Regional differences in triacylglycerol synthesis in adipose tissue and in cultured preadipocytes

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Abstract The initial suspicion that obesity increases coronary risk has been much sharpened with the demonstration that risk is more tightly linked to abdominal than to peripheral obesity, and tighter yet again when the mass of omental adipose tissue is taken into account. These data suggest that important metabolic differences might exist between adipocytes from different regions, and indeed, it has long been appreciated that triacylglycerol hydrolysis can be stimulated to a greater extent in omental than in subcutaneous adipocytes. The present study focuses on triacylglycerol synthesis in human subcutaneous and omental adipocytes, a process which, by contrast, has received relatively little attention. Experiments were done on adipose tissue removed at laparotomy and on cultured preadipocytes. With the former, triacylglycerol synthesis was measured in the presence and absence of oleate added to the medium using radiolabeled glucose and oleate as tracers. The results demonstrate that under all conditions examined triacylglycerol synthesis in subcutaneous adipose tissue exceeded that in deep omental adipose tissue. To study the cells in more detail, preadipocytes were cultured and triacylglycerol synthesis was examined again under basal conditions and with stimulation with insulin and acylation stimulating protein (ASP). Under basal conditions, particularly when oleate was added to the medium, clear differences were present such that triacylglycerol synthesis was substantially greater in subcutaneous preadipocytes than in omentally derived preadipocytes. These differences were more pronounced when the cells were stimulated with either insulin or acylation stimulating protein. Overall, triacylglycerol synthetic capacity in subcutaneous tissue exceeded that in omental tissue. As a consequence, omental tissue as compared to subcutaneous adipose tissue would have a limited capacity to prevent fatty acids from reaching the liver and stimulating hepatic lipoprotein synthesis.—**Maslowska, M. H., A. D. Sniderman, L. D. MacLean, and K. Cianflone.** Regional differences in triacylglycerol synthesis in adipose tissue and in cultured preadipocytes. *J. Lipid Res.* 1993. **34:** 219–228.

Supplementary key words omental • subcutaneous adipose tissue • human

A link between omental obesity and increased risk of coronary disease has now been established. Moreover, omental obesity has been related to a series of metabolic abnormalities that themselves have been linked to increased coronary risk. These include hypertriglyceridemia, increased apoB, small dense LDL, and hyperinsulinemia (review, 1). The most common explanation advanced at the present for these associations relates to differences in lipolytic rates between omental and subcutaneous adipocytes. Omental adipocytes have been shown to respond more intensely to beta agonists than subcutaneous adipocytes, whereas the inhibition induced by insulin is greater in subcutaneous tissue than in omental tissue (2–5). The increased release of fatty acids from omental adipocytes, it is argued, would then result in increased delivery of fatty acids to the liver and this in turn would result in all the adverse metabolic consequences just noted.

However, the fatty acid balance of the adipocyte is also determined by the rate of triacylglycerol synthesis, although little attention has been paid to this side of the equation. The purpose of the present study, therefore, was to compare triacylglycerol synthesis in human omental and subcutaneous adipocytes. We have done so using both tissue fragments and cultured preadipocytes and, in both instances, the data demonstrate less rapid triacylglycerol synthesis in omental as opposed to subcutaneous adipocytes. We believe these findings may provide new insights into the pathogenesis of the metabolic disorders associated with omental obesity.

METHODS

Materials

The following materials were obtained as indicated: [1-^{14}C]oleate and [6-(N)-^{3}H]glucose (NEN-DuPont, Mis-

Abbreviations: apo, apolipoprotein; ASP, acylation stimulating protein; BMI, body mass index; BSA, bovine serum albumin; FFA, free fatty acid; LPL, lipoprotein lipase; PBS, phosphate-buffered saline; TLC, thin-layer chromatography.

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sissauga, Canada), all tissue culture supplies including minimum essential medium and fetal calf serum (Flow-ICN, Mississauga, Canada), fatty acid-free bovine serum albumin (BSA), oleate sodium salt, collagenase Type II, and insulin (Sigma Chemicals, St. Louis, MO). Acylation stimulating protein (ASP) was partially isolated from human plasma as described previously (6, 7). [1-^{14}C]oleate complexed to BSA was prepared as described by Van Harken, Dixon, and Heimberg (8).

Subjects

All subjects were female, 30–60 years old. Body mass index (BMI) was measured as weight (kg) per height (meters squared). Blood samples were obtained fasting prior to abdominal surgery, and plasma triacylglycerol, plasma cholesterol, and HDL cholesterol were measured according to Lipid Research Clinic protocols (9). LDL cholesterol was calculated by the Friedewald formula (10). Plasma apoB and apoA-I were measured by immunonephelometry (Beckman Instruments, CA).

Tissue fragment experiments

Samples of subcutaneous and omental abdominal tissue were obtained with informed consent at the time of elective abdominal surgery. The tissue was carefully dissected to remove visible blood vessels and connective tissue, and the fat lobules were cut up manually into fragments of 10–20 mg wet weight (intra-sample variability was 25% as measured by soluble cell protein). Triacylglycerol synthesis was measured by a modification of previously described methods (11, 12). Briefly, each fragment was incubated with shaking in 1 ml medium (minimal essential medium) containing different concentrations of [^{3}H]glucose or [^{14}C]oleate complexed to BSA for the indicated time. Under these conditions, it has been shown that all of the [^{3}H]glucose is incorporated into the glycerol backbone with negligible incorporation into de novo synthesis of fatty acids (12, 13). Samples of tissue (10–20 mg) were also set aside for cell sizing as described below. After the incubation, the medium was set aside for free fatty acid (FFA) determination. The tissue was washed twice with phosphate-buffered saline (PBS) and the neutral lipids were extracted overnight in 1 ml of heptane–isopropanol 3:2. The tissue was re-extracted with an additional 1 ml and the two extracts were pooled. The lipid extract was set aside for analysis and soluble tissue protein in the tissue fragments was dissolved in 1 ml of 0.1 N NaOH.

Cell sizing

Fragments of subcutaneous and omental tissue about 5 mm in diameter (10–20 mg) were placed in 10% phosphate-buffered formalin to fix the tissue. Tissue was then fixed, embedded, and sliced with a microtome, and stained with hematoxylin and eosin staining. Cell sizing was measured by cell surface area, cell perimeter, and the maximum diameter using a Zeiss microscope with 40× magnification using Videoplan (Zeiss) computer-assisted data analysis. Fifty determinations were measured on each sample and an average was taken.

Isolation and culture of preadipocytes

Adipose tissue was obtained from laparotomy with informed consent and was carefully dissected as described above. Preadipocytes were cultured from fragments (5–10 mg) of subcutaneous and omental adipose tissue by a modification of the ceiling method (14) using isolated primary adipocytes (15) or by placing coverslips over the tissue fragments in 35-mm dishes to hold them in place during initial culture. Fragments were maintained in M199 medium with 20% fetal calf serum and 2% penicillin-streptomycin for 4 weeks with changes of medium twice a week. Cells were then passaged at confluence with a split ratio of 1:2 and used between passages 3–6. Both subcutaneous and omental cells appeared to grow at the same rate. For experiments, confluent subcutaneous and omental cells were trypsinized and re-suspended in medium to the same cell density and cells were plated into 24-well plates (17-mm wells) and used 1 week later. At the time of experiment there was no difference in soluble cell protein between the subcutaneous and omental cells, with less than 12% variation in soluble cell protein from dish to dish within each cell line. Cells were preincubated overnight in serum-free medium and then incubated for 24 h in minimum essential medium supplemented with varying concentrations of [^{3}H]glucose and [^{14}C]oleate complexed to BSA as for the tissue fragments. Previous work has shown that triacylglycerol synthesis is linear over this time period in human skin fibroblasts (6). The specific activity for the medium glucose and fatty acid was maintained constant. In addition, triacylglycerol synthesis was also stimulated with addition of insulin or ASP (isolated as described above) to medium already containing 5.5 mM [^{3}H]glucose and 100 μM [^{14}C]oleate. After incubation, the medium was removed, the cells were washed three times with PBS, and the neutral lipids were extracted twice with 1 ml heptane–isopropanol 3:2 for 30 min. The remaining soluble cell protein was dissolved in 1 ml of 0.1 N NaOH for 1 h.

Lipid and protein analyses

The neutral lipid cell extract was dried down and redissolved in a known volume of chloroform–methanol 2:1 (200 μl for tissue extracts, 100 μl for cell extracts). A known volume (20 μl) was applied to prewashed thin-layer chromatography (TLC) plates and developed in hexane–ether–acetic acid 75:25:1 to separate triacylglycerol, di-

acylglycerol, fatty acids, and polar lipids. Reference lipids were visualized by exposure to iodine vapor, and the triacylglycerol spots were scraped into scintillation vials containing 5 ml scintillation fluid and counted in a scintillation counter (Beckman Instruments, CA). Medium specific activity was maintained constant for all concentrations of glucose and fatty acid (average [^{3}H]glucose specific activity 3.17 dpm/pmol, average [^{14}C]oleate specific activity 4.29 dpm/pmol) and all values were corrected to nmol substrate incorporated per mg soluble cell protein or per mg soluble tissue protein. Soluble cell protein and tissue protein were measured by the method of Bradford (16) and medium free fatty acid mass by Novak (17). The dilution of medium [^{14}C]oleate by the free fatty acid released from the cells was corrected as suggested by Dole (18) as follows:

$$\text{corrected SA} = \frac{\text{calc[FA]} \times 2}{(\text{calc[FA]} + \text{final[FA]})} \times \text{calc SA}$$

where calc = calculated, final = final concentration free fatty acid mass measured as above, [FA] = concentration of oleate and SA = specific activity.

Statistical analysis

Each point is the average of triplicate determinations for each tissue and cell sample. Intra-sample variability for glucose incorporation into triacylglycerol, fatty acid incorporation into triacylglycerol, and medium free fatty acid release was 15 ± 4%, 17 ± 6%, and 31 ± 4%, respectively, for the tissue fragments. For the preadipocyte experiments, intra-sample variability for glucose incorporation into triacylglycerol was 7 ± 2% and for fatty acid incorporation into triacylglycerol was 4 ± 1%. Subcutaneous versus omental differences were compared by paired *t*-test for the adipose tissue fragments, and by unpaired *t*-test for two means for the cultured preadipocyte cells where pNS indicates no statistical difference. All values are expressed as nmol substrate incorporated into triacylglycerol per mg soluble cell protein or soluble tissue protein ± standard error of the mean (SEM).

RESULTS

Adipose tissue fragments

Adipose tissue fragments were obtained from eight subjects undergoing elective laparotomy. Five of the subjects were undergoing gastric bypass and the three others were undergoing cholecystectomy. None were taking hypolipidemic drugs or were on a special diet or were postmenopausal or diabetic. All were female and their average age was 41.5 ± 1.8 years. Three were of normal weight, while five had an elevated body mass index (overall average BMI = 34.2 ± 2.9). Their average lipid values were: plasma triacylglycerol 158 ± 33 mg/dl, plasma cholesterol 190 ± 14 mg/dl, LDL cholesterol 112 ± 14 mg/dl, HDL cholesterol 40.3 ± 5 mg/dl, plasma apoB 76 ± 11 mg/dl, plasma apoA-I 146 ± 12 mg/dl.

Cell size of subcutaneous and omental tissue did not differ substantially as measured by area, perimeter, or diameter: 4971 ± 606 subcutaneous area versus 4406 ± 1311 omental area, pNS). Although there was a wide range in cell size within each tissue fragment, the percent variation in size was the same between the subcutaneous and omental samples (24% subcutaneous and 23% omental). Additionally, soluble tissue protein was also the same between subcutaneous and omental tissue for each experiment (subcutaneous: 17.2 ± 4.5 vs. omental: 19.3 ± 7.0 mg protein; NS by paired *t* test).

Triacylglycerol synthesis was measured using minimal essential medium supplemented with either increasing amounts of [^{3}H]glucose or increasing amounts of ^{14}C-labeled fatty acid. The concentration range of glucose and fatty acid used in the experimental media are in the range of fasting to postprandial ranges (19). First, triacylglycerol synthesis was measured over 1–4 h to determine the effect of glucose concentrations on triacylglycerol synthesis. As shown in **Fig. 1,** glucose incorporation into triacylglycerol increased with time up to at least 4 h for all of the glucose concentrations examined and a 2-h time point was chosen for all subsequent experiments.

The effect of different concentrations of glucose on triacylglycerol synthesis in each of the paired omental and

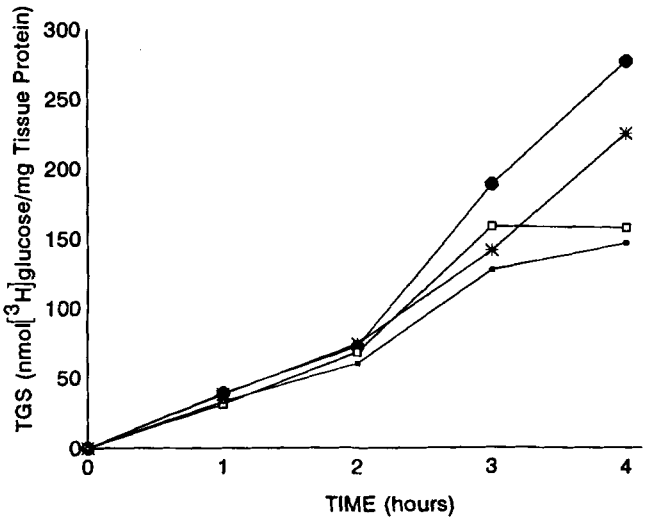

Fig. 1. Triacylglycerol synthesis in subcutaneous adipose tissue fragments over time. Adipose tissue fragments were incubated in medium supplemented with varying concentrations of [^{3}H]glucose: (■) 5.5 mM glucose, (□) 10 mM glucose, (*) 25 mM glucose and (●) 50 mM glucose. Triacylglycerol synthesis was measured from 1 to 4 h. Each point is the average of triplicate determinations and is expressed as nmol [^{3}H]glucose incorporated into triacylglycerol per mg soluble tissue protein.

subcutaneous tissues is shown in **Fig. 2.** With increasing concentrations of glucose, triacylglycerol synthesis using [^{3}H]glucose as tracer increased steadily. At any level, average triacylglycerol synthesis was always greater in the subcutaneous adipose tissue fragments than in the omental tissue fragments. However, these differences were statistically significant only at the two lower glucose concentrations and the average values are summarized in **Table 1.**

Triacylglycerol synthesis was then measured in medium supplemented with increasing amounts of [^{14}C]oleate complexed to BSA and analyzed at different time points. Triacylglycerol synthesis increased proportionally up to 3 h even at the highest concentration of fatty acid assayed as shown in **Fig. 3.** Therefore, as with the glucose experiments, a 2-h time period was used for all of the subsequent assays. The effects of different fatty acid concentrations on fatty acid incorporation into triacylglycerol

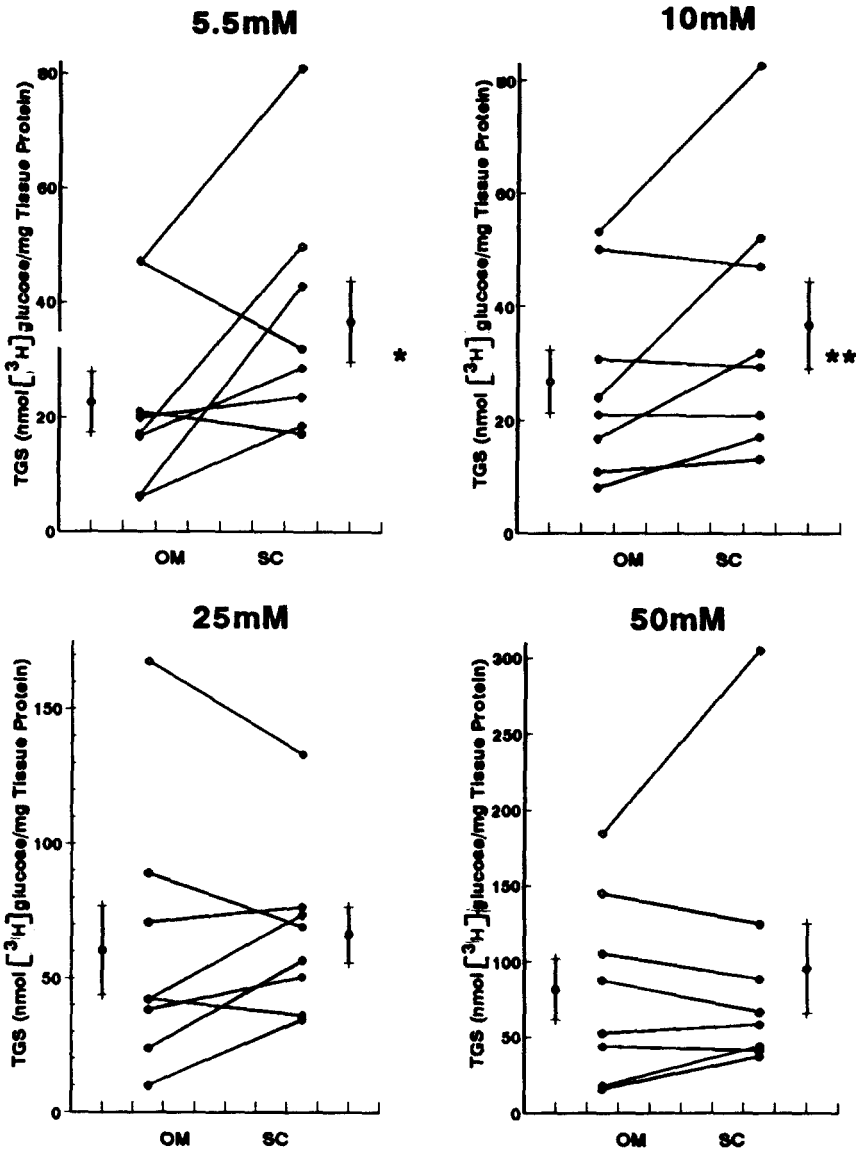

Fig. 2. Triacylglycerol synthesis in adipose tissue fragments. Adipose tissue fragments were incubated in medium supplemented with varying concentrations of [^{3}H]glucose (5.5 mM, 10 mM, 25 mM, and 50 mM). Triacylglycerol synthesis over 2 h was measured in paired omental (OM) and subcutaneous (SC) tissue fragments as nmol [^{3}H]glucose incorporation into triacylglycerol per mg soluble tissue protein. Each point is the average of triplicate values. Average ± SEM values are given in Table 1;*, $P < 0.05$; **, $P < 0.025$ by paired t test.

TABLE 1. Average [^{3}H]glucose incorporation into triacylglycerol in subcutaneous and omental tissue

Glucose	Incorporation into Triacylglycerol		P
	Subcutaneous	Omental	
mM	*nmol [^{3}H]glucose/mg tissue protein*		
5.5	36.7 ± 7.0	22.6 ± 5.3	<0.05
10	36.8 ± 7.6	26.8 ± 5.6	<0.025
25	66.2 ± 10.4	60.3 ± 16.5	NS
50	96.0 ± 29.6	81.5 ± 20.1	NS

Experimental conditions are as described in the legend to Fig. 2.

synthesis in each of the paired omental and subcutaneous adipose tissues are shown in **Fig. 4.** Note that at every concentration of fatty acid, triacylglycerol synthesis was higher in the subcutaneous adipose tissue than in omental adipose tissue. Again these differences were more pronounced at the lower two concentrations of fatty acids as summarized in **Table 2.** In these experiments, [^{3}H]glucose was also present in the medium and triacylglycerol synthesis using this tracer was estimated as well. Once again, this was greater in the subcutaneous than in omental adipocytes. Re-esterification estimated by the [^{14}C]oleate to [^{3}H]glucose ratio was identical in the subcutaneous and omental adipose tissue fragments (data not shown).

Lipolysis was measured by quantitating the mass of fatty acid released into the medium over the 2-h course of the experiments. As shown in **Fig. 5,** there was no significant difference in lipolysis between omental and subcutaneous tissue at any of the glucose concentrations. Similarly, there was also no difference in lipolysis between subcutaneous and omental tissues at any of the fatty acid concentrations assayed as shown in **Fig. 6.**

Cultured preadipocytes

Preadipocytes were cultured from adipose tissue obtained from eight women. Adipose tissue from four of these had also been examined in the first section of this study. Of the eight subjects, three were of normal weight and were undergoing cholecystectomy (one was postmenopausal) while five had an elevated BMI and were undergoing gastric bypass. Average age was 42 ± 2.6 yr and overall average BMI was 33.9 ± 3.1. Plasma lipid values were: plasma triacylglycerol 143 ± 29 mg/dl, plasma cholesterol 178 ± 16 mg/dl, LDL cholesterol 101 ± 18 mg/dl, HDL cholesterol 41.3 ± 4.4 mg/dl, plasma apoB 80 ± 13 mg/dl, plasma apoA-I 144 ± 12 mg/dl. Overall, this group of subjects did not differ from the group of subjects used for the tissue fragment experiments.

Triacylglycerol synthesis in the preadipocytes was measured as the incorporation of [^{3}H]glucose and [^{14}C]oleate into triacylglycerol in cells incubated in serum free medium for 16 h. Synthesis is linear over this time period as described previously in normal fibroblasts (6). The glucose and fatty acid concentrations used in the experiments were the same as used in the tissue fragment experiments and represent fasting to postprandial ranges. Average cell protein was no different between subcutaneous and omental cells (18.1 ± 7.7 μg soluble cell protein/2.3 cm^2 dish subcutaneous vs. 18.7 ± 9.8 μg soluble cell protein/2.3 cm^2 dish omental) and the cells were used at the same passage number.

Triacylglycerol synthesis was measured from 5.5 mM to 50 mM glucose. At each concentration of glucose, triacylglycerol synthesis was higher in the subcutaneous preadipocytes compared to the omentally derived preadipocytes as shown in **Fig. 7.** While a trend was evident, none of the differences were statistically significant. However, when the cells were incubated under conditions favoring triacylglycerol synthesis, namely with fatty acids added to the medium, then clear differences were found. The results in **Fig. 8** indicate that at each concentration of oleate, the subcutaneous preadipocytes synthesize significantly more triacylglycerol than do the omental preadipocytes. The same differences were observed when triacylglycerol synthesis was measured with two separate radiolabels: that is, with either [^{3}H]glucose incorporation into triacylglycerol backbone or with [^{14}C]oleate incorporation into triacylglycerol fatty acid. Overall, the subcutaneous preadipocytes synthesized 58% more triacylglycerol than the omental preadipocytes.

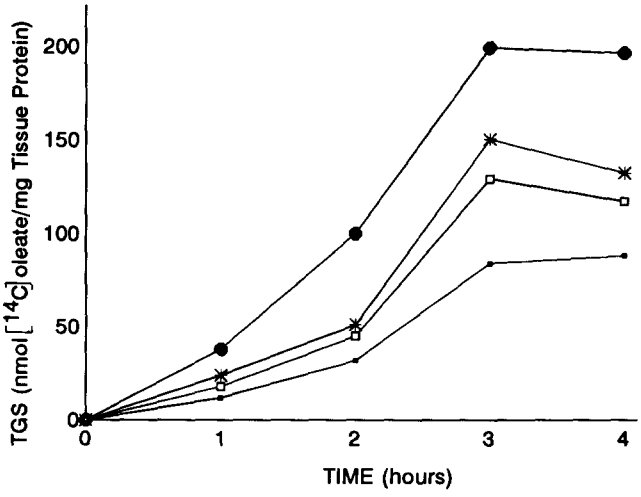

Fig. 3. Triacylglycerol synthesis in subcutaneous adipose tissue fragments over time. Adipose tissue fragments were incubated in medium supplemented with varying concentrations of [^{14}C]oleate complexed to BSA: (■) 50 μM oleate, (□) 100 μM oleate, (*) 200 μM oleate, and (●) 500 μM oleate. Triacylglycerol synthesis was measured from 1 to 4 h. Each point is the average of triplicate determinations and is expressed as nmol [^{14}C]oleate incorporated into triacylglycerol per mg soluble tissue protein.

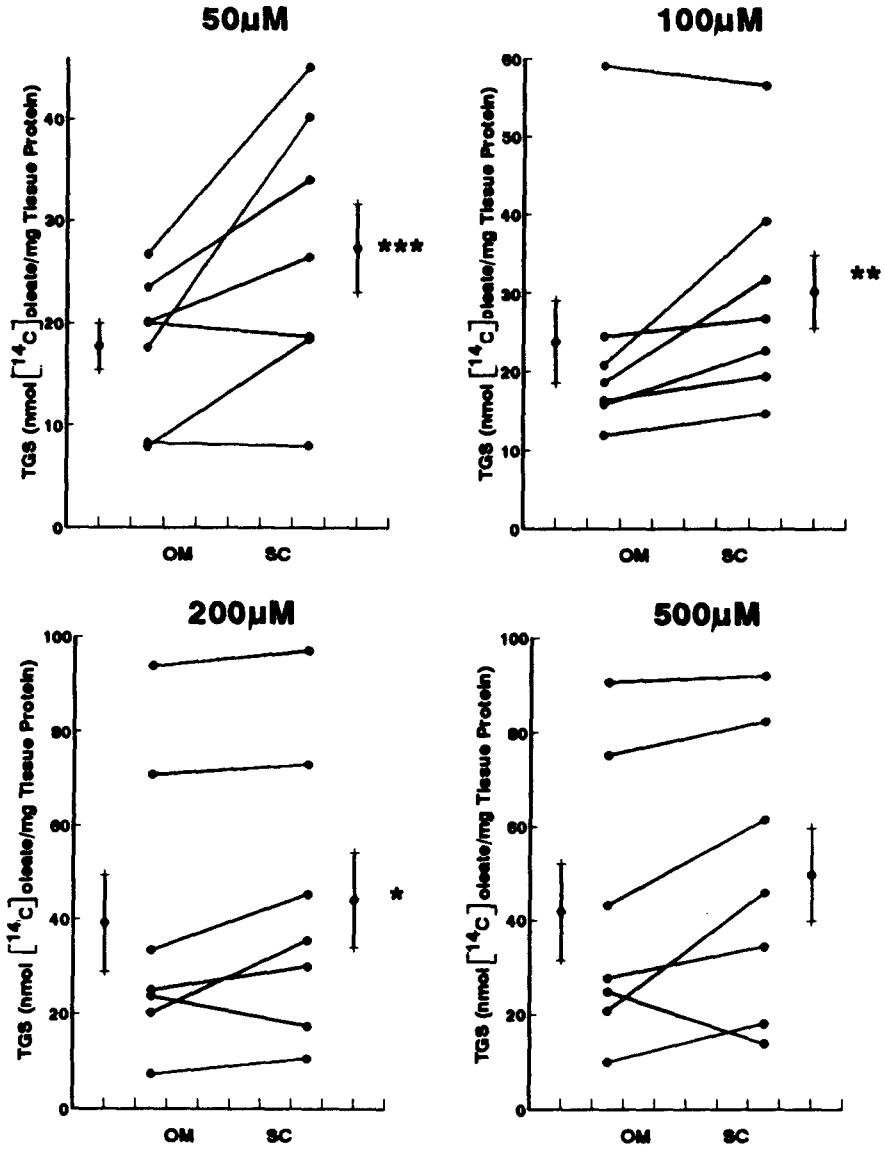

Fig. 4. Oleate stimulation of triacylglycerol synthesis in adipose tissue fragments. Adipose tissue fragments were incubated in 5.5 mM glucose medium supplemented with varying concentrations of [^{14}C]oleate complexed to BSA (50 μM, 100 μM, 300 μM, and 500 μM). Triacylglycerol synthesis over 2 h was measured in paired omental (OM) and subcutaneous (SC) tissue fragments as nmol [^{14}C]oleate incorporation into triacylglycerol per mg soluble tissue protein. Each point is the average of triplicate values. Averages ± SEM values are given in Table 2; *, $P < 0.05$; **, $P < 0.025$; ***, $P < 0.01$.

TABLE 2. Average [^{14}C]oleate incorporation into triacylglycerol in subcutaneous and omental tissue

	Incorporation into Triacylglycerol		
Oleate	Subcutaneous	Omental	*P*
μM	nmol [*^{14}C]oleate/mg tissue protein*		
50	27.3 ± 4.3	17.7 ± 2.3	<0.01
100	30.2 ± 4.6	23.8 ± 5.3	<0.025
200	44.2 ± 10.1	39.2 ± 10.2	<0.05
500	49.7 ± 9.9	41.8 ± 10.2	NS

Experimental conditions are as described in the legend to Fig. 4.

Finally, the effects of ASP and insulin on triacylglycerol synthesis in the preadipocytes were examined. These results are shown in **Fig. 9.** Again, triacylglycerol synthesis was estimated with two different tracers at concentrations of 5.5 mM [^{3}H]glucose and 100 μM [^{14}C]oleate and subcutaneous preadipocytes were compared to omental adipocytes. The differences are the same as in the previous experiments. Note that addition of oleate to the medium causes triacylglycerol synthesis to increase above basal (Fig. 9, left panel), the degree of increase being greater in subcutaneous compared to omental preadipo-

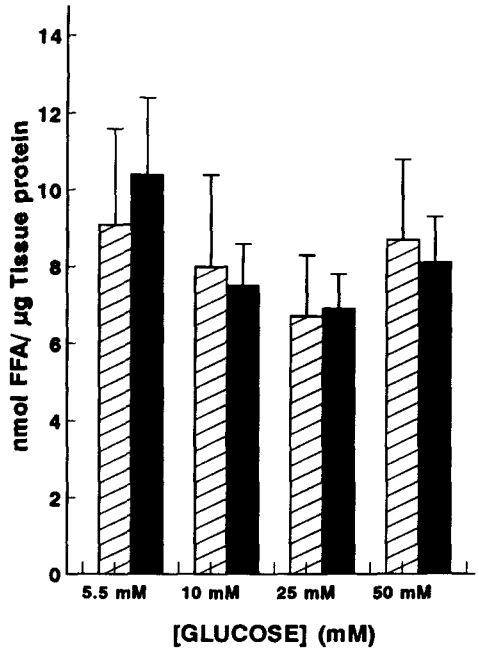

Fig. 5. Free fatty acid release in adipose tissue fragments. Adipose tissue fragments were incubated in medium supplemented with varying concentrations of ^{3}H]glucose. Free fatty acid (FFA) release over 2 h was measured in paired omental (hatched bars) and subcutaneous (solid bars) tissue fragments as nmol FFA per µg soluble tissue protein. Each tissue fragment was measured in triplicate. Results are given as average ± SEM of eight experiments. There is no significant difference between subcutaneous and omental tissues.

cytes. When insulin is also added, there is a further increase in triacylglycerol synthesis in both subcutaneous and omental preadipocytes (subcutaneous: 151% ± 16, $P < 0.01$ glucose incorporation and 141% ± 7, $P < 0.005$ oleate incorporation into triacylglycerol vs. omental: 178% ± 14, $P < 0.005$ glucose incorporation and 171% ± 13, $P < 0.005$ oleate incorporation into triacylglycerol). Finally when ASP is added to the medium there is a substantial increase in triacylglycerol synthesis, again to the same extent in both subcutaneous and omental cells (subcutaneous cells: 230% ± 36, $P < 0.02$ glucose incorporation, 206% ± 24, $P < 0.025$ oleate incorporation into triacylglycerol and omental cells: 229% ± 32, $P < 0.02$ glucose incorporation, 214% ± 23, $P < 0.02$ oleate incorporation into triacylglycerol). There was no statistically significant difference in the extent of response to either ASP or insulin in either subcutaneous or omental cells although in absolute quantities, triacylglycerol synthesis was always higher in the subcutaneous cells. When these same data were analyzed by paired *t*-test for the five paired samples, the results showed greater statistical significance.

DISCUSSION

In this study, we have examined the capacity of adipose tissue from two different sites in the abdomen to synthesize triacylglycerols. We chose to use tissue fragments that contain a normal distribution of both large and small cells, whereas primary isolated adipocytes contain only large cells (20). In addition, collagenase treatment, which is necessary to isolate primary adipocytes, may affect membrane integrity since it has been shown to destroy certain receptors (21). In order to evaluate cells under more controlled conditions, we have also chosen to use cultured preadipocytes as suggested by others (22, 23).

Previous studies have focused on triacylglycerol lipolysis in adipose tissue from different sites, the general consensus being that stimulated omental adipocytes release more fatty acids than do stimulated subcutaneous cells (2–5). By contrast, little attention has been paid to synthesis in adipose tissue, particularly under stimulated conditions such as the postprandial state. Here the consensus has been that triacylglycerol synthesis in adipose tissue is not rate-limiting but acts as an endless fatty acid acceptor and that the plasma enzyme lipoprotein lipase responsible for generation of the fatty acids is the rate-limiting step.

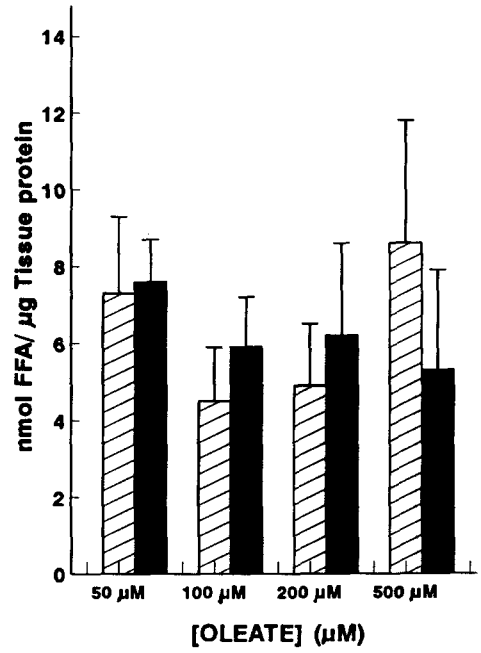

Fig. 6. Free fatty acid release in adipose tissue fragments. Adipose tissue fragments were incubated in medium supplemented with varying concentrations of [^{14}C]oleate complexed to BSA. Free fatty acid (FFA) release over 2 h was measured in paired omental (hatched bars) and subcutaneous (solid bars) tissue fragments as nmol FFA per µg soluble tissue protein. Each tissue fragment was measured in triplicate. Results are given as average ± SEM of eight experiments. There is no significant difference between subcutaneous and omental tissues.

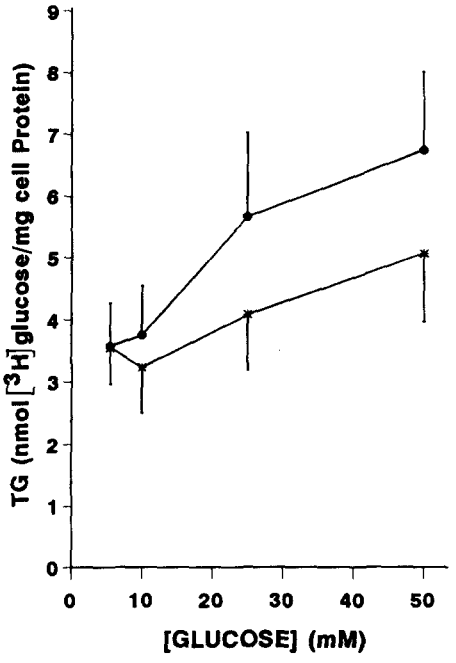

Fig. 7. Triacylglycerol synthesis in cultured preadipocytes. Triacylglycerol synthesis (nmol [^{3}H]glucose incorporated into triacylglcyerol per mg soluble cell protein) was measured in cultured omental (*; n = 7) and subcutaneous (●; n = 6) preadipocytes incubated in serum-free medium supplemented with varying concentrations of [^{3}H]glucose for 24 h. Each cell line was measured in triplicate; results are the average ± SEM of all of the cell lines.

In fact, Olivecrona and colleagues (24, 25) have reviewed the evidence relating triacylglycerol clearance to lipoprotein lipase, the step that has been thought to be rate-limiting, and shown that the correlation is poor. Moreover, the correlation between local lipoprotein lipase activity and corresponding triacylglycerol synthesis in different adipose tissue sites is also poor (26, 27). Indeed, although lipoprotein lipase is obligatory for normal clearance of chylomicron triacylglycerol, it is not essential for the formation of adipose tissue as adipose tissue mass tends to be normal in patients with homozygous lipoprotein lipase deficiency (28). Overall, the amount of lipoprotein lipase present appears to be well in excess of normal requirements (24). Taken together, these data suggest that tissue uptake of FFA is a key step and that failure of fatty acid uptake to proceed at the necessary rate will result in secondary inhibition of lipoprotein lipase activity due to formation of a lipoprotein lipase–FFA complex which is then swept away to the liver (25, 29). Until recently, only insulin was thought to increase adipose tissue triacylglycerol synthesis (30). However, we have shown that acylation stimulating protein (ASP) also has this property and indeed is even more potent in this respect than insulin (6, 31) and in consequence may play a physiologic role in the postprandial distribution of fatty acids.

Thus adipocytes from the two locations of interest were compared and triacylglycerol synthesis from exogenous substrates was estimated by two radiolabels under both basal and stimulated conditions. Postprandially, plasma glucose and fatty acid (derived from the action of lipoprotein lipase on plasma triacylglycerol) concentrations rise, and the concentration ranges of glucose and fatty acids used as substrates were within these physiological ranges (19). These substrates are then taken up by adipose tissue sites and used for triacylglycerol synthesis for storage, and this study specifically examines triacylglycerol synthesis from exogenous sources only. Previous work by Edens, Leibel, and Hirsch (32) has indicated that fatty acid freed by intracellular hydrolysis must exit the cell in order to be taken up and re-esterified and therefore the specific activity of the medium pool of fatty acid should represent the triacylglycerol synthetic pool. The results were similar throughout: in every situation examined, subcutaneous adipocytes synthesized more triacylglycerols from exogenous substrates, both fatty acid and glucose, than omental adipocytes although in the tissue fragments there was considerable variation from one subject to the next. Even more interesting are the results

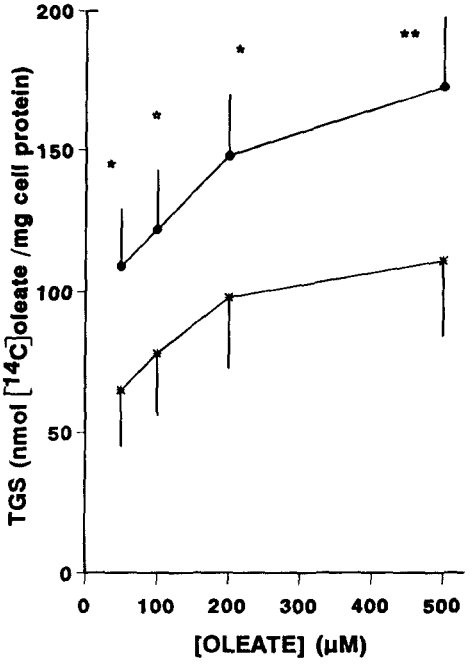

Fig. 8. Oleate stimulation of triacylglycerol synthesis in cultured preadipocytes. Triacylglycerol synthesis was measured in cultured omental (*; n = 7) and subcutaneous (●; n = 6) preadipocytes incubated with 5.5 mM [^{3}H]glucose in serum-free medium supplemented with increasing concentrations of [^{14}C]oleate: BSA for 24 h. Each cell line was measured in triplicate. Results are shown as nmol [^{14}C]oleate incorporated into triacylglycerol per mg soluble cell protein as an average + SEM of all of the cell lines; *, $P < 0.07$; **, $P < 0.05$).

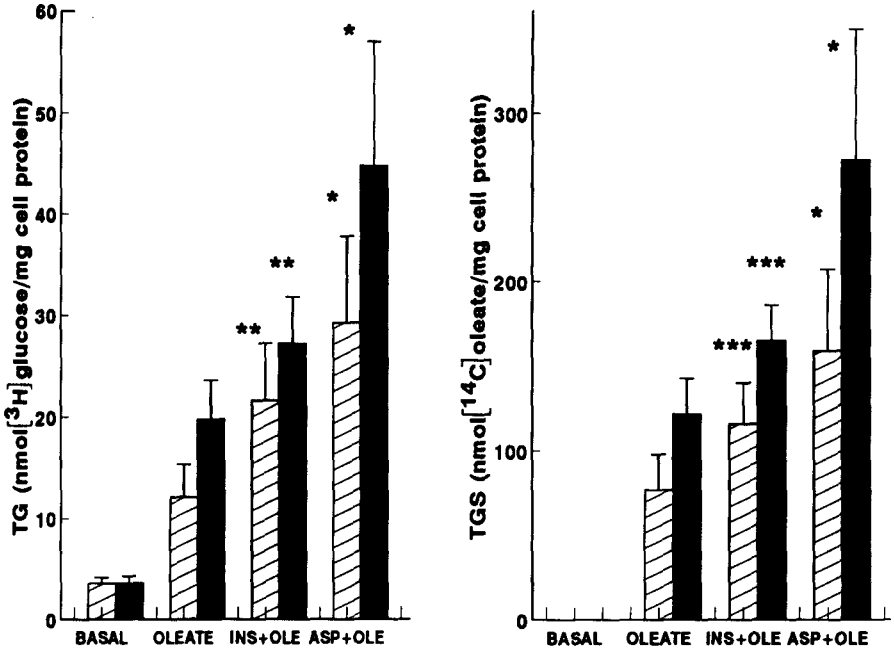

Fig. 9. Effect of insulin and ASP on oleate-stimulated triacylglycerol synthesis in cultured preadipocytes. Triacylglycerol synthesis was measured as [^{3}H]glucose incorporation into triacylglycerol (left panel) or [^{14}C]oleate incorporation into triacylglycerol (right panel) in omental (hatched bars, n = 7) or subcutaneous (solid bars, n = 6) preadipocytes per mg soluble cell protein. Cells were incubated in basal (serum-free medium with 5.5 mM [^{3}H]glucose) or supplemented with 100 μM [^{14}C]oleate, 100 μM [^{14}C]oleate plus insulin (10 μg/ml), or 100 μM [^{14}C]oleate plus ASP (40 μg/ml). Each cell line was measured in triplicate. Results are given as average ± SEM of all of the cell lines; *, $P < 0.02$; **, $P < 0.01$; ***, $P < 0.005$.

from the cultured preadipocytes. In spite of being maintained in an identical environment, and even after several passages, the subcutaneous and omental cells maintained their characteristic differences in triacylglycerol synthesis. At each concentration of glucose and fatty acid the subcutaneous cells synthesized more triacylglycerol from exogenous substrates than did the omental cells, and indeed the differences appeared to be even more marked than in the adipose tissue fragments. This maintenance of adipose tissue identity has also been demonstrated for adipose tissue androgen/estrogen metabolism (33) and in the case of obese versus nonobese preadipocyte mitogen secretion (34). The similarities demonstrated between freshly isolated adipose tissue and cultured preadipocytes are striking. This suggests that factors intrinsic to the specific type of adipose tissue are important in regulating overall capacity of the tissue to synthesize triacylglycerol and that not all adipose tissue is the same.

Numerous studies have shown strong relations between the degree of omental obesity and the level of plasma triacylglycerols, small dense LDL, apoB, and plasma insulin (1). These have usually been related to excess release of FFA from omental adipose tissue, and as a consequence, increased delivery of FFA to the liver (35). The present study suggests another mechanism, namely, that FFA removal from plasma will be less efficient in the omental vascular compartment than in the subcutaneous. If so, this would result in increased delivery of fatty acids either as FFA or as partially degraded chylomicrons to the liver. The present study does not resolve to what extent increased lipolysis as opposed to reduced synthesis of triacylglycerols in adipose tissue is responsible for the characteristic dyslipidemia associated with omental obesity. Nevertheless, given their consistency under the variety of experimental situations examined, we believe the present findings broaden the framework that may account for the relationships of omental obesity to dyslipoproteinemia.

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