Mechanism of free fatty acid re-esterification in human adipocytes in vitro

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Abstract Within adipose tissue, free fatty acids liberated by lipolysis may be re-esterified into newly synthesized triacylglycerol. We hypothesized that re-esterification may occur via an extracellular route, such that free fatty acids arising from lipolysis must leave the adipocyte and be taken up again before they can be re-esterified. We simultaneously measured rates of lipolysis, acylglycerol synthesis, and free fatty acid re-esterification in human adipose tissue and isolated adipocytes in vitro, utilizing a dual-isotopic technique. We manipulated incubations to increase mixing of released free fatty acids with the incubation medium. Such manipulations should decrease the probability that released free fatty acids would be taken up and re-esterified. We found that re-esterification was decreased in isolated adipocytes compared to fragments of tissue, in shaken compared to unshaken incubations, and in low adipocyte concentrations compared to high adipocyte concentrations. Rates of acylglycerol synthesis and lipolysis were unaltered by these manipulations, indicating that changes in free fatty acid re-esterification are not secondary to effects on these processes. ■■ The results are consistent with an extracellular route for free fatty acid re-esterification. Such a mechanism suggests that adipose tissue blood flow may play an important role in the regulation of free fatty acid release from adipose tissue. —**Edens, N. K., R. L. Leibel, and J. Hirsch.** Mechanism of free fatty acid re-esterification in human adipocytes in vitro. *J. Lipid Res.* 1990. **31:** 1423–1431.

Supplementary key words adipose tissue • triacylglycerol • isoproterenol • adenosine • lipid metabolism • adipose tissue blood flow • lipolysis

In adipose tissue, stored triacylglycerol (TG) undergoes continuous, simultaneous synthesis and breakdown. Glycerol arising from lipolysis cannot be reutilized, since human adipose tissue has negligible glycerokinase activity (1). However, newly hydrolyzed free fatty acids (FFA) can be activated and re-esterified into newly-synthesized TG. In this report, "FFA re-esterification" refers specifically to this cycle of TG hydrolysis and resynthesis within adipose tissue.

Previous investigations into the regulation of FFA re-esterification in vitro have used the balance technique (2), in which re-esterification is calculated from the difference between glycerol and FFA release (3–6). This method does not distinguish between FFA re-esterification and TG synthesis. Studies that measured both processes simultaneously did not provide FFA in the incubation medium (5, 7), and so do not accurately mimic the situation in vivo, in which FFA substrate may be derived from plasma, either from hydrolysis of circulating lipoproteins or from albumin-bound FFA. Another study in which both processes were measured simultaneously did not indicate how much FFA was provided by the human serum albumin used in the incubation medium (8) so that FFA re-esterification cannot be distinguished from total TG synthesis.

Whether FFA re-esterification may be regulated independently from TG synthesis is unknown but can be addressed by the recently developed dual-isotopic technique for measuring FFA re-esterification in adipose tissue (9). This method allows TG synthesis and re-esterification to be measured independently and simultaneously. Studies using this technique have suggested that the rate of FFA re-esterification is not regulated at the cellular level, but rather by changes in the extracellular environment (10). A model of FFA re-esterification consistent with this idea is that FFA are not re-esterified primarily by an intracellular pathway, but must leave the adipocyte and be taken up again before being re-esterified. To test this hypothesis, we examined the influence of degree of mixing of released FFA with incubation medium on the rate of re-esterification. Such mixing should specifically affect re-esterification only if FFA arising from lipolysis must leave the adipocyte and re-enter before being available for re-esterification.

The results of three different types of experiments designed to test this hypothesis indicated that *1*) the rate of re-esterification was higher in intact fragments of adipose tissue compared to isolated adipocytes, *2*) the rate of

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Abbreviations: TG, triacylglycerol; FFA, free fatty acid; ADA, adenosine deaminase; PIA, N^6-phenylisopropyl adenosine; BSA, bovine serum albumin.

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re-esterification is negatively correlated with the shaking speed of the incubation, and *3*) the rate of re-esterification is higher in adipocytes incubated at high cell concentration than low cell concentration. A final experiment showed that the addition of exogenous FFA, supplied in the incubation medium, is more effective in stimulating TG synthesis than is increasing the availability of endogenous FFA arising from lipolysis.

MATERIALS AND METHODS

Subjects

The subjects for these studies were 12 males and 10 females between 22 and 48 years of age. Body mass index [BMI; weight in kg/(height in m)^2] ranged from 19.1 to 50.3. Sex, age, degree of obesity, and site of tissue sampling were not independent variables in these studies. Adipose tissue samples were obtained from 17 subjects by needle aspiration from gluteal or abdominal sites following local anesthesia with 1% xylocaine (11) and from five subjects during hernia repair or obesity surgery. These studies were approved by the Rockefeller University Institutional Review Board and all subjects gave informed consent.

Materials

Fatty acid-poor bovine serum albumin was from Boehringer Mannheim (Indianapolis, IN). According to the manufacturer's label, the total fatty acid content of this albumin is less that 0.2 mg per g albumin. This would result in a fatty acid concentration of less than 0.04 mM in a 5% albumin solution. [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 the other components of the medium as well as diacylglycerol (both the 1,2 and 1,3 isomers), and rhodamine B were from Sigma (St. Louis, MO). Collagenase (CLS4196, lot #45N8919) was from Cooper Biomedical (Newark, NJ). Isoproterenol bitartrate was a gift from Sterling Winthrop Research Institute (Renssaeler, NY). N^6-phenylisopropyl adenosine and adenosine deaminase (~2000 U/ml, from calf intestine) were 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).

Incubations

Pre-incubation medium consisted of Krebs-Henseleit bicarbonate buffer containing 5 g/100 ml bovine serum albumin (BSA) and 4.17 mM glucose. Dual-isotope incubation medium was of composition identical to pre-incubation medium, except that it also contained 0.5 mM palmitic acid and both [U-^{14}C]glucose and [9,10-^{3}H]palmitic acid (12). Palmitic acid was complexed to albumin as described by Leibel et al. (12) with minor modifications. Briefly, a film of mixed unlabeled and [^{3}H]palmitic acid was dissolved with ethanol and a small (12%) molar excess of NaOH. This solution was heated at 60°C for 30 min with gentle shaking. It was then dried under a stream of N$_2$. Prewarmed (60°C) 10% BSA in distilled, deionized water was added. The 10% BSA was heated and gently shaken until clear. The 10% BSA (now containing 1 mM palmitic acid) was combined with an equal volume of double-strength Krebs-Henseleit bicarbonate buffer with glucose to make the incubation medium. The final medium specific activity of [^{14}C]glucose ranged from 0.4 μCi/μmol to 0.9 μCi/μmol; that of [^{3}H]palmitate was ~2.0 μCi/μmol. The final concentration of glucose in the incubation medium was ~5 mM. The gas phase above both pre-incubation and incubation media was 5% CO$_2$-95% O$_2$. Unless otherwise noted, all incubations were carried out for 2 h at a shaking speed of 80 cycles/min and temperature of 37°C. Zero time samples were run and all other values were corrected accordingly.

Experimental protocol

Re-esterification in tissue fragments versus isolated adipoyctes (Experiment 1). Adipose tissue samples (~3 g) were divided into two parts. One half was pre-incubated. During this pre-incubation (~1 h), adipocytes were isolated from the other half of the sample by collagenase digestion in the pre-incubation medium (13). The isolated adipocytes were suspended at a 50% concentration and aliquoted (200 μl) into 2 ml incubation medium. The tissue fragments were then removed from the pre-incubation medium, rinsed with 37°C 0.9% saline, and ~20 mg weighed fragments were aliquoted into 2 ml incubation medium. Incubations were done in 20 ml polypropylene, screw-top vials at 37°C, with shaking at 80 cycles/min. Incubations were done in the basal state (no additions) and with isoproterenol (10^{-7} M) to stimulate lipolysis. This experiment was performed on adipocytes derived from five subjects; four replicates were incubated in each condition in each experiment.

Effect of incubation shaking speed on re-esterification rate (Experiment 2). Fragments of human adipose tissue or isolated adipocytes, prepared and aliquoted as described for Experiment 1, were incubated in dual-isotope medium with no shaking, shaking at 80 cycles/min, or shaking at 160 cycles/min. Incubations were done in the basal state (no additions) and with isoproterenol (10^{-7} M). This experiment was performed on tissue fragments from two subjects and isolated adipocytes derived from five subjects; four replicates were incubated in each condition in each experiment.

Effect of adipocyte concentration on re-esterification rate (Experiment 3). Isolated adipocytes, prepared as described for Experiment 1, were incubated in dual isotope medium at a mean concentration of ~20,000 or ~300,000 cells/ml. In order to examine the effect of adipocyte concentration at both high and low rates of lipolysis, half of the incubations were done in the presence of adenosine deaminase (ADA, 0.4 U/ml) to remove adenosine released by broken adipocytes and prevent inhibition of lipolysis. The other half were done in the presence of ADA plus the nonhydrolyzable adenosine analog, N^6-phenylisopropyl adenosine (PIA, 10^{-7} M) to inhibit lipolysis (14).

In order to examine the role of dilution of medium-derived [^{3}H]palmitic acid with unlabeled FFA released by lipolysis, samples of incubation medium were taken at the beginning and end of the incubation for measurement of the specific activity of medium FFA. Aliquots of the medium were extracted by the method of Dole (15) and dried samples of the upper phase were assayed for FFA content by the enzymatic method of Miles et al. (16). Duplicate aliquots of medium were counted for ^{3}H. FFA re-esterification was calculated both from the medium FFA specific activity at the beginning of the incubation and from the geometric mean of medium FFA specific activities at the beginning and end of the incubation.

This experiment was performed on adipocytes derived from five subjects; at least two replicates were incubated in each condition in each experiment.

Relative effects of exogenous versus endogenous FFA on TG synthesis (Experiment 4). Isolated adipocytes, prepared and aliquoted as described for Experiment 1, were incubated in medium containing either no added FFA or 0.5 mM palmitic acid. These incubations were done in the basal state (no additions) and either with adenosine deaminase (ADA) to prevent inhibition of lipolysis or with isoproterenol to stimulate lipolysis. Because there was no difference in the results obtained with the two methods of increasing lipolysis, the results were combined for analysis. This experiment was performed on adipocytes derived from five subjects; four replicates were incubated in each condition in each experiment.

Extraction and separation of lipids

At the end of the incubations (2 h), tissue fragments or adipocytes and medium were poured into iced Falcon tubes containing PE90 catheters. The tubes were spun gently for 1–2 min at 0°C to float the tissue fragments or adipocytes. Incubation medium was withdrawn from beneath the tissue fragments or adipocytes through the catheter and frozen for subsequent glycerol assay by the method of Laurell and Tibbling (17). The fragments or cells remaining in the tube were extracted by the method of Dole (15). The extracted lipids were subjected to thin-layer chromatography to isolate triacylglycerol. The TG was recovered and counted simultaneously for both

^{3}H and ^{14}C. The incorporation of [^{14}C]glucose into lipid was used to calculate rates of triacylglycerol synthesis.

The relative incorporation of [^{14}C]glucose and [^{3}H]palmitate into TG was used to calculate the rate of re-esterification as described previously (9). Under the conditions of these experiments, and using human adipose tissue, ^{14}C is not incorporated into the fatty acid moiety of acylglycerols and ^{3}H is not incorporated into the glyceride moiety (9). FFA re-esterification can be measured because both unlabeled FFA arising from lipolysis and medium-derived [^{3}H]palmitate are esterified in newly synthesized [^{14}C]acylglycerols. The degree of dilution of [^{3}H]palmitate by unlabeled, endogenous FFA in [^{14}C]TG is an index of the rate of re-esterification. Re-esterification is calculated as the difference between the total theoretical moles of FFA in TG (μmol[^{14}C]TG × three) minus the actual total μmoles of [^{3}H]palmitate esterified in TG. Therefore, fatty acid re-esterification, or the esterification of unlabeled FFA in TG equals [(3 × μmol [^{14}C]TG) – μmol [^{3}H]TG]; where micromoles of [^{3}H]TG represents the amount of [^{3}H]palmitate esterified to [^{14}C]TG. The data are expressed as μmol FFA re-esterified per μmol newly synthesized TG {[(3 × μmol [^{14}C]TG – μmol [^{3}H]TG/μmol [^{14}C]TG}, a value that theoretically can vary between zero (no re-esterification) and three (all newly esterified fatty acids derived from lipolysis).

Adipocyte size was measured in aliquots of isolated adipocytes by microscopy. The lipid content of tissue fragments or aliquots of isolated adipocytes was determined gravimetrically in Dole extracts. Adipocyte concentration in the incubation was calculated by dividing lipid content by cell size (assumed to be 100% lipid) to obtain the number of cells in each incubation (18).

Statistics

Results are expressed as mean ± SEM. Data were evaluated with analysis of variance with repeated measures on incubation condition and, when appropriate, post-hoc Bonferroni t tests (19).

RESULTS

Re-esterification in tissue fragments versus isolated adipocytes

These experiments tested the possibility that adipocytes in a tissue fragment are exposed to an extracellular environment enriched in unlabeled FFA arising from lipolysis, since the extracellular matrix and other cells would limit diffusion of these FFA into the incubation medium. In contrast, it was hypothesized, any FFA released from isolated adipocytes would mix more readily with the relatively large pool of incubation medium and be less available for re-uptake and re-esterification. Thus, if FFA must leave the cell before they are re-esterified, then rates

of re-esterification of FFA arising from lipolysis should be higher in fragments than in isolated adipocytes.

As predicted, the rate of re-esterification was higher in tissue fragments compared to isolated adipocytes (F[1,4] = 42.47, $P < 0.005$; **Fig. 1**): in the basal state, in isolated adipocytes, ~0.6 μmol of fatty acid was re-esterified per μmol of newly synthesized TG, whereas in tissue fragments, ~1.6 μmol fatty acid was re-esterified per μmol of newly synthesized TG. Isoproterenol increased the rate of re-esterification (F[1,4] = 27.55, $P < 0.01$) in both tissue preparations.

Tissue preparation did not effect the basal rate of lipolysis. Isoproterenol (10^{-7} M) stimulated lipolysis to the same extent (~200%; F[1,4] = 34.35, $P < 0.005$) in both tissue preparations (**Fig. 2**, left). Neither tissue preparation nor isoproterenol affected the rate of TG synthesis (Fig. 2, right).

Effect of incubation shaking speed on re-esterification rate

Incubations of adipose tissue or isolated adipocytes are normally performed with shaking at about 80 cycles/min. The second set of experiments tested the hypothesis that, if FFA must leave adipocytes before being taken up again for re-esterification, then when tissue fragments or isolated adipocytes were incubated without shaking, released FFA would not mix with the entire volume of the incubation, and the rate of re-esterification would be increased. Conversely, if the shaking speed were doubled to 160 cpm, mixing of released FFA would be maximized and the rate of re-esterification would be decreased.

Consistent with our hypothesis, in basal incubations of isolated adipocytes, increasing shaking speed abolished FFA re-esterification, while in isoproterenol-stimulated
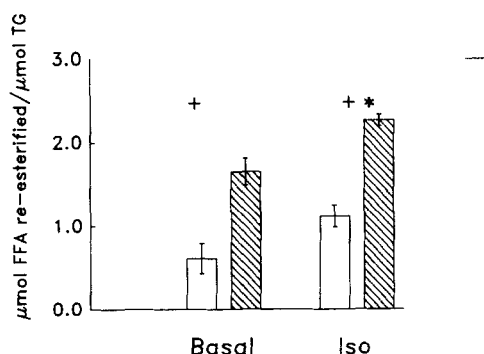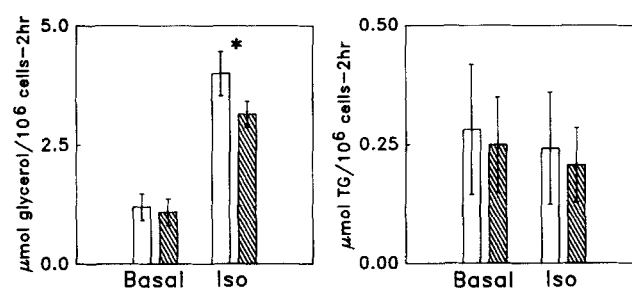

Fig. 2. Rates of lipolysis (left) and triacylglycerol synthesis (right) in tissue fragments vs. isolated adipocytes. Symbols as in Fig. 1.

adipocytes, increasing shaking speed decreased re-esterification to less than half the level seen in unshaken incubations (F[1,4] = 74.60, $P < 0.001$, **Fig. 3**). Isoproterenol increased the rate of re-esterification (F[1,4] = 118.84, $P < 0.001$) at all shaking speeds.

In isolated adipocytes, increasing the incubation shaking speed had no effect on the rate of lipolysis, although the basal rate of lipolysis tended to decrease with increasing shaking speed. Isoproterenol increased the rate of lipolysis by more than 300% at all shaking speeds (F[1,4] = 21.17, $P < 0.025$; **Fig. 4**, left). Neither shaking speed nor isoproterenol affected the rate of TG synthesis (Fig. 4, right).

Shaking speed had no effect on the rates of re-esterification, lipolysis, or TG synthesis in fragments of tissue (data not shown).

Effect of adipocyte concentration on re-esterification rate

These experiments tested the hypothesis that if re-esterification occurs primarily by an extracellular route, then increasing the concentration of adipocytes in the incubation should increase the rate of re-esterification, because more adipocytes would release more FFA to be taken up and re-esterified.

As predicted, incubating isolated adipocytes at high concentration increased the rate of re-esterification (F[1,4] = 32.96, $P = 0.005$). PIA decreased the rate of re-esterification at both adipocyte concentrations (F[1,4] = 102.43, $P = 0.001$; **Fig. 5**, left). Of course, incubating adipocytes at high concentration decreased the specific activity of [^{3}H]palmitate in the incubation more than did incubating adipocytes at low concentration. If this difference is taken into account by calculating re-esterification from the geometric mean of the specific activity of [^{3}H]palmitic acid at the beginning and end of the incubation, the rate of re-esterification in the presence of ADA is indistinguishable from zero in both high and low concentration incubations. Although PIA tended to decrease the rate of re-esterification, the effect was not significant (Fig. 5, right).
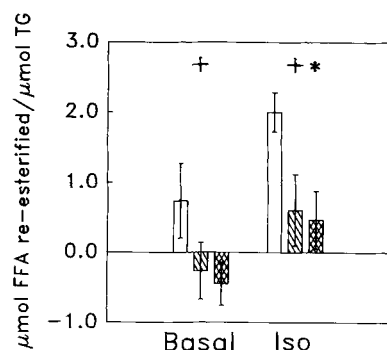

Fig. 1. Re-esterification in tissue fragments vs. isolated adipocytes. Open bars represent isolated adipocytes, hatched bars represent adipose tissue fragments. Values are mean ± SEM; *, $P < 0.05$ for the effect of isoproterenol (Iso); +, $P < 0.05$ for the effect of tissue preparation. Cell concentration was the same for both tissue preparations (fragments: 20,000 ± 6000 cells/ml; isolated adipocytes: 20,000 ± 7000 cells/ml). Mean adipocyte size was 0.690 ± 0.129 μg lipid/cell.

Fig. 3. Effect of incubation shaking speed on re-esterification rate. Open bars represent no shaking, one-way hatching represents shaking at 80 cycles/min, and cross-hatching represents shaking at 160 cycles/min; *, $P<0.05$ for the effect of isoproterenol (Iso); +, $P<0.05$ for the effect of shaking speed. Cell concentration averaged 43,000 ± 12,000 cells/ml. Mean adipocyte size was 0.418 ± 0.050 µg lipid/cell.

Fig. 5. The effect of adipocyte concentration on re-esterification rate. Open bars represent low adipocyte concentration incubations, hatched bars represent high adipocyte concentration incubations; *, $P<0.05$ for the effect of PIA; +, $P<0.05$ for the effect of adipocyte concentration. The left panel shows the influence of cell concentration and PIA on re-esterification when results are calculated using the specific activity of [3H]palmitic acid at the beginning of the incubation. The right panel shows the effect of these variables on re-esterification when the change in specific activity of [3H]palmitic acid during the incubation is accounted for. High cell concentration incubations averaged 313,000 ± 27,000 cells/ml, while low cell concentration incubations averaged 20,000 ± 3,000 cells/ml. Mean adipocyte size was 0.699 ± 0.077 µg lipid/cell.

PIA markedly inhibited lipolysis in all incubations, but because of wide variability in basal values, its effect was not significant when tested by ANOVA. However, the percent change in the rate of lipolysis induced by PIA was significantly different from zero by t-test at both high and low cell concentrations ($P<0.001$; **Fig. 6**, left). High adipocyte concentration tended to increase the rate of TG synthesis, although the effect was not statistically significant. PIA increased the rate of TG synthesis by 15 ± 4% in high concentration incubations and 57 ± 22% in low concentration incubations (F[1,4] = 12.97, $P<0.025$; Fig. 6, right).

Relative effects of exogenous versus endogenous FFA on TG synthesis

If FFA arising from lipolysis must leave the adipocyte and be taken up again before being re-esterified, they may be functionally compartmentalized. This implies that intracellularly, FFA arising from lipolysis have little or no access to the enzymes of esterification. We hypothesized that,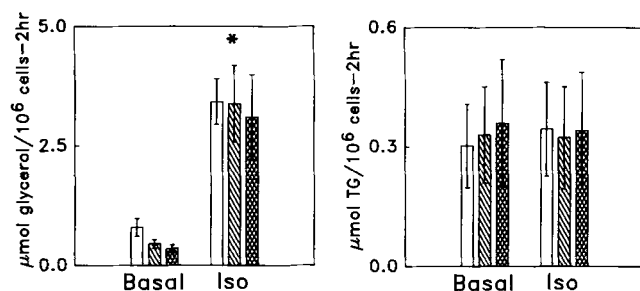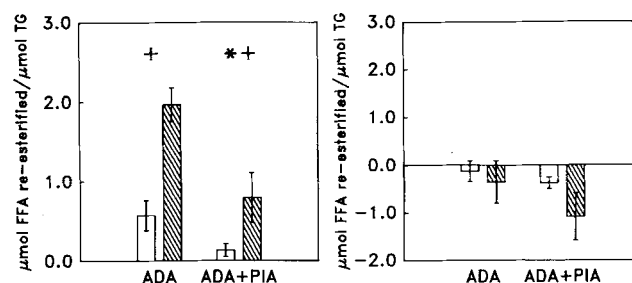 if functional compartmentation exists, exogenous FFA (those added to the incubation medium) would have a greater impact on the rate of TG synthesis than endogenous FFA arising from lipolysis. This was tested by incubating isolated adipocytes in the usual way, with 0.5 mM palmitic acid, or in the absence of exogenous FFA. Both types of incubations were done in the basal state (low rates of lipolysis) and when lipolysis was stimulated.

Consistent with our hypothesis, the addition of palmitic acid to the incubation medium increased the rate of acylglycerol synthesis by ~62% (F[1,4] = 47.53, $P<0.005$), whereas stimulation of lipolysis with isoproterenol or ADA had no effect on the rate of TG synthesis (**Fig. 7**, right).

The addition of palmitic acid (0.5 mM) to incubation medium did not affect the rate of lipolysis. Isoproterenol and ADA increased the rate of lipolysis by ~100% (F[1,4] = 7.64, $P = 0.05$; Fig. 7, left).

Fig. 4. Effect of incubation shaking speed on the rates of lipolysis (left) and TG synthesis (right). Symbols as in Fig. 3.
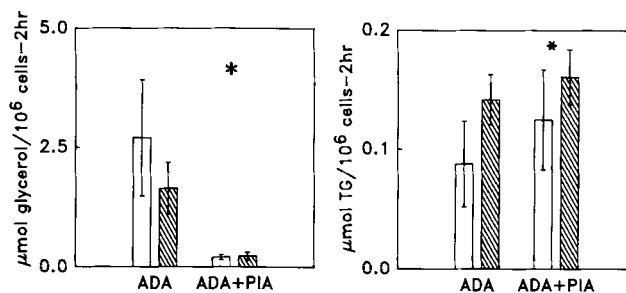

Fig. 6. The effect of adipocyte concentration on the rates of lipolysis (left) and TG synthesis (right). Symbols as for Fig. 5.
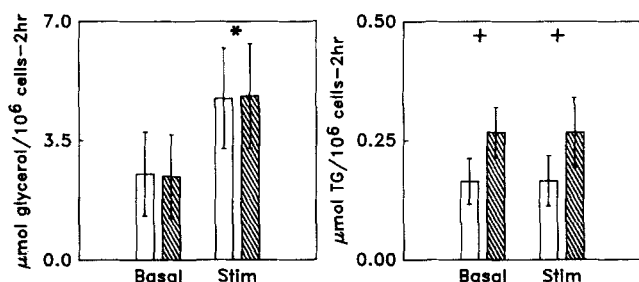
Fig. 7. Relative effects of exogenous vs. endogenous FFA on the rates of lipolysis (left) and acylglycerol synthesis (right). Open bars represent incubations done in the absence of exogenous FFA and hatched bars represent incubations carried out in medium containing 0.5 mM palmitic acid; Stim, incubations carried out in the presence of either ADA (0.4 U/ml) or isoproterenol (10^{-7} M); *, $P < 0.05$ for the effect of ADA and isoproterenol; +, $P < 0.05$ for the effect of palmitic acid. Cell concentration in these incubations averaged $34,000 \pm 6,000$ cells/ml. Mean adipocyte size was 0.661 ± 0.177 μg lipid/cell.

DISCUSSION

Free fatty acid re-esterification was decreased in isolated adipocytes compared to fragments of adipose tissue, in incubations of isolated adipocytes that were shaken compared to those that were not, and in isolated adipocytes incubated at low concentrations compared to high concentrations. To interpret these results, total esterification, which is measured by [^{14}C]glucose incorporation into TG, must be distinguished from FFA re-esterification, which refers here specifically to reutilization of FFA released by lipolysis and is measured by dilution of medium [^{3}H]palmitic acid by unlabeled, endogenous fatty acids in newly synthesized TG. Total esterification may be influenced by changes in glucose transport and metabolism. However, total esterification, as measured by [^{14}C]TG synthesis, was not affected by tissue preparation, shaking speed, or adipocyte concentration. This indicates that the manipulations did not impair the viability of the adipocytes and that changes in the rate of TG synthesis cannot account for changes in the rate of FFA re-esterification. The high variability in rates of [^{14}C]TG synthesis is probably due to the heterogeneity of the subject population for these studies; as noted in Materials and Methods, the BMI (an index of obesity) of these subjects ranged from 19.1 to 50.3.

Changes in the rate of lipolysis can influence the rate of re-esterification by increasing the supply of unlabeled FFA available for re-esterification. None of the experimental manipulations altered the rate of lipolysis, so their effects on FFA re-esterification cannot be attributed to changes in the rate of lipolysis per se. In contrast, the lipolytic agents ADA and isoproterenol stimulated lipolysis and thereby increased re-esterification by increasing the dilution of medium[^{3}H]palmitic acid with unlabeled, endogenous FFA.

The increased re-esterification in fragments of tissue compared to isolated adipocytes is best accounted for by enhanced availability of endogenous (lipolytically derived) FFA. If FFA released during lipolysis are trapped in the extracellular matrix of the tissue, raising the FFA concentration within the fragment rather than in the incubation medium, the trapped FFA would dilute medium[^{3}H]palmitic acid around the adipocytes within the fragment. In contrast, each isolated adipocyte is surrounded by albumin-containing medium, which can effectively bind and disperse FFA as they leave the adipocytes, making them less available for reuptake and re-esterification. It has been shown that the FFA concentration of human adipose tissue fragments is increased when lipolysis is stimulated (7), suggesting that the extracellular matrix of the tissue fragment may trap FFA released from adipocytes (20).

Previous investigators have reported that rates of lipolysis are higher in isolated adipocytes than in fragments of tissue (21). We did not replicate this result. However, this is not surprising, because the rate of lipolysis of isolated adipocytes is influenced by many variables. The source and lot of collagenase and BSA (22), as well as cell concentration (23) and variable removal of adenosine during isolation of adipocytes (24) may affect the so-called "basal" rate of lipolysis. Therefore, comparisons between isolated adipocytes and fragments of tissue are only meaningful under a carefully specified set of conditions.

The effect of shaking speed on FFA re-esterification may also be explained by differences in mixing of released FFA with incubation medium. When the incubation is not shaken, the adipocytes float into a layer on the surface of the medium. FFA released during lipolysis are not dispersed throughout the medium, and instead accumulate in the vicinity of the adipocyte layer, where they are likely to be taken up and re-esterified. In contrast, when the incubation is shaken, mixing of endogenous FFA in the entire volume of incubation medium makes them relatively unavailable for uptake and re-esterification. That shaking speed had no effect on re-esterification in fragments of tissue supports our hypothesis that FFA released by lipolysis accumulate within fragments rather than being released to the incubation medium.

It is unlikely that high rates of shaking damaged the adipocytes because: 1) no more floating oil (indicative of broken adipocytes) was observed after incubations at high shaking speed than at lower speeds; 2) adipocytes incubated at high shaking speed synthesized TG at the same rate as those incubated at lower speeds; and 3) other investigators use high shaking speeds (140 cycles/min) for isolation of human adipocytes without deleterious effects (25).

In the third experiment, high concentration of isolated adipocytes increased the rate of re-esterification by in-

creasing the dilution of medium-derived [³H]palmitic acid with unlabeled, endogenous FFA. When re-esterification calculations are adjusted for this change in specific activity, it is apparent that all of the re-esterification can be accounted for by FFA that have appeared in the incubation medium. That is, there is no evidence for a pathway of re-esterification that occurs entirely within the adipocyte.

High adipocyte concentration, with concomitantly higher medium FFA concentration, tended to decrease the rate of lipolysis, although the effect did not reach statistical significance. The final FFA:albumin molar ratio in high adipocyte concentration incubations was approximately four, compared to a ratio of one for low adipocyte concentration cells. In rat adipocytes, the lowest FFA: albumin molar ratio that inhibits adenylate cyclase is three (26), so the small effect of high adipocyte concentration may be due to the relatively small increment in the ratio above this critical value. Consistent with previous literature (27–29), PIA increased the rate of TG synthesis.

The final experiment indicates that adding exogenous FFA stimulates acylglycerol synthesis, while increasing the supply of endogenous FFA (those arising from lipolysis) has no effect. Other investigators have reported no effect of increasing medium FFA concentration above 0.3 mM on TG synthesis (30). However, our previous data suggest that the effect of added palmitic acid on TG synthesis in human adipocytes is maximal at 0.25 mM palmitic acid (31). This differential effect of endogenous and exogenous FFA on acylglycerol synthesis supports the idea that functional compartmentation of adipocyte FFA exists. This possibility has been raised by Dole (32) for rat adipose tissue. The nature of this compartmentation is speculative, but need not constitute a physical barrier. FFA derived from lipolysis may be channeled out of adipocytes so efficiently that they are unlikely to become diverted into the re-esterification pathway. Such channeling could result from stimulation of a plasma membrane fatty acid transporter (33, 34). Alternatively, newly hydrolyzed FFA may form lamellae that traverse the adipocyte plasma membrane, as described by Blanchette-Mackie and Scow (35).

The addition of 0.5 mM palmitic acid to the incubation did not affect the rate of lipolysis. This is probably due to the high BSA concentration in the incubation medium (5%) which gives a FFA:BSA molar ratio of ~0.7. Since BSA contains many binding sites for FFA per mole (36), the exogenous palmitic acid occupies only a small fraction of the available sites. Many residual sites are left free to bind FFA released by lipolysis, preventing intracellular accumulation of FFA and feedback inhibition on lipolysis (20).

The results of these experiments suggest the following model for FFA re-esterification (see **Fig. 8**). Normally, adipocytes in vivo esterify a mixture of endogenous FFA (those derived from lipolysis) and exogenous FFA (those

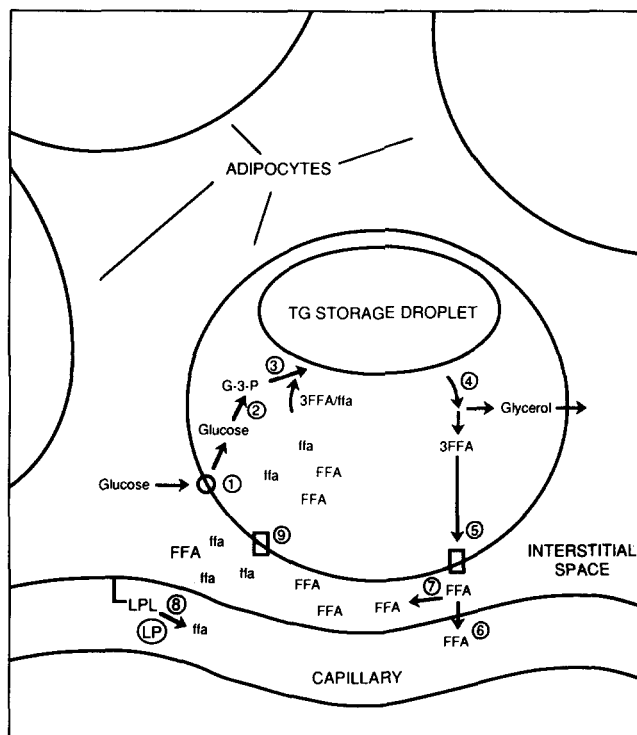## POSSIBLE MECHANISM FOR FFA RE-ESTERIFICATION

Fig. 8. Model for the control of free fatty acid (FFA) re-esterification by blood flow. Adipocytes are shown in situ next to a capillary. (1) Glucose enters the adipocyte by the glucose transporter. (2) Glucose undergoes glycolysis to glycerol-3-phosphate (G-3-P). (3) Glycerol-3-phosphate undergoes a series of acylations to form triacylglycerol (TG) which is stored in the central lipid droplet. (4) TG undergoes hydrolysis by hormone-sensitive lipase and monoacylglycerol lipase, forming glycerol and FFA. Glycerol exits the adipocyte. (5) FFA exit the adipocyte via the fatty acid transporter. (6) From the interstitial space FFA may enter the circulation. (7) Alternatively, newly released FFA may mix with (8) ffa arising from lipoprotein lipase (LPL) hydrolysis of circulating lipoproteins (LP). (9) A mixture of FFA newly released by adipocytes and those derived from plasma (ffa) may then be taken up again from the adipocyte into a pool of FFA destined for esterification. An increase in blood flow would tend to remove newly released FFA from the interstitial space into the circulation, while a decrease in blood flow would allow FFA to accumulate in the vicinity of the adipocyte, increasing the probability of reuptake and re-esterification.

derived from the circulation or from hydrolysis of circulating lipoproteins). Re-esterification takes place largely by an extracellular loop in which FFA leave the adipocyte and are taken up again. This extracellular loop is necessitated by a functional compartmentation of FFA within the adipocyte, which prevents access of lipolytically derived FFA to the enzymes of glycerolipid synthesis.

This model raises the possibility that the rate of reuptake and re-esterification of FFA within adipose tissue may be controlled by tissue perfusion. Experimental support for this idea is derived from studies of the in situ perfused canine inguinal fat pad, which indicate that FFA release from adipose tissue is more dependent than glycerol release upon perfusion rate and albumin concentra-

tion of the perfusate (37). In vivo, the rate of FFA re-esterification may vary with changes in adipose tissue blood flow. While this model suggests that acute control of FFA re-esterification occurs on the physiologic rather than the cellular level, it does not rule out the possibility that chronic alterations in nutritional or hormonal status, such as weight loss (38), may induce changes in the capacity of the adipocyte to re-esterify FFA over the long term. However, the present findings suggest that the extracellular environment may be important in determining the rate of FFA re-esterification and, thus, efflux from adipocytes. 🔳

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