

# Transcriptome Changes and Metabolic Outcomes After Bariatric Surgery in Adults With Obesity and Type 2 Diabetes

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## Abstract

**Context:** Bariatric surgery has been shown to be effective in inducing complete remission of type 2 diabetes in adults with obesity. However, its efficacy in achieving complete diabetes remission remains variable and difficult to predict before surgery.

**Objective:** We aimed to characterize bariatric surgery-induced transcriptome changes associated with diabetes remission and the predictive role of the baseline transcriptome.

**Methods:** We performed a whole-genome microarray in peripheral mononuclear cells at baseline (before surgery) and 2 and 12 months after bariatric surgery in a prospective cohort of 26 adults with obesity and type 2 diabetes. We applied machine learning to the baseline transcriptome to identify genes that predict metabolic outcomes. We validated the microarray expression profile using a real-time polymerase chain reaction.

**Results:** Sixteen patients entered diabetes remission at 12 months and 10 did not. The gene-expression analysis showed similarities and differences between responders and nonresponders. The difference included the expression of critical genes (*SKT4*, *SIRT1*, and *TNF* superfamily), metabolic and signaling pathways (Hippo, Sirtuin, ARE-mediated messenger RNA degradation, MSP-RON, and Huntington), and predicted biological functions ( $\beta$ -cell growth and proliferation, insulin and glucose metabolism, energy balance, inflammation, and neurodegeneration). Modeling the baseline transcriptome identified 10 genes that could hypothetically predict the metabolic outcome before bariatric surgery.

**Conclusion:** The changes in the transcriptome after bariatric surgery distinguish patients in whom diabetes enters complete remission from those who do not. The baseline transcriptome can contribute to the prediction of bariatric surgery-induced diabetes remission preoperatively.

**Key Words:** obesity, type 2 diabetes, bariatric surgery, microarray, transcriptomics, genetic predictors

**Abbreviations:** ARE, AU-rich elements; BMI, body mass index; cDNA, complementary DNA; CRP, C-reactive protein; DEG, differentially expressed gene; FDR, false discovery rate; LR, logistic regression; mRNA, messenger RNA; MSP, macrophage-stimulating protein; NR, nonresponder; P BMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; R, responder; RFECV, Recursive Feature Elimination with Cross-Validation; TNF, tumor necrosis factor.

Bariatric surgery is now considered the standard therapy for adults with obesity and type 2 diabetes who do not respond to a healthy lifestyle and optimal medical therapy [1]. However, it does not achieve complete remission of type 2 diabetes in approximately a third of patients at 1 year [2, 3]. Furthermore, the proportion of patients in remission can decline after several years of follow-up, although the rate varies between studies [3, 4]. Therefore, identifying adults with obesity and type 2 diabetes who will achieve short- and long-

term remission of diabetes after bariatric surgery is essential for informed decisions before surgery.

Most standard bariatric surgery procedures, including laparoscopic adjustable gastric banding, sleeve gastrectomy, Roux-en-Y gastric bypass, and biliopancreatic diversion, promote weight loss and metabolic improvement, albeit with variable magnitude [1]. Gastric bypass (Roux-en-Y gastric bypass and biliopancreatic diversion) has been shown to be superior to restrictive procedures (gastric banding and sleeve gastrectomy)

in leading to diabetes remission [5]. However, the likelihood of complete remission of type 2 diabetes after bariatric surgery remains difficult to predict at the individual level, regardless of the surgical procedure [2, 6-8]. Consequently, several studies have attempted to identify predictors of metabolic outcomes using patients' demographic, clinical, and biochemical factors to help clinicians select patients and surgical procedures [2, 6-8]. However, despite improving the short-term prediction of diabetes remission at 1 year, the specificity of these scores has remained insufficient to accurately identify individuals who will enter diabetes remission [9]. Furthermore, these remission scores have limited predictive capacity for long-term metabolic outcomes after bariatric surgery [10, 11].

Genetic variants associated with adults with obesity and type 2 diabetes have been shown to influence weight loss and metabolic response to bariatric surgery [12-14]. Few studies have evaluated the ability of single or multiple variants to predict diabetes remission after bariatric surgery [12, 13]. The findings have shown that patients with these variants are prone to better metabolic outcomes than noncarriers, suggesting that genetic factors can contribute to improving the preoperative prediction of diabetes remission after bariatric surgery. However, this approach may apply only to selected populations with specific variants. Therefore, these clinical and genetic studies have led us to hypothesize that identifying genetic predictors that can be integrated with clinical parameters may improve the prediction of individual response to bariatric surgery and form the basis of personalized care.

Clinical and experimental studies suggest that the transcriptional response to bariatric surgery is critically implicated in restoring metabolic homeostasis [15-24]. Type 2 diabetes in adults with obesity is characterized by low-grade inflammation, insulin resistance, and progressive loss of  $\beta$  cells [25-28]. In rodent models that recapitulate obesity-linked type 2 diabetes, duodenal-jejunal bypass surgery results in reduced inflammation, improved glucose tolerance and insulin sensitivity, and increased regeneration and proliferation of  $\beta$  cells [20-22]. These phenotypic changes have been attributed to decreased expression of proinflammatory cytokine genes [21], modulation of gene expression in the insulin signaling pathway [20], and activation of cell cycle regulator genes [22] in the liver, jejunum, adipose tissue, and Langerhans islets. Similarly, in humans, gene expression studies using whole blood, mononuclear cells, and adipose tissue reported a reversal of chronic inflammation simultaneously with a reduction of proinflammatory cytokine and chemokine gene expression after bariatric surgery [19, 23, 24]. Furthermore, the gene expression of *SIRT1*, a known regulator of glucose and lipid homeostasis, is suppressed in obesity and activated by caloric restriction and bariatric surgery concomitantly with weight loss and improvement in glucose metabolism [29, 30]. Together, these studies suggest that the beneficial effect of bariatric surgery can be achieved through a rapid restoration of altered molecular mechanisms underlying the pathogenesis of type 2 diabetes and their downstream effects on biological functions.

In this prospective study, our objective was to analyze the molecular changes specific to adults with obesity and type 2 diabetes who enter complete remission after bariatric surgery and to distinguish them from those who do not. We hypothesized that genes that promote restoration of metabolic homeostasis can serve as potential predictors of the efficacy of bariatric surgery in inducing complete remission of type 2 diabetes. We used whole-genome microarrays of peripheral blood mononuclear

cells (PBMCs) and applied machine-learning models to test this hypothesis. We designed the study to capture early and late dynamic changes in gene expression that begin rapidly after surgery and continue for more than a year. We selected PBMCs because the cells are easily accessible. Furthermore, clinical and experimental studies of gene expression of diet modifications showed that PBMCs reflect an early alteration of lipid and carbohydrate metabolism and inflammation of white adipose and liver tissues [31-35]. Gene expression changes in PBMCs have also been shown to reflect the pathogenesis [36] and pathophysiology [37] of type 2 diabetes associated with obesity. These include genes related to insulin signaling, inflammation, oxidative stress, DNA damage, and lipid metabolism [36, 37]. These studies support using PBMCs as a surrogate to investigate the biology of obesity and diabetes.

## Materials and Methods

### Patients

The institutional review board of King Abdullah International Medical Research Center approved the study protocol. We performed all methods following the ethical principles of the Declaration of Helsinki of the World Medical Association for medical research involving human subjects.

We estimated that the size of the study population is between 10 and 30 patients based on similar transcriptomic studies in adults with obesity and type 2 diabetes treated with bariatric surgery [15-17, 19, 38]. We conducted the study from February 2013 to April 2017 at the King Abdulaziz Hospital in Al Ahsa and the Imam Abdulrahman Bin Faisal Hospital in Dammam, Saudi Arabia. We obtained the written informed consent of all participants. We included adult patients (aged  $\geq 18$  years), men and women with obesity (body mass index [BMI]  $\geq 35$ ), and type 2 diabetes (glycated hemoglobin A<sub>1c</sub> level [HbA<sub>1c</sub>  $\geq 7.0\%$ ]) of less than 5 years. We excluded patients with pregnancy, malignancy, psychiatric disorders, and immunosuppressive therapy. Similarly, we did not include patients who did not consent or could not sign informed consent. Before surgery, we collected demographic information, coexisting hypertension and dyslipidemia, and medication history. We recorded vital signs, height, and body weight and calculated BMI.

### Diabetes Outcomes After Surgery

We considered patients in complete remission of type 2 diabetes to be responders (Rs) if their HbA<sub>1c</sub> level was 5.7% or less without diabetes medication 12 months after bariatric surgery. We considered patients as nonresponders (NRs) if they were in partial remission (HbA<sub>1c</sub> level between 5.8% and 6.4% without diabetes medication) or in no remission (HbA<sub>1c</sub> level of 6.5% or higher or remaining on diabetic medications). We did not choose the recommended HbA<sub>1c</sub> threshold of less than 6.5% for diabetes remission [39] as it falls within the classification of a prediabetic state [40]. However, the strict criteria chosen here reflect a true remission of diabetes, increasing the likelihood of identifying an accurate gene expression response.

### Biochemical Analysis

We obtained blood samples at baseline before surgery (T0), at 2 months (T1), and 12 months (T2) after bariatric surgery. We drew blood by venipuncture in sterile BD vacutainer EDTA

tubes (BD Biosciences) at each time point. We measured fasting glucose, liver, kidney, lipid profiles, and complete blood count using the automated Alinity-CI series system (Abbott). We determined plasma levels of HbA<sub>1c</sub> by high-performance liquid chromatography using the D-100 system (Bio-Rad Laboratories). We measured C-peptide and insulin levels using a sandwich chemiluminescent immunoassay. We determined C-reactive protein (CRP) activity using a turbidimetric method using the Alinity-CI series (Abbot). We isolated PBMC from whole blood using Ficoll-Paque (Ficoll-Paque PLUS; GE Healthcare, Bio-Sciences AB) according to the manufacturer's standard protocol. We stored the PBMCs at  $-80^{\circ}\text{C}$  until analysis.

### Microarray Analysis

According to the manufacturer's instructions, we used the SV Total RNA Isolation Kit (Promega) to extract the total RNA from the PBMCs. We measured the quantity and quality of the RNA using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc). We performed gene-expression profiling of the samples using the Gene Chip Human Genome U133 Plus 2.0 Array (Thermo Fisher Scientific). The array includes 54,000 probe sets to analyze 47,000 transcripts and variants [41]. Briefly, we reverse-transcribed 500 ng of total RNA into double-stranded complementary DNA (cDNA) and then labeled with biotin in cRNA (IVT PLUS reagent kit, Thermo Fisher Scientific). We hybridized approximately 11  $\mu\text{g}$  of labeled and fragmented cRNA in the arrays, then stained and washed the sample on the GeneChip Fluidics Station 450 (Thermo Fisher Scientific) using the GeneChip Hybridization, Wash, and Stain Kit (Thermo Fisher Scientific). We scanned the arrays using an Affymetrix 7000 G scanner (Thermo Fisher Scientific).

### Validation of Microarray Data by Quantitative Real-time Polymerase Chain Reaction

We reverse-transcribed 1000 ng of RNA to cDNA using the high-capacity cDNA reverse-transcription kit (Thermo Scientific) from 59 samples, consisting of T0 ( $n = 21$ ), T1 ( $n = 17$ ), and T2 ( $n = 21$ ), in good quantity and quality following the manufacturer's procedure. We used PowerUp SYBR Green reagent (Thermo Scientific) and the FAST QuantStudioTM 12K Flex system from Applied Biosystems to validate 12 selected genes identified in the transcriptomic analysis according to company instructions (Supplementary Table 1, data set 2 [42]). We used the 18S RNA to normalize the results and mean fold changes in each gene. We calculated the mean fold changes in each gene for each sample using the  $2^{-\Delta\Delta\text{Ct}}$  method as previously described [43].

### Bioinformatics Analysis

#### Quality control and preprocessing of HG-U133 Plus 2.0 microarray data

Before starting the downstream analysis of the microarray oligo-chip data, we first analyzed the data to assess the relative quality of the array chips used in the experiment. We used the pseudo-image plots to evaluate the spatial distribution of the data on the chips. All HG-U133 Plus 2.0 chips were good in terms of the spatial distribution of the data. We evaluated the quality of the CEL files for each time point using the Bioconductor array Quality Metrics program [44]. We used the program for outlier detection in MA plots and differences

between arrays at the two time points. None of the arrays was considered an outlier. Moreover, we used other quality measures such as relative log expression and normalized unscaled standard errors to ensure the quality of the chips and probes therein (Supplementary data set 1 [42]).

#### Downstream analysis of the microarray

After quality control, we processed the raw CEL files using freely available updated chip definition files (CDF) for HG-U133 Plus 2.0 arrays based on Entrez genes (Supplementary data set 1 [42]). We adopted 2-step processes to identify gene sets differentially expressed between 2 time points, using time T0 as a baseline. In the first step, we calculated the variance of gene expression values at all time points (T0, T1, and T2) for each gene in the gene-expression matrix. We analyzed the differentially expressed genes between 2 time points in the second step, considering T0 as a baseline, using the significance analysis of microarrays (SAM) method [45]. We also compared the gene expression data between R and NR patients at the start of T0. The filtered data were analyzed using a linear model to identify genes differentially expressed in R in contrast to NR. We kept the false discovery rate (FDR) threshold at less than 0.05 (Supplementary data set 1 [42]).

#### Gene enrichment analysis

We uploaded to the IPA online application ([www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity)) the differentially expressed genes (DEGs) after bariatric surgery in T1 and T2 for the entire cohort ( $n = 26$  patients) and for R ( $n = 16$ ) and NR ( $n = 10$ ). We analyzed the association of DEGs with canonical pathways and biological functions and their predicted activation states. IPA calculates an overlap  $P$  value associated with each function or pathway using a right-tailed Fisher exact test. This estimates the probability that the association between our data set of DEGs and a given pathway is due to random chance. A  $P$  value less than .05 indicates a statistically significant nonrandom association. IPA also predicts the activation state of canonical pathways by calculating a  $Z$  score independent of the overlap  $P$  value. A  $Z$  score greater than 1 indicates that a canonical pathway is significantly activated, and a  $Z$  score less than  $-1$  designates inhibition. We used the application's comparison tool to identify similarities and differences between Rs and NRs. Comparison analysis allows for the visualization of the canonical pathways side by side across time points.

#### A predictive model of response to bariatric surgery

We developed a machine-learning-based model to predict the outcome of bariatric surgery preoperatively. We formulated the gene expression data at baseline for Rs and NRs into a supervised 2-class classification problem. We performed all data training and testing performance procedures using the Python module called "Scikit-learn" [46] (Supplementary data set 1 [42]). This model development aimed to select a few genes (or features) that may predict the outcome of bariatric surgery in obese patients before surgery. We adopted a combined approach to feature selection using i) wrapper-based and ii) model-based methods simultaneously. Recursive Feature Elimination with Cross-Validation (RFECV) from Scikit-learn was used as a wrapper method around logistic regression (LR) as a classifier with a default parameter (penalty "l2"; solver = "lbfgs"). RFE initially trains the model on all the features, and the coefficient or feature importance of each

feature is obtained by the classifier or estimator (here, LR). The least important features are then eliminated from the feature set, and this procedure is repeated until the desired number of features are selected. The “SelectFromModel” meta-transformer in Scikit-learn was used to select model-based features, along with an estimator that can produce sparse solutions. Linear models, especially “logistic regression” penalized with L1 norm, is the method of choice to reduce the dimensionality of the data due to its ability to produce sparse solutions, that is, shrink the feature coefficients to zero and select the nonzero coefficients. Here, we used LR (penalty = ‘L1’, solver = ‘liblinear’) as an estimator with “SelectFromModel” to choose the nonzero coefficients and informative features or genes.

To adopt a combined approach of developing a predictive model to classify Rs and NRs using baseline gene expression data, we applied two separate feature selection methods in parallel (as described earlier) along with the computation of DEGs in Rs (vs NRs). The final gene set was selected based on the consensus of these three methods.

### Statistical Analysis

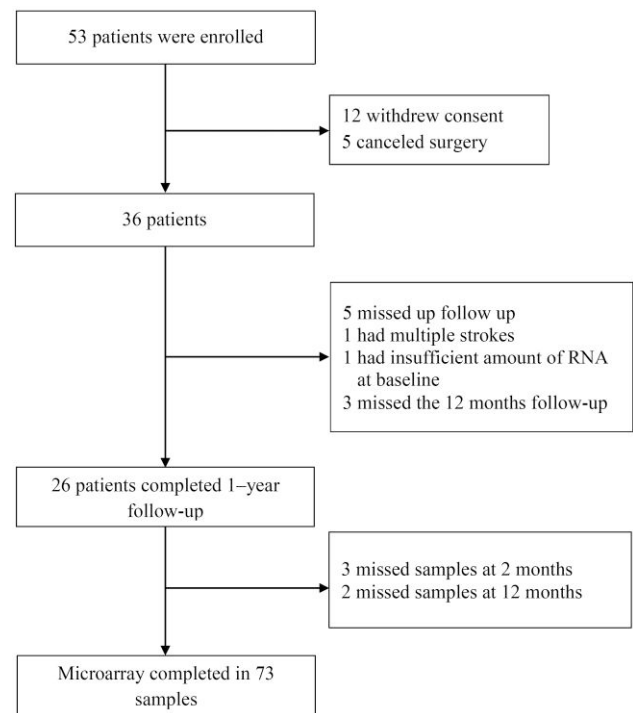
We reported continuous variables as means  $\pm$  SD and summarized categorical variables using frequencies. We modeled the primary end points to analyze the change over time and to compare the R and NR groups using mixed linear models with the main effects of group, time, and interaction terms. The reported *P* values were based on the time-effect and interaction terms from the linear mixed-effects model. Comparisons between groups at a single time point were made using the *t* test for continuous variables with normal distribution or Wilcoxon rank sum tests for skewed distribution and the chi-square or Fisher exact test for categorical variables. We performed all analyses using SAS software, version 9.4 (SAS Institute Inc).

## Results

### Baseline Characteristics

Fifty-three patients met the inclusion criteria and signed the informed consent to participate in the study (Fig. 1). Twelve patients withdrew after signing their consent and 5 did not have surgery. We did not include 10 of the 36 remaining patients in the final analysis. Eight did not complete their 12-month follow-up, 1 patient developed multiple strokes 6 months after bariatric surgery, and 1 had insufficient RNA. Therefore, information on the primary end point was available before and 2 and 12 months after bariatric surgery in 26 patients. Three samples were missing in T1 and 2 in T2.

We show the patients’ characteristics at baseline in Table 1. There were 11 men and 15 women with obesity, a BMI of  $48.8 \pm 8.4$ , and diabetes (fasting blood sugar  $8.9 \pm 2.2$  and HbA<sub>1c</sub>  $7.8 \pm 1.3$ ), all except one receiving 1 to 4 oral antidiabetic medications combined with insulin in 4 patients. Other clinical and metabolic alterations included hypertension, dyslipidemia, inflammation, and hyperuricemia (Tables 1 and 2). The patients underwent various bariatric laparoscopic surgical procedures. These consisted of Roux-en-Y gastric bypass (*n* = 14), biliopancreatic diversion (*n* = 6), sleeve gastrectomy (*n* = 5), and mini-gastric bypass (*n* = 1) (see Table 1).



**Figure 1.** Flow of patients during the study period. CONSORT diagram of 53 patients who signed informed consent and were enrolled. Twenty-nine patients completed all procedures, and 26 completed follow-up at 1 year.

### Metabolic Outcomes

One year after bariatric surgery, we classified *n* = 16 (61.5%) patients as Rs and *n* = 10 (38.4%) as NRs (Fig. 2). We did not find statistically significant differences in demographic, clinical, and biochemical variables, antidiabetic medications, and type of surgery between Rs and NRs before surgery (see Tables 1 and 2).

### Weight and Metabolic Changes After Bariatric Surgery

#### Weight and glucose metabolism

Bariatric surgery reduced the average weight by 15% and 31% at 2 and 12 months from baseline. The weight change was reflected by changes in BMI, which decreased by 15% and 34%, respectively (see Table 1). Fasting blood sugar, plasma HbA<sub>1c</sub>, C-peptide, and insulin levels decreased significantly with time, indicating a progressive improvement in glucose metabolism and restoration of insulin sensitivity. The reduction in body weight was significantly different between Rs and NRs, but not for HbA<sub>1c</sub> and fasting blood sugar (see Table 1). The improvement in glycemic control was paralleled by a progressive decrease over time in antidiabetic medications until their discontinuation in Rs and in 4 of 10 NRs.

#### Metabolic syndrome

Bariatric surgery progressively reversed all biomarkers of metabolic syndrome, including a significant decrease in triglycerides and high-sensitivity CRP levels, an increase in high-density lipoprotein cholesterol, and a reduction in the number of medications needed to treat hypertension (see Table 2). However, antilipid medications decreased in Rs but not in

**Table 1. Demographic data, weight, and glucose metabolism variables at baseline (before surgery) and at 2 and 12 months (after surgery)<sup>a</sup>**

Variables	Total n = 26	R n = 16	NR n = 10
Age, y	40.2 ± 7	40.4 ± 7.6	39.8 ± 6.2
Sex, M/F	11/15	8/8	3/7
Height, cm	162 ± 9.7	163 ± 9.7	159.4 ± 9.6
Weight, kg			
Baseline	128 ± 25.9	133 ± 28	121 ± 19.8
2 mo	109 ± 21.5	112 ± 23.9	104 ± 17.7
12 mo	88 ± 18.8	88 ± 19.2	87 ± 19.3
P	<.0001 <sup>c</sup>	.048 <sup>d</sup>	
BMI			
Baseline	48.8 ± 8.4	49.8 ± 8.6	47.4 ± 8.2
2 mo	41.4 ± 6.7	41.9 ± 6.9	40.7 ± 6.7
12 mo	33.9 ± 6.3	33.2 ± 5.3	35.4 ± 7.8
P	<.0001 <sup>c</sup>	.146 <sup>d</sup>	
Fasting blood glucose mmol/L			
Baseline	8.9 ± 2.2	7.9 ± 2.2	8.7 ± 2.3
2 mo	6.3 ± 0.9	6.0 ± 0.7	6.7 ± 0.9
12 mo	5.2 ± 0.9	4.9 ± 0.4	5.8 ± 1.1
P	<.0001 <sup>c</sup>	.942 <sup>d</sup>	
HbA <sub>1c</sub> , %			
Baseline	7.8 ± 1.3	7.7 ± 1.4	7.9 ± 1.2
2 mo	6.2 ± 0.8	5.9 ± 0.7	6.7 ± 0.7
12 mo	5.5 ± 0.7	5.3 ± 0.6	6.1 ± 0.7
P	<.0001 <sup>c</sup>	.491 <sup>d</sup>	
Insulin level, mmol/L			
Baseline	18.8 ± 11.8	15.9 ± 10.6	23.6 ± 12.9
2 mo	9.6 ± 8.2	8.9 ± 8.2	10.6 ± 8.6
12 mo	5.3 ± 3.1	5.6 ± 3.6	4.9 ± 2.4
P	<.0001 <sup>c</sup>	.102 <sup>d</sup>	
C-peptide, ng/mL			
Baseline	3.3 ± 1.4	3.3 ± 1.3	3.5 ± 1.5
2 mo	3.0 ± 1.8	3.0 ± 2.2	2.9 ± 0.9
12 mo	1.7 ± 0.9	1.5 ± 0.8	1.9 ± 1.1
P	<.0001 <sup>c</sup>	.619 <sup>d</sup>	
Medications, No.			
Antidiabetic			
Baseline	25	16	9
2 mo	14	7	7
12 mo	6	0	6
		.385 <sup>b</sup>	
Type of surgery <sup>e</sup>			
RYGB	14	8	6
BPD	6	5	1
SLG	5	3	2

(continued)

**Table 1. Continued**

Variables	Total n = 26	R n = 16	NR n = 10
LGB	1	0	1
		.428 <sup>b</sup>	

Abbreviations: BMI, body mass index; BPD, biliopancreatic diversion without duodenal switch; F, female; HbA<sub>1c</sub>, glycated hemoglobin A<sub>1c</sub>; LGB, laparoscopic mini gastric bypass; M, male; NR, nonresponders; R, responders; RYGB, Roux-en-Y gastric bypass; SLG, sleeve gastrectomy.

<sup>a</sup>Mean ± SD.

<sup>b</sup>P values are determined by the *t* test for continuous variables and the chi-square or Fisher exact test for categorical variables.

<sup>c</sup>P values for the main effect of time (baseline, 2, and 12 months after bariatric surgery).

<sup>d</sup>P values of the interaction effect of time (baseline, 2, and 12 months after bariatric surgery) and groups, R (responders) vs NR (nonresponders).

NRs. There were no significant differences in any metabolic syndrome variables examined in this study between Rs and NRs (see Table 2).

### Transcriptional Changes After Bariatric Surgery

The analysis identified 2150 DEGs in T1 and 2711 in T2, compared to baseline (T0), using a *P* value less than .05 and an FDR of less than 0.05 after Benjamini-Hochberg correction for multiple tests. The percentage of DEGs in T1 and T2 was 3.98% and 5.02% of the total genes included in the GeneChip array, respectively [41]. Pathway enrichment analysis showed that DEGs were related to proteolysis and stress response, senescence, and apoptosis (ubiquitination signaling, sumoylation signaling, and EIF2 signaling), immune response, and cytokine signaling (T-cell receptor signaling, Cdc 42, Th1 signaling, and antigen presentation signaling) both in T1 and T2 after surgery (Supplementary Fig. 1, data set 1 [42]). In the context of type 2 diabetes, ubiquitination, sumoylation, Cdc 42, and immune signaling are involved in glucose metabolism, insulin regulation, and the development of insulin resistance in obesity [47-50].

### Transcriptional Changes in Responders and Nonresponders After Bariatric Surgery

The analysis identified 1217 DEGs in Rs and 1648 in NRs in T1 compared to their respective baseline expression data. In T2, there were 1191 DEGs in Rs and 1650 in NRs. The heat map representing an unsupervised hierarchical cluster analysis (Fig. 3A) and principal component analysis (Fig. 3B) revealed different responses of gene expression to bariatric surgery between Rs and NRs, supporting the hypothesis that molecular alterations over time distinguish patients in whom diabetes enters complete remission vs those in which it did not. The Venn diagram (Fig. 3C) displays the number of distinct and shared genes between Rs and NRs in T1 and T2.

### Difference in Gene and Canonical Pathways Between Responders and Nonresponders

The DEGs were significantly associated with 55 and 230 canonical pathways in Rs and NRs in T1 (Supplementary Tables 2A and 2B, data set 2 [42]). In T2, DEGs were associated with 73 pathways in Rs and 184 pathways in NRs (Supplementary Tables 3A and 3B, data set 2 [42]) using a

**Table 2. Effects of bariatric surgery on metabolic syndrome variables at baseline (before surgery) and 2 and 12 months (after surgery)<sup>a</sup>**

Variables	Total n = 26	R n = 16	NR n = 10
Triglycerides, mmol/L			
Baseline	1.75 ± 1.5	1.83 ± 1.88	1.61 ± 0.56
2 mo	1.50 ± 0.8	1.53 ± 0.90	1.44 ± 0.69
12 mo	1.05 ± 0.5	0.99 ± 0.48	1.16 ± 0.43
<i>P</i>	.036 <sup>b</sup>	.485 <sup>c</sup>	
HDL, mmol/L			
Baseline	1.06 ± 0.3	1.05 ± 0.4	1.07 ± 0.2
2 mo	0.96 ± 0.2	0.92 ± 0.3	1.02 ± 0.3
12 mo	1.19 ± 0.3	1.16 ± 0.4	1.23 ± 0.3
<i>P</i>	<.0001 <sup>b</sup>	.751 <sup>c</sup>	
Uric acid, mmol/L			
Baseline	347 ± 112	346 ± 113	347 ± 115
2 mo	361 ± 136	361 ± 127	358 ± 155
12 mo	295 ± 98	296 ± 93	292 ± 109
<i>P</i>	<.0001 <sup>b</sup>	.924 <sup>c</sup>	
CRP, mg/L			
Baseline	13.4 ± 9.4	12.1 ± 8.6	16 ± 10.9
2 mo	8.2 ± 5.2	7.4 ± 3.6	9.8 ± 7.6
12 mo	3.7 ± 3.8	3.5 ± 2.8	4.1 ± 5.3
<i>P</i>	<.0001 <sup>b</sup>	.846 <sup>c</sup>	
Medications, No.			
Antilipid			
Baseline	14	10	4
2 mo	13	8	5
12 mo	10	4	6
		.422 <sup>d</sup>	
Antihypertensive			
Baseline	9	4	5
2 mo	8	4	4
12 mo	5	3	2
		.234 <sup>d</sup>	

Abbreviations: CRP, C-reactive protein; NR, nonresponders; R, responders.  
<sup>a</sup>Mean ± SD.

<sup>b</sup>*P* values for the main effect of time (baseline, 2, and 12 months after bariatric surgery).

<sup>c</sup>*P* values of the interaction effect of time (baseline, 2, and 12 months after bariatric surgery) and groups, R (responders) vs NR (nonresponders).

<sup>d</sup>*P* values determined by *t* test.

threshold value of *P* less than .05. Comparison analysis revealed that Rs and NRs shared the same predicted activation or inhibition patterns in the most enriched metabolic and signaling canonical pathways. However, 5 canonical pathways differed between Rs and NRs in directionality, expression changes in critical genes, and functional biological end points (Fig. 4). These pathways are related to the regulation of β-cell survival, proliferation, and regeneration (Hippo signaling pathway), regulation of energy metabolism and insulin signaling (sirtuin and *MSP* [macrophage-stimulating protein]-*RON* [recepteur d'origine nantais] signaling pathways), inflammation and oxidative stress (sirtuin, inhibition of ARE [AU-rich elements]-mediated messenger RNA (mRNA) degradation, and *MSP-RON* signaling pathways), and neurodegeneration and neuronal cell death (Huntington disease signaling pathways).

The heat map of the Hippo signaling pathway gene revealed that 18 genes were differentially regulated between R and NR, leading to increased cell proliferation (Supplementary Table 4, data set 2 [42]). Among these genes, *SKT4* (serine/threonine kinase 4), also known as mammalian sterile 20-like kinase (*MST1*) 1, a critical mediator of β-cell apoptotic death and failure in diabetes, was strongly upregulated in NRs but not in Rs (Fig. 5).

Seventy-two genes, including *SIRT1* (sirtuin 1), were differentially regulated between Rs and NRs in the sirtuin signaling pathway, leading to opposite downstream biological functions (Supplementary Table 5, data set 2 [42]). *SIRT1* was upregulated in Rs but not in NRs, leading to a signaling cascade with the activation of glycolysis, lipolysis, adipogenesis, oxidative stress, and Alzheimer disease (Fig. 6).

Inhibition of ARE-mediated mRNA degradation and *MSP-RON* signaling in macrophage pathways revealed different regulatory mechanisms of inflammation after bariatric surgery (Supplementary Tables 6 and 7, data set 2 [42]). For example, genes in the tumor necrosis factor (TNF) superfamily were upregulated in NRs but not in Rs, leading through two signaling cascades to enhance mRNA stability and thus sustained inflammation, suggesting a role for regulated mRNA decay in homeostasis after bariatric surgery (Supplementary Fig. 2, data set 1 [42]). However, the signaling pathway of *MSP-RON* in macrophages predicted a dual response of increased anti-inflammatory response. It decreased inflammation mediated by upregulation of signal transducer and activator of transcription 3 (*STAT3*) and downregulation of nuclear factor kappa B (*NFκB*), respectively (Supplementary Fig. 3, data set 1 [42]).

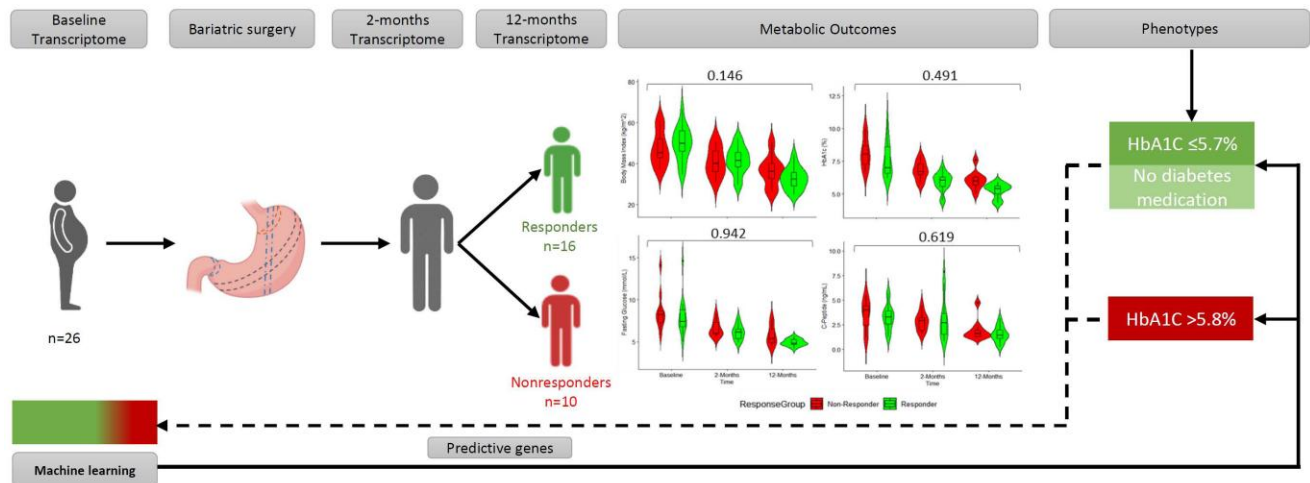
Huntington signaling pathways were predicted to be upregulated in NRs but not in Rs in T1 and T2 (Supplementary Table 8, data set 2 [42]). This led to a signaling cascade that included an increase in the calpain, heat shock protein 70 (*HSP70*), ubiquitin, and proteasome 26 genes, resulting in a predicted increase in neuronal cell death and neurodegeneration (Supplementary Fig 4, data set 1 [42]).

### Difference in Transcriptome Between Responders and Nonresponders at Baseline

Using the characterized metabolic response phenotypes after bariatric surgery, we compared the baseline transcriptome of Rs and NRs before surgery (see Fig. 2). We identified a significant difference in DEGs and their predicted downstream biological functions between Rs and NRs. Notably, genes associated with DNA replication, recombination, and repair and involved in various cancer signaling pathways were distinct (Supplementary Fig. 5, data set 1 [42]).

### Early Prediction of Response to Bariatric Surgery

With baseline gene expression data for Rs and NRs, we developed predictive models to identify Rs and NRs before surgery. We selected the best set of genes that could contribute the most to this classification (Fig. 7). Model-based LR with L1 regularization (feature selection 1) yielded 35 genes (GeneSet A) with nonzero coefficients. *RFECV* with LR (feature selection 2) selected 10 genes (GeneSet B). The differential analysis of Rs vs NRs identified 4200 DEGs (GeneSet C) with an FDR cutoff of 0.05. The consensus of these sets of genes produced the final set of genes, which included 10 genes (*ARAF* [A-Raf proto-oncogene, serine/threonine



**Figure 2.** Graphical summary of the study design. Twenty-six patients with obesity and type 2 diabetes who underwent bariatric surgery were studied. Plasma and peripheral blood mononuclear cells before surgery and at 2 and 12 months after surgery were used to determine fasting blood sugar, glycated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>), C-peptide, and transcriptome. Body mass index, fasting blood sugar, HbA<sub>1c</sub>, and C-peptide before and after surgery are shown as violin plots. The responders vs nonresponders phenotypes were based on HbA<sub>1c</sub> and antidiabetic medications 12 months after surgery. The machine-learning method applied to a pool of 20,183 genes at baseline after classification into responders and nonresponders was used to identify genes that can predict the metabolic outcome preoperatively.

kinase], *DNMT3A* [DNA methyltransferase 3 alpha], *GMDS* [GDP-mannose 4,6-dehydratase], *HUWE1* [HECT, UBA and WWE domain containing E3 ubiquitin protein ligase 1], *KLF7* [KLF transcription factor 7], *LGMN* [legumin], *PEX14* [peroxisomal biogenesis factor 14], *PP1E* [peptidyl-prolyl isomerase E], *RNF157* [ring finger protein 157], and *STX6* [syntaxin 6]) (Table 3). The feature selection was applied to the training set (containing 60% of the samples). The performance of these selected genes was evaluated on an independent test set (containing 40% of the samples), resulting in a precision, recall, F1 score, and accuracy of 1.0 (Fig. 7A). In conclusion, the model based on these final 10 genes was able to correctly predict the response to bariatric surgery.

To show that feature selection and predictive modeling were not random, the expression values of 10 selected genes were plotted (Fig. 7B) and another 10 genes were randomly selected to see the distribution of these genes in the R and NR samples. The distribution of expression between two groups (R and NR) contrasts with the selected genes, whereas for randomly selected genes, it is homogeneous between R and NRs.

## Discussion

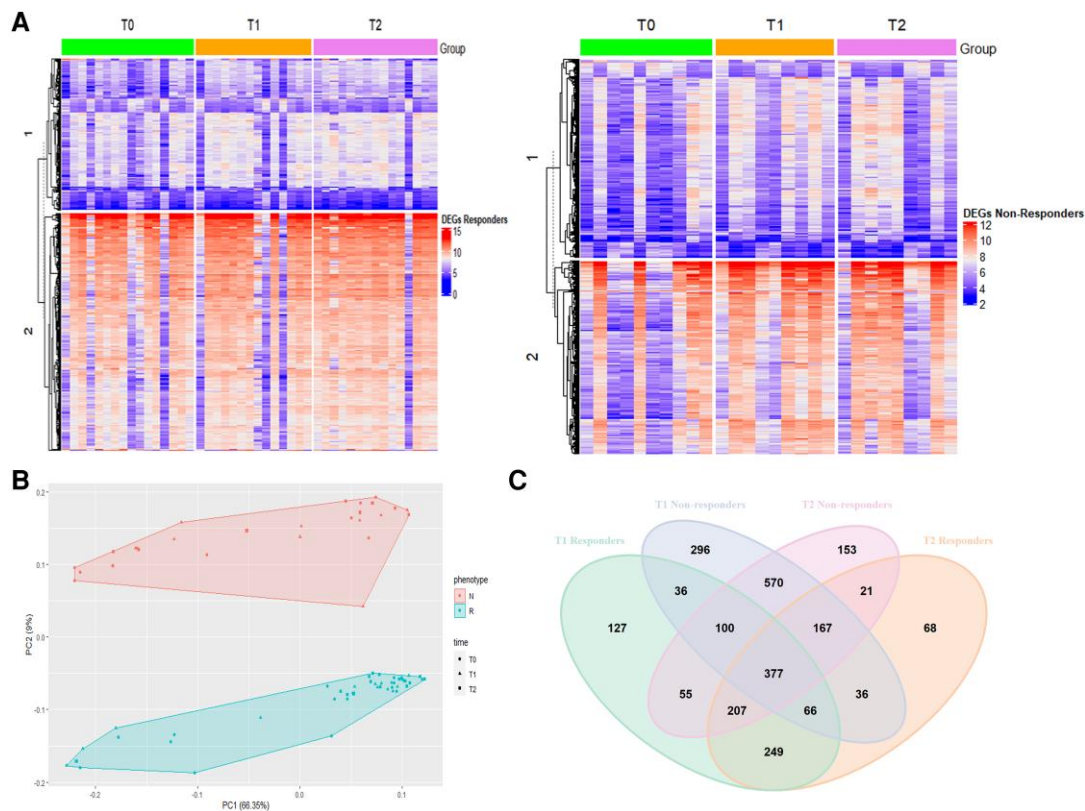
Previous studies have described molecular changes secondary to bariatric surgery in adults with obesity and type 2 diabetes [15, 19, 63-66]. However, none have evaluated the molecular modifications at the whole-genome level associated with different phenotypes after bariatric surgery to understand why diabetes enters complete remission in some but not in others. This knowledge is essential for identifying predictive molecular biomarkers and potential therapeutic targets. Here, we use genome-wide transcriptional profiling combined with machine-learning methods in a prospective cohort of adults with obesity and type 2 diabetes who were followed for a year after bariatric surgery to identify gene signatures and pathways that can distinguish patients who achieved complete diabetes remission (R) from those who did not (NR). We found that bariatric surgical therapy effectively induced

complete remission of type 2 diabetes at 1 year in approximately two-thirds of the patients, in agreement with previously published data [3, 4].

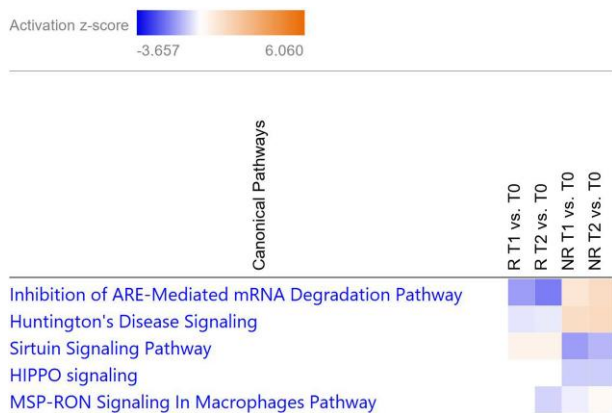
We showed that the transcriptomic changes in peripheral blood mononuclear cells after surgery differed between Rs and NRs in the expression of critical genes (*STK4*, *SIRT1*, and *TNF* superfamily), key metabolic and signaling pathways (Hippo, Sirtuin, MSP-RON, inhibition of ARE-mediated mRNA degradation, and Huntington), and predicted functional biological end points ( $\beta$ -cell survival and regeneration, regulation of energy metabolism and insulin signaling, inflammation and neurodegeneration). However, more studies are needed with a larger population and comparable bariatric surgical procedures to validate these findings. This is because one cannot rule out the possibility that the observed transcriptomic changes between Rs and NRs may be related to the difference in weight loss and type of surgery, regardless of metabolic outcomes.

We observed notably higher rates of type 2 diabetes remission among patients who underwent biliopancreatic diversion. This is consistent with previous studies showing that biliopancreatic diversion achieved greater weight loss and a higher diabetes remission rate than other bariatric surgery [67, 68]. Rodent studies have reported differential alterations in molecular pathways according to the type of surgery, including biliopancreatic diversion [69, 70]. Therefore, one cannot exclude that specific surgical procedures may exert differential effects on regulating key metabolic genes and signaling pathways. Given that our study did not control the particular types of bariatric surgery performed, future investigations would benefit from employing a more homogenized surgical approach or categorizing patients based on their surgical procedure.

Loss of pancreatic  $\beta$ -cell mass, dysfunction, and failure to produce insulin to meet the progressively increased metabolic load due to obesity are the ultimate events that lead to type 2 diabetes [26]. It is reflected clinically, as in our patients, by the increasing number of oral antidiabetic medications over time and, finally, the prescription of insulin therapy. On the



**Figure 3.** Transcriptomic changes in adults with obesity in complete (r) and incomplete (NR) diabetes remission after bariatric surgery. A, Heat map of unsupervised hierarchical clustering (only on rows, ie, genes) showing the differentially expressed genes (DEGs) in R and NR separately across different time points (T0, T1, and T2). Each column represents the gene expression profile of a patient. Horizontal color bars at the top indicate the study time points T0 (baseline, before surgery), T1 (2 months after surgery), and T2 (12 months after surgery) for each group responders (R) or nonresponders (NR). The color scale is the normalized expression values from microarray data. B, Principal component analysis of gene expression data (containing approximately 20,000 genes) in R and NR. The first two principal components (explaining ~75% of variance) were able to separate R and NR samples. C, The Venn diagram shows the number of different and shared DEGs in R (n = 16) and NR (n = 10) in T1 and T2 after bariatric surgery.



**Figure 4.** Differential metabolic and signaling pathways between complete and incomplete diabetes remission after bariatric surgery. Comparison analysis of canonical pathways based on the Z score in responders (R) and nonresponders (NR) at 2 months (T1) and 12 months (T2) after bariatric surgery relative to before surgery (T0). The yellow and blue colors indicate the pathways that are predicted to be activated (Z score > 1) or inhibited (Z score < -1), respectively.

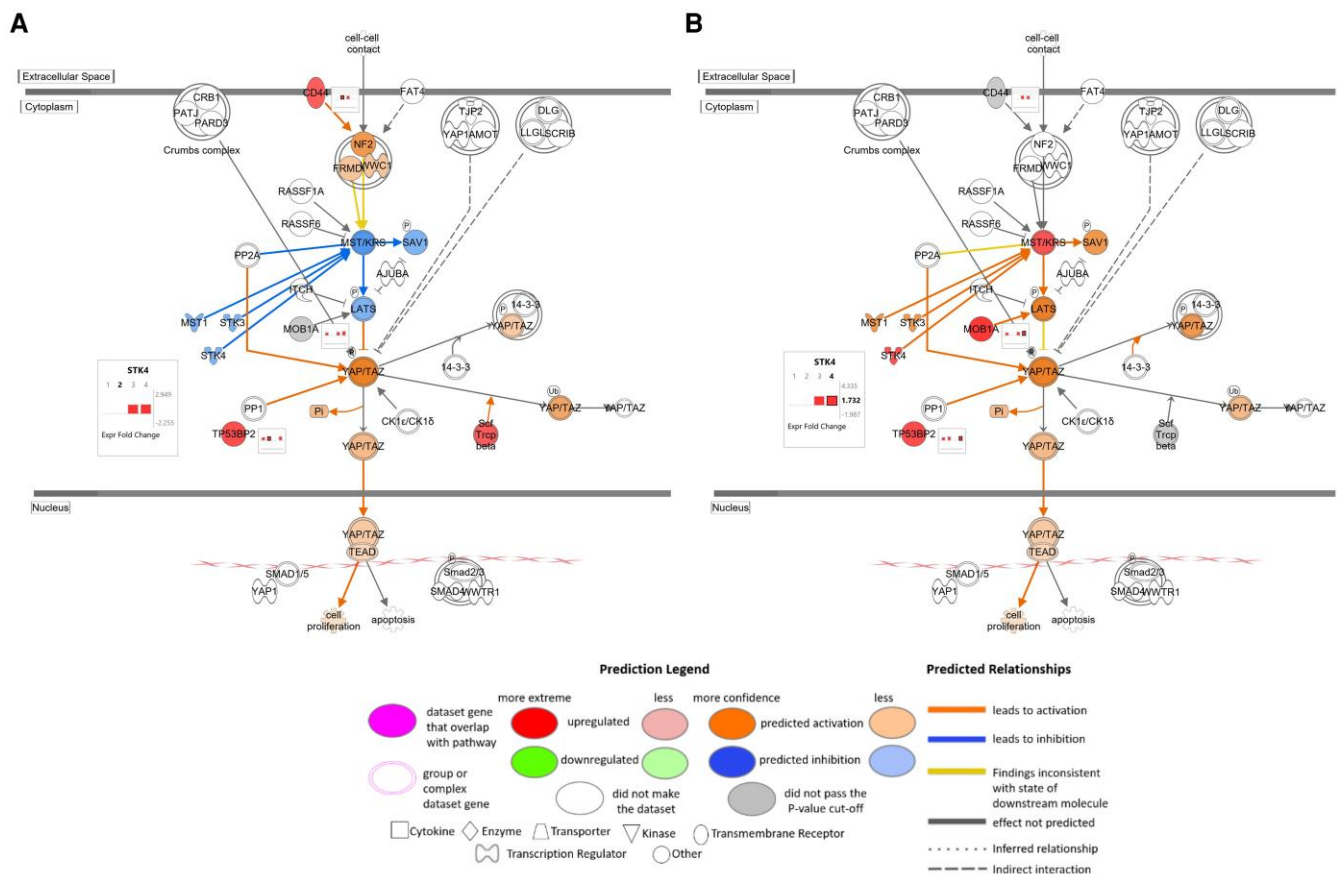
contrary, the reduction of antidiabetic medications until their total discontinuation, as in our Rs after bariatric surgery, indicates a restoration of  $\beta$ -cell function. The mass and function of  $\beta$  cells depend on complex signaling pathways that regulate the regeneration, proliferation, and survival of  $\beta$  cells [71].

Our transcriptomic analysis shows that the Hippo signaling pathway, which controls organ size in various species, including the pancreas in humans, is predicted to be activated in Rs and inhibited in NRs [72]. *STK4* (*MST1*), a central gene that encodes a kinase and mediates  $\beta$ -cell dysfunction and death in diabetes, was highly expressed in NRs [72]. *STK4* inhibition is known to restore  $\beta$ -cell mass and function, thus contributing to the normalization of glycemia [72, 73]. Therefore, the findings suggest that bariatric surgery may restore metabolic homeostasis by modulating the Hippo signaling pathway for the first time.

The sirtuin family, including *SIRT1*, are NAD<sup>+</sup> protein-dependent deacylases that function as energy sensors to maintain energy homeostasis [30]. *SIRT1* expression is reduced in obesity and restored by caloric restriction, including bariatric surgery [29, 30]. Consistent with these observations, the present study showed that the *SIRT1* gene is upregulated in Rs but not in NRs, although the reason behind this dichotomous response remains unclear. However, these findings indicate that the failure of bariatric surgery to restore bioenergetics balance through *SIRT1* signaling could have contributed to the incomplete remission of diabetes.

Insulin resistance is a characteristic feature of type 2 diabetes and obesity [27]. One of the mechanisms that contributes to insulin resistance is chronic inflammation [28]. Consequently, caloric restriction and bariatric surgery have been used to mitigate inflammation, despite contrasting outcomes [19, 74]. In





**Figure 5.** Hippo signaling pathway with prediction of overlaid molecular activity in A, responders, and B, nonresponders after bariatric surgery. A, Diagram of the canonical Hippo signaling pathway showing 6 genes upregulated (red) and 12 genes that did not reach statistical significance (gray) 12 months after bariatric surgery (T2) in responders (R), and B, 13 genes upregulated (red) and 5 genes that did not reach statistical significance (gray) in nonresponders (NR), along with predictions of biological functions. Cell proliferation (colored orange) is predicted to increase both in R and NR. The inset square shows that the *STK4* gene is upregulated in T1 (3) and T2 (4) in NR but not in R (1 and 2). Pathway and molecular activity prediction analyses were generated using QIAGEN Ingenuity Pathway Analysis (IPA, QIAGEN, [www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity)).

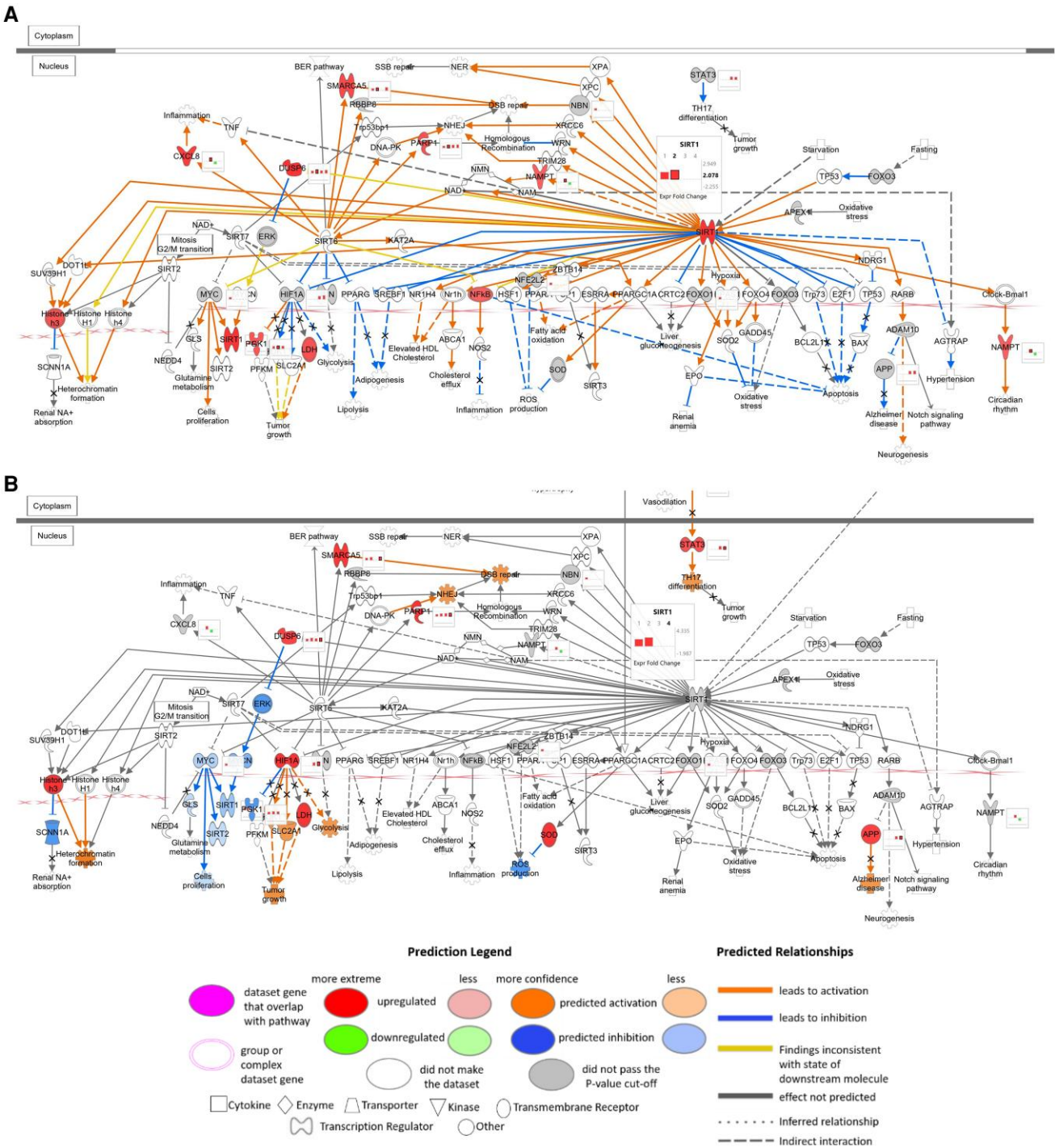
the present study, we identified several significantly enriched signaling pathways for inflammation, including control of mRNA stability (inhibition of the ARE-mediated mRNA degradation signaling pathway). AREs target mRNAs that play a critical role in regulating gene transcription during cell growth, differentiation, and the immune response to rapid degradation [75]. The failure of ARE-mediated decay has been associated with abnormal cell proliferation, leading to several cancers or sustained inflammation in conditions such as polyarthritis [76]. Here, we show that NRs, but not Rs, exhibit an increased expression of the TNF superfamily gene related to the failure of the mRNA decay mechanism to degrade TNF mRNA. To our knowledge, this is the first study to suggest a possible role of ARE-mediated mRNA decay in the inflammation of type 2 diabetes. Accordingly, the finding requires further confirmation.

MSP functions through the RON transmembrane receptor kinase tyrosine kinase/receptor protein to modulate the M1/M2 polarization of macrophages. Activation of Ron shifts the M1/M2 balance toward M2, or an anti-inflammatory phenotype, by inhibiting proinflammatory tissue-resident M1 macrophages, reducing tissue injury and damage, and promoting a repair phenotype [77, 78]. However, studies using Ron knockout mice showed that Ron plays both a protective [78] and deleterious [79] role in diet-induced obesity, atherosclerosis, and hepatic steatosis, although the cause of

this discrepancy remains unclear [78, 79]. In the present study, we show that this pathway is activated in NRs, promoting the anti-inflammatory response and inhibiting NF $\kappa$ B-mediated inflammation compared to Rs. Taken together, the results suggest that bariatric surgery modulates the inflammatory state through diverse and complex signaling pathways both in Rs and NRs; however, the net effect remains challenging to appreciate.

Based on distinct phenotypes after bariatric surgery, we compared the baseline transcriptome of Rs and NRs before surgery. We identified a significant difference between Rs and NRs, notably genes associated with DNA replication, recombination, and repair involved in various cancer signaling pathways. Many studies have shown that increased DNA damage and altered DNA repair signaling mechanisms may contribute to the pathogenesis of type 2 diabetes and its multi-organ complications [80]. Therefore, these findings suggest that genes involved in DNA damage repair before surgery may be critical determinants of the metabolic outcome of bariatric surgery.

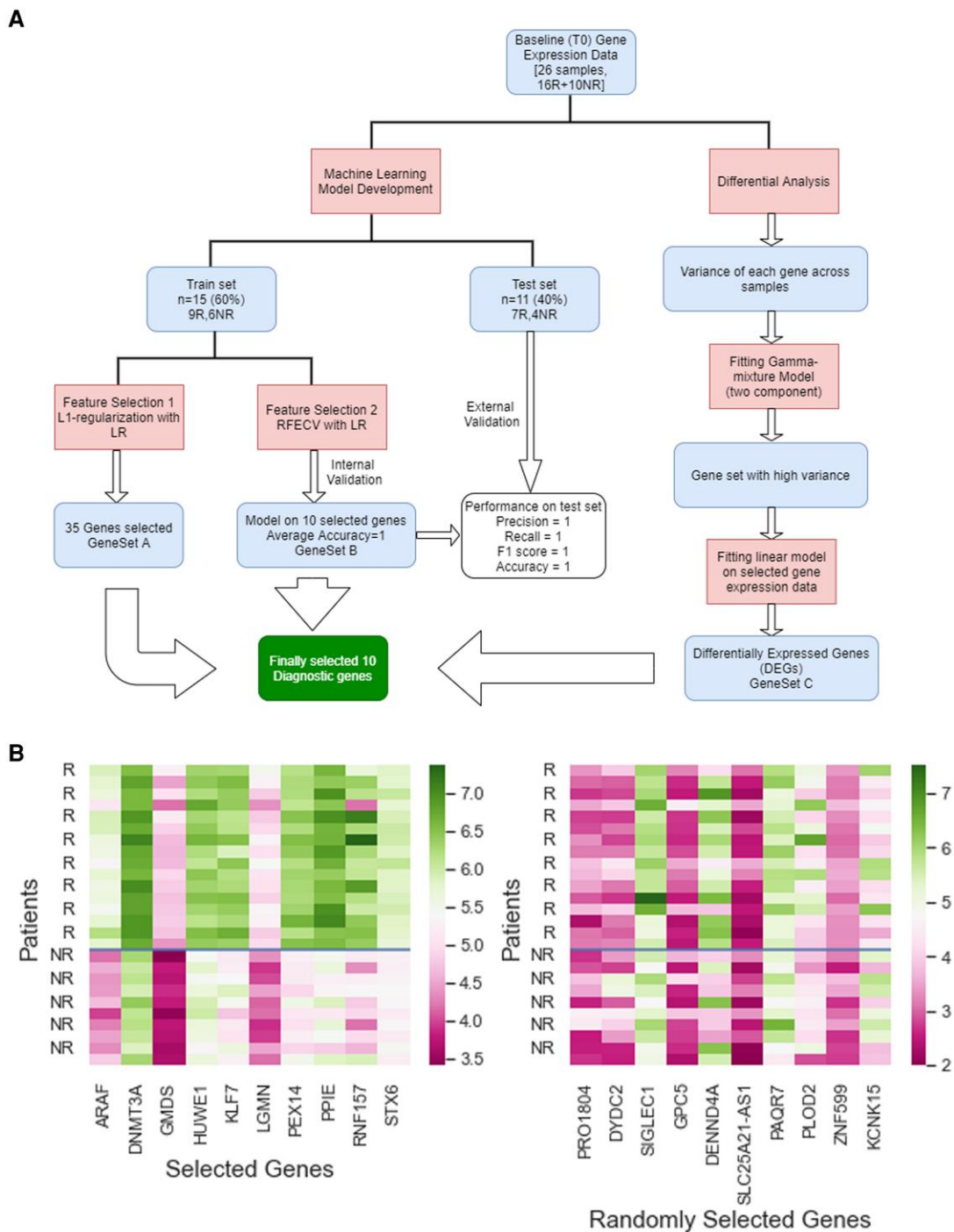
This study also investigated the role of the baseline transcriptome in predicting the metabolic response to bariatric surgery. Unlike previous studies that used genetic variants, we chose genes from the baseline transcriptome associated with the phenotypes R vs NR after bariatric surgery [12, 13]. We



**Figure 6.** Diagram of the SIRT1 signaling pathway with predicted molecular activity overlaid on A, responders, and B, nonresponders after bariatric surgery. A, Diagram of the canonical SIRT1 signaling pathway showing 23 genes upregulated (red) and 1 downregulated (green) and 48 genes that did not reach statistical significance (gray) 12 months after bariatric surgery (T2) in responders (R). B, In nonresponders (NR), 50 genes were upregulated and 22 genes did not reach statistical significance, along with predictions of biological function. Cell proliferation, inflammation, neurogenesis, and elevated high-density lipoprotein (HDL) cholesterol (colored orange) are predicted to increase, while apoptosis, oxidative stress, hypertension, Alzheimer disease, and renal anemia (colored blue) are predicted to decrease in A, responders, and B, the inverse in NR. The inset square shows that SIRT1 is upregulated in T1 (1) and T2 (2) in R but not in NR (3 and 4). Pathway and molecular activity prediction analyses were generated using QIAGEN Ingenuity Pathway Analysis (IPA, QIAGEN, [www.qiagen.com/ingenuity](http://www.qiagen.com/ingenuity)).

modeled the baseline expression data for these genes using state-of-the-art machine-learning methods. This has resulted in an accurate model comprising 10 genes that predict the metabolic outcome of bariatric surgery in adults with obesity and type 2 diabetes before surgery. These include *ARAF*,

*DNMT3A*, *GMDS*, *HUWE1*, *KLF7*, *LGMN*, *PEX14*, *PPIE*, *RNF157*, and *STX6*. Notably, most of these genes have previously been associated with type 2 diabetes and/or obesity, hence underlining their relevance [51, 52, 54-58, 60]. This finding supports the notion that genes related to type 2 diabetes



**Figure 7.** Modeling of baseline transcriptome using machine learning. A, A predictive model to classify responders (R) and nonresponders (NR) using baseline gene expression data. Two different selection methods were applied in parallel using machine learning and computation of differentially expressed genes in R vs NR. Data training and testing performance were performed using the Python module “Scikit-learn.” The final gene set was selected based on the consensus of these methods. B, Heat map shows normalized gene expression profiles for the 10 most significant genes selected by the machine-learning approach (left) and for 10 randomly sampled genes (right) from a pool of 20,183 genes in 26 patients before bariatric surgery. A blue horizontal line separates responders (R) from nonresponders (NR).

and obesity before surgery could potentially aid in the prediction of the type 2 diabetes metabolic response to bariatric surgery. However, due to the small number of patients used in this model, more studies are needed to replicate it.

The present study has limitations. The number of NRs included in the transcriptomic analysis was relatively low. Consequently, additional genes and biological mechanisms

underlying the metabolic response of bariatric surgery could be identified by studying a more substantial number of Rs and NRs. Moreover, a study of a larger cohort of different populations is warranted to establish whether our approach can be generalized. The present study was designed to assess molecular changes at 2 and 12 months after bariatric surgery; however, during this period, we had no control over

**Table 3. Genes established at baseline differentiate responders vs nonresponders after bariatric surgery**

Gene	Association with type 2 diabetes and obesity	References
<i>ARAF</i> (A-Raf proto-oncogene, serine/threonine kinase)	Contribute to survival of $\beta$ cells	[51]
<i>DNMT3A</i> (DNA methyltransferase 3a)	An epigenetic mediator of insulin resistance in vitro and in vivo	[52]
<i>GMDS</i> (GDP-mannose 4,6-dehydratase)	Association between a variant of GMDS and childhood obesity	[53]
<i>HUWE1</i> ( <i>HECT</i> , <i>UBA</i> , and <i>WWE</i> domains that contain E3 ubiquitin protein ligase 1)	Expression in pancreas is essential to determine mass of $\beta$ cells	[54]
<i>KLF7</i> (Kruppel-like factor 7)	Metabolic regulator modulating insulin sensitivity in pancreatic $\beta$ cells and skeletal muscle cells	[55]
<i>LGMN</i> ( <i>Legumain</i> )	The gene encodes a cysteine protease that may be involved in the regeneration of $\beta$ cells after pancreatectomy	[56]
<i>PEX14</i> (peroxisomal biogenesis factor 14)	Peroxisomes are involved in metabolism of reactive oxygen species and lipids	[57]
<i>PPIE</i> <sup>a</sup> (peptidylprolyl isomerase E)	Normal peroxisome metabolism is essential for the structure and function of $\beta$ cells	[58]
<i>RNF157</i> (ring finger protein 157)	Association with gestational diabetes mellitus	[59]
<i>STX6</i> (Syntaxin 6)	Association between variant of <i>RNF157</i> and type 2 diabetes	[60]
	Regulation of autophagy in adipose tissue	[61]
	Circadian regulation of the pancreatic beta cell	[62]
	Regulation of glucose transporters in adipocytes	

<sup>a</sup>No association of PPIE with type 2 diabetes and obesity has been reported.

each patient's energy intake and physical activity, so we cannot rule out their potential influence on the outcome. Last, the predicted biological functions would need to be validated, as genes that were significantly expressed in this study undergo further posttranscription regulation, which makes the prediction of their downstream biological effects uncertain.

The study has strengths: the clear segregation of the outcome of bariatric surgery into two distinct phenotypes that allow further downstream analysis of diabetes remission and nonremission and its relationship to changes in the transcriptome. Similarly, strict predefined criteria were used to define diabetes remission, which maximizes the chances of finding gene expression changes linked to an accurate diabetes response. While our study focuses on the utility of transcriptomic data in predicting diabetes remission following bariatric surgery, it is important to consider the synergistic value of combining these molecular insights with established clinical factors. Previous research has demonstrated the predictive power of clinical variables such as disease duration [81, 82] and baseline C-peptide levels [83, 84]. A more holistic model that integrates transcriptomic data and these clinical markers could offer enhanced predictive accuracy and clinical utility. This integrative approach could form the basis of future research to refine predictive models for diabetes remission post-bariatric surgery.

In conclusion, the analysis of mononuclear cell gene expression after bariatric surgery revealed similarities, but also differences, in critical genes and pathways between adults with obesity and type 2 diabetes who enter complete remission of diabetes vs those who do not. It also suggested that gene expression patterns before surgery can serve as a framework to identify genes that could help predict preoperatively the metabolic outcomes of bariatric surgery.

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## Author Contributions

Study conception and design: AB, CL, AAQ, SM. Data collection: SAM, MLA, RAA. Analysis and interpretation of results: AB, AAQ, MR, SM, RAA, SAM, OA, AK. Draft manuscript preparation: AB, AAQ, MR, SM, SAM. All authors reviewed the results and approved the final version of the manuscript.

## Disclosures

The authors declare that no relationships or activities might bias or be perceived to bias their work.

## Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author at a reasonable request.

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