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Direct Free Fatty Acid Storage in Different Sized Adipocytes from the Same Depot

Tamim I. Rajjo, **Debra A. Harteneck**, and **Michael D. Jensen***
Endocrine Research Unit, Mayo Clinic, Rochester, MN 55905

Abstract

Objective—Human adipocytes take up free fatty acids (FFA) directly from the circulation, even at times of high lipolytic activity. Whether these processes occurs simultaneously within the same cells or are partitioned between different cells, for example large and small cells, is unknown.

Design and Methods—We measured direct FFA storage in subcutaneous fat in 13 adults using a continuous infusion of [U-^{13}C]palmitate and a bolus of [1-^{14}C]palmitate followed 30 min later by abdominal and femoral adipose biopsies. The adipocytes were isolated by digestion procedures and separated into small, medium and large populations by differential floatation.

Results—We were able to isolate populations of adipocytes that were statistically and clinically (~3 fold different) in size. Adipocyte lipid SA was not different between small, medium and large cells, therefore, FFA storage per unit lipid was not different. However, FFA storage rates were significantly (2-4 times) greater per cell in large than small cells ($P < 0.005$). In summary, relative to lipid content, FFA storage rates are not different in large and small adipocytes, however, large cells have greater storage rates per cell.

Conclusions—This suggests that the processes of FFA release and storage are taking place simultaneously in adipocytes.

Keywords

adipose biopsy; free fatty acid isotopic tracers; femoral adipose tissue; abdominal adipose tissue

Introduction

We recently found that adipocytes take up and store free fatty acids (FFA) directly from the circulation independent of the lipoprotein lipase mechanism (1). Surprisingly, this process occurs even in the postabsorptive state when adipocytes are actively releasing FFA. In the postabsorptive state ~9% and 3% of systemic FFA are directly re-stored in subcutaneous fat in women and men, respectively (1,2). Even more unexpectedly, plasma FFA concentrations are the best predictor of direct FFA storage rates (2), which would indicate that greater

*Corresponding author: Michael D. Jensen, M.D., Mayo Clinic, Endocrine Research Unit, 200 1st Street SW, Rm 50194 Joseph, Rochester, MN 55905, telephone - 507 255-6515, fax - 507 255-4828, jensen.michael@mayo.edu. .

Conflicts of interest: None

lipolysis is associated with greater direct FFA storage. We had previously believed that at times of active FFA release from adipocytes an unfavorable concentration gradient would be generated such that FFA would not be simultaneously taken up and stored. We considered a variety of explanations as to how these two processes could occur simultaneously.

Increased adipocyte FFA storage rates in the face of increased lipolysis could be explained if release and uptake are partitioned within the same cell. FFA uptake might largely occur via facilitated transport in the caveolae through interactions between CD36, FATP1, and ACS, whereas the release processes might occur in other parts of the cell - mediated by adipose tissue triglyceride lipase (ATGL), hormone sensitive lipase (HSL), adipose lipid binding protein (aP2/ALBPF), and FATP4. If this hypothesis is correct, cells with high rates of uptake also have high rates of release and cell size remains stable. For example, large adipocytes are reported to be more lipolytically active in vitro (3,4). If this is true in vivo, then large cells would also have greater storage than smaller cells in proportion to cell size in order to maintain fat cell size. An alternative hypothesis is that some cells preferentially release FFA while others take up FFA – this would obviate the potential issue of unfavorable intra-adipocyte fatty acid concentration gradients. In this scenario, small adipocytes are disproportionately contributing to adipose tissue FFA storage and large adipocytes account for FFA release. In support of this theory, if small cells eventually become larger cells then fatty acid storage exceeds FFA release at some point in time.

Cell size is an important correlate/predictor of cell function. The storage of dietary fat in fat cells of different sizes from the same depot in humans was first described by Dr. Björntorp (5). He measured subcutaneous adipose tissue meal fatty acid storage using radiotracers in three men undergoing surgery and found no significant differences in triglyceride storage/ lipid weight of different cell sizes within the same depot. More recently, by using refined techniques for separating large and small cells investigators have been able to discover substantial differences between cells of different size within the same depot (6-9). We exploited these strategies to separate adipocytes by size to test the hypothesis that in vivo FFA storage is greater in smaller cells than larger cells within the same depot.

Research Design and Methods

Participants

After approval from the Mayo Clinic Institutional Review Board, 5 men and 8 premenopausal women age 35 ± 9 years gave informed written consent to participate in the study. Volunteers were healthy, non-smokers, weight-stable for at least 2 months prior to the study, and on no medications known to influence lipid metabolism.

Experimental Design

All volunteers consumed an isoenergetic diet, eating all meals from the Mayo GCRC for 3 days prior to the study to assure consistency of energy intake and nutrient composition prior to the studies. The macronutrient content of the meals was 45% carbohydrate, 20%protein and 35% fat. Body composition was assessed using DEXA and a single slice CT abdomen to measure subcutaneous and visceral fat mass (10). The volunteers were admitted to the

Clinical Research Unit (CRU) the evening prior to the study and given a standardized meal at 1800. The next morning an intravenous catheter was placed in a forearm vein to allow for infusion of isotopic tracers. A second, retrograde hand vein IV catheter was inserted for the sampling of arterialized venous blood (11). At ~0700 h, after collection of a baseline blood sample, a continuous infusion of [U-^{13}C]palmitate (Cambridge Isotope Laboratories, Andover, MA) was started at ~100 nmol·min^{-1}. After 50 min for isotopic equilibration, we began collecting arterialized blood samples to measure plasma palmitate kinetics. At ~0800 volunteers received an intravenous bolus of ~160 PCi of [1-^{14}C]palmitate (NEN Life Science Products, PerkinElmer, Boston, MA)) bound to human albumin. Thirty minutes after the bolus of [1-^{14}C] palmitate abdominal and femoral adipose biopsies were collected under local anesthesia using sterile technique as previously described (1). The adipose tissue samples (≥ 2 g from each site) was immediately rinsed of blood and transported to the laboratory. The volunteers were dismissed from the CRU after appropriate care for the adipose tissue biopsy sites.

Adipose tissue digestion

The adipose tissue specimens for each biopsied site were divided into smaller aliquots and placed into HEPES solution containing collagenase (Type II C-6885; Sigma Chemical Co. St Louis, MO) for digestion. These aliquots were then placed into a gently shaking 37° C water bath for 15-25 minutes. The resulting cell suspension was filtered through 250 micron nylon mesh and centrifuged at room temperature for 5 min at 300g to bring the cells to the top. The HEPES/collagenase solution below the cells was pipetted off and the cells were re-suspended in fresh HEPES solution. This procedure was repeated once in order to wash the cells. Final re-suspended volume was in 10 ml of HEPES. Cell sizing (12) was done before and after separating the sample into small, medium and large cells.

Adipocytes separation by size

When suspended in an aqueous solution, larger adipocytes float to the surface faster than small adipocytes (6). Using this approach we separated three populations of different size cells. Briefly, the adipocyte mixture and 40 ml HEPES were placed in a 250 cc separation funnel, gently mixed, and allowed to float for 50 seconds, at which time the lower 35 ml was drained from the separation funnel into a 50 ml Falcon tube to collect small cell fraction. We replaced 35 ml of HEPES buffer to the funnel and repeated this same procedure step in an effort to collect the maximum number of the smallest cells. To obtain the medium size cell fraction, we repeated the addition of 35 ml of HEPES, gently mixed and used a 35 second floatation time. The final portion that contained the large cell size fraction was then drained from the funnel and collected in a third 50 ml Falcon tube. To retrieve any remaining cells, the funnel was rinsed with HEPES solution, which we collected and combined with the large cell fraction. All tubes were briefly centrifuged at 300g, and a small aliquot of the cell suspension (100-200 μl) was removed from the original whole and each fraction for cell sizing with the remainder set aside for lipid extraction. A typical set of photomicrographs following this procedure are shown in Figure 1.

Lipid extraction

Lipid extraction was performed on the original whole and each fraction of adipocytes. The HEPES solution was removed from beneath the floating cell layer and 15 ml of CHCL3:MEOH (2:1) added to each tube. The mixtures were transferred to a 20 ml glass tube and placed in a dark cold room for two days. We added 3.75 ml of 0.88% KCL to each fraction of cells and after 20 minutes the samples were centrifuged for 10 minutes at 2000 rpm. The lower layer was removed and filtered into a pre-weighed scintillation vial. Samples were dried down using the evaporator system. After drying, the vials were reweighed in order to calculate actual lipid weight. Water and liquid scintillation cocktail was added to each vial before being placed on the scintillation counter to measure radioactivity. We have previously shown, using this experimental design, that virtually all ^{14}C-fatty acid tracer detected in isolated adipocytes is in the triglyceride fraction (1).

Assays

Plasma palmitate concentration and enrichment at steady state, and [U-^{13}C]palmitate infusate concentration and enrichment were measured as previously described (13). Screening laboratory studies were done using standard clinical laboratory methods.

Calculations

Systemic palmitate turnover [rate of appearance (Ra) = rate of disappearance (Rd)] was calculated by dividing the [U-^{13}C]palmitate infusion rate by the steady-state plasma [U-^{13}C]palmitate enrichment throughout the infusion after steady state was achieved. The rate of palmitate storage into adipocytes was calculated as previously described (2). We also calculated the rate of palmitate storage into small, medium and large abdominal and thigh fat cells both on per gram lipid basis and per million adipocytes.

Power Calculations and Statistics

We used the following information to develop the study design: 1) the lipid weight from the cell fractions can be accurately measured to the nearest 0.1 mg; 2) each sample is counted a sufficient period of time to reduce the counting error to < 2%. Our a priori estimate was that a difference in adipose lipid SA of 10% or greater between the small and large cell fractions would be meaningful in terms of the balance of FFA storage. To have 90% power to detect this difference with a $P<0.05$ we need only 3 participants. To account for possible technical problems we included 13 participants. With this number of total subjects we had 95% power to detect a 2% difference between large and small cells (assuming a normal distribution of differences).

Values are expressed as means ± SD or mean ± SEM. Statistical comparisons between different size cells using a repeated measures ANOVA followed by paired t tests if the ANOVA was significant. Statistical analyses were performed with JMP 9.0.1 (SAS Institute Inc. Cary, NC).

Results

Subject characteristics

Table 1 provides the anthropometric and biochemical characteristics of our participants. None of the volunteers were suffering from acute or chronic metabolic illnesses. Plasma palmitate concentrations averaged 140 ± 24 μmol/L and palmitate flux averaged 121 ± 25 μmol/min during the interval between the [^{14}C]palmitate bolus and the adipose biopsies.

Adipocyte size and specific activity

Table 2 provides the mean (mean of 95% confidence intervals of adipocyte size and mean ± SEM of adipocyte lipid specific activity. For both abdominal and femoral sites, adipocytes in the small, medium and large fractions were significantly (at least $P < 0.005$) different in size. Compared with the average adipocyte size for non-fractionated tissue, small and medium femoral adipocytes were significantly smaller (P at least < 0.01), whereas the large adipocyte fraction was not significantly larger ($P = 0.10$) than non-fractionated femoral adipocytes. The small abdominal adipocytes were significantly smaller than the non-fractionated abdominal adipocytes ($P < 0.001$), but medium ($P = 0.06$) and large ($P = 0.25$) abdominal adipocytes were not significantly different from non-fractionated abdominal adipocytes. The adipocyte lipid specific activity was not significantly different between any of the adipocyte fractions from the abdominal or femoral depots.

Palmitate storage rates

Table 3 provides the mean ± SEM of palmitate storage rates per g lipid and per million cells. Per gram adipocyte lipid, palmitate storage rates were not different between all cells, small, medium and large cells. However, palmitate storage per million cells was 60-80% less in small than large cells ($P \leq 0.005$ for both abdomen and thigh) and also less in medium than large cells ($P = 0.005$ for both abdomen and thigh).

Discussion

We tested the hypothesis that FFA storage differs between small, medium and large cells by performing carefully timed adipose tissue biopsies after a bolus of [^{14}C]palmitate and then separating adipocytes by size using differential floatation. We found that the adipocyte lipid SA was not different between small, medium and large cells from the same depots of the same individuals. Palmitate storage rates per gram of adipose tissue lipid were not different between the three cell size populations, but storage rates per million cells was greater in larger than smaller cells. Thus, we were able to disprove out hypothesis that direct storage of FFA in human adipocytes occurs preferentially in small cells.

Our findings are consistent with the report of Björntorp et al (5), who collected tissue from 3 men undergoing elective intra-abdominal surgery after they consumed a meal containing ^{14}C-palmitate. They found greater meal fatty acid uptake in large compared with small adipocytes per cell, but not per g lipid. Combined, our results and Björntorp's (5) compliment the findings of Laurencikiene et al (8), who found lipolysis rate to be equal in large and small cells isolated from the same liposuction samples when expressed per g lipid.

However, when expressed per cell, lipolysis rates were 50-100% greater in large adipocytes. Together, a clear picture is emerging where large adipocytes have up-regulated both the release and storage pathways to maintain a relatively stable cell size. This is as opposed to a circumstance where large adipocytes might attain a size where there is a shift towards net fatty acid loss and reduced size. Our findings of greater uptake, combined with the literature describing greater lipolysis from large adipocytes in in-vitro (8), suggests large adipocytes likely are relatively stable in size.

These combined results also imply that partitioning of storage and release occurs within cells rather than between cells. Rates of FFA storage (and probably release) per cell appears to be proportional to lipid content. Thus, on average, lipid inflow and outflow are balanced in cells of different sizes, although to the extent small fat cells eventually become larger, there must be some imbalance. We believe this indicates that fatty acid release and storage pathways are functionally distinct within adipocytes. It has been suggested that fatty acids released as a result of ATGL and HSL action are shuttled by fatty acid binding proteins (14,15) to the cell surface for release, whereas fatty acids that enter the cell are quickly esterified to long chain acyl-CoA's, thereby maintaining the concentration gradient for uptake. We have reported that the activities of acyl-CoA synthetase (ACS) or diacylglycerol acetyl-transferase (DGAT) are predictive of direct FFA storage rates even after accounting for the effects of plasma FFA concentrations (2,16). It may be that inter-individual and inter-depot differences in proteins and enzymes of the fatty acid storage pathway become the rate determining steps when uptake of fatty acids increases to a critical level.

One limitation of this study is that we did not have sufficient tissue from our research biopsy material to measure CD36, ACS and DGAT as we have in previous studies (2,16). Larger amounts of tissue are needed to be able to sort the samples into small, medium and large cells for measurement of lipid SA. This left us with insufficient material for additional assays. Investigators working with surgically-obtained samples or animals can readily collect enough adipose tissue to perform numerous assays, even after cell sorting is complete (6-8). However, an advantage of collecting samples under outpatient research settings is the ability to study both abdominal subcutaneous and femoral fat from carefully phenotyped volunteers and to measure rates of FFA storage using combined stable and radioisotope tracers. We were able to collect sufficient tissue to make accurate direct FFA storage measurements from individuals with BMI's of 24-27.

In summary, per g of adipose tissue lipid, direct FFA storage rates are equal in small, medium and large cells from abdomen and thigh adipose tissue. Storage rates per cell are much greater in large than small adipocytes. These results indicate that our findings of direct FFA storage in human adipose tissue is not a phenomenon of cell specialization within fat – small cell storing and large cells releasing FFA – but that adipocytes of all sizes have functionally distinct pathways of storage and release that do not appear to be in conflict.

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What is already known about this subject:

- Adipose tissue can take up and release FFA simultaneously
- Whether this is because some adipocytes take up FFA while others release FFA is unknown
- Large and small adipocytes within the same depot have different characteristics

What this study adds:

- Direct FFA storage rates per g lipid are the same in small and large adipocytes in the same person and same depot
- Direct FFA storage rates are greater in larger than smaller cells in proportion to adipocyte size
- Direct FFA storage appears to occur simultaneously with FFA release in adipocytes.

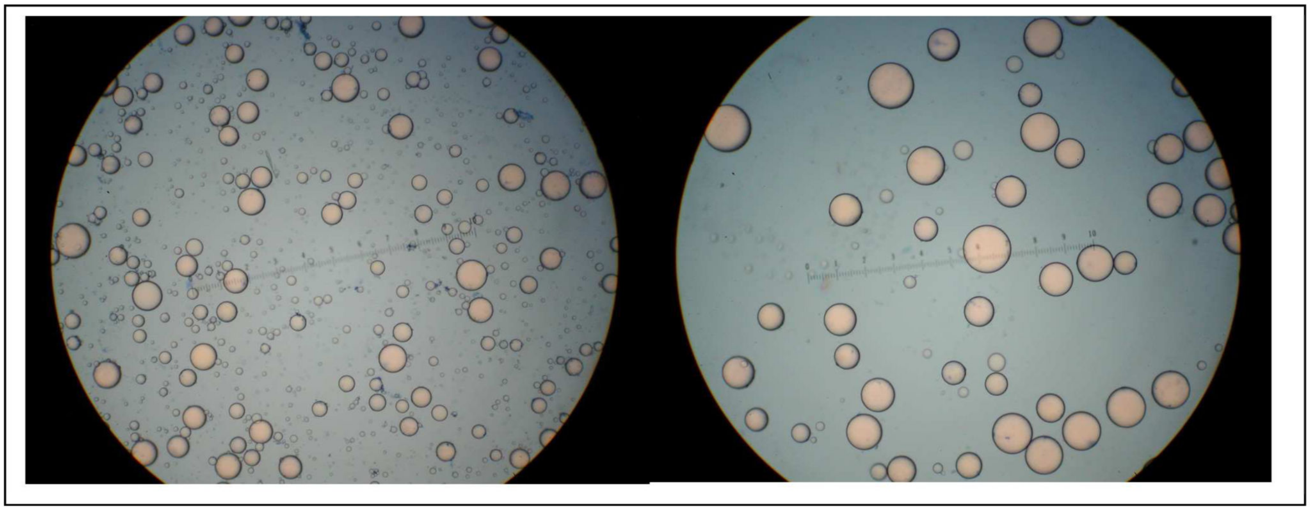

Figure 1.
Fat cell separation by size. Representative photographs of thigh adipocytes isolated from the same individual after their separation into different size populations. The left panel shows a photograph of the small adipocytes (average size 0.16 μg lipid/cell) and the right panel shows a photograph of the large adipocytes (average size 0.75 μg lipid/cell).

Table 1

Subject Characteristics

BMI (kg/m^2)	30.0 ± 3.7
Weight (kg)	93.2 ± 18.3
Body fat (%)	37 ± 6
Leg fat (kg)	11.3 ± 3.3
Upper body subcutaneous fat (kg)	17.8 ± 5.0
Visceral fat (kg)	4.7 ± 4.2
LDL-cholesterol (mg/dL)	109 ± 25
HDL-cholesterol (mg/dL)	53 ± 17
Triglycerides (mg/dL)	103 ± 36
Glucose (mg/dL)	88 ± 8

Table 2

Adipocyte size and lipid specific activities

	All cells	Small	Medium	Large
	Adipocyte size (µg lipid/cell)			
Abdomen	0.71 (0.66-0.76)	0.27 (0.23-0.31)*	0.52 (0.48-0.56)†	0.77 (0.72-0.81)
Thigh	0.79 (0.74-0.85)	0.25 (0.20-0.29)*	0.57 (0.52-0.61)†	0.90 (0.85-0.95)
	Adipocyte lipid specific activity (dpm/g)			
Abdomen	486 ± 78	535 ± 81	506 ± 80	484 ± 75
Thigh	463 ± 79	442 ± 95	465 ± 84	508 ± 86

Values are mean (mean of the 95% confidence intervals) for each adipocyte fraction. Adipocytes from abdomen and thigh were separated using differential flotation.

*P < 0.001 vs. medium and large cells;

†P < 0.005 vs. large cells.

Table 3

Palmitate storage rates

	All cells	Small	Medium	Large
	Palmitate storage (nmol·g lipid^{-1}·min^{-1})			
Abdomen	0.25 ± 0.04	0.27 ± 0.033	0.26 ± 0.03	0.25 ± 0.03
Thigh	0.24 ± 0.04	0.22 ± 0.05	0.23 ± 0.04	0.26 ± 0.04
	Palmitate storage (pmol·million cells^{-1}·min^{-1})			
Abdomen	0.166 ± 0.027	0.074 ± 0.012*	0.134 ± 0.023†	0.186 ± 0.030
Thigh	0.192 ± 0.040	0.054 ± 0.015*	0.144 ± 0.033†	0.247 ± 0.056

Values are mean ± SEM. Palmitate storage rates in small, medium and large adipocytes from isolated from abdomen and thigh are expressed per g adipocyte lipid and per million cells.

*P ≤ 0.005 vs. medium and large cells;

†P ≤ 0.005 vs. large cells.