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Application of fourier transform infrared vibrational spectroscopy in identifying early biochemical changes in lipid profiles of individuals undergoing Roux-en-y gastric bypass

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Abstract

Background Fourier transform infrared spectroscopy (FTIR) is an analytical technique increasingly applied in biological analysis. This study investigates the application of FTIR to identify early biochemical changes, particularly in lipid profiles, in individuals undergoing Roux-en-Y gastric bypass (RYGB).

Methods An observational study involving patients from a university hospital's Bariatric and Metabolic Surgery Program, with evaluations performed before (T0) and two months after (T1) RYGB. Biochemical parameters, anthropometric data, and body composition were assessed. FTIR spectra were pre-processed and analyzed using Principal Component Analysis and Partial Least Squares Discriminant Analysis. The normality of the data was evaluated using the Kolmogorov-Smirnov test, followed by paired T-tests or Wilcoxon tests as appropriate. Spearman correlation analysis of spectral information with biochemical parameters was also performed. A significance level of $p < 0.05$ was set for all tests. The university hospital's Research Ethics Committee approved the study (protocol CAAE 59075722.7.0000.5071).

Results The study evaluated 29 individuals (86.2% female) with a mean age of 41.2 ± 7.8 years. Significant differences were observed in anthropometric parameters and body composition ($p < 0.001$). Additionally, early improvements in the lipid profile were noted, with significant decreases in triglycerides, total cholesterol, and LDL cholesterol ($p < 0.05$) just two months post-surgery. FTIR identified correlations between biochemical parameters and specific spectral regions at T0 and T1. Notably, serum triglycerides showed a significant correlation with the lipid-specific spectral region (1796–1685 cm^{-1}) at both time points ($p < 0.05$).

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Conclusion FTIR can effectively monitor biochemical changes in RYGB surgery patients. The spectral range associated with lipid functional groups (1796–1675 cm^{-1}) showed a significant relationship with serum triglyceride levels before and after RYGB. Additionally, various biochemical parameters exhibited strong correlations with other spectral regions, implying that the serum spectral profile can indicate biochemical variations at different post-surgery stages.

Trial registration Brazilian Registry of Clinical Trials (Rebec), September 5, 2022, protocol RBR-26chs2g.

Keywords FTIR, Obesity, Bariatric-metabolic surgery, Lipid profile, Biochemical changes

Background

Bariatric-metabolic surgery, specifically Roux-en-Y gastric bypass (RYGB), continues to be indicated for the treatment of severe obesity [1]. RYGB involves modifications to the digestive system and consistently results in significant weight loss and improved quality of life [2]. However, weight loss after surgery can vary significantly between individuals, making it challenging to predict precisely how much weight someone will lose, especially in the long term [3]. Moreover, dietary restrictions and nutrient malabsorption resulting from RYGB can lead to substantial changes in anthropometric, biochemical, and metabolic parameters, potentially compromising nutritional status [4, 5].

In this context, methodological approaches for nutritional and prognostic assessment of patients undergoing RYGB, particularly in the first months after surgery, are imperative [6]. Early monitoring is crucial to promptly identify and address potential issues, improving long-term outcomes. Fourier transform infrared spectroscopy (FTIR) has been increasingly applied in biological analysis and is a possible tool for analyzing clinical parameters and detecting molecular alterations in health biomarkers [7]. This technique absorbs infrared radiation through covalent bonds in organic and inorganic molecules, generating characteristic molecular vibrations of specific functional groups. The resulting spectrum acts as a biochemical fingerprint of the sample, providing detailed molecular information [8]. The infrared (IR) spectrum is divided into three regions based on wavenumbers: far-infrared (far-IR, <400 cm^{-1}), mid-infrared (mid-IR, 400–4000 cm^{-1}), and near-infrared (near-IR, 4000–13000 cm^{-1}). The mid-IR region is the most commonly used for sample analysis, which was used in this research. The distinctive pattern produced by mid-IR radiation occurs because the sample absorbs specific frequencies corresponding to its chemical structure. The peaks of the mid-IR spectrum are unique for each sample, making this type of spectroscopy valuable for a wide range of applications [9].

Recent studies demonstrate the effectiveness of FTIR in identifying molecular alterations in blood samples associated with diseases such as metabolic syndrome and cancer [10, 11]. Multivariate data analysis methods, such as Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), are crucial for extracting meaningful information from FTIR spectra. These techniques help identify relevant patterns and highlight molecular changes [12, 13].

A study using FTIR to analyze serum samples from individuals with and without obesity found differences in absorbance between the spectra of the two groups, particularly in the functional groups of proteins and lipids [14]. Positive correlations have also been observed between FTIR spectra and biochemical parameters, such as aspartate aminotransferase, alanine transaminase, triglycerides, glucose, total cholesterol, high-density lipoprotein, low-density lipoprotein, and insulin.

Continuous patient engagement and effective post-operative monitoring are crucial for the success of these procedures. Monitoring with FTIR spectroscopy facilitates the timely identification of lipid profile changes, providing essential insights into patients' metabolic states post-surgery. This enhances adherence to post-surgical guidelines and enables prompt interventions when necessary. While most studies present results six months to a year after bariatric surgery [1, 15], communicating initial findings is critical for understanding trends and making timely adjustments to treatment approaches, which can significantly impact long-term outcomes. Hence, this study aims to evaluate the use of FTIR in early monitoring to identify alterations in the biochemical parameters of individuals undergoing RYGB.

Methods

Study design and participants

This prospective study was conducted with participants from the Bariatric and Metabolic Surgery Program of a university hospital in Brazil. From February to June 2020, individuals were invited to participate in the study voluntarily. The convenience sample included patients aged 18 to 60 with a BMI >40 kg/m^2 or >35 kg/m^2 with comorbidities. Pregnant women and patients with pacemakers or other metallic structures were excluded, following the recommendations of the European Society for Clinical Nutrition and Metabolism (ESPEN) [16]. Evaluations were performed at an average of 24.0 ± 20.5 days before (T0) and 72.0 ± 19.5 days after (T1) RYGB. The study was

approved by the Research Ethics Committee of the university hospital, number CAAE 59075722.7.0000.5071, following the Code of Ethics of the World Medical Association (Declaration of Helsinki), to ensure the ethical conduct and protection of participants' rights. Participants were fully informed of the study objectives and methodology, and informed consent was obtained from all individuals included.

Anthropometry and body composition

Body weight was measured using an anthropometric scale with an accuracy of 0.05 kg, and height was measured using a wall-mounted stadiometer with an approximation of 0.1 cm. BMI was calculated by dividing weight (kg) by height (m^2).

Fat-free mass (FFM) and fat mass (FM) (kg) were obtained by bioelectrical impedance analysis using the Biodynamics 450® analyzer (Biodynamics Co., Shoreline, WA, USA) at a single frequency of 50 kHz. FFM was calculated using the formula for people with obesity proposed by Segal & colleagues [17] and expressed in kg. FM was calculated using the following formula: FM = total body weight – FFM, also expressed in kg.

Biological material and biochemical parameters

Blood samples were collected after an 8–12 h fast, and the serum was collected immediately after centrifugation and stored in a freezer at -80 °C for later analysis.

The following parameters were analyzed using commercial kits (Wiener Lab, Santa Fé, Argentina): albumin; transthyretin (TTR); alpha-1-acid glycoprotein (AGP); C-reactive protein (CRP); aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP); glucose; triglycerides; total cholesterol (TC); high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

Sample preparation and FTIR spectrum acquisition

Samples were thawed at room temperature (22 °C ± 0.2 and 51% ± 2.1 humidity) for 30 min and homogenized using a vortex mixer. Then, 10 µL were pipetted onto aluminum plates and dried at room temperature for at least two hours in triplicate. Spectra were acquired in triplicate using the ALPHA II spectrometer (Bruker, Germany) in the spectral range of 4000 to 400 cm^{-1}, with an attenuated total reflectance (ATR) accessory with a diamond crystal, resolution of 4 cm^{-1} and 32 scans for each background and sample. For each analysis, the diamond sampling window was cleaned with Milli-Q® water and 70% (v/v) ethanol and dried with absorbent paper tissue.

Spectral data processing

The spectra were evaluated using Matlab software (R2023b). The mean of the spectral triplicates was calculated, followed by baseline correction (adaptive iteratively reweighted Penalized Least Squares algorithm - airPLS) [18] and smoothing with a Savitzky-Golay filter (5-point window). In addition, other preprocessing methods were tested to generate models with better group discrimination ability, such as vector normalization, multiplicative scatter correction (MSC), and second derivative (15-point window and second-degree polynomial).

Multivariate analysis

Multivariate analysis was conducted to discriminate the samples. For this purpose, the spectra were truncated and analyzed in the 3800–900 cm^{-1} wavenumber range. PCA was used to explore interactions between samples and variables, identifying patterns and information that explain the most significant variation in the data [12].

A supervised PLS-DA approach was used to classify samples from groups T0 and T1. For the development of classification models, the data were randomly divided into training sets (70%) and test sets (30%), keeping T0 and T1 from the same patient in either the training or test set.

The training samples were used in training and cross-validation (Monte Carlo method) to determine the model's optimal number of latent variables (LV). External test samples, however, were not included in training and were reserved for evaluating the model's predictive ability. To evaluate the performance of the PLS-DA models, the sensitivity (SENS), specificity (SPEC), error rate (ER) and accuracy (ACC) parameters were used, measuring the ability to identify positive cases correctly, the ability to correctly identify negative cases, the false positive rate, the overall error rate and the overall accuracy of the classifications, respectively.

Furthermore, the PLS-DA model enables the interpretation of relevant variables through an analysis known as the VIP score. A VIP value above 1 is commonly used as a threshold to identify variables with significant contribution [19, 20].

Statistical analysis

Analyses were performed using GraphPad Prism 8 software. Data normality was tested using the Kolmogorov-Smirnov test and expressed as mean ± standard deviation or median (25–75%). For comparative tests, data were analyzed by paired t-test or Wilcoxon test, with a significance level of 5%.

Spearman's correlation coefficient was calculated between the set of biochemical parameters and the correspondingly selected spectral regions of interest, which are indicated in Table 1.

For each selected region, the area under the curve (AUC) of the mean spectra, pre-processed by second derivative (Savitzky-Golay method, 21-point window),

Table 1 Spectral regions and molecular assignments

Spectral region	Wavenumber (cm^{-1})	Assignments	Reference
1	3358–3188	Bond N-H (Amide A and B), bond O-H cholesterol	[21–23]
2	3011–2816	Bond C-H, lipids, proteins	[21, 22]
3	1796–1685	C = O from lipids, aldehyde groups	[21, 22, 24]
4	1687–1574	Amide I	[21]
5	1574–1478	Amide II	[21]
6	1484–1420	Lipids, cholesterol	[21, 22]
7	1427–1356	Proteins	[21]
8	1332–1211	Amide III, proteins	[21]
9	1195–1133	Carbohydrates, phosphate groups (phospholipids), nucleic acids and amino acids	[21, 22]
10	1106–1046	Carbohydrates, phosphate groups (phospholipids), nucleic acids and amino acids	[21, 22]
11	964–905	Carbohydrates, phosphate groups (phospholipids), nucleic acids and amino acids	[21, 22]

was calculated [25]. The correlations found were classified as weak (0.30–0.50), moderate (0.50–0.70) or strong (0.70-1.00) [26].

Results

The final sample comprised 29 participants (86.2% female, $n = 25$) who attended both scheduled evaluations before and after the surgery. Participants had a mean age of 41.2 ± 7.8 years and a mean height of 162.0 ± 8.7 cm. The most prevalent comorbidities found in the participants at baseline (T0) were hypertension (70.3%), type 2 diabetes mellitus (42.3%), and dyslipidemia (60.4%).

Table 2 indicates that all anthropometric and body composition parameters decreased significantly between the two time points ($p < 0.001$). Regarding biochemical parameters, serum albumin, TTR, AGP, CRP, ALP, glucose, triglycerides, TC, and LDL decreased significantly ($p < 0.05$). At the same time, AST increased significantly ($p < 0.05$). Notably, only serum TTR was below the reference value after RYGB.

Principal Component Analysis (PCA) with the first two main components accounted for 43.84% of the variance in the original data, indicating a tendency for separation between the groups. Most T0 samples were located on the negative side of PC1, while T1 samples were predominantly on the positive side of PC1 (Fig. 1a). The loadings of the PCA model for the biochemical parameters (Fig. 1b) indicated that the main variables influencing the T0 samples towards the negative side of PC1 were TC, albumin, CRP, high-density lipoprotein (HDL), glucose, and triglycerides, which decreased after RYGB.

The spectral profile with the average spectra of the T0 and T1 groups is shown in the Fig. 2. The spectra display

Table 2 Biochemical, anthropometric, and body composition parameters before and after RYGB

Parameters	T0	T1	*p*-value	Reference values
Weight (kg)	114.1 (104.8–129.1)	97.3 (90.2 -106.4)	**< 0.001****	-
BMI (kg/m^2)	43.9 (41.1–49.6)	37.5 (33.9–42.5)	**< 0.001****	-
FFM (kg)	64.2 (57.7–71.1)	57.1 (53.7–63.3)	**< 0.001****	-
FM (kg)	52.8 (46.8–65.1)	39.4 (34.9–46.5)	**< 0.001****	-
Albumin (g/dL)	4.3 (4.0–4.5)	4.1 (3.9–4.2)	**0.002****	3.5–4.8 g/dL
TTR (mg/dL)	23.1 ± 4.7	17.9 ± 5.6	**< 0.001***	20–40 mg/dL
AGP (mg/dL)	108.6 ± 38.3	90.3 ± 32.5	**0.05***	50–120 mg/dL
CRP (mg/L)	10.9 (5.4–15.6)	3.6 (2.1–6.4)	**< 0.001****	< 5 mg/L
AST (U/L)	18.0 (15.5–23.0)	24.0 (20.0–28.0)	**< 0.001****	0–32 U/L
ALT (U/L)	25.4 ± 9.3	27.9 ± 10.7	0.271*	0–31 U/L
ALP (U/L)	200.5 (166.0–232.5)	192.2 (169.5–216.5)	**0.019****	68–240 U/L
Glucose (mg/dL)	100.0 (91.0–118.5)	90.5 (85.0–100.5)	**< 0.001****	78–99 mg/dL
Triglycerides (mg/dL)	149.3 (95.5–180.0)	93.0 (75.5–136.0)	**0.002****	< 150 mg/dL
TC (mg/dL)	192.5 (154.5–216.5)	154.5 (134.5–190.5)	**0.002****	< 190 mg/dL
HDL (mg/dL)	42.5 (36.0–50.5)	41.0 (34.0–48.0)	0.117**	> 40 mg/dL
LDL (mg/dL)	113.9 (89.2–138.9)	91.9 (76.4–120.7)	**0.006****	< 100 mg/dL

$N = 29$. RYGB: Roux-en-y gastric bypass. T0: 29.3 ± 23.9 days before RYGB; T1: 81.3 ± 32.9 days after RYGB. BMI: Body Mass Index; FFM: Fat-Free Mass; FM: Fat Mass; TTR: transthyretin; AGP: alpha-1-acid glycoprotein; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein. *Paired t-test. **Wilcoxon Signed Rank Test. The values are shown as median (interquartile range) or mean ± standard deviation, according to the data distribution. $p \leq 0.05$ was considered statistically significant

characteristic bands of biological matrices attributed to proteins (mainly amide I and II, 1687 –1478 cm^{-1}), carbohydrates and amino acids (1195 –905 cm^{-1}), and lipids (3358 –2816 cm^{-1}, 1796 –1685 cm^{-1}, 1484 –1420 cm^{-1}, and 1195 –1133 cm^{-1}). A PCA model of the spectra, using the scores plot with the first two principal components, explained 72.48% of the total variance of the original data. However, it did not show a separation of the samples from T0 and T1 in the principal component space, which is why this analysis was not presented.

However, employing Partial Least Squares Discriminant Analysis (PLS-DA) yielded a model with an accuracy

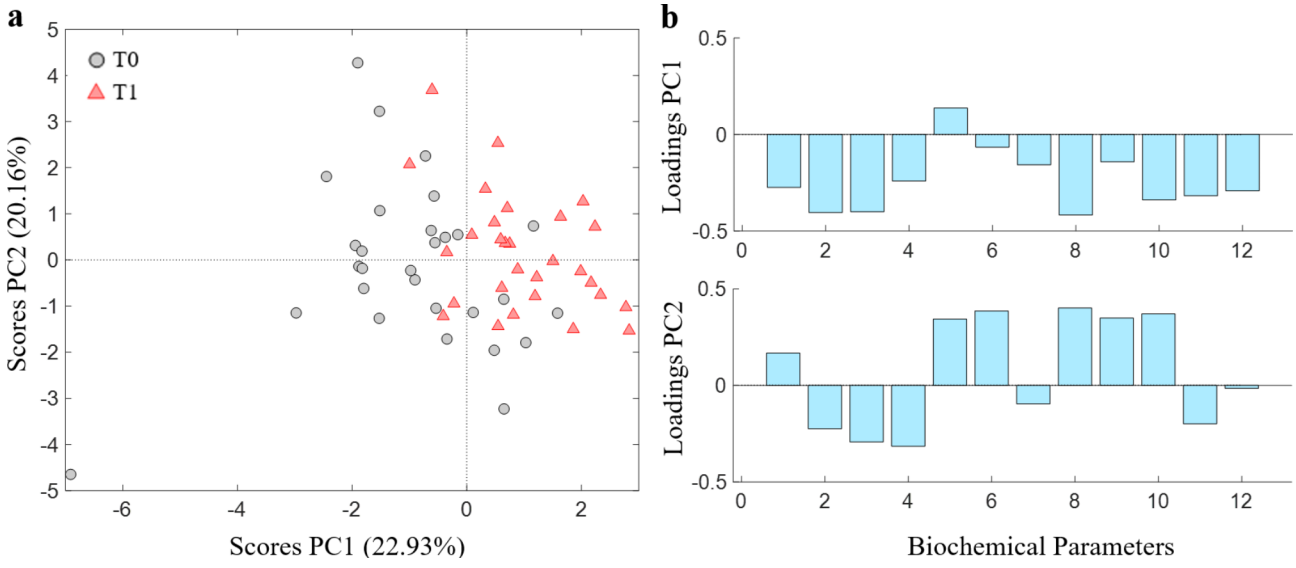

Fig. 1 Score plots and loading biochemical parameters before and after RYGB. RYGB: Roux-en-y gastric bypass. T0: 29.3 ± 23.9 days before RYGB; T1: 81.3 ± 32.9 days after RYGB. Score plots PCA model (**a**). Loadings of PCA model with scaled biochemical parameter data (**b**). 1: Transthyretin (TTR); 2: Albumin; 3: C-reactive protein (CRP); 4: alpha-1-acid glycoprotein (AGP); 5: aspartate aminotransferase (AST); 6: alanine aminotransferase (ALT); 7: alkaline phosphatase (ALP); 8: total cholesterol (TC); 9: high-density lipoprotein (HDL); 10: low-density lipoprotein (LDL); 11: glucose; 12: triglycerides

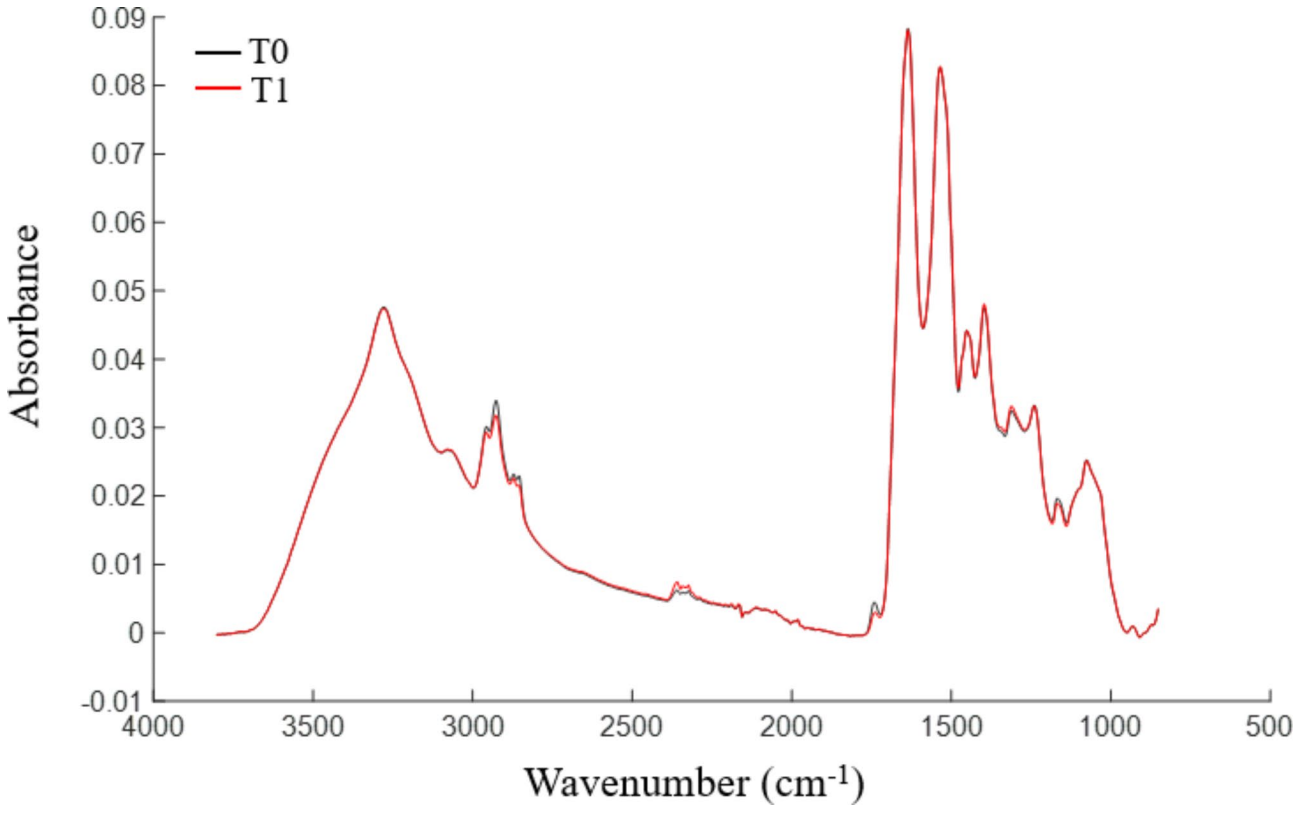

Fig. 2 Average spectra from FTIR of serum samples before and after RYGB. RYGB: Roux-en-y gastric bypass. T0: 29.3 ± 23.9 days before RYGB; T1: 81.3 ± 32.9 days after RYGB

Table 3 Performance measures and characteristics of the PLS-DA model for before and after RYGB

LV	SENS (%)	SPEC (%)	ER (%)	ACC (%)
7	**95.0**	**95.0**	**5.0**	**95.0**
	88.9	66.7	22.2	77.8

RYGB: Roux-en-y gastric bypass. LV: latent variables SENS: Sensitivity; SPEC: Specificity; ER: Error Rate; ACC: Accuracy. Bold line: training subset; unbold line: testing subset

of 77.8%. The model demonstrated an error rate of 5.0% for the training set and 22.2% for the test set (Table 3).

From the PLS-DA model, it was possible to evaluate the most relevant spectral variables for distinguishing between groups through the VIP score. Figure 3 shows regions with a VIP score > 1, indicating that regions with significant VIP scores are primarily attributed to lipids, proteins, and carbohydrates, demonstrating the contribution of the entire spectral information.

Table 4 shows the correlation coefficients of the selected regions with the biochemical parameters. At T0, there was a positive correlation of regions 2, 3, and 9 with serum AGP, regions 4, 7, and 10 with serum AST, and regions 2, 3, 6, and 9 with triglycerides concentrations. At T1, there was a significant correlation between region 2 and serum total and LDL cholesterol, and region 3 with serum TTR, ALT, AST, and triglycerides concentrations. Only triglycerides concentrations significantly correlated with region 3 at both time points.

Discussion

The spectral region characteristic of lipid functional groups, located at 1796 –1675 cm^{-1} (region 3), showed a significant correlation with serum triglycerides concentration both before and after RYGB. This region also includes aldehyde groups related to oxidative stress [24]. Although the correlation between spectral regions and oxidative stress parameters was not evaluated in this study, these findings open new perspectives for investigations into oxidative stress in obese patients undergoing

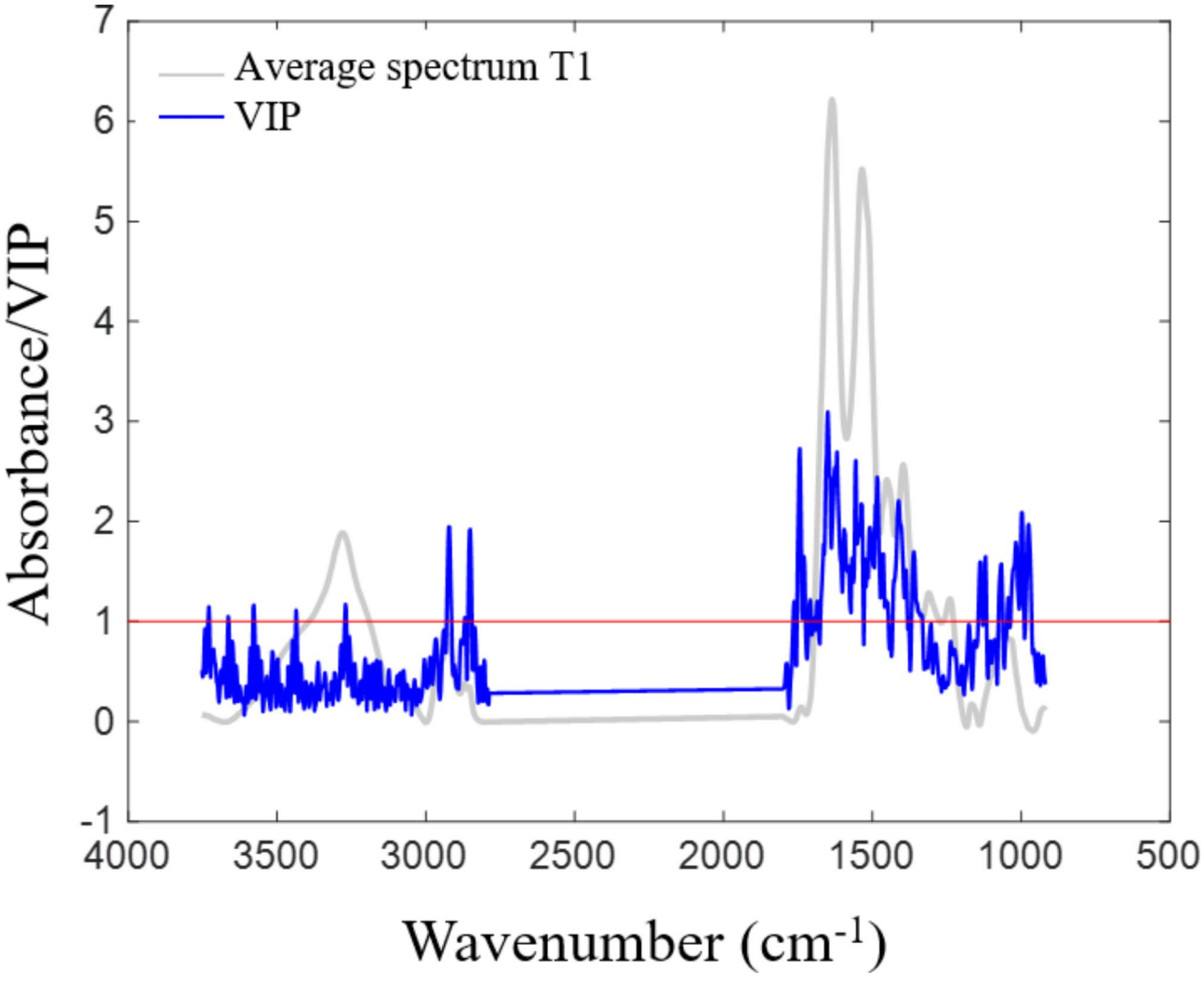

Fig. 3 VIP score analysis of spectral variables using the PLS-DA model

Table 4 Pearson correlation values between biochemical parameters and spectral regions before and after RYGB

	Spectral region																			
	1		2		3		4		5		6		7		8		9		10		11	
Parameter	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1	T0	T1
Albumin (g/dL)	0.02	0.21	0.05	0.11	0.06	-0.04	0.02	0.23	0.03	0.27	0.06	0.15	0.01	0.32	0.04	0.28	0.13	0.19	-0.07	**0.37**	0.02	0.28
TTR (mg/dL)	0.14	0.11	0.05	0.32	0.01	**0.42**	0.12	0.11	0.12	0.12	0.12	0.13	0.12	0.09	0.13	0.10	0.07	0.16	0.12	0.06	0.18	0.07
AGP (mg/dL)	0.16	0.08	**0.37**	-0.09	**0.40**	-0.31	0.25	0.04	0.24	0.04	0.30	-0.02	0.22	0.06	0.27	0.06	**0.37**	-0.01	0.25	0.09	0.11	-0.00
PCR (mg/L)	-0.02	-0.03	-0.08	-0.03	-0.10	-0.01	0.011	0.03	0.00	0.01	-0.04	0.02	0.03	0.00	0.00	-0.00	-0.02	-0.01	-0.01	0.07	-0.10	-0.06
AST (U/L)	-0.31	0.01	-0.18	0.10	-0.02	**0.38**	**-0.37**	-0.05	-0.36	-0.04	-0.26	0.03	**-0.43**	-0.07	-0.36	-0.07	-0.24	-0.01	**-0.44**	-0.12	-0.30	0.04
ALT (U/L)	-0.16	-0.07	0.05	-0.04	0.16	**0.41**	-0.23	-0.10	-0.20	-0.08	-0.09	-0.05	0.26	-0.10	-0.19	-0.10	-0.01	-0.04	-0.30	-0.15	-0.18	-0.03
ALP (U/L)	-0.02	0.04	0.20	-0.12	0.20	-0.12	0.04	-0.07	0.01	-0.07	0.06	-0.13	0.01	-0.06	0.03	0.00	0.10	-0.05	0.08	0.03	-0.15	-0.10
Glucose (mg/dL)	0.05	-0.03	0.02	0.18	-0.06	0.31	0.08	0.04	0.09	0.03	0.04	0.12	0.11	0.00	0.05	-0.04	0.08	0.01	-0.05	-0.03	0.10	0.03
Triglycerides (mg/dL)	0.13	-0.25	**0.76**	0.14	**0.85**	**0.63**	0.22	-0.22	0.21	-0.14	**0.43**	-0.27	0.13	-0.27	0.24	-0.22	**0.56**	-0.16	0.10	-0.30	0.07	-0.20
TC (mg/dL)	0.25	0.24	0.18	**0.49**	0.09	0.36	0.17	0.25	0.17	0.25	0.27	0.33	0.06	0.21	0.18	0.25	0.22	0.31	0.08	0.11	0.17	0.23
HDL (mg/dL)	-0.23	0.18	-0.35	0.23	-0.27	-0.02	-0.32	0.21	-0.32	0.22	-0.30	0.27	-0.35	0.20	-0.33	0.16	-0.36	0.22	-0.36	0.06	-0.20	0.18
LDL (mg/dL)	0.29	0.30	0.06	**0.45**	-0.09	0.23	0.20	0.30	0.21	0.29	0.25	0.35	0.12	0.26	0.215	0.03	0.18	0.30	0.17	0.16	0.21	0.30

N = 29. RYGB: Roux-en-y gastric bypass. T0: 29.3 ± 23.9 days before RYGB; T1: 81.3 ± 32.9 days after RYGB. TTR: transthyretin; AGP: alpha-1-acid glycoprotein; CRP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein. Bold numbers are significant (p-value ≤ 0.05)

surgical treatment, using mid-infrared spectroscopy to explore specific absorption bands.

Other biochemical parameters also showed significant correlations with specific spectral areas, although not in the same region at both time points. The correlations identified in region 2 (3011–2816 cm^{-1}) with triglycerides, TC, and LDL highlight the relevance of this region for investigating lipid dynamics and are consistent with literature data reporting changes in lipid CH_2 antisymmetric bands (2975–2952 cm^{-1}) in obese individuals enrolled in a bariatric surgery program compared to eutrophic individuals ($p = 0.037$), associating these bands with structural and metabolic changes in lipids [27]. Previous studies observed a significant correlation between serum triglycerides and the absorbance peak at 1457 cm^{-1}, corresponding to region 6 (1484–1420 cm^{-1}), as well as changes in the wavelength range of 1181–1131 cm^{-1}, corresponding to region 9 (1195–1133 cm^{-1}), between obese individuals and controls (BMI < 25) [14, 27].

FTIR demonstrated its ability to detect early changes in the lipid profile of individuals undergoing RYGB. This can be crucial for re-evaluating and potentially adjusting treatment approaches, as well as guiding early therapeutic decisions in clinical settings. In a study that investigated FTIR spectra of blood serum from individuals with and without obesity, significant differences in lipid composition were observed between the groups, identified by analysis of absorbance and peak positions associated with lipid functional groups, highlighting specific variations in wavenumbers 1750 cm^{-1} (lipid C = O bonds) and 2970–2950 cm^{-1} (lipid CH_3 bonds) [14]. The authors also observed a significant correlation between serum triglycerides and specific wavenumbers 1750 cm^{-1} and 2700–3000 cm^{-1}, respectively, to lipid C = O and CH_3 bonds. These findings are similar to the present study and contribute to a more comprehensive understanding of the use of FTIR for detecting alterations in lipid profiles in obesity.

The significant correlations of spectral information with serum triglycerides concentrations observed before and after RYGB may be partly explained by the ability of FTIR to detect vibrations of functional groups such as C = O and CH and, consequently, changes in the spectral regions associated with these compounds [21, 22, 28]. Lipids, especially triglycerides, represent a significant portion of blood serum content, and alterations in their molecular composition resulting from RYGB include modifications in the quantity and composition of circulating lipids [29, 30]. Alterations in nutrient absorption and lipid metabolism induced by RYGB might be reflected in FTIR spectra.

Concerning the PCA analysis, a clear separation of serum samples from patients before and after RYGB was

not observed. Factors such as intra- and inter-individual variability may hinder the achievement of a clear spectral distinction at different time points [31, 32]. In the context of RYGB, this suggests that variations in spectral characteristics exist even within a single individual before and after RYGB. Before surgery, factors such as inadequate diet, sedentary lifestyle, and health status may be reflected in FTIR spectra. After RYGB, despite improving health status, metabolic and hormonal changes and alterations in post-surgical diet may contribute to the complexity of spectra and specific sample characteristics [2, 4, 33, 34]. However, the results from the PLS-DA analysis suggest that considering the different spectral regions and their characteristics, FTIR was effective in differentiating individuals before and after RYGB, identifying true positives (sensitivity), true negatives (specificity), and the overall rate of correct classification (accuracy). In a study evaluating the use of FTIR in the diagnosis of COVID-19, a sensitivity rate of 85% and a specificity rate of 83% were observed, suggesting that FTIR is an essential tool for rapid, low-cost, and non-invasive diagnosis [35].

It is important to note that other significant correlations were also observed, although with variations at different time points. These variations may be attributed to the complex metabolic and physiological changes induced by RYGB, which may affect the serum concentrations of biochemical parameters and, in turn, the spectral characteristics of serum samples, disrupting the relationships between spectral characteristics and evaluated parameters [2, 4, 31, 32].

The significant changes in anthropometric and body composition parameters, as well as the changes in biochemical parameters, are consistent with the expected effects of RYGB [36, 37]. We observed that serum concentrations of CRP, glucose, TC, and LDL, altered before RYGB, were within the normal range after surgery. These findings highlight the efficacy of RYGB in improving metabolic and lipid profiles, contributing to an overall improvement in health status. However, it is essential to note that the serum concentration of TTR, a protein potentially used to identify nutritional risk [38], was below the reference value at T1. In this context, although not observed in this study, new perspectives could be opened for FTIR in evaluating molecular changes characteristic of protein nutritional status.

We acknowledge the importance of biochemical analysis as the gold standard for assessing the health conditions of bariatric patients. However, our data demonstrate that, even in the absence of established reference values, FTIR spectroscopy emerges as a promising approach. It offers valuable insights that can complement traditional methods, serving as an auxiliary tool for analyzing molecular changes in response to RYGB.

This study has certain limitations, as the small sample size may have reduced the statistical power of some analyses, limiting the generalizability of the results and constraining the interpretation of the evaluated sample. Additionally, the chemometric model may have been potentially influenced by patient comorbidities. Furthermore, data collection was affected by the suspension of elective surgeries funded by the Brazilian Unified Health System during the COVID-19 pandemic and the lack of follow-up during the first two years, resulting in deviations from the planned time points. Participant dropouts for personal reasons further contributed to the reduced sample size.

The study followed hospital protocols requiring an 8-to-12-hour fasting before lipid profile measurement. Although variations within this fasting range may introduce minor fluctuations in lipid levels, evidence suggests that these differences are minimal and unlikely to significantly impact the overall lipid profile interpretation [39, 40]. At the same time, evidence highlights the importance of standardizing the fasting timing before sample collection to manage variability [40].

While FTIR provides a rapid and efficient method for lipid analysis, it has intrinsic limitations should be considered, such as potential interference from overlapping absorbance bands, variations in sample types [41], and the need for calibration to ensure accurate results. Nevertheless, the laboratory where the analysis was conducted adhered strictly to the equipment manufacturer's guidelines for sample preparation, handling, storage conditions, and regular equipment calibration and validation to ensure data accuracy and reliability.

Conclusion

The results indicate that FTIR can effectively monitor biochemical changes in patients undergoing RYGB surgery. The spectral range associated with lipid functional groups (1796–1675 cm^{-1}) showed a significant relationship with serum triglyceride levels before and after RYGB. Additionally, various biochemical parameters exhibited strong correlations with other spectral regions, implying that the serum spectral profile can indicate biochemical variations at different stages post-surgery.

Abbreviations

AGP	Alpha-1-acid glycoprotein
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
BMI	Body mass index
CRP	C-reactive protein
ESPEN	European society for clinical nutrition and metabolism
FFM	Fat-free mass
FM	Fat mass
FTIR	Fourier transform infrared
HDL	High-density lipoprotein

LDL	Low-density lipoprotein
PCA	Principal component analysis
PLSA-DA	Partial least squares discriminant analysis
RYGB	Roux-en-Y gastric bypass
SENS	Sensitivity SPEC: specificity ER: error rate ACC: accuracy
T0	Preoperative period, approximately one month before surgery
T1	Postoperative period, approximately two months after surgery
TC	Total cholesterol
TTR	Transthyretin

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Author contributions
AM de B wrote the main manuscript text. AM de B, MHCN, BEGD, BB de B, and LRP data curation, formal analysis, and investigation. PRF, ABL, VGB, and FKH conceptualization, data curation, methodology, and supervision. VGB and FKH designed the study, funding acquisition, project administration, and resources. All authors reviewed the manuscript.

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Data availability
The data supporting the findings of this research are securely maintained under the supervision and responsibility of the Head of the Department of Laboratory of Obesity and Nutrition Studies at the Federal University of Espírito Santo, Brazil. For further details regarding the data and materials used, including the raw data, please contact Dr. Fabiano Kenji Haraguchi (fabiano.haraguchi@ufes.br).

Declarations

Ethics approval and consent to participate
The study was approved by the Research Ethics Committee of the university hospital, number CAAE 59075722.7.0000.5071, following the Code of Ethics of the World Medical Association (Declaration of Helsinki). Trial Registration: Brazilian Registry of Clinical Trials (Rebec) on September 5, 2022, under the number RBR-26chs2g. Participants were fully informed of the study objectives and methodology, and informed consent was obtained from all individuals included.

Consent for publication
Participants were fully informed of the study objectives and methodology, and informed consent was obtained from all individuals included.

Competing interests
The authors declare no competing interests.

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