

## STRUCTURE-FUNCTION RELATIONSHIPS IN THE ADIPOSE CELL

### III. Effects of Bovine Serum Albumin on the Metabolism of Glucose and the Release of Nonesterified Fatty Acids and Glycerol by the Isolated Adipose Cell

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#### ABSTRACT

Serum albumin stimulates the uptake of U-glucose- $^{14}\text{C}$  and the incorporation of  $^{14}\text{C}$ -counts into triglyceride glycerol and inhibits the incorporation of  $^{14}\text{C}$ -counts into triglyceride fatty acids by isolated adipose cells; insulin and epinephrine enhance these effects. In the absence of hormones, these responses to albumin increase with increasing albumin concentration. In the presence of insulin, a qualitatively similar pattern of increasing responses to albumin is observed; the enhancement of each response by insulin is, however, only slightly potentiated by higher albumin concentrations. In contrast, in the presence of epinephrine, these responses to albumin are maximal at the lowest albumin concentration tested, 0.1%; the enhancement of each response by epinephrine is similarly maximal at 0.1% albumin, but decreases rapidly as the albumin concentration is raised. Increasing serum albumin concentrations do, however, stimulate the release of fatty acids and glycerol by epinephrine-treated cells increasingly until a plateau, determined by the epinephrine dose, is reached. These data support the suggestion that intracellular fatty acid levels function in the regulation of adipose cell activity, and further suggest that serum albumin plays a role in determining the metabolic fate of these fatty acids.

#### INTRODUCTION

The first paper in this series demonstrated an extensive network of vesicles and smooth-surfaced endoplasmic reticulum in the cytoplasm of the adipose cell suggestive of a membrane-bound transport and metabolic system (1). The second paper in this series confirmed the existence of an extracellular-intracellular vesicular transport system with the demonstration of an epinephrine-sensitive pinocytotic activity in the adipose cell (2).

Correlations between the adipose cell's metabolism and its pinocytosis suggested that intracellular nonesterified fatty acid levels might play a role in the regulation of both of these activities and that pinocytosis in the adipose cell might function in nonesterified fatty acid transport (2).

The possible role of a pinocytotic system in the transport of nonesterified fatty acids (2-4), the potential capacity of such a pinocytotic system for

the uptake of serum albumin from the extracellular space (2, 5, 6), and the well established function of albumin as a fatty acid carrier in serum (7-10) have suggested that alterations in the availability and fatty acid-binding capacity of serum albumin, provided in the incubation medium, would considerably influence the metabolic function of the adipose cell. This paper describes the effects of serum albumin on the metabolism of the isolated adipose cell.

#### METHODS

Isolated adipose cell suspensions were prepared from rat epididymal fat pads and were incubated experimentally as previously described (1, 2). Since the concentration of defatted serum albumin (1) in the incubation medium was to vary, albumin was added only to the experimental vessels, and the adipose cells were isolated in Krebs-Ringer-bicarbonate (KRB) buffer alone.

U-glucose- $^{14}\text{C}$ , insulin, and epinephrine were added to the incubation vessels, and  $^{14}\text{C}$ -counts incorporated from U-glucose- $^{14}\text{C}$  into  $\text{CO}_2$  by the isolated adipose cells were measured as previously described (1).  $^{14}\text{C}$ -counts incorporated from U-glucose- $^{14}\text{C}$  into total lipid by the cells were measured by preparing total lipid extracts of the cells in heptane (10, 11), washing the extracts with 0.6% NaCl to free them of contaminating U-glucose- $^{14}\text{C}$ , and transferring portions of the washed extracts to tared, glass scintillation vials. The heptane was evaporated with a stream of warm air from a hair dryer, the vials were reweighed, 18 ml of scintillation fluid (1) were added to each vial, and  $^{14}\text{C}$ -counts were obtained. Results were expressed as total lipid  $^{14}\text{C}$ -counts per minute per ml of incubated cell suspension.

In preparing the total lipid extract, cells and incubation medium were processed together. In addition to triglyceride, this extract contained any fatty acids present within both the cells and the medium. However, even under conditions which should have increased intracellular fatty acid levels, such as treatment with epinephrine, the total fatty acid content of the cells, studied separately, remained below the limits of detection, 0.05  $\mu$  equivalents. Fatty acids present in the total lipid extract could, therefore, be equated with fatty acids released. These were quantitated by titration as described by Dole and Meinertz (12) and modified by Stone et al. (13) who used sodium ethoxide as the titrant and thymol blue in absolute ethanol as the indicator solution. This modification made it possible to carry out the titration in one phase. Results were expressed as  $\mu$  equivalents fatty acid per volume of incubated cell suspension.

$^{14}\text{C}$ -counts incorporated from U-glucose- $^{14}\text{C}$  into triglyceride fatty acids by the isolated adipose cells were measured as described by Rodbell (11), except

that saponification was carried out by incubating the dried total lipid extract with the saponification mixture at 60°C overnight in glass-stoppered test tubes. Results were expressed as triglyceride fatty acid  $^{14}\text{C}$ -counts per minute per ml of incubated cell suspension.  $^{14}\text{C}$ -counts incorporated from U-glucose- $^{14}\text{C}$  into triglyceride glycerol by the isolated adipose cells were taken to be the difference between total lipid and triglyceride fatty acid  $^{14}\text{C}$ -counts (11). Results were expressed as triglyceride glycerol  $^{14}\text{C}$ -counts per minute per ml of incubated cell suspension.

Glycerol released into the incubation medium by the isolated adipose cells could be measured only after cells and incubation medium had been separated.<sup>1</sup> Separation was carried out either by centrifuging portions of incubated cell suspension for 10 min in a clinical centrifuge at high speed in the cold, or by passing portions through nylon Millipore filters (Millipore Filter Corp., Bedford, Mass., NCWP-02500) supported on the plastic filter-support portion of Swinnex-25 filter units (Millipore Filter Corp., SW0002500) to which had been attached short sections of polyethylene tubing. Glycerol was assayed by coupling the enzymatic reactions of glycerokinase and  $\alpha$ -glycerophosphate dehydrogenase as described by Wieland (14). Results were expressed as  $\mu$ moles glycerol per ml of incubated cell suspension.

#### RESULTS

Serum albumin stimulates the incorporation of  $^{14}\text{C}$ -counts from U-glucose- $^{14}\text{C}$  in the incubation medium into total lipid by untreated and both insulin-treated and epinephrine-treated isolated adipose cells (Figs. 1, 2, and 4). In addition, albumin enhances the proportion of total lipid  $^{14}\text{C}$ -counts accounted for by triglyceride glycerol at the expense of that accounted for by triglyceride fatty acids (Figs. 1, 3, and 5).

In the absence of hormones, these effects of serum albumin increase in magnitude with increasing albumin concentration (up to one and one-half-fold at 4% for  $^{14}\text{C}$ -counts incorporated into total lipid [Fig. 1]) but take place primarily over the lower range, between 0 and 2%. In the presence of 100  $\mu\text{U}$  insulin per ml, a qualitatively similar pattern of increasing responses to albumin is observed (Figs. 2 and 3). The enhancement of each response by insulin relative to the basal response is, however, only slightly potentiated by higher albumin concentrations. No effect of albumin on  $^{14}\text{CO}_2$  evolution by the isolated cells is observed in the absence of hormones nor does

<sup>1</sup> A. E. Renold and B. Jeanrenaud. Personal study.

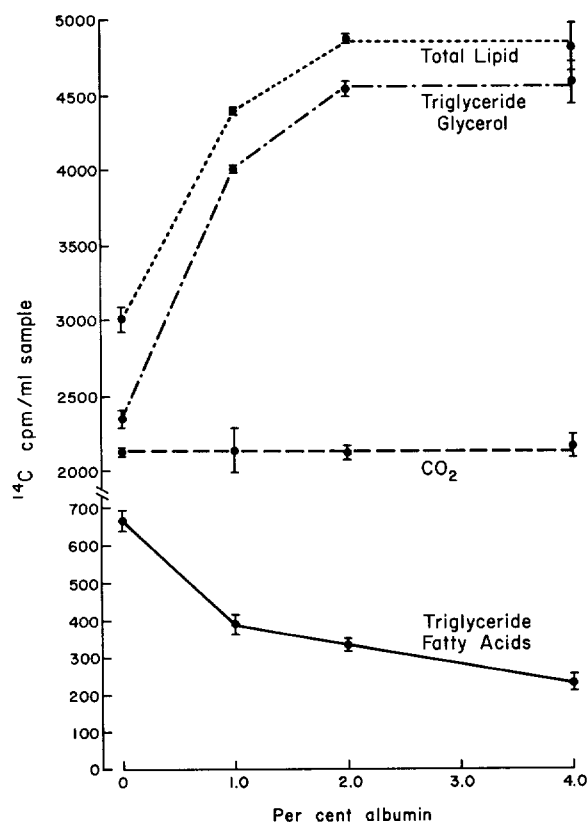


FIGURE 1  $^{14}\text{CO}_2$  evolution and total lipid- $^{14}\text{C}$ , triglyceride glycerol- $^{14}\text{C}$ , and triglyceride fatty acid- $^{14}\text{C}$  production *versus* serum albumin concentration. Isolated adipose cells incubated 60 min in KRB buffer in the presence of 0.1 mg glucose per ml and in the absence of hormones. Values  $\pm$  standard deviation.

albumin have an effect on the cell's response to insulin in  $^{14}\text{CO}_2$  evolution. In the complete absence of albumin, isolated adipose cells respond to insulin as they do in the presence of albumin. Nonesterified fatty acid release remains below the limits of detection at all albumin concentrations examined.

In contrast, in the presence of 0.1  $\mu\text{g}$  epinephrine per ml, the stimulation of the incorporation of  $^{14}\text{C}$ -counts into total lipid and triglyceride glycerol, and the inhibition of the incorporation of  $^{14}\text{C}$ -counts into triglyceride fatty acids by serum albumin in the isolated adipose cells are maximal (twofold for total lipid- $^{14}\text{C}$  production) at the lowest albumin concentration tested, 0.1%, and decrease slightly with increasing albumin concentration above 0.1% (Figs. 4 and 5). The enhancement of each response by epinephrine relative to the basal response is similarly maximal at 0.1% but decreases rapidly until an almost

negligible effect of epinephrine is observed at 4% (Table I, Figs. 4 and 5). Albumin, at 0.1%, also stimulates  $^{14}\text{CO}_2$  evolution by the epinephrine-treated cells (Table I); this effect of albumin disappears at albumin concentrations above 1% (Fig. 4). In the complete absence of albumin, isolated adipose cells do not respond to epinephrine in any of the parameters examined (Table I, Figs. 4 and 5).

Increasing serum albumin concentrations do, however, stimulate increasingly the release of nonesterified fatty acids into the incubation medium by the epinephrine-treated isolated adipose cells (Table I, Figs. 5 and 6). At a concentration of 0.1  $\mu\text{g}$  epinephrine per ml, this stimulation is linearly dependent upon albumin concentration up to 1% albumin (Table I, Figs. 5 and 6) and at 1.0  $\mu\text{g}$  epinephrine per ml, up to an albumin concentration of 4% (Fig. 6). Serum albumin

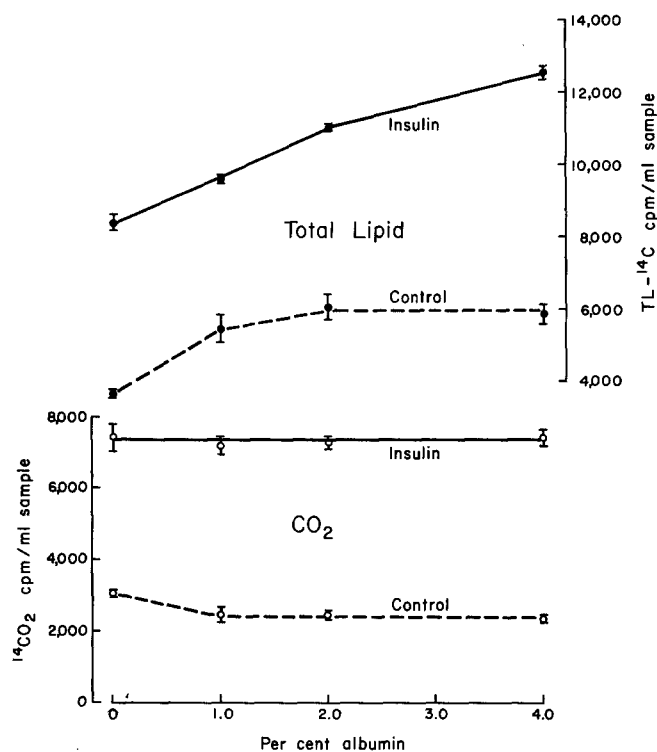


FIGURE 2  $^{14}\text{CO}_2$  evolution and total lipid (TL)- $^{14}\text{C}$  production versus serum albumin concentration in the presence of  $100\ \mu\text{U}$  insulin per ml or in the absence of hormones. Isolated adipose cells incubated 60 min in KRB buffer in the presence of  $0.1\ \text{mg}$  glucose per ml.

stimulates the release of glycerol by the epinephrine-treated cells in a similar fashion (Fig. 6).

Over the range of albumin concentrations in which fatty acid release increases linearly with albumin concentration, 0–1% and 0–4% in the presence of  $0.1\ \mu\text{g}$  and  $1.0\ \mu\text{g}$  epinephrine per ml, respectively, the molar ratio of fatty acids released to the amount of albumin present remains roughly constant and independent of epinephrine dose (Table I, Figs. 5 and 6). Over this same range, the molar ratio of fatty acids released to glycerol released increases only very gradually with increasing albumin concentration (Fig. 6). Over the range of albumin concentrations which does not affect fatty acid release, 1–4% in the presence of  $0.1\ \mu\text{g}$  epinephrine per ml, the molar ratio of fatty acids released to albumin present decreases rapidly (Figs. 5 and 6). Over this same range, the fatty acid to glycerol ratio remains constant and elevated (Fig. 6).

Maxima in the epinephrine-produced alterations in glucose metabolism correlate with albu-

min-limited rates of fatty acid and glycerol release, and roughly constant fatty acid to albumin and fatty acid to glycerol ratios. When the effect of epinephrine on glucose metabolism decreases as the result of increases in albumin concentration, fatty acid to albumin ratios decrease, fatty acid to glycerol ratios remain constant at their maximal levels, and rates of fatty acid and glycerol release are unchanged.

#### DISCUSSION

Increasing serum albumin concentrations stimulate the rate at which fatty acids are esterified into triglyceride by untreated and insulin-treated adipose cells as reflected in the increase in the incorporation of extracellular glucose carbons into triglyceride glycerol or total lipid (15, 16). Simultaneously, increasing albumin concentrations inhibit the rate of fatty acid synthesis by the cell as reflected in the decrease in the incorporation of glucose carbons into triglyceride fatty acids (16,

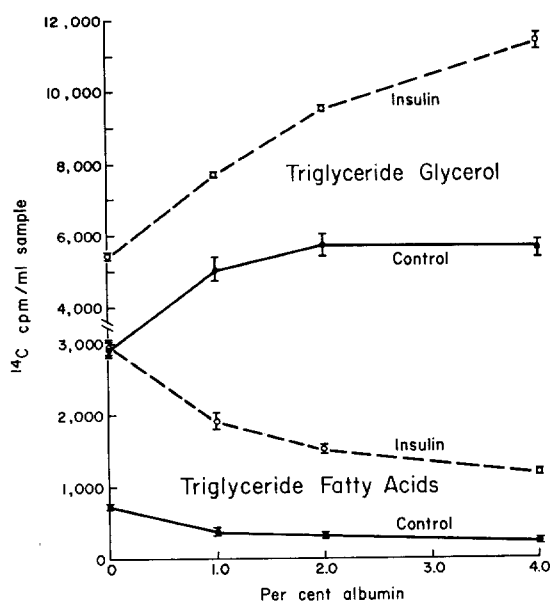


FIGURE 3 Triglyceride glycerol- $^{14}\text{C}$  and triglyceride fatty acid- $^{14}\text{C}$  production versus serum albumin concentration in the presence of  $100\ \mu\text{U}$  insulin per ml or in the absence of hormones. Isolated adipose cells incubated 60 min in KRB buffer in the presence of  $0.1\ \text{mg}$  glucose per ml.

17). No release of fatty acids into the incubation medium is detectable under these conditions. In the presence of decreased fatty acid synthesis and in the absence of fatty acids in the medium, the observed increase in the rate of esterification must reflect a corresponding increase in the rate of fatty acid liberation from triglyceride via lipolysis (17, 18).

In the complete absence of serum albumin in the incubation medium, isolated adipose cells respond to insulin just as they do in the presence of albumin. This response to insulin consists of a stimulation of the incorporation of extracellular glucose carbons into total lipid, triglyceride glycerol and fatty acids, and  $\text{CO}_2$ . Such a stimulation of each parameter by insulin relative to the basal level is observed at each albumin concentration.

Serum albumin is an absolute requirement for a metabolic response to epinephrine by the adipose cells as reflected in the absence of epinephrine-produced alterations in any of the parameters measured in the absence of albumin. Serum albumin, at the lowest concentration tested, simultaneously stimulates the rate of esterification and

inhibits the rate of fatty acid synthesis by the epinephrine-treated adipose cells maximally. Although albumin at this concentration stimulates fatty acid and glycerol release only slightly, those fatty acids released saturate the binding capacity of that albumin present as reflected in the maximal fatty acid to albumin ratio (8, 9, 19, 20). On the other hand, the efficiency of fatty acid release, that is, the proportion of fatty acids, liberated by lipolysis, which is released relative to that which is reesterified, is low as reflected in the submaximal fatty acid to glycerol ratio (17, 18).

Increasing serum albumin concentrations have little additional effect on the rates of esterification and fatty acid synthesis and on the efficiency of fatty acid release as long as they stimulate the rate of lipolysis, as reflected by an increase in glycerol release (17, 18), such that released fatty acids saturate the binding capacity of the albumin present. The response of the cell to epinephrine depends upon epinephrine concentration only when albumin is not so limiting for fatty acid release. The epinephrine concentration appears to determine only the ultimate maximal rate of lipolysis.

At and above that albumin concentration where the epinephrine dose becomes rate-limiting for lipolysis, increasing albumin concentrations reduce the proportion of fatty acid binding sites occupied.

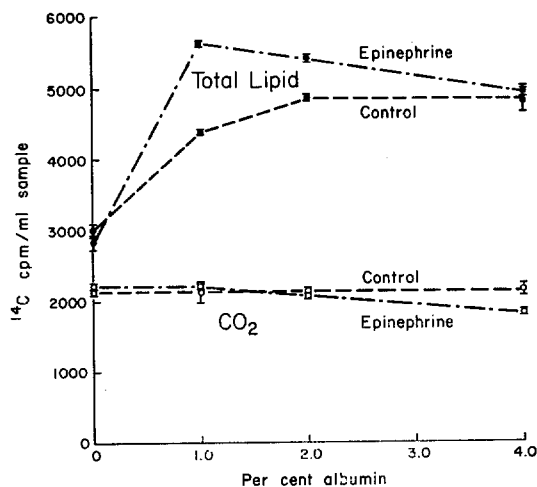


FIGURE 4  $^{14}\text{CO}_2$  evolution and total lipid- $^{14}\text{C}$  production versus serum albumin concentration in the presence of  $0.1\ \mu\text{g}$  epinephrine per ml or in the absence of hormones. Isolated adipose cells incubated 60 min in KRB buffer in the presence of  $0.1\ \text{mg}$  glucose per ml.

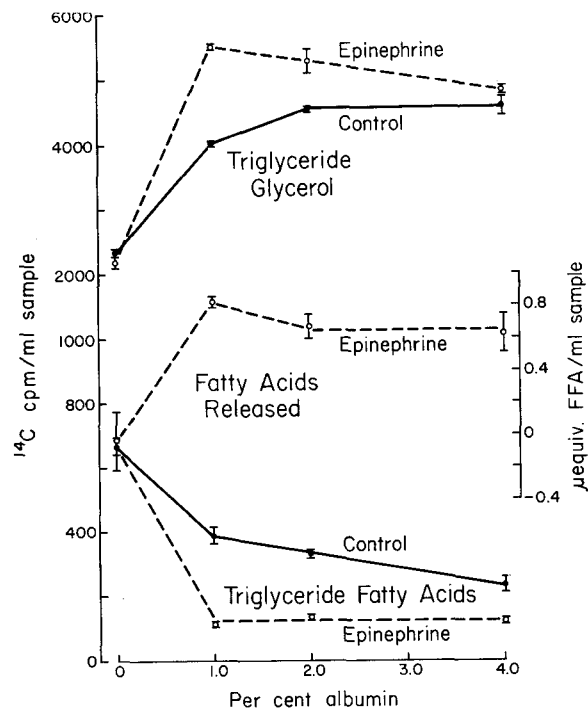


FIGURE 5 Triglyceride glycerol- $^{14}\text{C}$  and triglyceride fatty acid- $^{14}\text{C}$  production in the presence of  $0.1\ \mu\text{g}$  epinephrine per ml or in the absence of hormones and nonesterified fatty acid (FFA) release in the presence of  $0.1\ \mu\text{g}$  epinephrine per ml versus serum albumin concentration. Isolated adipose cells incubated 60 min in the presence of  $0.1\ \text{mg}$  glucose per ml.

TABLE I

*Response to Epinephrine versus Serum Albumin Concentration*

Responses to epinephrine of nonesterified fatty acid release, of molar ratio of fatty acids released to albumin in the medium, of  $^{14}\text{CO}_2$  evolution, and of total lipid- $^{14}\text{C}$ , triglyceride glycerol- $^{14}\text{C}$ , and triglyceride fatty acid- $^{14}\text{C}$  production versus serum albumin concentration. Isolated adipose cells incubated 60 min in KRB buffer in the presence of  $0.1\ \text{mg}$  glucose per ml and in the presence of  $0.1\ \mu\text{g}$  epinephrine per ml or the absence of hormones.

	Albumin, %			
	0.00	0.10	0.50	1.00
$\mu$ equiv.* FA released/ml (epi.-con.)	0.00	0.14	0.69	1.31
Moles FA released/mole albumin	—	9.33	9.32	8.91
$^{14}\text{C}$ -cpm- $\text{CO}_2$ , epi./ $^{14}\text{C}$ -cpm- $\text{CO}_2$ , con.	1.01	1.45	1.34	1.20
$^{14}\text{C}$ -cpm-TL, epi./ $^{14}\text{C}$ -cpm-TL, con.	0.99	2.16	1.89	1.61
$^{14}\text{C}$ -cpm-TGG, epi./ $^{14}\text{C}$ -cpm-TGG, con.	0.99	2.60	2.19	1.81
$^{14}\text{C}$ -cpm-TGFA, epi./ $^{14}\text{C}$ -cpm-TGFA, con.	1.00	0.28	0.33	0.39

\* equiv., equivalents; FA, fatty acid; epi., epinephrine; con., control; TL, total lipid; TGG, triglyceride glycerol; TGFA, triglyceride fatty acid.

This optimizes the efficiency of fatty acid release and reduces the rate of esterification but continues to inhibit the rate of fatty acid synthesis maximally.

The well-known fatty acid binding properties

of serum albumin (7-10) suggest that the effects of albumin on the metabolism of the adipose cell, reported here, reflect effects of albumin on the level of intracellular nonesterified fatty acids.

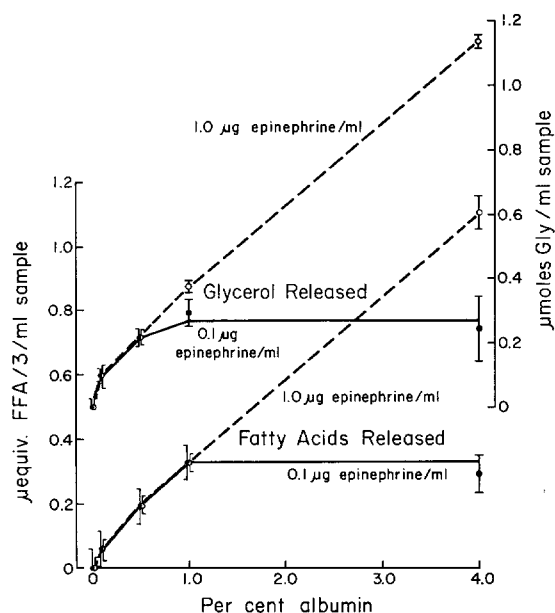


FIGURE 6 Nonesterified fatty acid (FFA) and glycerol (Gly) release *versus* serum albumin concentration in the presence of 0.1 or 1.0  $\mu\text{g}$  epinephrine per ml. Isolated adipose cells incubated 60 min in KRB buffer in the presence of 0.1 mg glucose per ml. The results of two experiments have been plotted together after subtraction of small control glycerol release values.

Such fatty acid levels have been implicated in the regulation of many adipose cell activities (2, 19–25). The chemical state of the cell's intracellular fatty acids is, however, unknown. The solubility of fatty acids in aqueous media is essentially negligible. In those cell types where the uptake and metabolism of fatty acids have been studied morphologically, fatty acids taken up are initially localized to the lumina of the endoplasmic or sarcoplasmic reticulum (26, 27). The enzymes of fatty acid activation, triglyceride synthesis, and triglyceride lipolysis are localized to smooth-surfaced membranes (28–32).

Alterations in the fatty acid binding capacity of albumin in the extracellular space might lead, passively and indirectly, to alterations in the intracellular fatty acid level and, thereby, to alterations in the cell's metabolism (33). However, the continued effects of albumin under experimental conditions where fatty acids in the incuba-

tion medium are neither radioisotopically (34) nor chemically detectable, suggest that albumin has direct access to metabolic compartments within the adipose cell and lend support to the suggestion that fatty acid metabolism in the adipose cell is mediated by a vesicular fatty acid transport system within which nonesterified fatty acids are bound to serum albumin of extracellular origin (2).

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