Triacylglycerol turnover in large and small rat adipocytes: effects of lipolytic stimulation, glucose, and insulin

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Abstract Rates of lipolysis and reesterification were determined under various conditions in adipocytes from epididymal fat pads of old, spontaneously obese rats and compared to cells from younger, leaner animals. No differences were observed in lipolytic responsiveness to several concentrations (2–50 μM) of the β-adrenergic agent ritodrine compared to cells from younger, leaner rats. The large cells showed diminished rates of lipolysis, reesterification, and glyceride-glycerol synthesis from glucose of submaximal but not maximal insulin concentrations, probably reflecting decreased large cell receptor numbers. In both cell types reesterification measured in the presence of ritodrine progressively rose with increasing concentrations of glucose in the medium. At each glucose concentration (0.5–25 mM) rates of reesterification were similar in each cell type. When maximal concentrations of insulin (5 nM) were also added, at low glucose concentrations (<5 mM) there was a similar increase in reesterification in large and small cells. No insulin effect in either cell type was observed at high concentrations of glucose. Although fatty acid synthesis from glucose in the large cells was markedly diminished, glyceride-glycerol synthesis was well maintained, correlating well with calculated reesterification rates. In fact, reesterification was found to be quantitatively very important in determining total triacylglycerol turnover in both cell types. High rates of reesterification might not only allow maintenance of triacylglycerol stores, but could also increase metabolic sensitivity to changes in hormonal or substrate concentrations.—**May, J. M.** Triacylglycerol turnover in large and small rat adipocytes: effects of lipolytic stimulation, glucose, and insulin. *J. Lipid Res.* 1982. **23:** 428–436.

Supplementary key words insulin resistance • obesity

Adipose tissue metabolism in old, spontaneously obese rats has been extensively studied as a model for human obesity (1–3). Although comparisons between adipose tissue or cells from older fatter and younger leaner rats may be criticized in that, with unrestricted feeding, the lean rats will eventually become obese, major metabolic differences are evident which appear to characterize established obesity. Thus it has been repeatedly shown that, compared to small cells from lean rats, large adipocytes prepared from fatter rats have diminished insulin-stimulated glucose metabolism, with profound impairment of both basal and insulin-stimulated de novo fatty acid synthesis (1–3). Despite these very low rates of fatty acid synthesis in the fatter animals, triglyceride accretion persists (1, 4). This could be related to continued esterification of exogenous free fatty acids supplied by plasma lipoproteins, or to reesterification of free fatty acids released during lipolysis. In the basal state, there is indirect evidence that both of these mechanisms may be operative. Adipose tissue from large rats has increased lipoprotein lipase activity (5), increased basal rates of esterification of *sn*-[^{14}C]glycerol-3-phosphate with free fatty acids (6), and increased activities of enzymes involved in triacylglycerol esterification (7). Since basal rates of lipolysis have also been reported to be elevated in large rat cells (8–10), total triacylglycerol turnover may be increased.

Although elevated basal rates of triacylglycerol synthesis from fatty acids may help to explain why these rats continue to deposit triacylglycerol in spite of already increased stores, one must also consider triacylglycerol turnover in response to various stimuli present in vivo, including glucose, lipolytic hormones, and insulin. Such studies would provide information regarding both the sensitivity and maximal responsiveness of triacylglycerol turnover. Stimulated rates of lipolysis in large adipocytes have been reported to be similar (8, 9, 11) or greater than (2, 10, 12, 13) rates in small cells. Previous studies (11, 14) have shown that large cells are less sensitive to the antilipolytic effects of low, but not high, insulin concentrations. Comparisons of rates of glyceride-glycerol synthesis from glucose (which under certain conditions may reflect reesterification (15)) have suggested that large cells may have increased absolute basal and insulin-stimulated rates of reesterification (1, 16), but direct measurements of the balance between lipolysis and reesterification are lacking. In particular, it would be important to determine whether the insulin resistance observed in large cells extends to free fatty acid reesterification, a process that is markedly increased by insulin in the presence of glucose in smaller cells (17). The present studies were therefore performed to compare rates

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of lipolysis and reesterification in large and small cells under various conditions, as well as to ascertain factors important in regulating triacylglycerol turnover. The results indicate that reesterification is not suppressed in the large cells and that reesterification plays a major role in triacylglycerol turnover in both large and small cells.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River Laboratories, Boston, MA) were used for all experiments. The younger, leaner animals weighed 140–160 g, and the older, fatter animals weighed over 500 g. The animals were fed standard rat chow ad libitum up to the time of killing.

Isolation of adipocytes

Animals were stunned by a blow to the head and decapitated, and the epididymal fat pads were removed. Adipocytes were isolated according to the method of Rodbell (18). The pads were slightly minced and portions (about 1 g) were digested with 1 mg/ml collagenase (Worthington Biochemicals, Freehold, NJ) and 40 mg/ml bovine serum albumin (Reheis Chemical Co., Elkhart, IN) in 4 ml of Krebs-Ringer bicarbonate buffer, pH 7.4. The digestion was performed at 37°C with mild shaking for 45–60 min. Cells from large rats usually were digested for the shorter time period to avoid breakage. After digestion, the cells were filtered through 250 μm nylon mesh and washed three times by centrifugation with Krebs-Ringer bicarbonate buffer containing 40 mg/ml bovine serum albumin. Cells were counted in duplicate by the hemocytometer method of Gliemann (19). Sizing of cells was performed with a calibrated microscope. The average diameter of the small rat cells studied was 58 ± 10μm, while that of the large rat cells was 105 ± 20 μm.

Incubations and assays

Low concentrations of cells (0.5–1.5 × 10^5/ml) were used in the incubations to minimize possible inhibitory effects of adenosine on lipolysis (11, 20–22). Cells were incubated with mild shaking in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 40 mg/ml bovine albumin for 1 h at 37°C, except as noted. Where indicated, glucose, ritodrine, and insulin were added at the beginning of each incubation. Under such conditions there was less than 10% cell loss in either cell type during a 1-hr incubation (not shown).

Glucose metabolism to glyceride-fatty acid and glyceride-glycerol was assessed in parallel with the studies of lipolysis and free fatty acid release. For the measurement of glucose metabolism, each incubation (1 hr) performed in triplicate contained 0.5 μCi of D-[U-^{14}C]glucose (Amersham) with the indicated amount of unlabeled glucose and other additions as noted. Blank samples containing no cells were treated similarly to experimental samples, and the values obtained were subtracted from appropriate experimental values. Incubations were terminated by removing 1.8 ml of the swirled cell suspension into 4 ml of chloroform–methanol 1:1 (23), followed by vigorous vortexing for 10 sec. The extraction was allowed to proceed overnight at −20°C. The aqueous upper layer was removed and discarded, and 2 ml of an upper phase wash was added. This wash solution consisted of methanol–aqueous KCl solution (7.4 mg/ml) 1:1 and effectively lowered background counts in the lipid fractions. The sample was vortexed vigorously for 10 sec, centrifuged for 5 min at 800 *g*, and the aqueous layer was again removed and discarded. After repeating the upper phase wash once more, the chloroform layer was carefully removed and taken to dryness, either overnight or for about 30 min at 55°C, both in a fume hood. The residue was saponified for 2 hr at 60°C with 2 ml of saturated ethanolic KOH. After the samples had cooled, they were acidified with 4 N HCl to at least pH 1 and extracted three times with 2-ml volumes of heptane. The heptane extract was dried and the residue was taken up in 10 ml of toluene scintillant (24) for counting at 84% efficiency. The remaining ethanolic solution was centrifuged 5 min at 800 *g*, and the supernatant was taken to dryness overnight. The residue was dissolved in 1 ml of water followed by 10 ml of ACS (Amersham) and counted at 60% efficiency. All liquid scintillation counting was done in a Chicago Nuclear Mark II liquid scintillation spectrometer.

Assays of glycerol released into the medium and total free fatty acids in cells and buffer were performed as follows. At the completion of incubation (1 hr), cells and buffer were swirled and an aliquot (0.1 or 0.2 ml) was removed for free fatty acid analysis. The remaining cells were allowed to float to the surface (1 min) and 0.25 ml of buffer was carefully removed from underneath for assay of glycerol by the method of Chernick (25). Cell and buffer free fatty acid contents were determined by the method of Laurell and Tibling (26). Intracellular free fatty acids were determined in duplicate by the method of Angel, Desai, and Halperin (27), using [^{3}H]inulin to correct for free fatty acids bound to albumin remaining in the cell "float".

Rates of reesterification were calculated by the balance method described by Vaughn (28), in which nmoles of free fatty acid reesterified equal three times glycerol release minus the net amount of free fatty acids generated. Several underlying assumptions and limitations of this method have been discussed in detail (29). Since glycerol

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is released quantitatively into the medium (28), only buffer glycerol was measured. It was necessary to correct for the glycerol and free fatty acids present in cells and albumin at the start of the incubations (29). The balance method does not account for fatty acids synthesized de novo and esterified. However, it is apparent from calculations in the Discussion that under lipolytic stimulation even in the small cells this is relatively small compared to rates of reesterification. Rates of glycerol and free fatty acid production were linear for 60–90 min.

The β-adrenergic lipolytic agent ritodrine hydrochloride used in all studies was provided by Phillips-Duphar, B.V. This agent was used because it has been found to be resistant to degradation in incubations with adipocytes (30).

Statistical comparisons of treatment responses of one cell type (large or small cells) were made with the Student's paired "t" test, while those between the large and small cells were made with the non-paired "t" test. Linear regression was calculated in the standard manner by the method of least squares. Error bars show standard error of the mean calculated for the indicated number of experiments.

RESULTS

The lipolytic responses to varying doses of the β-adrenergic agent ritodrine are shown in **Fig. 1.** Large and small cells released similar amounts of glycerol at all ritodrine concentrations. Although not shown, lipolytic responses of large and small cells were also similar in the presence of 5 nM insulin in each cell type. A sub-

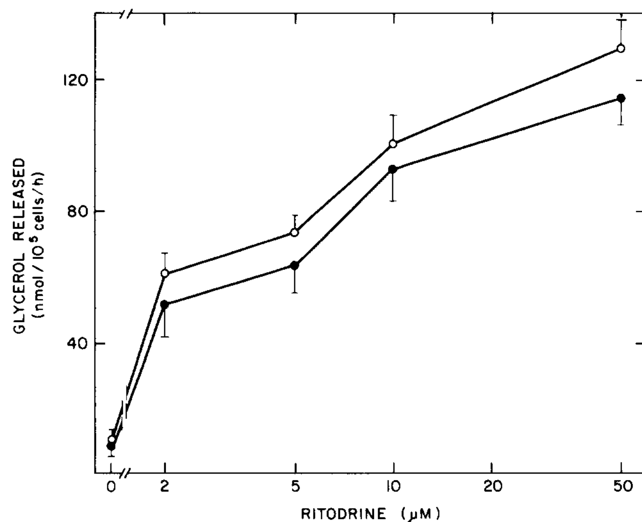

Fig. 1. Stimulation of lipolysis by increasing concentrations of ritodrine in large (○) and small (●) adipocytes. Data represent means of four experiments with each cell type.

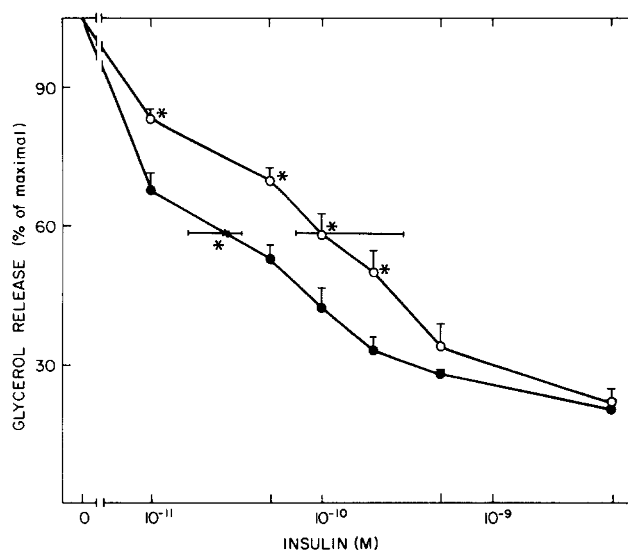

Fig. 2. Antilipolysis in large (○) and small (●) cells in response to increasing concentrations of insulin. Glycerol release was expressed as a percentage of rates with ritodrine (5 μM) alone for six experiments with each cell type. Rates of lipolysis stimulated by ritodrine alone were 99 ± 15 nmol/10^5 cells/hr in the large cells, and 95 ± 11 nmol/10^5 cells/hr in the small cells. An asterisk denotes $P < 0.05$ compared to cells of the other type. Insulin concentrations of half-maximal activity ± SE are shown for each curve. These were calculated from individual experiments and averaged together.

maximal ritodrine concentration of 5 μM was selected for use in subsequent studies.

Although glycerol release at 5 μM ritodrine was similar in both cell types (Fig. 1), this was not the case when submaximal concentrations of insulin were also added (**Fig. 2**). The antilipolytic effect of insulin at lower concentrations was diminished in the large cells, resulting in a higher concentration of insulin required for a half-maximal effect. The concentrations of half-maximal insulin effects are shown for each cell type.

The relative insulin resistance of the large cells was also apparent in studies of fatty acid reesterification. The experiments shown in **Fig. 3** were performed in the presence of 5.0 mM glucose, 5 μM ritodrine, and various concentrations of insulin as noted. Free fatty acid reesterification rates were calculated by the balance method. The maximal stimulation of reesterification of high insulin concentrations was similar (about twofold) in large and small cells. At lower concentrations the large cells displayed a significantly reduced response to the hormone, resulting again in a higher hormone concentration for half-maximal activity. In these experiments insulin decreased both glycerol release and free fatty acid contents of cells and buffer, as expected. However, the decline in free fatty acid contents was relatively greater, with the net result that both total and percent reesterification rose compared to reesterification with glucose and ritodrine alone. Further evidence that insulin ac-

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tually did increase reesterification is derived from measurement of glyceride-glycerol synthesis as shown in **Fig. 4.** Both large and small cells had about a threefold increase in glyceride-glycerol synthesis in response to maximal insulin concentrations in the presence of 5 μM ritodrine. Once again, the insulin concentration at which half-maximal activity occurred was higher in the large cells, as noted in the figure. Comparison with the curves for antilipolysis (see Fig. 2) which were obtained in parallel incubations shows that the half-maximally effective concentration of insulin was 2.5- to 3-fold less for lipolysis than for glyceride-glycerol synthesis. Although cells from large rats had decreased responsiveness of lipolysis and reesterification to submaximal doses of insulin, in each instance the rates in large and small cells were comparable at maximal insulin concentrations.

Fig. 5 plots reesterification rates as a function of glucose concentration in the medium. In both cell types reesterification increased progressively to a similar extent with increasing glucose concentrations in the presence of ritodrine. When maximal insulin concentrations were also added to the incubations, there was a further rise in rates of reesterification at each glucose concentration except the highest. The glycerol and free fatty acid responses that generated the ritodrine-stimulated reesterification curve in small cells of Fig. 5 are shown in **Fig. 6.** At subphysiologic glucose concentrations both glycerol release and free fatty acid contents were increased. At 10 mM glucose and above glycerol release plateaued, while free fatty acid content declined. These changes generated the net rise in calculated rates of reesterification shown in Fig. 5. Similar patterns were observed

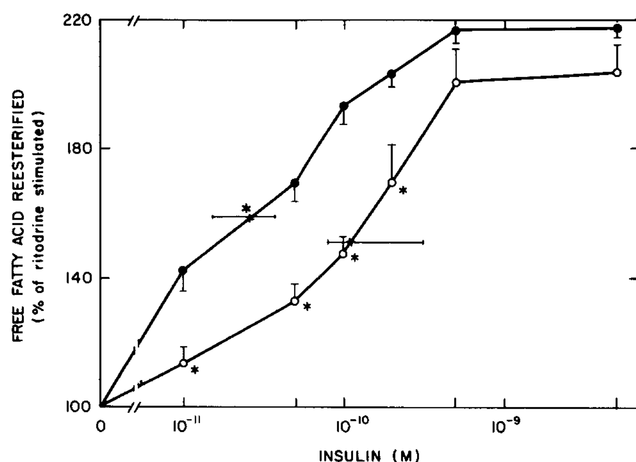

Fig. 3. Insulin-stimulated reesterification in large (O) and small (●) cells in the presence of 5 μM ritodrine and 5 mM glucose. Reesterification was expressed as a percentage of that observed with ritodrine and glucose. Rates of reesterification in the presence of ritodrine and glucose were 90 ± 11 nmol/10^5 cells/hr in the large cells, and 78 ± 11 nmol/10^5 cells/hr in the small cells. The insulin concentration producing half-maximal effect is shown for each cell type ($P < 0.05$).

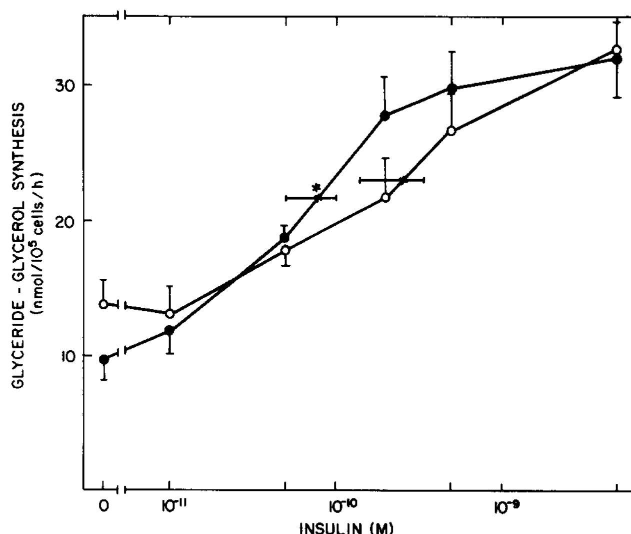

Fig. 4. Insulin responsiveness of glyceride-glycerol synthesis from D-[U-^{14}C]glucose (5 mM) in large (O) and small (●) adipocytes. Ritodrine (5 μM) was also present in each sample. The data are from six experiments in each cell type. The insulin concentrations of half-maximal effect are indicated on each curve, with an asterisk (*) indicating $P < 0.05$ between values in the respective cell types.

with insulin added, although of course glycerol release and free fatty acid contents were generally lower due to the antilipolytic effect of the hormone (not shown).

Fig. 7 presents the de novo production of glyceride-fatty acid from [^{14}C]glucose expressed as a function of increasing medium glucose. As expected, the large cells had very low rates of fatty acid synthesis even in the presence of insulin. The small cells synthesized increasing amounts of glyceride-fatty acid with increasing medium glucose, and a 2- to 5-fold stimulation by insulin was observed (Fig. 7).

Synthesis of glyceride-glycerol, on the other hand, was maintained in the large cells compared to the small cells over a wide range of glucose concentrations (**Fig. 8**). Maximal insulin-responsiveness was also similar in the two cell types, at each glucose concentration (Fig. 8). These results again provide evidence that reesterification was proceeding at comparable rates in each cell type regardless of the glucose concentration.

The parallel effects of glucose on ritodrine-stimulated free fatty acid reesterification (Fig. 6) and glyceride-glycerol synthesis from glucose (Fig. 8) are consistent with the interpretation that under these conditions reesterification is dependent upon glucose availability. Comparison of rates of reesterification with rates of glyceride-glycerol synthesis (with and without insulin) showed excellent correlation for both the small adipocytes ($r = 0.98$, $P < 0.01$) and large adipocytes ($r = 0.98$, $P < 0.01$).

Intracellular free fatty acid concentrations were studied under various conditions as shown in **Table 1.** Basal

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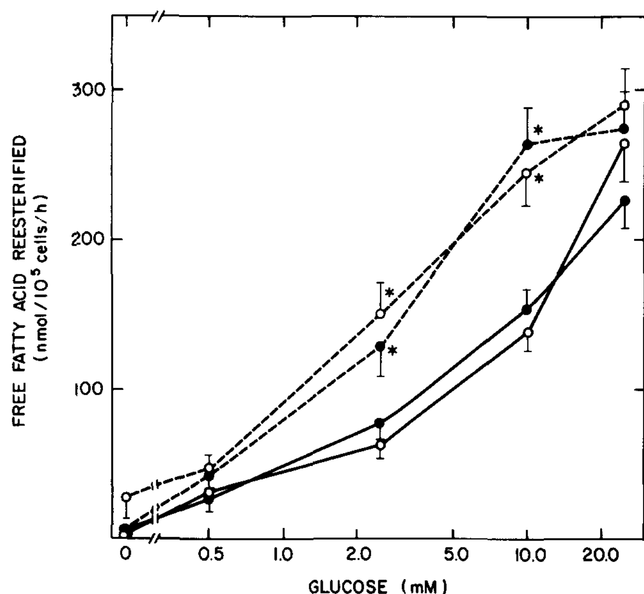

Fig. 5 Enhancement of calculated rates of reesterification in large (O) and small (●) adipocytes by increasing concentrations of glucose. The solid lines show the results of ritodrine (5 μM) treatment alone, while the broken lines indicate responses with ritodrine (5 μM) and insulin (5 nM). The data are from seven experiments in each cell type. An asterisk (*) indicates $P < 0.05$ compared to cells of the same type not exposed to insulin.

free fatty acid contents were similar, but ritodrine stimulation resulted in significantly higher intracellular free fatty acid concentrations in the small than in the large cells. This effect appeared to be preserved under the other conditions tested, except with both insulin and glucose present (Table 1). The effect of high insulin concentrations on the decrease of intracellular free fatty acids relative to ritodrine alone was similar in the two cell types (between 37 and 39% of control) with or without glucose. Glucose had no effect on free fatty acid concentrations. Since reesterification was occurring with glucose present, it is apparent that intracellular free fatty acid contents remained remarkably constant, in spite of marked changes in free fatty acid fluxes induced by the agents.

DISCUSSION

Triacylglycerol turnover in adipose tissue is determined by rates of lipolysis and free fatty acid incorporation into triacylglycerol; the balance leads to either fat deposition or removal (29). In the present studies triacylglycerol turnover was compared in adipocytes from obese and lean rats. With regard to lipolysis, it was found that basal and stimulated rates were similar in each cell type (Fig. 1). Basal lipolysis has been reported to be higher in cells from large rats in most (1, 9, 12, 37) but not all (11) studies. In the present work and in studies by Olefsky (11), low cell numbers were used to avoid inhibitory effects of adenosine (20–22) under stimulated conditions. At least in this work basal lipolysis was often undetectable. This probably accounted for the observed lack of difference between the two cell types. Stimulated rates of lipolysis have been found to be either the same (8, 9, 11) or increased (2, 10, 12, 13) in the large rat cells.

Evaluation of rates of esterification of newly synthesized or preformed fatty acids is complicated by a requirement for glucose (Fig. 5) (15, 17, 31–33). When glucose was present in increasing amounts, both large and small cells showed progressive and comparable rises in rates of free fatty acid incorporation into triacylglycerol, when assessed either by a balance method (which measures reesterification) (Fig. 5) or by glyceride-glycerol synthesis from glucose (which reflects both esterification of newly synthesized fatty acids and reesterification) (Fig. 8). One might have expected higher rates of glyceride-glycerol synthesis in the large cells than in the small cells on the basis of previous findings (1, 3, 16). The lack of such a difference in this study may have been related to the presence of 5 μM ritodrine in all incubations in Figs. 4 and 8. Thus, stimulated triacylglycerol turnover is well maintained in the cells from large rats, in spite of the virtual absence of de novo fatty acid synthesis.

On the other hand, it was possible to demonstrate that large rat adipocytes had decreased sensitivity to several biologic effects of insulin. At submaximal concentrations, the large cells were found to be less sensitive to effects

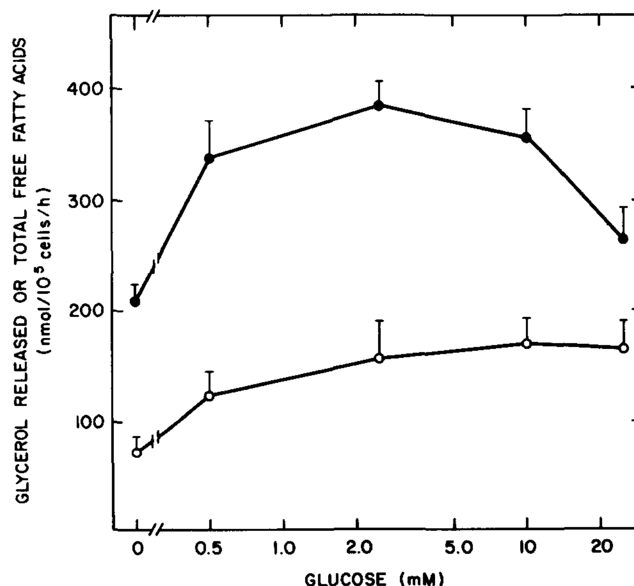

Fig. 6. Free fatty acid (O) and glycerol (●) responses which generated the data shown in Fig. 5 for small cells in the absence of insulin.

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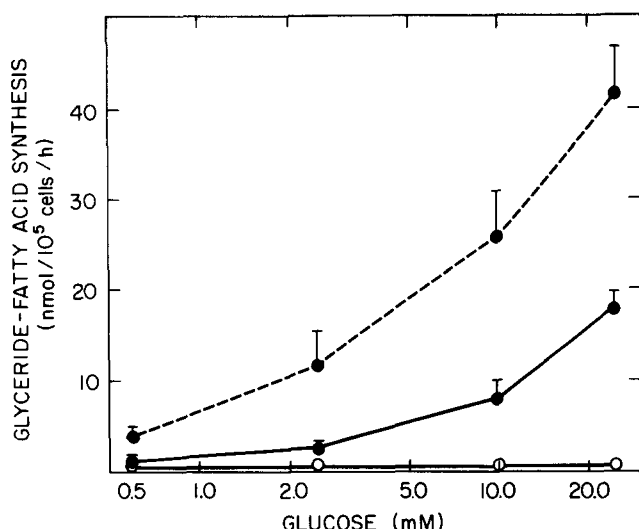

Fig. 7. Glyceride-fatty acid synthesis from increasing concentrations of D-[U-^{14}C]glucose in large (○) and small (●) cells incubated with 5 μM ritodrine. The broken line indicates small cell responses in the presence of 5nM insulin. In the large cells, basal and insulin-stimulated rates were very low and are shown here by the single solid line. Data are from six experiments in each cell type.

of the hormone on lipolysis, reesterification, and glyceride-glycerol synthesis from glucose (Figs. 2–4). The decreased insulin sensitivity of large cell lipolysis (11) and glucose metabolism (11, 34) has been attributed to decreased large cell insulin receptors by Olefsky (34), although others did not find decreased receptors (35, 36). If insulin receptor numbers are decreased on the large cells, this would also provide an explanation for the effects of insulin in large cell reesterification, which is determined at least in part by glucose metabolism. At high insulin concentrations there was no impairment of large cell antilipolysis, reesterification, or glyceride-glycerol synthesis (Figs. 2–4). Since all receptors should be occupied at such high insulin concentrations, biologic effects of the hormone should be similar unless there is also a defect in metabolism beyond the receptor (3, 34). A marked post-receptor block does appear to be present in large cell fatty acid synthesis from glucose (Fig. 7) (3, 34), but the present studies show that this does not extend to antilipolysis and reesterification.

In both large and small cells there was a dependence of reesterification and glyceride-glycerol synthesis on glucose availability in the presence of a lipolytic agent. This is shown by the progressive increases in rates of reesterification when glucose concentrations in the medium were raised (Fig. 5), by the further increase when insulin was also present (Fig. 5), and by the close correlation between glucose incorporation into glyceride-glycerol and calculated rates of reesterification. The large cells in particular might have had higher rates of reesterification if more α-glycerophosphate had been available from glucose metabolism, since these cells have been reported to have increased enzymatic capacity for fatty acid esterification (7). On the other hand, considering the profound impairment in large cell fatty acid synthesis as well as total glucose metabolism documented by others (1, 8), one might have expected lower rates of reesterification in the large cells. However, there appears to be preferential utilization of glucose and other substrate carbon for glyceride-glycerol synthesis in the large cells (16)[1]. It might even be speculated that more glucose carbon was available for reesterification because of diversion from other pathways with impaired flow.

Although basal contents of free fatty acids were similar in both cell types and rose with ritodrine stimulation, there was a smaller increment in the large cells (Table 1). The significance of this finding is uncertain, since at 1 hr the total free fatty acid contents were similar in each cell type (Fig. 5). It should also be noted that DiGirolamo et al. (37) found several-fold higher free fatty acid contents in adipocytes than reported herein, and that fatty acid contents in untreated cells were a function of cell size. The cause of these discrepancies is not known, but may be related to differences in transfer of free fatty acids in the adipocyte system, since free fatty acids may be rapidly lost to the external medium. This may depend on such factors as cell surface area, albumin concentrations, and free fatty acid binding capacity of the albumin. Nevertheless, both large and small cells had similar rel-

[1] May, J. M. Unpublished results.

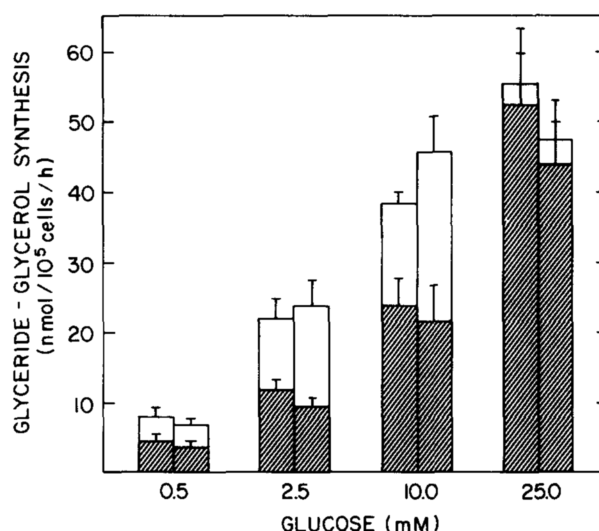

Fig. 8. Glyceride-glycerol synthesis in large (first bar of each pair) and small (second bar) cells incubated with increasing concentrations of D-[U-^{14}C]glucose. The hatched bars show responses with 5 μM ritodrine and open bars denote responses with 5 nM insulin also present. Data are from five experiments in each cell type.

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TABLE 1. Intracellular free fatty acid contents of large and small rat adipocytes

Treatment	Intracellular Free Fatty Acid Contents		
	Small Rat Cells	P^a	Large Rat Cells
		(nmol/10⁵ cells)	
None	3.32 ± 0.45	NS	2.60 ± 0.3
Ritodrine	9.48 ± 0.92[b]	<0.01	5.30 ± 0.66[b]
Ritodrine + insulin	5.87 ± 0.69[c]	<0.01	3.36 ± 0.49[c]
Ritodrine + glucose	8.67 ± 0.82	<0.05	5.88 ± 0.83
Ritodrine + insulin + glucose	5.28 ± 0.98[c]	NS	3.66 ± 0.55[c]

The data shown are from six separate experiments in each cell type. Ritodrine (5 μM), glucose (5 mM), and insulin (5 nM) were included where noted from the beginning of the incubations (30 min).
[a] Comparisons between animals.
[b] Indicates $P < 0.01$ compared to control cells of the same cell type.
[c] Indicates $P < 0.01$ compared to ritodrine-treated cells of the same cell type.

ative declines in intracellular free acids in the presence of ritodrine and insulin.

Quantitatively, the balance between lipolysis and reesterification may be much more important in determining acute changes in triacylglycerol mass than the effects of de novo fatty acid synthesis, even in small rat cells. For example, one can consider the amount of fatty acid synthesized from glucose by the small rat cells under ritodrine and insulin stimulation at 2.5 mM glucose (see Fig. 7). Thus 11.5 nmol of glucose were incorporated into glyceride-fatty acid per 10^5 cells in 1 hr under these conditions. Assuming an average chain length of 16 carbons, and that only glucose carbon is used in the synthesis, 11.5 nmol of glucose carbon would produce 4.3 nmol of fatty acid per 10^5 cells in 1 hr. The amount of glyceride-glycerol synthesized under the same conditions (Fig. 8) would be 48 nmols per 10^5 cells in 1 hr, since 24 nmol of glucose were incorporated into glyceride-glycerol, and since two glyceride-glycerol molecules can be formed from each glucose molecule. The total amount of free fatty acid esterified would be 3×48 nmol or 144 nmol/10^5 cells in 1 hr, since each glyceride-glycerol may be esterified with three free fatty acids. With certain assumptions (15, 29), glyceride-glycerol synthesis from glucose provides a reasonable estimate of reesterification. Although substantial amounts of labeled glycerol may be released into the medium under lipolytic stimulation (38), the loss of glyceride-glycerol radioactivity appeared to be minimal under the present conditions. It can be noted that the 144 nmol derived above compares favorably with the 128 nmol calculated for reesterification by the balance method (Fig. 5). Thus, in small cells under stimulated conditions, greater than 33 times more free fatty acid is synthesized into triacylglycerol than provided by de novo fatty acid synthesis, the difference being made up by reesterification of free fatty acids. In the large cells essentially all of the glyceride-glycerol synthesis represents reesterification, since rates of fatty acid synthesis are negligible (Fig. 7).

The process of free fatty acid release during lipolysis with subsequent reesterification may be considered a "substrate" or "futile" cycle (39, 40). Such cycles may play important roles in regulation of metabolic processes. As defined by Newsholme and Crabtree (41), increased rates of cycling increase the sensitivity of a system to regulatory influences. This effect is evident in the triacylglycerol cycle as diagrammed in **Fig. 9.** The rate of cycling is equal to the rate of reesterification as defined by the balance method, since glycerol release accurately reflects lipolysis (ie., glycerol is not further metabolized once released) and since metabolism of free fatty acid by pathways other than reesterification is minimal (for review, see Ref. 29). The rate of flux through this system is the rate of free fatty acid production. The effects of substrate cycling on flux through the system are exemplified by data derived from Figs. 5 and 6 from small rat cells. In the absence of glucose (ritodrine present) there was minimal reesterification (Fig. 9). Insulin at maximal doses inhibited free fatty acid production by about 70% in the small cells (Fig. 9). When 10 mM glucose was present, substrate cycling or reesterification increased as did the rate of flux. When insulin was added, cycling was further increased, but flux was markedly decreased to less than 10% of rates without insulin. Thus, when glucose was present cycling increased, and the effect of insulin on the decrease in the flux of free fatty acids was enhanced. That is, the increase in substrate cycling induced by glucose appeared to increase the responsiveness of the system to insulin (41). Had we considered lower doses of insulin, the sensitivity of the system

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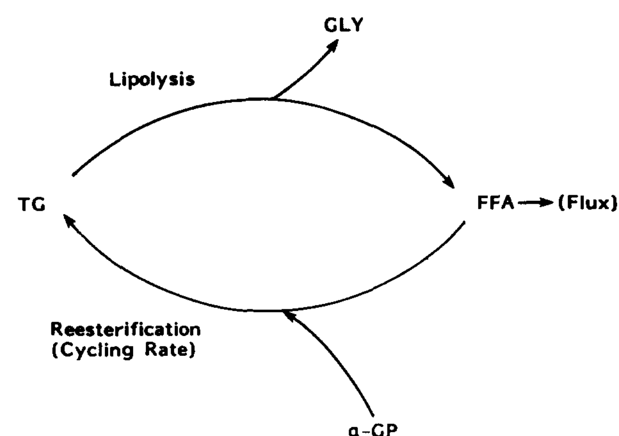

Condition	Insulin	Cycling Rate	Flux
Ritodrine	-	3	207
	+	9	79
Ritodrine +	-	154	356
glucose	+	264	30

Fig. 9. Substrate cycling in triacylglycerol turnover. Data are derived from Figs. 6 and 7 in small adipocytes. The units of reesterification and flux are nmol/10^5 cells per hr. Abbreviations: TG, triacylglycerol; GLY, glycerol; α-GP, α-glycerophosphate; FFA, free fatty acids.

might also have been increased (41). Substrate cycling in the triacylglycerol cycle has also been shown to consume metabolic energy (42), another process which may regulate the expansion or contraction of triacylglycerol stores.

One additional feature of triacylglycerol turnover that merits discussion is the finding that low glucose concentrations actually increased stimulated lipolytic rates in both large and small cells (cf. Fig. 6). Such effects have been observed by others and have been correlated with the metabolism of glucose (31–33). The effect had been attributed to lowering of intracellular free fatty acids by glucose-induced reesterification, which in turn would release the lipolytic process from feedback inhibition by free fatty acids (31). However, as was evident in two previous reports (34, 37), and confirmed in the present study, intracellular free fatty acid contents are not affected by physiologic glucose concentrations (Table 1). Total cell and buffer free fatty acid contents actually increase over the glucose concentration range when enhanced lipolysis occurs (Fig. 6). It therefore does not appear that a fall in intracellular free fatty acids induced by glucose accounts for the observed changes in lipolysis. One is left, therefore, with the possibility that glucose or a metabolite of glucose directly affects the lipolytic process (31).

In conclusion, in spite of markedly decreased rates of fatty acid synthesis in adipocytes prepared from obese rats, triacylglycerol turnover is well maintained in these cells by reesterification rates comparable to those found in small rat adipocytes. Furthermore, depending on the degree of lipolytic stimulation and substrate provision in vivo, lipolysis and reesterification may play major roles in regulating the rate and direction of adipose tissue triacylglycerol turnover.

This work was supported by a Clinical Investigator Award (AM 00651) from the NIH, and by a grant from the American Diabetes Association. The author appreciates the technical assistance of Terre Williams and the typing of the manuscript by Mary Graham and Jessie Reynolds. The author also appreciates review of the manuscript by Drs. Franklin J. Zieve and Gordon C. Weir.

Manuscript received 17 June 1981 and in revised form 6 November 1981.

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