Uptake of Long Chain Free Fatty Acids Is Selectively Up-regulated in Adipocytes of Zucker Rats with Genetic Obesity and Non-insulin-dependent Diabetes Mellitus*

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To examine whether fatty acid transport is abnormal in obesity, the kinetics of [14C]oleate uptake by hepatocytes, cardiac myocytes, and adipocytes from adult male Wistar (+/+), Zucker lean (fa+/+), and fatty (fa/fa), and Zucker diabetic fatty (ZDF) rats were studied. A tissue-specific increase in oleate uptake was found in fa/fa and ZDF adipocytes, in which the V_{max} was increased 9-fold (p < 0.005) and 13-fold (p < 0.001), respectively. This increase greatly exceeded the 2-fold increase in the surface area of adipocytes from obese animals, and did not result from trans-stimulation secondary to increased lipolysis. Adipocyte tumor necrosis factor-α mRNA levels, assayed by Northern hybridization, increased in the order +/+ < fa/fa < ZDF. Oleate uptake was also studied in adipocytes from 20–24-day-old male +/+, fa/+, and fa/fa weanlings. These animals were not obese, and had equivalent plasma fatty acid and glucose levels. Tumor necrosis factor-α mRNA levels in +/+ and fa/fa cells were also similar. Nevertheless, V_{max} was increased 2.9-fold (p < 0.005) in fa/fa compared +/+ cells. These studies indicate 1) that regulation of fatty acid uptake is tissue-specific and 2) that up-regulation of adipocyte fatty acid uptake is an early event in Zucker fa/fa rats. These findings are independent of the role of any particular fatty acid transporter. Adipocyte mRNA levels of three putative transporters, mitochondrial aspartate aminotransferase, fatty acid translocase, and fatty acid transporting protein (FATP) were also determined; mitochondrial aspartate aminotransferase and FATP mRNAs correlated strongly with fatty acid uptake.

Altered disposition of free fatty acids (FFA) is common in obesity and non-insulin-dependent diabetes mellitus (NIDDM) and is manifested, e.g. by adipocyte resistance to the antilipolytic effects of insulin, increased lipolysis, and increased plasma levels of FFA (e.g. see Refs. 1 and 2). Indeed, such changes may be the primary metabolic disturbance in these conditions (3–5). The Zucker fatty (fa/fa) rat is a widely used model of genetic obesity and exhibits many of the pathophysiologic alterations observed in obese humans (reviewed in Ref. 6). Although older fa/fa animals may exhibit hyperglycemia, several strains derived from the original Zucker stock become overtly diabetic. Males of the Zucker diabetic fatty (ZDF) GmiTM-fa/fa strain, for example, have marked hyperglycemia (7), and develop further metabolic and even pathologic features (8–10) resembling NIDDM in man. Although “lipotoxicity” due to altered FFA disposition is central to the pathophysiology of these animals as well as the human disorders (11, 12), studies of tissue FFA uptake mechanisms have not heretofore been reported.

FFA are critical energy substrates, building blocks for components of cell membranes, precursors of mediators such as prostaglandins, and important intracellular mediators of gene expression (13, 14). These multiple roles suggest that careful regulation of all aspects of FFA disposition, including cellular uptake, would be advantageous. However, the conventional view has been that cellular FFA uptake occurs by a passive, unregulated mechanism (reviewed in Refs. 15 and 16). Many studies (e.g. see Refs. 17–19) have found that FFA cross synthetic membranes at rates which greatly exceed rates of cellular uptake, leading to the argument that there was no need for a facilitated uptake mechanism to meet cellular FFA requirements (15, 16). However, FFA uptake rates in living cells observed by the same investigators (20, 21) were orders of magnitude slower than those reported with synthetic membranes. These and other data (22) suggest that synthetic membranes are not good models for cellular plasma membranes and call into question the biological relevance of FFA transport rates measured in synthetic liposomes.

Recent studies report that FFA uptake in liver, fat, and cardiac and skeletal muscle exhibits all the kinetic properties of facilitated transport, specifically saturation, trans-stimulation, cis-inhibition, stereospecificity, and counter transport (23–31). These features cannot be explained by diffusion. Although both saturable (facilitated) and nonsaturable (passive) uptake processes occur simultaneously in such cells (27), more than 90% of total FFA uptake at typical basal unbound FFA concentrations is via the facilitated pathway. By contrast, the major uptake mechanism in fibroblasts is diffusion (32).

A complex FFA transport system including a transmembrane transporter has been well described in Escherichia coli (33). Five putative mammalian FFA transporters also have been identified. Of these, three, plasma membrane fatty acid
binding protein (34), which has proven to be identical to mitochondrial aspartate aminotransferase (mAspAT) (35, 36), fatty acid translocase (FAT) (37), and fatty acid transport protein (FATP) (38), have already been cloned (reviewed in Ref. 39). The other two, a 22-kDa membrane protein identified in 3T3-L1 adipocytes (40) and a 56–60-kDa protein from kidney and cardiac muscle (41), have not yet been either cloned or extensively characterized.

We report studies of FFA uptake by isolated hepatocytes, adipocytes, and cardiac myocytes of Zucker fatty and diabetic animals. The data demonstrate tissue-specific up-regulation of FFA uptake in adipocytes of these two strains. Studies in weanling animals indicate that up-regulation of adipocyte FFA transport occurs early, precede increases in plasma FFA concentration or up-regulation of tumor necrosis factor-α (TNF-α) (42, 43). Adipocyte mRNA levels for two of the putative transporters, mAspAT and FATP, closely parallel the V_max for FFA transport.

**EXPERIMENTAL PROCEDURES**

**Animals**—Normal male Wistar rats (+/+; +/fa) were obtained from Charles River Laboratories (Wilmington, MA). Male Zucker lean (+/fa) and fatty homozygotes (fa/fa) were purchased from the Animal Resources Program at Vassar College (Poughkeepsie, NY), and male Zucker diabetic fatty (ZDF) rats from Genetic Models (Indianapolis, IN). Adult +/+ animals were 8–12 weeks old, and fa/+ and fa/fa, and ZDF animals were 8–14 weeks old at the time of study. Studies were performed in 21–24-day-old weanling +/+ and fa/+ and fa/fa animals from the same sources.

**Materials**—10 mM HEPES, pH 7.4, 500 μM BSA, and insulin by immunoassay (Lifescan, Milpitas, CA). Plasma FFA were assayed enzymatically (49) for use as probes in subsequent hybridization analyses.

**Cell Isolation**—Suspensions of hepatocytes (23, 51), adipocytes (24, 52), and cardiac myocytes (25, 53) were prepared by collagenase digestion of tissues, as previously reported. All preparations used in subsequent studies met established viability criteria (23–25). In particular, >98% of hepatocytes and adipocytes and >95% of cardiac myocytes excluded trypan blue.

**Cellular Uptake of Oleate**—The initial oleate uptake rate by hepatocytes (23, 26), adipocytes (24), and cardiac myocytes (25) was determined by rapid filtration. This parameter principally reflects transmembrane transport, relatively independent of subsequent intracellular binding or metabolism (23, 26). Briefly, cell preparations with known cell counts (adult animals) or protein concentrations (weanlings) were incubated for up to 30 s at 37 °C in Hanks’ buffer containing 10 mM HEPES, pH 7.4, 500 μM BSA, and with [3H]oleate concentrations, and then filtered and washed with ice-cold stop solution (23–26). The filters with the cells were placed in biodegradable counting scintillant (BXS, Amersham Corp.) 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 square fit. At the 500 μM BSA concentration employed, the observed kinetics again reflect membrane transport (54–56), 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 (57, 58). In studies with the anti-lypotic agent R80267 (44), one aliquot of adipocytes was preincubated in KRH (Krebs Ringer buffer containing 10 mM HEPES, pH 7.4), 10 μM R80267 for 15 min at 37 °C prior to the uptake study, while control cells were preincubated with KRH alone (39).

**Cellular Lipid Analysis**—To determine the fate of oleate taken up by hepatocytes, adipocytes, and cardiac myocytes of Zucker fatty and diabetic animals, the data demonstrate tissue-specific up-regulation of FFA uptake in adipocytes of these two strains. Studies in weanling animals indicate that up-regulation of adipocyte FFA transport occurs early, preceding increases in plasma FFA concentration or up-regulation of tumor necrosis factor-α (TNF-α) (42, 43). Adipocyte mRNA levels for two of the putative transporters, mAspAT and FATP, closely parallel the V_max for FFA transport.

**Up-regulation of Fatty Acid Uptake in Zucker Rat Adipocytes**

| Table I | Body weights, blood glucose, and FFA levels in 8–14-week-old adult rats |
|---------|------------------------------------------------------------------------|
| Rat     | n   | Weight (g) | Glucose (mg/dl) | FFA (μM) |
|---------|-----|------------|-----------------|----------|
| Normal Wistar | 6 | 225 ± 20^a | 92 ± 6 | 114 ± 4 |
| Zucker lean (fa/+) | 5 | 230 ± 15 | 113 ± 8 | 166 ± 19 |
| Zucker obese (fa/fa) | 5 | 505 ± 60 | 172 ± 28 | 229 ± 60 |
| Zucker diabetic (ZDF) | 5 | 485 ± 40 | 309 ± 31 | 358 ± 62 |

Values are mean ± S.E.

p < 0.005 compared to normal Wistar controls.

p < 0.001 compared to normal Wistar controls.

Adipocytes, cells from 5-wk-old fa/ or fa+ and fa+/+ animals were incubated with [3H]oleate in 500 μM BSA (p = 0.5–1.1) for 5 to 10 min prior to rapid filtration. [3H]Oleate uptake was determined in triplicate from one group of filters as above. Cellular lipids were extracted with chloroform: methanol (2:1) from cells on replicate filters, separated by thin-layer chromatography, visualized by exposure to iodine vapor, and quantitated as previously reported in studies with hepatocytes (23, 60) and Xenopus laevis oocytes (61). Individual compounds were identified by comparing their observed Rf values with those of commercial standards (60).

**Computations and Data Fitting**—The bound oleate concentration (Ou) was calculated from the oleate:BSA molar ratio (76) (62), using the FFA:BSA binding constants of Spector et al. (63). Although recent reports (64, 65) suggest that these constants overestimate FFA:BSA binding, other investigators have continued to use those of Spector et al. (63), to permit comparison of these studies with the large body of related earlier work. Based on previous analyses (27), curves relating initial oleate uptake velocity and Ou were fitted to several potential functions of Ou using the Simulation, Analysis, and Modeling (SAAM) program of Berman and Weiss (66). SAAM uses a fourth order Runge-Kutta procedure to compute, from the data, fit values for the parameters of the function being tested and their variances and covariances. The function which best described the data was selected using established criteria for goodness of fit (27). For purposes of comparing curves represented by equivalent mathematical functions, the computed statistical parameters are equivalent to the standard error of the slope of a linear regression (66). Accordingly, computed values for physiologic variables are expressed as mean ± S.E. Differences between groups were evaluated with two-tailed Student’s t tests. Using the Bonferroni correction for multiple comparisons (67), differences were considered significant if p ≤ 0.01.

**RESULTS**

**Glucose and FFA Concentrations in Adult Animals**—Body weights were increased in fa/fa and ZDF animals compared with the +/+ and fa/+ groups (Table 1). In the total population of 6–14-week-old animals, the blood glucose concentration was significantly elevated only in the ZDF group. The mean blood glucose in fa/fa animals 6–10 weeks old was 95 ± 3 mg/dl. However, as hyperglycemia was seen in some fa/ fa animals older than 10 weeks, the trend toward a progressive increase in
blood glucose from +/- to fa/fa to fa/fa to ZDF animals, assessed by a modified Bartholomew's test for ordered means (70), was highly significant (p << 0.01). There was a similar trend toward progressive elevation of plasma FFA levels (p << 0.01). However, due to variability in the FFA data, previously noted (e.g. see Refs. 71 and 72), the increase in the fa/fa animals per se did not achieve significance.

**FFA Uptake Kinetics**—The relationship between Ou and the [3H]oleate uptake velocity (UT(Ou)) by adult hepatocytes, cardiac myocytes, and adipocytes from all groups studied was best described, in each case, by the sum of a saturable and a nonsaturable component of the form

$$UT(Ou) = V_{max} \cdot Ou(K_m + Ou) + k \cdot Ou,$$

(Eq. 1)

(Fig. 1) (27). The kinetic constants are presented in Table II. $V_{max}$ values were equivalent in hepatocytes from all four groups; similarly, there were no significant differences in $K_m$ values. In cardiac myocytes, $V_{max}$ values were unchanged in fa/+ compared with +/- controls; modest increases in fa/fa animals to 1.6 and in ZDF to 1.9 times control values did not achieve statistical significance. There were, again, no significant differences in $K_m$ values among the groups. In adipocytes, $V_{max}$ values in fa/fa and ZDF animals were increased to 9 (p < 0.005) and 13 times (p < 0.001) the control value. Although the 1.4-fold increase in fa/+ heterozygotes was not statistically significant, the trend toward a progressive increase in $V_{max}$ from +/- to fa/+ to fa/fa to ZDF animals was highly significant (p << 0.001) (70). Thus, the modest increase in FFA uptake $V_{max}$ observed in fa/+ adipocytes may reflect a co-dominant effect of a single fa allele (see e.g. Ref. 70). $K_m$ values were significantly increased only in ZDF animals.

In selected studies with both +/- and fa/fa adipocytes, [3H]oleate-specific activity in the medium at the end of the 30-s incubation was determined and averaged 98 ± 3% of that at zero time. Thus the observed differences in $V_{max}$ did not reflect depletion or dilution of the [3H]oleate in the medium over the course of an experiment. In further studies, oleate uptake kinetics in intra-abdominal fa/fa adipocytes were compared with those in adipocytes from the corresponding epididymal fat pads. FFA uptake in the two cell populations was similar ($V_{max}$ intra-abdominal, 64 ± 11 pmol/s/50,000 cells; epididymal, 55 ± 8, p = NS).

In contrast to $V_{max}$, there were no statistically significant differences among groups in the value of $k$ for any cell type studied, although there was a trend toward higher $k$ values in fa/fa and ZDF compared with +/- and fa/+ adipocytes. These data suggest that possible obesity-related alterations in the lipid composition of plasma membranes in Zucker rats do not have a major impact on the rates of passive transmembrane FFA flux. We considered whether the increase in FFA uptake by fa/fa and ZDF adipocytes might simply be a reflection of a larger surface area. Using a method based on direct microscopic determination of the diameter of isolated adipocytes, the distribution frequency of the diameters of adipocytes isolated from +/- and fa/fa animals was determined as described previously (74). Since isolated adipocytes are virtually spherical, the distribution of surface areas and the mean surface area of each population was readily calculated (74). Although some fa/fa adipocytes are very large, the mean diameter of adult fa/fa adipocytes (94 μm) was 1.48 times that of normal adipocytes (64 μm), and the calculated surface area of a population of fa/fa adipocytes was 217% of that in +/- adipocytes. This is inadequate to explain the 9–13-fold increases in the $V_{max}$ for FFA uptake observed in fa/fa and ZDF adipocytes, although it may contribute to the smaller, 1.7-fold increase observed for $k$.

As in normal adipocytes (75), the lipolytic inhibitor RGS0267 at 10 μM had only a small effect on FFA uptake in fa/fa adipocytes, reducing the $V_{max}$ from 75 ± 4 to 61 ± 7 pmol/s/50,000 cells (p = NS). Hence, although facilitated FFA uptake may be trans-stimulated under some conditions (25), the increased rate of FFA uptake in fa/fa adipocytes is not simply a trans effect secondary to increased lipolysis within these cells (72, 76). Thus, the increase in the $V_{max}$ for FFA uptake in fa/fa and ZDF adipocytes is not a nonspecific consequence of changes in membrane lipid composition, lipolysis, or cell size, but rather reflects up-regulation of specific plasma membrane transport mechanism(s).

**Cellular Lipids**—In this experiment, the initial uptake oleate velocity in fa/fa adipocytes, 33 ± 1.3 pmol/s/50,000 cells, was 5.5 times that in +/- cells. Accumulation of radioactivity in specific, identifiable spots on the TLC plates paralleled cellular uptake; between 15 s and 5 min of incubation, radioactivity in oleate and in tri-, di-, and monoglycerides accounted for an average of 96% of total cellular radioactivity in both fa/fa and +/- cells. As in hepatocytes (60) and X. laevis oocytes (61), TLC analysis demonstrated that most radioactivity in the initial samples from both fa/fa and +/- adipocytes migrated with the Rf of unmetabolized oleate. However, by 1 min ≥87% and by 5 min ≥95% of the label within adipocytes migrated with the Rf of other lipids, principally triglycerides. These data indicate that oleate uptake, as measured, reflects transport into a metabolically active intracellular pool and that differences in FFA uptake between fa/fa and +/- adipocytes are followed by nearly quantitative conversion of the sequestered FFA into triglycerides and other cellular lipids.

**Studies in Weanling Animals**—[3H]Oleate uptake kinetics were also studied in 20–24-day-old male +/-, fa/+ and fa/fa animals. None was visibly obese, and mean body weights were comparable. FFA levels (μM) in the +/- (181 ± 44), fa/+ (182 ± 20), and fa/fa (166 ± 61) animals, as well as blood glucose levels, were also equivalent. A small increase in mean plasma insulin levels in the fa/fa pups (76 microunits/ml (range, 42–142)) compared with fa/+ (51 microunits/ml (range, 22–104)) and +/- (54 microunits/ml (range, 49–89)) was not statistically significant (77). By contrast, FFA uptake was appreciably
Up-regulation of Fatty Acid Uptake in Zucker Rat Adipocytes

**TABLE II**

| Cell type/strain       | \( V_{\text{max}} \) (pmol/s/50,000 cells) | \( K_m \) (nM) | \( K_h \) (pmol/s/50,000 cells) |
|------------------------|-------------------------------------------|----------------|--------------------------------|
| **Hepatocytes**         |                                           |                |                                |
| Normal Wistar           | 2.7 ± 0.6                                 | 137 ± 32       | 0.0005 ± 0.0009                |
| Zucker lean (fa/+)      | 2.7 ± 0.6                                 | 156 ± 43       | 0.0005 ± 0.0002                |
| Zucker obese (fa/fa)    | 2.6 ± 0.2                                 | 46 ± 5         | 0.0010 ± 0.0005                |
| Zucker diabetic (ZDF)   | 2.6 ± 0.4                                 | 96 ± 18        | 0.0012 ± 0.0007                |
| **Cardiac myocytes**    |                                           |                |                                |
| Normal Wistar           | 1.9 ± 0.2                                 | 17 ± 3         | 0.0014 ± 0.0009                |
| Zucker lean (fa/+)      | 1.7 ± 0.4                                 | 38 ± 10        | 0.0010 ± 0.0010                |
| Zucker obese (fa/fa)    | 3.2 ± 0.3                                 | 49 ± 7         | 0.0010 ± 0.0005                |
| Zucker diabetic (ZDF)   | 3.7 ± 0.3                                 | 40 ± 7         | 0.0010 ± 0.0005                |
| **Adipocytes**          |                                           |                |                                |
| Normal Wistar           | 6.3 ± 0.4                                 | 9 ± 1          | 0.0043 ± 0.0009                |
| Zucker lean (fa/+)      | 8.8 ± 0.7                                 | 11 ± 2         | 0.0050 ± 0.0016                |
| Zucker obese (fa/fa)    | 57.8 ± 8.3\(^a\)                         | 31 ± 7         | 0.0075 ± 0.0025                |
| Zucker diabetic (ZDF)   | 78.9 ± 41\(^b\)                          | 55 ± 6\(^c\)   | 0.0070 ± 0.0010                |

\( ^a p < 0.005; ^b p < 0.001; ^c p < 0.01; \) compared to normal Wistar.

\[ \text{[\textsuperscript{3}H]-OLEATE UPTAKE KINETICS IN ADIPOCYTES FROM WEANLING RATS OF VARIOUS STRAINS} \]

![Graph showing uptake kinetics](image)

**FIG. 2.** Oleate uptake by isolated adipocytes from male weanling rats, including normal Wistar (+/+), Zucker lean heterozygotes (fa/+), and Zucker fatty homozygotes (fa/fa). See text for computed \( V_{\text{max}} \) values.

accelerated in adipocytes from weanling fa/fa animals (Fig. 2); the \( V_{\text{max}} \) for FFA uptake by fa/fa adipocytes (710 ± 80 pmol/s/mg of cell protein) was highly significantly increased compared with fa/+ (302 ± 35, \( p < 0.01 \)) or +/+ controls (248 ± 15, \( p < 0.005 \)). As with the adult animals, studies of adipocyte cell size distribution demonstrated that, while 5% of fa/fa adipocytes were markedly enlarged, the mean diameter of the population, 42 \( \mu \)m, was increased by only 12–14% compared with that in fa/+ (37.6 \( \mu \)m) and +/+ (36.8 \( \mu \)m) cells. These data are very similar to those reported in similarly aged animals based on Coulter particle-sizing methods (78). Accordingly, the corresponding increase in surface area was 35 and 42% compared with fa/+ and +/+ cells, respectively. This is inadequate to explain the 2.9-fold increase in \( V_{\text{max}} \) in fa/+ adipocytes. As sufficient adipocytes to perform complete uptake studies, cell counts with size distributions, and protein determinations could not always be obtained from each individual +/+ and fa/+ weanling, FFA uptake data in these animals are expressed per mg protein. Comparisons of cell counts and protein content of suspensions of epidymal fat pad adipocytes from additional 20–21-day-old +/+, fa/+, and fa/fa weanlings indicate that the cellular protein content of adipocytes from the three groups did not differ significantly and that variations in cell size between groups reflect principally differences in lipid content (79). Therefore, the differences in \( V_{\text{max}} \) observed between groups of weanlings would be similar if the data were expressed per cell number.

**Expression of TNF-\( \alpha \), LPL, and Leptin mRNAs—**By Northern hybridization of a TNF-\( \alpha \) probe with total cellular RNA, a strong signal was obtained from activated rat peritoneal macrophages, which served as a positive control (data not shown). TNF-\( \alpha \) message was detectable in adult +/+ adipocytes, and progressively more strongly expressed in fa/fa and ZDF adipocytes (Fig. 3). In contrast, TNF-\( \alpha \) mRNA expression in adipocytes from +/+ and fa/ta weanling animals was equivalent (Fig. 4). In preliminary studies in three 20–22-day-old animals in each group, expression of LPL mRNA was increased 2.3-fold and leptin mRNA 17-fold in fa/ta compared with that of +/+ adipocytes (Fig. 4).

**Expression of Putative FFA Transporters—**As with FFA uptake \( V_{\text{max}} \), mAspAT mRNA levels, estimated by slot blotting, were similar in hepatocytes from adult +/+ and fa/ta and ZDF animals. In adipocytes, mAspAT mRNA levels increased in the order +/+ < fa/ta < ZDF and were highly correlated with the corresponding \( V_{\text{max}} \) values (\( r = 0.99, p < 0.01 \)). Subsequently, Northern blotting was also used to compare levels of mAspAT, FAT, and FATP mRNAs in adipocytes. In adult animals (Fig. 3), mAspAT and FATP mRNAs increased, again in the order +/+ < fa/ta < ZDF, and were highly correlated with the corresponding \( V_{\text{max}} \) values for adipocyte FFA uptake (\( r = 0.96, p < 0.01 \) and \( r = 0.90, p < 0.01 \), respectively). In contrast, FAT mRNA was increased only in fa/ta adipocytes; its level in ZDF adipocytes was less than that in control animals. Thus, adipo-
mRNA Levels in Weanling Rats

Fig. 4. Relative mRNA levels in adipocytes from weanling male normal Wistar (N) and Zucker homozygous fatty (F) rats, determined by Northern hybridization. Experimental details as in Fig. 3.

cyte FAT mRNA and FFA uptake $V_{max}$ correlated poorly ($r = 0.16, p = NS$). In weanlings, mAspAT, FAT and FATP mRNAs all increased in $fa/fa$ adipocytes compared with $+/+$ controls (Fig. 4).

DISCUSSION

While other aspects of FFA metabolism have been widely studied in Zucker rats, no previous studies of plasma membrane FFA transport have been reported. This study documents a striking, 9–13-fold increase in the $V_{max}$ for FFA uptake by adipocytes from adult $fa/fa$ and ZDF animals, whereas uptake is unchanged in hepatocytes and only modestly altered between fat deposition and utilization. Our data in weanlings evidence (these animals are functionally leptin-deficient. Several lines of evidence (e.g. see Ref. 86) indicate that, besides central effects on appetite, leptin has peripheral effects that alter the balance between fat deposition and utilization. Our data in weanlings indicate that, in the Zucker models of obesity and NIDDM, up-regulation of adipocyte FFA uptake is an early event that precedes obesity, increased plasma FFA, or increased expression of TNF-α mRNA. Related abnormalities, e.g. up-regulation of LPL (87), also occur early in these strains. The resulting diversion of FFA from tissues where they are oxidized to adipose tissue, where they are stored as fat, has been confirmed experimentally (88). The diversion of a potential energy source into fat explains not only the development of obesity, but also the almost universal finding that the reversal of established obesity is very difficult. Moreover, once increased fat accumulation has occurred, it leads to a cascade of events including, sequentially, up-regulation of TNF-α (42, 43); inhibition of adipocyte insulin receptor signaling (43); adipocyte resistance to the antilipolytic effects of insulin; increased lipolysis; increased plasma FFA levels; hyperinsulinemia; resistance of muscle and liver to the effects of insulin on glucose metabolism, mediated at least in part by the Randle cycle; further hyperinsulinemia; and, ultimately, frank NIDDM (reviewed in Refs. 1, 89, and 90).

The data and conclusions just discussed are independent of any hypotheses about particular plasma membrane FFA transporters. Although some investigators still dispute the concept that FFA uptake is a facilitated process, five putative FFA transporters have been identified (reviewed in Ref. 39). The first, plasma membrane fatty acid binding protein (FABPpm), was isolated in our laboratory in 1985 from rat hepatocyte plasma membranes (34). FABPpm is identical to the mitochondrial isoform of aspartate aminotransferase (mAspAT) (35, 36). As reviewed elsewhere (39), evidence that mAspAT functions at the plasma membrane as an FFA transporter is highly compelling and includes both antibody inhibition studies and transfection/expression studies in 3T3 fibroblasts (91) and X. laevis oocytes (39). The demonstration that both FFA uptake and efflux are subject to trans-stimulation (25, 60, 92) and that both processes are inhibitable by anti-mAspAT antibodies (39, 92) suggests that mAspAT may mediate a bidirectional transport process not unlike the bidirectional, GLUT2-mediated transmembrane transport of glucose. In addition to mAspAT, two other candidate transporters, designated FAT (37) and FATP (38), respectively, have also been cDNA cloned and extensively characterized. Evidence has been presented that each does, in fact, contribute to facilitated FFA uptake in particular cell types. The remaining two putative FFA transporters (40, 41) have not yet been cloned, and only limited data in support of their proposed function in FFA transport have been reported.

In the studies described above, expression of mAspAT, FATP, and FAT mRNAs in relation to the $V_{max}$ for FFA transport is consistent with the hypothesis that both the mAspAT and FATP genes are up-regulated as part of the genetically programmed evolution of obesity and NIDDM that occurs in Zucker $fa/fa$ and ZDF rats. The function of FAT in this setting requires further clarification.

The relevance of this study to human obesity remains to be established. Most cases of human obesity do not reflect simple Mendelian inheritance. Moreover, although obese patients have increased leptin levels, mutations in leptin or its receptor have not been identified in human obesity (82, 93). Whether alterations in adipocyte FFA transport similar to those observed in the present study also occur in animal models of dietary obesity or in obese humans is unknown. However, in preliminary studies, an adipocyte-specific increase in FFA uptake has accompanied weight gain in Harlan Sprague Dawley rats fed a high fat diet. We speculate that abnormal up-regulation of adipocyte FFA uptake, possibly reflecting an acquired abnormality in the leptin/leptin-receptor system, occurs in many forms of obesity and represents a final common pathway for diversion of FFA away from oxidation and into storage as fat.

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