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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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Abstract

Objective—This study examined whether changes in adipocyte LCFA uptake kinetics explain the weight regain increasingly observed post bariatric surgery.

Design—Three groups (10 patients each) were studied: patients who were not obese (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 & subcutaneous fat biopsies during

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Conflict of interest disclosure: Drs. Walewski and Berk disclose that they are co-inventors on a patent application for the use of Spexin for the treatment of obesity and obesity-related disorders. All other authors report that they have no competing interests to declare.

Author contributions: PDB designed the study, obtained all necessary approvals, and oversaw the project. MHT and FEE were the patient coordinators. GD, AP, MB, BS, AU-W, and WBI recruited patients, performed surgery, and obtained biological specimens. EC-T and DA created and maintained the data base. FG, HL-IV, CH, and SZ processed the specimens and performed biochemical and physiologic analyses on them; JLW performed and analyzed all molecular studies. PDB, DA and TF performed the statistical analyses and DA graphed all of the results. PDB and JLW wrote the manuscript, but all authors reviewed and critiqued it as it evolved.

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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 & [^{3}H]-LCFA uptake kinetics were determined in all biopsies.

Results—Vmax 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 2nd 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, Vmax (40.6±11.5) in this weight-reduced group remained ~2X that predicted from the BMI:Vmax regression among NO, O, & SO patients.

Conclusions—Facilitated adipocyte LCFA uptake remains significantly up-regulated ≥1 year after bariatric surgery, possibly contributing to weight re-gain.

Keywords

obesity; bariatric surgery; weight loss; adipocytes; fatty acid uptake; spexin

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 they are responsible for clinical consequences such as nonalcoholic 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], showed that regulation of LCFA uptake in adipocytes was a control point for adiposity [12-14], and that up-regulation of facilitated LCFA uptake in hepatocytes [1] & cardiomyocytes [2,15] was a key element in pathogenesis of obesity- and EtOH-associated hepatic steatosis & 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 2-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 (Vmax) of facilitated (saturable) LCFA uptake into human adipocytes is highly correlated with BMI and % body fat, increasing in obesity and decreasing with weight loss.

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Study Aims

(1) to compare the Vmax for LCFA uptake with BMI in four patient groups: [1] patients who were not obese (NO), [2] patients with obesity (O), [3] patients classified as super-obese (SO), and (2) to compare Vmax 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 ca. 2 months following recommended dietary modifications, SO patients (BMI >50 kg/m^2) at Weil-Cornell & Columbia University Medical Centers underwent an initial laparoscopic sleeve gastrectomy. When their weight subsequently stabilized, after significant weight loss often accompanied by remission of diabetes and/or hypertension, 10 returned for a second operation [17].

We received omental and subcutaneous fat biopsies and a venous blood sample at both surgical procedures in order to examine the effects of surgical weight loss on aspects of adipocyte biology and adipocyte LCFA uptake. Power calculations indicated that at least 10 patients would have to complete both operations to reach our desired end-points with appropriate statistical assurance. Accordingly, the study remained open to enrollment until 10 patients, now designated as SOr, had completed second stage surgery. By chance, those 10 patients consisted of 5 men and 5 women. As anticipated by prior experience, a total of 35 SO patients were enrolled to meet the study needs.

Patients who were Not-Obese (NO) (BMI <30)—NO patients consented to donate a venous blood sample and omental and subcutaneous fat biopsies during a clinically indicated laparoscopic abdominal surgical procedure. Of 18 NO patients studied, 5 men and 5 women were selected to age-match this cohort as closely as possible with the SO patients. Of the 10, 7 had undergone donor nephrectomy, 2 laparoscopic cholecystectomy, and 1 an inguinal herniorraphy.

Patients with Obesity (O) (BMI > 35)—Of 34 O male and 49 O female bariatric surgery patients studied by our laboratory, 5 men and 5 women were similarly chosen on the basis of age for inclusion in this analysis. The minimum BMI of 35 in this group reflects the lower limits of BMI currently acceptable for bariatric surgery.

Materials

[^{3}H]-oleic acid (OA) was from NEN Life Science Products (Boston, MA, USA), type I collagenase from Sigma (St Louis, MO, USA), 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).

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Body Fat—Percent body fat was measured in SO & 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 & 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 & 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 μ^2 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 ν [23], using the LCFA:BSA binding constants of Spector et al [24]. Measurements of initial OA uptake velocity (V_0) at values of ν from 0.25-2.0 were fitted to the sum of saturable & nonsaturable functions of the corresponding [OAu][7] according to the equation: UT([OAu]) = Vmax • [OAu] /(Km + [OAu]) + k • [OAu]. UT([OAu]) is the experimental measurement of uptake, in pmol/sec/50,000 cells, at the stated concentration of unbound OA ([OAu]); Vmax & Km 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 & Modeling (SAAM) program of Berman and Weiss [25, 26] to compute for each data set values of Vmax (pmol/sec/50,000 cells), Km (nM), and k (ml/sec/50,000 cells), and their variances & covariances. Prior studies showed that under the conditions employed, V_0 and derived parameters such as Vmax are measures of actual transmembrane transport [3, 5, 27] Further studies demonstrated that *an increase in Vmax preceded* an increase in adipocyte size early in the development of obesity [13], and *a decrease in Vmax preceded* a reduction in adipocyte size and body weight during leptin-induced weight loss [14], showing that changes in Vmax do not simply reflect changes in cell size.

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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 $p \leq 0.05$.

Spexin and leptin gene expression and serum assays

Circulating Spexin was measured by competitive enzyme immunoassay (EIA) & 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_r, 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 triglycerides (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 obese patients, 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 super-obese 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 **Supplemental 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 (**Supplemental Table 1**). Initially 8 of the 10 SO patients had hypertension and 6 had diabetes. SO patients had a mean of 3 comorbidities each; 1 patient had only steatohepatitis, and only 1 patient had none.

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Adipocyte Dimensions

Diameters of both omental and subcutaneous adipocytes from the 30 NO, O & 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±4.1 to 34.8±2.0 to 39.1±4.5 X 10^3 μ^2 per cell and cell volume from 437±74 to 616±53 to 749±124 pl per cell in NO, O & SO patients. Subcutaneous adipocytes showed very similar trends.

Adipocyte LCFA Uptake Studies

The Vmax for facilitated LCFA uptake by omental adipocytes increased exponentially as a function of BMI across the 3 patient groups (**Figure 2A**). Mean values averaged 8.3±0.6, 20.9±1.4, and 68.7±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 Vmax for omental and subcutaneous adipocytes in individual patients in each of the three patient groups were also very similar, and were linearly related (r= 0.96) (**Figure 3A)**. Effects of surgical weight loss on Vmax (**Figure 3B**) are examined below. Omental Vmax values in SO & O patients were highly correlated with BMI (p<<0.01) (**Supplemental Figure 1A**), which in turn was highly correlated with Body Fat % (p<0.01) (**Supplemental 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±2.2 mos after their initial surgery had lost a mean of 113±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 (**Supplemental Table 2**).

LCFA Uptake Kinetics After Weight Loss—SOr patients exhibited appreciable reductions in the Vmax for LCFA uptake in both omental and subcutaneous adipocytes when compared to values at initial surgery. At their 2nd surgery, BMIs had fallen from their initial 62.6±2.8 kg/m^2 to 44.4±2.4 kg/m^2, a value not significantly different from the 50.1±1.1 kg/m^2 in the O group (**Figures 4A, 4B**). There were corresponding reductions in Body Fat % (**Supplemental 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, Vmax's for facilitated adipocyte LCFA uptake (Omental: 42.1±6.4 pmol/sec/50,000 cells; subcutaneous: 37.7±6.2 pmol/sec/50,000 cells) remained significantly increased to ~2X that predicted for their BMI by the BMI:Vmax regression among NO, O, & SO patients **(Figure 4A,4B)**, 2X the value observed in the O patient group and ca. 5-fold compared to the NO range **(Figure 6A)**, indicating persistent up-regulation of both omental and subcutaneous facilitated adipocyte LCFA uptake in SOr patients. As illustrated in **Figure 6B**, the omental Vmax fell appreciably in the transition from SO to SOr status in 9 of 10 individual patients, the

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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], Vmax' is defined as a measure of the density of LCFA transporters per unit of cell surface area (Vmax' = Vmax/Cell Surface Area) (pmol/sec/ μ^2 X 10^{-8} cell surface) (**Figure 6C**). Vmax', 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 Obese adipocytes **(Figure 6C)**. By contrast, in a few SOr patients, Vmax' 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 Vmax' require further exploration **(see Supplemental Materials)**. By contrast to Vmax', 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, SOr groups were 0.0034±0.0005 X 10^{-8} & 0.0033±0.0004 X 10^{-8} ml/sec/μ^2 cell surface area for omental and subcutaneous adipocytes, respectively.

Effects on Serum Leptin and Spexin Concentrations

Human serum leptin concentrations have a *positive*, non-linear 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 (mean ± SE, p = 0.009) while serum Spexin increased from 1.65 ± 0.41 to 2.4 ± 0.36 ng/mL (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 were 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 Vmax for LCFA (**Figure 6A**) uptake both decreased as SO patients' BMIs fell after initial bariatric surgery, they did not decrease in parallel. SOr cell sizes often fell into the normal range and were therefore small relative to BMI, but Vmax 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*, Vmax' of omental

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adipocytes actually *increased* 28%, from 3.6 ± 0.5 to 4.6 ± 0.9 pmol/sec/μ^2 X 10^{-8}, a value nearly 4-fold higher than the 1.2 ± 0.1 pmol/sec/μ^2 X 10^{-8} typical of O patients (**Figure 6C**). These findings are graphed (**Figure 7**) and their implications discussed in detail in **Supplemental Materials**. Our cell size and Vmax' 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.

Since up-regulation of adipocyte LCFA transport is closely associated with obesity in man [16], its up-regulation predicts weight gain in multiple animal obesity models [1, 12, 13], & 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 post bariatric surgery in 2458 obese patients [36], of whom 1738 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, 5 weight trajectory groups were identified for each procedure. The 5 RYGB trajectories all showed initial weight loss for 6 months after surgery, but by the third post-operative year, trajectories for all 5 groups demonstrated weight regain, accompanied by some recurrences of co-morbidites. 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 up-regulation 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 up-regulation of adipocyte LCFA uptake, and of the present results, it is tempting to speculate that persistent up-regulation 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 post-operatively, 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

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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 up-regulation 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 were previously obese, who then lost weight by dietary restriction, to later regain the lost weight [33, 34, 38]. The role for persistently up-regulated 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 up-regulation of LCFA uptake reflects abnormal hormonal patterns or a more complex pathogenesis.

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. Since 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 & other mechanisms on weight regain and long-term obesity management should be actively pursued, and relevant processes, including increased adipocyte LCFA uptake, identified & studied in detail for their possible therapeutic benefits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Ge F, Zhou S, Hu C, Lobdell H IV, Berk PD. Insulin- and leptin-regulated fatty acid uptake plays a key causal role in hepatic steatosis in mice with intact leptin signaling but not in ob/ob or db/db mice. Am J Physiol Gastrointest Liver Physiol. 2010; 299:G855–866. [PubMed: 20595619]
2. Ge F, Hu C, Hyodo E, Arai K, Zhou S, Lobdell H IV, et al. Cardiomyocyte triglyceride accumulation and reduced ventricular function in mice with obesity reflect increased long chain fatty acid uptake and de novo fatty acid synthesis. J Obes. 2012; 2012:205648. [PubMed: 22132320]
3. Sorrentino D, Robinson RB, Kiang CL, Berk PD. At physiologic albumin/oleate concentrations oleate uptake by isolated hepatocytes, cardiac myocytes, and adipocytes is a saturable function of

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the unbound oleate concentration. Uptake kinetics are consistent with the conventional theory. J Clin Invest. 1989; 84:1325–1333. [PubMed: 2794064]

4. Sorrentino D, Stump DD, Van Ness K, Simard A, Schwab AJ, Zhou SL, et al. Oleate uptake by isolated hepatocytes and the perfused rat liver is competitively inhibited by palmitate. Am J Physiol. 1996; 270:G385–392. [PubMed: 8779983]
5. Stremmel W, Berk PD. Hepatocellular influx of [^{14}C]-oleate reflects membrane transport rather than intracellular metabolism or binding. Proc Natl Acad Sci U S A. 1986; 83:3086–3090. [PubMed: 3458166]
6. Stremmel W, Strohmeyer G, Berk PD. Hepatocellular uptake of oleate is energy dependent, sodium linked, and inhibited by an antibody to a hepatocyte plasma membrane fatty acid binding protein. Proc Natl Acad Sci U S A. 1986; 83:3584–3588. [PubMed: 3459144]
7. Stump DD, Fan X, Berk PD. Oleic acid uptake and binding by rat adipocytes define dual pathways for cellular fatty acid uptake. J Lipid Res. 2001; 42:509–520. [PubMed: 11290822]
8. Berk PD, Wada H, Horio Y, Potter BJ, Sorrentino D, Zhou SL, et al. Plasma membrane fatty acid-binding protein and mitochondrial glutamic-oxaloacetic transaminase of rat liver are related. Proc Natl Acad Sci U S A. 1990; 87:3484–3488. [PubMed: 2185471]
9. Bradbury MW, Stump D, Guarnieri F, Berk PD. Molecular modeling and functional confirmation of a predicted fatty acid binding site of mitochondrial aspartate aminotransferase. J Mol Biol. 2011; 412:412–422. [PubMed: 21803047]
10. Schwieterman W, Sorrentino D, Potter BJ, Rand J, Kiang CL, Stump D, et al. Uptake of oleate by isolated rat adipocytes is mediated by a 40-kda plasma membrane fatty acid binding protein closely related to that in liver and gut. Proc Natl Acad Sci U S A. 1988; 85:359–363. [PubMed: 3277174]
11. Stremmel W, Strohmeyer G, Borchard F, Kochwa S, Berk PD. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. Proc Natl Acad Sci U S A. 1985; 82:4–8. [PubMed: 3881757]
12. Berk PD, Zhou S, Kiang C, Stump DD, Fan X, Bradbury MW. Selective up-regulation of fatty acid uptake by adipocytes characterizes both genetic and diet-induced obesity in rodents. J Biol Chem. 1999; 274:28626–28631. [PubMed: 10497230]
13. Berk PD, Zhou SL, Kiang CL, Stump D, Bradbury M, Isola LM. 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. J Biol Chem. 1997; 272:8830–8835. [PubMed: 9079720]
14. Fan X, Bradbury MW, Berk PD. Leptin and insulin modulate nutrient partitioning and weight loss in ob/ob mice through regulation of long-chain fatty acid uptake by adipocytes. J Nutr. 2003; 133:2707–2715. [PubMed: 12949354]
15. Hu C, Ge F, Hyodo E, Arai K, Iwata S, Lobdell H IV, et al. Chronic ethanol consumption increases cardiomyocyte fatty acid uptake and decreases ventricular contractile function in c57bl/6j mice. J Mol Cell Cardiol. 2013; 59:30–40. [PubMed: 23422163]
16. Petrescu O, Fan X, Gentileschi P, Hossain S, Bradbury M, Gagner M, et al. Long-chain fatty acid uptake is upregulated in omental adipocytes from patients undergoing bariatric surgery for obesity. International journal of obesity. 2005; 29:196–203. [PubMed: 15570311]
17. Gagner, M.; Inabnet, WB.; Pomp, A. Laparoscopic sleeve gastrectomy with second stage biliopancreatic diversion and duodenal switch in the superobese.. In: Inabnet, WB.; DeMaria, EJ.; Ikramuddin, S., editors. Laparoscopic baratric surgery. Lippincott Williams and Wilkins; Philadelphia: 2002. p. 143-150.
18. Abumrad NA, Park JH, Park CR. Permeation of long-chain fatty acid into adipocytes. Kinetics, specificity, and evidence for involvement of a membrane protein. J Biol Chem. 1984; 259:8945–8953. [PubMed: 6746632]
19. Abumrad NA, Perkins RC, Park JH, Park CR. Mechanism of long chain fatty acid permeation in the isolated adipocyte. J Biol Chem. 1981; 256:9183–9191. [PubMed: 7263707]
20. Di Girolamo M, Mendlinger S, Fertig JW. A simple method to determine fat cell size and number in four mammalian species. Am J Physiol. 1971; 221:850–858. [PubMed: 5570342]

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21. Ge F, Lobdell H IV, Zhou S, Hu C, Berk PD. Digital analysis of hepatic sections in mice accurately quantitates triglycerides and selected properties of lipid droplets. Exp Biol Med (Maywood). 2010; 235:1282–1286. [PubMed: 20975077]
22. Berk PD, Stump DD. Mechanisms of cellular uptake of long chain free fatty acids. Mol Cell Biochem. 1999; 192:17–31. [PubMed: 10331655]
23. Wosilait WD, Nagy P. A method of computing drug distribution in plasma using stepwise association constants: Clofibrate acid as an illustrative example. Comput Programs Biomed. 1976; 6:142–148. [PubMed: 1000973]
24. Spector AA, Fletcher JE, Ashbrook JD. Analysis of long-chain free fatty acid binding to bovine serum albumin by determination of stepwise equilibrium constants. Biochemistry. 1971; 10:3229–3232. [PubMed: 5165844]
25. Berman M, Weiss MF. Users' manual for SAAM. 1967
26. SAAM Institute. SAAM ii user guide. 1998
27. Sorrentino, D.; Berk, PD. Free fatty acids, albumin and the sinusoidal plasma membrane: Concepts, trends and controversies.. In: Tavoloni, N.; Berk, PD., editors. Hepatic transport and bile secretion. Raven Press; New York: 1993. p. 197-210.
28. Snedecor, GW.; Cochran, WG. Statistical methods. 6th edition. Iowa State University Press; 1967.
29. Walewski JL, Ge F, Lobdell H IV, Levin N, Schwartz GJ, Vasselli JR, et al. Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. Obesity (Silver Spring). Jul; 2014 22(7):1643–52. [PubMed: 24550067]
30. Kleiner DE, Berk PD, Hsu JY, Courcoulas AP, Flum D, Khandelwal S, et al. Hepatic pathology among patients without known liver disease undergoing bariatric surgery: Observations and a perspective from the longitudinal assessment of bariatric surgery (LABS) study. Semin Liver Dis. 2014; 34:98–107. [PubMed: 24782263]
31. Walewski JL, Ge F, Gagner M, Inabnet WB, Pomp A, Branch AD, et al. Adipocyte accumulation of long-chain fatty acids in obesity is multifactorial, resulting from increased fatty acid uptake and decreased activity of genes involved in fat utilization. Obes Surg. 2010; 20:93–107. [PubMed: 19866242]
32. Mirabeau O, Perlas E, Severini C, Audero E, Gascuel O, Possenti R, et al. Identification of novel peptide hormones in the human proteome by hidden markov model screening. Genome Res. 2007; 17:320–327. [PubMed: 17284679]
33. Maclean PS, Bergouignan A, Cornier MA, Jackman MR. Biology's response to dieting: The impetus for weight regain. Am J Physiol Regul Integr Comp Physiol. 2011; 301:R581–600. [PubMed: 21677272]
34. Weiss EC, Galuska DA, Kettel Khan L, Gillespie C, Serdula MK. Weight regain in U.S. Adults who experienced substantial weight loss, 1999-2002. American journal of preventive medicine. 2007; 33:34–40. [PubMed: 17572309]
35. Sjostrom L. Bariatric surgery and reduction in morbidity and mortality: Experiences from the SOS study. International journal of obesity. 2008; 32(Suppl 7):S93–97. [PubMed: 19136998]
36. Courcoulas AP, Christian NJ, Belle SH, Berk PD, Flum DR, Garcia L, et al. Weight change and health outcomes at 3 years after bariatric surgery among individuals with severe obesity. JAMA. 2013; 310:2416–2425. [PubMed: 24189773]
37. Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, et al. Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. N Engl J Med. 2014; 370:2002–2013. [PubMed: 24679060]
38. Blomain ES, Dirhan DA, Valentino MA, Kim GW, Waldman SA. Mechanisms of weight regain following weight loss. ISRN obesity. 2013; 2013:210524. [PubMed: 24533218]
39. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med. 2011; 365:1597–1604. [PubMed: 22029981]
40. Adams TD, Avelar E, Cloward T, Crosby RD, Farney RJ, Gress R, et al. Design and rationale of the utah obesity study. A study to assess morbidity following gastric bypass surgery. Contemporary clinical trials. 2005; 26:534–551. [PubMed: 16046191]

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What is already known about this subject:

- The regulation of facilitated LCFA uptake by adipocytes, mediated by several putative LCFA transporters, is an important control point for body adiposity. However, the specific roles of individual transporters in these processes are not yet definitively established.
- Most patients who lose weight via diet and lifestyle changes regain it within 5 years. Persistence of weight gain-promoting hormone patterns; increased insulin sensitivity, rates of glucose transport, and LPL activity; and a multiplicity of metabolic abnormalities are factors believed to contribute to weight regain.
- In year 1 after bariatric surgery, many patients with obesity lose significant amounts of weight, and experience reversals of co-morbidities such as Type 2 diabetes mellitus.

What this study adds:

- The Vmax for facilitated LCFA uptake into human omental and subcutaneous adipocytes correlates with BMI.
- Vmax decreases appreciably with weight loss after sleeve gastrectomy in SO patients, but remains significantly upregulated relative to BMI. By post-operative year 3 weight plateaus for many patients, is replaced by weight regain for some, and some comorbidities re-emerge.
- LCFA uptake kinetics are nearly identical in subcutaneous and omental adipocytes, allowing long term LCFA uptake studies in sequential subcutaneous fat biopsies following bariatric surgery to be compared directly with weight changes in the same patients.

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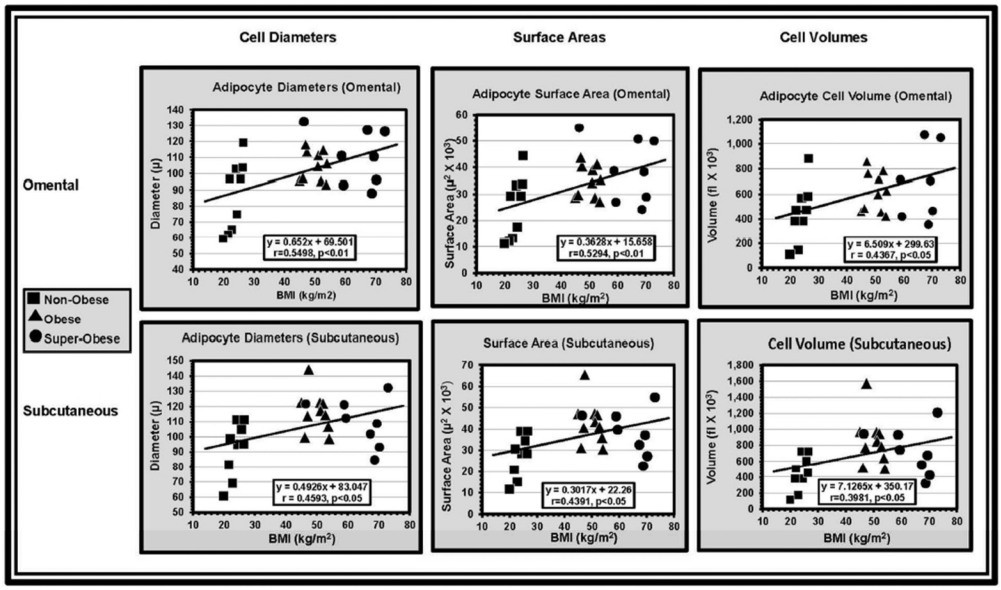

Figure 1. Measured values for adipocyte cell diameters (left panels) and resulting calculated values for mean adipocyte surface areas (center panels) and cell volumes (right panels) in omental (above) and subcutaneous (below) adipocytes from Non-Obese (■), Obese (▲), and Super-Obese (●) patients (n = 10/group)

Across the three patient groups, all of the parameters illustrated increased as statistically significant linear functions of BMI. Samples were obtained during bariatric surgery in the Obese and Super-Obese patients, and during other, clinically indicated abdominal surgical procedures in Non-Obese patients.

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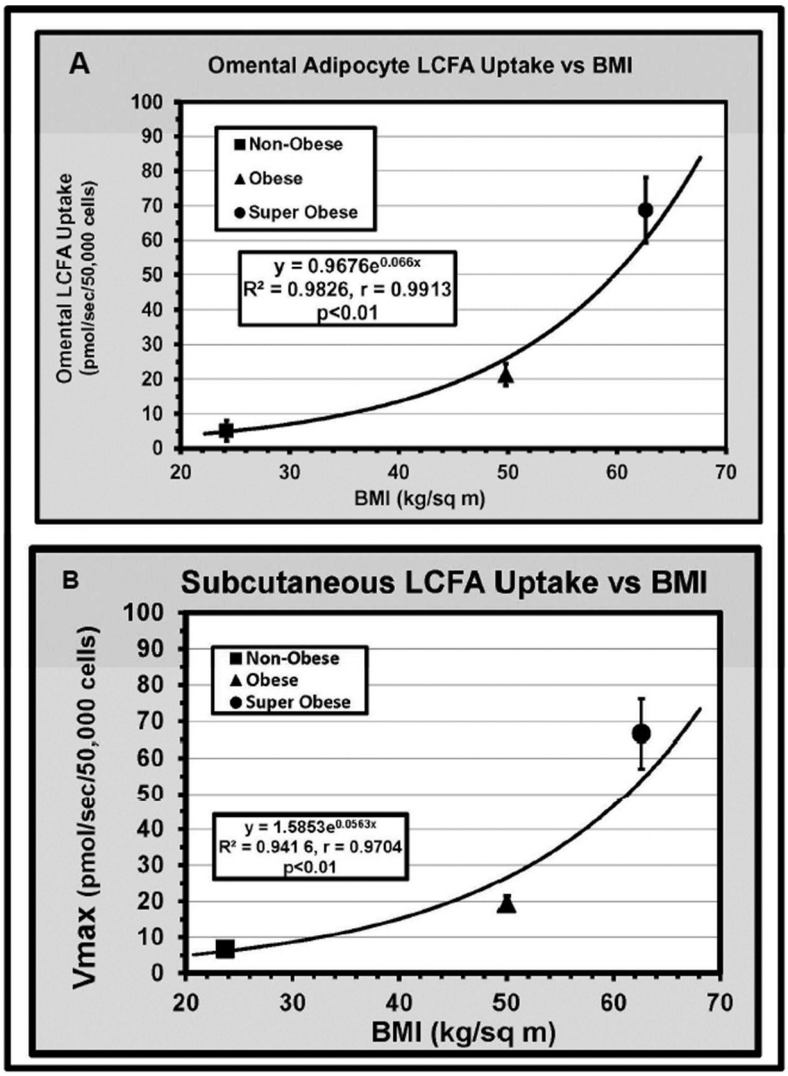

Figure 2.

Across the Non-Obese, Obese, and Super-Obese patient groups, Vmax (± 1 SE) for adipocyte uptake of LCFA increases as an exponential function of BMI in both omental (A) and subcutaneous (B) adipocytes.

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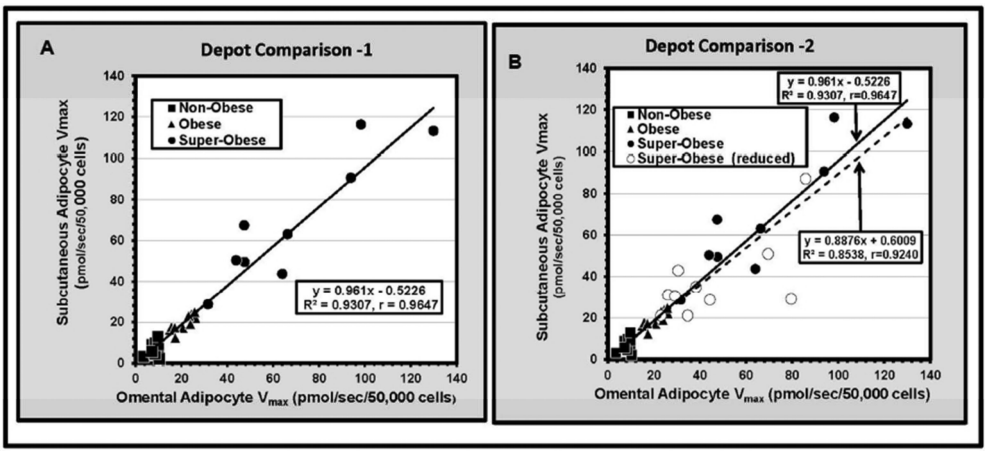

Figure 3. Comparisons of Vmax for LCFA uptake by omental (abscissa) and subcutaneous (ordinate) adipocytes in individual NO, O, and SO patients

A. Results for Vmax from the two depots were very similar in each individual patient, so that the slope of the regression line (0.96) is close to unity and the correlation coefficient r = 0.96. **B.** Data for **SOr** group were added to those in Panel A. Omental and subcutaneous Vmax values for 9 of the 10 **SO** patients decreased to a similar amount in association with a mean weight loss of 113 lbs, so that the data points essentially moved downward along the initial regression line (solid line). Consequently, both the slope (0.89) and correlation coefficient (r=0.92) of the new regression line (dashed line) are very similar to those in panel A. Differences in these two parmeters are entirely attributable to results in a single patient.

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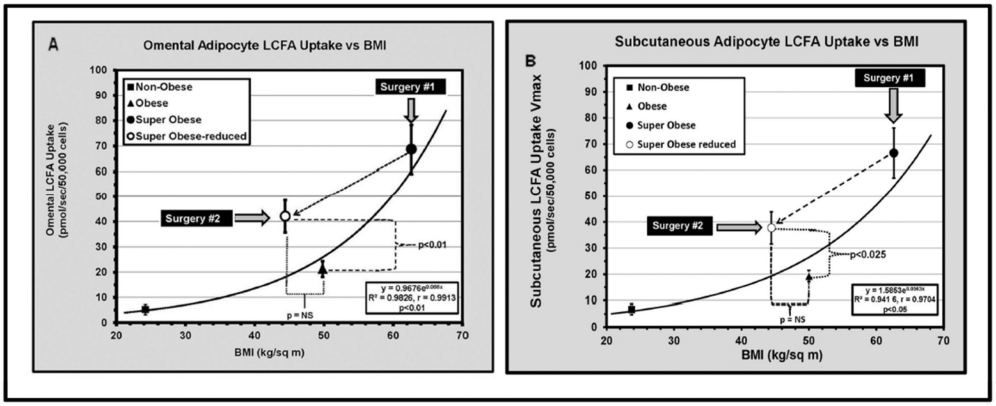

Figure 4. Effects of sleeve gastrectomy on BMI and on the Vmax for LCFA uptake by isolated [A] omental and [B] subcutaneous adipocytes

Exponentially increasing curves in both panels reflect the mean value ± 1 SE of the Vmax for LCFA uptake by isolated adipocytes in Non-Obese (■), Obese (▲), and Super-Obese (●) patients, taken from Figure 2. A sleeve gastrectomy was performed as the initial bariatric surgical procedure in the SO patients, after which both BMI and the Vmax for LCFA uptake decreased. After a mean of 16 months and loss of 113 pounds, the BMIs in the SO patients (now designated SOr) had fallen to 44.4 kg/m^2, similar to that in the Obese group. However, Vmax (40.6±11.5) in this weight-reduced group remained almost twice that predicted from the BMI:Vmax regression among NO, O, and SO patients. A second bariatric surgical procedure was performed in the SOr patients, during which additional fat biopsies were obtained for further studies of LCFA uptake kinetics.

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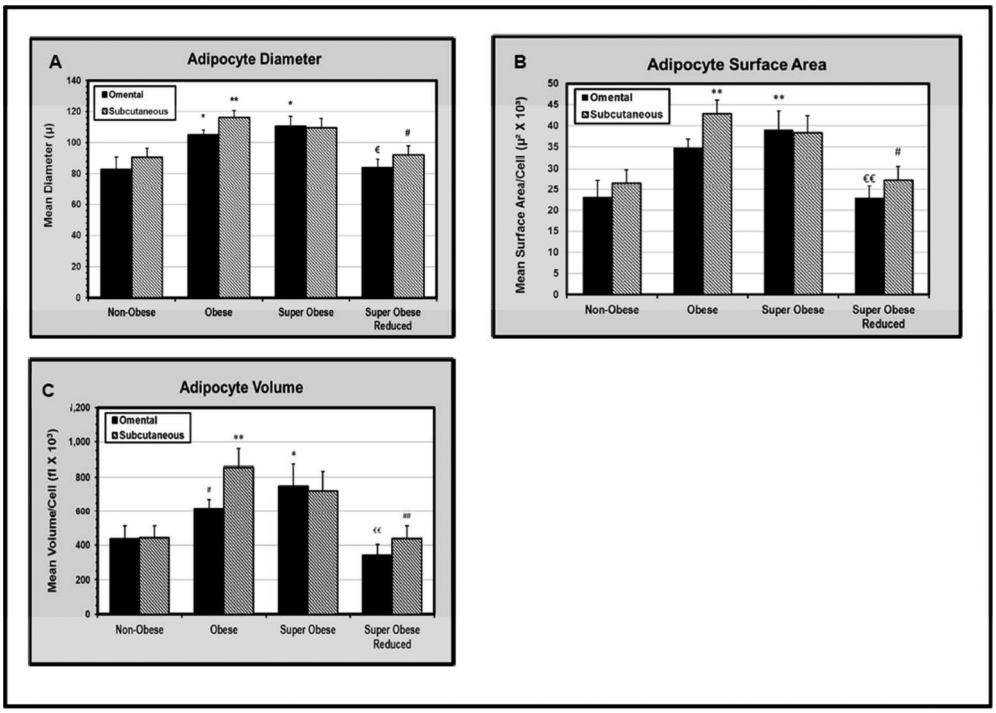

Figure 5. Dimensions of isolated omental and subcutaneous adipocytes in Non-Obese, Obese, Super-Obese and Super-Obese$_{reduced}$ patients

A. Measured diameters. **B.** Calculated cell surface areas. **C.** Calculated cell volumes. Data are mean ± 1 SE. In the SOr group, values for all variables had fallen into the NO range although the BMIs for the SOr patients were similar to those of the Obese group. Statistical Tests: * $p < 0.05$, ** $p < 0.01$ compared to the control (NO) group; # $p < 0.05$, # # $p < 0.01$ (obese versus super obese reduced); € $p<0.05$, €€ $p<0.01$, (super obese versus super obese reduced).

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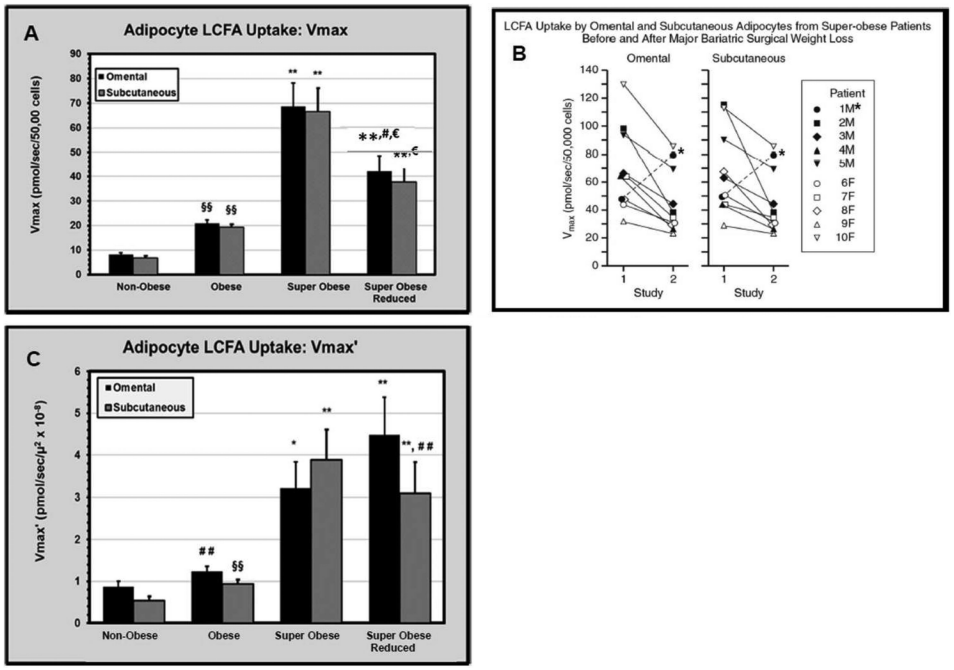

Figure 6. [A] Mean LCFA uptake Vmax ± 1 SE in adipocytes from omental & subcutaneous fat biopsies in the 4 patient groups of this study

Data in Panels [A] & [B] are expressed on a per cell basis. While significantly reduced compared to SO, Vmax in SOr remains significantly greater than in O patients, who have comparable BMIs. **[B]** Vmax values in both omental & subcutaneous adipocytes from individual SO patients, each of whom was studied during both an initial sleeve gastrectomy (Study 1) and during a second bariatric procedure conducted after a mean weight loss of 113 lbs (Study 2). Vmax at the second study was decreased appreciably in both depots in 9 of the 10 patients. The exception was patient 1M, denoted by *****, who was found, after the fact, to have drunk alcohol heavily between his two surgeries. Ethanol is known to increase LCFA uptake. **[C]** Adipocyte LCFA uptake Vmax'. Data in this panel are expressed per unit of cell surface area. In contrast to panel A, because cell diameter and calculated cell surface area in the SOr patients have decreased to an extent similar to or greater than the overall cellular expression of LCFA transport "machinery", Vmax' has remained constant or even increased to some degree in association with weight loss. **Statistical tests, Panels A and C:** * $p<0.05$, ** $p<0.01$ compared to the control (NO) group; § $p<0.05$, §§ $p<0.01$ (obese vs super obese; # $p<0.05$, ## $p<0.01$ (obese vs super obese reduced; € $p<0.05$, €€ $p<0.01$, (super obese vs super obese reduced).

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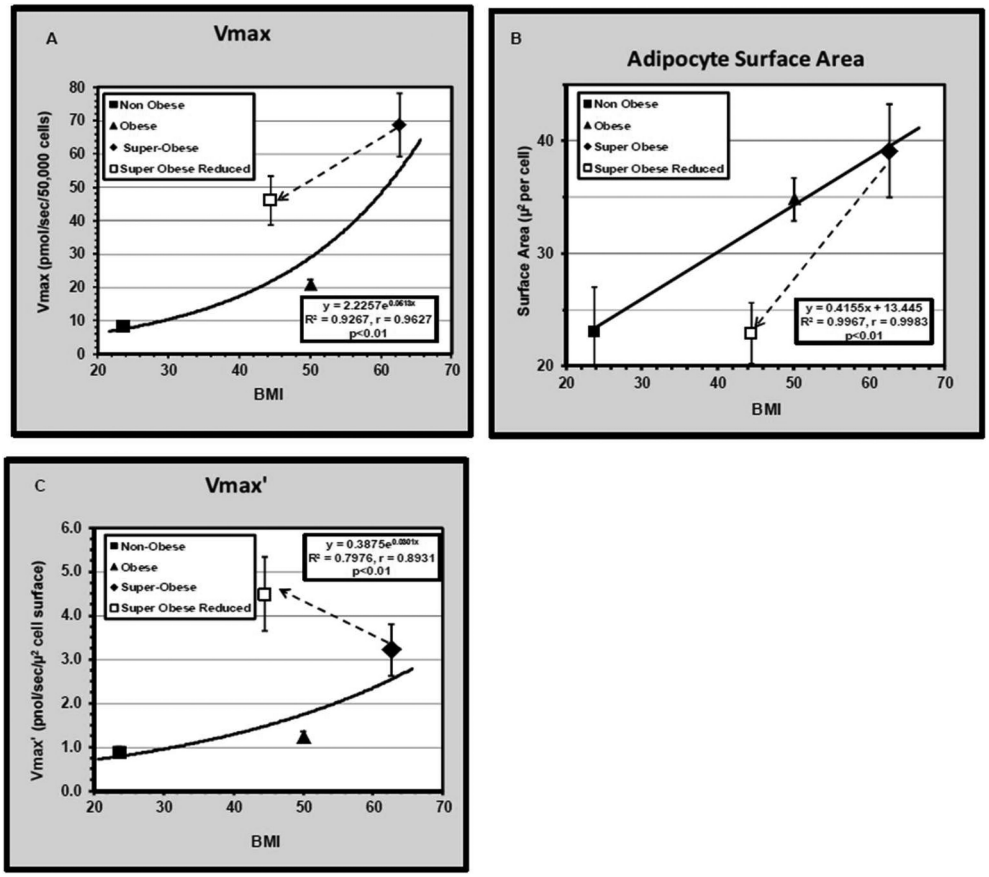

Figure 7. Contributions of Adipocyte Surface Area and Vmax to Vmax'

Vmax, the maximal rate of saturable LCFA uptake per cell, is in turn dependent on (i) Vmax', the maximal saturable LCFA uptake rate per u^2 of cell surface and (ii) the number of u^2 per cell. Ten SO patients in a 2-phase bariatric surgical protocol lost 113±13 lbs in 16±2 months between an initial sleeve gastrectomy and a 2nd operation. Omental adipocyte LCFA uptake kinetics and cell surface area (SA) were studied at both surgeries. Ten NO and 10 O surgical patients served as controls. The 3 panels illustrate means ±SE in these patient groups for [A] mean LCFA uptake Vmax, [B] adipocyte surface area, and [C] Vmax', as calculated from the 2 measured variables. Dashed lines indicated changes in these parameters in SO patients between their initial and second (post weight loss) operations. Both Vmax and SA were decreased in the weight reduced (SOr) patients.However, the proportional reduction in SA was greater than that in Vmax. Consequently, Vmax', **defined as Vmax/SA**, actually increased in these patients. In other settings Vmax' may change in parallel with Vmax. These and other studies indicate that Vmax and SA are independently regulated.

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Table 1

Clinical and Biochemical Characteristics of the Four Patient Groups

	[1]Age (yrs)	BMI (kg/M[2])	Glucose (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)	Albumin (mg/dL)	Bilirubin (mg/dL)	AST (U/L)	ALT (U/L)	Alk Phos (U/L)
NON OBESE												
Male	44.8 ± 7.0	24.1 ± 1.3	110.3 ± 17.3	179.3 ± 13.5	47.3 ± 5.7	96.8 ± 7.28	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 ± 16.89	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	[2]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**
OBESE												
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	46.0 ± 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	p<0.01	NS	NS	p<0.025	NS	p<0.025	NS
Total vs NO	**48.6 ± 1.7** NS	**50.0 ± 2.7** p<0.001	**92.2 ± 3.5** NS	**156 ± 15.9** NS	**39.2 ± 2.9** p<0.01	**111.9 ± 14.3** NS	**136.3 ± 15.1** p<0.05	**4.2 ± 0.1** p<0.05	**0.7 ± 0.1** NS	**22.8 ± 2.5** NS	**30.8 ± 5.0** NS	**70.5 ± 4.7** NS
SUPER OBESE												
Male	51.6 ± 2.9	61.5 ± 6.0	88.8 ± 3.7	175.2±19.4	41 ± 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 ± 12.8
Female	38.5 ± 3.9	63.8 ± 2.1	103.5 ± 14.6	185.8 ± 17.4	43.3 ± 5.6	122.8 ± 14.9	142.3 ± 43.7	3.5 ± 0.2	0.5 ± 0.1	26 ± 4.3	28.4 ± 5.2	70.2 ± 6.8
M vs F	p<0.025	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Total vs NO	**44.5 ± 3.1** NS	**62.8 ± 2.9** p<0.001	**95.3 ± 5.6** NS	**180.5 ± 11.7** NS	**42.0 ± 2.4** p<0.05	**113.1 ± 8.5** NS	**142.6 ± 26.4** p<0.025	**3.7 ± 0.1** p<0.001	**0.6 ± 0.1** NS	**26.3 ± 2.2** p<0.005	**30.4 ± 4.3** p<0.025	**68.1 ± 6.8** 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	NS	NS	p<0.05	NS	NS	NS	NS	p<0.005	NS
Total vs NO	**46.0 ± 3.3** NS	**45.2 ± 2.7** p<0.001	**81.3 ± 5.3** NS	**184.9 ± 18.6** NS	**47.9 ± 4.5** NS	**114.0 ± 14.3** NS	**117.9 ± 25.3** NS	**3.9 ± 0.1** p<0.001	**0.6 ± 0.1** NS	**21.5 ± 2.4** NS	**16.9 ± 1.9** NS	**63.5 ± 8.5** NS

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[1] **All data Mean ± SE**

[2] **NS = not significantly different (p>0.05)**