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

(Received for publication, June 3, 1999)

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 [3H]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. Vmax and Km were compared with controls of the appropriate background strain (C57BL/6J or C57BLKS) or diet (13% of calories from fat). Vmax 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

* Supported in part by Grants DK-26438 and DK-52401 from the NIDDK, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† 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@smplink.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; FABPpm, plasma membrane fatty acid-binding protein; FAT, fatty acid translocase; FATP, fatty acid transporting protein; Ou, unbound oleate concentration; v, oleate:BSA molar ratio.
orally 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 ob-
tained from Laboratory Animals, Inc., St. Louis, MO) containing 13% of calories from fat or a high fat diet
containing 35% lard (BioServ, Frenchtown, NJ), which provides 55% of
The unbound oleate concentration
Computations and Data Fitting—The unbound oleate concentration
in the mutant strains were 2.9
animals, was similar to the increase in adipocyte surface area
pericellular unstirred water
the cumulative uptake versus time curves, representing initial uptake ve-
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
ob animals, were studied in mice 12–13 weeks of age, at which
time they weighed 2.0 and 1.8 times as much as their
corresponding, age-matched controls are shown in 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.
Olate Uptake Kinetics—Representative LCFFA uptake curves from
each of the mutant strains and from those in the corresponding,
Table I). Obese mice considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.

RESULTS

Adipocytes
Animals Studied—[3H]Olate 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 (Table I), serum leptin
levels in mutant mice were considered significantly increased if densi-
tometric measurements of the appropriate autoradiographic bands
were 1.5-fold those in controls, after normalization for the correspond-
ing actin signal.
mRNA levels were marginal in all four mutant strains. (mean ratio, mutant/control 1.2) mice. Changes in FATP kinetic parameters for hepatocyte oleate uptake and the appropriate control mice with respect to any of the comparisons with the mutant animals. As indicated in Table II, the four mutant strains were essentially unchanged between the ages of 5 and 34 weeks, and the data were therefore pooled for purposes of weighted ratios of these message levels and that of LPL in adipocyte RNA from individual mice are illustrated in Fig. 3. Although the magnitude of the changes showed considerable inter-animal variability (Fig. 4), adipocyte FABP<sub>p</sub> 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<sub>p</sub> 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**

<sup>3</sup>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/J 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

### Table I

| Strain (n) | Age (weeks) | Weight (g) | V<sub>max</sub> (pmol/s/50,000 cells) | K<sub>m</sub> (mM) | k (ml/s/50,000 cells) | Diameter (arbitrary units) | Surface area (arbitrary units) | Serum leptin (ng/ml) |
|-----------|-------------|------------|------------------------------------|-----------------|----------------------|---------------------------|------------------------|-------------------|
| ob (4)    | 12.9 ± 1.3  | 51.9 ± 3.8 | 33.9 ± 5.8                         | 182 ± 7.4       | 0.0119 ± 0.0040     | 13.3                      | 591                    | Undetectable      |
| C57BL/6J (3) | 12.9 ± 0.2 | 25.5 ± 0.3 | 6.2 ± 1.3                           | 20 ± 10         | 0.0066 ± 0.0065     | 6.8                       | 141                    | 4.2 ± 0.8*        |
| tub (3)   | 35.3 ± 5.3  | 51.5 ± 4.1 | 37.4 ± 2.8                         | 83 ± 12         | 0.0122 ± 0.0048     | 10.2                      | 408                    | 35.5 ± 3.3        |
| C57BL/6J (3) | 41.0 ± 1.9 | 29.8 ± 1.1 | 8.3 ± 0.5                           | 20 ± 3          | 0.0043 ± 0.0012     | 6.3                       | 139                    | —                 |
| db (5)    | 12.8 ± 2.8  | 44.0 ± 4.3 | 43.3 ± 3.2                         | 65 ± 8          | 0.0340 ± 0.0048     | 12.9                      | 582                    | 40.3 ± 6.12       |
| 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*        |
| fat (6)   | 26.6 ± 5.3  | 41.3 ± 3.6 | 64.2 ± 4.1*                         | 116 ± 12        | 0.0359 ± 0.0049*    | 13.1                      | 563                    | 29.0 ± 11.1       |
| C57BLKS/J (3) | 25.0 ± 0.5 | 31.3 ± 0.2 | 5.1 ± 1.7                           | 155 ± 51        | 0.0109 ± 0.0027     | 6.7                       | 155                    | —                 |

<sup>a</sup> n = number of studies.

<sup>b</sup> 1 arbitrary unit = 9.6 microns.

<sup>c</sup> p < 0.001 compared with control strain.

<sup>d</sup> p < 0.05 compared with control strain.

<sup>e</sup> 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.

<sup>f</sup> 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<sub>p</sub>, 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<sub>p</sub> 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<sub>p</sub> 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.

[FIG. 1. Size distributions of epididymal fat pad adipocytes from homozygous male ob and tub mice and C57BL/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).]
final four weeks of the study, fat-fed animals gained 139.6 ± 9g at an average rate of 4.8 ± 0.3 g/day, compared with 92.6 ± 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 mM, 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 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 pathogenic 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<sub>pm</sub> 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<sub>max</sub> 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 fatty acid transport process(es) might represent an important new approach to therapy.

**REFERENCES**

1. Tataranni, P. A. (1998) *Diabetes Metab.* 24, 108–115
2. Björntorp, P. (1998) in *Handbook of Obesity* (Bray, G. A., Bouchard, C., and James, W. P. T., eds) pp. 573–600, Marcel Dekker, New York
3. Ravussin, E., Lilljefa, S., Knowler, W. C., Christin, L., Freymond, D., Abbott, W. G., Boyce, V., Howard, B. V., and Bogardus, C. (1988) *N. Engl. J. Med.* 318, 467–472
4. Schemmel, R., Mickelsen, O., and Gill, J. L. (1970) *J. Nutr.* 100, 1041–1048
5. Zhang, Y., Proenca, R., Maffeï, M., Barone, M., Leopold, L., and Friedman, J. M. (1994) *Nature* 372, 425–431
6. Chen, H., Charlat, O., Tartaglia, L. A., Woolf, E. A., Weng, X., Ellis, S. J., L bey, N. D., Culpepper, J., Moore, K. J., Breibart, R. E., Dufy, G. M., Tepper, R. I., and Morgenstern, J. P. (1996) *Cell* 84, 491–495
7. Kim, G.-H., Proenca, R., Montez, J. M., Carroll, K. M., Davishzadeh, J. G., Lee, J. I., and Friedman, J. M. (1996) *Nature* 380, 612–635
8. Noben-Trauth, K., Nagert, J. K., Noth, M. A., and Nishina, P. M. (1996) *Nature* 380, 534–538
9. Kley, P. W., Fan, W., Korats, S. G., Lee, J. J., Pulido, J. C., Wu, Y., Berdiel, L. R., Misini, D. J., Holmgren, L., Charlat, O., Woolf, P. A., Tai ber, O., Brody, T., Shu, P., Hawkins, F., Kennedy, B., Baldini, L., Ebeling, O., Alperin, G. D., Deeds, J., Leary, N. D., Culpepper, J., Chen, H., Gluckmann-Kuis, M. A., Moore, K. J., et al. (1996) Cell 85, 281–290
10. Miller, M. W., Duhl, D. M., Vrielig, H., Cordes, S. P., Ollmann, M. M., Winkes, B. M., and Barsh, G. S. (1993) *Genes & Dev.* 7, 454–467
11. Chua, S. C., Jr., Chung, W. K., Wu Peng, S., Zhang, Y., Liu, S.-M., Tartaglia, L. A., and Leibel, R. L. (1996) *Science* 271, 994–996
12. Chua, S. C., Jr., White, D. W., Sharon Wu-Peng, S., Liu, S.-M., Okada, N., Kershaw, E. E., Chung, W. K., Power-Kehoe, L., CXhua, M., Tartaglia, L. A., and Leibel R. L. (1994) *Diabetes* 43, 1141–1143
13. Phillips, M. S., Liu, Q., Hammond, H. A., Dugan, V., Hey, P. J., Caskey, C. T., Hess, J. F. (1996) *Nat. Genet.* 12, 18–19
14. Astrup, A., Bahren, A., Buemann, B., and Tourobo, S. (1997) *Ann. N. Y. Acad. Sci.* 827, 417–430
15. Paglia, S. M., Gayles, E. C., and Hill, J. O. (1997) *Ann. N. Y. Acad. Sci.* 827, 141–147
16. Kajigaya, R., Greenwood M. R. C., and Johnson, P. R. (1990) ILAR News 22, 4–8
17. Berk, P., Zhou, S.-L., Kiang, C.-L., Stump, D., Bradbury, M., Isola, L. M. (1997) *J. Biol. Chem.* 272, 8850–8855
18. Mattingly, J. R., Rodriguez, F. J., Gordon, J., Iriarte, A., and Martinez-Carrion, M. (1987) *Biochem. Biophys. Res. Commun.* 149, 859–865
19. Ahammad, N. A., Rafaat el-Maghrabi, M., Amri, K.-Z., Lopez, E., and Grimaldi, P. A. (1993) *J. Biol. Chem.* 268, 17665–17668
20. Schaffter, J. E., and Lodish, H. F. (1994) *Cell* 79, 427–436
21. Wion, K. L., Kirchgesner, T. G., Luise, A. J., Scholtz, M. C., and Laws, R. M. (1994) *J. Biol. Chem.* 269, 1141–1143
22. Murakami, T., and Shima, K. (1995) *Biochem. Biophys. Res. Commun.* 209, 944–952
23. Feinberg, A. P., and Vogelstein, B. (1983) *Anal. Biochem.* 136, 6–13
Up-regulation of Adipocyte Fatty Acid Uptake in Obesity

24. Stremmel, W., and Berk, P. D. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3086–3090
25. Berry, M. N., and Friend, D. S. (1969) J. Cell Biol. 43, 506–520
26. Schwieterman, W., Sorrentino, D., Potter, B. J., Rand, J., Kiang, C.-L., Stump, D., and Berk, P. D. (1986) Proc. Natl. Acad. Sci. U. S. A. 85, 3584–3588
27. Whitesell, R. K., and Abumrad, N. A. (1985) J. Biol. Chem. 260, 2894–2899
28. DiGirolamo, M., Mendlinger, S., and Fertig, J. W. (1971) Am. J. Physiol. 221, 850–858
29. Stremmel, W., Strohmeyer, G., and Berk, P. D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 359–363
30. Sorrentino, D., Robinson, R. B., Kiang, C.-L., and Berk, P. D. (1989) J. Clin. Invest. 84, 1325–1333
31. Sorrentino, D., Zifroni, A., Van Ness, K., and Berk, P. D. (1994) Am. J. Physiol. 266, G425–G432
32. Sorrentino, D., Van Ness, K., Stump, D. D., and Berk, P. D. (1994) J. Hepatol. 21, 551–559
33. Richieri, G. V., and Kleinfeld, A. M. (1995) J. Lipid Res. 36, 229–240
34. Rose, H., Conventz, M., Fischer, Y., Jüngling, E., Hennecke, T., and Kammermeier, H. (1994) Biochim. Biophys. Acta 1215, 321–326
35. Berman, M., and Weiss, M. F. (1967) Users’ Manual for SAAM, U. S. Public Health Service Publication 1703, Department of Health and Human Services, U. S. Government Printing Office, Washington, D. C.
36. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–169
37. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5201–5205
38. Mafiei, I., Hallaaj, J., Rauvusin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Lallone, R., Ranganathan, S., Kern, P. M., and Friedman, J. A. (1995) Nat. Med. 1, 1155–1161
39. Farese, R. V., Jr., Yost, T. J., and Eckel, R. H. (1991) Metabolism 40, 214–216
40. Kiens, B., Lithell, H., Mikines, K. J., and Richter, E. A. (1989) J. Clin. Invest. 84, 1124–1129
41. Eckel, R. H., Jensen D. N., Schlaepper, I. R., and Yost, T. J. (1996) Am. J. Physiol. 271, R1280–R1286
42. Weisiger, R. A. (1993) in Hepatic Transport and Bile Secretion (Tavoloni, N., and Berk, P. D., eds) pp. 171–196, Raven Press, New York
43. Weinstock, P. H., Levak-Frank, S., Hudgins, L. C., Radner, H., Friedman, J. M., Zechnier, R., and Breslow, J. L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10261–10266
44. Berk, P. D., and Stump, D. D. (1999) Mol. Cell. Biochem. 192, 17–31
45. Noy, N., and Zakim, D. (1993) in Hepatic Transport and Bile Secretion (Tavoloni, N., and Berk, P. D., eds) pp. 313–335, Raven Press, New York
46. Hamilton, J. A. (1998) J. Lipid Res. 39, 467–481
47. Hamilton, J. A. (1998) J. Lipid Res. 39, 467–481
48. Berk, P. D., and Stump, D. D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11367–11370
49. Berk, P. D., and Hamilton, J. A. (1993) Biochemistry 32, 11074–11086
50. Zakim, D. (1996) Proc. Soc. Exp. Biol. Med. 212, 5–14
51. Kamp, F., and Hamilton, J. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11367–11370
52. Weinstock, P. H., Levak-Frank, S., Hudgins, L. C., Radner, H., Friedman, J. M., Zechnier, R., and Breslow, J. L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10261–10266
53. Zakim, D. (1996) Proc. Soc. Exp. Biol. Med. 212, 5–14
54. Hamilton, J. A. (1998) J. Lipid Res. 39, 467–481
55. Odain, A. A., Shneider, B. L., Bennett, M. J., Pober, B. L., Reyes-Mujica, M., Friedman, A. L., Suchy, F. L., and Rinaldo, P. (1998) N. Engl. J. Med. 339, 1752–1757
56. Hamilton, J. A. (1998) J. Lipid Res. 39, 467–481