Revista Brasileira de ENGENHARIA BIOMÉDICA
BRAZILIAN JOURNAL OF BIOMEDICAL ENGINEERING
Volume 28, Número 3, p. 272-277, 2012

Artigo Original
http://dx.doi.org/10.4322/rbeb.2012.026

Methodology for the study of metabolic syndrome by heart rate variability and insulin sensitivity

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Abstract This work presents a methodology for studying the Heart Rate Variability (HRV) and Insulin Sensitivity (IS) during the Oral Glucose Tolerance Test (OGTT) in subjects with Metabolic Syndrome (MS). For this, it was designed a clinical protocol that includes the acquisition of ECG signals during OGTT for 15 subjects with MS and 10 subjects for control group. HRV parameters were obtained from electrocardiographic recordings. Significant differences in RR values were found between groups in the 30 minutes stage of the OGTT. In control subjects the RR values were higher compared with subjects with MS. The values of normalized low frequencies between baseline stages and the stage at 30 minutes in subjects with MS showed a significant increase in sympathetic tone during the OGTT, that was not observed in group control. The following methods were implemented for quantification of IS: integral equation, insulin sensitivity index, insulin sensitivity, metabolic, HOMA and QUICKI. We found significantly lower values of IS in the group with MS. It was concluded that there are changes in the HRV and the IS in the subjects with MS, which is a pre-diabetic disease. These findings suggest that HRV and IS can be used for diagnosis of MS and prevention of Diabetes Mellitus.

Keywords Diabetes mellitus, Metabolic syndrome, Heart rate variability, Insulin sensitivity, Oral glucose tolerance test.

Metodologia para o estudo da síndrome metabólica partindo da variabilidade da frequência cardíaca assim como da sensibilidade à insulina

Resumo *Este trabalho apresenta uma metodologia para o estudo da Variabilidade da Frequência Cardíaca (VFC) e Sensibilidade à Insulina (SI) durante o Teste de Oral de Tolerância à Glicose (TOTG) em indivíduos com Síndrome Metabólica (SM). Para isso, foi projetado um protocolo clínico que inclui a aquisição de sinais ECG durante o TOTG em 15 pacientes com SM e 10 indivíduos do grupo controle. Os parâmetros da VFC foram obtidos a partir de registros eletrocardiográficos. Diferenças significativas nos valores de RR foram encontradas entre os grupos na fase de 30 minutos do TOTG. No grupo controle os valores de RR foram maiores em comparação com indivíduos com SM. Os valores normalizados de baixas frequências entre as fases da linha de base e a fase de 30 minutos em pacientes com SM mostraram um aumento significativo no tônus simpático durante o TOTG, não observado no grupo controle. Os seguintes métodos foram implementados para a quantificação de SI: integral, índice de sensibilidade à insulina, sensibilidade à insulina, metabólicos, HOMA e QUICKI. Valores significativamente menores de IS foram encontrados no grupo com MS. Podemos concluir que existem alterações na VFC e SI nos indivíduos com MS, caracterizando uma doença pré-diabética. Estas descobertas sugerem que a VFC e SI podem ser usados para o diagnóstico da SM e prevenção da Diabetes Mellitus.*

Palavras-chave *Diabetes mellitus, Síndrome metabólica, Variabilidade da frequência cardíaca, Sensibilidade à insulina, Teste oral de tolerância à glicose.*

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Recebido: 09/01/2012 / Aceito: 29/02/2012

Introduction

Diabetes mellitus is a disease characterized by the accumulation of glucose in the blood. This is the result of poor performance of the pancreas to produce insulin. The causes of this disease are still unclear, however, there are pre-diabetic conditions that predispose or prelude the development of the disease. Among these conditions is the Metabolic Syndrome (MS). Therefore, early diagnosis and treatment of MS can prevent the development of diabetes, as well as associated diseases such as hypertension and neuropathy (Zimmet and Alberti, 2005).

Several studies reveal the importance of some parameters, such as physiological signs and symptoms, in the diagnosis of diabetes mellitus. Among these we can highlight the Heart Rate Variability (HRV), which, in the early stages of diabetes, show changes including increased sympathetic tone and decreased parasympathetic tone, which could indicate also cardiovascular disorders (Weissman *et al.*, 2006). It was also reported low values of insulin sensitivity in diabetic and hypertensive subjects (Burattini *et al.*, 2009). The methods to quantify the Insulin Sensitivity (IS) are numerous and there are not consensual borders to discriminate MS subjects from normal subjects.

This work proposes a methodology for the study of HRV and IS during Oral Glucose Tolerance Test (OGTT) in subjects with MS. For this, it was designed a clinical protocol that allowed the construction of a database. This database includes the acquisition of Electrocardiographic (ECG) signals during the OGTT, in order to study HRV. This allowed us to determine possible relationships between blood glucose levels and HRV, as well as the determination of IS in both populations.

Materials and Methods

Selection of subjects

Subjects were chosen for this study by the following criteria: male, age between 20 and 44 years old, non-smoker, not on drug treatment, without disabilities and without known cardiovascular diseases. Each subject signed an informed consent form, which explains the conditions of test, importance and usefulness.

For the diagnosis of MS it was used the criteria of National Cholesterol Education Program-Adult Treatment Panel (NCEP ATP III), which consist in the presence of two or more of the following characteristics: abdominal circumference in men greater than 102 cm and in women greater than 88 cm, blood pressure equal or higher than 130/85 mmHg, triglycerides higher than 150 mg/dL, HDL-cholesterol in men lower than 40 mg/dL and in women lower than 50 mg/dL, fasting blood glucose equal to or higher than 110 mg/dL.

Clinical protocol

Table 1 shows the tests, measurements and recordings made during the clinical protocol, which consists of the following steps:

- ECG signal acquisition before the OGTT;
- Extraction of blood sample at baseline, with the subject on fasting;
- Ingestion of 75 grams of liquid glucose; and
- Four blood samples extracted in intervals of 30 minutes and, before each of these samples, a fifteen-minute ECG signal was acquired.

The Cardiosoft® software was used for the acquisition of ECG signals and extraction of the RR and QRS intervals. The ECG recordings were

Table 1. Description of tests and measurements during the clinical protocol.

Tests and measurements	Description
Blood tests	Includes complete hematology, fasting blood glucose, urea, creatinine, cholesterol, triglycerides, high density lipids (HDL), low density lipids (LDL), very low density lipid (VLDL), uric acid and alanine transaminase (ALT).
Anthropometric variables measurement	Height, weight, waist circumference and Body Mass Index (BMI).
Clinical variables measurement	Blood pressure.
Oral Glucose Tolerance Test (OGTT)	It consists of oral intake of 75 grams of liquid glucose, followed by the extraction of blood samples at intervals of 30 minutes and laboratory measurements of insulin and glucose for each sample. The test lasts 2 hours and, at the end, two curves are generated: insulin concentration and glucose concentration (these data are used in different methods for the quantification of insulin sensitivity).
ECG recording	The ECG recordings were performed before each blood sampling of the OGTT, lasting fifteen minutes each, for a total of five records per subject.

Rev. Bras. Eng. Biom., v. 28, n. 3, p. 272-277, set. 2012
Braz. J. Biom. Eng., 28(3), 272-277, Sept. 2012

performed at sampling frequency of 1 kHz, resolution of 16 bits and 12-lead records (CardioSoft, 2012).

Heart rate variability

Measurement of HRV is the oscillation during the interval between consecutive heartbeats. This is an established quantitative method, allowing the assessment of cardiac autonomic activity through the study spectral HRV. Based on HRV values, it can be studied the autonomic nervous system and its fluctuations, and, thereby, cardiovascular disorders and diabetes (Chan *et al.*, 2007).

Analysis of the RR series

The CardioSoft® program was used for the detection of the QRS complex and construction of the RR series. The RR series for each time interval in the OGTT were validated and resampled at 2 Hz. The Wilcoxon and Kruskal-Wallis tests were used in order to find differences between groups and stages of the OGTT in relation to HRV parameters. In all cases, 'p' values '<0.05' were considered significant.

HRV parameters

From the RR series, SD and RMSSD were calculated in the time domain, and the Low Frequency (LF), High Frequency (HF) and LF/(LF + HF) were calculated in the frequency domain. All calculations and algorithms used were implemented and executed in MATLAB. Table 2 shows the description of the parameters.

The RR series extracted were resampled at 2 Hz to make them a uniform sampled series. From the RR uniform sampled series, the temporal domain parameters (SD and RMSSD) and the frequency domain parameters (LF, HF, and LF/(LF + HF)) were calculated. Frequency domain parameters were calculated using the autoregressive Burg's algorithm.

Insulin sensitivity

Insulin sensitivity (IS) is defined as the ability of the cells to react to the presence of insulin (Clausen *et al.*, 1996). The IS can be quantified by existing methods that use data from the OGTT; the methods used in this study for the quantification are: integral equation, insulin sensitivity index, insulin sensitivity, metabolic, $HOMA_{IR}$, $HOMA\beta_{CELL}$ and QUICKI. The formulation of these methods is specified in Table 3.

Results

Database

The database obtained consisted in 25 subjects, of which 15 correspond to a group with MS and 10 to the control group. Significant differences were found between groups in weight and abdominal circumference. However, no significant differences were found in age and height. The MS group presents obesity (with a Body Mass Index (BMI) above 30), which is located mainly in the abdomen. Table 4 shows the anthropometrics data for the MS and control groups.

Parameters obtained from the study of HRV

The study was performed on HRV data obtained during the OGTT. The following parameters were obtained from the electrocardiographic recordings: RR, HF, LF, RMSSD, SD and LF/(HF + LF). Those values were recorded for each one of the OGTT stages, for both groups.

Significant differences were found between groups in the values of RR during the 30 minutes stage of the OGTT (control group 966.286 ± 160.146 ms *versus* MS group 843.200 ± 137.478 ms). Subjects with MS showed an increase in sympathetic tone during the OGTT, which was not observed in the control group. Significant

Table 2. HRV parameters in time and frequency domain found in this study (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Frequency domain parameters	Units	Description	Frequency range
VLF	ms^2	Very low frequency	<0.04 Hz
LF	ms^2	Low frequency	0.04-0.05 Hz
LF normalized		LF/LF + HF	
HF	ms^2	Power range of high frequencies	0.15-0.4 Hz
HF normalized		HF/LF + HF	
Time domain parameters	**Units**	**Description**	
SDRR	ms	Standard deviation of all RR intervals	
RMSSD	ms	The root square of the mean of the sum of squared differences between adjacent RR intervals.	

Table 3. Insulin Sensitivity Quantification Methods (Di Nardo *et al.*, 2006; Fernandez *et al.*, 2003).

Methods	Description
Integral equation	$SI_{EI} = \dfrac{f\dfrac{D}{W}\dfrac{ABC\left[\Delta G(t)/G(t)\right]_0^{to} - ABC\left[\Delta G(t)/G(t)\right]_{to}^{T}}{ABC\left[\Delta G(t)\right]_0^{to} - ABC\left[\Delta G(t)\right]_{to}^{T}} - GEABC\left[\Delta G(t)/G(t)\right]}{ABC\left[\Delta I(t)\right]}$ (1)
	ABC: area under the curve; G(t): glucose (mg/dL); I (t): insulin (mUI/mL); ΔG(t): glucose values above basal glucose; ΔI(t): insulin levels above basal insulin; GE: glucose effectiveness $(dL/\min\cdot Kg)$; D/W: amount of glucose consumed per unit weight (mg/kg); f: fraction of ingested glucose appearing in the circulatory system; t_0: time at which the values of ΔG(t) becomes negative; T: total duration of the OGTT.
Insulin sensitivity	$IS_{OGIS120} = \frac{1}{2}\left[B + \sqrt{B^2 + 4p_5p_6(G_{90}G_{CLAMP})CL_{OGTT}}\right]$ (2)
	$CL_{OGTT} = p_4\dfrac{\dfrac{p_1D_o - V(G_{120} - G_{90})}{G_{90}} + \dfrac{p_3}{G_0}}{I_{120} - I_0 + p_2}$ (3)
	$B = (p_5(G_{90} - G_{CLAMP}) + 1)CL_{OGTT}$ (4)
	$SC = \sqrt{\dfrac{PT}{3600}}$ (5)
	G_0, G_{120}, G_{90}: glucose concentration in the instants 0, 120 and 90 minutes respectively (mg/dL); I_0, I_{120}: insulin concentration at the instants 0 and 120 minutes respectively (mUI/mL); D_0: dose of glucose per unit of body surface area (g/m²); V: total volume of distribution (mL/kg); G_{CLAMP} = 90 (mg/dL); p_1 = 650 (min^{-1}); p_2 = 325 (μUI/mL); p_3 = 81.3 10^3 ($mg\cdot min^{-1}\cdot m^{-2}$); p_4 = 132 (μUI/m); p_5 = 652 10^{-6} (mL/mg) y p_6 = *173* ($mL\cdot min^{-1}\cdot m^{-2}$); P: weight (Kg); T: subject's height (cm).
Insulin sensitivity index	$SI_{ISI} = \dfrac{10^4}{\sqrt{G_0I_0G_mI_m}}$ (6)
	G_0 and G_m: glucose concentrations at the instant 0 minutes and mean glucose values at instants 30, 60, 90 and 120 minutes; I_0 and I_m: insulin concentrations at the instant 0 minutes and the average of insulin values at the instants 30, 60, 90 and 120 minutes.
Metabolic	$SI_{IM} = 18.8 - 0.271BMI - 0.0052I_{120} - 0.27G_{90}$ (7)
	BMI: expressed as the weight divided by height squared (kg/m²); I_{120}: insulin concentration at 120 minutes of OGTT (pmol/L); G_{90}: glucose concentration at 90 minutes of the OGTT (mmol/L).
HOMA QUICKI	$HOMA_{IR} = \dfrac{G_0I_0}{405}$ (8)
	$HOMA_{\beta CELL} = \dfrac{360I_0}{G_0 - 63}$ (9)
	$QUICKI = \dfrac{1}{\log G_0 - \log I_0}$ (10)
	G_0: Glucose at the instant 0 minutes (mg/dL); I_b: Insulin at the instant 0 minutes (μUI/mL).

differences were observed for LF/LF + HF in the MS group between the baseline stage (0.692 ± 0.093) and the 30 minutes stage (0.757 ± 0.067).

The significant differences between groups and stages found in this study might indicate a very early stage of cardiac dysfunction induced by dysfunctional metabolism of sugars.

Insulin sensitivity obtained from the methods

Significant differences between groups were found in the following methods: integral equation, insulin sensitivity, index of insulin sensitivity, $HOMA\beta_{CELL}$, $HOMA_{IR}$ and QUICKI. The insulin sensitivity in the control group is higher than in MS group, indicating that for control group the quality of insulin metabolism is better than in MS group. Table 5 shows the values of IS obtained from each method, for both groups.

Discussion

This study shows the design of a clinical protocol for the study of heart rate variability and insulin sensitivity

Rev. Bras. Eng. Biom., v. 28, n. 3, p. 272-277, set. 2012
Braz. J. Biom. Eng., 28(3), 272-277, Sept. 2012

Table 4. Mean anthropometric data and clinical variables for both study groups.

Anthropometric data		**Control group**	**MS group**	***p* values**
Age (years)		26.90 ± 4.18	31.40 ± 6.98	NS
Height (m)		1.77 ± 0.09	1.74 ± 0.07	NS
Weight (Kg)		73.01 ± 13.56	104.66 ± 23.14	0.001
Abdominal circumference (cm)		83.51 ± 10.75	113.63 ± 19.35	0.001
Clinical variables measurement		**Control group**	**MS group**	***p* values**
Arterial pressure (mmHg)	Systolic	117.60 ± 9.23	135.13 ± 7.10	0.001
	Diastolic	72.40 ± 7.23	85.67 ± 10.59	0.006
Body mass index (Kg/m^2)		23.30 ± 3.48	34.27 ± 6.68	0.0003

Table 5. Insulin sensitivity according to the following methods: Integral equation, insulin sensitivity index, insulin sensitivity, metabolic, HOMA and QUICKI.

Insulin Sensitivity methods	**Control group**	**MS group**	***p* values**
	Mean	**Mean**	
Integral equation (dL Kg^{-1} min^{-1}/(μUI/mL))	$6.31.10^{-3} \pm 5.62.10^{-3}$	$5.93.10^{-4} \pm 6.21.10^{-4}$	0.001
Insulin sensitivity (mL/min m^2)	403.71 ± 26.44	307.86 ± 56.31	0.00006
Insulin sensitivity index	9.57 ± 4.70	3.48 ± 2.90	0.001
Metabolic (mg/min Kg)	6.99 ± 2.66	4.29 ± 2.83	0.012
$HOMA\beta_{CELL}$	40.86 ± 24.22	106.00 ± 59.75	0.006
$HOMA_{IR}$	0.98 ± 0.64	3.15 ± 1.77	0.001
QUICKI	0.4006 ± 0.0412	0.3349 ± 0.0399	0.001

in patients with MS. The database obtained consists of electrocardiographic signals collected during OGTT for a MS group and a control group. Data were analyzed from the point of view of HRV and IS.

The study included 25 subjects - 10 formed the control group and 15 the MS group. The database includes, in addition to traditional variables in the study of subjects with metabolic syndrome (clinical, laboratory and anthropometric variables), the ECG recording during the OGTT. This ECG provides more points of comparison between groups, facilitating the diagnosis of MS. There are alterations in HRV and IS that can appear in a pre-diabetic stage as Metabolic Syndrome. These parameters, HRV and IS, may constitute diagnostic features in the evolution of pre-diabetic patient. This may contribute for easier prevention, diagnosing and managing of important illnesses, such as diabetes and its complications (Navarro and Vargas, 2008).

The ECG acquisition, allowed the calculation of HRV parameters in the time domain (SD and RMSSD) and in the frequency domain (RR, LF, HF and LF/(LF + HF)). Significant differences were found in the MS group between stages of OGTT for the values of LF/(LF + HF) – the same was not observed in the control group. This indicates an increase in sympathetic tone during the OGTT in the MS group.

In the study of insulin sensitivity, the following methods were used: integral equation, insulin sensitivity index, insulin sensitivity, metabolic, HOMA and QUICKI. Significant differences between groups in all methods were found. The MS group showed lower insulin sensitivity compared with the control group, which represents a metabolic dysfunction reflected in the poor metabolism of glucose.

Conclusion

Globally, the metabolic syndrome is increasing among the population and should be monitored carefully, since it is a precursor to diabetes and cardiovascular disease. The main contribution of this work is the introduction of a methodology that allows, from a noninvasive test such as the electrocardiogram, to obtain parameters that provide a better understanding of the mechanisms associated with MS. It is expected that the results obtained in this pilot study provide a basis for future research.

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