid synthesis in omental fat cells and pieces of omental adipose tissue (12, 26–28) may well be more apparent than real. Here, we have corrected the results for differences in the numbers of subcutaneous and omental fat cells, whereas this was not the case with earlier studies. The fact that subcutaneous fat cells were generally larger than omental fat cells indicates that data expressed per unit weight of tissue or per unit weight of adipose lipid will yield a relative overestimate of the metabolic activity of omental fat. It is also likely that the extent to which the metabolism of omental adipose tissue is overestimated will vary from one study to another because the absolute differences in volume between subcutaneous and omental fat cells were not constant, but varied inversely with the age of the subject and directly with the volume of subcutaneous fat cells.

The finding of higher rates of lipolysis and synthesis of glyceride-glycerol from glucose in large fat cells was unexepected because similar observations have not been reported previously in human adipose tissue. The most consistent data were positive relationships of cell volume to the absolute rates of basal and theophylline-stimulated glycerol production. These correlations were not only similar in omental and subcutaneous fat cells, but also were of a surprisingly high order of magnitude considering the variability of biological systems. Furthermore, partial correlation analysis indicated that the associations were independent of age and relative obesity. We emphasize that the basic limitations of the present experimental model do not permit us to conclude that enlargement of a fat cell directly influences its rate of lipolysis. Nevertheless, the data clearly indicate that the rate of lipolysis is altered in enlarged fat cells. It is more difficult to evaluate the validity of the high-order positive interrelationships which were demonstrated between the volume of subcutaneous fat cells and their rates of basal lipolysis and basal incorporation of glucose into glyceride-glycerol. Whereas these findings indicate an increased turnover of glyceride-glycerol in enlarged subcutaneous fat cells, we have no explanation for the absence of a relationship between the volume of omental fat cells and their basal rates of incorporation of glucose into glyceride-glycerol. This discrepancy in our data is underscored by the inability of other investigators to demonstrate a relationship between the lipid content of cells in human subcutaneous adipose tissue and the rate of neutral lipid synthesis from glucose (4), whereas glyceride-glycerol synthesis from glucose apparently increases in parallel with enlargement of fat cells prepared from rats, guinea pigs, and hamsters (29).

A prime objective of the present investigation was to evaluate the importance of cellular enlargement on the responsiveness of human adipose tissue to insulin and lipolytic agents. Our failure to demonstrate a resistance to the effects of insulin on glucose metabolism in large fat cells is at variance with the observations of Salans, Knittle, and Hirsch (4) on glucose-1-^{14}C oxidation by aspirated fragments of human subcutaneous adipose tissue. Since the isolated fat cell preparation is at least as responsive to this hormone as are fragments of adipose tissue, one explanation for our negative findings is the rather restricted range of relative body weights and adipose cell volumes of the subjects studied here. Nevertheless, none of the investigations on human adipose tissue has confirmed the findings in rodent preparations of a diminished effect of insulin on glyceride-glycerol and glyceride–fatty acid synthesis from glucose in enlarged fat cells (29). In view of the inherently greater responsiveness of rodent adipose tissue to the stimulatory effects of insulin on glucose metabolism, the possibility remains that technical difficulties with human preparations are responsible for these apparent species differences. By comparison with studies on glucose metabolism, it is evident from this and other reports (9, 16, 25) that the effects of agents such as catecholamines, insulin, and theophylline on lipolysis are readily demonstrable in human fat cells. Hence, the observation that adipose cell volume bore no relationship to the lipolytic effects of theophylline, or the inhibition of epinephrine-stimulated lipolysis by insulin appears to be valid within the limitations of the group of subjects examined. In contrast, paired comparisons of subcutaneous and omental fat cells indicated a decreased responsiveness of enlarged fat cells to stimulation of lipolysis by epinephrine. The reason why correlation analysis failed to reveal a formal relationship between cell volume and response to epinephrine in either type of fat cell is uncertain. However, these negative findings may mean

that some critical degree of cellular enlargement is associated with a decreased response to epinephrine. Such a phenomenon would be consistent with the finding of an association between cellular lipid content and resistance to the lipolytic effects of epinephrine in the subcutaneous fat of nonobese children and the absence of this relationship in the subcutaneous adipose tissue of obese children (30).

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