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Pre-operative circulating succinate levels as a biomarker for diabetes remission after bariatric surgery

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Short running title: succinate levels predict diabetes remission

ABSTRACT

Objective: To determine the potential use of baseline circulating succinate to predict type 2 diabetes remission following bariatric surgery.

Research Design and Methods: Forty-five obese diabetic patients were randomly assigned to Roux-en-Y gastric bypass (RYGB), sleeve gastrectomy (SG) or laparoscopic greater curvature plication. Anthropometrical parameters were evaluated and a complete biochemical analysis including circulating serum succinate concentrations was performed at baseline and 1-year after surgery. The results were externally validated in a second cohort including 88 obese diabetic patients assigned to RYGB or SG based on clinical criteria.

Results: Succinate baseline concentrations were an independent predictor of diabetes remission after bariatric surgery. Patients achieving remission after 1 year had lower levels of baseline succinate (47.8[37.6–64.6]μM vs. 64.1[52.5–82.9]μM; p=0.018). Moreover, succinate concentrations were significantly decreased 1-year after surgery (58.9[46.4–82.4]μM vs. 46.0[35.8–65.3]μM, p=0.005). In multivariate analysis, the best logistic regression model showed that baseline succinate (OR=11.3, p=0.031) and the type of surgery (OR=26.4, p=0.010) were independently associated with remission. The C-statistic for this model was 0.899(95%CI: 0.809–0.989) in the derivation cohort, which significantly improved the prediction of remission when compared with current available scores, and 0.729(95% CI: 0.612–0.846) in the validation cohort. Interestingly, patients had a different response to the type of surgery according to baseline succinate, with significant differences in remission rates.

Conclusions: Circulating succinate is reduced after bariatric surgery. Baseline succinate levels have predictive value for diabetes remission independently of

previously described pre-surgical factors and improve upon the current available scores to predict remission.

Keywords: succinate, bariatric surgery, type 2 diabetes, remission

Bariatric surgery has emerged as an effective treatment for obesity, leading to marked weight loss and the improvement of related comorbidities such as type 2 diabetes and cardiovascular disease, and reducing mortality (1). Sleeve gastrectomy (SG) is the most commonly performed bariatric procedure, because of its simplicity and low rate of complications, accounting for 53.6% of worldwide surgical procedures, followed by Roux-en-Y gastric bypass (RYGB) (30.1%) (2). However, it remains unclear whether RYGB or SG benefits patients with type 2 diabetes equally with regards to glycemic homeostasis (3; 4).

Remission rates of type 2 diabetes observed across published series vary, likely due to heterogeneity in the preoperative characteristics of patients. Against this background, several predictive factors for remission have been identified including age, disease duration, baseline C-peptide concentrations, HbA_{1c}, or previous insulin treatment (5-9). Further, remission rates have been associated with greater weight loss after bariatric surgery independently of initial body mass index (BMI) (10). Accordingly, several predictive outcome scores for type 2 diabetes remission have been developed based on these variables to help identify those patients most likely to benefit from surgery (11-14). However, there is limited evidence regarding the optimal candidate for each surgical procedure and relatively few studies have tested these scores in cohorts to predict type 2 diabetes remission following different surgical procedures, such as RYGB and SG, with contradictory results (14-17). Thus, efforts are needed to improve the accuracy to better predict diabetes remission according to the surgical procedures considering malabsorptive or restrictive techniques.

Bariatric surgery is a known potent modifier of key metabolic intermediates, as has been recently demonstrated in animal models and in patients submitted to

RYGB (18-21). In this context, specific circulating and cardiac tricarboxylic acid cycle metabolites have been identified as potential key players of bariatric cardioprotection through the so-called “enterocardiac axis” (19). Among them, succinate has recently emerged as an extracellular signaling metabolite governing local stress and inflammatory processes *via* engagement with its cognate receptor SUCNR1/GPR91 (22). Remarkably, elevated levels of circulating succinate have been detected in several high-risk cardiovascular disease states such as hypertension (23), ischemic heart disease (24) and type 2 diabetes (25; 26). Along these lines, we recently demonstrated that BMI is a determinant of circulating succinate levels, which are directly linked to specific constituents of fecal microbiota (25). Interestingly, microbiota-produced succinate has also been related to intestinal glucose metabolism (27; 28) and metabolic activity of brown adipose tissue (29), which are major pathophysiological mechanisms mediating beneficial metabolic effects of bariatric surgery (30).

Several studies have attempted to identify robust biological and clinical predictors of type 2 diabetes remission after surgery (5-9; 31); however, there is still insufficient evidence to define cut-off values able to quantitatively predict remission over time. Here, we postulated that succinate, which occupies a pivotal position in metabolism as both an energetic and a signaling metabolite, plays an important role in glucose metabolism and hence in clinical response following bariatric surgery. Our study aimed to assess whether pre-operative circulating levels of succinate might have an impact on type 2 diabetes remission.

RESEARCH DESIGN AND METHODS

Study design and patients

The study was completed in the setting of a previously published prospective single center, non-blinded, randomized controlled trial including obese patients with type 2 diabetes (9). The trial was registered at clinicaltrials.gov (NCT14104758). In brief, forty-five patients were consecutively recruited among patients undergoing bariatric surgery at the Department of Endocrinology of the Bellvitge University Hospital (Barcelona, Spain). The study was conducted according to the principles of the Declaration of Helsinki. The local ethics committee study approved the study and all subjects gave written informed consent before study entry.

Study protocol

The study design and protocol have been previously reported in detail (9; 31). From May 2012 to February 2014, patients were consecutively randomized to RYGB (n=15), SG (n=15) and laparoscopic greater curvature plication (LGCP) (n=15). The procedures were performed by the same surgeon and at the same center. A physical examination with determination of anthropometrical parameters and a complete biochemical analysis was performed at baseline and at 1-year following surgery.

Laboratory determinations

Whole blood was stored at 4ºC and was centrifuged (2500 rpm for 20 minutes) within 2 hours to collect serum.

Glucose, cholesterol, and triglycerides were determined using standard enzymatic methods. Plasma insulin was analyzed by immunoassay (Coat-A-Count Insulin; Diagnostic Products Corp., Los Angeles, CA). Fasting C-peptide levels were

also analyzed by immunoassay (Immulite 2000 XPi, Siemens Healthcare, Munich, Germany). Circulating serum succinate levels were measured at baseline and 1-year after bariatric surgery using the EnzyChrom™ Succinate Assay Kit (BioAssay Systems, Hayward, CA). The assay sensitivity was 12 μM, the intra- and inter-assay co-efficient of variance (CV) was less than 3.5 and 6.95%, respectively (25) and the accuracy ranged from 1% to 11.5% error (Supplementary Table 1). Circulating succinate levels measured by this fluorimetric assay were previously validated by liquid chromatography-mass spectrometry and nuclear magnetic resonance analysis (25).

Outcomes

The primary outcome was to evaluate the rate of type 2 diabetes remission at 1-year following bariatric surgery. To evaluate the rate of remission after surgery, we used Buse's consensus criteria, considering complete type 2 diabetes remission if HbA_{1c} <6% and fasting glucose <100 mg/dl, in the absence of pharmacologic therapy or ongoing procedures, for a duration of at least 1 year (32).

Models

To assess the suitability of the current scores to predict remission after bariatric surgery, the data were used to calculate the ABCD (11), DiaRem (12), AdDiaRem (13) and the DiaBetter (14) scores.

Validation cohort

The prediction model was externally validated in a second independent cohort of patients with obesity recruited in three different Spanish centers (Hospital

Germans Trias i Pujol [Badalona, Spain], Hospital Arnau de Vilanova [Lleida, Spain] and Hospital Josep Trueta [Girona, Spain]). The local ethics committee study of each center approved the study and all subjects gave written informed consent before study entry. The validation population consisted of 88 patients with obesity aged between 18 and 60 years, BMI >=35 kg/m^2, type 2 diabetes on hypoglycemic agents alone, insulin or both. Type 2 diabetes remission was also evaluated at 1 year following bariatric surgery and using Buse's consensus criteria. The indication for the type of surgical procedure (SG or RYGB) was based on clinical criteria.

Sample size

Taking into account the available sample size of the initial randomized controlled trial of 45 subjects, to obtain an absolute difference of type 2 diabetes remission rates of 50% between groups (low vs. high succinate) and allowing for a type 1 error of 0.05, the number of subjects included in our study provided a statistical power of 94%.

Statistical analysis

All data were tested for normality using the Shapiro-Wilk test. Data were presented as percentage, mean and standard deviation (SD) for normally distributed quantitative variables, or median and 25th-75th percentiles for non-normally distributed quantitative variables. Non-normally distributed quantitative variables were used after performing a $\log_{10}$ transformation. Differences between groups were analyzed using the $\chi 2$ test for comparisons of proportions, and the unpaired t-test or the Mann-Whitney U test for comparisons of normally and non-normally distributed quantitative variables, as needed. One-way analysis of variance was used to

compare groups in the cross-sectional study. The least square difference test was used for post hoc analyses. Paired t test and the Wilcoxon signed-rank test were used for paired analysis of the prospective data. Pearson's and Spearman's correlation coefficients were used to analyze the relationship between parameters, as described. Two-tailed p-values <0.05 were considered statistically significant.

To identify the potential role of circulating succinate and the other factors independently related to type 2 diabetes remission after bariatric surgery, logistic regression analyses were performed. All associated variables in the univariate analyses (0.67 < odds ratio [OR] >1.67 and p<0.2) and those variables known or likely to be associated with remission (based on previous literature) were included in the logistic regression models as potential independent variables. Receiver operating characteristic (ROC) curves, in which sensitivity is plotted as a function of 1-specificity, were developed to assess the predictive value of circulating succinate and to compare it with the different scores for the prediction of type 2 diabetes remission. Subsequently, the equality between the different ROC areas obtained was tested. In addition, we used classification and regression tree (CART) analysis, which splits the data into segments that are as homogenous as possible with respect to the dependent variable.

The calculations, figures and statistical analysis were made using STATA v.13.1 for Mac (StataCorp LP, College Station, TX) and GraphPad Prism (GraphPad Software Inc., San Diego, CA). Diagrams of the evolution of type 2 diabetes status were constructed using a Sankey diagram generator (https://sankey.csaladen.es/). CART analysis was performed with the Statistical Package for the Social Sciences software, version 19 (SPSS; Chicago, IL, USA).

RESULTS

Follow-up study. Design, clinical and anthropometric variables and type 2 diabetes evolution

A total of 45 obese patients with type 2 diabetes were included in a 1-year follow-up prospective study. Baseline characteristics (**Table 1**) were similar between the 3 surgery groups except for BMI, which was slightly but significantly higher in the LGCP group than in the RYGB and SG groups. As expected, weight, BMI, waist circumference and waist-to-hip ratio were all reduced after bariatric surgery in the three groups, with marked weight loss and reduced BMI in the RYGB group as compared with the SG and LGCP groups. Fasting plasma glucose, insulin concentration and HbA_{1c} were also improved in the three groups, but especially so in the RYGB and SG groups. Moreover, dyslipidemia improved at 1-year after bariatric surgery, with a decrease of triglycerides levels and an increase of high-density lipoprotein-cholesterol (**Table 1**). Regarding the evolution of type 2 diabetes status 1-year after bariatric surgery, the overall remission rate was 51.1% (n=23): 26.7% in RYGB (n=12), 17.8% in SG (n=8) and 6.7% (n=3) in LGCP. In addition, at the 2-year clinical follow-up, the total remission rate was 46.7% (n=21): 24.4% in RYGB (n=11), 15.6% in SG (n=7) and 6.7% (n=3) in LGCP (**Fig. 1A**).

No differences were found between the three types of surgery for circulating concentrations of succinate at baseline (**Table 1**). Considering the whole population in the study, circulating concentrations of succinate were significantly reduced at 1-year of follow-up (58.9 [interquartile range, IQR 46.4–82.4] μM vs. 46.00 [IQR 35.8–65.3] μM, p=0.005). When the results were analyzed according to the type of surgery, the reduction was only significant for the RYGB group (58.1 [IQR 37.6–82.8] μM vs. 38.6 [IQR 32.4–50.1] μM, p=0.025). Consistent with our previous

observations in obese subjects (25), circulating concentrations of succinate at baseline were positively associated with glucose (r=0.296, p=0.048), HbA_{1c} (r=0.341, p=0.022) and triglycerides (r=0.421, p=0.004) (**Fig. 1B**).

Pre-operative levels of circulating succinate are linked to type 2 diabetes remission rates at 1-year after bariatric surgery

We next sought to further explore the potential association between serum succinate and type 2 diabetes remission. Notably, those patients achieving remission at 1-year after surgery had lower circulating concentrations of succinate at baseline (47.8 [IQR 37.6–64.6] μM vs. 64.1 [IQR 52.5–82.9] μM; p=0.018) (**Fig. 2A**). To test the value of the basal succinate level to predict remission, we developed the corresponding ROC curve. The C-statistic of basal succinate was 0.710 (95% confidence interval [CI]: 0.554–0.865) for predicting remission 1-year after bariatric surgery (**Fig. 2B**). The best cut-off point of succinate, selected based on the Youden Index calculation, was 48.1 μM (sensitivity, 91% and specificity, 52%), which was used to classify the cohort.

To assess the validity of succinate as a biomarker to predict diabetes remission, we compared the accuracy of the existing risk scores for predicting type 2 diabetes remission after bariatric surgery with that of circulating sucinate. Accordingly, the ABCD, DiaRem, AdDiaRem and DiaBetter scores were calculated for each patient and their ROC curves were developed. The C-statistic for the prediction of remission for each of these ROC curves was 0.610 (95% CI: 0.447–0.772) for the ABCD score, 0.760 (95% CI: 0.616–0.904) for the DiaRem score, 0.728 (95% CI: 0.586–0.807) for the AdDiaRem score and 0.751 (95% CI: 0.606–

0.896) for the DiaBetter score, which were all similar to the predictive value of baseline circulating succinate alone (**Fig. 2B**).

To evaluate the independent factors associated with type 2 diabetes remission at 1-year in our cohort, we developed logistic regression models. In univariate analysis, and in accord with previous studies (5-8), we found that the variables associated with remission were the type of surgery (malabsorptive vs. restrictive) (OR=6.909; p=0.010), previous insulin treatment (OR=0.146; p=0.011), baseline HbA_{1c} (OR=0.606, p=0.011) and baseline circulating succinate <48.1 μM (OR=10.909; p=0.005). The best logistic regression model obtained showed that baseline circulating succinate <48.1 μM (OR=11.3, p=0.031) and the type of surgery (OR=26.4, p=0.010), were independently associated with remission after adjusting for potential confounders (**Table 2**). Remarkably, the C-statistic for this model (which we term DiaSuc) was 0.899 (95% CI: 0.809–0.989) (**Fig. 2C**), which significantly improves upon type 2 diabetes prediction of the ABCD score (0.610 vs. 0.899, p=0.001) and the AdDiaRem score (0.728 vs. 0.899; p=0.018), and was slightly but not significantly better than the DiaRem score (0.760 vs. 0.899, p=0.061) and the DiaBetter score (0.751 vs. 0.899, p=0.052) (**Fig. 2D**).

To confirm these results, we performed a regression tree (CART) analysis (Supplementary Fig. 1). We included all the previously selected variables as predictors of type 2 diabetes remission (see the logistic regression model, **Table 2**). The first node was split based on the most important predictor, which was considered the pre-operative levels of circulating succinate. According to this model, those patients with low baseline succinate (<48.1 μM) had a higher percentage of remission at 1-year after bariatric surgery compared with those patients with high baseline succinate (85.7% vs. 35.5%; p=0.003) (Supplementary Fig. 1). The second

node was split based on the type of surgery (restrictive vs. malabsorptive). Interestingly, those patients with low baseline succinate had a similar rate of remission independently of the type of surgery (100.0% vs. 75.0%; p=0.473). By contrast, those patients with high baseline succinate who underwent malabsorptive surgery had a higher rate of remission at 1-year after bariatric surgery than those who underwent a restrictive procedure (66.7% vs. 22.7%; p=0.038) (Supplementary Fig. 1).

Next, we extended the clinical follow-up to 2 years after bariatric surgery to confirm the potential role of basal succinate levels as a predictor of remission. As mentioned above, 46.7% of patients achieved complete type 2 diabetes remission (n=21). At this time-point, and according to the results at 1-year, those patients that achieved remission at 2 years after surgery had lower circulating concentrations of succinate at baseline (47.6 μM (IQR 37.6–64.7) vs. 63.8 μM (IQR 55.0–82.9) p=0.013). The C-statistic of DiaSuc for prediciting remission at 2-years after bariatric surgery, including baseline circulating succinate concentrations and type of surgery, was 0.912 (95% CI: 0.821–1.000), which again significantly improved upon the ABCD score (0.620 vs. 0.912, p<0.001), the DiaBetter score (0.710 vs. 0.912, p=0.025) and the DiaRem score (0.755 vs. 0.912, p=0.042), and was slightly better (but not significantly) than the AdDiaRem score (0.774 vs. 0.912; p=0.055) (**Fig. 2E**).

Finally, the results were externally validated in a second independent cohort (baseline characteristics are shown in Supplementary Table 2). In the validation cohort, the baseline circulating concentration of succinate was 53.2 μM (IQR 32.0–73.9). Circulating concentrations of succinate 1-year after surgery were available in 55 out of 88 patients included in the validation cohort. The results obtained in the validation cohort (n=55, 100% SG) also showed a decrease in succinate

concentrations 1-year after bariatric surgery (IQR 42.3 [26.6–63.7] μM vs. 34.9 [26.9–48.2] μM; p=0.029). The external validation of the DiaSucc model in this second cohort showed a very good discrimination, with a C-statistic of 0.729 (95% CI: 0.612–0.846) (**Fig. 2F**).

CONCLUSIONS

In the present study, we provide the first demonstration that baseline circulating concentrations of succinate are an independent predictor of type 2 diabetes remission after bariatric surgery in obese patients. Further, baseline circulating concentrations of succinate together with the type of surgery improves upon the accuracy of established predictive diabetes remission scores (11-14).

Bariatric surgery is well established as an effective weight loss strategy for people with obesity that also improves comorbidities. Increasing evidence indicates that it can be a more effective treatment for a subset of type 2 diabetes patients than standard medical therapy, achieving greater metabolic effects such as improving glucose homeostasis, insulin sensitivity, β-cell function and incretin response, which has led to the term "metabolic surgery" (30; 33-35). Using surgery as an intervention for type 2 diabetes, however, implies conceptual and practical differences from the traditional practice of bariatric surgery for obesity, for instance, in the criteria used to select candidates for metabolic surgery including metrics of metabolic disease severity and predictors of treatment success. In this sense, some predictive pre-surgical factors of metabolic outcomes have been identified, such as younger age, shorter disease duration, pre-operative C-peptide levels and the absence of insulin treatment prior to surgery, which are all associated with higher remission rates (5-9). Accordingly, several predictive remission scores (ABCD, DiaRem, Ad-DiaRem and DiaBetter) have been developed incorporating these factors (11-14). Nevertheless, the predictive models do not always consider the different types of surgery. Thus, there is still the need to discover novel biomarkers to better predict remission outcomes.

We have previously demonstrated a link between circulating succinate and poor metabolic control in patients with type 2 diabetes (25). In the present study, baseline circulating succinate was associated with type 2 diabetes remission at 1- and 2-years after bariatric surgery (the lower the value, the higher the remission rate), and the predictive value of succinate alone was similar to all the aforementioned established scores. Moreover, baseline circulating succinate was independently associated with remission after adjusting for the remainder of the predictive pre-surgical factors, and the logistic regression model tested (which includes baseline succinate and type of surgery) improved the score accuracy to predict remission rates when compared with the other models. Considering that the established scores to predict remission generally combine clinical variables mostly related to diabetes severity, our results point to succinate as a metabolite capable of representing several components of metabolic status, rendering a broader view of homeostasis in an easily measurable serum biomarker. Accordingly, succinate could be a new preoperative predictive factor of type 2 diabetes remission after metabolic surgery. Along this line, succinate has been recognized as a predictor of mortality in critically injured patients and as a marker of hypoxic metabolic reprogramming in cancer, ischemia, inflammation and immunomodulation (36).

Succinate is traditionally considered as an energetic metabolite produced by both host (mitochondria) and microbiota (25; 27). Unfortunately, fecal samples were not available to explore the relationship between circulating succinate and the gut microbiome in the context of bariatric surgery. Nonetheless, one might speculate that mitochondrial dysfunction of metabolic tissues also underlies changes in circulating levels of succinate. Indeed, this metabolite is a good marker of cellular status, as it occupies a pivotal position in metabolism as the only direct link between the

tricarboxylic acid cycle and the mitochondrial respiratory chain through complex II activity. When cells rely on certain stress stimuli, mitochondrial levels and consequently extracellular levels of succinate might increase (37). While intracellular succinate is commonly considered a pro-inflammatory stimulus (22), we recently demonstrated that engagement with its receptor SUCNR1 has a critical role in the anti-inflammatory responses in macrophages; but this interaction is disrupted in obesity. Obesity is defined by high circulating levels of succinate; however, adipose tissue-resident macrophages from obese subjects show decreased expression of SUCNR1, suggesting a 'succinate-resistant state' (38). Succinate resistance in the context of obesity might contribute to the increased baseline succinate levels and to the inability to resolve inflammation and glucose homeostasis following bariatric surgery.

Metabolic functions are reduced in insulin-responsive tissues (muscle and adipose tissue) in obesity and type 2 diabetes. No consensus has thus far been reached with regards to whether insulin resistance is a result of reduced mitochondrial density or whether it is the cause or consequence of mitochondrial dysfunction. The debate on mitochondrial dysfunction as a contributor to type 2 diabetes pathogenesis continues and drives the need for more studies on mitochondrial function in this patient group (39). Moreover, it has been reported that destabilized mitochondrial metabolism in type 2 diabetes and obesity can be influenced by bariatric surgery (40). Therefore, despite the unresolved question concerning the source of circulating succinate levels, our results point to this energetic metabolite as a key player in type 2 diabetes remission after bariatric surgery and a new variable to reflect metabolic disease severity.

It remains unclear whether RYGB or SG has equal benefits for patients with type 2 diabetes regarding glycemic homeostasis. Given that both techniques are different with regard to the surgical procedures, post-operative care, long-term comorbidities and economic costs, it is important to uncover novel biomarkers to adopt a personalized approach to bariatric surgery by identifying which patients will benefit most from malabsorptive vs. restrictive procedures. In that sense, we found that the remission rates were similar independently of the type of surgery (malabsorptive vs. restrictive) for those patients with low baseline circulating succinate (100% vs. 75% at 1 year and 83% vs. 75% at 2 years) (**Fig. 2G**). By contrast, for those patients with high baseline circulating succinate, the remission rates clearly differed according to the surgical procedure (66.7% vs. 22.7% at 1 year and 67.7% vs. 13.6% at 2 years) (**Fig. 2G**). Thus, our results suggest that pre-operative concentrations of circulating succinate could be a useful metric to determine the best-suited surgical procedure.

We are aware that our study has some limitations. It was a single-center, single-surgeon study and the sample size was small, although this was supported by the sample size calculations. Importantly, the results were validated in a second independent cohort. Despite randomization, RYGB patients had a tendency towards a more favorable initial metabolic profile, although not reaching statistical significance, and this fact may have conditioned the better metabolic outcomes. In addition, we acknowledge that remission of type 2 diabetes can be also associated with the initial BMI and with a greater weight loss after bariatric surgery. In this sense, we did not find any association between baseline BMI and type 2 diabetes remission. We aimed to identify a potential biomarker that might help to develop a clinical decision tree to select the best-suited surgical procedure, focusing only in

pre-surgical factors. Finally, the follow-up was at 2-years; however, the cohorts allocated to the different bariatric procedures will be closely followed at long-term.

In conclusion, the present study shows that baseline circulating concentrations of succinate have predictive value for type 2 diabetes remission, independently of previously described pre-surgical factors, improving upon the current available scores to predict remission. Furthermore, we propose a cut-off value of succinate as a new criterion to select the best candidates for restrictive surgical procedures over the more aggressive malabsorptive surgical techniques in type 2 diabetes. We are fully aware that despite the good agreement of the succinate cut-off values in two independent cohorts from 4 different centers, further randomized controlled studies will be needed as a next step to establish the use of succinate as a clinical decision support tool for selecting the best-suited bariatric surgical procedures. Furthermore, as with any diagnostic test, it will be essential to standardize blood collection and processing protocols to incorporate succinate measurements into routine clinical practice. Our findings underscore the importance of circulating metabolites as potentially valuable predictive factors of diabetes remission after bariatric surgery. In this sense, while other metabolites cannot be excluded, our results, together with the unique and specific attributes of succinate – its pivotal position in energy metabolism, its role as a fuel substrate and signaling metabolite, and the fact that it is produced by both host and gut microbiota – make it an attractive target for management of diabetes.

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JV and SF-V conceived, designed and supervised the research project and wrote the manuscript. VC-M and GLL participated in the conception and design of the study, sample collection, statistical analysis and wrote the manuscript. NK and BA analyzed serum succinate levels. EB constructed diagrams of the evolution of type 2 diabetes. LM, SP, JMG-C and AM provided scientific discussion and revised the manuscript. NV participated in the human sample recruitment and conducted the clinical trial. AR, JMF-R and AL participated in the human sample recruitment of the validation cohort. SF-V and JV are the guarantors of this work.

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Table 1. Clinical and laboratory data for the obese patients with type 2 diabetes before and at 1 and 2 years after bariatric surgery

	All	RYGB	SG	LGCP	p for trend
n	45	15	15	15	-
Age (years)	49.4 (8.0)	51.1 (7.7)	49.2 (9.2)	49.7 (8.1)	0.827
Gender (M/F)	15/30	7/8	5/10	3/12	0.361
Type 2 diabetes					
Type 2 diabetes (n, %)					
Baseline	45 (100)	15 (100)	15 (100)	15 (100)	1.000
1 year	22 (48.9)	3 (20.0)	7 (46.7)	12 (80.0)	**0.005**
2 years	24 (53.3)	4 (26.7)	8 (53.3)	12 (80.0)	**0.016**
Type 2 diabetes treatment (n, %)					
Baseline	45 (100)	15 (100)	15 (100)	15 (100)	1.000
1 year	13 (28.9)	0 (0.0)	6 (40.0)	7 (46.7)†	**0.005**
2 years	15 (33.3)	0 (0.0)	6 (40.0)	9 (60.0)†	**0.001**
Insulin (n, %)					
Baseline	17 (37.8)	5 (33.3)	6 (40.0)	6 (40.0)	0.910
1 year	5 (11.1)*	0 (0.0)*	3 (20.0)*	2 (13.3)	0.343
Type 2 diabetes duration (years)	7.4 (3.6-13.0)	4.5 (3.1-10)	10.0 (4.2-15.3)	6.9 (3.4-12.9)	0.209
Anthropometric measurements					
Weight (kg)					
Baseline	103.6 (11.0)	103.0 (10.8)	102.3 (10.8)	105.5 (11.9)	0.710
1 year	75.1 (13.4)*	66.6 (10.7)*	74.9 (10.1)*	83.6 (13.6)*,†	**0.001**
2 years	78.7 (13.8)	70.4 (11.6)	79.3 (12.5)	86.4 (12.9)†	**0.004**
BMI (kg/m^2)					
Baseline	39.5 (1.9)	38.6 (1.9)	39.0 (1.7)	40.9 (1.4)†,‡	**0.001**
1 year	28.6 (4.1)*	24.9 (3.0)*	28.6 (2.5)*,∫	32.3 (2.7)†,‡	**<0.001**
2 years	30.0 (4.0)	26.3 (3.0)	30.2 (3.1)∫	33.4 (2.2)†,‡	**<0.001**
Waist (cm)					
Baseline	117.9 (7.9)	118.9 (7.6)	117.5 (7.6)	117.3 (8.9)	0.852
1 year	96.0 (9.9)*	91.3 (7.9)*	93.6 (10.0)*	103.5 (7.7)*	0.822
WHR					
Baseline	0.96 (0.07)	0.97 (0.09)	0.98 (0.07)	0.93 (0.06)	0.184
1 year	0.91 (0.07)*	0.93 (0.07)*	0.89 (0.07)*	0.92 (0.06)	0.217
Blood pressure					
SBP (mmHg)					
Baseline	134.0 (18.5)	140.8 (19.7)	132 (13.9)	129.1 (20.4)	0.199
1 year	129.0 (19.4)	128.8 (18.7)*	126.8 (25.4)	131.2 (14.3)	0.836
DBP (mmHg)					

Baseline	81.1 (14.3)	84.5 (13.6)	77.9 (13.7)	80.7 (15.7)	0.457
1 year	77.2 (13.0)	77.7 (12.9)	74.2 (13.5)	79.6 (13.1)	0.543
Glucose homeostasis					
Glucose (mmol/L)					
Baseline	8.5 (6.4-11.6)	8.0 (5.6-11.1)	8.5 (6.4-12.4)	8.8 (6.3-11.6)	0.583
1 year	5.1 (4.5-6.2)*	4.5 (4.4-5.0)*	5.1 (4.9-6.5)*	6.3 (5.5-8.6)*,†	**0.029**
2 years	5.8 (5-6.9)	5.1 (4.8-5.7)	6.0 (5.3-8.0)	6.9 (5.8-9.3) †	**0.006**
Insulin (pmol/L)					
Baseline	115 (80-137)	132 (115-143)	94 (63-117)	106 (67-128)	0.779
1 year	50 (35-74)*	37 (28-71)*	42 (36-88)*	72 (59-84)†	**0.021**
C-peptide (nmol/L)					
Baseline	1.1 (0.8-1.3)	1.1 (0.7-1.3)	0.8 (0.7-1.4)	1.0 (0.8-1.5)	0.566
1 year	0.6 (0.5-0.8)	0.5 (0.4-0.6)	0.6 (0.5-1.0)	0.8 (0.6-0.9) †	**0.013**
HbA1c (%)					
Baseline	7.2 (6.3-9.3)	6.7 (5.9-9.5)	7.2 (6.4-9.3)	7.5 (6.3-9.8)	0.634
1 year	5.9 (5.4-6.5)*	5.4 (4.7-5.5)*	5.9 (5.5-6.8)*,∫	5.9 (6.3-7.5)*,†	**<0.001**
2 years	5.8 (5.4-7.5)	5.2 (4.7-5.6)	6.2 (5.5-7.8)	7.4 (5.8-10.2)†	**<0.001**
HbA1c (mmol/mol)					
Baseline	55 (45-78)	50 (41-80)	55 (46-78)	58 (45-84)	0.634
1 year	41 (36-48)*	36 (28-37)*	41 (37-51)*,∫	41 (45-58)*,†	**<0.001**
2 years	40 (36-58)	33 (28-38)	44 (37-62)	57 (40-88)†	**<0.001**
Lipid profile					
Total cholesterol (mmol/L)					
Baseline	4.76 (4.27-5.38)	4.88 (3.74-5.44)	4.53 (3.89-4.99)	4.88 (4.41-5.66)	0.456
1 year	4.61 (3.95-5.26)	4.04 (3.09-4.37)*	5.02 (4.1-5.28) ∫	5.18 (4.77-5.99)†	**<0.001**
HDL-cholesterol (mmol/L)					
Baseline	1.13 (1.01-1.33)	1.18 (1.02-1.37)	1.12 (1.04-1.30)	1.06 (0.96-1.46)	0.731
1 year	1.46 (1.23-1.60)*	1.35 (1.18-1.46)*	1.48 (1.27-1.64)*	1.54 (1.25-1.69)*	0.075
LDL-cholesterol (mmol/L)					
Baseline	2.75 (2.28-3.32)	3.02 (2.14-3.45)	2.53 (2.26-2.89)	2.71 (2.47-3.74)	0.572
1 year	2.60 (2.05-3.26)	2.15 (1.37-2.61)*	2.91 (2.33-3.36)	3.03 (2.43-3.72)†	**0.017**
Triglycerides (mmol/L)					
Baseline	1.70 (1.17-1.99)	1.70 (1.14-1.99)	1.54 (1.15-1.75)	1.89 (1.22-2.40)	0.424
1 year	0.96 (0.73-1.38)*	0.78 (0.65-1.21)*	0.98 (0.78-1.36)*	1.16 (0.78-2.31)*,†	**0.045**
Succinate					
Circulating succinate (μM)					
Baseline	58.9 (46.4-82.4)	58.1 (37.6-82.8)	48.6 (42.4-79.5)	64.5 (52.3-85.8)	0.318
1 year	46.0 (35.8-65.3)*	38.6 (32.4-50.1)*	44.6 (36.1-54.6)	64.2 (42.0-73.7)	0.056

Data are presented as mean (SD) or median (25th-75th percentiles), as appropriate. RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; LGCP, laparoscopic greater curvature plication; BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *p<0.05 for the comparison between results 1-year after bariatric surgery vs. baseline. ∫p<0.05 for least-square difference (LSD) post-hoc test comparing SG vs. RYGB. †p<0.05 for LSD post-hoc test comparing LGCP vs. RYGB. ‡p<0.05 for LSD post-hoc test comparing LGCP vs. SG.

Table 2. Logistic regression model for the prediction of complete type 2 diabetes remission

LR χ^2 27.51, p=0.001

	Coefficient	SE	95%CI	OR	p
Baseline succinate	2.423	1.126	0.217-4.269	11.3	0.031
Type of surgery	3.274	1.272	0.781-5.768	26.4	0.010

Variables included in the model: age, gender, previous treatment on insulin, baseline HbA_{1c}, circulating baseline succinate <48.1µM (No/Yes) and type of surgery (restrictive vs. malabsorptive).

FIGURE LEGENDS

Figure 1. Follow-up study. Type 2 diabetes evolution and baseline succinate associations

A: Evolution of type 2 diabetes status throughout the 2-year clinical follow-up, before, 1 year and 2 years after bariatric surgery. The cohort is distributed according to the type of surgery (RYGB: Roux-en-Y gastric bypass; SG: sleeve gastrectomy and LGCP: laparoscopic greater curvature placation). Complete diabetes remission (CDR) and no diabetes remission (NDR) at 1 and 2 years was defined according to Buse's consensus group criteria. Percentage values are represented vs. total (n=45). Green represents patients achieving CDR, and red those who remained NDR during the post-bariatric surgery follow-up. **B:** Positive correlation between baseline succinate levels and glucose, HbA_{1c} and triglycerides in the entire cohort. Spearman's correlation analysis was used.

Figure 2. Pre-operative levels of circulating succinate are an independent predictor of type 2 diabetes remission after bariatric surgery

A: Baseline serum succinate in patients with complete diabetes remission (CDR) and patients with no diabetes remission (NDR) at 1 year after bariatric surgery. Results are expressed as median with interquartile range; *$p<0.05$ (Mann Whitney test). **B–F:** The receiver operating characteristic (ROC) curves for succinate (**B**), DiaSuc (succinate and type of surgery) (**C**) and for DiaSuc, DiaRem, AdDiaRem, DiaBetter and ABCD in predicting diabetes 1 year after bariatric surgery (**D**) and in predicting diabetes 2 years after bariatric surgery (**E**). ROC curves for DiaSuc in predicting diabetes 1-year after bariatric surgery in the validation cohort (**F**). **G:** Evolution of type 2 diabetes status throughout the 2-year clinical follow-up, before, 1-

year and 2-years after bariatric surgery. The cohort is distributed according to the cut-off point of circulating baseline succinate, 48.1 μM (low/high) and the type of surgery (restrictive/malabsorptive). Percentage values are represented vs. the number of patients for each group. Green represents patients achieving CDR, and red those who remained NDR during the post-bariatric surgery follow-up.

Pre-operative circulating succinate levels as a biomarker for diabetes remission after bariatric surgery

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Short running title: succinate levels predict diabetes remission

ABSTRACT

Objective: To determine the potential use of baseline circulating succinate to predict type 2 diabetes remission following bariatric surgery.

Research Design and Methods: Forty-five obese diabetic patients were randomly assigned to Roux-en-Y gastric bypass (RYGB), sleeve gastrectomy (SG) or laparoscopic greater curvature plication. Anthropometrical parameters were evaluated and a complete biochemical analysis including circulating serum succinate concentrations was performed at baseline and 1-year after surgery. The results were externally validated in a second cohort including 88 obese diabetic patients assigned to RYGB or SG based on clinical criteria.

Results: Succinate baseline concentrations were an independent predictor of diabetes remission after bariatric surgery. Patients achieving remission after 1 year had lower levels of baseline succinate (47.8[37.6–64.6]μM vs. 64.1[52.5–82.9]μM; p=0.018). Moreover, succinate concentrations were significantly decreased 1-year after surgery (58.9[46.4–82.4]μM vs. 46.0[35.8–65.3]μM, p=0.005). In multivariate analysis, the best logistic regression model showed that baseline succinate (OR=11.3, p=0.031) and the type of surgery (OR=26.4, p=0.010) were independently associated with remission. The C-statistic for this model was 0.899(95%CI: 0.809–0.989) in the derivation cohort, which significantly improved the prediction of remission when compared with current available scores, and 0.729(95% CI: 0.612–0.846) in the validation cohort. Interestingly, patients had a different response to the type of surgery according to baseline succinate, with significant differences in remission rates.

Conclusions: Circulating succinate is reduced after bariatric surgery. Baseline succinate levels have predictive value for diabetes remission independently of

previously described pre-surgical factors and improve upon the current available scores to predict remission.

Keywords: succinate, bariatric surgery, type 2 diabetes, remission

Bariatric surgery has emerged as an effective treatment for obesity, leading to marked weight loss and the improvement of related comorbidities such as type 2 diabetes and cardiovascular disease, and reducing mortality (1). Sleeve gastrectomy (SG) is the most commonly performed bariatric procedure, because of its simplicity and low rate of complications, accounting for 53.6% of worldwide surgical procedures, followed by Roux-en-Y gastric bypass (RYGB) (30.1%) (2). However, it remains unclear whether RYGB or SG benefits patients with type 2 diabetes equally with regards to glycemic homeostasis (3; 4).

Remission rates of type 2 diabetes observed across published series vary, likely due to heterogeneity in the preoperative characteristics of patients. Against this background, several predictive factors for remission have been identified including age, disease duration, baseline C-peptide concentrations, HbA_{1c}, or previous insulin treatment (5-9). Further, remission rates have been associated with greater weight loss after bariatric surgery independently of initial body mass index (BMI) (10). Accordingly, several predictive outcome scores for type 2 diabetes remission have been developed based on these variables to help identify those patients most likely to benefit from surgery (11-14). However, there is limited evidence regarding the optimal candidate for each surgical procedure and relatively few studies have tested these scores in cohorts to predict type 2 diabetes remission following different surgical procedures, such as RYGB and SG, with contradictory results (14-17). Thus, efforts are needed to improve the accuracy to better predict diabetes remission according to the surgical procedures considering malabsorptive or restrictive techniques.

Bariatric surgery is a known potent modifier of key metabolic intermediates, as has been recently demonstrated in animal models and in patients submitted to

RYGB (18-21). In this context, specific circulating and cardiac tricarboxylic acid cycle metabolites have been identified as potential key players of bariatric cardioprotection through the so-called “enterocardiac axis” (19). Among them, succinate has recently emerged as an extracellular signaling metabolite governing local stress and inflammatory processes *via* engagement with its cognate receptor SUCNR1/GPR91 (22). Remarkably, elevated levels of circulating succinate have been detected in several high-risk cardiovascular disease states such as hypertension (23), ischemic heart disease (24) and type 2 diabetes (25; 26). Along these lines, we recently demonstrated that BMI is a determinant of circulating succinate levels, which are directly linked to specific constituents of fecal microbiota (25). Interestingly, microbiota-produced succinate has also been related to intestinal glucose metabolism (27; 28) and metabolic activity of brown adipose tissue (29), which are major pathophysiological mechanisms mediating beneficial metabolic effects of bariatric surgery (30).

Several studies have attempted to identify robust biological and clinical predictors of type 2 diabetes remission after surgery (5-9; 31); however, there is still insufficient evidence to define cut-off values able to quantitatively predict remission over time. Here, we postulated that succinate, which occupies a pivotal position in metabolism as both an energetic and a signaling metabolite, plays an important role in glucose metabolism and hence in clinical response following bariatric surgery. Our study aimed to assess whether pre-operative circulating levels of succinate might have an impact on type 2 diabetes remission.

RESEARCH DESIGN AND METHODS

Study design and patients

The study was completed in the setting of a previously published prospective single center, non-blinded, randomized controlled trial including obese patients with type 2 diabetes (9). The trial was registered at clinicaltrials.gov (NCT14104758). In brief, forty-five patients were consecutively recruited among patients undergoing bariatric surgery at the Department of Endocrinology of the Bellvitge University Hospital (Barcelona, Spain). The study was conducted according to the principles of the Declaration of Helsinki. The local ethics committee study approved the study and all subjects gave written informed consent before study entry.

Study protocol

The study design and protocol have been previously reported in detail (9; 31). From May 2012 to February 2014, patients were consecutively randomized to RYGB (n=15), SG (n=15) and laparoscopic greater curvature plication (LGCP) (n=15). The procedures were performed by the same surgeon and at the same center. A physical examination with determination of anthropometrical parameters and a complete biochemical analysis was performed at baseline and at 1-year following surgery.

Laboratory determinations

Whole blood was stored at 4ºC and was centrifuged (2500 rpm for 20 minutes) within 2 hours to collect serum.

Glucose, cholesterol, and triglycerides were determined using standard enzymatic methods. Plasma insulin was analyzed by immunoassay (Coat-A-Count Insulin; Diagnostic Products Corp., Los Angeles, CA). Fasting C-peptide levels were

also analyzed by immunoassay (Immulite 2000 XPi, Siemens Healthcare, Munich, Germany). Circulating serum succinate levels were measured at baseline and 1-year after bariatric surgery using the EnzyChrom™ Succinate Assay Kit (BioAssay Systems, Hayward, CA). The assay sensitivity was 12 μM, ~~and~~ the intra- and inter-assay co-efficient of variance (CV) was less than 3.5 and 6.95%, respectively (25) and the accuracy ranged from 1% to 11.5% error (Supplementary Table 1). Circulating succinate levels measured by this fluorimetric assay were previously validated by liquid chromatography-mass spectrometry and nuclear magnetic resonance analysis (25).

Outcomes

The primary outcome was to evaluate the rate of type 2 diabetes remission at 1-year following bariatric surgery. To evaluate the rate of remission after surgery, we used Buse's consensus criteria, considering complete type 2 diabetes remission if HbA_{1c} <6% and fasting glucose <100 mg/dl, in the absence of pharmacologic therapy or ongoing procedures, for a duration of at least 1 year (32).

Models

To assess the suitability of the current scores to predict remission after bariatric surgery, the data were used to calculate the ABCD (11), DiaRem (12), AdDiaRem (13) and the DiaBetter (14) scores.

Validation cohort

The prediction model was externally validated in a second independent cohort of patients with obesity recruited in three different Spanish centers (Hospital

Germans Trias i Pujol [Badalona, Spain], Hospital Arnau de Vilanova [Lleida, Spain] and Hospital Josep Trueta [Girona, Spain]). The local ethics committee study of each center approved the study and all subjects gave written informed consent before study entry. The validation population consisted of 88 patients with obesity aged between 18 and 60 years, BMI >=35 kg/m^2, type 2 diabetes on hypoglycemic agents alone, insulin or both. Type 2 diabetes remission was also evaluated at 1 year following bariatric surgery and using Buse's consensus criteria. The indication for the type of surgical procedure (SG or RYGB) was based on clinical criteria.

Sample size

Taking into account the available sample size of the initial randomized controlled trial of 45 subjects, to obtain an absolute difference of type 2 diabetes remission rates of 50% between groups (low vs. high succinate) and allowing for a type 1 error of 0.05, the number of subjects included in our study provided a statistical power of 94%.

Statistical analysis

All data were tested for normality using the Shapiro-Wilk test. Data were presented as percentage, mean and standard deviation (SD) for normally distributed quantitative variables, or median and 25th-75th percentiles for non-normally distributed quantitative variables. Non-normally distributed quantitative variables were used after performing a $\log_{10}$ transformation. Differences between groups were analyzed using the $\chi 2$ test for comparisons of proportions, and the unpaired t-test or the Mann-Whitney U test for comparisons of normally and non-normally distributed quantitative variables, as needed. One-way analysis of variance was used to

compare groups in the cross-sectional study. The least square difference test was used for post hoc analyses. Paired t test and the Wilcoxon signed-rank test were used for paired analysis of the prospective data. Pearson's and Spearman's correlation coefficients were used to analyze the relationship between parameters, as described. Two-tailed p-values <0.05 were considered statistically significant.

To identify the potential role of circulating succinate and the other factors independently related to type 2 diabetes remission after bariatric surgery, logistic regression analyses were performed. All associated variables in the univariate analyses (0.67 < odds ratio [OR] >1.67 and p<0.2) and those variables known or likely to be associated with remission (based on previous literature) were included in the logistic regression models as potential independent variables. Receiver operating characteristic (ROC) curves, in which sensitivity is plotted as a function of 1-specificity, were developed to assess the predictive value of circulating succinate and to compare it with the different scores for the prediction of type 2 diabetes remission. Subsequently, the equality between the different ROC areas obtained was tested. In addition, we used classification and regression tree (CART) analysis, which splits the data into segments that are as homogenous as possible with respect to the dependent variable.

The calculations, figures and statistical analysis were made using STATA v.13.1 for Mac (StataCorp LP, College Station, TX) and GraphPad Prism (GraphPad Software Inc., San Diego, CA). Diagrams of the evolution of type 2 diabetes status were constructed using a Sankey diagram generator (https://sankey.csaladen.es/). CART analysis was performed with the Statistical Package for the Social Sciences software, version 19 (SPSS; Chicago, IL, USA).

RESULTS

Follow-up study. Design, clinical and anthropometric variables and type 2 diabetes evolution

A total of 45 obese patients with type 2 diabetes were included in a 1-year follow-up prospective study. Baseline characteristics (**Table 1**) were similar between the 3 surgery groups except for BMI, which was slightly but significantly higher in the LGCP group than in the RYGB and SG groups. As expected, weight, BMI, waist circumference and waist-to-hip ratio were all reduced after bariatric surgery in the three groups, with marked weight loss and reduced BMI in the RYGB group as compared with the SG and LGCP groups. Fasting plasma glucose, insulin concentration and HbA_{1c} were also improved in the three groups, but especially so in the RYGB and SG groups. Moreover, dyslipidemia improved at 1-year after bariatric surgery, with a decrease of triglycerides levels and an increase of high-density lipoprotein-cholesterol (**Table 1**). Regarding the evolution of type 2 diabetes status 1-year after bariatric surgery, the overall remission rate was 51.1% (n=23): 26.7% in RYGB (n=12), 17.8% in SG (n=8) and 6.7% (n=3) in LGCP. In addition, at the 2-year clinical follow-up, the total remission rate was 46.7% (n=21): 24.4% in RYGB (n=11), 15.6% in SG (n=7) and 6.7% (n=3) in LGCP (**Fig. 1A**).

No differences were found between the three types of surgery for circulating concentrations of succinate at baseline (**Table 1**). Considering the whole population in the study, circulating concentrations of succinate were significantly reduced at 1-year of follow-up (58.9 [interquartile range, IQR 46.4–82.4] μM vs. 46.00 [IQR 35.8–65.3] μM, p=0.005). When the results were analyzed according to the type of surgery, the reduction was only significant for the RYGB group (58.1 [IQR 37.6–82.8]

μM vs. 38.6 [IQR 32.4–50.1] μM, p=0.025). Consistent with our previous observations in obese subjects (25), circulating concentrations of succinate at baseline were positively associated with glucose (r=0.296, p=0.048), HbA_{1c} (r=0.341, p=0.022) and triglycerides (r=0.421, p=0.004) (**Fig. 1B**).

Pre-operative levels of circulating succinate are linked to type 2 diabetes remission rates at 1-year after bariatric surgery

We next sought to further explore the potential association between serum succinate and type 2 diabetes remission. Notably, those patients achieving remission at 1-year after surgery had lower circulating concentrations of succinate at baseline (47.8 [IQR 37.6–64.6] μM vs. 64.1 [IQR 52.5–82.9] μM; p=0.018) (**Fig. 2A**). To test the value of the basal succinate level to predict remission, we developed the corresponding ROC curve. The C-statistic of basal succinate was 0.710 (95% confidence interval [CI]: 0.554–0.865) for predicting remission 1-year after bariatric surgery (**Fig. 2B**). The best cut-off point of succinate, selected based on the Youden Index calculation, was 48.1 μM (sensitivity, 91% and specificity, 52%), which was used to classify the cohort.

To assess the validity of succinate as a biomarker to predict diabetes remission, we compared the accuracy of the existing risk scores for predicting type 2 diabetes remission after bariatric surgery with that of circulating sucinate. Accordingly, the ABCD, DiaRem, AdDiaRem and DiaBetter scores were calculated for each patient and their ROC curves were developed. The C-statistic for the prediction of remission for each of these ROC curves was 0.610 (95% CI: 0.447–0.772) for the ABCD score, 0.760 (95% CI: 0.616–0.904) for the DiaRem score, 0.728 (95% CI: 0.586–0.807) for the AdDiaRem score and 0.751 (95% CI: 0.606–

0.896) for the DiaBetter score, which were all similar to the predictive value of baseline circulating succinate alone (**Fig. 2B**).

To evaluate the independent factors associated with type 2 diabetes remission at 1-year in our cohort, we developed logistic regression models. In univariate analysis, and in accord with previous studies (5-8), we found that the variables associated with remission were the type of surgery (malabsorptive vs. restrictive) (OR=6.909; p=0.010), previous insulin treatment (OR=0.146; p=0.011), baseline HbA_{1c} (OR=0.606, p=0.011) and baseline circulating succinate <48.1 μM (OR=10.909; p=0.005). The best logistic regression model obtained showed that baseline circulating succinate <48.1 μM (OR=11.3, p=0.031) and the type of surgery (OR=26.4, p=0.010), were independently associated with remission after adjusting for potential confounders (**Table 2**). Remarkably, the C-statistic for this model (which we term DiaSuc) was 0.899 (95% CI: 0.809–0.989) (**Fig. 2C**), which significantly improves upon type 2 diabetes prediction of the ABCD score (0.610 vs. 0.899, p=0.001) and the AdDiaRem score (0.728 vs. 0.899; p=0.018), and was slightly but not significantly better than the DiaRem score (0.760 vs. 0.899, p=0.061) and the DiaBetter score (0.751 vs. 0.899, p=0.052) (**Fig. 2D**).

To confirm these results, we performed a regression tree (CART) analysis (Supplementary Fig. 1). We included all the previously selected variables as predictors of type 2 diabetes remission (see the logistic regression model, **Table 2**). The first node was split based on the most important predictor, which was considered the pre-operative levels of circulating succinate. According to this model, those patients with low baseline succinate (<48.1 μM) had a higher percentage of remission at 1-year after bariatric surgery compared with those patients with high baseline succinate (85.7% vs. 35.5%; p=0.003) (Supplementary Fig. 1). The second

node was split based on the type of surgery (restrictive vs. malabsorptive). Interestingly, those patients with low baseline succinate had a similar rate of remission independently of the type of surgery (100.0% vs. 75.0%; p=0.473). By contrast, those patients with high baseline succinate who underwent malabsorptive surgery had a higher rate of remission at 1-year after bariatric surgery than those who underwent a restrictive procedure (66.7% vs. 22.7%; p=0.038) (Supplementary Fig. 1).

Next, we extended the clinical follow-up to 2 years after bariatric surgery to confirm the potential role of basal succinate levels as a predictor of remission. As mentioned above, 46.7% of patients achieved complete type 2 diabetes remission (n=21). At this time-point, and according to the results at 1-year, those patients that achieved remission at 2 years after surgery had lower circulating concentrations of succinate at baseline (47.6 μM (IQR 37.6–64.7) vs. 63.8 μM (IQR 55.0–82.9) p=0.013). The C-statistic of DiaSuc for prediciting remission at 2-years after bariatric surgery, including baseline circulating succinate concentrations and type of surgery, was 0.912 (95% CI: 0.821–1.000), which again significantly improved upon the ABCD score (0.620 vs. 0.912, p<0.001), the DiaBetter score (0.710 vs. 0.912, p=0.025) and the DiaRem score (0.755 vs. 0.912, p=0.042), and was slightly better (but not significantly) than the AdDiaRem score (0.774 vs. 0.912; p=0.055) (**Fig. 2E**).

Finally, the results were externally validated in a second independent cohort (baseline characteristics are shown in Supplementary Table 2~~1~~). In the validation cohort, the baseline circulating concentration of succinate was 53.2 μM (IQR 32.0–73.9). Circulating concentrations of succinate 1-year after surgery were available in 55 out of 88 patients included in the validation cohort. The results obtained in the validation cohort (n=55, 100% SG) also showed a decrease in succinate

concentrations 1-year after bariatric surgery (IQR 42.3 [26.6–63.7] μM vs. 34.9 [26.9–48.2] μM; p=0.029). The external validation of the DiaSucc model in this second cohort showed a very good discrimination, with a C-statistic of 0.729 (95% CI: 0.612–0.846) (**Fig. 2F**).

CONCLUSIONS

In the present study, we provide the first demonstration that baseline circulating concentrations of succinate are an independent predictor of type 2 diabetes remission after bariatric surgery in obese patients. Further, baseline circulating concentrations of succinate together with the type of surgery improves upon the accuracy of established predictive diabetes remission scores (11-14).

Bariatric surgery is well established as an effective weight loss strategy for people with obesity that also improves comorbidities. Increasing evidence indicates that it can be a more effective treatment for a subset of type 2 diabetes patients than standard medical therapy, achieving greater metabolic effects such as improving glucose homeostasis, insulin sensitivity, β-cell function and incretin response, which has led to the term "metabolic surgery" (30; 33-35). Using surgery as an intervention for type 2 diabetes, however, implies conceptual and practical differences from the traditional practice of bariatric surgery for obesity, for instance, in the criteria used to select candidates for metabolic surgery including metrics of metabolic disease severity and predictors of treatment success. In this sense, some predictive pre-surgical factors of metabolic outcomes have been identified, such as younger age, shorter disease duration, pre-operative C-peptide levels and the absence of insulin treatment prior to surgery, which are all associated with higher remission rates (5-9). Accordingly, several predictive remission scores (ABCD, DiaRem, Ad-DiaRem and DiaBetter) have been developed incorporating these factors (11-14). Nevertheless, the predictive models do not always consider the different types of surgery. Thus, there is still the need to discover novel biomarkers to better predict remission outcomes.

We have previously demonstrated a link between circulating succinate and poor metabolic control in patients with type 2 diabetes (25). In the present study, baseline circulating succinate was associated with type 2 diabetes remission at 1- and 2-years after bariatric surgery (the lower the value, the higher the remission rate), and the predictive value of succinate alone was similar to all the aforementioned established scores. Moreover, baseline circulating succinate was independently associated with remission after adjusting for the remainder of the predictive pre-surgical factors, and the logistic regression model tested (which includes baseline succinate and type of surgery) improved the score accuracy to predict remission rates when compared with the other models. Considering that the established scores to predict remission generally combine clinical variables mostly related to diabetes severity, our results point to succinate as a metabolite capable of representing several components of metabolic status, rendering a broader view of homeostasis in an easily measurable serum biomarker. Accordingly, succinate could be a new preoperative predictive factor of type 2 diabetes remission after metabolic surgery. Along this line, succinate has been recognized as a predictor of mortality in critically injured patients and as a marker of hypoxic metabolic reprogramming in cancer, ischemia, inflammation and immunomodulation (36).

Succinate is traditionally considered as an energetic metabolite produced by both host (mitochondria) and microbiota (25; 27). Unfortunately, fecal samples were not available to explore the relationship between circulating succinate and the gut microbiome in the context of bariatric surgery. Nonetheless, one might speculate that mitochondrial dysfunction of metabolic tissues also underlies changes in circulating levels of succinate. Indeed, this metabolite is a good marker of cellular status, as it occupies a pivotal position in metabolism as the only direct link between the

tricarboxylic acid cycle and the mitochondrial respiratory chain through complex II activity. When cells rely on certain stress stimuli, mitochondrial levels and consequently extracellular levels of succinate might increase (37). While intracellular succinate is commonly considered a pro-inflammatory stimulus (22), we recently demonstrated that engagement with its receptor SUCNR1 has a critical role in the anti-inflammatory responses in macrophages; but this interaction is disrupted in obesity. Obesity is defined by high circulating levels of succinate; however, adipose tissue-resident macrophages from obese subjects show decreased expression of SUCNR1, suggesting a 'succinate-resistant state' (38). Succinate resistance in the context of obesity might contribute to the increased baseline succinate levels and to the inability to resolve inflammation and glucose homeostasis following bariatric surgery.

Metabolic functions are reduced in insulin-responsive tissues (muscle and adipose tissue) in obesity and type 2 diabetes. No consensus has thus far been reached with regards to whether insulin resistance is a result of reduced mitochondrial density or whether it is the cause or consequence of mitochondrial dysfunction. The debate on mitochondrial dysfunction as a contributor to type 2 diabetes pathogenesis continues and drives the need for more studies on mitochondrial function in this patient group (39). Moreover, it has been reported that destabilized mitochondrial metabolism in type 2 diabetes and obesity can be influenced by bariatric surgery (40). Therefore, despite the unresolved question concerning the source of circulating succinate levels, our results point to this energetic metabolite as a key player in type 2 diabetes remission after bariatric surgery and a new variable to reflect metabolic disease severity.

It remains unclear whether RYGB or SG has equal benefits for patients with type 2 diabetes regarding glycemic homeostasis. Given that both techniques are different with regard to the surgical procedures, post-operative care, long-term comorbidities and economic costs, it is important to uncover novel biomarkers to adopt a personalized approach to bariatric surgery by identifying which patients will benefit most from malabsorptive vs. restrictive procedures. In that sense, we found that the remission rates were similar independently of the type of surgery (malabsorptive vs. restrictive) for those patients with low baseline circulating succinate (100% vs. 75% at 1 year and 83% vs. 75% at 2 years) (**Fig. 2G**). By contrast, for those patients with high baseline circulating succinate, the remission rates clearly differed according to the surgical procedure (66.7% vs. 22.7% at 1 year and 67.7% vs. 13.6% at 2 years) (**Fig. 2G**). Thus, our results suggest that pre-operative concentrations of circulating succinate could be a useful metric to determine the best-suited surgical procedure.

We are aware that our study has some limitations. It was a single-center, single-surgeon study and the sample size was small, although this was supported by the sample size calculations. Importantly, the results were validated in a second independent cohort. Despite randomization, RYGB patients had a tendency towards a more favorable initial metabolic profile, although not reaching statistical significance, and this fact may have conditioned the better metabolic outcomes. In addition, we acknowledge that remission of type 2 diabetes can be also associated with the initial BMI and with a greater weight loss after bariatric surgery. In this sense, we did not find any association between baseline BMI and type 2 diabetes remission. We aimed to identify a potential biomarker that might help to develop a clinical decision tree to select the best-suited surgical procedure, focusing only in

pre-surgical factors. Finally, the follow-up was at 2-years; however, the cohorts allocated to the different bariatric procedures will be closely followed at long-term.

In conclusion, the present study shows that baseline circulating concentrations of succinate have predictive value for type 2 diabetes remission, independently of previously described pre-surgical factors, improving upon the current available scores to predict remission. Furthermore, we propose a cut-off value of succinate as a new criterion to select the best candidates for restrictive surgical procedures over the more aggressive malabsorptive surgical techniques in type 2 diabetes. We are fully aware that despite the good agreement of the succinate cut-off values in two independent cohorts from 4 different centers, further randomized controlled studies will be needed as a next step to establish the use of succinate as a clinical decision support tool for selecting the best-suited bariatric surgical procedures. Furthermore, as with any diagnostic test, it will be essential to standardize blood collection and processing protocols to incorporate succinate measurements into routine clinical practice. Our findings underscore the importance of circulating metabolites as potentially valuable predictive factors of diabetes remission after bariatric surgery. In this sense, while other metabolites cannot be excluded, our results, together with the unique and specific attributes of succinate – its pivotal position in energy metabolism, its role as a fuel substrate and signaling metabolite, and the fact that it is produced by both host and gut microbiota – make it an attractive target for management of diabetes.

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JV and SF-V conceived, designed and supervised the research project and wrote the manuscript. VC-M and GLL participated in the conception and design of the study, sample collection, statistical analysis and wrote the manuscript. NK and BA analyzed serum succinate levels. EB constructed diagrams of the evolution of type 2 diabetes. LM, SP, JMG-C and AM provided scientific discussion and revised the manuscript. NV participated in the human sample recruitment and conducted the clinical trial. AR, JMF-R and AL participated in the human sample recruitment of the validation cohort. SF-V and JV are the guarantors of this work.

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Table 1. Clinical and laboratory data for the obese patients with type 2 diabetes before and at 1 and 2 years after bariatric surgery

	All	RYGB	SG	LGCP	p for trend
n	45	15	15	15	-
Age (years)	49.4 (8.0)	51.1 (7.7)	49.2 (9.2)	49.7 (8.1)	0.827
Gender (M/F)	15/30	7/8	5/10	3/12	0.361
Type 2 diabetes					
Type 2 diabetes (n, %)					
Baseline	45 (100)	15 (100)	15 (100)	15 (100)	1.000
1 year	22 (48.9)	3 (20.0)	7 (46.7)	12 (80.0)	**0.005**
2 years	24 (53.3)	4 (26.7)	8 (53.3)	12 (80.0)	**0.016**
Type 2 diabetes treatment (n, %)					
Baseline	45 (100)	15 (100)	15 (100)	15 (100)	1.000
1 year	13 (28.9)	0 (0.0)	6 (40.0)	7 (46.7)†	**0.005**
2 years	15 (33.3)	0 (0.0)	6 (40.0)	9 (60.0)†	**0.001**
Insulin (n, %)					
Baseline	17 (37.8)	5 (33.3)	6 (40.0)	6 (40.0)	0.910
1 year	5 (11.1)*	0 (0.0)*	3 (20.0)*	2 (13.3)	0.343
Type 2 diabetes duration (years)	7.4 (3.6-13.0)	4.5 (3.1-10)	10.0 (4.2-15.3)	6.9 (3.4-12.9)	0.209
Anthropometric measurements					
Weight (kg)					
Baseline	103.6 (11.0)	103.0 (10.8)	102.3 (10.8)	105.5 (11.9)	0.710
1 year	75.1 (13.4)*	66.6 (10.7)*	74.9 (10.1)*	83.6 (13.6)*,†	**0.001**
2 years	78.7 (13.8)	70.4 (11.6)	79.3 (12.5)	86.4 (12.9)†	**0.004**
BMI (kg/m^2)					
Baseline	39.5 (1.9)	38.6 (1.9)	39.0 (1.7)	40.9 (1.4)†,‡	**0.001**
1 year	28.6 (4.1)*	24.9 (3.0)*	28.6 (2.5)*,∫	32.3 (2.7)†,‡	**<0.001**
2 years	30.0 (4.0)	26.3 (3.0)	30.2 (3.1)∫	33.4 (2.2)†,‡	**<0.001**
Waist (cm)					
Baseline	117.9 (7.9)	118.9 (7.6)	117.5 (7.6)	117.3 (8.9)	0.852
1 year	96.0 (9.9)*	91.3 (7.9)*	93.6 (10.0)*	103.5 (7.7)*	0.822
WHR					
Baseline	0.96 (0.07)	0.97 (0.09)	0.98 (0.07)	0.93 (0.06)	0.184
1 year	0.91 (0.07)*	0.93 (0.07)*	0.89 (0.07)*	0.92 (0.06)	0.217
Blood pressure					
SBP (mmHg)					
Baseline	134.0 (18.5)	140.8 (19.7)	132 (13.9)	129.1 (20.4)	0.199
1 year	129.0 (19.4)	128.8 (18.7)*	126.8 (25.4)	131.2 (14.3)	0.836
DBP (mmHg)					

Baseline	81.1 (14.3)	84.5 (13.6)	77.9 (13.7)	80.7 (15.7)	0.457
1 year	77.2 (13.0)	77.7 (12.9)	74.2 (13.5)	79.6 (13.1)	0.543
Glucose homeostasis					
Glucose (mmol/L)					
Baseline	8.5 (6.4-11.6)	8.0 (5.6-11.1)	8.5 (6.4-12.4)	8.8 (6.3-11.6)	0.583
1 year	5.1 (4.5-6.2)*	4.5 (4.4-5.0)*	5.1 (4.9-6.5)*	6.3 (5.5-8.6)*,†	**0.029**
2 years	5.8 (5-6.9)	5.1 (4.8-5.7)	6.0 (5.3-8.0)	6.9 (5.8-9.3) †	**0.006**
Insulin (pmol/L)					
Baseline	115 (80-137)	132 (115-143)	94 (63-117)	106 (67-128)	0.779
1 year	50 (35-74)*	37 (28-71)*	42 (36-88)*	72 (59-84)†	**0.021**
C-peptide (nmol/L)					
Baseline	1.1 (0.8-1.3)	1.1 (0.7-1.3)	0.8 (0.7-1.4)	1.0 (0.8-1.5)	0.566
1 year	0.6 (0.5-0.8)	0.5 (0.4-0.6)	0.6 (0.5-1.0)	0.8 (0.6-0.9) †	**0.013**
HbA1c (%)					
Baseline	7.2 (6.3-9.3)	6.7 (5.9-9.5)	7.2 (6.4-9.3)	7.5 (6.3-9.8)	0.634
1 year	5.9 (5.4-6.5)*	5.4 (4.7-5.5)*	5.9 (5.5-6.8)*,∫	5.9 (6.3-7.5)*,†	**<0.001**
2 years	5.8 (5.4-7.5)	5.2 (4.7-5.6)	6.2 (5.5-7.8)	7.4 (5.8-10.2)†	**<0.001**
HbA1c (mmol/mol)					
Baseline	55 (45-78)	50 (41-80)	55 (46-78)	58 (45-84)	0.634
1 year	41 (36-48)*	36 (28-37)*	41 (37-51)*,∫	41 (45-58)*,†	**<0.001**
2 years	40 (36-58)	33 (28-38)	44 (37-62)	57 (40-88)†	**<0.001**
Lipid profile					
Total cholesterol (mmol/L)					
Baseline	4.76 (4.27-5.38)	4.88 (3.74-5.44)	4.53 (3.89-4.99)	4.88 (4.41-5.66)	0.456
1 year	4.61 (3.95-5.26)	4.04 (3.09-4.37)*	5.02 (4.1-5.28) ∫	5.18 (4.77-5.99)†	**<0.001**
HDL-cholesterol (mmol/L)					
Baseline	1.13 (1.01-1.33)	1.18 (1.02-1.37)	1.12 (1.04-1.30)	1.06 (0.96-1.46)	0.731
1 year	1.46 (1.23-1.60)*	1.35 (1.18-1.46)*	1.48 (1.27-1.64)*	1.54 (1.25-1.69)*	0.075
LDL-cholesterol (mmol/L)					
Baseline	2.75 (2.28-3.32)	3.02 (2.14-3.45)	2.53 (2.26-2.89)	2.71 (2.47-3.74)	0.572
1 year	2.60 (2.05-3.26)	2.15 (1.37-2.61)*	2.91 (2.33-3.36)	3.03 (2.43-3.72)†	**0.017**
Triglycerides (mmol/L)					
Baseline	1.70 (1.17-1.99)	1.70 (1.14-1.99)	1.54 (1.15-1.75)	1.89 (1.22-2.40)	0.424
1 year	0.96 (0.73-1.38)*	0.78 (0.65-1.21)*	0.98 (0.78-1.36)*	1.16 (0.78-2.31)*,†	**0.045**
Succinate					
Circulating succinate (μM)					
Baseline	58.9 (46.4-82.4)	58.1 (37.6-82.8)	48.6 (42.4-79.5)	64.5 (52.3-85.8)	0.318
1 year	46.0 (35.8-65.3)*	38.6 (32.4-50.1)*	44.6 (36.1-54.6)	64.2 (42.0-73.7)	0.056

Data are presented as mean (SD) or median (25th-75th percentiles), as appropriate. RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; LGCP, laparoscopic greater curvature plication; BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *p<0.05 for the comparison between results 1-year after bariatric surgery vs. baseline. ∫p<0.05 for least-square difference (LSD) post-hoc test comparing SG vs. RYGB. †p<0.05 for LSD post-hoc test comparing LGCP vs. RYGB. ‡p<0.05 for LSD post-hoc test comparing LGCP vs. SG.

Table 2. Logistic regression model for the prediction of complete type 2 diabetes remission

LR χ^2 27.51, p=0.001

	Coefficient	SE	95%CI	OR	p
Baseline succinate	2.423	1.126	0.217-4.269	11.3	0.031
Type of surgery	3.274	1.272	0.781-5.768	26.4	0.010

Variables included in the model: age, gender, previous treatment on insulin, baseline HbA_{1c}, circulating baseline succinate <48.1µM (No/Yes) and type of surgery (restrictive vs. malabsorptive).

FIGURE LEGENDS

Figure 1. Follow-up study. Type 2 diabetes evolution and baseline succinate associations

A: Evolution of type 2 diabetes status throughout the 2-year clinical follow-up, before, 1 year and 2 years after bariatric surgery. The cohort is distributed according to the type of surgery (RYGB: Roux-en-Y gastric bypass; SG: sleeve gastrectomy and LGCP: laparoscopic greater curvature placation). Complete diabetes remission (CDR) and no diabetes remission (NDR) at 1 and 2 years was defined according to Buse's consensus group criteria. Percentage values are represented vs. total (n=45). Green represents patients achieving CDR, and red those who remained NDR during the post-bariatric surgery follow-up. **B:** Positive correlation between baseline succinate levels and glucose, HbA_{1c} and triglycerides in the entire cohort. Spearman's correlation analysis was used.

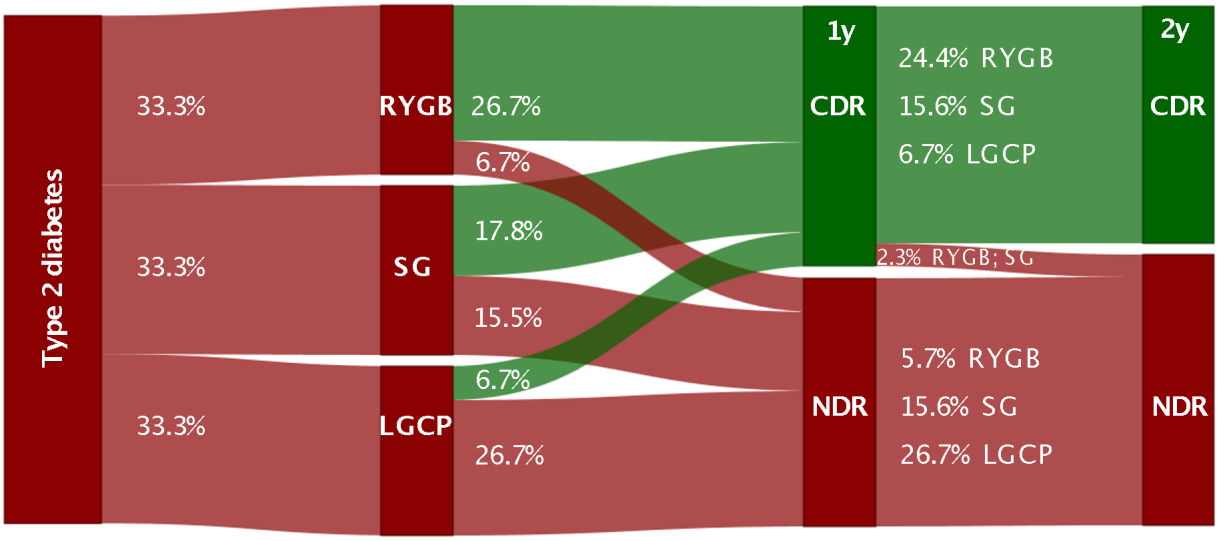
Figure 2. Pre-operative levels of circulating succinate are an independent predictor of type 2 diabetes remission after bariatric surgery

A: Baseline serum succinate in patients with complete diabetes remission (CDR) and patients with no diabetes remission (NDR) at 1 year after bariatric surgery. Results are expressed as median with interquartile range; *$p<0.05$ (Mann Whitney test). **B–F:** The receiver operating characteristic (ROC) curves for succinate (**B**), DiaSuc (succinate and type of surgery) (**C**) and for DiaSuc, DiaRem, AdDiaRem, DiaBetter and ABCD in predicting diabetes 1 year after bariatric surgery (**D**) and in predicting diabetes 2 years after bariatric surgery (**E**). ROC curves for DiaSuc in predicting diabetes 1-year after bariatric surgery in the validation cohort (**F**). **G:** Evolution of type 2 diabetes status throughout the 2-year clinical follow-up, before, 1-

year and 2-years after bariatric surgery. The cohort is distributed according to the cut-off point of circulating baseline succinate, 48.1 μM (low/high) and the type of surgery (restrictive/malabsorptive). Percentage values are represented vs. the number of patients for each group. Green represents patients achieving CDR, and red those who remained NDR during the post-bariatric surgery follow-up.

Figure 1.

A.


Type 2 diabetes
33.3%
33.3%
33.3%
RYGB
SG
LGCP
26.7%
6.7%
17.8%
15.5%
6.7%
26.7%
1y
CDR
NDR
24.4% RYGB
15.6% SG
6.7% LGCP
2y
CDR
2.3% RYGB; SG
5.7% RYGB
15.6% SG
26.7% LGCP
NDR

B.

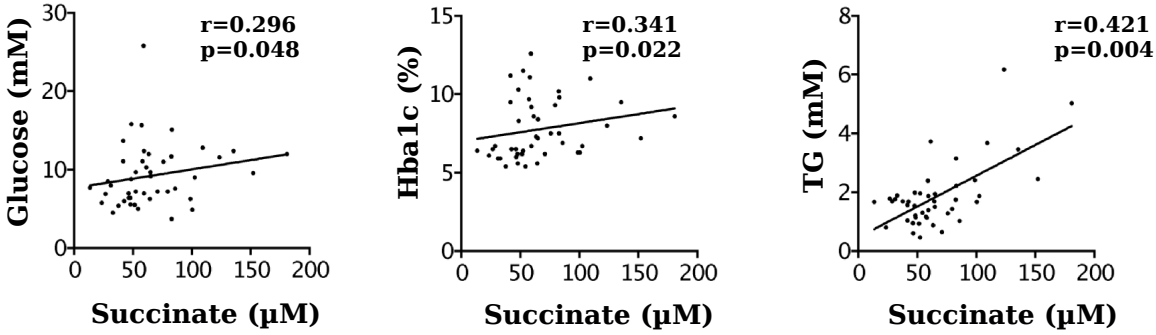
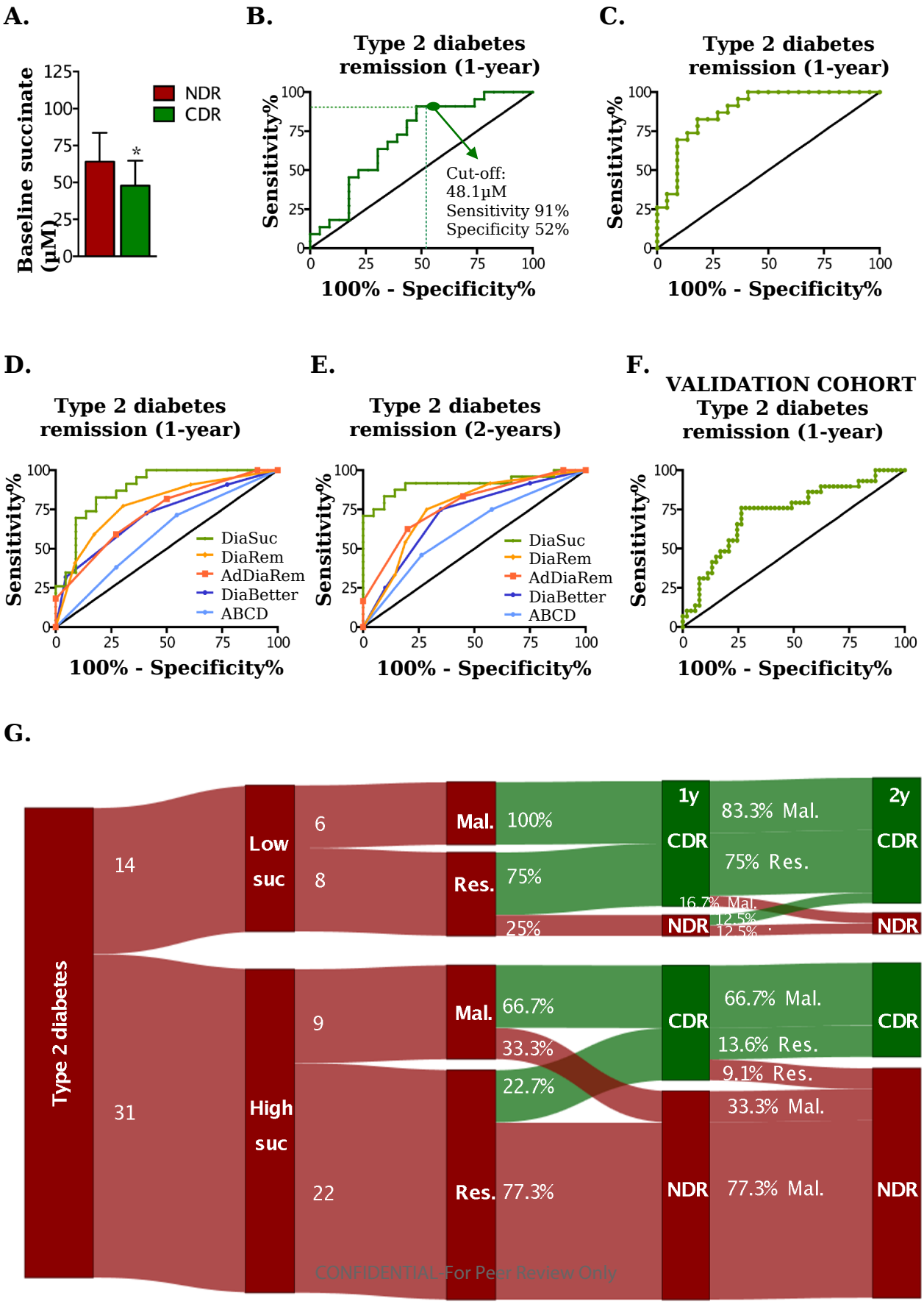

Glucose (mM)
r=0.296
p=0.048
Succinate (µM)
Hba1c (%)
r=0.341
p=0.022
Succinate (µM)
TG (mM)
r=0.421
p=0.004
Succinate (µM)

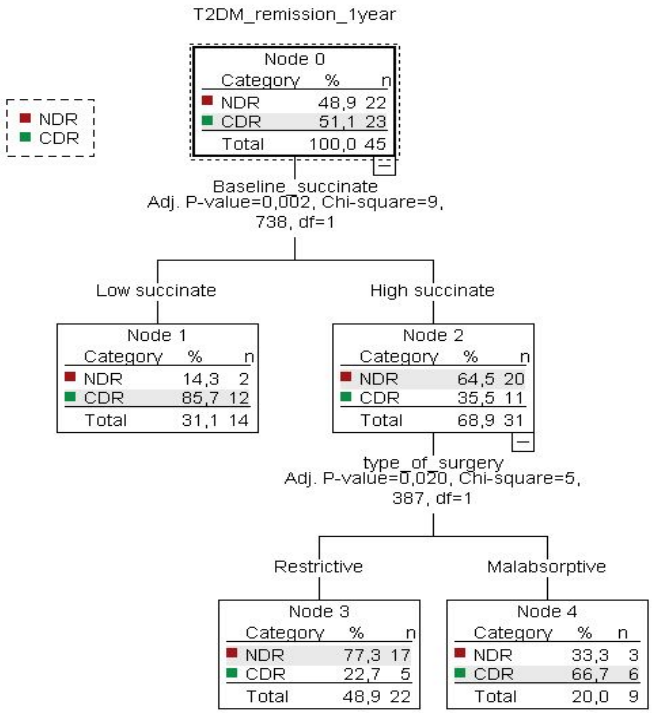
Figure 2.



SUPPLEMENTARY DATA

Supplementary Table 1. Accuracy. Measured value (concentrations of succinate by fluorimetric assay), theoretical value and the percent error (theoretical-measured/theoretical x 100) of four samples (pre-quantified) with different concentrations of succinate standard added.

	SUCCINATE (µM)			
SAMPLE	SUCCINATE (STANDARD) ADDED	MEASURED VALUE	THEORETICAL VALUE	ERROR (%)
1	0	115.17	-	-
1	10	120.72	125.17	3.56
1	30	139.95	145.17	3.60
1	100	239.19	215.17	11.16
2	0	89.2	-	-
2	25	127.30	114.20	11.47
2	50	129.40	139.20	7.04
3	0	27.6	-	
3	2,5	29,.7	30.1	0.96
4	0	8.75	-	-
4	10	16.89	18.75	9.92



Supplementary Figure 1. CART analysis. Classification and regression tree (CART) analysis for type 2 diabetes remission rates based on baseline succinate levels and type of surgery. Complete diabetes remission (CDR) and no diabetes remission (NDR) at 1 year after bariatric surgery.

Supplementary Table 2. Baseline characteristics of patients included in the validation cohort.

n	88
Age (years)	52.6 (6.8)
Gender (M/F)	26/62
Type of surgery (n, %) RYGB SG	 16 (18.1) 72 (81.8)
Weight (kg)	115.5 (18.6)
BMI (kg/m^2)	44.1 (6.4)
Type 2 diabetes (n, %)	88 (100%)
Insulin (n, %)	27 (30.7%)
Glucose (mmol/L)	116 (97–151)
HbA1c (%)	6.7 (6.2–7.7)
Triglycerides (mmol/L)	137 (98–175)
HDL-cholesterol (mmol/L)	42 (37–47)
LDL-cholesterol (mmol/L)	94 (66–119)
1-year type 2 diabetes remission (n, %)	54 (62.1%)

Data are presented as mean (SD) or median (25th-75th percentiles), as appropriate. RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.