Facilitated Long Chain Fatty Acid Uptake by Adipocytes Remains Upregulated Relative to BMI for More than a Year After Major Bariatric Surgical Weight Loss

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**Objective:** This study examined whether changes in adipocyte long chain fatty acid (LCFA) uptake kinetics explain the weight regain increasingly observed following bariatric surgery.

**Methods:** Three groups (10 patients each) were studied: patients without obesity (NO: BMI 24.2 ± 2.3 kg m⁻²); patients with obesity (O: BMI 49.8 ± 11.9); and patients classified as super-obese (SO: BMI 62.6 ± 2.8). NO patients underwent omental and subcutaneous fat biopsies during clinically indicated abdominal surgeries; O were biopsied during bariatric surgery, and SO during both a sleeve gastrectomy and at another bariatric operation 16 ± 2 months later, after losing 113 ± 13 lbs. Adipocyte sizes and [³H]-LCFA uptake kinetics were determined in all biopsies.

**Results:** \( V_{\text{max}} \) for facilitated LCFA uptake by omental adipocytes increased exponentially from 5.1 ± 0.95 to 21.3 ± 3.20 to 68.7 ± 9.45 pmol/sec/50,000 cells in NO, O, and SO patients, respectively, correlating with BMI (\( r = 0.99, P < 0.001 \)). Subcutaneous results were virtually identical. By the second operation, the mean BMI (SO patients) fell significantly (\( P < 0.01 \)) to 44.4 ± 2.4 kg m⁻², similar to the O group. However, \( V_{\text{max}} \) (40.6 ± 11.5) in this weight-reduced group remained ~2X that predicted from the BMI: \( V_{\text{max}} \) regression among NO, O, and SO patients.

**Conclusions:** Facilitated adipocyte LCFA uptake remains significantly upregulated ≥1 year after bariatric surgery, possibly contributing to weight regain.

Introduction

Obesity is the accumulation of excess fat, principally triglycerides (TG), in adipocyte depots throughout the body. Excessive TG, typically within discrete droplets, also accumulates in the liver and heart, where it is responsible for clinical consequences such as non-alcoholic fatty liver disease (1) and obesity cardiomyopathy (2). In the late 1980s, skeptical of the concept that long chain fatty acids (LCFA) entered cells exclusively by diffusion, we postulated that the principal uptake process would prove to be regulatable, facilitated transport, and used studies of cellular LCFA uptake kinetics in...
rodents to prove that (3-7). We identified the first LCFA transporter (8-11) and showed that regulation of LCFA uptake in adipocytes was a control point for adiposity (12-14) and that upregulation of facilitated LCFA uptake in hepatocytes (1) and cardiomyocytes (2,15) was a key element in pathogenesis of obesity- and EtOH-associated hepatic steatosis and cardiomyopathies in rodents.

Translational studies confirmed key findings in man, especially up-regulation of adipocyte LCFA uptake in patients with obesity (16). The present study extends these observations to patients classified as super-obese participating in a two-stage bariatric surgical protocol beginning with a sleeve gastrectomy (17). After major weight loss over the first post-operative year, weight typically stabilized during year 2, leading to a second procedure, usually a biliopancreatic diversion with duodenal switch (BPD-DS), in patients requiring further weight loss. Fat biopsies from these procedures facilitated studies of the effects of weight loss on multiple aspects of adipocyte biology.

Study hypothesis
The rate (V_{max}) of facilitated (saturable) LCFA uptake into human adipocytes is highly correlated with BMI and % body fat, increasing in obesity and decreasing with weight loss.

Study aims
1. To compare the V_{max} for LCFA uptake with BMI in four patient groups: [1] patients without obesity (NO), [2] patients with obesity (O), [3] patients classified as super-obese (SO).
2. To compare V_{max} with BMI in SO patients both at the time of an initial sleeve gastrectomy and at a second surgical procedure after major weight loss, when they were classified as [4] super-obese reduced (SOr).

Methods
Patients and protocol
Patients classified as super-obese (SO). After stabilizing their weight for about 2 months following recommended dietary modifications, SO patients (BMI >50 kg m^{-2}) at Weil-Cornell and Columbia University Medical Centers underwent an initial laparoscopic sleeve gastrectomy (17). After major weight loss over the first post-operative year, weight typically stabilized during year 2, leading to a second procedure, usually a biliopancreatic diversion with duodenal switch (BPD-DS), in patients requiring further weight loss. Fat biopsies from these procedures facilitated studies of the effects of weight loss on multiple aspects of adipocyte biology.

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Materials
[^-1H]-oleic acid (OA) was from NEN Life Science Products (Boston, MA), type I collagenase from Sigma (St Louis, MO), and fatty acid-free bovine serum albumin (BSA) from Boehringer Mannheim (Indianapolis, IN). Circulating Spexin was assayed with a competitive EIA kit and leptin by an antigen capture ELISA kit from Phoenix Pharmaceuticals (Burlingame, CA).

Body fat. Percent body fat was measured in SO and SOr patients using Tanita scales equipped with Bioelectrical Impedance technology.

Isolation of adipocytes. Biopsies of 5-10 g were obtained from all enrolled patients from omental and anterior abdominal wall subcutaneous fat depots at each operation. Approximately 1/3 of each biopsy was frozen at −80°C in RNAlater for subsequent qRT-PCR gene expression and biochemical studies. Adipocyte single cell suspensions meeting established viability criteria were prepared with collagenase from the remainder of each biopsy and counted as described (10,16,18,19).

Cell size studies. Diameter distributions in each adipocyte preparation were determined as reported by digital analysis of suspended cells using a Nikon Eclipse 80i microscope and Nikon Digital DXM 1200C camera. Digital images were analyzed using Nikon NIS-Elements (NE) Br software, generating mean diameters (with distribution), in micrometers (μ), for each preparation. The mean cell surface area and cell volume, in μ² and pl, respectively, were computed from the diameters (20,21).

Adipocyte LCFA uptake studies. Aliquots from each cell preparation were incubated at 37°C in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 500 μM BSA and one of five different concentrations of OA, such that the OA:BSA molar ratio (ν) was 0.25, 0.5, 1.0, 1.5, or 2.0:1 (3). The initial velocity (V_{0}) of cellular OA uptake from each test solution was determined by a rapid filtration technique from four samples obtained in triplicate over the initial 30 s of incubation, during which uptake was a linear function of time (3,5,7,10,14,16,22).

Computations and data fitting. The unbound oleate concentration ([OAu]) in each test solution was calculated from V (23), using the LCFA:BSA binding constants of Spector et al. (24). Measurements of initial OA uptake velocity (V_{0}) at values of V from 0.25-2.0 were fitted to the sum of saturable and non-saturable functions of the corresponding [OAu] (7) according to the equation:

\[ UT([OAu]) = V_{max} \cdot [OAu] / (K_a + [OAu]) + k \cdot [OAu] \]

UT([OAu]) is the experimental measurement of uptake, in pmol/sec/50,000 cells, at the stated concentration of unbound OA ([OAu]);
TABLE 1 Clinical and biochemical characteristics of the four patient groups

|                  | Agea (yrs) | BMI (kg m⁻²) | Glucose (mg dL⁻¹) | Cholesterol (mg dL⁻¹) | HDL (mg dL⁻¹) | LDL (mg dL⁻¹) | TG (mg dL⁻¹) | Albumin (mg dL⁻¹) | Bilirubin (mg dL⁻¹) | AST (U L⁻¹) | ALT (U L⁻¹) | Alk phos (U L⁻¹) |
|------------------|------------|--------------|-------------------|-----------------------|---------------|---------------|--------------|-------------------|---------------------|-------------|-------------|-----------------|
| **NON-OBSESE**   |            |              |                   |                       |               |               |              |                   |                     |             |             |                 |
| Male             | 44.8 ± 7.0 | 24.1 ± 1.3   | 110.3 ± 17.3      | 179.3 ± 13.5          | 47.3 ± 5.7    | 96.8 ± 7.3    | 109.5 ± 30.9 | 4.6 ± 0.1         | 0.5 ± 0.1            | 17.8 ± 2.1 | 23.8 ± 6.9  | 68.8 ± 4.3      |
| Female           | 40.8 ± 4.8 | 23.3 ± 0.5   | 79.3 ± 1.6        | 178.3 ± 19.1          | 59.3 ± 5.4    | 106.8 ± 16.9  | 61.5 ± 11.1   | 4.3 ± 0.1         | 0.5 ± 0.1            | 18.0 ± 2.9 | 15.5 ± 3.4  | 79.0 ± 18.7      |
| M vs. F          | NS         | NS           | NS                | NS                    | NS            | NS            | NS           | NS                | NS                  | NS          | NS          | NS              |
| Total            | 42.8 ± 4.0 | 23.7 ± 0.7   | 94.8 ± 9.9        | 178.8 ± 10.6          | 53.3 ± 4.3    | 101.8 ± 8.72  | 85.5 ± 17.7  | 4.5 ± 0.1         | 0.5 ± 0.1            | 17.9 ± 1.6 | 20.1 ± 3.8  | 73.3 ± 8.1      |
| **OBSESE**       |            |              |                   |                       |               |               |              |                   |                     |             |             |                 |
| Male             | 47.6 ± 1.6 | 49.5 ± 5.5   | 90.4 ± 6.1        | 107.6 ± 7.1           | 33.8 ± 3.4    | 84.8 ± 5.8    | 144.6 ± 24.7 | 4.1 ± 0.1         | 0.8 ± 0.1            | 25.2 ± 3.4 | 40.2 ± 7.9  | 68.8 ± 5.4      |
| Female           | 49.6 ± 3.2 | 50.5 ± 1.5   | 94 ± 4.0          | 216.5 ± 17.9          | 46 ± 1.8      | 145.8 ± 17.8  | 123.8 ± 18.5 | 4.2 ± 0.2         | 0.5 ± 0.1            | 20.0 ± 3.4 | 19.4 ± 3.6  | 72.6 ± 8.3      |
| M vs. F          | NS         | NS           | NS                | P < 0.001             | P < 0.01      | NS            | NS           | NS                | P < 0.025            | NS          | NS          | NS              |
| Total            | 48.6 ± 1.7 | 50.0 ± 2.7   | 92.2 ± 3.5        | 156 ± 15.9            | 39.2 ± 2.9    | 111.9 ± 14.3  | 136.3 ± 15.1 | 4.2 ± 0.1         | 0.7 ± 0.1            | 22.8 ± 2.5 | 30.8 ± 5.0  | 70.5 ± 4.7      |
| vs. NO           | NS         | P < 0.001   | NS                | NS                    | P < 0.01      | NS            | P < 0.05     | NS                | NS                  | NS          | NS          | NS              |
| **SUPER-OBSESE** |            |              |                   |                       |               |               |              |                   |                     |             |             |                 |
| Male             | 51.6 ± 2.9 | 61.5 ± 6.0   | 88.8 ± 3.7        | 175.2 ± 19.4          | 41.0 ± 3.3    | 105.4 ± 12.8  | 142.8 ± 45.6 | 3.9 ± 0.1         | 0.7 ± 0.2            | 26.6 ± 4.9 | 32.4 ± 8.1  | 66.0 ± 12.8     |
| Female           | 38.5 ± 3.9 | 63.8 ± 2.1   | 103.5 ± 14.6      | 185.5 ± 17.4          | 43.3 ± 5.6    | 122.8 ± 14.9  | 142.3 ± 43.7 | 3.5 ± 0.2         | 0.5 ± 0.1            | 26.0 ± 4.3 | 28.4 ± 5.2  | 70.2 ± 6.8      |
| M vs. F          | P < 0.025  | NS           | NS                | P < 0.05              | NS           | NS            | NS           | NS                | NS                  | NS          | NS          | NS              |
| Total            | 44.5 ± 3.1 | 62.8 ± 2.9   | 95.3 ± 5.6        | 180.5 ± 11.7          | 42.0 ± 2.4    | 113.1 ± 8.5   | 142.6 ± 26.4 | 3.7 ± 0.1         | 0.6 ± 0.1            | 26.3 ± 2.2 | 30.4 ± 4.3  | 68.1 ± 6.8      |
| vs. NO           | NS         | P < 0.001   | NS                | NS                    | P < 0.05     | NS            | P < 0.025   | NS                | P < 0.005            | NS          | NS          | NS              |
| **SUPER-OBESE reduced** |            |              |                   |                       |               |               |              |                   |                     |             |             |                 |
| Male             | 52.8 ± 2.7 | 42.8 ± 4.0   | 73.2 ± 5.9        | 165.4 ± 25.7          | 47.2 ± 4.7    | 96.6 ± 17.4   | 112.2 ± 39.1 | 4.9 ± 0.2         | 0.6 ± 0.1            | 24.6 ± 2.9 | 21.2 ± 2.3  | 61.0 ± 10.4     |
| Female           | 39.2 ± 4.3 | 46.7 ± 4.5   | 89.4 ± 8.0        | 217.3 ± 18.9          | 49.0 ± 12.5   | 143.0 ± 17.2 $| 127.3 ± 40.1 | 3.7 ± 0.2         | 0.6 ± 0.1            | 18.4 ± 3.7 | 12.6 ± 1.3  | 66.0 ± 12.8     |
| M vs. F          | P < 0.05   | NS           | NS                | P < 0.05              | NS           | NS            | NS           | NS                | NS                  | NS          | NS          | NS              |
| Total            | 46.0 ± 3.3 | 45.2 ± 2.7   | 81.3 ± 5.3        | 184.9 ± 18.6          | 47.9 ± 4.5    | 114.0 ± 14.3  | 117.9 ± 25.3 | 3.9 ± 0.1         | 0.6 ± 0.1            | 21.5 ± 2.4 | 16.9 ± 1.9  | 63.5 ± 8.5      |
| vs. NO           | NS         | P < 0.001   | NS                | NS                    | NS           | NS            | NS           | NS                | P < 0.001            | NS          | NS          | NS              |

$a$ All data mean ± SE.

$^b$ NS = not significantly different (P > 0.05).
$V_{\text{max}}$ and $K_m$ are the maximal velocity of saturable OA uptake and the value of [OAu] at one-half the maximal uptake velocity; $k$ is the rate constant for nonsaturable uptake.

Data fitting used the SAAM II version of the Simulation, Analysis and Modeling (SAAM) program of Berman and Weiss (25,26) to compute for each data set values of $V_{\text{max}}$ (pmol/sec/50,000 cells), $K_m$ (nM), and $k$ (ml/sec/50,000 cells), and their variances and covariances. Prior studies showed that under the conditions employed, $V_{\text{max}}$ and derived parameters such as $V_{\text{max}}$ are measures of actual transmembrane transport (3,5,27). Further studies demonstrated that an increase in $V_{\text{max}}$ preceded an increase in adipocyte size early in the development of obesity (13), and a decrease in $V_{\text{max}}$ preceded a reduction in adipocyte size and body weight during leptin-induced weight loss (14), showing that changes in $V_{\text{max}}$ do not simply reflect changes in cell size.

**Statistical methods.** Relationships between parameters were assessed by both linear and nonlinear correlations (28). For group comparisons, results are expressed as mean ± SE, with $n = 10$ per group. Each of the experimental groups was compared to the control group with two-tailed Student’s $t$ tests. The other groups were also compared with each other by one way ANOVA as previously described (15). In addition, the effects on changes in LCFA uptake rates in response to weight loss, of age, gender, ethnicity, baseline weight, % body fat, metabolic status (as reflected in e.g., HbA1c and cholesterol), and the presence of specific co-morbidities or medication use, were explored by effect adjustments in the ANOVA. In all statistical testing, significance was set at $P ≤ 0.05$.

**Spexin and leptin gene expression and serum assays**

Circulating Spexin was measured by competitive enzyme immunoassay (EIA) and leptin by antigen capture ELISA using Phoenix Pharmaceuticals kits (Burlingame, CA) (29). Sera were diluted 1/20 in assay buffer, and quantified by comparison to within-assay standard curves according to the manufacturer’s instructions.

**Results**

**Patients**

Demographic and clinical laboratory data for the 10 participants in each of the NO, O, and SO groups are summarized in Table 1, as are analogous data for the group designated as SO, which were obtained from SO patients at the time of their second bariatric procedure. Mean ages, initial BMIs and clinical and laboratory data for the 10 SO patients who completed a second operation are very similar to corresponding data from all 35 SO patients enrolled in the study. Overall, the NO, O, and SO patient groups were similar in age (Table 1). O and SO weighed more than NO patients and had higher BMIs ($P < 0.001$). While high-density lipoprotein (HDL) values were lower and TG higher in the O and SO patients than in the NO controls (Table 1), there were no significant increases in glucose or cholesterol in these two groups of patients with obesity, possibly
reflecting ongoing treatment for hyperglycemia and/or hypercholesterolemia. Albumin was marginally reduced and aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) marginally increased in the SO and O groups (Table 1). Overall, the abnormalities in aminotransferases were on the mild end of the spectrum observed in larger populations of SO patients (30). Gender differences were observed for several clinical and laboratory parameters summarized in Table 1. However, as there were only 5 patients of each gender per group, these differences were not analyzed further.

Additional data from the 10 individual SO/SOr patients that were, inter alia, the basis for our ANOVA effects adjustment testing are presented in Supporting Information Tables 1 and 2. Among these 10 SO patients, 8 were Caucasian-non-Hispanic, 1 Caucasian-Hispanic, and 1 self-described as a Caucasian/Hispanic/African American (Supporting Information Table 1). Initially 8 of the 10 SO patients had hypertension and 6 had diabetes. SO patients had a mean of three comorbidities each; one patient had only steatohepatitis, and only one patient had none.

Adipocyte dimensions

Diameters of both omental and subcutaneous adipocytes from the 30 NO, O and SO patients increased linearly with increasing BMI (omental: \( r = 0.55, P < 0.01 \); subcutaneous: \( r = 0.46, P < 0.05 \)) (Figure 1).

Computed adipocyte surface areas and volumes were similarly correlated with BMI. When averaged by group, the calculated omental adipocyte surface area increased from 23.2 \( \pm 4.1 \) to 34.8 \( \pm 2.0 \) to 39.1 \( \pm 4.5 \times 10^3 \) \( \mu \text{m}^2 \) per cell and cell volume from 437 \( \pm 74 \) to 616 \( \pm 53 \) to 749 \( \pm 124 \) pl per cell in NO, O, and SO patients. Subcutaneous adipocytes showed very similar trends.

Adipocyte LCFA uptake studies

The \( V_{\text{max}} \) for facilitated LCFA uptake by omental adipocytes increased exponentially as a function of BMI across the three patient groups (Figure 2A). Mean values averaged 8.3 \( \pm 0.6 \), 20.9 \( \pm 1.4 \), and 68.7 \( \pm 9.6 \) pmol/sec/50,000 cells in the NO, O, and SO patients, respectively. Data from subcutaneous adipocytes were very similar (Figure 2B). The \( V_{\text{max}} \) for omental and subcutaneous adipocytes in individual patients in each of the three patient groups were also very similar, and were linearly related (correlation coefficient \( r = 0.96 \)) (Figure 3A). Effects of surgical weight loss on \( V_{\text{max}} \) (Figure 3B) are examined below. Omental \( V_{\text{max}} \) values in SO and O patients were highly correlated with BMI (\( P < 0.01 \)) (Supporting Information Figure 1A), which in turn was highly correlated with Body Fat % (\( P < 0.01 \)) (Supporting Information Figure 1B). Results in subcutaneous adipocytes were virtually identical.

Effects of post-surgical weight loss

SOr participants who returned for a second operation 16.3 \( \pm 2.2 \) mos after their initial surgery had lost a mean of 113 \( \pm 13 \) lbs. As indicated by comparing SO with SOr results in Table 1, weight loss was associated with modest changes in clinical laboratory parameters, including glucose, lipids, and liver tests. Medications for control of T2DM, hyperlipidemia, or cardiovascular disorders including hypertension, prescribed by referring physicians, were also little changed (Supporting Information Table 2).

**LCFA uptake kinetics after weight loss.** SOr patients exhibited appreciable reductions in the \( V_{\text{max}} \) for LCFA uptake in both omental and subcutaneous adipocytes when compared to values at initial surgery. At their second surgery, BMIs had fallen from their initial 62.6 \( \pm 2.8 \) kg \( \text{m}^{-2} \) to 44.4 \( \pm 2.4 \) kg \( \text{m}^{-2} \), a value not significantly different from the 50.1 \( \pm 1.1 \) kg \( \text{m}^{-2} \) in the O group (Figures 4A,4B). There were corresponding reductions in Body Fat % (Supporting Information Table 1). Both omental and subcutaneous adipocyte dimensions (Figure 5A-C) fell into the NO range in most SOr patients following initial bariatric surgery, and were reduced compared to those of steady-state O adipocytes. However, \( V_{\text{max}} \)'s for facilitated adipocyte LCFA uptake (omental: 42.1 \( \pm 6.4 \) pmol/sec/50,000 cells; subcutaneous: 37.7 \( \pm 6.2 \) pmol/sec/50,000 cells) remained significantly increased to \( \sim 2 \times \) that predicted for their BMI by the BMI:\( V_{\text{max}} \) regression among NO, O, and SO patients (Figure 4A,4B), \( 2 \times \) the value observed in the O patient group (Figure 6A), and about fivefold compared to the NO range, indicating persistent upregulation of both omental and subcutaneous facilitated adipocyte LCFA uptake in SOr patients. As illustrated in Figure 6B, the omental \( V_{\text{max}} \) fell appreciably in the transition from SO to SOr status in 9 of 10 individual patients, the exception being a patient who—unknown to his surgeon at the time—began to drink heavily in the period between his two bariatric surgical procedures.

In our model of LCFA transport (7,13,14,16), \( V_{\text{max}} \) is defined as a measure of the density of LCFA transporters per unit of cell surface area.
$V_{\text{max}}$, on average, increased with weight loss in omental SOr vs. SO adipocytes, and were elevated 5-fold compared to the NO range and 3.7-fold compared to that in O adipocytes (Figure 6C). By contrast, in a few SOr patients, $V_{\text{max}}$ decreased rather than increasing with weight loss (Figure 7). Other than being a means of classifying adipocytes in terms of the response of their fatty acid uptake process to weight loss, the precise physiological implications of the nature of the change in $V_{\text{max}}$ require further exploration (see Supporting Information). By contrast to $V_{\text{max}}$, $k'$, a measure of non-saturable (passive) uptake per unit surface area, was not significantly changed in any group, consistent with its previously ascribed role as a measure of cell membrane permeability to passive LCFA diffusion (14,16). The means of the 40 values of $k'$ in the NO, O, SO, and SOr groups were $0.0034 \pm 0.0005 \times 10^{-8}$ and $0.0033 \pm 0.0004 \times 10^{-8}$ ml s$^{-1}$ µm$^{-2}$ cell surface area for omental and subcutaneous adipocytes, respectively.
Effects on serum leptin and Spexin concentrations

Human serum leptin concentrations have a positive, nonlinear correlation with BMI; in contrast, Spexin concentrations and BMI are negatively correlated (29). These and others findings raise the possibility that leptin and Spexin are counter-acting regulators of adipocyte LCFA uptake (29). In this study leptin concentrations in SO patients fell from 79.61 ± 22.05 to 37.72 ± 14.23 ng ml⁻¹ (mean ± SE, P < 0.01) while serum Spexin increased from 1.65 ± 0.41 to 2.4 ± 0.36 ng ml⁻¹ (P = 0.043) between their two bariatric surgeries, consistent with the previously identified trend (29).

Modulators of changes in LCFA uptake with weight loss

No significant effects of age, gender, ethnicity, baseline weight, metabolic status (e.g., HbA1c, cholesterol), presence of specific co-morbidities or medication use on the observed changes in LCFA uptake rates in response to weight loss were detected by the effect adjustments in the ANOVA. However, this conclusion must be qualified because of the small group sizes available for these analyses.

Discussion

The complexity of changes in adipose gene expression was not appreciated (31) and Spexin (32) and its association with weight regulation (29) had not been discovered when the project began in 2006. Our initial expectations were that adipocyte size and saturable LCFA uptake would fall in parallel during bariatric surgery-induced weight loss.

While omental adipocyte sizes (Figure 5) and the $V_{max}$ for LCFA (Figure 6A) uptake both decreased as SO patients’ BMIs fell after initial bariatric surgery, they did not decrease in parallel. SO cell sizes often fell into the normal range and were therefore small relative to BMI, but $V_{max}$ did not consistently fall even into the O range in these patients, whose BMIs remained in the 40s. Consequently, when expressed per unit surface area, $V_{max}$ of omental adipocytes actually increased 28%, from 3.6 ± 0.5 to 4.6 ± 0.9 pmol s⁻¹ µ⁻² × 10⁻³, a value nearly fourfold higher than the 1.2 ± 0.1 pmol s⁻¹ µ⁻² × 10⁻³ typical of O patients (Figure 6C). These findings are graphed (Figure 7) and their implications discussed in detail in Supporting Information. Our cell size and $V_{max}$ data are consistent with recent models (33) which propose that small for body weight adipocytes are an important stimulus to weight regain via several complex pathways.
Because upregulation of adipocyte LCFA transport is closely associated with obesity in man (16), its upregulation predicts weight gain in multiple animal obesity models (1,12,13), and this study documents a significant correlation between LCFA uptake and BMI (Figure 2), it is tempting to speculate that persistent upregulation of LCFA uptake in our SOr patients following bariatric surgical weight loss is a harbinger of weight regain. Weight regain has become an increasingly important issue in obesity management, after both dietary weight loss (34) and bariatric surgery (35). The LABS consortium recently published the outcomes over 3 years following bariatric surgery in 2458 patients with obesity (36), of whom 1,738 underwent Roux-en-Y gastric bypass (RYGB), 610 laparoscopic placement of an adjustable gastric band (LAGB), and 110 other procedures including 59 sleeve gastrectomies. RYGB and LAGB patients experienced most of their total weight loss in the first post-operative year. To evaluate weight patterns, five weight trajectory groups were identified for each procedure. The five RYGB trajectories all showed initial weight loss for 6 months after surgery, but by the third post-operative year, trajectories for all five groups demonstrated weight regain, accompanied by some recurrences of co-morbidities. Modest weight regain was reported during the second and third post-operative years among patients who had undergone sleeve gastrectomy or RYGB in another trial comparing intensive medical therapy alone vs. intensive medical therapy plus bariatric surgery for treatment of diabetes (37). The prevalence of weight regain in these and earlier studies (35) became increasingly evident by the middle of the second post-operative year, corresponding to when we noted persistent upregulation of adipocyte LCFA uptake in our SOr patients.

Persistence of weight gain-promoting hormone patterns; increased insulin sensitivity, rates of glucose transport, and LPL activity; and a multiplicity of persistent metabolic abnormalities are factors believed to contribute to weight regain (33,35,38). Given our earlier demonstration of the association between weight gain and upregulation of adipocyte LCFA uptake, and of the present results, it is tempting to speculate that persistent upregulation of adipose tissue LCFA uptake is, at the least, another potential mechanism contributing to weight regain after initial weight loss induced by bariatric surgery. However, to prove that, it will be necessary to study it, other potential causes of weight regain, and weight regain per se in the same cohort. This was
impossible in the current study because our protocol mandated a second omental fat biopsy, which could only be obtained during a second bariatric surgical procedure. This second procedure led to further weight loss, averaging 50 ± 13.5 lbs by a mean of 11 months postoperatively, making any tendency to more modest weight regain from the first surgery undetectable. However, the finding that adipocyte dimensions and LCFA uptake kinetics in subcutaneous adipocytes are virtually identical to those in omental fat is important, since subcutaneous fat can be obtained by aspiration during routine outpatient visits. Correlating serial aspiration biopsies of subcutaneous fat and simultaneous weight determinations after a single bariatric surgical procedure in each patient will provide a much stronger assessment of the relationship between LCFA kinetics and weight regain, indicate whether any observed upregulation of adipocyte LCFA uptake persists with longer follow-up or whether it eventually normalizes, and the extent to which it occurs with all bariatric surgical procedures or is a unique consequence of sleeve gastrectomy. Adaptive responses lead the majority of patients who previously had obesity, who then lost weight by dietary restriction, to later regain the lost weight (33,34,38). The role for persistently upregulated adipocyte LCFA uptake in that process is an open question.

Body weight and energy balance are principally regulated by integration of numerous signals, including concentrations of hormones released mainly from the gut and adipose tissues (39). The details of these processes are still being elucidated, as is the extent to which the persistent upregulation of LCFA uptake reflects abnormal hormonal patterns or a more complex pathogenesis.

Figure 7 Contributions of adipocyte surface area and $V_{\text{max}}$ to $V'_{\text{max}}$. $V_{\text{max}}$, the maximal rate of saturable LCFA uptake per cell, is in turn dependent on (i) $V'_{\text{max}}$, the maximal saturable LCFA uptake rate per $\mu^2$ of cell surface and (ii) the number of $\mu^2$ per cell. Ten SO patients in a two-phase bariatric surgical protocol lost 113 ± 13 lbs in 16 ± 2 months between an initial sleeve gastrectomy and a second operation. Omental adipocyte LCFA uptake kinetics and cell surface area (SA) were studied at both surgeries. Ten NO and ten O surgical patients served as controls. The three panels illustrate mean ± SE in these patient groups for (A) mean LCFA uptake $V_{\text{max}}$, (B) adipocyte surface area, and (C) $V'_{\text{max}}$, as calculated from the two measured variables. Dashed lines indicate changes in these parameters in SO patients between their initial and second (post weight loss) operations. Both $V_{\text{max}}$ and SA were decreased in the SO patients. However, the proportional reduction in SA was greater than that in $V_{\text{max}}$. Consequently, $V'_{\text{max}}$, defined as $V_{\text{max}}/\text{SA}$, actually increased in these patients. In other settings $V'_{\text{max}}$ may change in parallel with $V_{\text{max}}$. These and other studies indicate that $V_{\text{max}}$ and SA are independently regulated.
Several large studies (35,36,40) show that bariatric surgery is the most effective current approach to short- and medium-term weight reduction and, often, remission of co-morbidities. However, its longer term efficacy and the role of weight regain in modulating its benefits are still uncertain. Because effective anti-obesity drugs developed in response to improved understanding of obesity pathophysiology are likely to become available in the future, appropriate therapeutic choices for optimal weight management will require improved understanding of the underlying physiology. Accordingly, evaluating the impact of persistently increased LCFA uptake and other mechanisms on weight regain and long-term obesity management should be actively pursued, and relevant processes, including increased adipocyte LCFA uptake, identified and studied in detail for their possible therapeutic benefits.

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