103 - ASSOCIATION BETWEEN BODY MASS INDEX AND BODY ADIPOSITY WITH FT-IR SPECTRA OF FASTING BLOOD OF PATIENTS STRATIFIED BY GLYCATED HEMOGLOBIN AND FASTING GLUCOSE

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1.INTRODUCTION

In many countries, the prevalence of type 2 diabetes mellitus (DM2) has increased fastly and is expected to increase even more. It is estimated that in the year 2030 the number of people with DM2 is 300 milllion (WILD 2004; SARTORELLI et al., 2006).

Metabolic controls of individuals with de diseases evolving into one of the biggest challenges of public health services Americans and Brazilians. Therefore, the development of efficient and viable programs to public health services for the primary prevention of type 2 diabetes in at-risk population is needed both the control the incidence of DM2 as well as for secondary prevention of metabolic complications (SARTORELLI et al., 2006).

Obesity and/or overweight are present in most patients (DM2), but its prevalence varies depending on genetic and environmental factors (educational and cultural). Ethnicity plays an important role in the different prevalence of obesity exist in countries with the same level of economic development, however a common fact seems to be the higher prevalence of obesity in females (CORRÊA et al., 2003).

The BMI is calculate by dividing the body weight in kilograms by the square of height in meters, in order to classify individuals as underweight, normal weight, overweight and obesity. Although not a parameter with complete accuracy due to over-or underestimate values of brevilineal and longilineal individuals, BMI becomes a tool to be a simple, practical, fast, easy to apply and measurement, and requires, less training and cheaper equipment. Thus, the BMI, has great advantage over methods of body composition assessment, and the choose the most routine clinical and epidemiologic study is the method currently used by WHO to classify obesity (COSTA, GUISELINI, FISBERG 2007; NUNES et al., 2009).

Obesity is a chronic metabolic disorder characterized by excess body fat. There are several methods used to evaluate this excess being used more BMI. Obesity is defined as a BMI equal to or greater than 30kg/m2, and is characterized by excessive body fat in relation to lean body mass (CORRÊA et al., 2003).

Visceral obesity results in several physiopathological changes that may result in different degrees of glucose intolerance in individuals with DM2, will, reflected by higher levels influence glycemic control of glycated hemoglobin (A1c). This fact should be considered in the context of recent studies have demonstrated that glycemic control in these patients is critical to reducing the risk of progression to microvascular complications. Moreover, some studies further suggest that the control of other parameters such as blood pressure, serum lipids, such as an increase in total cholesterol, triglycerides and apolipoprotein B, HDL cholesterol reduction would be of significant importance to minimize the risk of emergence of macrovascular complications of diabetes, which represents 65% of causes of deaths in this population (CORRÊA et al., 2003).

In this context, and specially concerning to the diagnosis is where emerges the infrared spectroscopy with Fourier Transform (FT-IR) associated with programs of multivariate analysis. With this methodology it has been possible to obtain simultaneous quantification data, fast, and low cost and without specific reagents (LIQUN WANG, 2008; MAHMOUD, 2010).

Thus the present study aimed to evaluate the association between FT-IR spectra of whole blood fasting and anthropometric parameters BMI and body adiposity (BA) of individuals euglycemic and dysglycemic (pre-diabetics and diabetics).

2. MATERIALS AND METHODS

2.1 Study design

The experiment was conducted in an outpatient department of Clinical Medicine Hospital Santa Cruz and forwarded the Basic Health Units (BHU) in the municipality of Santa Cruz do Sul, in the period from march to october 2011.

2.2 Inclusion and exclusion criteria

The study included individuals of both sexes, aged 18 to 59 years, who presented fasting glucose between 100-126 mg/dL and/or oral glucose tolerance test with 75 g less than 140 and less than 200 mg/dL, or in pre-diabetic patients.

Exclusion criteria were: previous diagnosis of DM2, use of oral hypoglycemic agents or insulin, kidney disease clinic (creatinine> 1.5 mg/dL), defined cardiovascular disease (stroke, coronary artery disease or atherosclerotic disease clinic) and diagnosis neoplasms in the last 12 months.

2.3 Classification of individuals

Individuals were classified into Control, Pre-diabetics and diabetics, according to criteria of the ADA (American Diabetes Association) in 2011, which include fasting glucose and A1c as diagnostic parameters. Patients with fasting glucose levels between 70 and 99 mg/dL and A1c less than 5.7% were included in the control group. Patients with fasting glucose between 100 and 125 mg/dL or A1c between 5.7 and 6.5% were diagnosed with pre-diabetes. Finally, patients with fasting glucose above 126 mg/dL or A1c above 6.5% were classified as diabetic.

2.4 Anthropometric measurements

All anthropometric measurements were performed at the Laboratory of Physical Activity (LAFISA) University of Santa Cruz do Sul, by trained personnel, in accordance with the recommended techniques. The following variables were measured: weight, height and calculated the body mass index (BMI). BA was estimated by measurements of seven skinfolds different for men and women, according to the protocol of Jackson and Pollock (JACKSON POLLOC& WARD, 1980), using the compass Langue. Measurements were taken three times in rotational order, taking an average of the results.

2.5 Laboratory analysis

Blood samples were taken via antecubital venipuncture after a 12 h overnight fast. All blood tests were performed in the same laboratory and in triplicate. Were asked the following tests: glucose, glycated hemoglobin (A1C), total cholesterol, HDL (high density lipoprotein), triglycerides, creatinine. LDL cholesterol (low density lipoprotein) was calculated by the Friedewald equation (FRIEDEWALD, 1972).

2.6 Analysis by FT-IR

Upon antecubital venipuncture, aliquots were separated 5 uL in triplicate without anticoagulant for analysis by FT-IR. The blood samples were stored at 2-8 °C until processing. The material used for the collection and storage of blood was discarded as routine disposal of biological materials LAFISA. Triplicate samples of 5 uL of whole blood (peripheral or antecubital) were mixed with 150 mg of potassium bromide, lyophilized for 2 h 15 min 1.10-4 torr, -50 °C (Labcomco Lyofilyzer ®), analyzed in FT-400 spectrometer Spectrum IR / FT-NIR Spectrometer (Perkin Elmer ®) HeNe laser of 633 nm accessory diffuse reflectance spectroscopy in the infrared (Pike Technologies, Madison) Fourier Transform in the range 4000-600 cm-1, 4 cm-1 resolution and 16 scans.

2.7 Statistical analysis

The spectra were acquired in absorbance, normalized, converted into extension *. CSV, organized in Microsoft Office Excel 2010, and submitted to multivariate regression analysis via the method of Partial Least Squares (PLS) to transform multiple scattering correction (MSC) and preprocessing self-scaled in software Pirouette 4.0 (Infometrix). The calibration models were validated by leave one out cross-validation. The calibration models were optimized using as criterion the value of correlation coefficient R2 above 0.99 and lowest root mean square error of cross validation (RMSECV).

2.8 Ethical aspects

The research project was approved by the research ethics committee of the University of Santa Cruz do Sul, process 2686/10. Participants were properly oriented and signed an informed consent.

3. RESULTS AND DISCUSSION

This study consisted of 46 individuals. These being subdivided into three groups: control, pre-diabetics and diabetics, according to criteria of the ADA (American Diabetes Association) in 2011, which includes A1c (glycated hemoglobin) as a diagnostic parameter. Thus patients with fasting glucose levels between 70 and 99 mg/dL and A1c less than 5.7% was included in the control group, euglycemic. Patients with fasting glucose levels between 100 and 125 mg/dL and A1c between 5.7 and 6.5%, receive the diagnosis of pre-diabetes and thus included in the respective group. Finally, patients with fasting glucose above 126 mg/dL and A1C above 6.5% included in the diabetic group. Is important to remember that just one parameter changed so that the diagnosis is confirmed (ADA, 2011).

Table 1 characterizes the sample group, taking into account anthropometric and biochemical parameters.

As can be seen in (Table 1), it is a very heterogeneous group, but when stratified by A1c and fasting subgroups show characteristic patterns of diagnosis through both biochemical and anthropometric parameters. It can be observed the association of these groups of individuals with dyslipidemia and obesity, these being the most likely causes of insulin resistance and consequently DM2. These differences also appear in the average spectrum of each group (Figure 1).

Table 1 - Characteristics of the sample of subjects investigated

	Control (n=12)	Pre-diabetic (n=26)	Diabetic (n=8)	Overall (n=46)
Age	47.21 (8.81)	49.07 (8.53)	48.77 (8.94)	48.73 (8.52)
Sex				
Male	6	5	5	16
Female	6	21	3	30
BMI	27.98 (3.81)	29.87 (4.47)	33.58 (4.87)	30.05 (4.61)
BA	27.6 (5.3)	30.5 (3.7)	31.5 (3.6)	30.0 (4.3)
GLI	82.4 (6.4)	87.5 (9.2)	111 .0 (13.3)	90.4 (13.4)
A1c	5.5 (0.1)	6.0 (0.3)	6.7 (0.3)	6.0 (0.5)
Lipid profile				
TC	190.6 (27.7)	212.8 (39.0)	222.6 (42.1)	209.3 (37.7)
TRI	109.5 (32.3)	149.1 (80.3)	248.1 (86.3)	155.0 (83.7)
HDL	53.1 (4.5)	52.8 (4.8)	52.9 (6.1)	53.1 (4.9)
LDL	115.7 (22.3)	130.2 (33.5)	125.4 (29.1)	125.3 (30.2)

Age: mean in years; BMI: Body mass index (kg/m²); BA: body adiposity (%); GLI: fasting glucose (mg/dL); A1c: glycated hemoglobin (%), TC = Total Cholesterol (mg/dL); TRI: Triglycerides (mg/dL); HDL: HDL Cholesterol (mg/dL), LDL: LDL cholesterol (mg/dL). Standard deviation in parentheses.

From the average spectra for each of the groups: controls, pre-diabetics and diabetics, differences were found, particularly in spectral regions of 3200-2800 cm-1 and 1500-1200 cm-1 spectral regions where there is a greater contribution lipids and carbohydrates respectively. In both regions, the control group showed a lower absorbance in relation to the group of pre-diabetic and diabetic consistent with the average total cholesterol and triglycerides (Table 1). Thus it can be inferred that in relation to lipids, the spectrum can differentiate a major factor in diabetes, dyslipidemia, and this together with DM2, elements of the metabolic syndrome. Regarding carbohydrates, this difference may be due to the direct relationship of serum glucose. Thus, patients with higher serum glucose levels or longer exposure to hyperglycemia, the absorbance change in this spectral range.

The difference between pre-diabetic and diabetic individuals was not clear, or due to similar behavior in relation to the metabolism of diabetic and pre-diabetic or due to the sample group of diabetics be very small, and one could present no greater perceived differences.

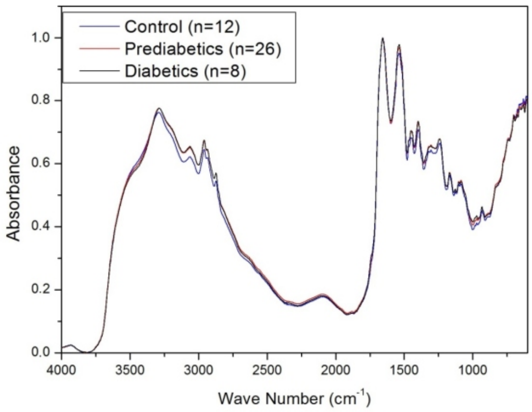

Figura 1Average spectra of diffuse reflectance infrared Fourier transform of whole blood from control, pre-diabetic and diabetic groups.

The application of linear regression between the set of FT-IR spectra of whole blood and its BMI, and BA is shown in Table 2.

Table 2 – Figures of merit of PLS-DRIFTS modeling between whole blood and BMI and BA of individuals investigated.

Parameter	Spectral region (cm^{-1})	R²	RMSECV	CRV	Factor number
BMI	4000-2401, 2300-600	0.996*	0.39	30.4	3
BA	4000-2401, 2300-600	0.893*	1.96	46.8	15

RMSECV = root mean square error of standard cross-validation R^2 = correlation coefficient of cross-validation; CRV = cumulative relative variance (%), LV = latent variables, BMI = Body mass index (kg / m²); BA = Body adiposity (%); * = p < 0.001.

The R^2 values are found show a strong association with BMI and may be subject to further studies in order to predict this anthropometric parameter from the infrared spectrum and reinforces the anthropometric changes are strongly associated with blood composition. With BA this correlation decreases but still remains strong. This is due to the difficulty of accurately measuring skin folds in obese patients more compromising estimate of that parameter.

4. CONCLUSIONS

Before the present study it was concluded that infrared spectroscopy with Fourier Transform (FT-IR) can characterize and differentiate the three groups: control, pre-diabetics and diabetics according to the current diagnostic criteria (ADA, 2011), from the average spectra for each group. The differences are sharper at higher contribution of lipids and carbohydrates and especially among euglycemic inidviduals with respect to pre-diabetics and diabetics is therefore an alternative to screening patients. The differences are sharper at higher contribution of lipids and carbohydrates and especially among euglycemic individuals with respect to pre-diabetics and diabetics and, it can be used as an alternative to screening of disglycemic individuals. Thus, in addition to using the existing dosage biochemical parameters which are of great benefit for patients with multiple comorbidities as well as in DM2, there is the option of using FT-IR as a tool for monitoring anthropometric being an aid in combating the main risk factor for this disease: the obesity.

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ASSOCIATION BETWEEN BODY MASS INDEX AND BODY ADIPOSITY WITH FT-IR SPECTRA OF FASTING BLOOD OF PATIENTS STRATIFIED BY GLYCATED HEMOGLOBIN AND FASTING GLUCOSE

ABSTRACT

This study evaluated the association via partial least squares regression between body mass index (BMI) and body adiposity (BA) with infrared spectra of blood from 46 individuals (controls = 12; prediabetic = 26; diabetic = 8) stratified by fasting blood glucose and glycated hemoglobin (A1c). It was observed a greater absorption at wavelengths related to the spectral regions of 3200-2800 cm^{-1} and 1500-1200 cm^{-1} due to contributions of lipids, proteins and carbohydrates in prediabetic and diabetic patients. A strong association between the infrared spectra of blood with BMI (R^2 = 0.996, RMSECV = 0.39 kgm^{-2}) and lower intensity with BA (R^2 = 0.893; RMSECV = 1.96%) was found.

KEY-WORDS: body adiposity, body mass index, FT-IR spectroscopy

ASSOCIATION ENTRE L'INDICE DE MASSE CORPORELLE ET LA ADIPOSITÉ CORPORELLE AVEC LES SPECTRES FT-IR DE JEÛNE SANG DE PATIENTS EN STRATIFIÉ PAR LE L'HÉMOGLOBINE GLYQUEÉ E LE GLUCOSE

RÉSUMÉ

Cette étude a évalué l'association par les moindres carrés partielle régression entre l'indice de masse corporelle (IMC) et la adiposité corporelle (AC) avec les spectres infrarouge de sang á partir de 46 personnes (témoins = 12 ; prédiabétique = 26 ; diabétique = 8) stratifieés par le glucose et l'hémoglobine glyquée (A1c) à jeun. Il y avait une plus grande absorption à des longueurs d'onde associées à des régions spectrales de 3200-2800 cm^{-1} et 1500-1200 cm-1 due à la contirubution des lipides, des protéines et des glucides chez les personnes pré-diabétiques et diabétiques. Une forte association entre les spectre infrarouges de sang avec l'IMC (R^2 = 0,996, RMSECV = 0,39 $kg.m^{-2}$) et avec moins de force avec AC (R^2 = 0,893, RMSECV = 01,96 $kg.m^{-2}$) a été trouvé.

MOTS-CLÉS: adiposité corporelle, indice de masse corporelle, spectroscopie IR-TF

ASOCIACIÓN ENTRE EL ÍNDICE DE MASA CORPORAL Y LA ADIPOSIDAD CORPORAL CON FT-IR DE LOS ESPECTROS DE SANGRE DE PACIENTES EN AYUNO CLASIFICADOS POR LA HEMOGLOBINA GLICOSILADA Y GLUCOSA

RESUMEN

Este estudio evaluó la asociación a través de mínimos cuadrados parciales de regresión entre el índice de masa corporal (IMC) y la adiposidad corporal (CA) con espectros de infrarrojo de sangre de 46 individuos (controles = 12; prediabéticos = 26; diabéticos = 8) estratificados por la glucosa y la hemoglobina glicosilada (A1c) de ayuno. Ocurrió una mayor absorción en las longitudes de onda en relación con las regiones espectrales de 3200-2800 cm^{-1} y 1500-1200 cm^{-1} debido a las contribuciones de los lípidos, proteínas y carbohidratos en individuos prediabéticos y diabéticos. Una fuerte asociación entre los espectros de la sangre se encontró en el infrarrojo con el IMC (R^2 = 0,996, RMSECV = 0,39 kgm^{-2}) y con menor intensidad con la AC (R^2 = 0,893; RMSECV = 1,96%).

PALABRAS-CLAVE: adiposidad corporal, índice de massa corporal, espectroscopía IR-TF

ASSOCIAÇÃO ENTRE ÍNDICE DE MASSA CORPORAL E PORCENTAGEM DE GORDURA COM ESPECTROS FT-IR DE SANGUE DE JEJUM DE PACIENTES ESTRATIFICADOS POR HEMOGLOBINA GLICADA E GLICOSE

RESUMO

Este trabalho avaliou a associação via regressão por mínimos quadrados parciais entre índice de massa corporal (IMC) e porcentagem de gordura (%G) com espectros no infravermelho de sangue de 46 indivíduos (controles = 12; pré-diabéticos = 26; diabéticos = 8) estratificados por glicose e hemoglobina glicada (A1c) de jejum. Observou-se uma maior absorção em comprimentos de onda relacionados com as regiões espectrais de 3200-2800 cm^{-1} e 1500-1200 cm^{-1} devido a contribuições de lipídios, proteínas e carboidratos em indivíduos pré-diabéticos e diabéticos. Foi encontrada uma forte associação entre espectros de sangue no infravermelho com IMC (R^2 = 0,996, RMSECV = 0,39 kgm^{-2}) e com menor intensidade com %G (R^2 = 0,893; RMSECV = 1,96%).

PALAVRAS-CHAVE: porcentagem de gordura, índice de massa corporal, espectroscopia FT-IR