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Metabonomic model for the assessment of type 2 diabetes remission after bariatric/metabolic surgery

Modelo metabonômico para avaliação da regressão do diabetes mellitus tipo 2 após cirurgia bariátrica

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A B S T R A C T

Purpose: To evaluate the differences in the metabonomic profile of patients who achieved remisison of Type 2 diabetes mellitus (T2DM) after bariatric surgery in relation to those who presented maintenance or recurrence of this condition after surgery. **Methods:** Thirthy-three patients with obesity and T2D were submitted to bariatric/metabolic surgery, among which, 22 experienced complete remission of T2D, and 11 did not experience remission in the postoperative period. Blood samples were taken in order to assess the serum profiles through a 1H NMR-based metabonomic study. **Results:** The metabonomic model for the assessment of T2D recurrence presented an accuracy of 93.9%, sensibility of 81.8%, specificity of 100%, positive predictive value of 100% and a negative predictive value of 91.7%. **Conclusion:** bariatric surgery provide specific effects on the distribution of metabolites in those patients who achieved remission of T2DM, and this new distribution can be assessed through a metabonomic model.

HEADINGS: Bariatric Surgery. Diabetes Mellitus, Type 2. Obesity. Metabolomics. Metabolism.

INTRODUCTION

Obesity is a disease characterized by chronic and excessive accumulation of adipose tissue due to the long-lasting imbalance between energy intake and expenditure, often resulting in serious damages to the health, such as dysfunctions in metabolic and endocrine activity and nutritional changes[1-6]. Obesity has been identified as one of the biggest public health issues worldwide, with an estimation that in 2025 there will be approximately 2.3 billion overweight adults and more than 700 million obese[2].

Researchers have been demonstrating that diet-induced weight loss appears to be less effective than bariatric surgery when it comes to controlling blood glucose levels. In fact, after bariatric surgery, remission of type 2 diabetes mellitus (DM2) can be observed regardless of weight loss[6-9]. Current metabolic/bariatric surgical techniques result in significant and lasting weight loss, as well as major metabolic improvements[7-10]. Surgical treatment of obesity appears to be superior to drug treatment regarding glycemic control, given that bariatric surgery can reduce or slow the progression of diabetic nephropathy[11-12].

It is a challenge for the academic community to understand the molecular mechanisms involved in the metabolic outcomes of bariatric surgery and its strong association with DM2 remission. The analysis of metabolites, through metabonomics, helps understand different phenotypes. It has also the potential to help diagnose metabolic diseases, as well as to assess severity, monitor progression and treatment results and prevent weight regain[13-14].

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By definition, metabonomics is a quantitative measure of the dynamic and multiparametric metabolic response of living organisms to pathophysiological or genetic changes[15]. It involves the determination of the time-dependent dynamic profile of the metabolites within the organism and how they change as a result of biological processes[16]. Metabonomics refers to the broad profile of metabolites present within the cells, tissues or whole organisms. It has a multifaceted role and involves tools such as nuclear magnetic resonance spectroscopy (NMR), and multivariate statistical formalism, such as principal component analysis (PCA), discriminant analysis (DA) and least-square regression (LSR)[17].

Metabolization techniques have already been used to study metabolites and metabolic pathways derangements in patients with type 2 diabetes mellitus. However, few studies have evaluated the role of metabolites as prognostic factors for remission or relapse after surgical treatment of obesity, in diabetic patients. In this pilot study, we hypothesized that patients whom achieved remission of DM2 after bariatric surgery would have a different metabolic profile from those who did not achieve such an outcome and that this difference could be assessed by a metabonomic model. Therefore, the present study aimed to evaluate the differences between the metabolic profile of patients who achieved remission of DM2 after bariatric surgery compared to those who presented maintenance or recurrence of this condition after surgery.

METHODS

This is a cross-sectional study conducted at the General Surgery outpatient clinic of the Hospital das Clínicas and at the Central Analytical Laboratory of the Department of Fundamental Chemistry (Universidade Federal de Pernambuco).

Were included in the 33 obese and type 2 diabetic patients who underwent bariatric surgery (Roux-en-Y gastric bypass or vertical gastrectomy), and 2 years after the procedure, achieved complete remission of DM2 or had recurrence or maintenance of DM2 in the postoperative period. It was defined as a recurrence of diabetes when patients remained on insulin therapy or presented with glycated hemoglobin >7mg/dL. Pathological weight regain was defined as an increase of more than 15% in relation to the minimum weight achieved after surgery.

The research project was approved by the Ethics Committee of the Federal University of Pernambuco, with CAAE 03919118.7.0000.8807. All participants signed a consent form.

Blood samples were collected at the Central Laboratory of UFPE Hospital das Clínicas, where all routine tests were performed. Serum samples from each patient were sent to the Analytical Center of the Fundamental Chemistry Department at UFPE to obtain the ^{1}H NMR spectrum.

A research model was established to investigate the recurrence of diabetes mellitus 2 after bariatric surgery:

- Group I: Patients who presented remission of diabetes mellitus after the surgical intervention;
- Group II: Patients who did not present remission of diabetes mellitus after surgical intervention.

Thirty three samples from patients undergoing bariatric surgery were used. From those, 11 (33.3%) patients had diabetes recurrence and 22 did not. Of those, 5 (15.2%) had both weight regain and diabetes recurrence. Hence, a metabonomic model was created, using the LDA (Linear Discriminants Analysis).

The model was validated by LOOCV (Leave-One-Out Cross Validation), with the values of accuracy, sensitivity, specificity, VPN and VPN extracted from the classification matrix after cross-validation. The cross-validation method does not require a test group, reducing the number of analyzes.

The samples were prepared using 400 µL of serum blood and 200 µL of deuterated water. The 1H NMR spectra were obtained using a 9.0 T NMR spectrometer, operating at 400 MHz for the 1H core. The HPRESAT pulse sequence, which suppresses the water signal, and then CPMG was used to obtain the spectra, using the following parameters: 4.807 Hz spectral window, acquisition time equal to 1.704 s, waiting equal to 2 s, RF pulses of 90° equal to 13,200 and 128 repetitions. The spectra were processed with line broadening equal to 0.3 Hz and divided into regions equal to 0.04 ppm, within the interval between d 0.0 to 4.5 ppm. The region after d 4.5 ppm was excluded. The baseline and phases of the spectra were corrected manually. Each spectrum was processed with a line broadening equal to 0.3 Hz and divided into regions of the same width (0.04 ppm (bins)), within the interval between d 0 to 4.5 ppm, giving a total of 113 bins, using the MestReNova 12.0 software. Regions after 4.5 ppm, which contain the water signal, were excluded. The baselines were automatically corrected by the Bernstein Polynomial method and the phases of the spectra were corrected manually.

A matrix was built with the data from these bins, where the bins of each sample were converted into a matrix line (each line in the matrix represents a sample, that is, a spectrum), in which the elements are filled with the integral of the bin corresponding to each column (variables), resulting in a matrix with 34 lines (33 of the samples plus the classification line) and 166 variables (113 of the bins plus the class variables) that was subjected to chemo metric treatment by the multivariate statistical analysis.

The data matrix was pre-processed using normalization by the sum (Standard Normal Variate - SNV) to perform the LDA formalism, according to Equation 1 below. This processing aims to minimize any type of error, such as variation in concentration, error associated with the analyst, etc. Pre-processing prevents samples with associated systematic errors from having a greater relevance in the construction of the model. This is done without changing the information contained in the variable, so that the samples can be compared with each other. To build the model, five main component scores were used, chosen by the Wilks' Lambda variable selection method.

$$x=(x_i- \bar{x})/s \qquad \text{Equation (1)}$$

x is the signal strength at a given chemical shift, x_i is the intensity in the original spectrum (that is, the area of integration of variable i), $\bar{x}$ is the mean of x for that sample and s is the standard deviation of x in the sample.

RESULTS

The sample consisted of 33 individuals, 3 men (12%) and 30 women (88%), with an average age of 51 years, among whom 22 achieved remission of DM2, and 11 presented relapse or maintenance of DM2 after surgery. The mean preoperative BMI was 46.05kg/m² and the postoperative was 31.3kg/m². Among those who presented recurrence of DM2, the average HbA1c was 7.45%, while among those who presented remission of DM2, the average value was 4.5% (Table 1).

To assess diabetes recurrence, 05 variables were selected to build a model, which resulted in on two discriminant functions presented in the equations below:

$$DF1= - 699{,}999.d_{0,56} - 257{,}116.d_{2,11} - 378{,}566.d_{3,03} - 703{,}369.d_{3,98} + 778{,}530.d_{4,02} - 247{,}463$$

$$DF2= 581{,}654.d_{0,56} - 212{,}437.d_{2,11} - 319{,}460.d_{3,03} - 602{,}170.d_{3,98} + 627{,}283.d_{4,02} - 186{,}909$$

DF1 and DF2 are the discriminant functions used to classify a sample in the diabetes relapse or non-diabetes groups, respectively. Table 2 shows the classification matrix, after cross-validation, for the diagnosis of diabetes recurrence.

The model presented an accuracy of 93.9% (31/33), sensitivity of 81.8%, specificity of 100%, positive predictive value (PPV) equal to 100% and negative predictive value (NPV) equal to 91.7%. Fischer's F test was performed to investigate whether the model was statistically significant. 5 and 27 degrees of freedom were used, resulting in $F_{5.27}$=12.48 (p<0.001).

Figure 1 represents the spectrum obtained through 1H NMR after the processing described in the Methods.

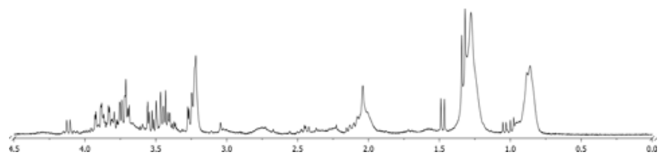

Figure 1. *1H RMN spectrum after manual processing.*

Figure 2 shows the graph of the values predicted by the metabolic model versus the values observed for each sample (0 = Remission; 1 = Recurrence).

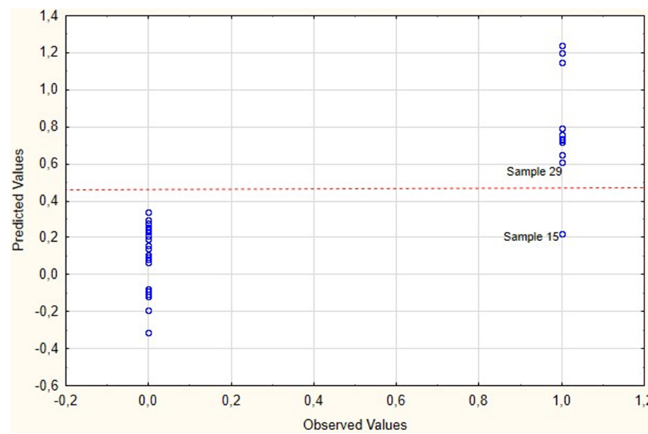

Figure 2. *Predicted versus observed values (Linear Discriminants Analysis).*

Table 1. *Anthropometric and laboratory data of patients in the pre and postoperative periods.*

Variable	Minimum	Maximum	Mean	Standard deviation
Pre-op BMI. (Kg/m²)	35.1	70	46.05	6.76
Pos-op BMI. (Kg/m²)	18	44.3	31.3	5.71
HbA1c (%) DM2 recurrence	5.5	10.5	7.45	1.24
HbA1c (%) without DM2 recurrence	4	6	4.5	0.53

Table 2. *Classification matrix of the metabolic model I (LDA, 5 variables, 33 samples).*

		Clinical diagnosis	
		Recurrence	Remission
MM1	Recurrence	9	0
	Remission	2	22

DISCUSSION

There is a latent need to identify methods that have a prognostic value for diabetes remission in patients who will undergo bariatric surgery, as a way to screen patients who will show, or not, diabetes remission after surgery.

It is well known that factors such as age, time since diagnosis of diabetes, sex, body mass index, glycated hemoglobin A1c and fasting C-peptide are prognostic factors for the treatment of diabetes[18].

However, there is a need to search for factors that are independent from those prognostic factors and that can signal patients who might be good responders to the proposed therapy.

In the current scenario of bariatric and metabolic surgery, surgical treatment of the metabolic syndrome is indicated for patients refractory to the clinical treatment of diabetes, with increasingly lower BMIs[19]. In this context, identifying a metabonomic technique, that is capable of assess the metabolic spectrum of patients who will be good responders to the proposed surgical treatment, is very relevant.

A previous study has shown that the metabolic impact of bariatric surgery could be accessed via metabonomics, demonstrating the surgical effects on various components and biochemical pathways of the body, although the exact pathophysiological mechanism is not completely elucidated.

The tool we used in our study was the 1H NMR. Although there are only a few studies, this is one the most used platforms for metabonomic analysis in studies regarding the effects of bariatric surgery. Some other studies, however, used mass spectrometry, a method that allows the detection, characterization and quantification of low molecular weight metabolites of the most varied classes, such as lipids, amino acids, peptides, nucleic acids, organic acids, vitamins and carbohydrates.

However, some other studies, used mass spectrometry, a method that allows the detection, characterization and quantification of low molecular weight metabolites of the most varied classes, such as lipids, amino acids, peptides, nucleic acids, organic acids, vitamins and carbohydrates.

Even with the current lack of consensus and the need for further investigation, some groups of metabolites stand out, showing greater importance in the pathophysiological process[20]. Amino acids are one of the main groups to undergo changes, BCAA levels, for example, are correlated with insulin resistance in the postoperative period[20,21]. In addition to amino acids, the lipid metabolism is also altered, especially when it comes to the levels of fatty acids, triglycerides, LDL and HDL[20-22]. Another important factor is the modulation of the intestinal microbiota, bacteroids and firmicutes, whose levels in the postoperative period show a connection with weight loss and DM2 remission[20-23].

In diabetic patients, remission of DM2 is believed to occur regardless of weight loss[24], and the recent applications of metabomics in this area have enabled the discovery of markers. In these cases, the metabolic profile for 1HNMR showed changes in basal metabolism associated with energy homeostasis after bariatric surgery, with decreased glucose and increased acetate and lactate, decreased BCAA and changes in lipid metabolism.

In our study, we observed that DM2 remission occurred independently of weight loss. Even in patients with weight regain, there was a remission of diabetes during the follow-up period. The model for assessing diabetes remission showed an accuracy of 93.9% (31/33), sensitivity of 81.8%, specificity of 100%, positive predictive value (PPV) equal to 100% and negative predictive value (VPN) equal to 91.7%. Fisher's F test was performed to investigate whether the model has statistical significance. 5 and 27 degrees of freedom were used, resulting in $F5.27=12.48$ ($p<0.001$).

In addition, it is necessary to investigate the important spectral regions for discrimination, in order to identify the metabolites. However, the number of patients was very low in the diabetes recurrence group, considering that we had 11 patients, and five of them also had weight regain. In this sense, the investigation of possible metabolites associated with the observed discriminations should begin only when these biases in the samples distribution are corrected, increasing, for example, the number of patients assessed.

In this pilot study, it was possible to observe that patients undergoing bariatric surgery who achieved remission of DM2 have a metabolic profile different from those in which remission was not achieved. This data is important because it opens up space for future studies, which seek to prospectively assess the metabolic effects of bariatric surgery. Once a distinct metabolic profile between those who have achieved and those who have not achieved DM2 remission has been demonstrated, it will be plausible to delve deeper into the investigations, seeking to identify the altered metabolites and proceed with preoperative assessments, aiming at drawing a profile of good responders to surgery.

CONCLUSION

With the results of this study, the authors conclude that bariatric surgery promotes specific effects on the distribution of the metabolites of patients who have achieved remission of DM2, and that this new distribution can be evaluated through metabonomic models.

R E S U M O

Objetivo: *avaliar as diferenças no perfil metabonômico de pacientes que atingiram remissão de diabetes mellitus tipo 2 (DM2) após cirurgia bariátrica em relação aos que apresentaram manutenção ou recidiva dessa condição após a cirurgia.* ***Métodos:*** *participaram do estudo 33 pacientes obesos diabéticos tipo 2, dos quais 22 tiveram remissão completa da DM2 e 11 e tiveram recidiva da DM2 ou não apresentaram remissão da doença no pós-operatório. Amostras de sangue foram coletadas para avaliação dos perfis metabonômicos séricos através de um estudo metabonômico baseado em RMN de* ^{1}*H.* ***Resultados:*** *o modelo metabonômico para avaliação da recidiva da diabetes apresentou uma acurácia de 93,9%, sensibilidade de 81,8%, especificidade de 100%, valor preditivo positivo (VPP) igual a 100% e valor preditivo negativo (VPN) igual a 91,7%.* ***Conclusão:*** *a cirurgia bariátrica promove efeitos específicos na distribuição dos metabólitos de pacientes que atingiram remissão de DM2, e essa nova distribuição pode ser avaliada através de um modelo metabonômico.*

Descritores: *Cirurgia Bariátrica. Diabetes Mellitus Tipo 2. Obesidade. Metabolômica. Metabolismo.*

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