

Fat depot origin affects fatty acid handling in cultured rat and human preadipocytes

FRANK CASERTA,¹ TAMARA TCHKONIA,¹ VILDAN N. CIVELEK,¹ MARC PRENTKI,⁴ NICHOLAS F. BROWN,⁵ J. DENIS MCGARRY,⁶ R. ARMOUR FORSE,² BARBARA E. CORKEY,¹ JAMES A. HAMILTON,³ AND JAMES L. KIRKLAND¹

¹Obesity Research Center, Evans Department of Medicine, and Departments of Biochemistry,

²Surgery, and ³Biophysics, Boston University Medical Center, Boston, Massachusetts 02118;

⁴Department of Nutrition, University of Montreal, Montreal, Quebec, Canada H2L 4M1;

⁵Department of Internal Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261;

and ⁶Departments of Internal Medicine and Biochemistry, Gifford Laboratories, Center for Diabetes Research, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 91010

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Caserta, Frank, Tamara Tchkonina, Vildan N. Civelek, Marc Prentki, Nicholas F. Brown, J. Denis McGarry, R. Armour Forse, Barbara E. Corkey, James A. Hamilton, and James L. Kirkland. Fat depot origin affects fatty acid handling in cultured rat and human preadipocytes. *Am J Physiol Endocrinol Metab* 280: E238–E247, 2001.—Regional differences in free fatty acid (FFA) handling contribute to diseases associated with particular fat distributions. As cultured rat preadipocytes became differentiated, FFA transfer into preadipocytes increased and was more rapid in single perirenal than in epididymal cells matched for lipid content. Uptake by human omental preadipocytes was greater than uptake by abdominal subcutaneous preadipocytes. Adipose-specific fatty acid binding protein (aP2) and keratinocyte lipid binding protein abundance was higher in differentiated rat perirenal than in epididymal preadipocytes. This interdepot difference in preadipocyte aP2 expression was reflected in fat tissue in older animals. Carnitine palmitoyltransferase 1 activity increased during differentiation and was higher in perirenal than in epididymal preadipocytes, particularly the muscle isoform. Long-chain acyl-CoA levels were higher in perirenal than in epididymal preadipocytes and isolated fat cells. These data are consistent with interdepot differences in fatty acid flux ensuing from differences in fatty acid binding proteins and enzymes of fat metabolism. Heterogeneity among depots results, in part, from distinct intrinsic characteristics of adipose cells. Different depots are effectively separate miniorgans.

fatty acid binding protein; free fatty acid uptake; carnitine palmitoyltransferase 1; regional fat distribution

FAT DISTRIBUTION IN HUMANS varies considerably, even among individuals with the same total body fat content. Particular fat distributions are associated with distinct risks for diabetes, atherosclerosis, dyslipidemia, and hypertension (12, 24, 30, 31, 46, 58–60, 63, 66, 77). For several decades, it has been known that fat tissue is regionally heterogeneous with respect to met-

abolic function (5). These regional differences in metabolic function probably contribute to the risk profiles of particular fat distributions. Regional differences in endogenous free fatty acid (FFA) handling may be especially important in this regard (41, 42). Fat cells isolated from various rat and human depots differ in size, responses to insulin and lipolytic agents, lipoprotein lipase (LPL) release, lipoprotein binding, de novo lipid synthetic capacity, and fatty acid incorporation, each of which can affect fatty acid handling (5, 27, 29, 36, 40, 58, 59, 74, 80, 83). A particularly intriguing question concerns the fundamental basis of regional variation in fatty acid handling. Is interdepot variation solely a result of influences extrinsic to adipose cells (including their hormonal and paracrine microenvironment, local nutrient availability, innervation, and anatomic constraints), or do intrinsic differences in the innate characteristics of adipose cells also contribute to interdepot variation? Studies in preadipocytes cultured under identical conditions originating from various depots from the same individuals suggest that the latter may be the case (3, 25, 26, 37, 50, 54, 91).

To elucidate the fundamental mechanisms responsible for regional variation in fatty acid handling, we investigated the transfer of exogenous FFA into preadipocytes cultured from different fat depots. There are few reliable methods to measure the transfer of FFA across lipid bilayers. Partly as a result of methodological problems, there is controversy as to the mechanism and rate of transbilayer movement of FFA in plasma membranes (33). Some investigators have hypothesized that FFA require anion transporters to reach the cytoplasmic compartment after their delivery to the outer leaflet of the membrane (87). There is indirect evidence to support the hypothesis of protein-mediated transport, including the cloning of a “fatty acid trans-

Address for reprint requests and other correspondence: J. L. Kirkland, Rm. F435, Boston University Medical Center, 88 E. Newton St., Boston, MA 02118 (E-mail: kirkland@bu.edu).

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port protein" and association of its expression with increased FFA utilization (84, 86). However, this protein is a very long chain acyl-CoA synthetase (21). This may account for its promotion of very long chain fatty acid uptake rather than transporter activity. Two other potential fatty acid transport proteins, mammalian fatty acid binding protein and fatty acid transporter CD36, have since been described and may play a role in fatty acid uptake through direct or indirect mechanisms (1).

An alternative hypothesis is that FFA are capable of rapid diffusion ("flip-flop") across membranes and do not require protein-mediated transport, although membrane proteins may still affect FFA uptake through indirect mechanisms (35). Key tenets of this theory are that a significant fraction of FFA in a membrane is un-ionized and that this neutral form of FFA can flip-flop rapidly. If such passive FFA diffusion occurs across membranes, cytosolic pH changes should accompany FFA movement (45). This, in fact, occurs: pH changes in the cytosolic compartment of adipocytes and pancreatic β -cells consistent with the predictions of this model have been described (18, 34). The mechanisms of protein-mediated transport and passive diffusion are not necessarily mutually exclusive. Some investigators hypothesize that protein-dependent mechanisms are important in determining basal FFA levels, whereas for high levels of FFA, the diffusion mechanism becomes important (2). In addition to transbilayer movement, the amount of fatty acid entering a cell may also depend on the amount of intracellular fatty acid binding protein available as well as the rate at which fatty acids are esterified or utilized through β -oxidation (19).

Within the cell, carnitine palmitoyltransferase 1 (CPT-1) is thought to be a critical control point in fatty acid metabolism. After conversion of FFA to long-chain acyl-CoA (LC-CoA), CPT-1 activity determines whether β -oxidation or esterification will occur. There are two isoforms of CPT-1 whose relative expression varies in different cell types and as cells undergo differentiation (6, 14, 15, 75, 76, 89). Little is known about effects of fat depot origin on CPT-1 or LC-CoA levels.

To determine whether fat cell anatomic origin results in distinct patterns of fatty acid handling that result from mechanisms intrinsic to the cells themselves, we compared fatty acid uptake, adipose-specific fatty acid binding protein (aP2) and keratinocyte lipid binding protein (KLBP) expression, CPT-1 activity and expression, and LC-CoA levels in differentiating rat preadipocytes, as well as fatty acid uptake in human preadipocytes originating from different depots. We found that fatty acid uptake increased with differentiation, was greater in rat perirenal than in epididymal cells, and was greater in human visceral than in subcutaneous cells. Interdepot and differentiation-dependent differences in preadipocyte fatty acid binding protein expression, CPT-1 isoform expression and activity, and LC-CoA levels were found that could contribute to this intrinsic variation in fatty acid handling. Variation among depots in preadipocyte aP2 abundance in

young animals became evident in fat tissue in older animals. This supports the contention that preadipocytes continue to develop into fat cells in adulthood and that intrinsic differences in preadipocyte characteristics contribute to interdepot differences in fat tissue function.

METHODS

Rat preadipocyte cultures. The epididymal and caudal portion of the perirenal depots of 3- (225–250 g) and 24 (400–450 g)-mo-old, specific pathogen-free Fischer 344 rats were removed. We compared epididymal and perirenal rat cultures, because purity and recovery have been optimized for these depots and because large differences in the characteristics of cultures from these two regions have been described in previous studies (25, 26, 50, 54, 91). Fat tissue was minced into fragments, digested in 1 mg/ml of collagenase for 60 min at 37°C, and filtered (50, 54, 55, 91). After centrifugation of the digests, the pellets were resuspended in a basal medium [α -minimal essential Eagle's medium (α -MEM) containing 10% fetal bovine serum (FBS) and antibiotics] and were plated at $\sim 4 \times 10^4$ cells/cm² in culture dishes. After 12 h, a period during which no replication occurs (25), the adherent preadipocytes were washed, trypsinized, and replated at a density of 4×10^4 cells/cm² on coverslips coated with Cell-Tak (Collaborative Research, Bedford, MA) for single-cell imaging studies or into 25-cm² flasks for other studies. We found that replating 1) reduces mesothelial cell and macrophage contamination and 2) results in accurate plating densities [plating density affects capacity of preadipocytes to differentiate (92)]. Medium was changed every 48 h. We have previously demonstrated that, by use of these methods, preadipocyte recoveries are similar among depots and that our isolation procedures result in >90% pure populations of preadipocytes, irrespective of fat depot origin (50). Brown fat preadipocytes are not present in epididymal or perirenal (caudal portion) cultures, because uncoupling protein 1 is not evident in perirenal or epididymal cultures but is observed in interscapular preadipocyte positive controls (54, 62). We have previously shown that there are at least two types of preadipocytes in both rat and human fat, the one capable of more extensively differentiating than the other (50, 53, 91). The proportions of each of these subtypes are identical in epididymal and perirenal preadipocyte cultures ($42 \pm 3\%$ of perirenal and $43 \pm 2\%$ of epididymal preadipocytes were of the extensively differentiating subtype, and $58 \pm 3\%$ and $57 \pm 2\%$, respectively, were of the less extensively differentiating subtype). Confluent preadipocyte cultures were differentiated in an enriched medium containing α -MEM, 2 μ M insulin, 10 mM glucose, 5 μ l/ml 10% Liposyn III (Abbott, North Chicago, IL), and 20% Nuserum (DM3) or, in α -MEM, 2 μ M insulin, 10 mM glucose, 125 μ M indomethacin, and 10% FBS (DM1) (50, 51, 54).

For imaging studies, differentiated cells cultured on coverslips coated with Cell-Tak were maintained in a Krebs buffer (109 mM NaCl, 4.6 mM KCl, 0.15 mM Na₂HPO₄, 2.0 mM CaCl₂, 1.0 mM MgSO₄, 10 mM glucose, 10 mM HEPES, 0.4 mM KH₂PO₄, and 5 mM NaHCO₃, pH 7.4). For mRNA analyses, cultures were harvested by scraping into a guanidinium thiocyanate buffer (16). For Western analyses, cells were washed twice with phosphate-buffered saline and then were scraped into 200- μ l ice-cold radioimmune precipitation buffer (RIPA) buffer (96). For the other studies, cells were scraped into a sucrose-Tris-EDTA buffer and homogenized (57).

Human preadipocyte preparation and culture. Fat tissue was resected during abdominal surgical procedures from subjects who had given informed consent. This protocol was approved by the Boston University Institutional Review Board for Human Research. The investigators excluded subjects with malignancies. All subjects had fasted for ≥ 10 h. Two of the subjects were men and four were women. Subjects were 38.3 ± 2.4 yr of age (mean ± 1 SE; range 33–48). The subjects were moderately obese (body mass index 29 ± 3). Two to ten grams of abdominal subcutaneous (external to the fascia superficialis) and omental fat were obtained from each subject. These depots were chosen because major differences have been found in the characteristics of preadipocytes and fat cells isolated from them (3, 27, 40, 74, 80). The tissues were minced and digested in a 1 mg/ml collagenase solution for 30 min, and the resulting digests were treated with an erythrocyte lysis buffer to improve subsequent differentiation (38, 90). The cells were plated for 6 h using a low serum-plating medium [HEPES-buffered DMEM-Ham's F12 (1:1), 0.5% FBS, and antibiotics], washed extensively, and replated at a density of 4×10^4 cells/cm² (plating efficiency was 50%). The cells were then cultured on a gel [1.5% gelatin/phosphate-buffered saline (wt/vol) placed in culture dishes for 24 h that were then washed before cells were plated] in medium containing 1:1 DMEM-Ham's F12, 100 nM dexamethasone, 500 nM human insulin, 200 pM triiodothyronine, 30 μ M biotin, 17 μ M pantothenate, 0.5 μ M rosiglitazone, and antibiotics for 2 wk.

CPT-1 activity. Total cellular CPT-1 plus CPT-2 activity in homogenates was determined in the presence of saturating carnitine and LC-CoA while monitoring the production of free CoASH enzymatically (6, 22, 93, 94). CPT-1 activity was measured, as previously described (6), in mitochondrial fractions obtained after purification on a Percoll gradient. Specifically, cells were gently homogenized seven strokes in a glass ten broek homogenizer using a tight-fitting pestle and were then centrifuged at 10,000 *g* for 10 min. The pellet containing intact mitochondria was washed and resuspended in a 10 mM HEPES, 0.1 M mannitol, 0.25 M sucrose buffer, pH 7.2. CPT-1 activity was measured as the malonyl-CoA-inhibitable formation of palmitoyl carnitine from [³H]carnitine in the presence of 80 μ M palmitoyl-CoA and 1 mM carnitine at 30°C during the linear portion of the time course. The reaction was started by the addition of carnitine and was stopped after 6 min by addition of isobutanol plus saturated (NH₄)₂SO₄.

Messenger RNA analysis. RNA was isolated from preadipocytes and collagenase-digested fat cells by use of the guanidinium thiocyanate-phenol method (16). RNA integrity was verified by means of 2% agarose gels. Messenger RNA was measured by Northern blot hybridization as described previously (51, 52, 54, 56, 88).

Western analysis. SDS-PAGE was performed according to the method of Laemmli (65). Either the gels were stained to visualize banding, or the protein was transferred to nitrocellulose for probing. Western analysis was done basically as outlined in Majumder (70). Blotting paper was blocked overnight at 4°C in Tris-buffered saline containing 5% milk. Incubation with the primary polyclonal aP2 and KLBP antibodies (provided by Dr. D. A. Bernlohr, University of Minnesota, St. Paul, MN) was for 1 h at 25°C. The secondary antibody was conjugated to horseradish peroxidase with incubation for 30 min at room temperature. Visualization of the binding of the horseradish peroxidase-conjugated secondary antibody was performed by chemiluminescence. Over the range of protein levels measured, responses were linear for the loading densities employed.

CoA esters. Free CoASH and the total pool of LC-CoA esters were measured enzymatically using published TCA extraction and alkaline hydrolysis techniques (22, 93, 94).

pH measurement and imaging. The fluorescent indicator 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein (Molecular Probes, Eugene, OR) was used to measure intracellular pH (pH_i) in single cells, as described previously in experiments in which fatty acid movement in groups of cells was monitored (34, 43). Dispersed preadipocytes were loaded with the indicator by a method similar to that described previously (34). Digital imaging analysis of single cells was performed on cells grown on coverslips with the use of a filter changer, a dual wavelength excitation light source (Ionoptix, Milton, MA), and an emission light detection system comprised of an intensified video camera and video data storage (69). Fluorescence was calibrated in terms of pH with the method of Rink et al. (82). In spectrofluorometric studies, oleic acid (NuChekprep, Elysian, MN) in Krebs buffer was added to cells dropwise as complexes with BSA (Sigma, St. Louis, MO; initial fraction by cold alcohol precipitation, essentially fatty acid free; >97% purity by agarose electrophoresis) that had been dialyzed against Krebs buffer. Two oleic acid-to-albumin ratios were used to achieve net partitioning of fatty acids into cells, 8:1 or 6:1. The 8:1 complex delivers more oleic acid to cells than the 6:1 complex, because the 8:1 complex utilizes the low-affinity binding sites on BSA, whereas the 6:1 complex utilizes only the high- and medium-affinity binding sites (17).

Statistical analysis. Northern and Western analysis densitometry data were analyzed by ANOVA with post hoc comparisons by Duncan's multiple range test or paired *t*-tests as appropriate (44, 47). Statistical analysis of imaging study data was done using applied regression analysis (61) or a two-tailed, unpaired, unequal variance *t*-test (32). *P* < 0.05 was considered significant.

RESULTS

Intracellular pH changes were measured by fluorescence to compare FFA uptake by cells from different depots occurring within the first few seconds after exposure to FFA and before FFA were metabolized substantially. The pH experiments were based on the more rapid flip-flop of the un-ionized form of FA compared with the ionized form (45). The pH decrease reflects binding of FA to the plasma membrane, diffusion through the membrane, and dissociation of protons from the FA that reach the inner leaflet of the plasma membrane. The extent of pH change after exposure to FFA is a measure of the total amount of FFA that binds to the plasma membrane and diffuses to the cytosolic leaflet, as well as properties of the cytoplasmic component, such as the buffering capacity of the cell and possibly the concentration of fatty acid binding proteins (33, 34). In previous studies, we demonstrated pH changes in cell populations in response to FFA (18, 34), whereas this study is the first to examine single cells by means of a similar fluorescence approach. The use of single cells removes potential complications arising from a heterogeneous population of cells. In single-cell imaging experiments, we used cells from different depots from the same animals that were matched for lipid content (determined by measuring numbers and diameters of lipid inclusions by light microscopy). The extent of the pH decrease in differentiated rat perire-

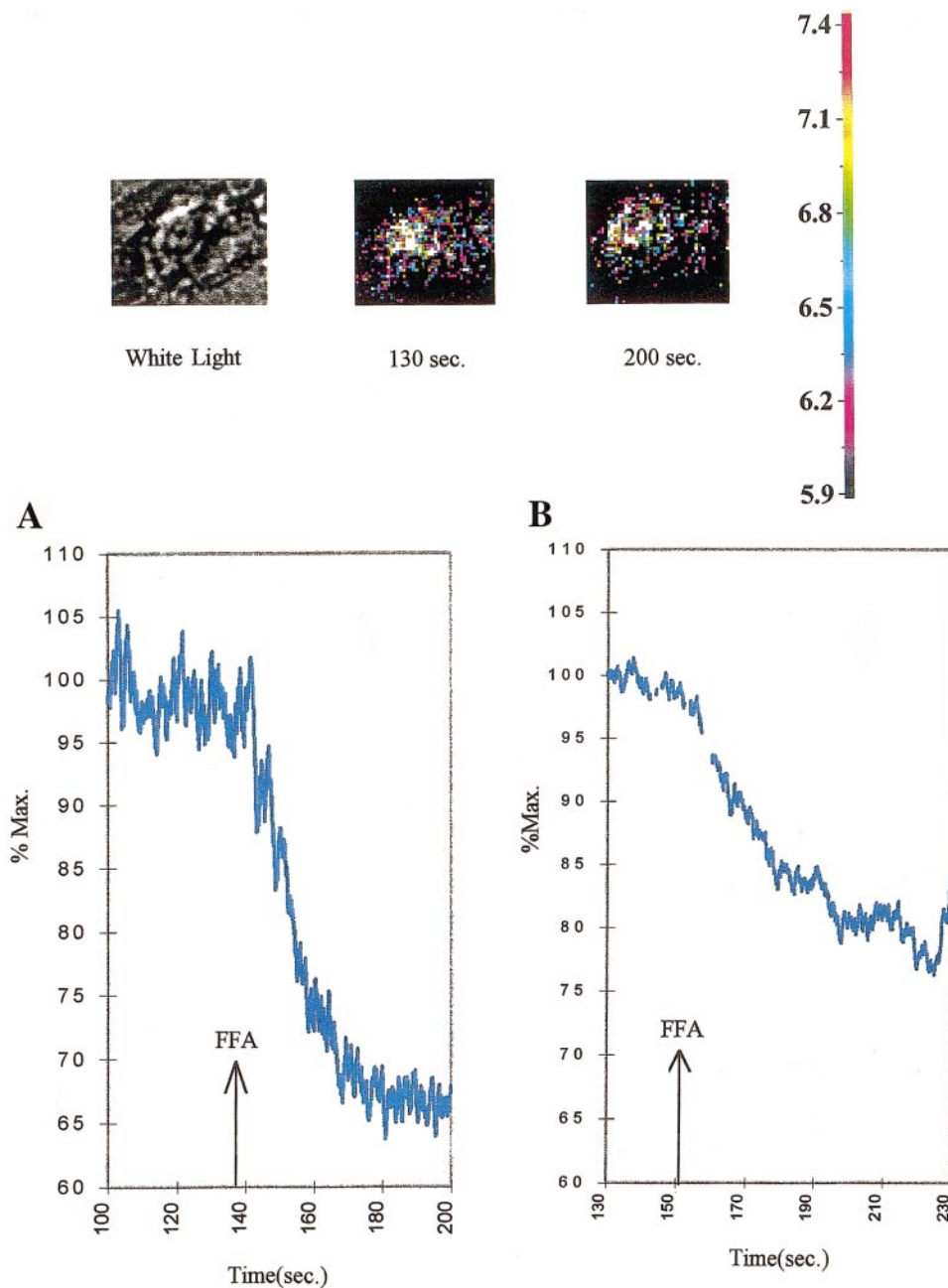


Fig. 1. Comparison of free fatty acid (FFA)-induced acidification in single, differentiated rat perirenal (A) and epididymal (B) preadipocytes loaded with 2',7'-bis(2-carboxyethyl)-5,(6)-carboxy-fluorescein (BCECF) and maintained in Krebs' buffer. Images of a cell before and after oleic acid addition (*top*, *center* and *right*, respectively) are shown above. pH in the images is indicated on the color bar. White light image of the cell studied is shown at *left*. Traces in (A) and (B) reflect the average fluorescence of BCECF in such cell images at a given time. A: large change in fluorescence in a perirenal cell (35%) on exposure to oleic acid complexed with BSA in a mole ratio of 8:1 (272 μ M oleic acid-34 μ M BSA). This high ratio, which encompasses low-affinity albumin binding sites, allows net transfer of significant amounts of fatty acid into the cell. In a parallel experiment in an epididymal cell (B), a smaller change (20%) occurred in fluorescence. Gaps in (B) are due to the removal of artifactual noise spikes. Similar results were obtained in 5 experiments, each using separate animals (total of 36 perirenal and 28 epididymal cells) in cells matched for number and size of lipid droplets. Signal intensity of 100% corresponds to pH 7.4; 60% signal intensity corresponds to pH 6.8.

nal preadipocytes exposed to oleic acid was greater than in epididymal cells (Fig. 1; 0.40 ± 0.02 and 0.26 ± 0.03 pH units in perirenal and epididymal cells, respectively; $P < 0.001$ by ANOVA; $n = 36$ perirenal cells from 5 animals and 28 epididymal cells from the same animals). The high ratios of FFA to BSA used in these experiments were intended to model the delivery of large amounts of FFA to adipocytes, as would occur during lipolysis of triglyceride-rich lipoproteins in adjacent capillary endothelial cells, rather than during basal conditions.

We also investigated FA uptake by human preadipocytes. In each of six experiments, differentiated human preadipocytes from the omental region took up oleic acid more rapidly and extensively than cells derived

from the subcutaneous region (Fig. 2). In cells from both depots, addition of 180 μ M albumin resulted in partial recovery of pH_i , as was observed previously in pancreatic β -cells and rat adipocytes (18, 34). With the assumption that the extent of pH change reflects total uptake by diffusion, these variations in FFA uptake confirm that site-specific differences occur in both the rat and the human.

As preadipocytes differentiate into fat cells, they accumulate multiple, small lipid droplets that coalesce into larger droplets. In perirenal preadipocyte cultures from four animals, individual cells were selected that contained large lipid droplets (diameter $>6 \mu\text{m}$). These cells were compared with cells in the same cultures selected at the same time that contained less lipid in

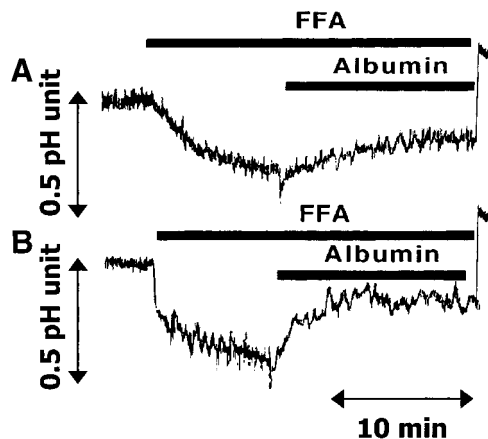


Fig. 2. Comparison of FFA-induced acidification in differentiated human subcutaneous (A) and omental (B) preadipocytes. Preadipocytes were isolated from abdominal subcutaneous and omental fat tissue from subjects undergoing abdominal surgery by collagenase digestion, filtration, and differential plating. Cells were cultured for 2 wk on a gel in a serum-free medium that promotes differentiation. Experiment shown is representative of those performed with cells cultured from 6 separate human subjects. The decrease in pH in omental cells exposed to oleic acid was greater than in abdominal subcutaneous cells (0.63 ± 0.09 vs. 0.38 ± 0.02 pH units in omental and abdominal subcutaneous cells, respectively; $P < 0.01$, paired *t*-test). Fatty acid was supplied to cells as a complex of 6 mol oleic acid-1 mol albumin ($272 \mu\text{M}$ oleic acid- $45 \mu\text{M}$ BSA). After pH achieved plateau, $180 \mu\text{M}$ BSA was added to the external medium.

multiple, small droplets (diameter $<3 \mu\text{m}$). FFA uptake was determined by following the intracellular pH decrease after oleic acid addition to the medium (8:1 complexes of oleic acid-albumin). The decrease in intracellular pH of preadipocytes with extensive lipid stores was 2.5 times that of cells with less lipid (0.23 ± 0.04 vs. 0.09 ± 0.03 pH units in cells with droplets $>6 \mu\text{m}$ vs. those with $3\text{-}\mu\text{m}$ droplets, respectively; $P < 0.005$ by applied linear regression analysis; $n = 18$ cells from 6 animals). Hence, initial FFA transfer was greater in preadipocytes that had progressed to the stage of having a few large droplets than in cells with multiple lipid droplets at earlier stages of differentiation.

The larger change in pH in perirenal than in epididymal cells could reflect, among other possibilities, differences in preadipocyte plasma membranes (including types and quantities of protein as well as lipid composition) and/or in the content of intracellular fatty acid binding proteins among sites. We investigated the latter possibility by examining the relationship between expression of the intracellular fatty acid binding proteins aP2 and KLBP and FFA uptake. In comparisons of cells of different anatomic origin, aP2 mRNA levels were 2.5-fold higher in differentiated perirenal than epididymal preadipocytes ($n = 12$; $P < 0.05$; Fig. 3). aP2 protein levels were also higher in differentiated perirenal than epididymal preadipocytes (2.5-fold; $n = 8$; $P < 0.01$; Fig. 4). KLBP expression was 2.2-fold greater in differentiated perirenal than in epididymal preadipocytes ($n = 5$; $P < 0.005$; Fig. 4). Indeed, in every experiment, fatty acid binding protein levels were higher in perirenal than in epididymal cells. As

indicated in Fig. 1, FFA uptake was more extensive in perirenal than in epididymal cells. The correlation among FFA uptake, aP2 expression, and KLBP levels also held when undifferentiated preadipocytes were compared with differentiated cells. Both aP2 and KLBP expression increased with differentiation [2.4-fold in the case of aP2 ($n = 8$, $P < 0.05$) and 2.5-fold in the case of KLBP ($n = 5$, $P < 0.05$)], as did FFA uptake (see above). Thus a positive correlation was found between uptake of FFA and levels of aP2 and KLBP, suggesting that content of FFA binding sites plays a role in FFA uptake.

To test the relevance of the interdepot differences in fatty acid binding protein levels we found in cultured preadipocytes to effects of depot origin on *in vivo* fat tissue characteristics, aP2 protein levels were determined in epididymal and perirenal fat tissue from 3-mo-old animals (Fig. 5). Although significant statistically, aP2 protein abundance was only slightly higher in perirenal than in epididymal fat in these animals. However, when aP2 protein abundance was compared between depots from 24-mo-old animals, levels were 2.6-fold higher in perirenal fat. Hence, interdepot differences in the characteristics of preadipocytes evident earlier in life are reflected in fat tissue in later life.

Changes in response to differentiation as well as variation between sites were suggested by a Northern

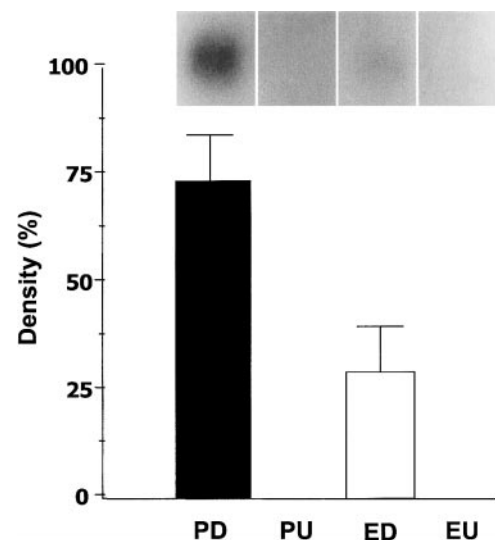


Fig. 3. Northern blots comparing expression of adipocyte lipid binding protein (aP2) in differentiated (D) and undifferentiated (U) perirenal (P) and epididymal (E) rat preadipocytes. Rat preadipocytes isolated from E and P depots were cultured in parallel for 1 wk in either a differentiation-promoting medium (DM1) or a basal medium not promoting differentiation. aP2 mRNA levels measured by Northern blotting and densitometry were 2.5-fold lower in E than in P cultures ($n = 12$ animals, $P < 0.05$) after adjusting for 28S rRNA levels in the same lanes on the same blots to correct for differences in RNA loading. aP2 was not detectable in U preadipocytes. We found that 28S rRNA levels are the same in EU, ED, PU, and PD preadipocytes when equal nos. of cells are analyzed (51). For each animal, densities of lanes (adjusted for 28S rRNA) representing the 4 conditions studied were expressed as %summed density. Vertical axis indicates the mean of these percentages and reflects relative cellular aP2 mRNA abundance.

blot of CPT-1 mRNA from rat epididymal and perirenal preadipocytes pooled from six animals (Fig. 6). Figure 6B (liver CPT-1) indicates that undifferentiated epididymal preadipocytes expressed low levels of the liver isoform of CPT-1. Similar data were obtained in undifferentiated perirenal preadipocytes (data not shown). Differentiation led to increased total CPT-1 expression. This was corroborated by measurement of CPT-1 activity, which increased from 0.93 ± 0.06 nmol·mg protein⁻¹·min⁻¹ in undifferentiated epididymal cells to 1.73 ± 0.15 nmol·mg⁻¹·min⁻¹ in differentiated cells ($P < 0.01$; Duncan's multiple range test; $n = 4$). Perirenal preadipocyte CPT-1 activity also increased during differentiation from 1.34 ± 0.01 to 1.94 ± 0.08 nmol·mg⁻¹·min⁻¹ ($P < 0.01$; Duncan's multiple range test; $n = 4$). As suggested by the Northern analysis in Fig. 6, total CPT-1 activity was higher in perirenal than in epididymal cells ($P < 0.01$; ANOVA; $n = 4$). The percentage of the liver isoform appeared greater in epididymal than in perirenal cells. Figure 6A (muscle CPT-1) shows that little muscle isoform was expressed in undifferentiated preadipocytes. Differentiation led to an increase in the expression of this isoform. The amount of the muscle CPT-1 isoform as a function of total CPT-1 was higher in perirenal than in epididymal cultures. We had previously found that mature fat cells expressed high levels of mainly the muscle isoform (14), confirming the increased expression of this isoform during differentiation suggested in Fig. 6. Figure

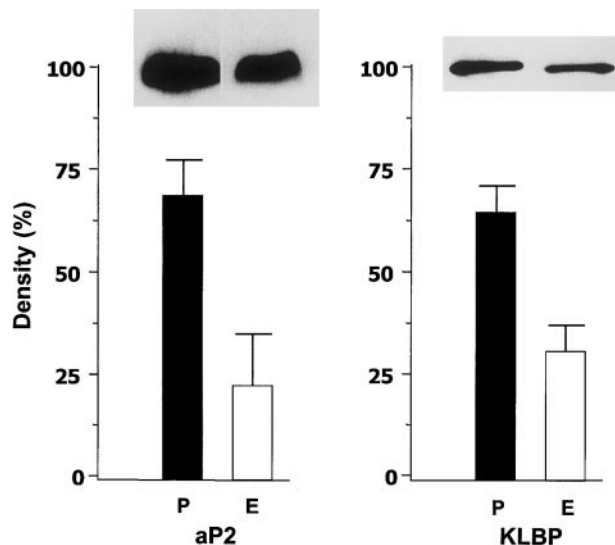


Fig. 4. Western blots comparing aP2 and keratinocyte lipid binding protein (KLBP) in differentiated P and E rat preadipocytes. aP2 protein levels measured by densitometry were 2.5-fold higher in P than in E preadipocytes cultured in DM3 for 48 h after confluence ($n = 8$ animals, $P < 0.01$, paired t -test). In similarly treated P preadipocytes, KLBP levels were 2.2-fold higher than in E preadipocytes ($n = 5$ animals, $P < 0.005$). Twenty micrograms of protein were loaded in each lane. Total protein contents (pg/cell) were 310 ± 38 in P and 349 ± 42 in E preadipocytes. For each animal, densities of lanes (adjusted for differences in cellular protein content among depots) representing the 2 depots studied for each binding protein were expressed as %summed density. Vertical axis indicates the mean of these percentages and reflects relative cellular aP2 and KLBP protein abundance.

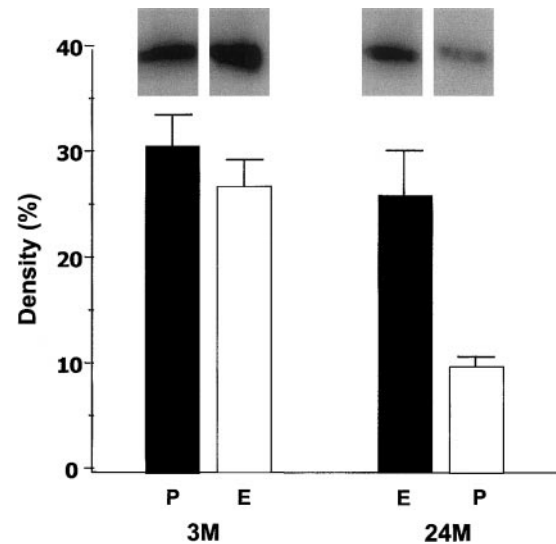


Fig. 5. aP2 protein abundance in fat tissue reflects differences among depots in its abundance in preadipocytes. In 3 parallel experiments, aP2 protein levels were determined in P and E fat tissue from 3- (3M) or 24-mo-old (24M) rats. In 3M animals, aP2 abundance in P fat tissue was only slightly greater than in E tissue ($P < 0.05$, Duncan's multiple range test). However, in 24M rats, P aP2 was 2.6-fold higher than in E tissue ($P < 0.01$, Duncan's multiple range test). aP2 decreased with age in both E and P preadipocytes ($P < 0.01$ and $P < 0.05$, respectively, Duncan's multiple range test). For each pair of animals (young and old), densities of lanes (adjusted for differences in cellular protein content among depots and ages) representing the 2 depots studied were expressed as %summed density. Vertical axis indicates the mean of these percentages and reflects relative cellular aP2 protein abundance.

6C (CPT-2) indicates that there were no site- or differentiation-specific differences in CPT-2 expression.

A predictable consequence of higher expression of the muscle isoform of CPT-1 is an increased level of cytosolic LC-CoA. Indeed, the amount of LC-CoA was 25% higher in all preparations of differentiated perirenal than epididymal adipocytes obtained from three different groups of rats (172 ± 8 vs. 143 ± 6 pmol/mg protein, respectively; $n = 3$, $P < 0.05$, paired t -test). Furthermore, perirenal isolated fat cell LC-CoA (280 ± 10 pmol/mg protein; $n = 3$, pooled samples from 3 rats) was more than twofold higher than epididymal fat cell LC-CoA (120 ± 10 pmol/mg protein; $n = 3$, pooled samples from 3 rats, $P < 0.05$, paired t -test). Hence, interdepot variation in LC-CoA levels became more pronounced once preadipocytes had fully differentiated into fat cells.

DISCUSSION

Differentiating preadipocytes exhibited distinct, depot-dependent patterns of fatty acid uptake and of factors involved in FFA handling (aP2, KLBP, CPT-1 activity and expression, and LC-CoA content). Although it is possible that the local microenvironment of preadipocytes in vivo or factors that contribute to variability of freshly isolated tissues (e.g., vascular, hormonal, and neural effects) have sustained effects on fatty acid handling, the cells were maintained in culture for at least 1 wk before analysis and had under-

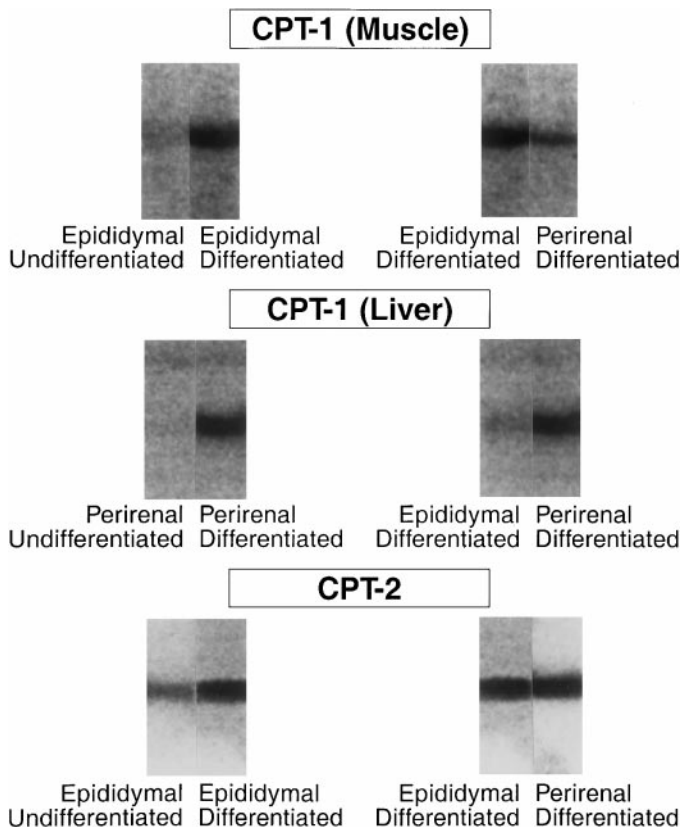


Fig. 6. Northern blots comparing carnitine palmitoyltransferase (CPT) mRNA levels in EU, ED, PU, and PD rat preadipocytes. Rat preadipocytes isolated from E and P depots were cultured for 1 wk in either a basal medium, in which cells remained undifferentiated, or in a differentiation-promoting medium (DM3). Cultures from 6 different animals were pooled to prepare each blot. *Top*: muscle isoform of CPT-1; *middle*: liver isoform of CPT-1; *bottom*: CPT-2.

gone at least two population doublings. Hence, effects of these factors seem unlikely to account for the interdepot variation in fatty acid handling that we observed. Although markers of differentiation are lower in epididymal than in perirenal rat mass cultures after the same period of exposure to adipogenic agents (25, 26, 50, 54, 91), differences in fatty acid uptake were observed at the single cell level in cells matched for lipid content. Therefore, although FFA uptake increases with differentiation, variation in extent of differentiation of cultured preadipocytes among depots is not the only factor contributing to interdepot variation in FFA uptake.

Our findings are in accord with those of previous studies that have suggested that interdepot variation in fat cell function reflects differences in the innate characteristics of adipose cells themselves, in addition to effects of variation in nutrient supply, innervation, and other extrinsic factors. Preadipocytes cloned from rat perirenal depots are capable of more extensive replication *in vitro* and *in vivo* than preadipocytes from epididymal depots (25, 26, 50, 73, 91). This interdepot variation in preadipocyte replicative capacity is reflected in the extent of increases in fat cell number during fat depot growth *in vivo* (91). Under a number of

different conditions that promote differentiation, perirenal preadipocytes accumulate more lipid and develop higher levels of expression of differentiation-dependent genes than epididymal preadipocytes (25, 50, 54, 91). In the human, abdominal subcutaneous preadipocytes differentiate more extensively than omental preadipocytes after thiazolidinedione treatment (3). Interdepot variation in cultured rat preadipocyte differentiation-dependent gene expression is reflected in differences among depots in fat cell expression of the same genes (54). These interregional differences do not result from variation among depots in preadipocyte recovery, admixture with nonadipose cell types or brown fat preadipocytes, or heterogeneity of preadipocyte subtypes (50, 54, 91). Despite exposure to similar hormonal manipulations *in vivo*, such as estrogen administration, hypophysectomy, or castration, preadipocytes cultured from various depots retain distinct cell-dynamic and biochemical responses, suggesting that there are site-dependent, intrinsic differences in preadipocyte hormone responsiveness (49, 64). Hence, there appear to be depot-dependent differences in the innate characteristics of adipose cells that could contribute to anatomic variation in function. Our current findings show that, together with differences in capacities for preadipocyte replication and differentiation, there are intrinsic interdepot differences in preadipocyte functional characteristics.

In humans, increased FFA and triglyceride turnover occurs in abdominal compared with peripheral obesity (4, 42, 71). Part of the basis of this difference in FFA flux *in vivo* may be related to the intrinsic differences in capacity for FFA uptake that we found in preadipocytes cultured from these depots. Because human omental and abdominal subcutaneous fat cells are of similar size in all but obese women (28), the greater capacity for hormone-stimulated lipolysis in omental cells that is associated with interdepot variation at the level of receptor function (67) may be balanced by the increased fatty acid transfer we found into these cells. These interdepot differences in fatty acid flux likely contribute to diminished antilipolytic effects of insulin and other agents in omental compared with abdominal subcutaneous fat cells and greater catecholamine-stimulated lipolysis in isolated omental than in subcutaneous fat cells (4, 5, 60). The insulin resistance associated with visceral obesity probably results, in part, from the FFA released from visceral depots traveling directly to the liver, where they stimulate gluconeogenesis and reduce extraction of insulin. The combination of these effects results in hyperinsulinemia (10, 58, 78, 79, 81).

Variation in LPL activity among depots would affect FFA availability to fat cells. In comparisons among differentiating preadipocytes, isolated fat cells, and fat tissue from various depots in humans and rats, interdepot differences in heparin-released LPL activity have been found, but they are generally small (29, 54, 68, 72). Any such differences in LPL action were bypassed in our studies by exposure of cells directly to fatty acids in serum-free media. Therefore, in addition to any direct effects of variation in LPL release by

adipose cells, mechanisms downstream from LPL contribute to interregional variation in FFA transfer.

We found higher levels of aP2 mRNA in differentiated perirenal than epididymal preadipocytes and that aP2 expression increased during differentiation in rat preadipocytes. Increased FFA transfer was correlated with lipid accumulation in differentiating preadipocytes. Hence, there may be an association between aP2 expression and FFA transfer into preadipocytes. Indeed, fatty acids are not taken up efficiently by cardiac myocytes in transgenic mice lacking the heart-type fatty acid binding protein (11). Interestingly, in adipose tissue from aP2 knockout mice, FFA content (as a percentage of adipose tissue mass) was noted to be around twice as great as in wild-type animals (7). It was hypothesized that the absence of aP2 may hamper flux of intracellular pools of FFA out of the adipocyte (7, 20). Also, no differences were found in FFA uptake into adipocytes from wild-type and aP2 knockout mice (20). Because of the species differences and differences in the uptake assay conditions (our assay used much higher FFA-to-albumin ratios, and cells were cultured under conditions that did not favor lipolysis), those results are not necessarily in conflict with ours. Additionally, in the aP2 knockout animals, a 13-fold compensatory upregulation of KLBP partially replaced the fatty acid binding proteins in wild-type animals. Although KLBP in the knockout mice was present in lower abundance than aP2 in wild-type animals, the fatty acid binding efficiency of KLBP is double that of aP2 (85) and may contribute to enhanced FFA uptake into perirenal cells. In cultured rat preadipocytes, lower expression of aP2 in epididymal than in perirenal preadipocytes was not offset by upregulation of KLBP. Indeed, KLBP expression was lower in epididymal than in perirenal cells, as was that of aP2.

Our data indicate that CPT-1 activity is higher in perirenal than in epididymal preadipocytes. Furthermore, LC-CoA levels were higher in differentiated perirenal preadipocytes and fat cells than in epididymal cells. In addition to the enhanced fatty acid uptake in perirenal compared with epididymal cells, the relatively higher CPT-1 muscle isoform expression in perirenal cells indicated in Fig. 6 could explain higher LC-CoA levels in the face of increased CPT-1 activity. Because the muscle isoform of CPT-1 is more sensitive than the liver isoform to malonyl-CoA [the inhibition constant (K_i) of the muscle isoform for malonyl-CoA is 20 nM; the K_i of the liver isoform is 2 μ M (23, 95)], differences in isoform expression could contribute to higher LC-CoA levels in perirenal cells. A testable hypothesis arising from this is that CPT-1 isoforms and levels of its allosteric regulators will determine steady-state cytosolic LC-CoA levels, which will in turn determine the rate of LC-CoA esterification.

In addition to the mechanisms discussed above, a number of other processes could contribute to interdepot variation in cultured preadipocyte fatty acid handling. Regional variation in protein-mediated transport [e.g., involving fatty acid transport protein (84), which is a very long chain acyl-CoA synthetase (21)] or

in fatty acid composition of cell membranes [which contribute to species differences in inner mitochondrial membrane proton permeability and hence fatty acid utilization (13)] are among these processes. These processes, in turn, may be links along a causal chain of events that begins with more fundamental differences among fat depots in cell programming.

PERSPECTIVE

From the foregoing discussion, it is likely that adipose tissue heterogeneity results, at least in part, from differences among depots in the intrinsic characteristics of preadipocytes and fat cells. Furthermore, differences among depots in preadipocyte characteristics earlier in life become more evident in fat cells later in life, supporting the contentions that new fat cells continue to develop from preadipocytes in adulthood and that intrinsic characteristics of preadipocytes are reflected in the characteristics of fat tissue that develops from them (8, 9, 39, 48). It is almost as if the preadipocytes (and fat cells derived from them) originating from different regions represent distinct miniorgans.

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