Lipolytic effects on diacylglycerol accumulation in human adipose tissue in vitro

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Abstract When fragments of rat or human adipose tissue, or isolated adipocytes, are incubated with [^{14}C]glucose in vitro, [^{14}C]diacylglycerol accumulates rapidly: it comprises 20–50% of newly synthesized (^{14}C-labeled) acylglycerols, compared to less than 1% diacylglycerol accumulated in the bulk lipid store in vivo. The experiments reported in this study were performed to test the possibility that agents that influence the rate of lipolysis might differentially affect the accumulation of di- and triacylglycerol in human adipose tissue, and perhaps account for the discrepancy between the early labeling and the later accumulation of diacylglycerol. Fragments of gluteal subcutaneous adipose tissue obtained from obese men and women were incubated with isoproterenol, epinephrine plus yohimbine, adenosine deaminase, or dibutyryl 3',5'-cyclic adenosine monophosphate to stimulate lipolysis. Tissue fragments were also incubated with clonidine, adenosine, or insulin to inhibit lipolysis. No agent had any effect on the rate of accumulation of newly synthesized triacylglycerol. The effects of these agents on the rate of lipolysis were negatively correlated with their effects on accumulation of newly synthesized diacylglycerol. Newly synthesized diacylglycerol may be preferentially hydrolyzed by hormone sensitive lipase. This increased susceptibility to lipolytic stimulation, compared to newly synthesized triacylglycerol, may account for the minute accumulation of diacylglycerol in adipose tissue in vivo. —**Edens, N. K., R. L. Leibel, and J. Hirsch.** Lipolytic effects on diacylglycerol accumulation in human adipose tissue in vitro. *J. Lipid Res.* 1990. **31:** 1351–1359.

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Adipocytes are repositories of energy stored in the form of lipid. Approximately 99% of this lipid is triacylglycerol (TG). Although diacylglycerol (DG) comprises less than 1% of the total bulk lipid store (1, 2), [^{14}C]DG constitutes 20–50% of newly synthesized [^{14}C]acylglycerols when adipose tissue fragments or isolated adipocytes are incubated for several hours in vitro with [^{14}C]glucose (3–8). The reason for the disproportionately high accumulation of [^{14}C]DG during incubations of up to 6 h is unknown, as are the mechanisms that prevent the accumulation of DG in the bulk lipid store of adipose tissue in vivo.

We have previously shown that in vitro incubation of human adipose tissue with epinephrine, a mixed alpha- and beta-adrenergic agonist, plus the beta blocker propranolol increases the relative proportion of DG in newly synthesized acylglycerols (8). We hypothesized, therefore, that adrenergic modulation of acylglycerol synthesis may play a role in maintaining DG at less than 1% of total adipocyte lipid in vivo.

Adrenergic effects on lipolysis have been well described. It is known, for instance, that the binding of endogenous catecholamines (epinephrine and norepinephrine) or synthetic agonists (e.g., isoproterenol) to beta-1 adrenergic receptors stimulates lipolysis by a 3',5'-cyclic adenosine monophosphate (cAMP)-dependent mechanism; conversely, agonist binding to alpha-2 adrenergic receptors inhibits lipolysis by decreasing adipocyte cAMP. The net effect of an adrenergic agonist on lipolysis in vitro depends upon the relative sensitivity and responsiveness of beta-1 and alpha-2 receptors in a given sample of adipose tissue (9).

Despite the detailed characterization of adrenergic effects on lipolysis, little is understood about the role of catecholamines in adipocyte lipid synthesis. The latter effects are likely to be complex, because acylglycerol synthesis may depend upon lipolysis for free fatty acid (FFA) substrate (10, 11), and catecholamines may increase the availability of glucose substrate by increasing glucose transport into human adipocytes (12). Although beta adrenergic stimulation increases the rate of total lipid (TG + DG) synthesis in both rat and human adipose tissue (10, 11), these effects are confounded by increases in the rate of lipolysis, such that a specific effect on TG synthesis cannot be inferred.

Abbreviations: TG, triacylglycerol; DG, diacylglycerol; FFA, free fatty acid; DGAT, diacylglycerol acyltransferase; LF, lipolysis factor; HSL, hormone-sensitive lipase.

Adrenergic stimulation may also influence acylglycerol synthesis by modulating the activity of the enzyme that catalyzes the conversion of diacylglycerol to triacylglycerol, diacylglycerol acyltransferase (DGAT). In rat adipose tissue, for example, beta adrenergic stimulation acutely decreases the activity of DGAT (13). The effect of adrenergic stimulation on DGAT activity in human adipose tissue is unknown. However, taken together, these observations suggest that adrenergic modulation of glucose metabolism, TG synthesis, and/or lipolysis may influence the accumulation of DG in human adipose tissue, either by altering the rate of conversion of DG to TG or by some other, uncharacterized, mechanism.

The experiments described below were performed to delineate adrenergic influences on the relative rates of di- and triacylglycerol accumulation in human adipose tissue. Additional experiments were done with insulin, adenosine deaminase, and adenosine to determine whether the observed interactions between the rates of lipolysis and acylglycerol accumulation were specific to adrenergic agonists.

MATERIALS AND METHODS

Subjects

Subjects for the study were 16 obese patients in good health at their lifetime maximum body weight (**Table 1**). Adipose tissue samples were obtained in the morning after an overnight fast. All samples were taken by needle aspiration (14) from the gluteal region following local anesthesia with 1% xylocaine. These procedures were approved by the Rockefeller University Institutional Review Board and written informed consent was obtained from each subject.

TABLE 1. Clinical characteristics of subjects

Subject	Sex	Age	Height	Body Weight	BMI[a]	FCS[b]
		yr	*m*	*kg*		*μg*
1	F	21	1.64	87.1	32.4	0.833
2	F	38	1.61	129.3	49.9	1.168
3	F	48	1.63	96.5	36.3	0.878
4	F	35	1.48	75.7	34.6	0.635
5	F	33	1.68	143.3	50.8	0.886
6	F	42	1.66	113.8	41.3	0.726
7	F	20	1.67	156.5	56.1	1.100
8	F	38	1.68	89.0	31.7	1.096
9	F	37	1.62	123.7	47.1	1.336
10	F	37	1.60	80.6	31.3	0.646
Mean		35	1.63	109.6	41.2	0.930
11	M	32	1.86	172.0	49.7	0.745
12	M	23	1.83	156.0	46.6	NA[c]
13	M	25	1.72	130.1	44.0	0.549
14	M	30	1.74	154.7	51.1	0.644
15	M	28	1.83	243.8	72.8	0.569
16	M	30	1.81	180.0	54.9	1.050
Mean		28	1.80	172.8	53.2	0.711

[a]Body Mass Index = weight in kg/ (height in m)^2.
[b]Gluteal Fat Cell Size in μg lipid/cell.
[c]Not available; data for subject 12 were referenced to sample wet weight (g).

Materials

Fatty acid-poor bovine serum albumin (BSA) was from Boehringer Mannheim (Indianapolis, IN). [U-^{14}C]glucose and [9,10-^{3}H]palmitate were from New England Nuclear Research Products (Boston, MA). Unlabeled palmitic acid was from Alltech (Deerfield, IL). All other components of the medium as well as epinephrine bitartrate, dibutyryl cAMP (Na salt), adenosine, yohimbine HCl, diacylglycerol (both the 1,2 and 1,3 isomers), and rhodamine B were from Sigma (St. Louis, MO). Humulin R brand of recombinant DNA human insulin was from Eli Lilly and Company (Indianapolis, IN). Isoproterenol bitartrate was a gift from Sterling Winthrop Research Institute (Renssaeler, NY). Clonidine-HCl was a gift from Boehringer Ingelheim Ltd. (Pearl River, NY). Adenosine deaminase (~2000 U/ml, from calf intestine) was purchased from Boehringer Mannheim. Aluminum-backed, silica-coated (0.2 mm) thin-layer chromatography plates were from E. M. Science (Cherry Hill, NJ). Ultrafluor scintillation cocktail was from National Diagnostics (Somerville, NJ). All organic solvents were redistilled.

Incubations

Gluteal adipose tissue samples (~2 g) obtained by needle aspiration were transported to the laboratory in 0.9% saline at 37°C and incubated as described previously (8). Briefly, the tissue was pre-incubated in Krebs-Henseleit bicarbonate buffer containing 4 g/100 ml bovine serum albumin and 4.17 mM glucose. The gas phase above the medium was 5% CO_2-95% O_2. After 1 h of pre-incubation, the tissue was rinsed with 0.9% saline at 37°C and weighed fragments of about 20 mg each were aliquoted into incubation medium of composition identical to that of the pre-incubation medium, except that it also contained 0.5 mEq/l palmitic acid and both [U-^{14}C]glucose and [9,10-^{3}H]palmitic acid. The final medium specific activity of [^{14}C]glucose ranged from 0.4 mCi/mmol to 0.9 mCi/mmol; that of [^{3}H]palmitate was about 2.0 mCi/mmol. The final concentration of glucose in the incubation medium was about 5 mM. Incubations were done in 7-ml polypropylene, screw-top vials at 37°C, with shaking at 70 cycles/min. The gas phase above the incubation medium was 5% CO_2-95% O_2. Incubations in isotope-containing medium were carried out for 2 h. Previous studies have shown that glyceride-glycerol accumulation is linear for at least 4 h (3) and di- and triacylglycerol accumulate in constant proportions to each other for at least 5 h of incubation (8). Thus, di- and

triacylglycerol accumulation may be expressed as rates of accumulation over 2 h. Each vial contained adrenergic agonists, antagonists, insulin, adenosine, or adenosine deaminase in the concentrations indicated below. Four replicates were incubated with each reagent in each experiment.

Extraction and separation of lipids

At the end of the 2-h incubation in isotopically labeled medium, tissue samples were washed with distilled, deionized water on glass filters in a vacuum filter apparatus (Millipore). The washed tissue and filter were extracted with chloroform–methanol 2:1 and the extract was washed 2 × with a large excess of distilled, deionized water. Diacylglycerol (both the 1,2- and 1,3- isomers) were added to the extract as carriers for newly synthesized labeled diacylglycerol. The extract was spotted on aluminum-backed, silica-coated thin-layer chromatography plates which were then developed in chloroform–methanol–acetic acid 200:4:3. The plates were sprayed with rhodamine B and lipid spots were visualized under UV light. The triacylglycerol, 1,2- and 1,3-diacylglycerol spots were cut from the plate with scissors, placed in scintillation vials, and the lipids were eluted from the silica gel with diethyl ether. Dried samples were counted simultaneously for both ^{3}H and ^{14}C in a Packard Tri-Carb 300 with automatic quench correction. Recovery of ^{14}C in TG and DG always exceeded 70 % of the counts applied to the plate and was usually near 95 %.

Mean adipocyte size was measured by the Coulter counter method of Hirsch and Gallian (15).

Calculation of rates of acylglycerol accumulation and lipolysis

Because incubation with adrenergic agonists may alter the rate of acylglycerol breakdown as well as synthesis, the results represent net rates of accumulation (balance between synthesis and breakdown) of newly synthesized TG and DG, rather than absolute rates of synthesis. Previous experiments have shown that [^{14}C]glucose is incorporated exclusively into the glyceride-glycerol backbone of newly synthesized acylglycerols in human adipose tissue under these incubation conditions (8). Thus, rates of TG and DG accumulation could be calculated from ^{14}C dpm in each acylglycerol, using the specific activity of [^{14}C]glucose in the medium (i.e., μmol glucose incorporated into each acylglycerol × 2).

The inclusion of both [^{14}C]glucose and [^{3}H]palmitate in the incubation medium allowed determination of the rate of lipolysis by the dual isotope method of Leibel et al. (16). This method takes advantage of the fact that the rate of lipolysis is strongly correlated with the rate of re-esterification of newly hydrolyzed FFA. These FFA, which are unlabeled, dilute [^{3}H]palmitic acid and reduce the specific activity of fatty acids esterified in newly synthesized TG. Thus, the relative incorporation of [^{14}C]glucose and [^{3}H]palmitate into newly synthesized TG is an index of the rate of lipolysis. This index is expressed as "lipolysis factor" (LF), a unitless value, proportional to the rate of lipolysis, which is calculated from the relative incorporation of isotopes into TG, corrected for the specific activities of precursors in the incubation medium. The use of this method for measuring lipolysis is justified because the lipolytic response to an agonist, expressed as the change in LF(ΔLF), is stongly correlated with the increment in the rate of lipolysis measured by glycerol release ($r = 0.90$, $P < 0.001$) (16). Although a correlational analysis has not been performed between glycerol release and LF for antilipolysis, it has been shown that incubations with epinephrine and propranolol (resulting in pure alpha-2 antilipolytic action) produce identical percent decreases in glycerol release and the ^{14}C:^{3}H ratio from which LF is calculated (16). In addition, this method has been used to document differences in lipolytic responsiveness to adrenergic agonists based on anatomic site (17, 18) and sex (17) which are in good agreement with differences based on glycerol release reported by other investigators (19–21). The rate of lipolysis, as measured by glycerol release, is linear for at least 2 h under the conditions of these experiments (8).

Statistics

Results are expressed as means ± SEM in the text. Individual data points are shown in figures. The results of various incubation conditions were assessed with Wilcoxon matched pairs signed rank test (two-tailed) or, for experiments with five subjects, the sign test (one-tailed). Comparisons between males and females were made with the Mann-Whitney test. Spearman's rank correlation coefficient (r_s) was used to assess correlations between variables.

RESULTS

Clinical characteristics of the subjects

Sex, age, height, weight, body mass index, and gluteal adipocyte size of the sixteen subjects are shown in Table 1.

Effects of gender

Gender did not affect basal lipolysis (mean ± SEM of lipolysis factor: women, 24.0 ± 1.1 vs. men, 24.9 ± 1.7). The rate of accumulation of newly synthesized TG in adipose tissue obtained from women (n = 10) tended to be higher than in that obtained from men (n = 6) (women, 0.379 ± 0.042 vs. men, 0.264 ± 0.056 μmol/10^6 cells per 2 h), but the effect was not statistically significant. In contrast, the rate of accumulation of newly synthesized

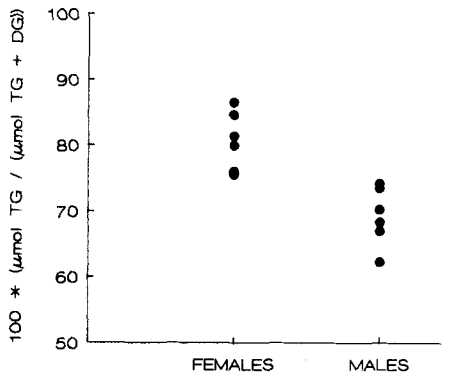

Fig. 1. Percent of total acylglycerol accumulation as TG when gluteal subcutaneous adipose tissue fragments from men (n = 6) and women (n = 7) were incubated for 2 h in Krebs-Henseleit bicarbonate buffer with 4 g/100 ml bovine serum albumin, ~5 mM glucose, [^{14}C]glucose, and 0.5 mM sodium palmitate. Acylglycerol accumulation was estimated from total [^{14}C]glucose incorporation into DG and TG. Adipose tissue from women converted a higher percentage of total acylglycerol to TG ($P<0.01$, Mann-Whitney test).

DG tended to be lower in adipose tissue obtained from women than in that obtained from men (women, 0.090 ± 0.009 vs. men, 0.114 ± 0.018 μmol/10^6 cells per 2 h), but again the effect was not statistically significant. However, the percentage of total acylglycerols converted to TG during the 2-h incubation (100 TG/(TG + DG)), was higher in women than in mean (women, 80.2 ± 1.4% vs. men, 69.3 ± 2.0%; $P<0.01$; **Fig. 1**).

Agents that increase the rate of lipolysis

Beta adrenergic stimulation with isoproterenol (10^{-7} to 10^{-5} M, n = 12) doubled lipolysis from a basal LF value of 24.8 ± 1.2 to 47.8 ± 3.3 ($P<0.01$; **Fig. 2**). Beta adrenergic stimulation produced by epinephrine (10^{-6} M) plus the alpha-2 blocker yohimbine (10^{-5} M) increased lipolysis by 86 ± 14% over basal (n = 5; $P<0.01$; **Table 2**). Similarly, dibutyryl cAMP, a nonhydrolyzable analog of cAMP, increased lipolysis by 107 ± 12% over basal (n = 3; Table 2).

None of these agents had any effect on the rate of accumulation of newly synthesized TG (Table 2 and Fig. 2).

In contrast, isoproterenol decreased accumulation of newly synthesized DG in all experiments by 46 ± 5% below basal rates of accumulation ($P<0.01$; Fig. 2). Similarly, epinephrine plus yohimbine decreased the accumulation of newly synthesized DG by 53 ± 7% below basal ($P<0.05$; Table 2). Dibutyryl cAMP (10^{-3} M) mimicked the effect of beta adrenergic agonists, decreasing newly synthesized DG accumulation by 52 ± 7% below basal (Table 2).

The isoproterenol (10^{-11} to 10^{-6} M; n = 5) dose-response curves (expressed as percent maximal response) were very similar for stimulation of lipolysis and reduction of newly synthesized DG accumulation (**Fig. 3**). That is, the more lipolysis was stimulated, the more DG was depleted. The congruence of the two dose-response curves suggests that the depletion of DG may result from the stimulation of lipolysis.

Finally, adenosine deaminase (n = 5), which hydrolyzes endogenous adenosine and thus prevents inhibition of lipolysis, increased the rate of lipolysis by 13 ± 7% (**Table 3**). Adenosine deaminase had no effect on TG accumulation, but decreased accumulation of newly synthesized DG by 22 ± 3% below basal ($P<0.05$).

Agents that inhibit lipolysis

The alpha-2 agonist, clonidine (10^{-6} to 10^{-5} M; n = 12), decreased lipolysis from a basal LF of 25.2 ± 1.1 to 18.6 ± 1.0 ($P<0.01$; **Fig. 4**). Similarly, insulin (500 μU/ml; n = 5) and adenosine (10^{-6} M; n = 5) decreased

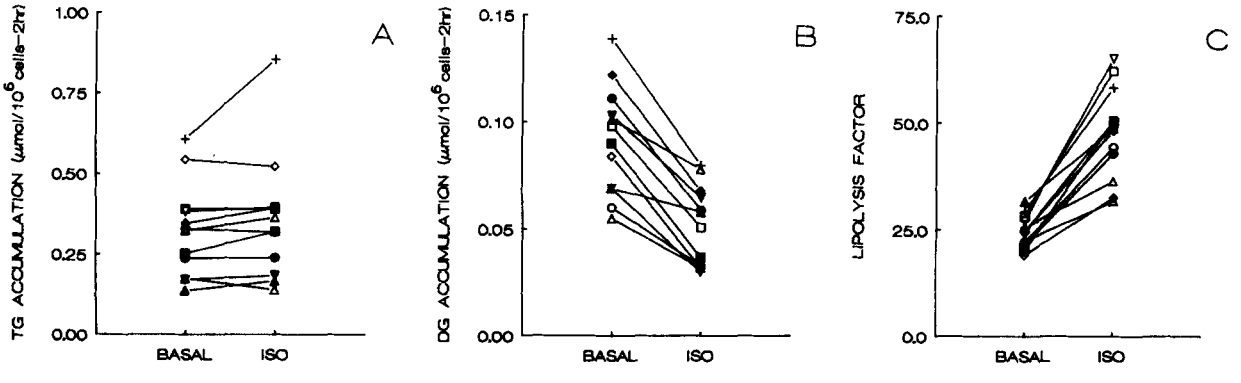

Fig. 2. Effect of isoproterenol (ISO; 10^{-7} M; n = 12) on (A) triacylglyerol accumulation, (B) diacylglycerol accumulation, and (C) lipolysis in human adipose tissue fragments incubated, as described in the legend to Fig. 1, without additions or in the presence of isoproterenol. Open symbols represent data from females and closed symbols data from males. Isoproterenol increased lipolysis and decreased DG accumulation ($P<0.01$, Wilcoxon test).

TABLE 2. Effects of epinephrine + yohimbine versus yohimbine alone (n = 5) and dibutyryl cAMP versus basal (no additions) (n = 3) on lipolysis and accumulation of newly synthesized acylglycerols

	Yohimbine	Epinephrine (10^{-6} M) + Yohimbine (10^{-5} M)	Basal	Dibutyryl Cyclic AMP (10^{-3} M)
Lipolysis factor[a]	25.7 ± 2.3	47.6 ± 4.8*	22.2 ± 1.6	46.9 ± 5.7*
Triacylglycerol[b]	0.367 ± 0.064	0.350 ± 0.064	0.221 ± 0.031	0.229 ± 0.025
Diacylglycerol[c]	0.101 ± 0.014	0.048 ± 0.010*	0.101 ± 0.007	0.050 ± 0.010*

[a]See Methods and ref. 16.
[b]Triacylglycerol accumulation (μmol per 10^6 cells per 2 h).
[c]Diacylglycerol accumulation (μmol per 10^6 cells per 2 h).
*Different from control with $P < 0.05$.

lipolysis, by 24 ± 3 and 21 ± 4% below basal, respectively ($P<0.05$; Table 3).

Clonidine increased accumulation of newly synthesized DG by 30 ± 4% above basal levels ($P<0.01$), but had no effect on accumulation of newly synthesized TG (Fig. 4).

Insulin did not significantly affect newly synthesized TG accumulation, but increased newly synthesized DG accumulation by 40 ± 10% ($P<0.05$; Table 3). Similarly, while exogenous adenosine had no consistent effect on newly synthesized TG accumulation, it increased newly synthesized DG accumulation by 23 ± 6% above basal ($P<0.05$; Table 3).

Epinephrine

The naturally occurring, mixed adrenergic agonist, epinephrine (10^{-6} to 10^{-5} M, n = 8), had a variable effect on lipolysis, which was not statistically significant (basal LF = 24.2 ± 1.4, epinephrine-stimulated LF = 25.7 ± 2.5). Epinephrine also had no effect on newly synthesized TG accumulation, but increased the accumulation of newly synthesized DG by 42 ± 10% above basal levels ($P<0.01$; **Fig. 5**). The degree to which epinephrine inhibited lipolysis (decrement vs. basal LF) was positively correlated with the change in newly synthesized DG accumulation (r_s = 0.73; $P<0.05$).

DISCUSSION

The net accumulation of newly synthesized diacylglycerol in human adipose tissue is extremely sensitive to agents that affect lipolysis, while the accumulation of newly synthesized triacylglycerol is unaffected. Beta-1 adrenergic activation (isoproterenol or epinephrine plus yohimbine), dibutyryl cAMP, and adenosine deaminase increased the rate of lipolysis and decreased DG accumulation. Conversely, alpha-2 adrenergic stimulation (clonidine), insulin, and adenosine inhibited lipolysis and increased DG accumulation. The mixed agonist, epinephrine, increased DG accumulation in proportion to its ability to inhibit lipolysis. The variable effect of epinephrine on lipolysis (Fig. 5) is accounted for by the fact that epinephrine is a mixed adrenergic agonist, acting at both stimulatory (beta-1) and inhibitory (alpha-2) receptors (9). Whether the net effect of epinephrine is to stimulate or inhibit lipolysis depends upon the relative preponderance and sensitivity of the two types of adrenergic receptors in a given sample of adipose tissue. Previous studies, using both glycerol release (21–23) and LF (17; R. L. Leibel, unpublished observations) have documented the relatively high alpha-2 adrenergic responsiveness of human adipose tissue (especially gluteal tissue) in vitro. This high alpha-2 response may in some cases be sufficient to result in a net inhibition of lipolysis by epinephrine.

Although our results were obtained in tissue from obese subjects, they seem to be relevant to lean individuals as well. In experiments carried out on isolated adipocytes from seven lean subjects (mean adipocyte size = 0.425 μg lipid) for other purposes, we found the same pattern of results: isoproterenol depleted newly synthesized DG by about 50% without affecting the accumulation of newly synthesized TG (N. K. Edens, unpublished observations).

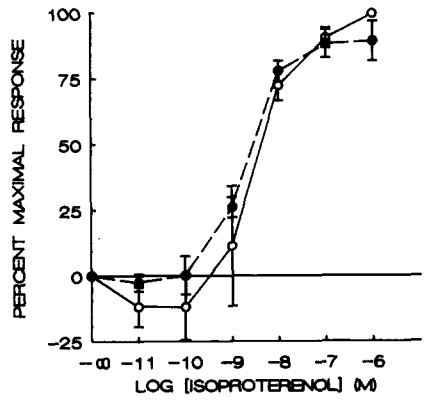

Fig. 3. Human adipose tissue fragments were incubated (n = 5) as described in the legend to Fig. 1 in the absence or presence of a range of concentrations of isoproterenol (ISO). The effects of ISO on lipolysis (closed circles) and DG accumulation (open circles) are expressed as the percent maximal response of the change over basal. Percent maximal response was calculated as: 100 × [(stimulated – basal)/(maximally stimulated – basal)]. For lipolysis, the change is an increase in ΔLF, representing stimulation of lipolysis with increasing concentration of ISO. For DG, this change is a decrease in the rate of accumulation of DG (μmol/10^6 cells per 2 h), compared to basal, with increasing concentration of ISO.

TABLE 3. Effects of adenosine deaminase (ADA), adenosine (ADN), and insulin on lipolysis and accumulation of newly synthesized acylglycerols (n = 5)

	Basal	ADA (0.4 U/ml)	ADN (10^{-6} M)	Insulin (500 μU/ml)
Lipolysis factor[a]	23.1 ± 1.2	26.6 ± 3.0*	18.5 ± 1.0*	18.0 ± 1.0*
Triacylglycerol[b]	0.341 ± 0.025	0.327 ± 0.014	0.355 ± 0.015	0.385 ± 0.021
Diacylglycerol[c]	0.104 ± 0.011	0.079 ± 0.006*	0.134 ± 0.015*	0.144 ± 0.012*

[a]See Methods and ref. 16.
[b]Triacylglycerol accumulation (μmol per 10^6 cells per 2 h).
[c]Diacylglycerol accumulation (μmol per 10^6 cells per 2 h).
*Different from basal with $P < 0.05$, sign test.

Beta adrenergic stimulation did not affect TG accumulation in these experiments, probably because palmitic acid was provided in the incubation medium, and therefore FFA supply was not rate limiting for TG synthesis (24, 25). The lack of insulin effect on TG synthesis is surprising, but may be accounted for by the small number of subjects, their extreme obesity, the presence of 5 mM glucose in the incubation medium, and the fact that aspirated fragments of tissue were used. Björntorp and Martinsson (26) have shown that insulin effects on adipocyte glucose metabolism are more marked in dissected pieces of tissue than in aspirated fragments, and in the presence of 1 mM glucose rather than 5 mM glucose.

These effects on DG accumulation in human adipose tissue are consistent with previous data from rat adipocytes, in which insulin increased DG accumulation and norepinephrine or epinephrine (which exert a nearly pure beta adrenergic effect in rat adipocytes (9)) decreased DG accumulation (7, 27). Similarly, Zinder, Eisenberg, and Shapiro (28) showed that stimulation of lipolysis in rat adipocytes prelabeled with [^{14}C]palmitic acid decreased the specific activity of [^{14}C]DG, suggesting that newly synthesized DG were hydrolyzed and that the total DG pool was replenished by partial hydrolysis of unlabeled TG.

What is the nature of the link between lipolysis and DG accumulation? It is unlikely that agents that stimulate lipolysis also increase the rate of conversion of DG to TG, because: *1*) TG accumulation was not affected by any treatment, and *2*) DGAT, which catalyzes the conversion of DG to TG, is inhibited by beta adrenergic stimulation in rat adipose tissue (13) and by cAMP in rat liver (29). The regulation of this enzyme in human adipose tissue has not been investigated; however, it is unlikely that the effect of cAMP in human tissue would be in the opposite direction of its effect in rat tissues.

An alternative, and more plausible, explanation is that changes in the rate of lipolysis may mediate changes in the rate of DG accumulation directly, as has been suggested by Winand et al. (7) for rat adipocytes. The dose-responses to isoproterenol for lipolysis and DG depletion were very similar, suggesting that newly synthesized DG may be preferentially hydrolyzed (compared to newly synthesized TG) when lipolysis is stimulated. Others (28, 30) have shown that the specific activity of the FFA released when lipolysis is stimulated in prelabeled adipo-

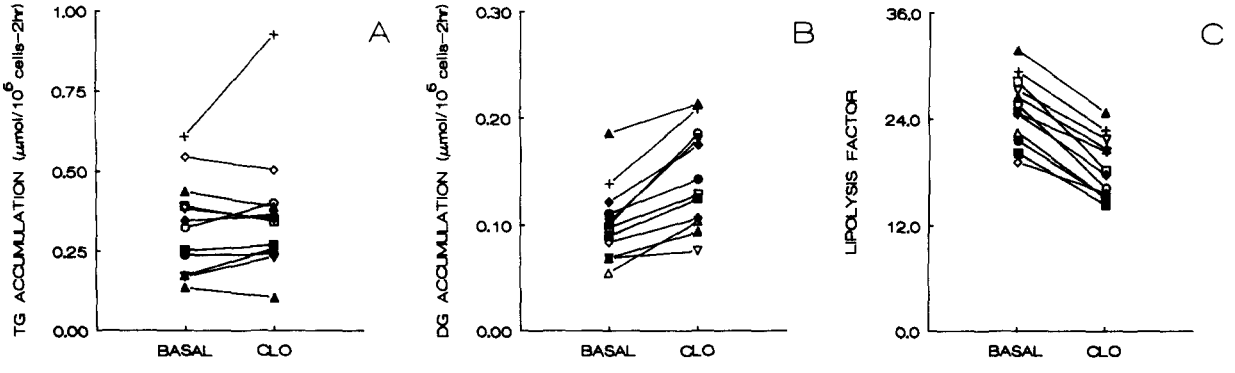

Fig. 4. Effect of clonidine (CLO; 10^{-6}–10^{-5} M; n = 12) on (A) triacylglycerol accumulation, (B) diacylglycerol accumulation, and (C) lipolysis in human adipose tissue fragments incubated, as described in the legend to Fig. 1, without additions or in the presence of clonidine. Open symbols represent data from females and closed symbols data from males. Clondine decreased lipolysis and increased DG accumulation ($P<0.01$, Wilcoxon test).

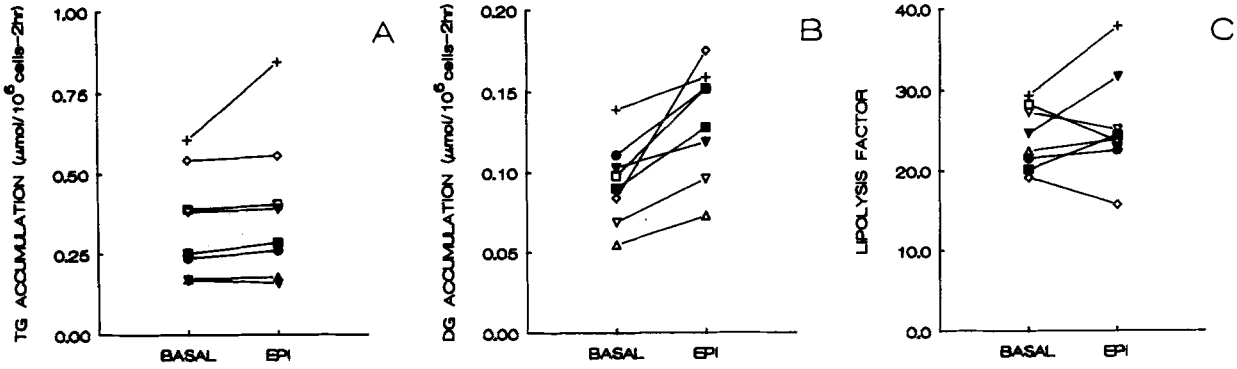

Fig. 5. Effect of epinephrine (EPI; 10^{-6}-10^{-5} M; n = 8) on (A) triacylglycerol accumulation, (B) diacylglycerol accumulation, and (C) lipolysis in human adipose tissue fragments incubated, as described in the legend to Fig. 1, without additions or in the presence of epinephrine. Open symbols represent data from females and closed symbols data from males. Epinephrine increased DG accumulation ($P<0.01$, Wilcoxon test).

cytes is higher than the specific activity of the bulk lipid. Since our data indicate that labeled, newly synthesized TG is not depleted when lipolysis is stimulated, newly synthesized DG seems to be—of the newly synthesized acylglycerols—the preferred substrate for lipolysis.

Specific lipolytic depletion of DG could occur if newly synthesized DG were more accessible to hormone-sensitive lipase (HSL) than newly synthesized TG. Such differential accessibility might result if newly formed TG droplets bore a surface layer of emulsifying DG as they pass through the aqueous cytoplasm from their site of synthesis in the smooth endoplasmic reticulum (31) to storage in the main lipid droplet. The mechanism and time course for mixing of newly synthesized acylglycerols with the bulk lipid droplet are controversial. Previous investigators (5), using evidence from electron microscopy studies, have concluded that newly synthesized acylglycerols mix immediately with the bulk lipid droplet, and that the disproportionate accumulation of DG results from separation of DG from DGAT. However, that study did not distinguish DG from TG, so it may be that newly synthesized TG mixed with the bulk lipid droplet, while newly synthesized DG formed a surface layer susceptible to lipolysis.

Other investigators have hypothesized that the transfer of a portion of newly synthesized DG to the main lipid droplet constitutes a sequestration of DG, which results in delayed conversion to TG (4, 27). Pulse-chase studies show that newly synthesized DG has a relatively long half-life of ~80 min (4). However, these studies did not demonstrate that the disappearance of newly synthesized DG actually resulted in the appearance of newly synthesized TG. Our results are consistent with the idea that this fraction of newly synthesized DG may be sequestered only from DGAT, and remains accessible to HSL. An alternative interpretation is that incubation with lipolytic stimuli selectively blocks the formation of a labeled DG pool which is not a substrate for TG synthesis. This idea seems relatively unlikely, however, because pulse-chase experiments of Zinder et al. (28) showed that counts in [^{14}C]DG, labeled during a pulse, decline when chased in unlabeled medium with epinephrine. The decrease in labeled DG was accompanied by an increase in total DG which is thought to result from partial hydrolysis of adipose tissue TG (1, 2). Partial glycerides, along with adipocyte glycogen, may make a small (15–20%) contribution to glyceride synthesis in human adipocytes (32, 33). However, the glyceride derived from these sources is distributed between DG and TG in the same proportion as glyceride derived from medium [^{14}C]glucose (N. K. Edens, unpublished observations). Thus, utilization of these sources of glyceride-glycerol would not affect the interpretation of the present experiments.

The susceptibility of newly synthesized DG to depletion by HSL may play a role in limiting adipocyte DG accumulation in vivo. Lipolytic stimulation in vivo may deplete newly synthesized DG, limiting its accumulation to the small proportion measured in the bulk lipid store. This could occur despite the fact that maximal lipolytic stimulation (~100%) in vitro depleted DG by only ~50% of basal accumulation, which is not adequate to reduce DG to less than 1%, as measured in the bulk lipid store. However, in vivo, lipolysis may vary through a much larger range; epinephrine infusions triple the rate of lipolysis in obese subjects fasted for 15 h (34). In addition, it is possible that the balance between rates of DG synthesis and degradation may vary with nutritional state in a way that accounts for low DG accumulation over the long term. For example, the increase in lipolytic rate that occurs in the post-absorptive state could deplete newly synthesized DG and prevent its accumulation. In the absence of lipolytic depletion, there may be a slow transfer of DG from the sequestered pool to the pool that serves as substrate for TG synthesis. This transfer could result in slow conversion of DG to TG.

If the fraction of newly synthesized DG that accu-

mulates during labeling incubations is sequestered from DGAT, then this labeled DG does not represent the substrate pool for further TG synthesis. That a 50% depletion of the newly synthesized DG pool has no effect on TG accumulation supports this idea. However, only pulse-chase studies, which measure the effect of depleting this labile DG pool on subsequent TG synthesis, will answer this question definitively. The present studies also indicate that newly synthesized DG and TG are not in rapid equilibrium, since if such an equilibrium existed, changes in DG accumulation would be rapidly reflected in changes in TG accumulation.

DG is also a substrate for phosphatidylcholine and phosphatidylethanolamine synthesis (35). It would be of interest to determine whether lipolysis-induced changes in DG accumulation affect the rate of phospholipid synthesis, with potential consequences for adipocyte structure and function.

The percentage of TG in newly synthesized acylglycerol was higher in gluteal adipose tissue obtained from women than from men. Since basal lipolysis did not differ between men and women, it is unlikely that this difference results from enhanced lipolytic depletion of DG in adipose tissue from women. The higher proportion of TG may represent higher activity of DGAT and enhanced efficiency of lipid storage in adipose tissue from women than from men. Such enhanced efficiency of lipid storage would be consistent with the larger gluteal adipocyte size in women than men (0.930 ± 0.079 vs. 0.711 ± 0.102 μg lipid/cell in this study, not significant). In light of the differences between men and women in the relative sizes of the abdominal and gluteal adipose tissue depots (36), it would be of interest to compare the proportion of TG in newly synthesized acylglycerols in abdominal adipose tissue of men and women; men might be more efficient at synthesizing lipid in abdominal adipose tissue than women. In addition to these possible sex- and site-related differences in the basal rate of conversion of DG to TG, these data indicate that the relatively greater alpha (antilipolytic) adrenergic responsiveness of abdominal adipocytes from males (17) and nutritionally induced changes in adrenergic responsiveness (37) may influence not only lipid mobilization but also lipid storage.

We have demonstrated that a variety of agents that affect lipolysis also deplete newly synthesized DG without affecting newly synthesized TG. The depletion of newly synthesized DG is likely to result from stimulation of lipolysis. Beta adrenergic stimulation does not increase the rate of TG synthesis in human adipose tissue, as it does in that of the rat, provided that FFA substrate is available in the incubation medium. The proportion of TG in newly synthesized glycerides appears to be influenced both by intrinsic activity of DGAT (hypothesized to be higher in women than in men) and by acute lipolytic effects on DG accumulation. It is clear from our data that depletion of DG by stimulation of lipolysis does not affect TG accumulation in the short term (2 h). Whether lipolytic depletion of DG by physiologic events (e.g., by adrenergic stimulation during exercise) might produce delayed or long-term deficits in TG synthesis is a matter for further investigation.

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