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Selective Up-regulation of Fatty Acid Uptake by Adipocytes Characterizes Both Genetic and Diet-induced Obesity in Rodents*

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P. D. Berk‡§¶, S.-L. Zhou‡‖, C.-L. Kiang‡, D. D. Stump‡, X. Fan‡, and M. W. Bradbury‡

From the ‡Department of Medicine, Division of Liver Diseases, and the §Department of Biochemistry, Mount Sinai School of Medicine, New York, New York 10029

Long chain fatty acid transport is selectively up-regulated in adipocytes of Zucker fatty rats, diverting fatty acids from sites of oxidation toward storage in adipose tissue. To determine whether this is a general feature of obesity, we studied [^{3}H]oleate uptake by adipocytes and hepatocytes from 1) homozygous male obese (*ob*), diabetic (*db*), fat (*fat*), and tubby (*tub*) mice and from 2) male Harlan Sprague-Dawley rats fed for 7 weeks a diet containing 55% of calories from fat. V_{max} and K_m were compared with controls of the appropriate background strain (C57BL/6J or C57BLKS) or diet (13% of calories from fat). V_{max} for adipocyte fatty acid uptake was increased 5–6-fold in *ob*, *db*, *fat*, and *tub* mice *versus* controls ($p < 0.001$), whereas no differences were seen in the corresponding hepatocytes. Similar changes occurred in fat-fed rats. Of three membrane fatty acid transporters expressed in adipocytes, plasma membrane fatty acid-binding protein mRNA was increased 9–11-fold in *ob* and *db*, which lack a competent leptin/leptin receptor system, but was not increased in *fat* and *tub*, *i.e.* in strains with normal leptin signaling capability; fatty acid translocase mRNA was increased 2.2–6.5-fold in *tub*, *ob*, and *fat* adipocytes, but not in *db* adipocytes; and only marginal changes in fatty acid transport protein 1 mRNA were found in any of the mutant strains. Adipocyte fatty acid uptake is generally increased in murine obesity models, but up-regulation of individual transporters depends on the specific pathophysiology. Leptin may normally down-regulate expression of plasma membrane fatty acid binding protein.

In normal man and most mammalian species, body weight is maintained within narrow limits through regulation of both caloric intake and energy expenditure (1, 2). If caloric intake persistently exceeds energy expenditure, obesity is an inevitable consequence. However, there are obvious differences in the tendency to obesity among individuals with seemingly equivalent caloric intake and similar degrees of physical activity (3). Likewise, there are differences among rat strains in the propensity to develop obesity on high fat diets (4). Finally, a number of single-gene mutations that lead to obesity in mice and rats have been identified and cloned (5–13), leading in several instances to elucidation of the mechanisms underlying phenotypic expression. Studies in these animal models and in obese humans have led to the concepts of energy efficiency and of nutrient partitioning as being important physiological mechanisms underlying individual or strain differences in the propensity to become obese (1, 14, 15).

Individuals with high energy efficiency require fewer calories to meet basal metabolic needs and accomplish a given level of physical work. Thus, on a given caloric intake, more calories are, in essence, left over, and are stored as fat. Individuals with low energy efficiency utilize more of their caloric intake for basal metabolism and physical work, leaving fewer calories for storage as fat. The factors responsible for differences in energy efficiency are incompletely understood. The concept of nutrient partitioning suggests that the body may preferentially shunt particular energy substrates either toward consumption as fuel or into storage as fat. Conceptually, nutrient partitioning might be one determinant of energy efficiency.

The homozygous obese Zucker fatty rat (*fa*/*fa*) is a well studied animal model in which obesity is a consequence of a mutation in the leptin receptor (11–13, 16). We have reported that the cellular uptake of long chain free fatty acids (LCFFA)[1] is selectively up-regulated in adipocytes of *fa*/*fa* animals but is unchanged in hepatocytes and cardiac myocytes (17). These changes have the effect of partitioning LCFFA away from tissues in which they would be burned as fuel into adipocytes, where they are stored as triglyceride. Adipocyte LCFFA uptake was already up-regulated in 19–21-day *fa*/*fa* weanlings prior to the development of obvious obesity (17). To determine whether the observed tissue-specific changes in LCFFA uptake are specific to the Zucker model or are a more general feature of obesity, studies of LCFFA uptake kinetics were conducted in homozygous adult male mice of four different strains bearing obesity-causing mutations and in adult male Harlan Sprague-Dawley rats fed a high fat diet in which 55% of calories were derived from lard. Appreciable tissue-specific up-regulation of LCFFA uptake was observed in adipocytes from all of these animal models of obesity. These results suggest that, in established obesity resulting from a variety of different underlying pathogenetic mechanisms, tissue-specific changes in cellular uptake mechanisms effectively partition LCFFA into storage within adipocytes, thereby perpetuating the obese phenotype.

EXPERIMENTAL PROCEDURES

Animals—Homozygous male obese (*ob*), diabetic (*db*), fat (*fat*), and tubby (*tub*) mice were obtained at 5–10 weeks of age, as available, from The Jackson Laboratory (Bar Harbor, ME), along with age-matched male C57BL/6J and C57BLKS control mice. The *ob* and *db* mice were

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¶ To whom correspondence should be addressed: Div. of Liver Diseases (Box 1633), Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029. Tel.: 212-241-6479; Fax: 212-348-3517; E-mail: paul_berk@smtplink.mssm.edu.

‖ Present address: Institute of Hematology, Beijing Medical University, 42 Bei-Li-Shi-Lu, Beijing 100044, People's Republic of China.

[1] The abbreviations used are: LCFFA, long chain free fatty acids; LPL, lipoprotein lipase; BSA, bovine serum albumin; mAspAT, mitochondrial aspartate aminotransferase; $FABP_{pm}$, plasma membrane fatty acid-binding protein; FAT, fatty acid translocase; FATP, fatty acid transporting protein; Ou, unbound oleate concentration; ν, oleate:BSA molar ratio.

overtly overweight on arrival and were studied soon thereafter, at approximately 9–13 weeks of age. *fat* and *tub* animals were studied at approximately 27–34 weeks of age, respectively, when they had achieved weights similar to those in the studied *ob* and *db* animals. Normal male Harlan Sprague-Dawley rats, 8 weeks of age, were obtained from Charles River Laboratories (Wilmington, MA).

Materials—9,10-[^{3}H]Oleic acid (2.6 Ci/mmol) was purchased from NEN Life Science Products, and all routine reagents were from Sigma. cDNA clones for mAspAT (18), FAT (19), FATP1 (20), and lipoprotein lipase (LPL) (21) were gifts of Drs. Joseph Mattingly, Nada Abumrad, Jean Schafer, and Susan Fried, respectively. A rat leptin cDNA was cloned as described previously (22). Appropriate fragments of these cDNAs were labeled by random priming with ^{32}P (23) for use as probes in Northern hybridization studies. Cell protein was measured by the bicinchoninic acid assay (BCA* kit, Pierce) and serum leptin with rat or mouse RIA kits (Linco, St. Charles, MO).

Dietary Studies in Rats—Twelve 8–10-week-old male Harlan Sprague-Dawley rats (295 ± 23 g) were randomly assigned to receive *ad libitum* either a standard laboratory chow diet (Ralston-Purina, St. Louis, MO) containing 13% of calories from fat or a high fat diet containing 35% lard (Bioserve, Frenchtown, NJ), which provides 55% of calories from fat. Rats were housed in individual cages with free access to water in a temperature-controlled facility with a 12-h light/dark cycle. Animals were weighed periodically and sacrificed after a mean of 50 ± 2 days for tissue harvest and cellular uptake studies as described below.

Cell Isolation and Characterization—Suspensions of rat and mouse hepatocytes (24, 25) and adipocytes (26, 27) were prepared by collagenase digestion of appropriate tissue samples, as previously reported. All preparations used in subsequent studies met established viability criteria (24, 26). In particular, ≥90% of hepatocytes and adipocytes excluded trypan blue. To achieve these viability levels in the fragile adipocytes of obese mouse strains, all mouse adipocytes were suspended in Dulbecco's modified Eagle's medium rather than KRH (Krebs-Ringer buffer containing 10 mM HEPES, pH 7.4) after isolation and maintained in this medium at room temperature until rewarmed to 37 °C for use. The size distribution of freshly isolated mouse adipocytes was determined by microscopy as described by Di Girolamo *et al.* (28).

Cellular Uptake of Oleate—The initial rate of [^{3}H]oleate uptake by hepatocytes and by epididymal fat pad adipocytes was determined by rapid filtration, as described previously (24, 26, 27, 29). This parameter has been shown principally to reflect transmembrane transport, relatively independent of subsequent intracellular binding or metabolism (24, 29). Briefly, cell preparations with known cell counts were incubated for up to 30 s at 37 °C in HH (Hanks' buffer containing 10 mM HEPES, pH 7.4) containing 500 μM BSA and varying [^{3}H]oleate concentrations and were then filtered and washed with ice-cold stop solution (24, 26, 29). The filters with the cells were placed in BCS scintillant and counted by liquid scintillation spectrometry. Oleate uptake by these cell types is linear within this time period. The slopes of the cumulative uptake *versus* time curves, representing initial uptake velocity, were calculated from this linear portion of the curve by a least mean squares fit. At the 500 μM BSA concentration employed, the observed kinetics again reflect membrane transport (30–32), largely unmodified by such pre-membrane phenomena as rate-limiting dissociation from albumin and the effects of the pericellular unstirred water layer on substrate availability at the cell surface (33, 34).

After confirming that observations in epididymal fat pad adipocytes were reflective of those in intra-abdominal fat pads (17), we subsequently conducted studies in adipocytes from the former site. This reduced the numbers of animals necessary for each study. Each oleate uptake study in hepatocytes and in adipocytes from the *ob*, *db*, *fat*, and *tub* mutants was performed with cells isolated from a single animal. To obtain sufficient cells, studies in control adipocytes still required pooled cells harvested from 2–4 mice.

Computations and Data Fitting—The unbound oleate concentration (Ou) was calculated from the oleate:BSA molar ratio (ν) (35) using the FFA:BSA binding constants of Spector *et al.* (36). Although recent reports (37, 38) suggest that these constants overestimate Ou, there is no general agreement on alternative values. Use of the more recent data would modify the computed values of K_m and k but would not change the conceptual interpretation of these studies. Therefore, we continue to use the binding constant values of Spector (36), to permit comparison of these studies with the large body of related earlier work. Based on prior analyses (39) for each group of animals and cell type studied, measurements of initial oleate uptake velocity obtained at values of ν from 0.1–2.0 were fitted to the sum of a saturable and a nonsaturable function of the corresponding Ou, using the Simulation, Analysis and Modeling (SAAM) program of Berman and Weiss (40). SAAM computes, for each data set, the values of the V_{max} (pmol/s/50,000 cells) and K_m (nM) of the saturable uptake function and the rate constant k (ml/s/50,000 cells) for the nonsaturable uptake process, as well as their variances and co-variances. For comparing parameter values obtained for different experimental groups, the computed statistical parameters are equivalent to the standard error of the slope of a linear regression (40). Accordingly, computed values for physiologic variables are also expressed as mean ± S.E. Differences between groups were evaluated with two-tailed Student's t tests.

RNA Isolation and Northern Hybridization—Cellular RNA was isolated with a guanidinium thiocyanate phenol-chloroform single-step extraction method (41) using a Stragagene (La Jolla, CA) kit. To isolate adipocyte RNA, cells were first disrupted and chilled to 4 °C. Aqueous cellular contents were then aspirated with a micropipette inserted through the layer of congealed lipid that rises to the top of the tube.[2] RNA extraction then continued as described. RNA samples were separated in 1.2% agarose-formaldehyde gels and transferred to Hybond-N nylon membranes (Amersham Pharmacia Biotech) in 20× SSC. The membranes were prehybridized for 3 h and then hybridized overnight to ^{32}P-labeled DNA probes of interest (42). Relative quantities of message in various samples were determined by autoradiography. Band intensity was quantitated by scanning densitometry using a *pdi* (Huntington Station, NY) Discovery Scanner, attached to a Sun SPARC work station. Quantity 1 (*pdi*) software was used to compute the area under the curve (OD × mm^2) for each band of interest. Results were normalized for lane loading by comparison with the signal intensity obtained with rodent β-actin. The variability of replicate scans of the same film was less than ±5%. In a series of duplicate studies with adipocyte RNA samples from paired control mice, the coefficient of variation for the ratio of bands of interest to actin was ±25%. Accordingly, message levels in mutant mice were considered significantly increased if densitometric measurements of the appropriate autoradiographic bands were >1.5-fold those in controls, after normalization for the corresponding actin signal.

RESULTS

Studies in Mice

Adipocytes

Animals Studied—[^{3}H]Oleate uptake by adipocytes from *ob* and *db* animals was studied in mice 12–13 weeks of age, at which time they weighed 2.0 and 1.8 times as much as their respective age-matched C57BL/6J and C57BLKS controls (Table I). The *tub* and *fat* animals became obese more slowly, weighing 1.7 and 1.3 times that of their corresponding control animals when studied at 35 and 27 weeks of age, respectively. Although adipocyte leptin message levels were markedly increased in all four mutant strains when compared with their C57BL/6J and C57BLKS controls (see below), serum leptin levels were undetectable in the *ob* animals when assayed with the antibody supplied by Linco. As reported (43), serum leptin levels were significantly increased in *db*, *fat*, and *tub* animals (Table I). Adipocytes isolated from each of the obese strains were appreciably larger than those of the control animals (Fig. 1). Calculated cell surface areas in the mutant strains were 2.9 to 4.2 (mean ± S.E., 3.7 ± 0.4) times those of the controls.

Oleate Uptake Kinetics—Representative LCFFA uptake curves from each of the mutant strains and from those in corresponding, age-matched controls are shown in Fig. 2. V_{max} values in all four mutant strains were significantly increased, ranging from 4.5 to 12.5 times those in the corresponding controls (Table I). The relative increase in V_{max} averaged 6.8-fold, which is nearly double the increase in surface area of the corresponding adipocyte populations. The rate constant for nonsaturable uptake (k) was also increased by an average of 2.9 ± 0.4-fold in all of the mutant strains. This increase, which was statistically significant ($p < 0.001$) only in the *db* and *fat* animals, was similar to the increase in adipocyte surface area

[2] We are indebted to Dr. Susan Fried for suggesting this procedure.

TABLE I

[^{3}H]Oleate uptake studies in adipocytes from mice with various obesity mutations

Adipocyte sizes are mean values for each strain. All other data are mean ± S.E.

Strain (n)[a]	Age	Weight	Adipocyte [^{3}H]oleate uptake			Adipocyte size[b]		Serum leptin
			V_{max}	K_m	k	Diameter	Surface area	
	weeks	*g*	*pmol/s/50,000 cells*	*nM*	*ml/s/50,000 cells*	*arbitrary units*	*arbitrary units^2*	*ng/ml*
ob (4)	12.9 ± 1.3	51.9 ± 3.8	33.9 ± 5.8[c]	193 ± 47[d]	0.0119 ± 0.0040	13.3	591	Undetectable
C57BL/6J (3)	12.9 ± 0.02	25.5 ± 0.3	6.2 ± 1.3	28 ± 10	0.0066 ± 0.0035	6.6	141	4.2 ± 0.8[e]
tub (3)	35.3 ± 5.3	51.5 ± 4.1	37.4 ± 2.8[c]	83 ± 12[c]	0.0122 ± 0.0048	10.2	408	35.5 ± 3.3[c]
C57BL/6J (3)	41.0 ± 1.9	29.8 ± 1.1	8.3 ± 0.5	20 ± 3	0.0043 ± 0.0012	6.3	139	—[e]
db (5)	12.8 ± 2.8	44.0 ± 4.3	43.3 ± 3.2[c]	65 ± 8	0.0340 ± 0.0048[c]	12.9	582	40.3 ± 6.12[c]
C57BLKS/J (3)	11.9 ± 2.3	25.0 ± 0.1	9.4 ± 1.2	101 ± 19	0.0098 ± 0.0015	6.3	137	3.0 ± 0.6[e]
fat (6)	26.6 ± 5.3	41.3 ± 3.6	64.2 ± 4.1[c]	116 ± 12	0.0359 ± 0.0049[c]	13.1	563	29.0 ± 11.1[f]
C57BLKS/J (3)	25.0 ± 0.5	31.3 ± 0.2	5.1 ± 1.7	155 ± 51	0.0109 ± 0.0027	6.7	155	—[e]

[a] n = number of studies.
[b] 1 arbitrary unit = 9.6 microns.
[c] $p < 0.001$ compared with control strain.
[d] $p < 0.005$ compared with control strain.
[e] No differences in serum leptin concentrations were observed between younger and older mice of either control strain. Accordingly, all samples from each control strain were pooled to generate a single set of serum leptin data for each strain.
[f] $p < 0.05$ compared with control strain.

($p > 0.1$), as would be anticipated for a passive, diffusive process.

Northern Hybridization—Representative Northern hybridizations of probes for leptin, $FABP_{pm}$, FAT, and FATP with adipocyte RNA from individual mice are illustrated in Fig. 3. The observed ratios of these message levels and that of LPL in mutant animals compared with those in controls are presented in Fig. 4. Expression of leptin mRNA was consistently up-regulated by a mean of 7.3–13.7-fold in adipocytes from each of the four mutant strains. Somewhat smaller and more variable increases (means, 5.1-fold each in *ob*, *db*, and *fat* and 1.9-fold in *tub*) were also observed in lipoprotein lipase message levels. Although the magnitude of the changes showed considerable inter-animal variability (Fig. 4), adipocyte $FABP_{pm}$ mRNA was increased in all *ob* (mean, 9-fold) and *db* (mean, 11-fold) mice, as it is in the Zucker rat (17). All of these mutants lack a competent leptin/leptin receptor system. By contrast, $FABP_{pm}$ mRNA was not increased in any of the *fat* or *tub* animals, *i.e.* in strains with normal leptin signaling capability. Adipocyte FAT mRNA was increased to lesser degrees in *fat* (mean, 6.5-fold), *ob* (mean, 4.2-fold), and *tub* (mean, 2.2-fold) mice, but not in *db* (mean ratio, mutant/control 1.2) mice. Changes in FATP mRNA levels were marginal in all four mutant strains.

Hepatocytes

[^{3}H]Oleate uptake in *ob* and *db* mice was studied in hepatocytes harvested from 8–10-week-old animals that weighed 1.8–2.0 times as much as their respective C57BL/6J and C57BLKS controls. Hepatocytes from *tub* and *fat* animals were studied at a mean of 31 and 34 weeks of age, when the animals weighed 2.0 and 1.3 times as much as their corresponding controls. In hepatocytes, the kinetic parameters in control animals of each strain were essentially unchanged between the ages of 5 and 34 weeks, and the data were therefore pooled for purposes of comparison with the mutant animals. As indicated in Table II, there were no differences between any of the mutant strains and the appropriate control mice with respect to any of the kinetic parameters for hepatocyte oleate uptake.

Rat Studies

Weight Gain

It took approximately 2 weeks for the fat-fed rats to adjust to the high fat diet, during which their weight gain was slow. However, they gained weight rapidly thereafter. During the

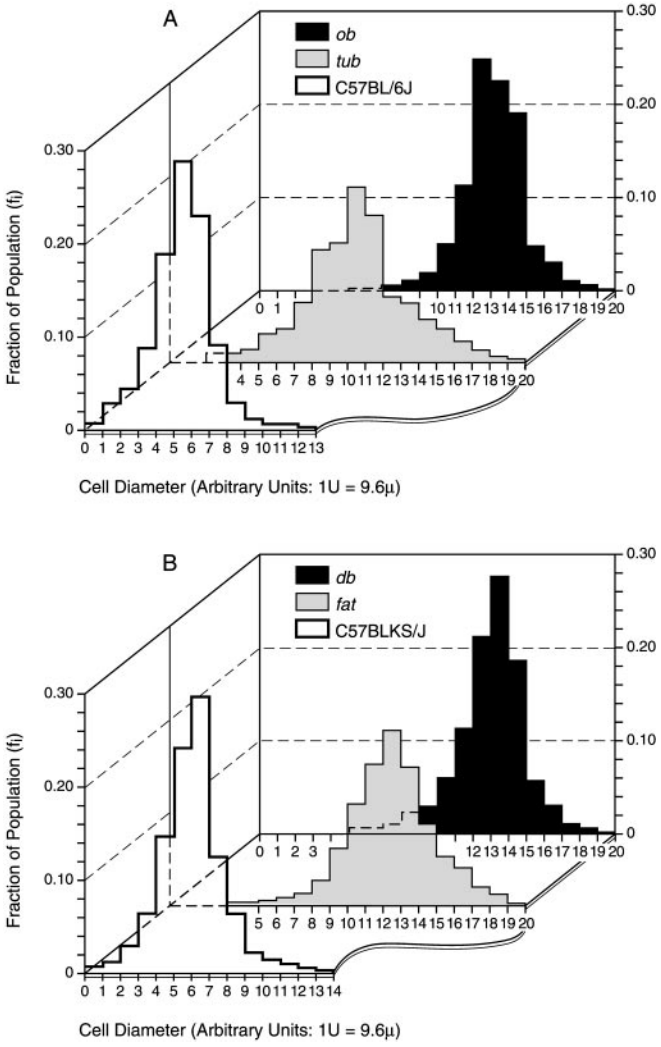

FIG. 1. **Size distributions of epididymal fat pad adipocytes from homozygous male *ob* and *tub* mice and C57BK/6J control mice (*A*) and homozygous male *db* and *fat* mice and C57BLKS/J controls (*B*).** Surface areas were computed from the measured diameters on the assumption that isolated adipocytes are essentially spherical (see Ref. 28).

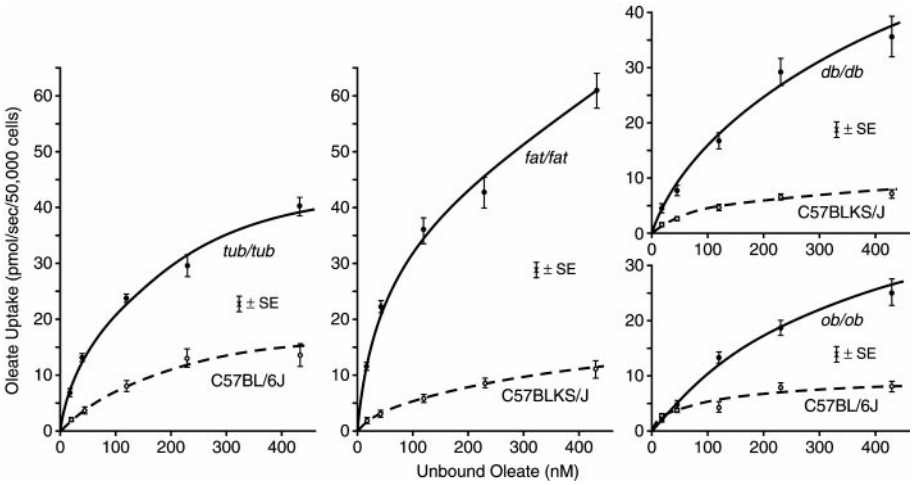

FIG. 2. **Representative [^{3}H]oleate uptake curves in isolated adipocytes from homozygous male *ob*, *db*, *tub*, and *fat* mice and the corresponding C57BL/6J and C57BLKS/J controls.** Each *curve* represents a single study. Data *points* depict the mean ± S.D. of triplicate determinations. Studies in the mutant mice were done on cells from a single animal. Studies in the control mice were done on cells obtained from two to four controls.

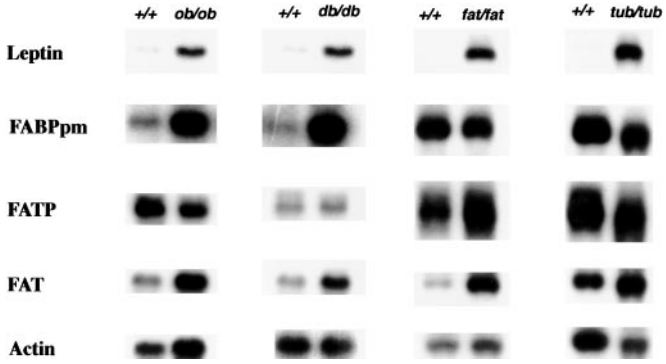

FIG. 3. **Representative Northern blots indicating mRNA levels in adipocytes from homozygous male *ob*, *db*, *tub*, and *fat* mice and the corresponding C57BL/6J and C57BLKS/J controls.** Each *band* illustrates one of three to seven replicate analyses of RNA preparations from different animals.

TABLE II
[^{3}H]Oleate uptake studies in hepatocytes from mice with various obesity mutations

All data are mean ± S.E.

Strain (*n*)	Hepatocyte [^{3}H]oleate uptake		
	V_{max}	K_m	*k*
	pmol/s/50,000 cells	*nM*	*ml/s/50,000 cells*
ob (4)	0.87 ± 0.18	125 ± 37	0.0009 ± 0.0002
tub (3)	1.18 ± 0.40	188 ± 84	0.0012 ± 0.0003
C57BL/6J (5)	0.83 ± 0.30	166 ± 57	0.0009 ± 0.0004
db (4)	1.03 ± 0.39	115 ± 43	0.0016 ± 0.0005
fat (3)	0.83 ± 0.35	161 ± 72	0.0009 ± 0.0005
C57BLKS/J (7)	0.88 ± 0.26	113 ± 37	0.0008 ± 0.0004

final four weeks of the study, fat-fed animals gained 139 ± 9 g at an average rate of 4.8 ± 0.3 g/day, compared with 92 ± 12 g ($p < 0.025$) at an average rate of 3.1 ± 0.4 g/day ($p < 0.01$) for those on the chow diet. In consequence, despite their slow initial weight gain, fat-fed rats weighed significantly more than chow-fed animals (533 ± 15 *versus* 483 ± 15 g, $p < 0.05$) at sacrifice. Non-fasting blood glucose levels were normal in both groups at sacrifice. Plasma FFA concentrations in the fat-fed animals at sacrifice were 298 ± 93 μM, but in view of the wide variability, these levels were not significantly increased.

Cellular Fatty Acid Uptake

At sacrifice, the V_{max} for adipocyte oleate uptake was significantly increased in the fat-fed animals compared with chow-fed controls (4.7 ± 0.6 *versus* 2.6 ± 0.3 pmol/s/50,000 cells; $p < 0.01$). By contrast, there were no significant differences in

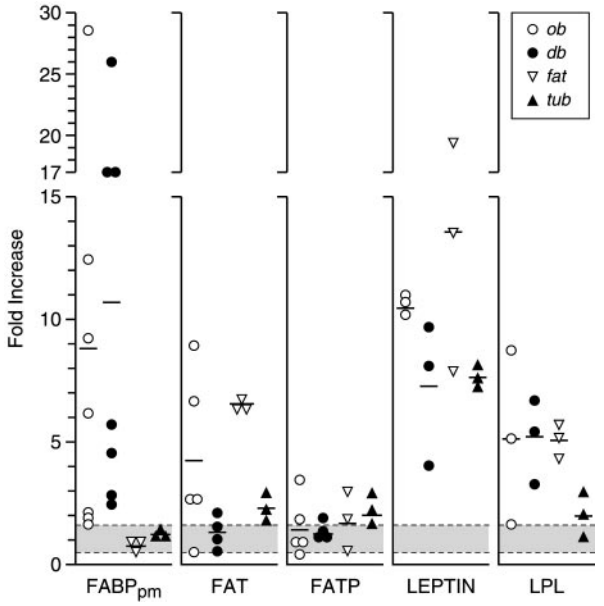

FIG. 4. **Results of Northern hybridization analyses of adipocyte mRNA levels.** For each of the indicated messages, the results are expressed as the ratio of measurements in mutant animals divided by corresponding measurements in the appropriate control strain (see text). Each data *point* represents a single analysis in one mutant animal. *Horizontal bars* indicate mean values for each strain. The *shaded band* indicates the estimated limits of variability in normal controls based on measured coefficients of variation in replicate control studies.

hepatocyte oleate uptake V_{max} between the fat-fed and chow-fed animals (0.45 ± 0.13 *versus* 0.43 ± 0.06 pmol/s/50,000 cells; $p > 0.5$). There were no appreciable differences between groups in either adipocyte or hepatocyte K_m or *k*.

DISCUSSION

The results presented above indicate that up-regulation of a saturable process mediating LCFFA uptake occurs selectively in adipocytes from animals with various models of obesity, whereas LCFFA uptake by hepatocytes is unaltered. These results parallel those reported earlier in the Zucker fatty (*fa/fa*) and Zucker diabetic fatty (*ZDF*) rat (17), in which selective up-regulation of adipocyte LCFFA uptake was observed with no change in uptake by either hepatocytes or cardiac myocytes. Although technical difficulties precluded accurate definition of LCFFA uptake by mouse cardiac myocytes, the implications of the current data are the same as those in the Zucker animals. Specifically, selective up-regulation of adipocyte LCFFA up-

take is a consistent finding in animals in which obesity results from a variety of pathogenetic mechanisms. Models exhibiting this finding include those with defective leptin signaling caused by mutations in the gene encoding leptin (*ob*/*ob* mouse) or the leptin receptor (*db*/*db* mouse, Zucker *fa*/*fa* rat), in genetic models in which the mutation does not involve the leptin system (*fat* and *tub* mouse) and in a widely used "normal" rat strain when fed a high fat diet.

The data suggest that, in each of these models, LCFFA are being diverted away from tissues such as liver and cardiac muscle, where they would be burned as fuel or otherwise utilized, and into adipose tissue, where they are stored as triglyceride. Thus, tissue-specific regulation of LCFFA uptake represents a form of nutrient partitioning. An analogous nutrient partitioning mechanism is effected by tissue-specific regulation of LPL activity. Adipose LPL activity in man is reportedly increased and that in muscle is decreased by insulin (44, 45), whereas isoproterenol selectively increases LPL activity in muscle (46). These effects favor either adipose tissue storage or muscle utilization of LCFFA under different hormonal environments. The importance for LCFFA disposition of tissue-specific LPL expression has also been shown in LPL-knockout mice or in transgenic animals expressing LPL exclusively in muscle (47). Because the enzymatic activity of LPL generates much of the LCFFA presented to cellular uptake mechanisms, coordinated expression of LPL and proteins involved in LCFFA uptake might be anticipated. In the present studies, the correlation of LPL mRNA levels with those of $FABP_{pm}$ in the five mice in which both were determined failed to achieve statistical significance ($r = 0.62$, $p > 0.1$), and correlations with FAT ($r = 0.42$) and FATP1 ($r = 0.21$) message levels were even weaker. Nevertheless, this issue merits more detailed attention.

Although this discussion presupposes that cellular uptake of LCFFA occurs by a facilitated, and therefore regulatable process, the mechanism(s) by which LCFFA enter cells have, in fact, been controversial. Nevertheless, as recently reviewed in detail (48), the model most consistent with all currently available data is one in which LCFFA enter cells by two distinct pathways: a rapid, facilitated process for the transmembrane movement of fatty acid anions, which predominates at physiologic concentrations of unbound LCFFA; and a much slower process reflecting passive flip-flop of the uncharged, protonated species. In the present study, the proportional increase in the rate constant for nonsaturable oleate uptake by adipocytes from the various mouse obesity mutants is quite similar to the increase in adipocyte surface area, consistent with a passive diffusive process. By contrast, the increase in V_{max} appreciably exceeds that in surface area, a finding consistent with up-regulation of a specific transport process. Some researchers still dispute the existence of facilitated LCFFA uptake mechanisms (49–57). However, a recent report of a new syndrome in which liver failure in children results from deficient hepatic uptake of LCFFA clearly indicates the existence of a membrane transport process that selectively mediates cellular LCFFA uptake (58).

Even as facilitated LCFFA transport was being disputed, seven plasma membrane proteins have been proposed as LCFFA transporters. The first of these described, plasma membrane fatty acid-binding protein ($FABP_{pm}$) (59), eventually proved identical to mitochondrial aspartate aminotransferase (60, 61). Despite initial skepticism, substantial evidence establishes that this prototypical mitochondrial enzyme is sorted in regulated fashion to the plasma membrane of selected cell types and functions there to facilitate LCFFA uptake. Fatty acid translocase (FAT) (19) and fatty acid transporting protein (FATP, now FATP1) (20, 62) were initially reported to be expressed mainly in adipose tissue and in skeletal and cardiac muscle, but later studies document appreciable hepatic expression under certain circumstances (63). Recently, homologues of FATP1, designated FATP2–FATP5, have been identified and shown to be members of a family of related transporters, differentially expressed in different tissues and highly conserved from mycobacteria to man (62). Of these proteins, FATP5 is expressed exclusively in liver and FATP2 in liver and kidney. All of these putative FFA transporters increase cellular FFA uptake following transfection of their cDNAs into non-expressing cell types. Thus, they all meet the currently accepted criteria for function as transporters.

In weanling Zucker rats up-regulation of adipocyte LCFFA uptake precedes overt obesity (17), suggesting that the increase in adipocyte LCFFA uptake may actually contribute to evolution of the obese phenotype. Weanling *ob*, *db*, *fat*, and *tub* mice were not available for the present studies, so that changes in LCFFA transport cannot at present be assigned a pathogenetic role in the corresponding obesity syndromes. What can be inferred is that the selective up-regulation of adipocyte LCFFA uptake in established obesity contributes to sustaining the obese phenotype. Furthermore, the finding that $FABP_{pm}$ is appreciably up-regulated in obesity models characterized by defective leptin signaling, but not in those in which the leptin system is normal, suggests that leptin may normally serve to down-regulate $FABP_{pm}$-mediated adipocyte LCFFA uptake. If selective up-regulation of LCFFA uptake contributes to the pathogenesis and/or maintenance of the obese phenotype, equally selective, pharmacologic down-regulation of the same transport process(es) might represent an important new approach to therapy.

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