Adipose Tissue Protein Glycoxidation is Associated with Weight-Loss Potential

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Objective: This study aimed to characterize the differences in protein oxidation biomarkers in adipose tissue (AT) as an indicator of AT metabolism and bariatric surgery weight-loss success.

Methods: A human model, in which sixty-five individuals with obesity underwent bariatric surgery, and a diet-induced obesity animal model, in which animals were treated for 2 months with normocaloric diets, were analyzed to determine the associations between AT protein oxidation and body weight loss. Protein oxidative biomarkers were determined by gas chromatography/mass spectrometry in AT from human volunteers before the surgery, as well as 2 months after a diet treatment in the animal model.

Results: The levels of carboxyethyl-lysine (CEL) and 2-succinocystein (2SC) in both visceral and subcutaneous AT before the surgery directly correlated with greater weight loss in both human and animal models. 2SC levels in subcutaneous AT greater than 4.7×10^6 µmol/mol lysine (95% CI: 3.4×10^6 to 6.0×10^6) may predict greater weight loss after bariatric surgery (receiver operating characteristic curve area $= 0.8222$; $P = 0.0047$). Additionally, it was observed that individuals with diabetes presented lower levels of CEL and 2SC in subcutaneous AT ($P = 0.0266$ and $P = 0.0316$, respectively) compared with individuals without diabetes.

Conclusions: CEL and 2SC in AT are useful biomarkers of AT metabolism and predict the individual's ability to reduce body weight after bariatric surgery.

Introduction

The use of bariatric surgery as an intervention for inducing weight loss among patients with morbid obesity has been clearly demonstrated to be the most effective in terms of time and body weight reduction. In a meta-analysis that included 11 studies, bariatric surgery induced greater weight loss (mean difference of −26 kg at 1- to 3-year follow-up) compared with nonsurgical treatment (1). However, 20% to 25% of the individuals operated on have had inadequate response (2). It has been suggested that the effectiveness of weight-loss reduction depends largely on some preoperative factors such as the age of onset of obesity, high preoperative BMI, diabetes, and hypertension; all have been associated with unsuccessful weight loss (3,4). To identify these individuals is a challenge, both for the physician and for health administrations.

Greater visceral adipose tissue (VAT) to subcutaneous adipose tissue (SAT) ratio, which has been associated with nonremission of diabetes, was shown to be a predictive parameter of poor outcome following bariatric surgery (5). The basis for the proposed harmful effect of VAT, in particular, is due to the draining of portal blood to the liver, affecting its glucose metabolism and thus whole-body glucose homeostasis, possibly via the secretion of adipokines. Furthermore, increased intra-abdominal fat has been associated with an adverse metabolic profile and has been found to predict the development of obesity-related diseases (6). However, the deleterious role of VAT per se in obesity pathophysiology is still far from clear. For example, from a metabolic point of view, VAT (per milligram of tissue) was shown to be more metabolically active than SAT and to have twice the number of mitochondria as SAT (7). In the same sense, in healthy individuals with obesity, adipose tissue perfusion and insulin-stimulated glucose uptake per gram

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of tissue were found to be significantly higher in VAT compared with SAT (8). Moreover, the ratio of VAT to SAT after bariatric surgery was shown to remain fairly constant regardless of the presence of metabolic syndrome or the loss of body weight (9). In the case of patients with type 2 diabetes, they lost less weight and visceral fat after bariatric surgery but more muscle mass compared with their nondiabetic counterparts (10).

Few studies have measured glucose oxidative bioenergetic function of adipocytes and its potential relationship with treatment success during weight-reduction programs. Adipocytes are a major site of lactate production (11), a process stimulated by insulin and glucose uptake, the inadequate release of which was altered in the adipose tissue of women with insulin resistance and obesity (12). Therefore, an adequate adipose tissue glucose metabolism and its consequent lactate production are proxies for the change in metabolic flux during the transition from the fasted to the fed state (13). Notwithstanding, it is not clear whether the increased glycolytic activity in adipose tissue induced by high blood levels of glucose and insulin, and the consequent generation of oxidation by-products, may influence its metabolic capacity. The aim of this study was to explain differences in weight loss through the characterization of protein oxidation biomarkers as a measure of glycolytic flux in adipose tissue of patients with morbid obesity undergoing bariatric surgery as well as in an animal model of obesity before and after dietary energy-restriction treatment.

Methods

Participants

A total of 65 consecutive patients of Caucasian origin who underwent bariatric surgery between July 2013 and September 2014 were enrolled at the time of a regular visit to the outpatient obesity unit. In line with previous studies (2), sample size was calculated by assuming that 25% of bariatric surgery patients may have an inadequate response to the surgery. A precision of 10% and a confidence level of 95% were established for sample size calculation. Written informed consent was obtained from all participants, and the human ethics committee of the Arnau de Vilanova University Hospital approved the study (CEIC 1176). The study was conducted according to the ethical guidelines of the Helsinki Declaration. The following were used as exclusion criteria: type 1 diabetes, requirements of corticosteroid treatment, positive clinical history of malignancy, chronic or acute renal failure, chronic liver disease, abuse of alcohol or any kind of drug, and hypothyroidism or endocrine diseases other than diabetes. The main anthropometric and clinical variables of the study population are described in Table 1.

Patient management before surgery and discharge

The preoperative evaluations included assessment by an endocrinologist, psychiatrist, pulmonologist, and anesthesiologist to identify and treat all comorbid medical conditions before the surgery according to standard guidelines. All patients were required to complete a preoperative low-calorie diet (800 kcal/d or less replacing all usual food intake) in order to decrease liver steatosis and mesenteric fat infiltration before bariatric surgery. The duration of this very low-calorie diet was adapted to the patient's BMI, and an almost 5-point reduction in BMI was mandated for all patients with a preoperative BMI higher than 50 kg/m^2. This diet was supplemented with a multivitamin complex in order to provide the daily allowances of all essential

TABLE 1 Characteristics of study participants ($N=65$)

Parameter	Mean ± SD (range)
Age (y)	48 ± 10 (24-68)
Sex	25 male; 40 female
BMI (kg/m^2)	44.0 ± 4.9 (36-59)
Type 2 diabetes mellitus (%)	37%
Hypertension (%)	66%
Dyslipidemia (%)	55%
Obstructive sleep apnea syndrome (%)	51%
Fasting glucose (mg/dL)	112 ± 43 (54-301)
HbA$_{1c}$ (%)	6.23 ± 1.34 (4.60-10.90)
LDL cholesterol (mg/dL)	48 ± 15 (20-100)
HDL cholesterol (mg/dL)	112 ± 30 (54-175)
Triglycerides (mg/dL)	189 ± 178 (59-1,141)
NEFA (mmol/L)	0.847 ± 0.398 (0.242-2.514)
Type of surgery	Bypass 26 cases; gastrectomy 39 cases

HbA$_{1c}$, hemoglobin A1c.

nutrition requirements. Laparoscopic Roux-en-Y gastric bypass or sleeve gastrectomy was performed using a five-trocar approach in 26 and 39 patients, respectively. During bariatric surgery, SAT and VAT biopsies were performed, quickly minced, frozen in liquid nitrogen, and stored at −80°C for further analysis. Demographic data (gender and age), BMI, waist circumference, and prior diagnosis of type 2 diabetes were also collected. Preoperative weight was defined as the weight on the day of surgery. Ideal body weight was estimated using the weight of the "large frame" from the corresponding height from the 1983 Metropolitan Life Insurance Company weight-height tables. The percentage of expected weight loss at month 12 (EWL12, in kilograms) was calculated by the following formula: (weight loss at month 12 × 100) ÷ (preoperative weight − ideal body weight). Type 2 diabetes and impaired fasting glucose were defined according to the criteria recommended by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (14). None of the patients with diabetes (24 patients) was being treated with insulin.

Animal experiment and diets

Three-week-old male CD-1 Swiss mice (initial weight, mean 22.1 g [SD 1.9]) obtained from Harlan Laboratories (Catalunya, Spain) were maintained at 23°C SD (2°C) under a 12 hour:12 hour light:dark cycle (lights on from 7:00 AM to 7:00 PM). All mice were allowed unlimited access to an in-house-produced high-fat diet (59% of energy from fat) for 4 months. After that period of time, half of the animals were exposed to a normocaloric diet (25% of energy from fat) for 2 months, and the other half continued with the high-fat diet as a control group. Body weight and food and beverage intake were measured weekly throughout the study. After the 2 months of treatment with the normocaloric diet, subcutaneous glucose and insulin tolerance tests were performed. Then all animals were euthanized, and VAT was collected, frozen in liquid nitrogen, and stored at −80°C. All animal procedures followed the approved protocols from the Institutional Animal Care and Use Committee and by the Ethics Committee of the University of Lleida (Approval Number CEEA 18-01/12).

Subcutaneous glucose and insulin tolerance tests

Mice were fasted for 12 hours or 2 hours and then injected subcutaneously with glucose (2 g/kg body weight) or insulin (0.5 U/kg body weight; insulin solution from bovine pancreas, #I0516; Sigma-Aldrich, St. Louis, Missouri), respectively, with a rest period of 5 days between tests. Blood samples were taken every 20 minutes (0-120 minutes) from the tail vein, and blood glucose levels were determined with a portable glucometer (Accu-Chek Aviva 05911982002; Roche Diagnostics, Basel, Switzerland). The area under the curve was calculated as the sum of trapezoids in both tests.

Adipose tissue homogenization

Samples were homogenized in a buffer containing 20mM Tris (pH 8), 150mM NaCl, 2 mM EDTA, 1% Triton X-100 (Sigma-Aldrich), 10% glycerol, 1μM butylated hydroxyl toluene, 10 μg/mL of aprotinin, 1mM NaF, 1mM Na_3VO_4, and a protease inhibitor mix (80-6501-23; GE Healthcare, Chicago, Illinois) (1% volume per volume) with a Potter-Elvehjem device at 4°C. After samples were centrifuged (14,000 rpm; 20 min; 4°C), the lower phase was collected for further analysis. Protein concentrations were measured using the Bradford protein assay (BioRad Laboratories, München, Germany).

Analysis of protein oxidative modifications

Glutamic semialdehydes (GSA) and aminoadipic semialdehydes (AASA), glycoxidation (carboxyethyl-lysine [CEL] and carboxymethyl-lysine [CML]), lipoxidation (malondialdehyde-lysine [MDAL]), and 2-succinocystein (2SC) were determined by gas chromatography-mass spectrometry (GS-MS) as trifluoroacetic acid methyl esters derivatives in acid-hydrolyzed de-lipidated and reduced protein samples using an HP 6890 Series II gas chromatograph (Agilent, Barcelona, Spain) with a 5975 Series MSD and a 7683 Series automatic injector with an Rtx-5MS Restek column (30 m×0.25 mm×0.25 μm). Quantification was performed by internal and external standardization using standard curves constructed from mixtures of deuterated and nondeuterated standards. Analyses were carried out by selected ion-monitoring GC-MS. The ions used were as follows: lysine and [$^2H_8$]lysine mass to charge ratio [m/z] 180 and 187, respectively), 5-hydroxy-2-aminovaleric acid and [$^2H_5$]5-hydroxy-2-aminovaleric acid (stable derivatives of GSA; m/z 280 and 285, respectively), 6-hydroxy-2-aminocaproic acid and [$^2H_4$]6-hydroxy-2-aminocaproic acid (stable derivatives of AASA; m/z 294 and 298, respectively), CML and [$^2H_4$]CML (m/z 392 and 396, respectively), CEL and [$^2H_4$]CEL (m/z 379 and 383, respectively), MDAL and d8-MDAL (m/z 474 and 482, respectively), and 2SC and d2-2SC (m/z 284 and 286, respectively). The amounts of product are expressed as the micromolar ratio of GSA, AASA, CML, CEL, MDAL, and 2SC per mol of lysine.

Mitochondrial DNA level quantification

Total DNA was extracted using Gentra Puregene Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A TaqMan real-time polymerase chain reaction (PCR) was performed within the ABI PRISM 7300HT sequence detection system using the TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, California). To quantify mitochondrial DNA (mtDNA) content, 100 ng of total DNA was used, and cytochrome b (Hs05296867-s1, Custom TaqMan Gene Expression Assays; Applied Biosystems, Warrington, UK) was used as an mtDNA marker. Quantification of mtDNA was referred to nuclear DNA as determined by the amplification 18S (Hs03003631-g1, Custom TaqMan Gene Expression Assays) using the comparative ($2^{-\Delta Ct}$) method, according to the manufacturer's instructions.

Tissue antioxidant capacity

Adipose tissue antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) method. Briefly, 900 μL of the FRAP reagent, containing 2,4,6-tripyridyl-S-triazine, $FeCl_3$, and acetate buffer, was mixed with 90 μL of distilled water and 30 μL of the test sample or the blank (solvents used for homogenization). Maximum absorbance values taken at 595 nm were taken every 15 seconds at 37°C. The readings at 30 minutes were selected for calculations of FRAP values. Solutions of known trolox (238813; Sigma-Aldrich) concentration were used for antioxidant capacity equivalents.

Statistical analysis

Data are presented as mean (SD). Analysis of Gaussian distribution of all variables was performed by D'Agostino-Pearson omnibus normality test. The statistical significance comparison of variables with normal Gaussian distribution was calculated by paired t test, two-way ANOVA analysis with least significant difference as a post hoc test, or Pearson correlation analysis. Non-normal Gaussian distribution was analyzed by Wilcoxon matched-paired signed rank test, Kruskal-Wallis test with Dunn as a post hoc test, or Spearman correlation analysis. In both cases, $P<0.05$ in all statistical tests employed was considered statistically significant. P values in correlation analyses were adjusted for multiple comparisons by false discovery rate method, and q-values for each correlation are indicated. The receiver operating characteristic (ROC) curve was calculated considering volunteers with a lower response (EWL12 below 59% - lower 95% CI) and volunteers with a higher response (EWL12 above 68% - higher 95% CI). GraphPad Prism version 5.0 (GraphPad Software, San Diego, California) and MetaboAnalyst (version 3.0; https://www.metaboanalyst.ca/faces/home.xhtml) were used for statistical analysis and graph plotting.

Results

The percentage weight loss observed at 12 months after the surgery in this study was between 14% and 57% with respect to the initial body weight (95% CI: 28.9%-37.3%), with a coefficient of variation of 32.7%. The evolution of percentage of EWL at 1 to 12 months is described in Supporting Information Figure S1A. No differences were observed in EWL12 between the two types of surgeries (EWL12 for bypass 65.2 kg [14.6] and gastrectomy 62.1 kg [14.0]; $P=0.4853$) or gender (EWL12 for males 65.2 kg [3.7] and females 62.3 kg [2.7]; $P=0.5549$). Similarly, no differences in weight loss have been observed between the two types of surgeries by other authors (15). In relation to pathologies prior to the surgery, a lower percentage of EWL12 was observed among individuals with type 2 diabetes mellitus ($P=0.0212$), hypertension ($P=0.0044$), and dyslipidemia ($P=0.0046$) (Supporting Information Figure S1B-S1D), all components of metabolic syndrome.

Based on these results, other markers at the cellular level that could explain the difference in weight loss were evaluated. Among them, two protein oxidative modification markers, specifically CEL and 2SC, showed a directly proportional correlation with percentage of EWL12 (Figure 1). Individuals with higher levels of CEL and 2SC prior to surgery (both in SAT and VAT) showed greater weight loss. To rule out whether the increase in glycoxidation markers was related to the incidence of diabetes, CEL and 2SC levels were evaluated according to the presence or absence of type 2 diabetes mellitus (Figure 2A-2B). Participants with type 2 diabetes mellitus showed reduced levels of CEL
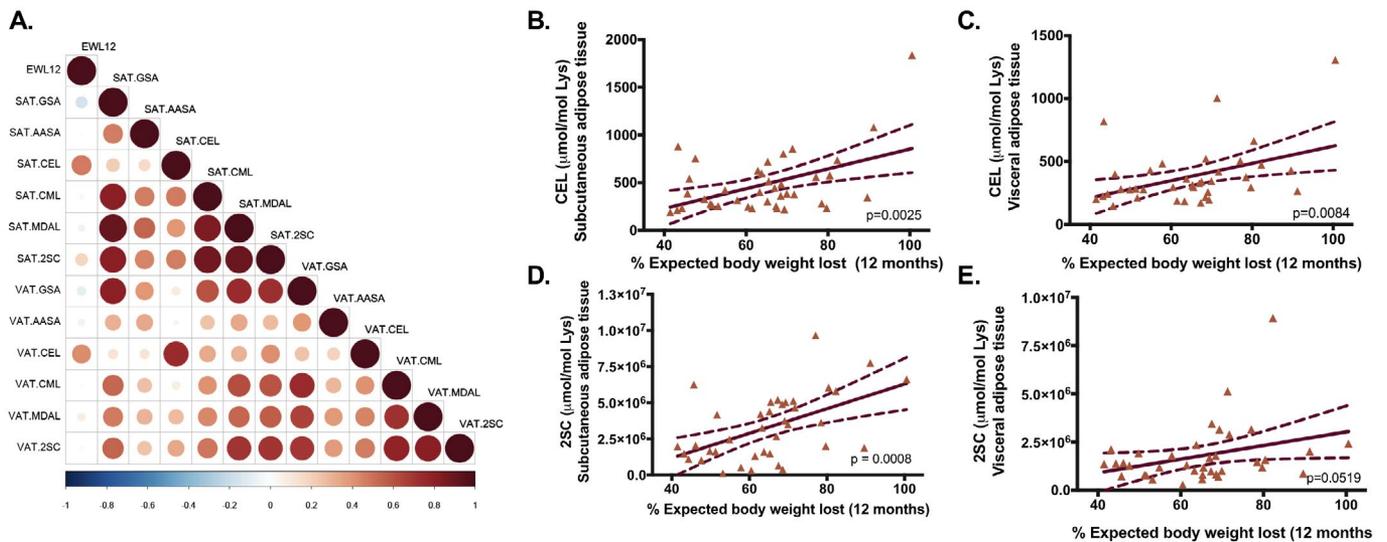
Figure 1 Adipose tissue protein oxidative damage markers. (**A**) Correlation table of the values of protein oxidative biomarkers in SAT and VAT. Color of circles indicates the Spearman correlation coefficient, such that the red color describes a direct proportion correlation while the blue color inverse proportion. Size of circle denotes the significance of the correlation. The larger the size, the closer the P values are to 0. (**B-E**) Correlation between EWL12 and the preoperative values of CEL and 2SC in SAT and VAT. B slope$=10.4\pm3.2$ ($r^2=0.2212$, $P=0.0025$, and q$=0.0048$); C slope$=6.8\pm2.4$ ($r^2=0.1775$, $P=0.0084$, and q$=0.0149$); D slope$=85,094\pm23,256$ ($r^2=0.2711$, $P=0.0008$, and q$=0.0012$); and E slope$=35,376\pm17,575$ ($r^2=0.1037$, $P=0.0495$, and q$=0.0495$). Direct proportional correlation was observed between the levels of CEL and 2SC in SAT and CEL in VAT with EWL. [Colour figure can be viewed at wileyonlinelibrary.com]
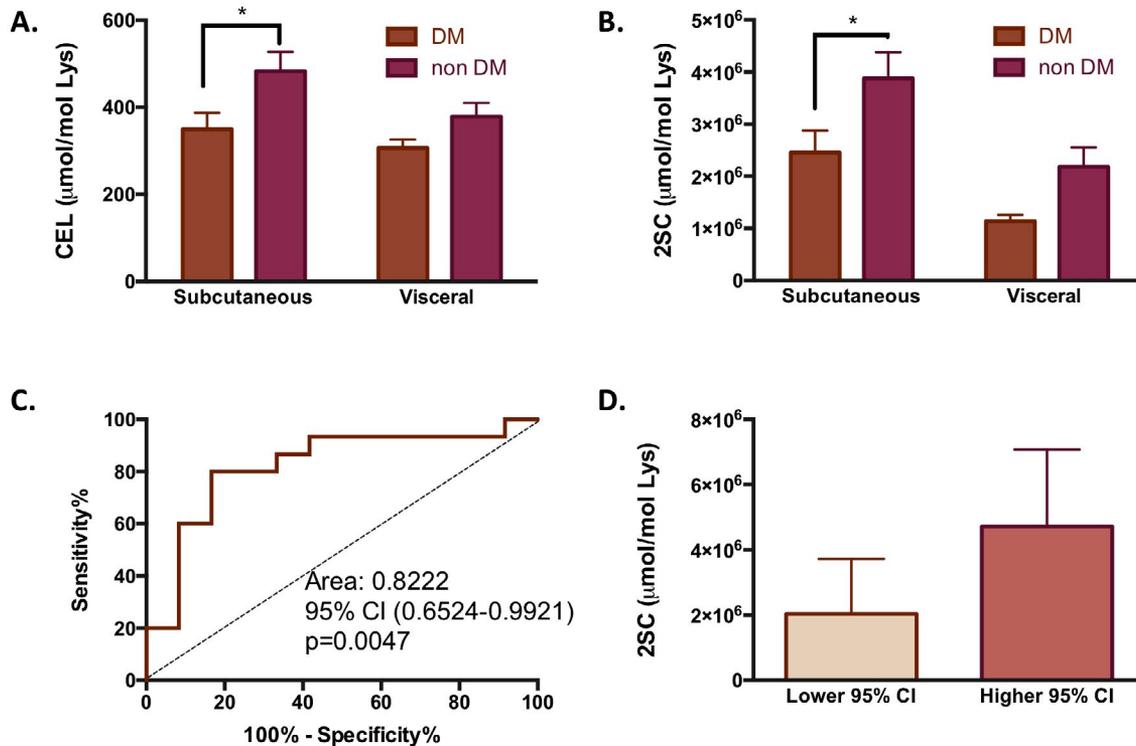

Figure 2 Differences in CEL and 2SC in SAT and VAT from participants with or without diabetes mellitus (DM) and ROC analysis for the use of SAT's 2SC levels as a predictor of weight loss. (**A,B**) Volunteers without DM presented higher values of CEL and 2SC in SAT compared with the participants with DM ($P=0.0266$ and $P=0.0316$, respectively). (**C**) ROC curve for SAT's levels of 2SC as a predictor of EWL12. (**D**) 2SC in SAT at lower and higher 95% CIs of volunteers' EWL12. "*" denotes statistical differences between the selected parameters. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Protein oxidation biomarkers, antioxidant capacity, and mitochondrial DNA in adipose tissue

Protein oxidation biomarker (μmol/mol of lysine)	SAT	VAT	*P* value
CML	$2.0 \times 10^5 \pm 1.0 \times 10^5$	$1.2 \times 10^5 \pm 4.8 \times 10^4$	<0.0001
CEL	571 ± 388	256 ± 122	0.0023
MDAL-	$2,051 \pm 985$	395 ± 241	0.0016
2SC	$3.2 \times 10^6 \pm 2.8 \times 10^6$	$1.7 \times 10^6 \pm 1.9 \times 10^6$	<0.0001
GSA	$7.5 \times 10^6 \pm 4.3 \times 10^6$	$6.0 \times 10^6 \pm 2.8 \times 10^6$	0.0001
AASA	$1.8 \times 10^5 \pm 1.2 \times 10^5$	$1.8 \times 10^5 \pm 1.0 \times 10^5$	0.9692
FRAP (μmol/g tissue)	$1,333 \pm 360$	$1,452 \pm 282$	0.0145
mtDNA/actin	85.9 ± 44.9	99.6 ± 61.5	0.0915

Data presented as mean $\pm$ SD. Paired *t* test performed to estimate individual differences within tissues.

and 2SC in both SAT and VAT. The difference was significant between tissues and for the presence or absence of diabetes for 2SC ($P=0.0015$ and $P=0.0088$, respectively) and for the presence or absence of diabetes for CEL ($P=0.0162$). The increase in CEL and 2SC levels could be attributed to the presurgery dietary treatment that all patients underwent; however, the reduction in body weight was scarce, with an average of 7 kg of weight reduction.

To evaluate the performance of adipose tissue levels of 2SC and CEL as a predictor of weight-loss success, ROC analyses were performed. Only the levels of 2SC in SAT showed an adequate sensitivity (Figure 2C-2D), in which values above 4.7×10^6 μmol/mol lysine (95% CI: 3.4×10^6 to 6.0×10^6) might be a good predictor of successful weight loss. 2SC and CEL levels in VAT and CEL levels in SAT showed lower sensitivity (ROC curve areas 0.6513, 0.6923, and 0.6974, respectively).

Differences in the levels of markers of oxidative modification between SAT and VAT were also observed. Higher levels of almost all protein oxidative biomarkers were observed in SAT compared with VAT (Table 2). In the same way, higher levels of antioxidant capacity, measured by the FRAP method, as well as higher mitochondrial density, were observed in VAT compared with SAT. Similarly, good correlation was found between SAT CEL levels and lipid metabolism by means of nonesterified fatty acid (NEFA) plasmatic levels (slope −252 [107]; $r^2=0.2702$; $P=0.0325$).

To verify this behavior in another model, the content of CEL was evaluated in the adipose tissue of mice with obesity subjected to weight-reduction treatments through a normocaloric diet for 2 months. The mean percentage weight loss observed was 9% (95% CI: 6.3%-11.6%). In the same way, as in humans, it was observed that lower body weight after dietary treatment was significantly associated with higher levels of CEL in adipose tissue (Figure 3A). Adipose tissue CEL levels in the control group (high-fat diet) were lower than observed in the weight-reduction group ($P=0.0378$; mean [lower-upper 95% CI] 329 [296-363] and 377 [350-403] μmol of CEL/mol of lysine, respectively). Similarly, mice with a lower area under the curve after a subcutaneous glucose and insulin tolerance test (Supporting Information Figure S2), which suggests better glucose homeostasis, also showed higher levels of CEL in adipose tissue (Figure 3B-3C), suggesting that the capacity to reduce body weight and a greater capacity for glycemic control could be related to the glycolytic capacity of adipose tissue. To determine whether the changes in biomarkers of protein oxidative damage in adipose tissue could be related to oxidative stress status at the body level, a correlation analysis was performed between several biomarkers of oxidative

modifications in three key tissues of metabolism (liver, skeletal muscle, and adipose tissue) (Figure 3D). No correlation was observed between the levels of the different biomarkers analyzed between tissues. In fact, biomarkers of protein oxidative modification were clustered by tissue type rather than by type of oxidative modification. That is, the elevated levels of CEL and 2SC in adipose tissue were not translated to higher levels of these biomarkers in other tissues such as liver and skeletal muscle (*P* values of Pearson correlation analysis for CEL $P=0.995$ and $P=0.139$ for muscle and liver, respectively; and for 2SC $P=0.875$ and $P=0.592$ for muscle and liver, respectively), which may suggest that each tissue presents different levels of oxidative status.

Discussion

Bariatric surgery has been the preferred treatment for individuals with morbid obesity. However, the percentage weight loss can vary considerably among individuals who undergo this type of surgery. Based on the results from this study, adipose tissue glycolytic capacity may be an important indicator of the individual's ability to reduce body weight after bariatric surgery. Therefore, protein glycoxidation biomarkers like CEL and 2SC can also be used as biomarkers of glycolytic flux in adipose tissue as an indicator of metabolic activity. Others have recognized that metabolism impacts or is impacted by virtually every other cellular process (16). And obesity-related cardiometabolic diseases are increasingly being recognized as disorders of metabolic inflexibility, in which nutrient overload and heightened substrate competition result in mitochondrial inflexibility, impaired fuel switching, and energy dysregulation (17), which will later result in a reduction in energy metabolic rate. Altogether, these could serve as predictors of the ability of individuals with obesity to respond to weight-reduction plans.

Figure 4 provides a scheme of the proposed mechanism by which higher levels of protein glycoxidation biomarkers may be related to insulin resistance and metabolic capacity. 2SC is a chemical modification of proteins formed by the reaction of the Krebs cycle intermediate fumarate with cysteine residues in proteins (18). Increased succination of proteins is a direct result of increased fumarate concentration, as a potential consequence of high glucose disposal in adipose tissue. Increased protein succination formation during high-fat diets or type 2 diabetes is a differential feature in adipose tissue compared with other organs such as skeletal muscle, liver, heart, lungs, and brain (19), suggesting that it could serve as a specific biomarker of adipose tissue glycolytic metabolism. CEL is considered an advanced glycation end product (AGE) formed during the reaction of methylglyoxal, a subproduct
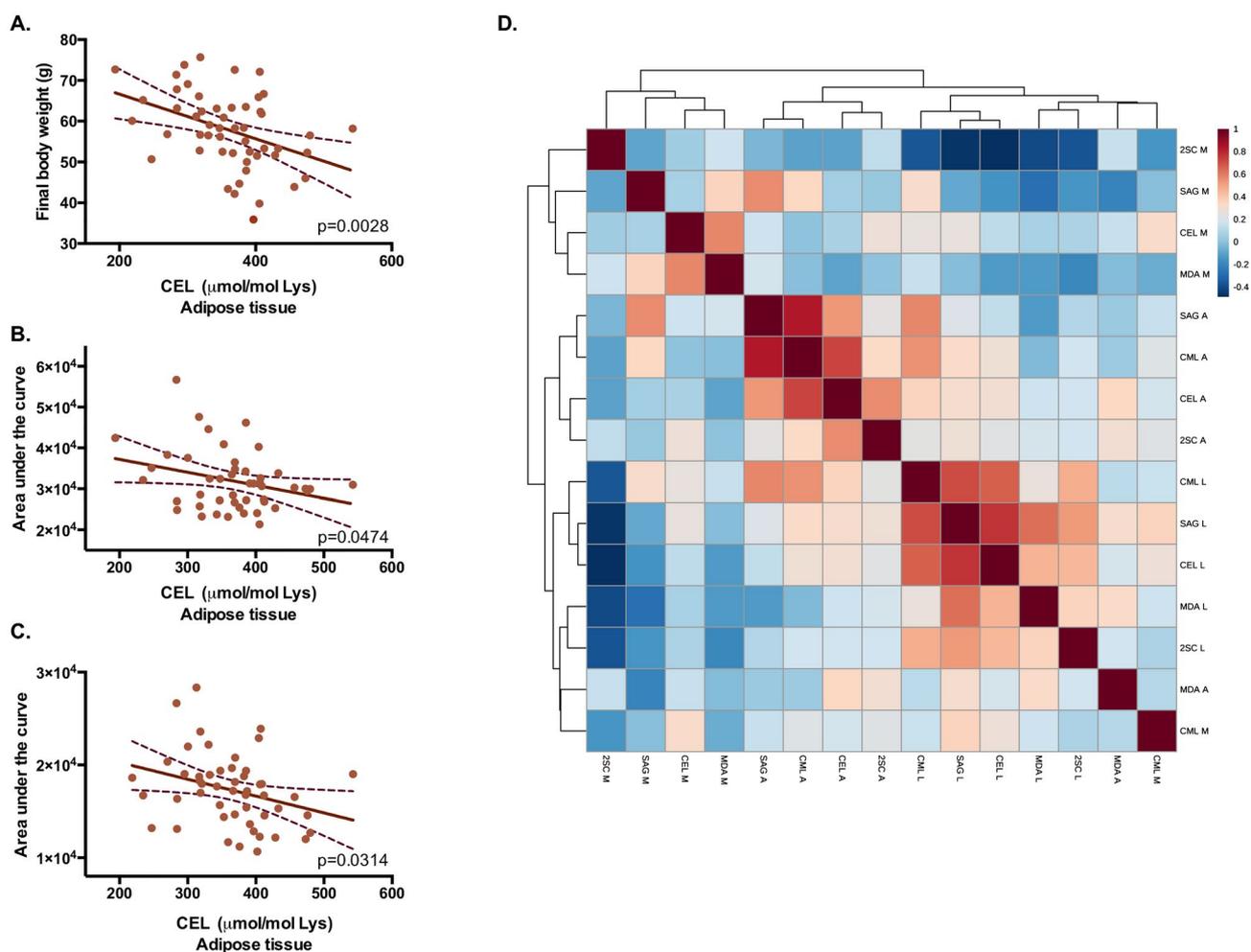
Figure 3 Correlation between CEL content in mice adipose tissue and postdietary treatment outcomes. (**A-C**) Inverse correlation was observed between CEL content and both final body weight and the area under the curve after a subcutaneous glucose tolerance and insulin test (slope -0.054 ± 0.017 [$r^2 = 0.1651$] for body weight; slope -31.6 ± 15.5 [$r^2 = 0.2702$, $P = 0.0474$, $q = 0.0686$] and slope -18.13 ± 8.18 [$r^2 = 0.0928$, $P = 0.0314$, $q = 0.0419$] for the area under the curve for glucose and insulin tests, respectively). (**D**) Correlation and cluster analysis between different biomarkers of protein oxidative damage (2SC, GSA, CEL, CML, and MDAL) in adipose tissue, muscle, and liver. Red squares denote positive correlations and blue squares negative correlations. Letters beside initials of biomarkers correspond to the following: A, adipose tissue; L, liver; M, muscle. [Colour figure can be viewed at wileyonlinelibrary.com]

derived from the enzymatic activity of glyceraldehyde-3-phosphate and cellular proteins (20). In this context, as some authors have suggested elsewhere, both CEL and 2SC are proportionally derived from the rate of cell glycolytic capacity (18,21,22), and in both cases, their increased production may be favored by glucose uptake in adipose tissue through insulin stimulation.

The correlation between CEL and plasma NEFA could be explained in part by the interaction between lipid and carbohydrate metabolism. Carbohydrate metabolism intersects with triglyceride storage through the generation of glycerol-3-phosphate by way of dihydroxyacetone phosphate produced by glucose partially passing through glycolysis. Additionally, lactate is considered an autocrine molecule that mediates part of the insulin-dependent inhibition of lipolysis (13). Adipose tissue is an important source of lactate, and it converts more than 50% of the metabolized glucose to lactate, a process stimulated by insulin and glucose uptake (23). In this context, it was observed

that the increase in lactate plasma concentrations, as a marker of glycolytic pathways, was accompanied by a decrease in NEFA plasma concentration (13). Similar findings were observed in this study, in which NEFA plasmatic levels were inversely correlated with CEL content in adipose tissue from individuals with diabetes. This feature may have important metabolic consequences because high circulating levels of NEFA are implicated with the ectopic accumulation of lipids in other tissues, translating the metabolic inflexibility to other tissues.

The lower CEL levels in adipose tissue in patients with diabetes might seem counterintuitive, based on high levels of circulating glycation products and the observed increase in adipose tissue protein carbonylation in individuals with obesity and lower insulin sensitivity (24,25). It is well known that circulating AGE correlates with hemoglobin A_{1c} levels (26-28), with *r* values ranging between 0.26 and 0.65. In our cohort, no significant relationships were found between adipose tissue
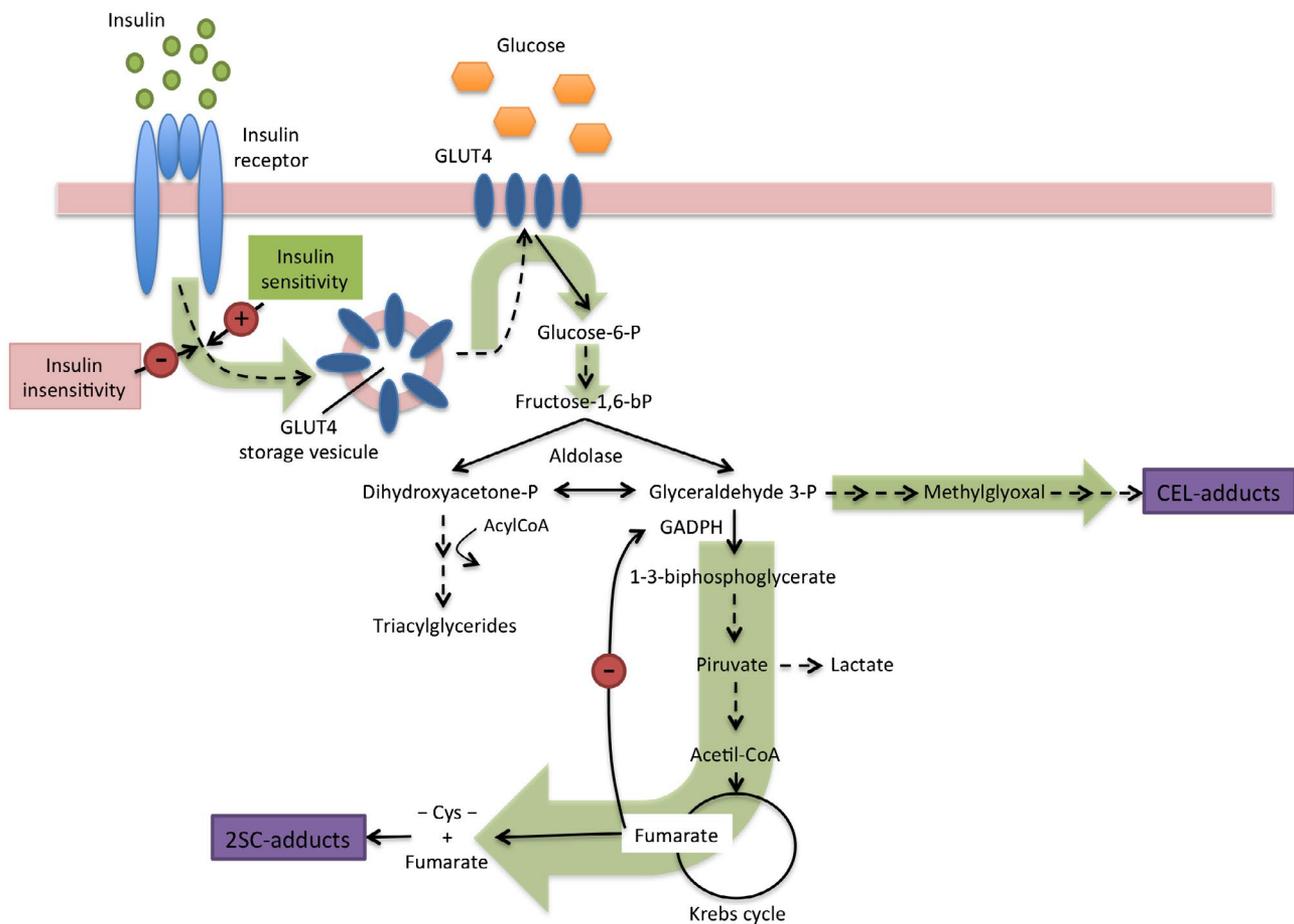
Figure 4 Proposed mechanism of the observed effects. Prior to the surgery, participants underwent a weight-reduction plan. Patients with metabolic flexibility and/or insulin sensitivity will have the ability to uptake glucose and metabolize it. Increase in glycolytic pathways may induce higher levels of fumarate, which is highly reactive to protein-cysteine, and 2SC adducts may be formed. As a side effect of the increase in the glycolytic pathways, methylglyoxal may be formed from glyceraldehyde-3-P. Because of the reactivity of methylglyoxal, CEL adducts may be formed. Patients without metabolic flexibility and/or insulin sensitivity will have a reduced flux in the glycolytic pathways, which in turn will show lower levels of 2SC and CEL adducts. [Colour figure can be viewed at wileyonlinelibrary.com]

levels of CEL or 2SC and hemoglobin A_{1c} (P values for correlation in either subcutaneous or visceral depots ranged between 0.2 and 0.6), suggesting that tissue determinants of AGE accumulation could differ from those indicating circulating AGE values. The latter suggest that changes of adipose tissue CEL could depend on several factors besides glycemic control. Of note, other researchers have found no increased circulating AGE values in patients with insulin resistance, at least at the impaired fasting glucose stage (29). Consequently, it is possible to speculate that decreased CEL tissue levels in the human cohort could be due to decreased levels of insulin-driven glycolysis and/or increased protein turnover, both factors that are due to insulin resistance.

The results presented in this study are in agreement with previous studies regarding the differences in oxidation markers between VAT and SAT in that this study observed greater metabolic activity in VAT. For example, although the relationship between mtDNA and nuclear DNA is the same in both tissues, VAT (per milligram of tissue) was shown to be more metabolically active than SAT (8). Similarly, it was observed that VAT contained more mitochondria per milligram of tissue than SAT, and it was concluded that VAT was bioenergetically more active and more sensitive to mitochondrial substrate supply than SAT (7). Together with the results of the present study, these findings may suggest that metabolic capacity in SAT can be crucial as a determinant of weight loss.

Some limitations can be raised, and additional confirmation of this behavior is necessary by other types of analysis like metabolism inflexibility assessment and energy expenditure after an insulin challenge (30) or physical activity (31). Additionally, all study participants were subjected to a dietary intervention with a hypocaloric diet (800 kcal/d) prior to the collection of adipose samples. Because of this, it is not possible to determine whether the increase in the observed protein oxidative modification was due to the previous dietary treatment or was a special feature of individuals with obesity who present an adequate metabolic capacity. In this context, further studies are recommended to identify which adipose tissue proteins are modified and the plausible cellular and physiological changes induced by their oxidative modification.

Conclusion

Based on the findings of this study, it is possible to conclude that protein glycoxidation content in SAT and VAT, such as CEL and 2SC adducts, may be useful biomarkers of adipose tissue metabolic capacity and, together, may help to identify "good" and "bad" responders to bariatric surgery.**O**

Acknowledgments

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