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Sociedade Brasileira de Cardiologia • ISSN-0066-782X • Volume 108, Nº 3, March 2017

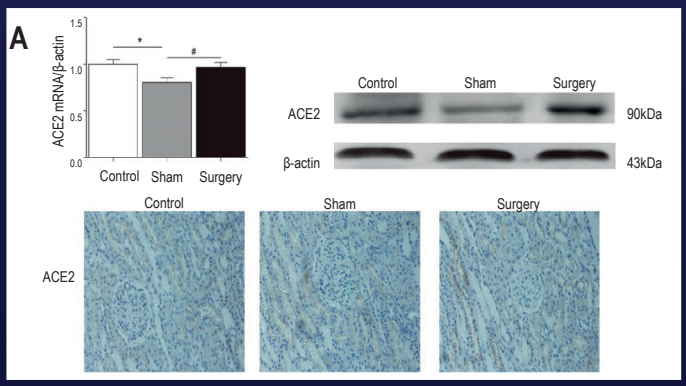

Figure 4 – *Effects of RDN on renal ACE2 mRNA and protein expression (A), Ang-(1-7) concentration (B) and Mas mRNA and protein expression(C) of beagle dogs. Values are mean±SEM. * p < 0.05 versus control group, # p < 0.05 versus sham surgery group. ACE2 mRNA: angiotensin-converting enzyme 2. Page 243*

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Arquivos Brasileiros de Cardiologia

Volume 108, Nº 3, March 2017

Indexing: ISI (Thomson Scientific), Cumulated Index Medicus (NLM), SCOPUS, MEDLINE, EMBASE, LILACS, SciELO, PubMed

Address: Av. Marechal Câmara, 160 - 3º andar - Sala 330
20020-907 • Centro • Rio de Janeiro, RJ • Brasil
Phone.: (21) 3478-2700
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Gramado Declaration: The Impact of 20 Years of Cardiovascular Prevention

Aloyzio Achutti,[1] Ricardo Stein,[1] Lúcia Pellanda,[2] Bruce B. Duncan[1]

Universidade Federal do Rio Grande do Sul (UFRGS);[1] Universidade Federal de Ciências da Saúde de Porto Alegre (UFSCPA),[2] Porto Alegre, RS – Brazil

From May 1st to 10th, 1997, the First Brazilian Seminar on Cardiovascular Epidemiology,[1] was held in the city of Gramado (RS), in the manner in which the World Federation of Cardiology has been promoting in various parts of the world since 1968 with The title Ten-Day International Didactic Seminars on Cardiovascular Epidemiology and Prevention.[2]

The initiative came from the Scientific Advisory Board of the Faculty of Medicine of the Universidade Federal of Rio Grande do Sul (UFRGS) and the Cardiology Clinical Department and of the Committe de Epidemiology and Public Health of the *Sociedade Brasileira de Cardiologia* (SBC), under the auspices of the Coordination of Higher Education for Personal Development (CAPES), the World Heart Federation (at that time still called the *International Society and Federation of Cardiology*) and the Inter-American Heart Foundation.

Together with the two coordinators, Aloyzio Achutti and Bruce Duncan, several national teachers (Annick Fontbonne, Eduardo de Azeredo Costa, Emilio Moriguchi, Jorge Pinto Ribeiro, Maria Inês Reinert Azambuja, Maria Inês Schmidt, Paulo Lotufo, Rosely Sichieri and Sérgio Bassanesi), and three international guests (Teri Manolio, Director of Epidemiology and Biometrics of the National Heart Lung and Blood Institute, Ulrich Grueninger, Head of Research and Medical Education of the Swiss Federal Office of Public Health, and Woody Chambless of the Department of Biostatistics at the University of North Carolina) ministered the activities. The 40 participants were from 10 Brazilian states.

In addition to basic concepts of epidemiology and statistics, and topics related to etiology and the prevention of cardiovascular diseases, were part of the program issues that, although currently consecrated, were new in Brazil at the time, as medicine based in evidence and systematic/meta-analysis review. At the time of the beginning of the implantation of the Unified Health System (SUS) and the concern with chronic noncommunicable diseases as a public health problem, this unique meeting enabled and encouraged Brazilian leaders in the field of cardiovascular prevention - several of whom later assumed positions of national leadership. There was a wide debate and, from the first day, time was made to the elaboration of a document that presented three different perspectives of prevention: individual, local and demographic. This document was called the Gramado Declaration[3] and was widely nationally and internationally disseminated.

For the consolidation of the document, an online discussion was conducted through e-mail - which, at that time, was used by only 23 participants. From this experience, with messages that began with the "dear friends of the heart" greeting, a social group was started that was named AMICOR, at the suggestion of Eduardo de Azeredo Costa.[4] In the course of time a website was created, and the AMICOR designation was also used by ProCOR, released two months later, during the Third International Conference on Preventive Cardiology, on the initiative of Professor Bernard Lown (Boston, USA). The name AMICOR was also adopted for some time by SBC on its website, under the name ProCOR / AMICOR, and later in 2004, as a blog named AMICOR.

Since then, much has happened, in terms of Brazilian public health. However, ischemic heart disease remains the main cause of morbidity and mortality in Brazil[5], and social inequalities continue to have direct and indirect impact on early mortality due to cardiovascular diseases in our country.[6-8] In the beginning of 2017, when the Declaration Of Gramado completes 20 years, some evils that affect Brazilian public health show that there is much to be done in the short, medium and long term to face with major achievement the overwhelming burden of cardiovascular disease in Brazil.

On the other hand, as it was already evident in the Seminar and it is increasingly clear nowadays, that cardiovascular diseases can be prevented by public health actions that involve the control of risk factors, as well as by the optimized clinical management of patients. When checking the website of the Global Burden of Disease, it is observed that the mortality standardized by cardiovascular diseases in Brazil from 1995 to 2015 fell by 36%.[9] Recent calculations using slightly different methodology suggest even greater decline - over 2% per year.[10] This reduction can be observed in different Brazilian studies, in various contexts and age groups.[11-15]

It is always difficult to assign causes for changes in disease incidence at the population level. However, improvements such as those that have been seen are, in part, the result of thousands of small gains from multiple actions and actors in the health sector. We would like to consider that the Gramado Seminar, held in the distant year of 1997, was one of these actions and may have contributed to the advances of practical impact seen in the cardiovascular health of the population.

The reduction of cardiovascular diseases in Brazil and in the world is a complex task that depends on numerous agents and continuous effort. Thus, in 2012, was published in the *Brazilian Cardiology Archives* the "Carta do Rio de Janeiro",[16] prepared under the auspices of SBC during the

Keywords

Cardiovascular Diseases / prevention & control; Cardiovascular Diseases / epidemiology; Cardiovascular Diseases / trends.

Mailing Address: Lucia Pellanda •
Av. Princesa Isabel, 370, 3º andar. Postal Code 99620-000, Santana, Porto Alegre, RS – Brazil
E-mail: pellanda.pesquisa@gmail.com

DOI: 10.5935/abc.20170040

III Brazil Prevent / I Latin America Prevent , endorsing the overall target of 25% reduction in early mortality from noncommunicable diseases up to 2025, set out in the World Health Assembly (WHA). The letter was signed by SBC, the *Sociedad Interamericana de Cardiologia*, the American Heart Association, the European Society of Cardiology and the World Heart Federation, and has made concrete decisions on how to achieve global goals.

Among these deliberations, many could already be observed as fundamental since the Gramado Declaration, such as "Implementing actions to acquire epidemiological information, including mortality and cardiovascular morbidity, execution and maintenance of existing registries in some of the signatories, aiming at development of strategies that promote the planning of health actions" and "Create an international permanent discussion forum to monitor the actions aimed at prevention, diagnosis and treatment of Cardiovascular risk factors in Latin America ", of which the AMICOR group could be considered an embryo.

As stated at the end of the Gramado Declaration.[3] "Finally, despite the enormous scientific and technological advances already achieved or prospective in cardiology, it is increasingly necessary to construct a paradigm of health and disease that allows the benefit of such achievements to the entire population. Therefore, a reform in medical education and education of other health professionals is necessary, along with a broad discussion in which popular culture participates, contributing to the evolution of the assistance model, from the traditional biomedical to the biopsychosocial, with emphasis In health and not only in disease".

Thus, it is up to all of us to maintain the mobilization for effective and evidence-based cardiovascular prevention, taking into account the values of society. Actions such as the Brazilian Seminar, with in-depth discussion of relevant topics and strategic objectives, can multiply and have a significant impact in the long run.

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Positioning about the Flexibility of Fasting for Lipid Profiling

Marileia Scartezini,[1] Carlos Eduardo dos Santos Ferreira,[2] Maria Cristina Oliveira Izar,[3] Marcello Bertoluci,[4] Sergio Vencio,[5] Gustavo Aguiar Campana,[2] Nairo Massakazu Sumita,[2] Luiz Fernando Barcelos,[1] André A. Faludi,[3] Raul D. Santos,[3,] Marcus Vinícius Bolívar Malachias,[3] Jerolino Lopes Aquino,[1] César Alex de Oliveira Galoro,[2] Cleide Sabino,[4] Maria Helane Costa Gurgel,[4] Luiz Alberto Andreotti Turatti,[5] Alexandre Hohl,[4] Tania Leme da Rocha Martinez[3]

Sociedade Brasileira de Análises Clínicas (SBAC),[1] Rio de Janeiro, RJ; Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial (SBPC/ML),[2] Rio de Janeiro, RJ; Sociedade Brasileira de Cardiologia (SBC),[3] Rio de Janeiro, RJ; Sociedade Brasileira de Endocrinologia e Metabologia (SBEM),[4] Rio de Janeiro, RJ; Sociedade Brasileira de Diabetes (SBD),[5] São Paulo, SP – Brazil

Justifications

The review of the need of fasting for lipid profile analysis (total cholesterol, LDL-C, HDL-C, non-HDL-C, and triglycerides [TG]) is based on the following grounds:

- Since the postprandial state predominates during most of the day, the patient is more exposed to the lipid levels in this condition when compared with the fasting state. Therefore, the postprandial state may represent more effectively the potential impact of the lipid levels on an individual's cardiovascular risk.
- Measurements in the postprandial state are more practical and provide the patient a greater access to the laboratory, decreasing the number of missed working days and medical appointments due to missed tests, allowing a better assessment of the individual's cardiovascular risk.
- Blood collection in the postprandial state is safer in several circumstances and may help prevent hypoglycemia secondary to the use of insulin in patients with diabetes mellitus, or due to prolonged fasting in pregnant women, children, and elderly individuals, minimizing complications and increasing the adherence to the tests and the attendance to medical appointments.
- There are no significant differences in measurements of total cholesterol, HDL-C, non-HDL-C, and LDL-C performed in the postprandial or fasting state. Levels of TG increase in the fed state, but such increase has little relevance as far as a regular meal without fat overload is considered, with the possibility of adjustment in the reference values.[1-7]
- With a flexibility for lipid profiling, there is a greater amplitude of schedules, thereby reducing congestion in the laboratories, especially early in the morning, bringing more comfort to the patient.
- With the technological advances in diagnostic methods, the main assays available have mitigated the interference caused by increased sample turbidity due to high TG concentrations. However, there are potential limitations, especially related to the calculation of LDL-C, in which performance studies of different methodologies have shown a need for a revision of the practical use of the adopted formulas.

Keywords

Fasting / metabolism; Postprandial; Lipid profile; Reference values; Risk category.

Mailing Address: Marileia Scartezini •
Rua Paulo Gorski, 1837 Apto 701 C, Mossunguê. Postal Code 81210-220, Curitiba, PR – Brazil
E-mail: marileiasca@hotmail.com, marileiascar@gmail.com

DOI: 10.5935/abc.20170039

Clinical and Laboratory Aspects in the Flexibility of Fasting for Lipid Profile Analysis

In easing the requirement for fasting in the collection of samples for lipid profile assessment, some clinical and laboratory recommendations become important.

Recommendations for the care of the patient in the laboratory

- Nonfasting sample collection for lipid profiling**:** may be done by the laboratory with the presence of the information about fasting at the time of sample collection in the laboratory report.
- A medical request without a definition of the fasting duration and without other tests known to require fasting: it is recommended to include in the laboratory report the fasting duration informed at the time of the sample collection.
- Presence in the same request of other tests that require fasting: the laboratory may define that the lipid profile should be collected with a 12-hour fasting when other laboratory tests, ordered on the same request, also require this period of fasting. The laboratory is recommended to specify whether or not fasting is required for each exam: no fasting, 12-hour fasting, or according to the definition set by the laboratory.
- When an indication of a specific fasting duration is present: if the request by the physician has a specific fasting duration, the laboratory should follow such recommendation. The calculation of hours of fasting by the "SIL" (Laboratory Information System, *Sistema de Informação Laboratorial*) may be used, based on the information of the time elapsed since the last meal.
- When the TG levels in the postprandial state are > 440 mg/dL, or in the presence of special situations such as the recovery from pancreatitis secondary to hypertriglyceridemia, or at the beginning of a treatment with drugs that cause severe hypertriglyceridemia, the prescribing physician is recommended to request a new TG evaluation with a 12-hour fasting and this will be considered as a new TG test by the laboratory.[1]
- When the second sample collection for TG measurement occurs: the use of the same code or another specific code for the TG measurement without fasting and after a 12-hour fast will be at the discretion of each laboratory, depending on its system and strategy.

Template recommendations for the laboratory's report

The report is a responsibility of the laboratory and its technical manager. With the purposes of alignment and harmonization among the institutions, it is recommended the adoption of the following information in the report:

- The reference values and therapeutic target for the lipid profile (adults aged > 20 years) according to the cardiovascular risk assessment estimated by the prescribing physician are described in Table 1.[1,8,9]
- Insertion of a note in the report referencing that the lipid profile results should be interpreted according to the clinical assessment and evolution of the patient. The following sentence is recommended: "The clinical interpretation of the results should take into account the reason for indication of the test, the metabolic state of the patient, and the stratification of risk for establishment of the therapeutic goals."
- The target reference values of the lipid profile for children and adolescents are shown in Table 2.[10,11]
- Patients with diabetes and no risk factors or evidence of subclinical atherosclerosis should maintain LDL-C levels below 100 mg/dL. Patients with risk factors or subclinical atherosclerotic disease should maintain LDL-C levels below 70 mg/dL. Patients with a history of acute myocardial infarction; stroke; coronary, carotid or peripheral revascularization; or history of amputation should maintain the LDL-C levels below 50 mg/dL.
- The inclusion of a specific note about the screening of familial hypercholesterolemia (FH) is left at the discretion of the laboratory. The following sentence is recommended: "Values of total cholesterol > 310 mg/dL in adults or ≥ 230 mg/dL in children and adolescents may be indicative of familial hypercholesterolemia, if secondary dyslipidemias are excluded."[14]

Recommendations about formulas and direct LDL-C measurement

The assessment of LDL-C can be performed by direct measurement or estimated by calculation based on Friedewald's or Martin's formula.[13] The following recommendations are suggested to the laboratories:

- Observe the limitations of nonfasting and TG values > 400 mg/dL when Friedewald's formula[15] is used to estimate LDL-C; in these cases, Martin's formula[16] or direct measurement should be used.
- When collecting postprandial samples, the LDL-C measurement can be performed by direct measurement or calculated using Martin's formula.[16]
- Include non-HDL-C in the calculation along with other results of the lipid profile in adults, even without fasting, since the TG levels do not interfere in such calculation. Reporting or not of the VLDL-C calculation may be done at the discretion of the laboratory.

The main purpose of this document is to standardize the clinical and laboratory actions in regards to the flexibility of fasting in the lipid profile analysis across the national territory, contributing to offer security to the decision-making process by physicians and laboratories, grounded by scientific evidence.

Author contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual contente: Scartezini M, Ferreira CES, Izar MCO, Bertoluci M, Vencio S, Campana GA, Sumita NM, Barcelos LF, Faludi AA, Santos RD, Malachias MVB, Aquino JL, Galoro CAO, Sabino C, Gurgel MHC, Turatti LAA, Hohl A, Martinez TLR

Table 1 – Reference values and therapeutic targets for adults > 20 years according to the patient's cardiovascular risk assessed by the physician requesting the lipid profile

Lipids	With fasting (mg/dL)	Without fasting (mg/dL)	Referential category
Total cholesterol*	< 190	< 190	Desirable
HDL-C	> 40	> 40	Desirable
Triglycerides**	< 150	< 175	Desirable
			Risk category
LDL-C	< 130	< 130	Low
	< 100	< 100	Intermediary
	< 70	< 70	High
	< 50	< 50	Very high
Non-HDL-C	< 160	< 160	Low
	< 130	< 130	Intermediary
	< 100	< 100	High
	< 80	< 80	Very high

** Total cholesterol > 310 mg/dL: consider the likelihood of familial hypercholesterolemia; **When the triglyceride levels are above 440 mg/dL (without fasting), the prescribing physician must request a new triglycerides measurement after 12-hour fasting and the laboratory should consider this as a new triglycerides test.*

Table 2 – Reference values of lipid profile for children and adolescents

Lipids	With fasting (mg/dL)	Without fasting (mg/dL)
Total cholesterol*	< 170	< 170
HDL-C	> 45	> 45
Triglycerides (0-9 years) **	< 75	< 85
Triglycerides (10-19 years) **	< 90	< 100
LDL-C	< 110	< 110

** Total cholesterol > 230 mg/dL: consider the likelihood of familial hypercholesterolemia; **When the triglycerides levels are above 440 mg/dL (without fasting), the prescribing physician must request a new triglycerides measurement after 12-hour fasting and the laboratory should consider this as a new triglycerides test.*

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

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MMP-9 Levels and IMT of Carotid Arteries are Elevated in Obese Children and Adolescents Compared to Non-Obese

Claudio Andrade, Adriana Bosco, Valeria Sandrim, Francisco Silva

Santa Casa de Misericórdia de Belo Horizonte – Núcleo de Pós-Graduação e Pesquisa, Belo Horizonte, MG – Brazil

Abstract

Background: Childhood obesity is associated with increased risk of atherosclerosis and cardiovascular disease in adulthood. Increased intima-media thickness (IMT) of the carotid artery is linked to the initiation and progression of the chronic inflammatory processes implicated in cardiovascular disease. Matrix metalloproteinase-9 (MMP-9) plays an important role in the degradation of the extracellular matrix and, consequently, in the development, morphogenesis, repair and remodeling of connective tissues.

Objectives: (i) to determine and compare the concentrations of MMP-9, tissue inhibitor of metalloproteinase -1 (TIMP-1), and MMP-9/TIMP-1 ratio in obese and non-obese children and adolescents; (ii) to investigate the association of these markers with common and internal IMT of carotid arteries.

Methods: Cross-sectional study involving 32 obese and 32 non-obese (control) individuals between 8 - 18 years of age.

Results: Significantly ($p < 0.05$) higher values of MMP-9 concentration, as well as a higher MMP-9/TIMP-1 ratio were detected in the obese group compared to control counterparts. Common and internal carotid IMT values were significantly higher ($p < 0.001$) in the obese group compared to the control group. Positive correlations were observed between the common carotid IMT values and MMP-9 concentrations as well as MMP-9/TIMP-1 ratio.

Conclusions: Our data demonstrate that obese children and adolescents present higher mean IMT values, plasma MMP-9 and MMP-9/TIMP-1 ratio compared to the non-obese. Thus, these findings indicate that this group presents a risk profile for early atherosclerosis. (Arq Bras Cardiol. 2017; 108(3):198-203)

Keywords: Pediatric Obesity; Biomarkers; Atherosclerosis; Tissue Inhibitor of Metalloproteinase

Introduction

Childhood obesity is a major health problem because of its association with an increased risk of atherosclerosis and cardiovascular disease in adulthood.[1] Obesity is correlated to an increased intima-media thickness (IMT) of the carotid artery, which, in turn, is linked to the initiation and progression of chronic inflammatory processes implicated in cardiovascular disease.[1-7] The increase in carotid IMT starts during childhood,[8,9] and nearly all children present fat deposits in these arteries by the age of three.[10] A study by Dawson et al.,[11] with 635 adolescents and young adults, has shown that carotid IMT is significantly correlated to coronary artery risk scores; therefore, early assessment of this parameter through non-invasive methods may assist in the identification of individuals most at risk of cardiovascular disease.

Mailing Address: Valeria Sandrim •
R Prof. Dr. Antônio Celso Wagner Zanin, 250 Bairro: Distrito de Rubião Junior, Postal Code 18618-689 – Botucatu, SP – Brazil
E-mail: valsandrim@yahoo.com.br
Manuscript received November 17, 2015, revised manuscript May 30, 2016, accepted June 09, 2016.

DOI: 10.5935/abc.20170025

Matrix metalloproteinase-9 (MMP-9) plays an important role in the degradation of the extracellular matrix and, consequently, in the development, morphogenesis, repair and remodeling of connective tissues.[13,13] Since MMP-9 activity is regulated primarily by tissue inhibitor of metalloproteinase-1 (TIMP-1), an imbalance between MMP-9 and TIMP-1 could lead to the uncontrolled degradation of extracellular matrix as seen in various pathological disorders, including cardiovascular diseases.[13,14] Thus, some studies in adults have correlated IMT values and circulating MMP-9/TIMP-1 concentrations;[15,16] however, to our knowledge, no study has evaluated these correlations in children and adolescents. Also, increased IMT values of the carotid artery are linked to chronic inflammatory processes in cardiovascular disease,[1-7] and this process involves the activation of MMP-9.

Therefore, we hypothesized that obese children and adolescents present higher concentrations of plasmatic MMP-9 and MMP-9/TIMP-1 ratio compared to the non-obese group, and that these concentrations are positively correlated to IMT values of common and internal carotid arteries. Thus, the aim of this study was to compare plasma MMP-9 and TIMP-1 levels and correlate these concentrations to IMT values of common and internal carotid arteries in obese and non-obese children and adolescents.

Methods

Study population and experimental design

Details of the cross-sectional study were presented to and approved by the Ethics Committee of the Hospital *Santa Casa de Misericórdia* in the city of Belo Horizonte (Belo Horizonte, MG, Brazil). Written informed consent was obtained from all participants and/or their legal guardians prior to the investigation.

Potential participants were recruited in the outpatient clinic of the Division of Endocrinology and Metabolism of *Santa Casa de Misericórdia* in the city of Belo Horizonte and included males and females between 8 and 18 years of age. Individuals presenting hypertension, metabolic, endocrine, autoimmune, neoplastic and infectious diseases were excluded from the study. Participants were assessed as obese (n = 32) or non-obese (n = 32; control group) according to their body mass index (BMI) referenced against the 2000 Centers for Disease Control and Prevention (CDC) sex-adjusted BMI-for-age growth charts with the cut-off point for obesity taken as ≥ 95th percentile.[17,18] Hypertension was defined by the *"VI Diretrizes de Hipertensão Arterial da Sociedade Brasileira de Cardiologia"* (VI Arterial Hypertension Guidelines from the Brazilian Society of Cardiology) and for children and adolescents, it was based on percentiles. Obese and non-obese groups were not on any medication. A minimum sample size of 23 individuals per group was calculated considering an alpha error of 0.05% and a test power of 90%. Data were collected between March 2010 and March 2012.

Anthropometrical, clinical and biochemical evaluations

Anthropometrical (weight, height and BMI), clinical (carotid IMT) and biochemical (TSH, MMP-9, TIMP-1, MMP-9/TIMP-1 ratio) parameters were collected for all selected individuals. Anthropometric measurements were performed with participants barefoot and with light clothes. Body weight was measured using portable digital scales (capacity 180 kg; sensitivity 100 g), while height was determined by portable stadiometer (non-extendable 2 m measuring tape graduated in 0.1 cm divisions) with the subject in the orthostatic position. Systolic (SBP) and diastolic (DBP) blood pressures were measured at least three times after 15 min of rest and hypertension was defined as SBP and/or DBP exceeding the 95th percentile.[19]

Serum TSH was estimated with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quibasa Química Básica, Belo Horizonte, MG, Brazil). Plasma was collected in tubes containing EDTA as anticoagulant, MMP-9 and TIMP-1 tests were performed using human MMP-9/TIMP-1 complex DuoSet kit (R&D Systems, Minneapolis, MN, USA).

IMT measurements

Common carotid artery: average measurement of the thickness on both sides, longitudinal projection, exactly 1 cm before the bifurcation. Internal carotid artery: average measurement of the thickness on both sides, longitudinal projection at the origin.

Measurements were performed using a Vivid i (GE Healthcare, Milwaukee, WI, USA) portable ultrasound system with the subject lying in the supine position and with the neck rotated (45°) to the side opposite to the undergoing examination.[20] All examinations were performed by a single physician with certified skills in diagnostic imaging.

Statistical analysis

Statistical analyses were performed with the aid of SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Student's t test was used to compare the mean values of the two groups regarding variables that were normally distributed, while the Mann Whitney test was used to compare variables that were not normally distributed. The χ^2 test was employed to assess the relationship between carotid IMT and independent variables. The correlations among plasma biomarkers and common and internal carotid IMT were analyzed using Spearman´s correlation. In all tests, statistical significance was set at 5% (0.05).

Results

Clinical and biochemical characteristics of subjects enrolled in study are shown in Table 1. Although both groups exhibited serum TSH values within the normal range, the mean value of this parameter in the obese group was significantly higher ($p < 0.05$) than that recorded in the non-obese group (2.7 ± 0.8 *vs* 2.0 ± 0.8 μIU/mL, $p < 0.05$). Plasma MMP-9 concentrations were significantly higher in the obese group compared to the non-obese group ($p < 0.05$), while plasma TIMP-1 concentrations were similar ($p > 0.05$) in both groups. Mean MMP-9/TIMP-1 ratio was significantly higher ($p < 0.05$) in the obese group in comparison to the non-obese. Mean IMT values of the common and internal carotid arteries of obese individuals were significantly greater ($p < 0.001$) than those of their control counterparts.

There was a direct and statistically significant correlation among plasma MMP-9, MMP-9/TIMP-1 ratio, and IMT values of the common carotid artery ($p = 0.02$ and $p = 0.04$, respectively: Figure 1, A and E). In contrast, there was no significant correlation between plasma TIMP-1 and IMTs of common and internal carotid arteries (Figure 1, C and D) or MMP-9 and IMT of internal carotid arteries (Figure 1B).

Discussion

To our knowledge, this is the first study to correlate plasma MMP-9 and TIMP-1 levels to common and internal IMT in obese and non-obese children and adolescents. Following an evaluation of matrix metalloproteinases in obese and non-obese children and adolescents, Głowińska-Olszewska et al.[12] reported high concentrations of the atherosclerosis marker MMP-9 in the obese group and even higher concentrations in hypertensive obese individuals. These authors argued that the abnormally high concentrations of MMP-9 could indicate modifications in the metabolism of the extracellular matrix of blood vessels and heart muscle, and that such alterations could speed up the atherosclerotic process. Additionally, the same research team described

Table 1 – Demographic, anatomical and biochemical characteristics of obese and non-obese children and adolescents recruited in the outpatient clinic of the Division of Endocrinology and Metabolism of *Santa Casa de Misericórdia de Belo Horizonte* (Belo Horizonte, MG, Brazil).

Variable	Obese group [n = 32]				Non-obese group [n = 32]			
	Minimum	Maximum	Mean/%	SD	Minimum	Maximum	Mean/%	SD
Age [years]	8	17	13	2	12	18	15*	2
Height [m]	1.28	1.79	1.57	0.13	1.52	1.84	1.63*	0.08
Weight [kg]	47	120	73	17	35	71	56*	9
BMI [kg/m²]	26	40	29	5	15	23	22*	2
SBP (mmHg)	90	120	103	6	90	110	103	6
DBP (mmHg)	50	70	60	7	50	80	63	7
Gender (% Female)	-	-	59	-	-	-	47	-
TSH [µIU/mL]	1.5	4.6	2.7	0.8	0.7	4.2	2.0*	0.8
Common carotid IMT [mm]	0.38	0.58	0.45	0.04	0.38	0.45	0.42*	0.02
Internal carotid IMT [mm]	0.36	0.46	0.42	0.03	0.37	0.44	0.40*	0.02
MMP-9 [ng/mL]	127	1208	343	249	92	925	246*	151
TIMP-1 [ng/mL]	322	1165	677	214	207	1522	709	284
MMP-9/ TIMP-1 ratio	0.15	1.47	0.48	0.25	0.11	1.59	0.41*	0.31

*BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TSH: thyroid-stimulating hormone; IMT: intima-media thickness; MMP-9: metalloproteinase-9; TIMP-1: tissue inhibitor of metalloproteinase-1; SD: standard deviation. * Significant differences p < 0.05 compared to obese group.*

that MMP-9 and TIMP-1 concentrations were elevated in obese children and adolescents, and that the values of these parameters increased even further when obesity was accompanied by hypertension.[12] Moreover, Belo et al.[21] reported that genotypes and haplotypes of MMP-9 gene modulate circulating MMP-9 levels in obese children and adoslecentes. In the present study, plasma MMP-9 and the ratio MMP-9/TIMP-1 were significantly higher in obese individuals compared to their control counterparts, but the two groups presented no statistical difference in plasma TIMP-1. Although weak, it was possible to demonstrate a direct relationship between the concentrations of MMP-9 and MMP-9/TIMP-1 ratio, but not those of TIMP-1 and IMT values of common carotid arteries, suggesting a potential participation of this gelatinase in artery remodeling. Furthermore, no such relationship could be established with internal carotid IMT. This difference of correlations could be explained by the magnitude of the IMT of the internal carotid that is lower than that of the common carotid; therefore, the difference of magnitude may have interfered in the correlation. It is important to note that plasma MMP-9 concentrations reflect the systemic MMP-9 production and not only the vascular production, which may reduce the magnitude of correlations between this biomarker and IMT.

In the present study, mean IMT values of the common and internal carotid arteries of the obese group (0.47 and 0.43 mm, respectively) were significantly increased ($p < 0.001$) compared to those of the control group (0.42 and 0.40 mm, respectively); a result that is in agreement with previous reports.[22,23] Thus, in a case-control study carried out in Belgium by Beauloye et al.,[23] involving healthy subjects between 8 and 18 years of age, the mean value of carotid IMT of the obese group (0.470 mm) was significantly greater than that of the non-obese control group (0.438 mm), even though the mean age of the two groups did not differ significantly. Furthermore, these authors were able to demonstrate a significant positive correlation between carotid IMT and relative BMI. Moreover, studying Brazilian adolescents, Silva et al.[24] demonstrated, in 35 obese and 18 non-obese subjects between 10-16 years old, that cIMT, triglycerides, HOMA-IR, insulin, and CRP values were higher, while high-density lipoprotein cholesterol (HDL-c), adiponectin, and VO_{2max} values were lower in the obese group than in the non-obese group.[24]

Based on mean IMT values of the common carotid artery determined in the obese and control groups in the present study, a cut-off point of 0.44 mm was established. A sonographic evaluation of common carotid and femoral arteries of 247 healthy subjects between 10 and 20 years of age[25] revealed that mean IMT values increased almost linearly from 0.38 to 0.40 mm with increasing age. Since the adopted cut-off point was considerably higher than the value previously ascribed to healthy individuals in the age range 18 to 20 years of age, it is possible to state that children and adolescents comprising the obese group in the present study exhibited abnormally increased carotid IMT values. Moreover, it was possible to estimate from the data obtained that the risk of the obese group exhibiting elevated common carotid IMT was 2 to 5 times higher than that of the control group, while the risk of increased internal carotid IMT was 1.5 to 4 times greater.

Non-invasive techniques are reliable tools for identifying adults with increased risk of atherosclerosis and cardiovascular risk, but for children and adolescents, such techniques have been reserved mainly for research purposes. Ultrasound imaging appears to be a reliable technique to estimate IMT values of human arteries *in vivo*, since Pignoli et al.[26] were able to confirm that there were no significant differences between B

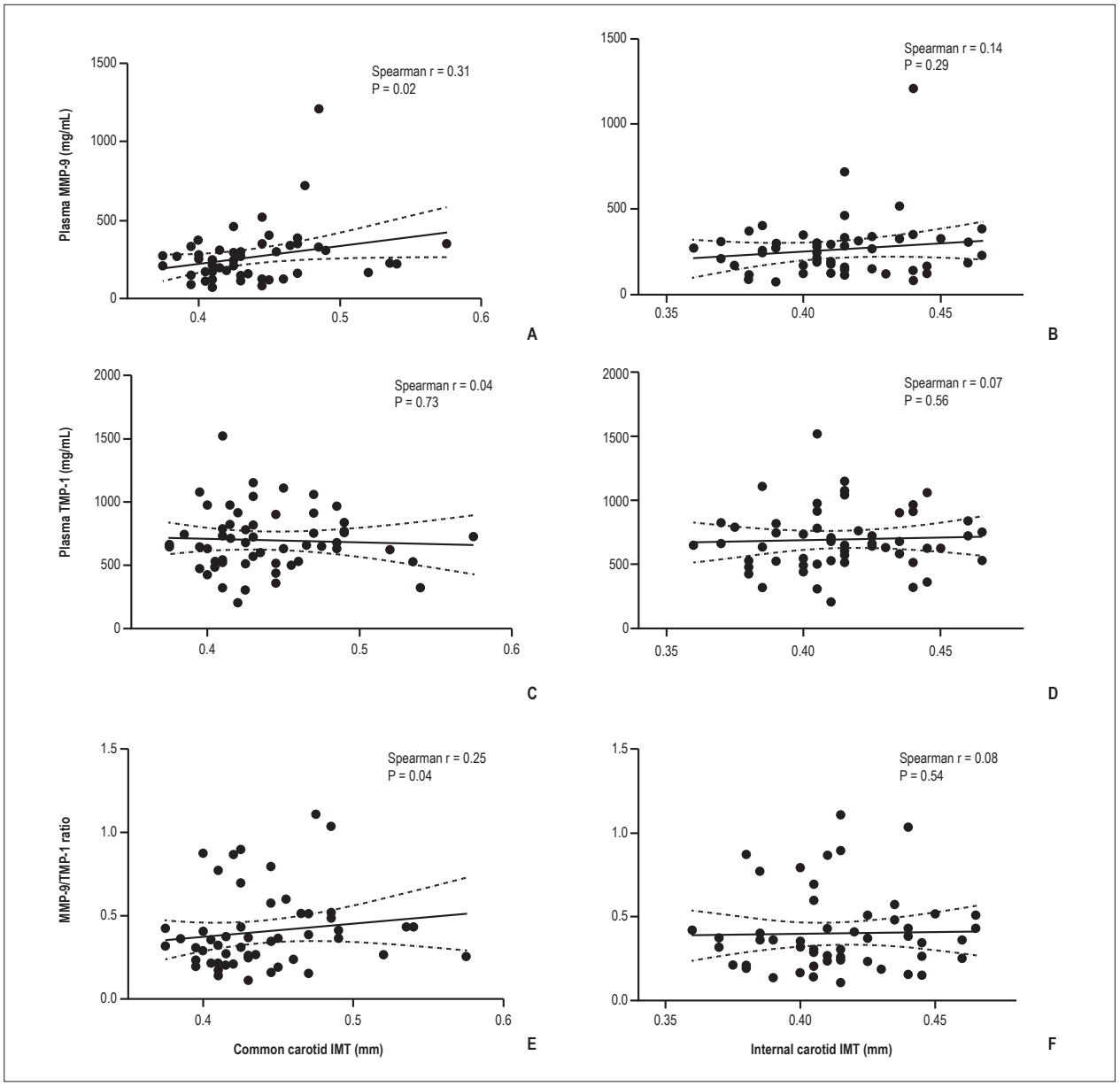

Figure 1 – *Correlations among biomarkers [MMP-9 (A,B), TIMP-1(C,D) and MMP-9/TIMP-1 ratio (E,F)] and common (A,C,E) and internal (B,D,F) carotid IMT. The correlations among plasma biomarkers and common and internal carotid IMT were analyzed using Spearman´s correlation.*

mode-determined IMTs of the common carotid arteries evaluated in pathogenic examination and those evaluated *in vivo* in young subjects. Moreover, while the analysis of IMT has often been used in cross-sectional studies, only a few clinical trials with children have employed this parameter.[20] The Cardiovascular Risk in Young Finns study,[27] which comprised a 21 year follow-up longitudinal investigation, suggested that obesity indices, such as BMI, skinfold, serum lipoproteins, insulin, glucose and blood pressure, measured in youth, are significantly associated to increased IMT and decreased elasticity of the carotid artery in adulthood. These findings emphasize the importance of weight control from youth to adulthood in reducing cardiovascular risk.

Although mean TSH value of the obese group was higher than that of the control group (2.85 versus 1.98 μIU/mL), no cases of hypothyroidism were diagnosed in obese participants. Conventionally, a serum TSH concentration of 4 to 5 μIU/mL is considered elevated; however, recent data from large population studies have indicated that a lower TSH cut-off point in the region of 2 to 2.5 μIU/mL would be more appropriate.[28] Likewise, the National Academy of Clinical Biochemistry has recommended an upper limit of 2.5 μIU/mL[29] for serum TSH, a value that is below the mean concentration of the obese group determined in the present study. However, it is not possible to state with certainty that cases of subclinical hypothyroidism were absent within the obese group of the present study.

In addition, numerous studies have revealed a positive association between measures of obesity and serum thyroid-stimulating hormone (TSH) concentrations, although the mechanisms responsible for this association require further elucidation,[30] it is proposed that variations in thyroid hormone could affect lipoproteins and oxidation steps contributing to vascular remodeling and endothelial function.[31] Interestingly, a significant correlation has also been demonstrated between carotid IMT and TSH values within normal reference values, suggesting an increased cardiovascular risk in subjects with low normal thyroid function.[31]

Yap and Jasul[32] found a positive correlation between serum TSH and BMI, and inferred that an increase in TSH concentration, even within the generally accepted limits, could contribute to weight problems. The present study demonstrated that the group of obese children and adolescents exhibited increased TSH concentrations, although the concentrations were within the normal range, similarly to findings previously reported by Aypak et al.[33] However, this problem clearly requires further investigation since hypothyroidism may be associated with markers of atherosclerosis and, consequently, with increased carotid IMT.[34,35] A limitation of our study is the small number of subjects enrolled.

Conclusion

Our data demonstrate that obese children and adolescents present higher mean IMT values, plasma TSH, plasma MMP-9 and MMP-9/TIMP-1 ratio compared to the non-obese. Thus, these findings indicate that this group presents a risk profile for early atherosclerosis.

Acknowledgements

The study was financed by IEP – *Santa Casa de Misericórdia de Belo Horizonte*, MG, Brazil and *Fundação de Amparo á Pesquisa do Estado de Minas Gerais*.

Author contributions

Conception and design of the research: Andrade C; Acquisition of data: Bosco A, Sandrim V; Analysis and interpretation of the data: Andrade C, Bosco A, Sandrim V, Silva F; Statistical analysis: Bosco A, Sandrim V; Writing of the manuscript: Andrade C, Bosco A, Sandrim V; Critical revision of the manuscript for intellectual content: Silva F.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Study Association

This article is part of the thesis of master submitted by Claudio Andrade, from Santa Casa de Misericórdia de Belo Horizonte.

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Ankle-Brachial Index as a Predictor of Mortality in Hemodialysis: A 5-Year Cohort Study

Jair Baptista Miguel, Jorge Paulo Strogoff de Matos, Jocemir Ronaldo Lugon
Universidade Federal Fluminense (UFF), Rio de Janeiro, RJ - Brazil

Abstract

Background: **Abnormal ankle-brachial index (ABI) has been found to be a strong predictor of mortality in some hemodialysis populations in studies with relatively short periods of follow-up, lower than 2 years.**

Objective: **This study aimed to assess the predictive value of abnormal ABI as a risk factor for death among patients on maintenance hemodialysis after a 5-year follow-up.**

Methods: **A total of 478 patients on hemodialysis for at least 12 months were included in the study. ABI measurement was performed using a mercury column sphygmomanometer and portable Doppler. Patients were divided into 3 groups according to ABI (low: <0.9; normal: 0.9 to 1.3; and high: >1.3) and followed for a 60-month period.**

Results: **The prevalence rates of low, normal and high ABI were 26.8%, 64.6% and 8.6%, respectively. The 5-year survival rate was lower in the groups with low ABI (44.1%, P<0.0001) and high ABI (60.8%, P= 0.025) than in the group with normal ABI (71.7%). Cox regression was used to evaluate the association between ABI and mortality, adjusting for potential confounders. Using normal ABI as reference, a low, but not a high ABI was found to be an independent risk factor for all-cause mortality (HR2.57; 95% CI, 1.84-3.57 and HR 1.62; 95% CI, 0.93-2.83, respectively).**

Conclusions: **long-term survival rates of patients with either low or high ABI were lower than the one from those with normal ABI. However, after adjustment for potential confounders, only low ABI persisted as an independent risk factor for all-cause mortality among hemodialysis patients. (Arq Bras Cardiol. 2017; 108(3):204-211)**

Keywords: **Ankle Brachial Index / mortality; Measures; Renal Dialysis; Renal Insufficiency, Chronic; Arterial Pressure; Cohort Studies.**

Introduction

The mortality rate among end-stage renal disease (ESRD) patients is still high and cardiovascular diseases (CVD) are responsible for approximately 50% of the deaths.[1-4] In addition to ischemic heart disease and cerebrovascular disease, peripheral arterial disease (PAD) is highly prevalent among dialysis patients and its presence is associated with high morbidity and mortality.[5-8] The ankle-brachial index (ABI) is a simple, inexpensive and non-invasive test[7-9] that has been shown to have a high sensitivity and specificity for the diagnosis of PAD when compared to angiography, the gold-standard diagnosis method.[10] This index is based on the fact that systolic blood pressure in the legs is usually equal to or slightly higher than in the upper limbs in healthy individuals. In the presence of artery stenosis, a reduction in pressure occurs distally to the lesion.[11] In addition, low ABI has a strong correlation with arterial disease in other sites and has been found to be a good predictor of mortality in the general population.[12,13] Moreover, both low and high ABI have been found to be strong predictors of death among hemodialysis patients.[6-9]

Considering that the usefulness of ABI has already been demonstrated in some hemodialysis populations with a mean follow-up lower than 2 years,[7,8] the present study aimed to assess the predictive value of ABI as an independent risk factor for death among patients on maintenance hemodialysis after a 5-year follow-up.

Methods

This is an observational prospective study, with a 5-year follow-up period, performed at six dialysis facilities in Rio de Janeiro State, Brazil. All patients aged 18 to 75 years, who had been on hemodialysis for at least 12 months, were eligible to be included in the study. Written informed consent was obtained and approved, as well the protocol of study, by the local ethical committee, number CEP 23/06. Patients with cancer, anti-HIV positive test, atrial fibrillation, bilateral lower-limb amputation, or dementia and those

Mailing Address: Jair Miguel •
Rua Bromélias, 100. Postal Code: 28470-000, Monte Líbano, Santo Antônio de Pádua, RJ - Brazil
E-mail: jairbaptista.miguel@hotmail.com; jair.miguel@cdrclinefron.com.br
Manuscript received January 18, 2016; revised manuscript February 26, 2016; accepted June 02, 2016.

DOI: 10.5935/abc.20170026

who refused to participate were excluded from the study. The ABI measurements were taken between March 2006 and September 2007.

The ankle-brachial index

ABI, defined as the ratio of ankle-to-arm systolic blood pressure, was measured once, at the entrance of the patients in the study, before hemodialysis session and after 5 minutes in supine position. In lower limbs, tibial posterior arteries were used, since the *dorsalis pedis* artery is congenitally absent in 4 to 12% of the population.[14] Systolic blood pressure was measured twice at each site, in rapid and alternate succession, to obtain a mean value. Standard blood pressure arm cuffs connected to a mercury column were applied to the arm and to each ankle (with the lower end of the bladder just above the malleoli). Ultrasound gel was applied, and a Doppler stethoscope (10 MHz, Super Dupplex, Huntleigh Technology Inc., Manalapan NJ, USA) was used to assess systolic blood pressure. Systolic blood pressure in the upper limb was measured on the brachial artery of the arm contralateral to the vascular access. To calculate ABI, the lowest mean from the ankles was divided by the mean in the arm. All ABI measurements were performed by 3 trained observers (one physician and two medical students) based on the information that inter- and intra-observer variability for Doppler blood pressure measurement is negligible.[5,15]

To evaluate the relationship between ABI and demographics, clinical and laboratory data, the population was divided into three groups according to ABI values: low ABI group (< 0.9), normal ABI group (0.9 to 1.3) and high ABI group (> 1.3).

Demographic, clinical and laboratory data

Demographics and clinical data were derived from both a structured clinical interview and a database used in all six dialysis facilities. These data included gender, age, race, time on dialysis, primary kidney disease, vascular access, and current smoking status. Comorbidities were defined as following: diabetes; hypertension (pre-dialysis systolic blood pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg and/or use of anti-hypertensive drugs); coronary artery disease (exertional angina, current use of coronary vasodilator, past myocardial infarction, and coronary artery bypass graft or percutaneous coronary intervention); stroke sequelae; PAD (current use of peripheral vasodilator, past lower limb artery bypass surgery, angioplasty or non-traumatic lower limb amputation); and hepatitis C seropositivity. Levels of C-reactive protein (CRP) were determined by ultra-sensitive immunoturbidimetric assay, specifically for the study, and the values shown were determined by the time ABI was measured. The remaining laboratory data – basal levels of hemoglobin, serum creatinine, blood urea nitrogen (BUN), equilibrated urea Kt/V (eKt/V), and albumin – were extracted from patients' medical records. To better estimate the impact of bone mineral disturbances on our findings, cumulative exposure was assessed through calculation of the mean of all values for serum calcium, phosphorus and intact parathyroid hormone (i-PTH) measurements along the 36-month period preceding the ABI evaluation or since hemodialysis initiation for patients on dialysis for less than 3 years, as described previously.[16] Calcium and phosphorus serum levels were measured on a monthly basis and i-PTH every six months. All routine blood analyses were performed in a central laboratory.

Statistical analysis

Continuous variables were expressed as mean ± SD if distribution was normal and as median and range in case of non-Gaussian distribution. Categorical variables were presented as frequency. Comparison of the means between the ABI groups were performed using analysis of variance (ANOVA) complemented by Bonferroni test or the nonparametric test of Kruskal-Wallis complemented by Dunn test as appropriated. Frequencies were compared by Fisher's exact test. The Kaplan-Meier test was used for analysis of survival, and comparison between curves was made by the Log-Rank test.

Based on a previous pilot study, we estimated that the prevalence of low, normal and high ABI would be approximately 30%, 60% and 10%, respectively. Our study was designed to have a statistical power of 0.8 to detect a difference in survival rate between low and normal ABI of 30%, with a two-sided alpha level of 5%. Thus, after accounting for an expected 20% of drop-out for reasons other than death, the minimal number of participants was estimated to be 450.

Associations of ABI group (low, normal and high) with death risk were analyzed by Cox-regression models: a non-adjusted model, which only included the variable of primary interest, ABI; a model adjusted for demographics and clinical data (gender, age, race, diabetes, time on dialysis, smoking, coronary disease, stroke sequelae) – "Model 1"; and finally, a model in which laboratory variables (serum albumin, hemoglobin, i-PTH, ionized calcium, phosphorus, eKt/V, and CRP) were also included as potential confounders – "Model 2".

The null hypothesis was rejected when P value was < 0.05. The software SPSS, version 18.0 (Chicago, Illinois, USA) was used for the statistical analysis.

Results

Of a total of 1,170 patients on maintenance hemodialysis in six dialysis facilities, 478 patients were enrolled in the study. Demographic and laboratory characteristics of patients are listed in Table 1. Median age was 54 (18 -75) years, 56% were males, and 14.9% and 50.6% had diabetes and hypertension as the primary kidney disease, respectively. Median time on dialysis was 59 (12 - 427) months, and longer than 3 years for 73% of patients.

The prevalence of low, normal and high ABI was 26.8%, 64.6% and 8.6%, respectively. Table 2 shows the characteristics of each ABI group. Male gender prevailed

Table 1 – Demographic and laboratory characteristics of the population (n = 478)

Male gender, (%)	268 (56%)
Age (years)	54 (18-75)
Race	
White	221 (46.2%)
Non-White	257 (53.8%)
Time on dialysis (months)	59 (12-427)
Primary kidney disease, (%)	
Diabetic nephropathy	71 (14.9%)
Hypertensive nephrosclerosis	242 (50.6%)
Chronic glomerulonephritis	41 (8.6%)
Unknown	62 (13.0%)
Polycystic kidney disease	21 (4.4%)
Lupus nephropathy	8 (1.7%)
Others	33 (6.9%)
Comorbidities, (%)	
Diabetes	81 (16.9%)
Hypertension	291 (60.9%)
Smoking	73 (15.3%)
Coronary artery disease	114 (23.9%)
Stroke sequelae	16 (3.3%)
Peripheral artery disease	86 (18%)
Patients < 3 years of hemodialysis, (%)	
Parathyroidectomy	29 (6.1%)
Anti-HCV positive test, (%)	101 (21.1%)
HB HBsAg positive test	13 (2.7%)
eKt/V	1,51 ± 0,40
Hemoglobin (g/dL)	11,4 ± 1,6
Albumin (g/dL)	3,8 ± 0,3
Calcium (mg/dL)	4,6 ± 0,3
Phosphorus (mg/dL)	5,4 ± 1,2
i-PTH (pg/mL)	370 (10-2,500)
CRP (mg/L)	4,7 (0,1 - 150)

CRP: C-reactive protein; eKt/V: equilibrated Kt/V; HCV: hepatitis C virus; i- PTH :Intact parathyroid hormone; HBsAg: Hepatitis B Surface Antigens; Values are expressed as median (range), mean ± SD or by frequency.

in the high ABI group, when compared to the low and normal ones. Patients in the low ABI group were significantly older than those in the normal and high ABI groups. The prevalence of diabetes, PAD and non-traumatic amputation was significantly lower in the normal ABI group, when compared to both low and high ABI groups. Coronary artery disease and stroke sequelae were more frequent in the low ABI group than in the normal ABI group. No difference was found regarding arm blood pressure measurements between the groups.

Laboratory findings of each group are shown in Table 3. The low ABI group had higher CRP and lower serum albumin than the normal ABI group. Serum creatinine was lower in the low ABI group than in normal and high ABI groups. The high ABI group had increased serum phosphorus levels and calcium × phosphorus product, when compared to the normal and low ABI groups. The high ABI group also had increased i-PTH levels compared to the low ABI group.

After a 5-year follow-up period, 158 of 478 patients died, 69 lost their follow-up due to a change of dialysis facility and 28 underwent kidney transplantation. The survival curves according to the ABI group are presented in Figure 1. When the 5-year survival rates were compared, values were lower in the groups of altered ABI (44.1% for low ABI and 60.8% for high ABI) than in the normal ABI group (78%), $P < 0.0001$ and $P = 0.025$, respectively.

The association of ABI with mortality risk in the Cox proportional hazard models is shown in Table 4. In the non-adjusted model, low ABI was associated with increased mortality risk (HR 2.57, 95%CI1.84-3.57), but the association of high ABI with death (HR 1.62, 95%CI0.93-2.83) was not significant. In the multivariate analysis, after adjustment for demographics and comorbidities (Model 1), low ABI persisted significantly associated with all-cause mortality (HR1.83, 95%CI1.28-2.63), accompanied by age (HR1.02, 95%CI1.01-1.04). After further adjustment for laboratory variables (Model 2), low ABI (HR 1.69, 95%CI 1.14-2.51) and age (HR 1.02[per year], 95%CI 1.01-1.04) again persisted as significantly associated with all-cause mortality. Here, stroke sequelae (HR 2.25, 95%CI 1.09-4.67) and CRP (HR 1.02[per mg/L], 95%CI 1.01-1.03) also were found to be significantly associated with increased mortality risk.

Discussion

ABI is an easy, reliable and non-invasive test, that has been used as a diagnostic tool for PAD, a condition highly prevalent among hemodialysis patients.[5,8] It has also been shown to be a useful marker of diffuse atherosclerotic disease as well as a predictor of mortality in patients on hemodialysis and in general population.[5,7,8,12,13] The relationship between ABI and CVD has also been demonstrated by a negative correlation between ABI and intimal medial thickness,[17] and by an inverse correlation between ABI and left ventricular mass in hypertensive patients without clinical manifestations of PAD.[18]

The survival curves were significantly different between the ABI groups in the current study. Survival was lower in both low and high ABI groups when compared to the normal one. These findings point to the importance usefulness of ABI as an important predictor of mortality in hemodialysis population. Low ABI was found to be associated with higher mortality rate in general population[12-15] as well as in patients with chronic kidney disease, stages 3 to 5[19] and hemodialysis patients.[7,8,20] High ABI has also been associated with increased mortality in studies involving hemodialysis patients.[8,20]

Abnormal values of ABI as predictors of death were assessed by Cox proportional hazards models. In the non-adjusted model, in which only three bands of ABI were taken into account, and the normal ABI band was taken as reference,

Table 2 – Demographics according to ankle-brachial index (ABI) classification

Variables	ABI		
	Low (n=128)	Normal (n=309)	High (n=41)
Male (%)	53.1	53.7	80.5*
Age (years)	62 (20 to 77)	49 (18 to 75)**	54 (27 to 71)**
Race (White), %	45	44	61†
Time on dialysis (months)	57 (13 to 321)	59 (12 to 292)	65 (13 to 427)
Primary kidney disease, f (%)			
Diabetes	25.0	8.4**	31.7†
Hypertension	51.6	52.1	36.6
Chronic glomerulonephritis	3.9	11.0**	4.9
Polycystic kidney disease	3.9	4.9	2.4
Lupus nephropathy	-	2.3	2.4
Others	8.6	6.5	4.9
Unknown	7.0	14.9	17.1
Comorbidities (%)			
Diabetes	30.5	9.4**	31.7†
Hypertension	65.6	60.5	48.8
Smoking	17.2	15.2	9.8
Coronary artery disease	25.0	15.2#	12.2
Stroke sequelae	8.6	1.6**	-
Peripheral artery disease	27.3	7.4**	24.4†
Nontraumatic amputation	7.8	1.3**	9.8†
Parathyroidectomy	4.7	6.5	7.3
Positive HBsAg	4.7	2.3	-
Positive anti-HCV test (%)	20.3	19.4	36.6††

HCV: hepatitis C virus; HBsAg: Hepatitis B Surface Antigens; Values are expressed by frequency and median (range); *p < 0.01 vs. low and normal ABI;†P < 0.01 vs. normal ABI;# p < 0.05 vs. low ABI;**p < 0.01 vs. low ABI; ††p < 0.05 vs. normal ABI.

Table 3 – Laboratory findings according to ABI classification

Parameters	ABI		
	Low (n=128)	Normal (n=309)	High (n=41)
CRP (mg/L)	6.4 (0.2-150)	3.9 (0.1-150)*	4.3 (0.2-41)
Albumin (g/dL)	3.74 ± 0.31	3.84 ± 0.30*	3.72 ± 0.36
BUN (mg/dL)	69 ± 22	68 ± 22	76 ± 22
Creatinine (mg/dL)	10.6 ± 2.8	11.9 ± 3.0*	12.2 ± 2.8*
eKt/V	1.51 ± 0.41	1.53 ± 0.42	1.36 ± 0.23†
Hemoglobin (g/dL)	11.6 ± 1.6	11.2 ± 1.7	12.2 ± 2.8
i-PTH (pg/mL)	297 (28 – 2,202)	386 (4 – 2,500)	489 (10 – 2,160)**
Ionic calcium (mg/dL)	4.6 ± 0.3	4.6 ± 0.3	4.6 ± 0.4
Phosphorus (mg/dL)	5.3 ± 1.2	5.4 ± 1.1	5.8 ± 1.4††
Ca x P product (mg^2/dL^2)	24.1 ± 5.7	24.7 ± 5.5	27.1 ± 6.3††

CRP: C-reactive protein; BUN: Blood urea nitrogen; eKt/V: Equilibrated Kt/V; i-PTH: Intact parathyroid hormone; Values are expressed by the median (limits) or by the mean ± SD; *p < 0.01 vs. low ABI; †p < 0.05 vs. normal ABI;**p < 0.05 vs. low ABI; ††p < 0.05 vs. low and normal ABI.

Table 4 – Predictors for overall mortality using Cox proportional hazards models

Variables	Non-adjusted	Model 1*	Model 2**
	HR (95%CI)	HR (95%CI)	HR (95%CI)
ABI			
Normal (ref.)	1.00	1.00	1.00
High	1.62 (0.93-2.83)	1.47 (0.83-2.60)	1.16 (0.60-2.26)
Low	2.57 (1.84-3.57)	1.83 (1.28-2.63)	1.69 (1.14-2.51)
Gender (male)	-	1.23 (0.89-1.71)	1.25(0.86-1.81)
Age (years)	-	1.02 (1.01-1.04)	1.02 (1.01-1.04)
Race (White)	-	0.98 (0.71-1.36)	0.95 (0.66-1.37)
Diabetes (y/n)	-	1.37 (0.93-2.03)	1.37 (0.88-2.13)
Time on dialysis (mo)	-	1.00 (0.98-1.00)	1.00 (0.99-1.00)
Smoking (y/n)	-	1.23 (0.83-1.82)	1.27 (0.84-1.92)
Coronary disease (y/n)	-	1.13 (0.77-1.67)	1.06 (0.69-1.63)
Stroke Sequelae (y/n)	-	1.73 (0.89-3.39)	2.25 (1.09-4.67)
Laboratory parameters			
Albumin (g/dL)	-	-	0.82 (0.44-1.52)
Hemoglobin (g/dL)	-	-	0.97 (0.87-1.09)
i-PTH (pg/mL)	-	-	1.00 (0.99-1.00)
Calcium (mg/dL)	-	-	1.06 (0.60-1.89)
Phosphorus (mg/dL)	-	-	0.93 (0.78-1,10)
eKt/V	-	-	0.85 (0.53-1.36)
CRP (mg/L)	-	-	1.02 (1.01-1.03)

Values expressed as hazard ratios (HR) and 95% confidence interval (CI); *Adjusted for demographics date and comorbidities; **Adjusted for demographics data, comorbidities and laboratory parameters; PTH -Intact parathyroid hormone; eKt/V -equilibrated Kt/V; CRP - C reactive protein.

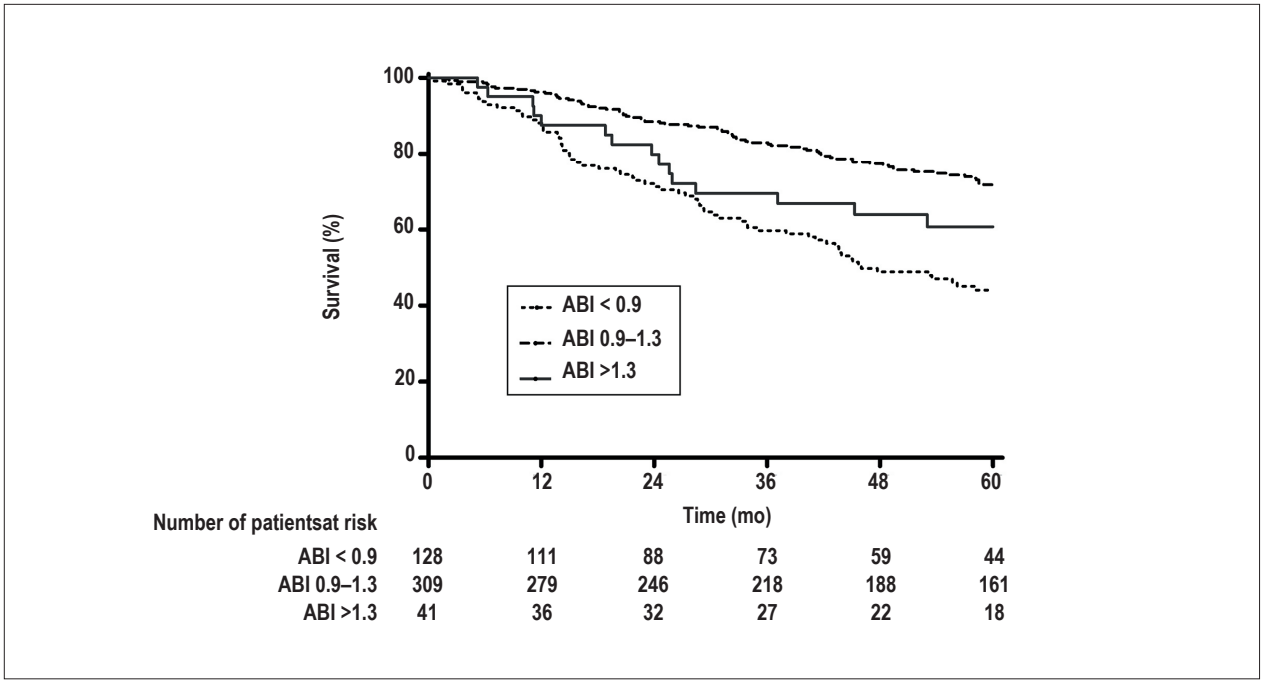

Figure 1 – *Survival curves for the first 5 years of follow-up according to ankle-brachial index (ABI) at baseline*

only low ABI was associated with a significant death risk. The multivariate analysis was performed in two steps. Firstly, we developed the Model 1, in which the association of ABI bands with mortality was adjusted for gender, age, race, diabetes status, time on dialysis, smoking, coronary disease, and stroke sequelae. On a second step, in Model 2, laboratory parameters were also added as potential confounding factors. Our findings showed that low ABI persisted as an independent risk factor for all-cause mortality even after adjustment for all demographics, comorbidities and laboratory variables. On the other hand, we found that high ABI did not represent an independent risk factor for all-cause mortality. This is in disagreement with previous studies,[8,20] but the small sample size in our analysis could have reduced the chance of detecting a true effect of high ABI due to the low statistical power.

Also interesting was the finding that diabetes, *per se,* did not represent an independent determinant for mortality. This finding is consistent with previous studies[21,22] suggesting that only diabetic patients on hemodialysis having arterial disease present a greater risk of death. Moreover, diabetes was not a risk factor for death in hemodialysis when patients with PAD were excluded from the sample.[23]

Age, baseline CRP levels and stroke sequelae were confirmed as independent risk factors for death during the 5-year follow-up period. The first two variables are well-known risk factors for death in hemodialysis[24,25], confirming the association between a single baseline CRP measurement and long-term mortality risk. Stroke sequelae may represent the association between low ABI and diffuse atherosclerotic disease, and could be seen as a link between low ABI and high mortality rate in hemodialysis patients.

Among 478 patients enrolled, the frequency of normal, low and high ABI was 64.6%, 26.8% and 8.6%, respectively. There was a predominance of males among patients with high ABI. Patients in the low ABI group were older than those in the other two groups. We found a higher prevalence of diabetes in both low and high ABI groups, in comparison to the group with normal ABI. The high prevalence of diabetes among low ABI patients could be attributed to the presence of macrovascular disease, whereas the predominance of diabetes in the group of high ABI could be explained by the greater prevalence of vascular calcification in diabetic patients.[8] Vascular calcification can cause arterial stiffness, and consequently increased ABI.

Regarding hypertension, we did not detect significant differences between the ABI groups. We also found no association between smoking and the risk of abnormal ABI. Perhaps, the low prevalence of smoking in our population could have mitigated such effect. Moreover, the absence of such correlation could be attributed to data collection strategy, since we considered only current smokers in our study. The association between smoking and PAD in hemodialysis patients was controversial in previous studies.[6,8,20,26]

The low ABI group showed a higher prevalence of coronary artery disease, stroke sequelae, PAD and non-traumatic amputation when compared to the normal ABI group. The association between low ABI and generalized atherosclerotic disease was also found in previous studies.[5,6,8,27] It should be underscored that the high ABI group also presented a higher prevalence of PAD and non-traumatic amputation in relation to the normal group.

The positive correlation between atherosclerosis and inflammation, demonstrated in previous studies in both general population and hemodialysis patients[28,29] could also be observed in our study, considering the variables CRP and serum albumin. The group of low ABI showed higher levels of CRP and lower serum albumin than the normal group. This finding is also consistent with studies evaluating specifically PAD in both general population and hemodialysis patients.[30-32] The lower serum creatinine levels in the low ABI group suggest that some degree of malnutrition was present in these patients, a comorbidity correlated with inflammation.

Ionized calcium, phosphorus and i-PTH levels were used to evaluate bone and mineral disturbances. The levels of ionized calcium were similar in the three groups, whereas phosphorus levels and the calcium × phosphorus product were higher in the high ABI group than in the other two groups reflecting, probably, a putative role of phosphorus in vascular calcification. These results are in agreement with a prior study, in which the association between serum phosphorus and Ca x P levels were observed only in patients with ABI >1.4 or incompressible ankle arteries.[33]

It should be stressed that, differently from other studies, we did not perform a merely cross-sectional analysis of the association between current ionized calcium and phosphorus levels and the presence of PAD. In fact, in our study, calcium and phosphorus data represent the mean of values of monthly measurements of these variables during a long period of up to 36 months preceding ABI evaluation. Thus, our data point against a direct association between hypercalcemia or hyperphosphatemia and low ABI.

i-PTH values were higher in the group with ABI >1.3 than in the group with ABI <0.9. A negative association of i-PTH levels with PAD, as well as with cardiac and aortic valve calcification was also found in previous studies.[27,34] The reasons for this negative association are not clear but might be related to a tendency toward a soft tissue calcification in low-turnover bone disease or to the association between low i-PTH levels and malnutrition.[35] It is worth pointing out, however, that the inverse association between i-PTH levels and the presence of PAD is not a uniform finding.

Considering the high prevalence of PAD, its consequences and the current lack of effective therapies for hemodialysis patients, we think that the routine measurement of ABI could identify patients in higher long-term risk of death, who could benefit from early detection of PAD and interventions on risk factors associated with low ABI, such as inflammation, in attempt to change the apparently inexorable course of this disease.

This study has some limitations that deserve consideration. Several established risk factors for PAD in general population, as smoking, could not be properly evaluated, since the data collection considered only the current state and not the total burden of exposition to it. We also could not distinguish between overall and cardiovascular cause of mortality due to the lack of accurate information. Another limitation is that the studied population could not be representative of the national one a nationwide feature, since all patients are from Rio de Janeiro State. On the other side, the strengths of our study include the assessment of ABI by Doppler, the gold-standard method, its prospective design and the long follow-up period. Most of similar studies observed patients for no more than 2 years. There is no standardized definition for "long-term" concerning the follow-up in clinical research, but its meaning can be viewed as dependent on the disease, treatment and populations studied.[36] Considering a mean annual mortality rate of 15% to 20% in hemodialysis population, it seems reasonable to label a period of 5 years in our population as a long-term follow-up.

Conclusions

Our findings showed a high frequency of abnormal ABI among patients in hemodialysis. Long-term survival rates of patients with either low or high ABI were lower than the one from those with normal ABI. However, after adjustment for potential confounders, only low ABI persisted as an independent risk factor for all-cause mortality among hemodialysis patients. In addition, the relatively higher risk of death for diabetic patients was reversed after adjustment for ABI.

Author contributions

Conception and design of the research, Analysis and interpretation of the data, Statistical analysis and Writing of the manuscript: Miguel JB, Matos JPS, Lugon JR; Acquisition of data: Miguel JB; Critical revision of the manuscript for intellectual content: Matos JPS, Lugon JR.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of Doctoral submitted by Jair Baptista Miguel, from Universidade Federal Fluminense.

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Effects of Chronic Exercise on Endothelial Progenitor Cells and Microparticles in Professional Runners

Célia Regina de Oliveira Bittencourt,[1] Maria Cristina de Oliveira Izar,[1] Carolina Nunes França,[2] Valdir Lauro Schwerz,[1] Rui Manuel dos Santos Póvoa,[1] Francisco Antonio Helfenstein Fonseca[1]

Universidade Federal de São Paulo (UNIFESP),[1] Universidade de Santo Amaro,[2] São Paulo, SP - Brazil

Abstract

Background: **The effects of chronic exposure to exercise training on vascular biomarkers have been poorly explored.**

Objective: **Our study aimed to compare the amounts of endothelial progenitor cells (EPCs), and endothelial (EMP) and platelet (PMP) microparticles between professional runners and healthy controls.**

Methods: **Twenty-five half-marathon runners and 24 age- and gender-matched healthy controls were included in the study. EPCs (CD34+/KDR+, CD133+/KDR+, and CD34+/CD133+), EMP (CD51+) and PMP (CD42+/CD31+) were quantified by flow-cytometry. All blood samples were obtained after 12 h of fasting and the athletes were encouraged to perform their routine exercises on the day before.**

Results: **As compared with controls, the CD34+/KDR+ EPCs (p=0.038) and CD133+/KDR+ EPCs (p=0.018) were increased, whereas CD34+/CD133+ EPCs were not different (p=0.51) in athletes. In addition, there was no difference in MPs levels between the groups.**

Conclusion: **Chronic exposure to exercise in professional runners was associated with higher percentage of EPCs. Taking into account the similar number of MPs in athletes and controls, the study suggests a favorable effect of exercise on these vascular biomarkers. (Arq Bras Cardiol. 2017; 108(3):212-216)**

Keywords: **Endothelial Progenitor Cells; Biomarkers; Athletes; Sports; Running**

Introduction

An appropriate number of circulating endothelial progenitor cells (EPCs) seems related with the maintenance of vascular homeostasis.[1,2] In fact, decreased number of EPCs has been associated with cardiovascular risk factors, cardiovascular mortality, and recurrent cardiovascular events in subjects with coronary heart disease,[3,4] despite some controversies regarding the measurement, characterization, origin and destiny of such cells.[5,6]

Microparticles (MPs) are small cell-derived anucleoid phospholipid particles (100-1000 nm) that can be identified by their origin from endothelium (EMP), platelets (PMP) or many other cells. Increased number of EMPs has been linked with endothelial injury or endothelial dysfunction.[7,8] Interestingly, PMPs, initially considered markers of thrombosis, are now considered relevant for some transcriptional signaling, for the interaction with monocytes and activation of inflammatory responses.[9]

Mailing Address: Francisco Antonio Helfenstein Fonseca •
Rua Loefgren, 1350. Postal Code: 04040-00, V. Clementino, São Paulo, SP - Brazil
E-mail: ffonseca@cardiol.br; fahfonseca@terra.com.br
Manuscript received May 11, 2016; revised manuscript May 23, 2016; accepted November 10, 2016.

DOI: 10.5935/abc.20170022

Regular exercise has been widely recommended for prevention of cardiovascular disease, but information regarding the effects of chronic and intense exposure to exercise on these vascular biomarkers is scarce.[10,11] Thus, the objective of this study was to evaluate the effects of chronic exercise in professional runners on EPCs and MPs.

Methods

Study population

Professional half-marathon runners (n=25) and age and gender-matched controls (n=24) without known cardiovascular diseases were prospectively included. Subjects with cardiovascular risk factors such as hypertension, diabetes, obesity, smoking, or hypercholesterolemia were excluded. The local ethics committee approved the study (# 1808/08) and all participants have signed the informed consent prior to their inclusion in the study protocol.

Laboratory analysis

Blood samples were obtained after 12 hours of fasting and the analyses were performed at the central laboratory of our university. All athletes were allowed to maintain their daily exercises program even on the day before blood sample collection. The athletes had very similar exercise training programs, corresponding to two long-distance running sessions

every day, 15 km in the morning and 10 km in the afternoon, and intensive training (100-1,000 meter shots, repeated many times)twice a week, on Tuesday and Thursday mornings. All blood samples were collected on Thursdays, before exercise.

Measurements of EPCs and MPs were performed as previously reported, using fresh blood samples in EDTA containing tubes.[12-15] For determination of EPCs, a minimum of 500,000 events was acquired by flow-cytometry (FACSCalibur, BD Biosciences, USA). Fluorescently labeled mouse anti-human antibodies were used for EPCs (CD34 FITC, BD Biosciences, USA; CD133 APC, Miltenyi Biotec, USA; KDR PE, R&D Systems, USA), PMPs (CD42 FITC and CD31 PE, BD Biosciences, USA) and EMPs (CD51 FITC, BD Biosciences). Disposable containers (BD Biosciences) were used to quantify the number of microparticles *per* microliter of platelet-poor plasma (PPP).

Statistical analysis

Results are presented as mean ± standard deviation (SD) or by median and interquartile range (IQR), for normal or non-Gaussian distributions, respectively. Categorical variables were compared by Pearson's Chi-square test. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess normality of continuous variables. Between-group comparisons of continuous variables were made by unpaired t-test or Mann-Whitney test, when appropriate. Spearman's rank correlation test was used to evaluate correlations of EPCs and MPs with variables of ergospirometry. All analyses were performed using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL) and significance was set at $p<0.05$.

Results

All athletes reported to have exercised on the day before (22.08 ± 2.67 km, mean ± SD), and the mean time between the last exercise session and blood collection was 16.5 ± 2.8 hours. Male and female athletes did not differ in both distance (124±25 vs. 128±29 km per week, p=0.88, respectively, mean ± SD, unpaired t test) and time spent in training (14±4 vs. 14±7 hours per week, mean±SD, p=0.53, respectively, unpaired t test). Despite exposure to the same training regimen, male athletes reported better mean time for 10,000 meters than female athletes (32.4±2.1 vs. 37.6±1.6 min, p<0.0001, mean±SD, unpaired t test). As compared with controls, athletes had lower weight, body mass index, abdominal circumference and percentage of body fat, lower heart rate, and higher body lean mass, but similar values of systolic and diastolic blood pressure. In addition, they presented lower serum levels of total cholesterol, LDL-C and triglycerides, and higher serum levels of HDL-C than controls.

Endothelial progenitor cells and microparticles

Compared to controls, the athletes presented higher percentage of two lineages of EPCs (CD34+/KDR+, and CD133+/KDR+) and similar percentage of CD34+/CD133+ cells (Figure 1).

The amount of EMPs and PMPs did not differ between the two groups (Figure 2).

No correlation between the percentage of EPCs or MPs with variables of ergospirometry was observed, including absolute and maximum rate of oxygen consumption (VO_2max) (data not shown).

Discussion

The present study revealed that the chronic exposure to exercise training among professional runners was associated with increased percentage of circulating EPCs without changes in the amount of EMPs or PMPs. These findings suggest that chronic exercise was not associated with endothelial cell apoptosis or thrombosis. In fact, it seemed to have a protective effect in these subjects, taking into account the observed increase in EPCs. In our athletes, blood samples were collected during their routine training program, since we wanted to evaluate EPCs and MPs in real-life context.

Several cardiovascular risk factors including diabetes,[3] hypertension,[16] smoking,[17] hypercholesterolemia,[18] and age.[19] have been related to reduced function of circulating EPCs. Conversely, exercise has been recognized as a promise tool to increase EPCs.[20,21] Early experimental and clinical studies[22,23] reported increased number of EPCs after regular exercise, although the effects of exercise on EPCs seemed to be influenced by training regimen, age of subjects, and concomitant presence of cardiovascular disease, such as coronary heart disease or heart failure.[20]

Circulating EMPs have been linked to several stimuli, including the transcription of interleukins, chemokines and chemoattractants mediated by activation of nuclear factor-κB (NF-κB), and associated with oxidative stress.[8,24] All these conditions have been long associated with classical cardiovascular risk factors, but more recently, new biological effects mediated by EMPs have been considered, including transport of mRNAs, microRNAs and other active molecules of physiologic relevance for angiogenesis and tissue repair.[25]

Cellular activation and apoptosis are linked to release of MPs. Of special interest, the amount of PMPs has been recognized as a possible marker of thrombosis, due to their high content of phospholipids and potential pro-thrombogenic roles because of thrombin generation.[26] Besides, high shear stress triggers platelet aggregation and release of platelet derived MPs.[27] In addition, circulating PMPs may carry tissue factor (TF), which can also generate thrombin and platelet activation. However, it is also true that MPs may transport some inhibitors of coagulation, such as the TF pathway inhibitor (TFPI) that can neutralize, in part, the procoagulant properties of these MPs.[28] More recently, interesting aspects linking PMPs to the signaling of inflammatory and immune responses have been proposed, considering the potential transcriptional factors in the platelets, that include nuclear factor kappa β (NF-κB) and peroxisome proliferator-activated receptor gamma (PPARγ).[29]

In our study, we found increased percentage of EPCs in athletes and similar number of EMPs and PMPs in comparison with healthy controls, despite the intensive training of these professional athletes. These promising findings are important because our understanding of the role of exercise on EPCs and MPs is mainly derived from acute exposure or in non-athletes.[10,11,30,31] Intermittent and high-intensity exercise induces catecholamine release and decreases highly differentiated T cells, but does not increase the amount of

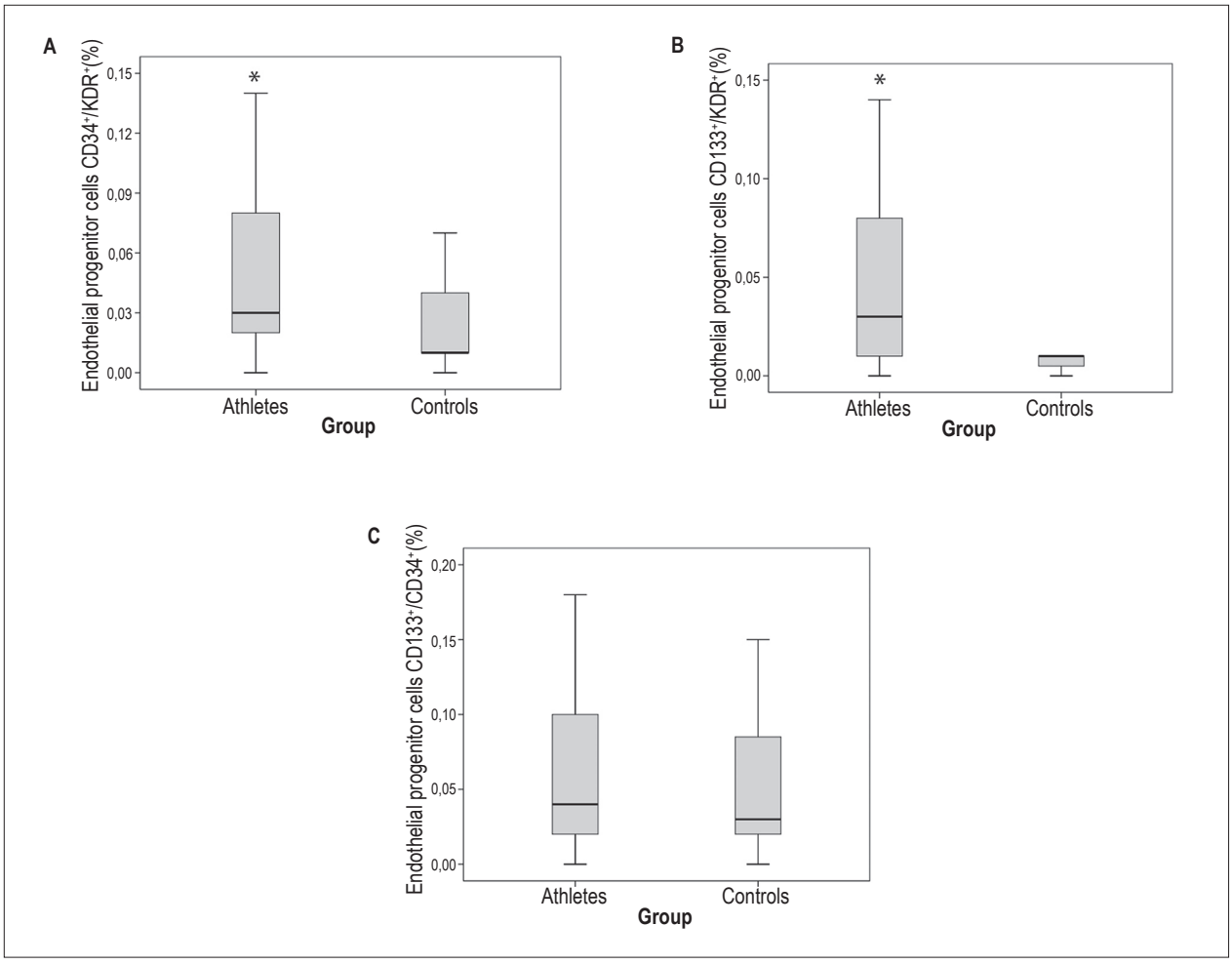

Figure 1 – *Box-plots showing the percentage of circulating endothelial progenitor cells (EPCs) determined by flow-cytometry. Higher percentage of CD34+/KDR+ EPCs (A) (p=0.038 vs. controls, Mann-Whitney U test), as well as CD133+/KDR+ EPCs (p=0.018 vs. controls, Mann-Whitney U test) (B) were found in athletes. No differences were observed between groups for CD133+/CD34+ (p=0.51) (C).*

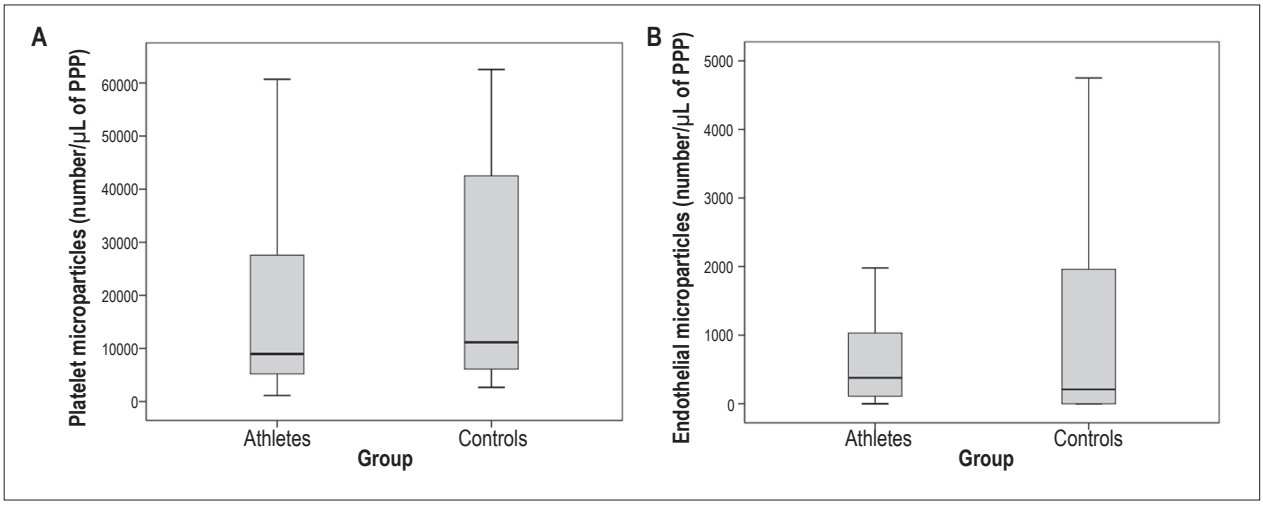

Figure 2 – *Box-plots representing the amount of circulating microparticles determined by flow-cytometry. The number of CD42+/CD31+ platelet microparticles (PMPs) (A) and CD51+ endothelial microparticles (EMPs) (B) was similar between the groups. (PMPs, p=0.695, Mann-Whitney U test; EMPs, p=0.496, Mann-Whitney U test). PPP - platelet-poor plasma.*

EPCs compared with continuous exercise[33]. In other article, despite increase in white blood cells count, the amount of EPCs observed in advanced-aged marathon runners was not modified when collected in the early period after the race.[33]

In addition, among other biochemical variables, C-reactive protein levels were lower in athletes than in controls, and creatine phosphokinase levels modestly increased, even with the routine training on the day before blood sample collection, reinforcing protective properties of high-performance exercise.

Study limitations

Although this was a cross-sectional, case-control study, our results cannot be considered as hypothesis generating, since we do not have baseline laboratory values of the athletes. Finally, these results are applicable to marathon runners and cannot be extrapolated to other sports.

Conclusions

Chronic exercise was associated with a favorable increase in EPCs, without affecting circulating levels of MPs in professional runners, suggesting a positive impact of prolonged exposure to chronic exercise on these vascular biomarkers.

Author contributions

Conception and design of the research: Izar MCO, Fonseca FAH; Acquisition of data: Bittencourt CRO, França CN, Schwerz VL; Analysis and interpretation of the data and Critical revision of the manuscript for intellectual contente: Bittencourt CRO, Izar MCO, França CN, Schwerz VL, Póvoa RMS, Fonseca FAH; Statistical analysis: Bittencourt CRO, Izar MCO, França CN, Fonseca FAH; Obtaining financing: Fonseca FAH; Writing of the manuscript: Izar MCO, França CN, Póvoa RMS, Fonseca FAH.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by FAPESP and was partially funded by CAPES.

Study Association

This article is part of the thesis of master submitted by José Henrique Cunha Figueiredo, from Universidade Federal do Rio de Janeiro.

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Major Depression and Acute Coronary Syndrome-Related Factors

Jose Henrique Cunha Figueiredo,[1] Nelson Albuquerque de Souza e Silva,[1] Basilio de Bragança Pereira,[1,2] Glaucia Maria Moraes de Oliveira[1,2]

Programa de Pós Graduação em Medicina (Cardiologia) – Universidade Federal do Rio de Janeiro;[1] Instituto do Coração Edson Saad – Universidade Federal do Rio de Janeiro,[2] Rio de Janeiro, RJ – Brazil

Abstract

Background: Major Depressive Disorder (MDD) is one of the most common mental illnesses in psychiatry, being considered a risk factor for Acute Coronary Syndrome (ACS).

Objective: To assess the prevalence of MDD in ACS patients, as well as to analyze associated factors through the interdependence of sociodemographic, lifestyle and clinical variables.

Methods: Observational, descriptive, cross-sectional, case-series study conducted on patients hospitalized consecutively at the coronary units of three public hospitals in the city of Rio de Janeiro over a 24-month period. All participants answered a standardized questionnaire requesting sociodemographic, lifestyle and clinical data, as well as a structured diagnostic interview for the DSM-IV regarding ongoing major depressive episodes. A general log-linear model of multivariate analysis was employed to assess association and interdependence with a significance level of 5%.

Results: Analysis of 356 patients (229 men), with an average and median age of 60 years (SD ± 11.42, 27-89). We found an MDD point prevalence of 23%, and a significant association between MDD and gender, marital status, sedentary lifestyle, Killip classification, and MDD history. Controlling for gender, we found a statistically significant association between MDD and gender, age ≤ 60 years, sedentary lifestyle and MDD history. The log-linear model identified the variables MDD history, gender, sedentary lifestyle, and age ≤ 60 years as having the greatest association with MDD.

Conclusion: Distinct approaches are required to diagnose and treat MDD in young women with ACS, history of MDD, sedentary lifestyle, and who are not in stable relationships. (Arq Bras Cardiol. 2017; 108(3):217-227)

Keywords: Acute Coronary Syndrome; Depressive Disorder, Major; Social Class; Life Style.

Introduction

Prior studies have attempted to understand the factors influencing the prognosis of an acute coronary event, and screening for symptoms of depression has been recommended as routine for acute coronary syndrome (ACS) patients.[1] Although the association between depression and a worse prognosis in ACS patients has been documented in a number of studies,[2-4] only recently did the American Heart Association recommend it be included as a risk factor for adverse ACS outcomes, even if they emphasize the heterogeneity of the studies employed in the systematic review on which the recommendation was based.[5]

The prevalence of depression in ACS patients in the USA was estimated at 20%, thus affecting 15.4 million adult coronary artery disease patients.[6] In São Paulo, Brazil, a study using the Beck Depression Inventory reports symptoms of depression in 43.5% of the patients hospitalized with ACS.[7] Another study[8] found a similar prevalence rate, 46.7%, and concluded that women, men under 50 years of age and people suffering from anxiety are more likely to show signs of depression when screened for depression [*Primary Care Evaluation of Mental Disorders* (Prime MD) and BDI], trait anxiety and state anxiety (IDATE), and alcohol consumption (AUDIT). These rates depict the greater sensitivity of screening tools.

The Danish National Patient Registry, which gathered a cohort of around 83,000 ACS patients, pointed to excessive mortality in those with marked inequalities in education, even when adjusted for prior comorbidities and depression,[9] raising the hypothesis that depression may fit into an adverse social context that is most common among women. Therefore, questions still remain as to the prevalence of depression where ACS is concerned, and its influence on prognosis and associated factors, especially in regard to the Brazilian population.

The aims of this study are to check the prevalence of Major Depressive Disorder (MDD) in patients diagnosed with ACS in three public hospitals in the city of Rio de Janeiro and analyze factors potentially associated with MDD within this setting.

Mailing Address: Gláucia Maria Moraes de Oliveira •
Universidade Federal do Rio de Janeiro. Rua João Lira 128, 101. Postal Code 22430-210, Leblon, Rio de Janeiro, RJ – Brazil
E-mail: glauciam@cardiol.br, glauciamoraesoliveira@gmail.com
Manuscript received June 17, 2016; revised manuscript August 04, 2016; accepted November 11, 2016.

DOI: 10.5935/abc.20170028

Methods

This is an epidemiological, observational, descriptive, cross-sectional study involving patients hospitalized with ACS who have been diagnosed using clinical, enzymatic and electrocardiographic criteria. The research protocol was submitted to and approved by the Research Ethics Committee, and the participants signed a Free and Informed Consent Form (HSE, nº 160/04).

We used the Acute Coronary Care (ACC) of three public hospitals used for both teaching and medical care in Rio de Janeiro City: a federal general hospital, a municipal general hospital, and a state hospital specializing in cardiology. The study was carried out over the course of 24 consecutive months. Together the three ACC had a total of 21 hospital beds.

Included in the study were male and female patients over the age of 20 admitted to the cardiology units of the three participating hospitals. Excluded were the patients clinically unable to answer the interview by their seventh day of hospitalization at the ACC, as well as patients clinically deemed unable to respond to the interview due to cognitive alterations or auditory deficiencies that would preclude an oral interview.

The interview and application of the research tools were carried out by the seventh day of hospitalization in the ACC. All the tools used were read by the patient and subsequently applied in a single interview by one of the authors. Patients responded to the Structured Clinical Interview of the *DSM-IV Axis I Disorders - Patient Edition (SCID-I/P, version 2.0)* (Appendix 1).[10-12] For this study, we applied the section on Major Depressive Episodes (MDE),[13] as well as a standardized questionnaire on sociodemographic, lifestyle and clinical data.

The sociodemographic variables contemplated were gender, age, marital status (married or in common-law marriages according to Brazilian law or living together in a stable relationship; not married when not living together in marital situation), level of education (years of schooling completed or not completed dichotomized into under four years or over four years), family income (monthly income of all family members sharing the overall common costs of living in three categories: group A, up to US 615 monthly wages; group B, between US 615 and US 1230 monthly wages; and group C, more than US 1230 monthly wages). Social support (two dimensions assessed: family and close friends) consolidated in two categories: patients who lived alone and/or had no friends were considered to have "no social support", and those who didn't live alone and did have friends were considered to "have social support".

The variables referring to lifestyle were tobacco smoking (a "smoker" is someone who reported smoking cigarettes up to one year prior to the current coronary event, and a "non-smoker" is someone who reported having quit smoking more than a year before the latest coronary event or who had never smoked before), and sedentary lifestyle ("sedentary" is someone who reported not practicing any regular physical activity – walking, jogging, riding a bicycle, practicing sports – for at least 30 minutes three times a week at the least).

The clinical variables found were dyslipidemia, hypertension and diabetes mellitus (when self-reported, with elevated or normal serum values associated with specific drugs used for treatment and confirmed on the medical records, and with increases in systolic and diastolic blood pressure in the case of hypertension); prior acute myocardial infarction (AMI - taken from the medical records); Killip class, dichotomized into Killip 1 and Killip ≥ 2; and ongoing major depression, verified by a structured interview (SCID-I/DSM-IV) to estimate point prevalence and throughout life.

The selection of variables was based on the association with coronary syndrome and observed in previous studies in non-Brazilian population.

Statistical analysis

We employed the chi-squared test to assess dependence between sociodemographic, lifestyle and clinical variables and MDD, and the Mantel-Haenszel test to assess dependence between sociodemographic, lifestyle and clinical variables and MDD when controlled by the variable gender. In our analysis of the results, besides statistical significance ($p < 0.05$), we considered clinical significance ($0.05 < p \leq 0.15$) to be a factor that explains the association in question. We used a general log-linear multivariate model of analysis to assess the level of association between variables of interest. The statistical program used was the R system, version 2.1.1.

Results

This study assessed 356 patients (229 men), whose ages varied from 27 to 89 years. The average and median age was 60 (SD ± 11.42) years. Average age for the women was 62 years, and for the men, 59, suggesting that women tend to suffer ACS later in life. Average time hospitalized at the ACC was 9.7 days.

The point prevalence we identified for the current MDD, according to DSM-IV diagnosis criteria, was 23% (82 of the 356-patient sample group).

The sociodemographic, lifestyle and clinical characteristics categorized by presence of MDD are shown in Table 1.

The MDD prevalence was higher in the patients ≤ 60 years of age (26.4% x 19.5%), though not statistically relevant. Regarding marital status, 226 (63.5%) of the patients were married or in a stable relationship, 31 (8.7%) were single, 48 (13.5%) were separated, divorced or legally separated, 51 (14.3%) were widowed, and the MDD prevalence among the unmarried ones was statistically significant. There was a greater prevalence of MDD in those with *less than 4 years* of schooling and those without *social support*, but without statistical significance, perhaps due to the size of the sample group in question. As for family income, subgroup A counted 102 (28.7%) patients, of whom 28 (27.5%) were depressed; subgroup B had 117 (32.9%) patients, with 27 (19.7%) of them depressed; and subgroup C comprised 137 (38.7%) patients, with 27 (19.7%) of them found to be depressed. Though there was no statistical difference between the subgroups, what stands out is the progressive drop in the rate of depressed patients according to how high the family income was (Table 1).

Table 1 – Sociodemographic, lifestyle and clinical characteristics according to the presence or absence of major depressive disorder (MDD)

	MDD n (%) 82 (23.0)	Non-MDD n (%) 274 (77.0)	Total (n) 356
Male gender*	34 (14.8)	195 (85.2)	229
> 60 years	34 (19.5)	140 (80.5)	174
Married***	44 (19.5)	182 (80.5)	226
Schooling > 4 years	40 (21.1)	150 (78.9)	190
With social support	63 (21.8)	226 (78.2)	289
Family income			
A (≤ US 615 m.w.)	28 (27.5)	74 (72.5)	102
B (US 615-1230 m.w.)	27 (23.1)	90 (76.9)	117
C (> US 1230 m.w.)	27 (19.7)	110 (80.3)	137
Tobacco smoking	30 (24.4)	93 (75.6)	123
Sedentary lifestyle**	69 (26.3)	193 (73.7)	262
Dyslipidemia	40 (26.0)	114 (74.0)	154
SH	58 (23.1)	193 (76.9)	251
Diabetes	23 (22.8)	78 (77.2)	101
History AMI	20 (20.8)	76 (79.2)	96
History MDD	33 (53.2)	29 (46.8)	62
Killip ≥ 2	10 (37.0)	17 (63.0)	27

Pearson chi-squared test, p < 0.0001, p < 0.01**, p < 0.05***, † clinical relevance. SH: systemic hypertension; m.w.: monthly wages; AMI: acute myocardial infarction.*

The presence of depression was significantly greater among sedentary patients but not among smokers, dyslipidemic, diabetic or hypertensive patients, nor those with a history of AMI. The Killip class ≥ 2 was found to have the greatest prevalence of MDD, and was clinically relevant. There were 62 (17.4%) patients with a history of MDD, of whom 33 (53.2%) were found to be depressed, and that was statistically relevant (Table 1).

In summary, this initial analysis of the variables gender, marital status, sedentary lifestyle, Killip class ≥ 2 and history of MDD associated significantly with MDD in the ACS index event (Table 1).

Table 2 shows the results controlled for gender. The frequency of depressed females was significantly greater than that found for males (37.8% x 14.8%), with a three-and-a-half-time greater likelihood of developing MDD than the male subgroup. Regarding age, for both sexes, the odds ratio was less than 1, and older age was found to be more protective against depression; the data suggest that this protection is greater in men than in women.

Depression was found at a greater frequency in women than in unmarried men. Despite percentage differences, type of marital relationship did not relate significantly to MDD when controlled for gender. We also found that, percentage-wise in this sample, women have lower levels of education than men, and those with less schooling had a greater likelihood, albeit not by much, of becoming depressed than those with more schooling. Among men there was no difference in this respect. Among women we found practically no difference between the with- or without-social support categories. In contrast, we found that men without social support were nearly two and a half times more likely to become depressed than those with social support. However, there was no statistical difference between genders when comparing the variable social support (Table 2).

Taking as a reference family income in subgroup A, we found that women in this subgroup are more likely to show signs of depression than those in subgroup B, and less likely than those in subgroup C. Among the men, those in subgroup A were more likely to become depressed than those in subgroup B and subgroup C. However, between men and women the odds ratio of subgroups B and C compared to subgroup A was not significant (Table 2).

Regarding smoking, the women had a much higher rate of depression than the men. Nevertheless, what became clear was that the likelihood of male smokers becoming depressed was greater than that of non-smokers, which was not the case with the women. The results pointed to a greater number of sedentary persons of both sexes, with the frequency among women being greater than among men. Sedentary persons were found to have greater odds of becoming depressed regardless of their gender. However, the sedentary men were around 2.5 times more likely to develop MDD than the non-sedentary ones. Unlike the men, the chance of a woman becoming depressed while sedentary was much lower (Table 2).

There appears to be a greater tendency toward becoming depressed among males, however slight, where having or not having a history of hypertension is concerned. We found that non-diabetic women were slightly more likely to become depressed than those with diabetes.

Table 2 – Sociodemographic, lifestyle and clinical characteristics according to the presence or absence of major depressive disorder (MDD) controlled for gender

	Female			Male		
Characteristics	**MDD N (%)**	**Non-MDD N (%)**	**OR**	**MDD N (%)**	**Non-MDD N (%)**	**OR**
Female gender[1]*	48 (37.8)	79 (62.2)		34 (14.8)	195 (85.2)	3.485
≤ 60 years[2]***	22 (40.7)	32 (59.3)	0.81	26 (20.3)	102 (79.7)	0.34
Unmarried[2]	30 (39.0)	47 (61.0)	0.88	8 (15.1)	45 (84.9)	0.98
Schooling > 4 years[2]	40 (38.5)	64 (61.5)	0.85	23 (12.4)	162 (87.6)	2.35
Family income > 6 m.w.[2]	13 (41.9)	18 (58.1)	0.84	14 (13.2)	92 (86.8)	1.57
Smoker[2]	13 (39.4)	20 (60.6)	0.91	17 (18.9)	73 (81.1)	0.6
Sedentary lifestyle[2]†	41 (38.7)	65 (61.3)	0.79	28 (17.9)	128 (82.1)	0.41
Dyslipidemia	28 (39.4)	43 (60.6)	0.85	12 (14.5)	71 (85.5)	1.05
SH[2]	38 (36.9)	65 (63.1)	1.22	20 (13.5)	128 (86.5)	1.34
Diabetes[2]	15 (34.1)	29 (65.9)	1.28	8 (14.0)	49 (86.0)	1.09
Killip ≥ 2[2]	6 (42.9)	8 (57.1)	0.81	4 (30.8)	9 (69.2)	0.36
History AMI[2]	13 (35.1)	24 (64.9)	1.18	7 (11.9)	52 (88.1)	1.4
History MDD[2]*	20 (64.5)	11 (35.5)	0.23	13 (41.9)	18 (58.1)	0.16

1 Chi-squared test, 2 Mantel-Haenszel test, p < 0.0001, p < 0.01**, p < 0.05***, † clinical relevance. SH: systemic hypertension; AMI: acute myocardial infarction; OR: odds ratio; m.w.: monthly wage.*

Among the men there was practically no difference between those with a history of diabetes and those without a history of diabetes. There was no statistically significant association between diabetes and MDD (Table 2).

Apparently, patients of either gender without a history of AMI had greater chances of becoming depressed than those *with* a history of AMI. We also noted that there were greater odds for men than for women. However, we did not find a statistically significant association with MDD. Regardless of gender, patients without a history of MDD have greater protection against depression in the index event (Table 2).

In summary, when controlled for gender, the association between MDD and the variables age ≤ 60 years, sedentary lifestyle and history of MDD is statistically significant (Table 2).

To assess the power of association in the general log-linear model, we chose to represent it in Figures 1 and 2, with a thick line that thins as the power of association progressively decreases according to estimates of (λ's) parameters of the log-linear model.

To perform the multivariate analysis we used the log-linear model that allows us to assess the associations of all the variables all together. We found direct relationship between the variable MDD and gender, age ≤ 60 years and marital status. Other variables were related to MDD conditioned by one or more of the variables directly related to MDD (Figure 1). (Appendix 2)

We also found that the power of association was greater (thicker line) between MDD and history of MDD (λ = 17.387) and MDD and gender (λ = –11.755), and was weaker (thinner line) between MDD and sedentary lifestyle (λ = 0.6026), and much weaker (thin line) between MDD and age ≤ 60 (λ = 0.3886). Gender and marital status (λ = –16.320) associated strongly (thicker line), while gender and sedentary lifestyle associated less strongly (thinner line) (λ = 0.7402). The association between MDD and marital status was conditioned to gender (Figure 2).

Discussion

In this study, 23% of the patients with ACS met the criteria for MDD. This evidence in coronary-patient sample groups is similar to that reported in the international literature, and has prognostic implications that show a rise in mortality from all causes, and cardiovascular mortality between 12 months to 5 years after the ACS index event, even when evaluating MDD.[14-17] It is noteworthy that in this study the use of a diagnostic measuring instrument – DSM-IV clinical diagnostic interview – that differs from tracking scales, and the time criterion for diagnosing MDD was maintained, which means that all the patients who met the MDD/DSM-IV criteria were already depressed at the time of the coronary event. Thus, the MDD prevalence having been found to be much higher than that of the overall population could suggest that there are common factors shared between the development of MDD and ACS. One study[9] pointed to social inequality, especially where education is concerned, and comorbidities as factors that are present in and associated with depression and ACS. On the other hand, MDD and ACS seem to share such inflammatory biomarkers as cytokines, alterations in oxidative stress, platelet alterations, and vascular reactivity, with an array of complex biological interactions that are so far not fully understood.[18-20]

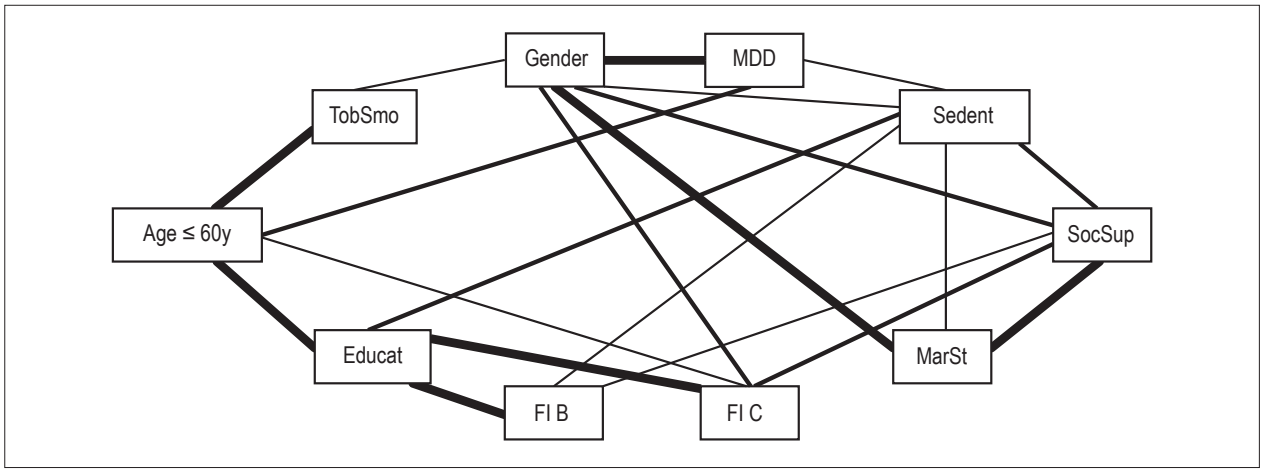

Figure 1 – *Graphical representation of the general log-linear model demonstrating the interdependent relationships between the sociodemographic variables and the powers of association without history of major depressive disorder (MDD). Sedent: sedentary lifestyle; SocSup: social support; MarSt: marital status; FI B: family income B (US 615-1230 monthly wages); FI C: family income C (> US 1230 monthly wages); Educat: education level; TobSmo: tobacco smoking.*

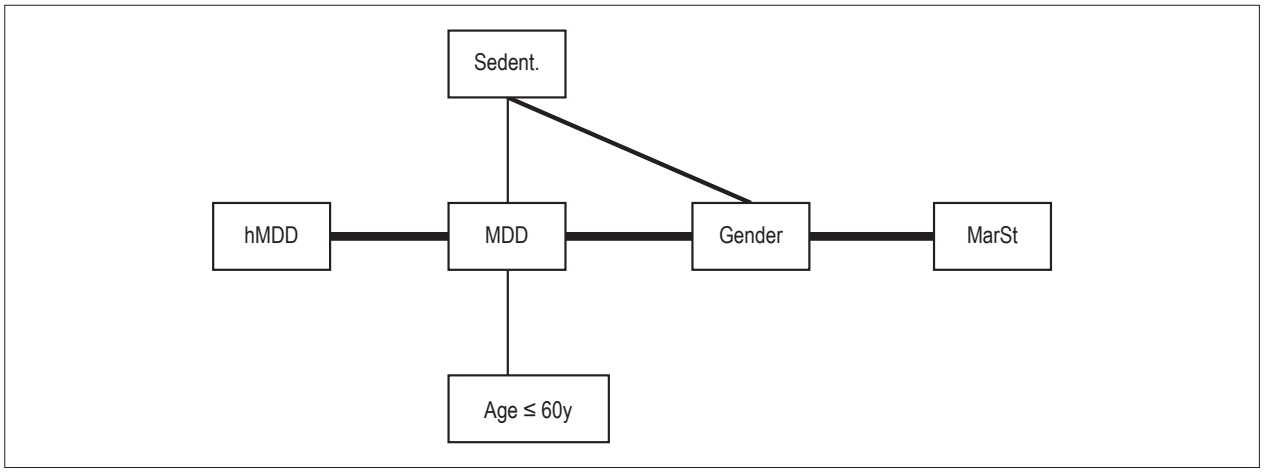

Figure 2 – *Graphical representation of the general log-linear model demonstrating the interdependent relationships between the variables examined and the powers of association. MDD: major depressive disorder; MarSt: marital status; Sedent.: sedentary lifestyle; hMDD: history of MDD.*

The rate of depression in women has varied from one and a half time to three times that of men.[21,22] This difference was found in this study, where the rate of MDD in men was 14.8% and in women, 37.8%. In the descriptive analysis controlling for gender, the women were at three-and-a-half-time greater risk of developing MDD than men. The reasons for women being more susceptible to MDD than men remain obscure, in spite of studies[23] that found an association between the neuroticism factor of personality (moodiness, worry and nervousness) and the female gender and severer depression.

Also noteworthy is the fact that we found a greater rate of depression in patients of both genders ≤ 60 years of age, which converges with the findings from another study.[24] One must remember that this stage of life is the most productive and is still early, in current terms, for the subjects to incur such subjective experiences as limitations or threat to their lives, or objective experiences that give rise to worries regarding socio-familial responsibilities, which make them more susceptible to depression.

Marital status influenced the rate of depression in patients with ACS.[25] The general log-linear model also showed that there is an association between the variable marital status and MDD conditioned to gender. What can be inferred from this is that this association pertained to the unmarried and females, who, as aforementioned, showed a greater tendency to MDD than married persons and males.

Regarding level of schooling, what needs to be put into perspective is that this sample group, having come from public hospitals and thus tending to be from lower socioeconomic classes, was expected to have a lower level of schooling, which proved to be the case. Females had a lower level of schooling than did men, and those from the less schooled subgroup were slightly more likely to become depressed than those with higher levels of schooling, which

was not the case with men. Less formal education among females is congruent with this generation who had less opportunity to study than to focus on their families.

Mankind, being essentially gregarious by nature, needs the company of others who would generally comprise part of a social support network. Falling ill and being hospitalized produce suffering, isolation and a feeling of solitude from being away from home, work, friends and family. This social support network is relevant because it is within it that the sick and hospitalized individual, oftentimes, seeks emotional support. There was an association between perceived social support and lower cardiovascular reactivity in depression and ACS sufferers,[26] with a decrease in cardiac mortality.[20,27] In this study, we found that patients without social support tended to become depressed more often that those with it, though the difference was not statistically significant. When the analysis was controlled for gender, we found that this tendency held true for men without social support, who were two and a half times more likely to become depressed than those with social support.

The rates of depression by income were similar to those found in the global sample, although it dropped from 27.5% to 19.7% as family income increased, suggesting that the lower-income strata of society have a greater tendency toward depression, though the difference is not statistically significant. When the analysis was controlled for gender, the female subgroup was found to change the order that was found without this control, and the much higher rates of depression remained stable in the lower-income and intermediary socioeconomic classes (37.8% and 35.3%), only slightly rising in the highest family-income class (41.9%). In the male subgroup the rates were much lower and inversely related to the female subgroup, having decreased (19.3%, 13.6% and 13.2%), without a statistically significant difference. The women of the highest income class tended to become depressed more often, though only slightly, unlike the men, probably because the causes of depression relating to this variable are distinct between genders.[28]

In the overall sample, we found higher rates of depression among sedentary persons than non-sedentary ones, with a statistically significant association. When the sample was controlled for gender, the statistical significance dropped, but the association remained relevant because of the male subgroup, where the sedentary men were around 2.5 times more likely to become depressed than the non-sedentary ones. This is understandable, as the symptoms of depression could explain the higher rate of sedentary lifestyle among depressed men.[29]

The significant association found between patients with MDD and with and without a history of MDD corroborates the findings from other studies.[1,2,4,22,30] This finding was not surprising, because a 50% rate of MDD relapse is expected to follow the initial episode, regardless of gender. By the same token, we found that having a history of MDD reflected in the female subgroup with around four-and-a-half-time greater likelihood of becoming depressed, and in the male subgroup with around six-time higher chance of becoming depressed than those who had never had MDD - a finding of major clinical importance.

Clinical implications

The highlight of this multivariate analysis was the capacity to evaluate the interdependence of various indistinct variables; that is, the fact that all the variables are response variables concedes to them the same importance and increases the likelihood of their being applied in clinical practice. Our findings point to a need for a distinct approach to diagnosing and treating MDD in female ACS patients, ≤ 60 years of age and with a history of MDD, sedentary lifestyle and who are not in a stable marital relationship. The prognostic implications of these findings need further analysis in future studies.

Limitations

If on the one hand the descriptive statistics controlling for gender were significant, on the other they were insignificant in the analysis of the subgroups for having produced results that must be considered with caution. The fact that the study was carried out in public hospitals precludes the possibility of generalizing the data, even though around 70% of the Brazilian population is treated in the Brazilian public healthcare system.

Although there are no Brazilian studies on the subject using an interview considered gold standard for the diagnosis of major depression, the authors recognize the sample size as small.

Conclusion

This study found a 23% prevalence of patients with ACS meeting the diagnostic criteria for MDD. Females were more susceptible to developing MDD in the sample group of ACS patients, with a three-and-a-half-time greater likelihood than males.

Social support, sedentary lifestyle and Killip class ≥ 2 were variables that directly related to the male gender, the subgroup being around two-and-a-half-time more likely to develop MDD than the female subgroup.

History of MDD, regardless of gender, strongly associated with the current MDD, with the chances for women being a little more than four times, and for men, around six times.

The general log-linear multivariate analysis suggests that history of MDD, gender, sedentary lifestyle and age ≤ 60 years are the variables with the greatest power of association with MDD in this sample of ACS patients.

Acknowledgments

The directors of the Coronary Units at Servidores do Estado do Rio de Janeiro Hospital, the Aloysio de Castro State Institute of Cardiology, and Miguel Couto Municipal Hospital.

Author contributions

Conception and design of the research: Figueiredo JHC, Souza e Silva NA; Acquisition of data: Figueiredo JHC; Analysis and interpretation of the data and Critical revision of the

manuscript for intellectual contente: Figueiredo JHC, Souza e Silva NA, Pereira BB, Oliveira GMM; Statistical analysis: Pereira BB; Writing of the manuscript: Figueiredo JHC, Oliveira GMM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by José Henrique Cunha Figueiredo, from Universidade Federal do Rio de Janeiro.

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APPENDIX 1

Structured Clinical Interview for DSM-IV (SCID-DSM IV) – Criteria for Major Depressive Episode.

A)	At least 5 of the following were present (+) during the same period of 2 weeks, representing a change in the previous functioning; at least 1 symptom is (1) depressed mood, or (2) loss of interest or pleasure.

In the last month...
(1) ... has there been a period of time when you were feeling depressed or down most of the day, nearly every day? How was that? If yes, how long did it last? (For as long as 2 weeks?)
(2) ... have you lost interest in things that you would usually enjoy? If yes, was it nearly every day? How long did it last? (For as long as 2 weeks?)
[For 2 weeks]...
(3) ... have you lost or gained weight? How much? Were you trying to lose weight? If not, how was your appetite? And how do you compare with your usual appetite? Have you forced yourself to eat? Have you eaten more/less than usual? Has this happened nearly every day?
(4) ... how have you been sleeping? Trouble falling asleep, waking frequently in the night or early morning, or have you been sleeping more than usual? How many hours per night, as compared to usual? Has this happened nearly every day?
(5) ... have you been feeling so fidgety or restless, that you feel unable to sit still? Was it so intense that people around you would notice? What did they notice? Has this happened nearly every day? If not, on the contrary – have you spoken or moved more slowly than usual? Was it so intense that people around you would notice? What did they notice? Has this happened nearly every day?
(6) ... how was your energy level? Have you felt fatigued all the time? Nearly every day?
(7) ... how have you been feeling about yourself? Worthless? Nearly every day? If not, have you felt guilty about things you have or have not done? Nearly every day?
(8) ... have you had problems concentrating or thinking? Has that interfered with anything? Nearly every day? If not, has it been hard to make decisions about everyday things?
(9) ... things were so bad that you had thoughts of dying, or that it would be better to die? And what about killing yourself? If yes, have you ever tried to kill yourself

APPENDIX 2

Results of the log-linear model to assess the relationship between the sociodemographic variables, including the variable major depressive disorder (MDD)

	Estimator (λ)	Standard error	Z Value	Pr (>|z|)	
(Intercept)	–2.688.449	0.635332	–4.232	2.32e–05	***
MDD	–0.821252	0.548862	–1.496	0.134580	
Gender	2.251.472	0.492674	4.570	4.88e–06	***
Age ≤ 60	–1.172.157	0.513623	–2.282	0.022481	*
Marital status	1.795.698	0.479341	3.746	0.000180	***
Schooling	–1.422.957	0.521306	–2.730	0.006341	**
Social support	1.885.126	0.521265	3.616	0.000299	***
Family income (B)	0.180327	0.552230	0.327	0.744013	
Family income (C)	–1.249.277	0.602538	–2.073	0.038139	*
Smoking	–2.349.186	0.556843	–4.219	2.46e–05	***
Sedentary lifestyle	1.277.152	0.508897	2.510	0.012085	*
MDD: Gender	–1.316.731	0.301854	–4.362	1.29e–05	***
MDD: Age ≤60	0.620317	0.303374	2.045	0.040882	*
MDD: Marital status	–0.046769	0.305908	–0.153	0.878488	
MDD: Schooling	–0.165475	0.298806	–0.554	0.579724	
MDD: Social support	–0.477188	0.349738	–1.364	0.172437	
MDD: Family income (B)	–0.058983	0.340688	–0.173	0.862550	
MDD: Family income (C)	0.007777	0.354681	0.022	0.982506	
MDD: Smoking	0.148600	0.303039	0.490	0.623873	
MDD: Sedentary lifestyle	0.612873	0.350552	1.748	0.080411	.
Gender: Age ≤ 60	0.357111	0.289478	1.234	0.217338	
Gender: Marital status	–1.782.421	0.282649	–6.306	2.86e–10	***
Gender: Schooling	0.173322	0.285969	0.606	0.544456	
Gender: Social support	–1.043.043	0.359433	–2.902	0.003709	**
Gender: Family income (B)	–0.185105	0.325247	–0.569	0.569273	
Gender: Family income (C)	0.755098	0.345023	2.189	0.028630	*
Gender: Smoking	0.470905	0.302531	1.557	0.119577	
Gender: Sedentary lifestyle	–0.483353	0.318571	–1.517	0.129203	
Age ≤ 60: Marital status	–0.332687	0.285541	–1.165	0.243974	
Age ≤ 60: Schooling	1.213.026	0.264028	4.594	4.34e–06	***
Age ≤ 60: Social support	–0.018670	0.330887	–0.056	0.955004	
Age ≤ 60: Family income (B)	–0.437650	0.320723	–1.365	0.172388	
Age ≤ 60: Family income (C)	–0.478175	0.328037	–1.458	0.144927	
Age ≤ 60: Smoking	1.667.095	0.268469	6.210	5.31e–10	***
Age ≤ 60: Sedentary lifestyle	0.174090	0.285680	0.609	0.542267	
Marital status: Schooling	0.236244	0.284772	0.830	0.406771	
Marital status: Social support	–1.853.831	0.332073	–5.583	2.37e–08	***
Marital status: Family income (B)	–0.253452	0.325323	–0.779	0.435933	
Marital status: Family income (C)	–0.321234	0.335701	–0.957	0.338614	
Marital status: Smoking	0.142201	0.293944	0.484	0.628551	

To be continued

Continuation

Marital status: Sedentary lifestyle	0.530015	0.312457	1.696	0.089833	.
Schooling: Social support	0.246803	0.331015	0.746	0.455912	
Schooling: Family income (B)	1.090.231	0.311334	3.502	0.000462	***
Schooling: Family income (C)	1.755.401	0.316313	5.550	2.86e–08	***
Schooling: Smoking	0.348653	0.275326	1.266	0.205395	
Schooling: Sedentary lifestyle	–0.741624	0.286969	–2.584	0.009757	**
Social support: Family income (B)	0.648041	0.362608	1.787	0.073911	.
Social support: Family income (C)	0.993385	0.388036	2.560	0.010466	*
Social support: Smoking	0.264417	0.344911	0.767	0.443305	
Social support: Sedentary lifestyle	0.709920	0.341477	2.079	0.037620	*
Family income (B): Smoking	–0.101796	0.332136	–0.306	0.759233	
Family income (C): Smoking	0.093087	0.328933	0.283	0.777179	
Family income (B): Sedentary lifestyle	–0.681732	0.354139	–1.925	0.054225	.
Family income (C): Sedentary lifestyle	–0.392269	0.359034	–1.093	0.274584	
Smoking: Sedentary lifestyle	–0.115416	0.285370	–0.404	0.685887	

Family incomes (B) and (C) – categories B and C defined in this study.

	Estimator (λ)	Standard error	Z Value	Pr (>|z|)	
(Intercept)	10.180	0.2687	3.788	0.000152	***
MDD	–16.204	0.3859	–4.199	2.68e–05	***
Gender	21.191	0.2832	7.482	7.31e–14	***
Sedentary lifestyle	14.193	0.2590	5.479	4.27e–08	***
History of MDD	–21.340	0.1964	–10.867	< 2e–16	***
Marital status	0.4318	0.1816	2.377	0.017437	*
Age ≤ 60	–0.0438	0.1209	–0.362	0.717020	
MDD: Gender	–11.755	0.2639	–4.454	8.43e–06	***
MDD: Sedentary lifestyle	0.6026	0.3401	1.772	0.076453	.
MDD: History of MDD	17.387	0.2988	5.819	5.92e–09	***
Gender: Sedentary lifestyle	–0.7402	0.2850	–2.598	0.009390	**
Gender: Marital status	–16.320	0.2399	–6.804	1.02e–11	***
MDD: Age ≤ 60	0.3886	0.2547	1.526	0.126975	

A Single Resistance Exercise Session Improves Aortic Endothelial Function in Hypertensive Rats

Thaís de Oliveira Faria, Jhuli Keli Angeli, Luiz Guilherme Marchesi Mello, Gustavo Costa Pinto, Ivanita Stefanon, Dalton Valentim Vassallo, Juliana Hott de Fúcio Lizardo

Universidade Federal do Espírito Santo (UFES), Vitória, ES – Brazil

Abstract

Background: **Physical exercise is an important tool for the improvement of endothelial function.**

Objective: **To assess the effects of acute dynamic resistance exercise on the endothelial function of spontaneously hypertensive rats (SHR).**

Methods: **Ten minutes after exercise, the aorta was removed to evaluate the expression of endothelial nitric oxide synthase (eNOS), phosphorylated endothelial nitric oxide synthase (p-eNOS1177) and inducible nitric oxide synthase (iNOS) and to generate concentration-response curves to acetylcholine (ACh) and to phenylephrine (PHE). The PHE protocol was also performed with damaged endothelium and before and after N^G-nitro-L-arginine methyl ester (L-NAME) and indomethacin administration. The maximal response (E_{max}) and the sensitivity (EC_{50}) to these drugs were evaluated.**

Results: **ACh-induced relaxation increased in the aortic rings of exercised (Ex) rats (E_{max} = –80 ± 4.6%, $p < 0.05$) when compared to those of controls (Ct) (E_{max} = –50 ± 6.8%). The E_{max} to PHE was decreased following exercise conditions (95 ± 7.9%, $p < 0.05$) when compared to control conditions (120 ± 4.2%). This response was abolished after L-NAME administration or endothelial damage. In the presence of indomethacin, the aortic rings' reactivity to PHE was decreased in both groups (EC_{50} = Ex –5.9 ± 0.14 vs. Ct –6.6 ± 0.33 log μM, $p < 0.05$ / E_{max} = Ex 9.5 ± 2.9 vs. Ct 17 ± 6.2%, $p < 0.05$). Exercise did not alter the expression of eNOS and iNOS, but increased the level of p-eNOS.**

Conclusion: **A single resistance exercise session improves endothelial function in hypertensive rats. This response seems to be mediated by increased NO production through eNOS activation. (Arq Bras Cardiol. 2017; 108(3):228-236)**

Keywords: **Exercise; Rats; Hypertension; Nitric Oxide; Endothelium Vascular.**

Introduction

The vascular endothelium has been considered to be a major target organ of arterial hypertension.[1] Several reports have shown that endothelial dysfunction is involved in the genesis or the development of arterial hypertension and may be either the cause or the consequence of the problem.[2,3] In the presence of arterial hypertension there is an imbalance in the production of endothelial factors, thus vasoconstrictors are produced in greater quantity than vasodilators. It explains the impaired endothelium-dependent relaxation in hypertensive animals and human subjects.[3-5]

The main cause of this endothelial dysfunction in arterial hypertension seems to be the decreased bioavailability of nitric oxide (NO).[2,3,6] It is well known that by interacting with NO, superoxide anions (O_2^-) form peroxynitrite, which decreases NO availability for smooth muscle relaxation.[7] Endogenous inhibitors of NO synthase (NOS) are also found in the blood of hypertensive individuals, and their increased expression has been associated with greater cardiovascular risk.[3]

Physical exercise is an important tool for the improvement of endothelial function, because improves the balance between the release of vasodilators and vasoconstrictors. It has already been shown that chronic or acute exercise protocols have important effects on the release of vasoactive substances resulting in better endothelium-dependent control of vascular tone.[8-11] Nevertheless, these data concerns endurance exercise. Thus, the effects of a single resistance exercise session on endothelial function are still poorly understood. We have previously demonstrated that a single resistance exercise session decreased the reactivity to phenylephrine (PHE) and increased the endothelium-dependent relaxation to acetylcholine (ACh) in the tail arteries of spontaneously hypertensive rats (SHR).[12] Cheng et al.[13] have also demonstrated similar response, however after endurance exercise.

The vascular function improvement after acute endurance exercise seems to be mediated by increased NO release.[8-11,13] Our results suggested that acute resistance exercise also potentiates the production of that vasoactive agent, and the response was associated with the release of vasodilator prostanoids. More studies are necessary to clarify the underlying mechanisms of endothelial function after acute resistance exercise.

Mailing Address: Juliana Hott de Fúcio Lizardo •
Rua Luíza Grinalda. Postal Code 29100-240, Centro, Vila Velha, ES – Brazil
E-mail: julianahfl@gmail.com, julianahott@yahoo.com
Manuscript received June 13, 2016; revised manuscript October 26, 2016; accepted September 27, 2016

DOI: 10.5935/abc.20170023

Thus, the present study aimed to investigate endothelial function after a single resistance exercise session in SHR.

Methods

Animals

The experiments were conducted using 22 male SHR that weighed 250-300 g. The rats were housed in an environment that was controlled for room temperature, humidity, light cycles (12 h light/dark). They had free access to tap water and were fed a standard rat chow ad libitum. The care and use of laboratory animals and all of the experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Ethics Committee Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória, Brazil (CEUA- EMESCAM).

Experimental design

Experimental groups

The animals were submitted to surgery for direct measurement of blood pressure. All of the surgical procedures were performed using aseptic techniques. Anesthesia was induced with chloral hydrate (400 mg/kg, i.p.) and supplementary doses were administered if the rat regained a blink reflex. The left carotid artery was carefully isolated to avoid damage to any nearby nerves. A tapered polyethylene cannula (PE 50) filled with heparinized saline (100 units/ml) was inserted into the left common carotid artery for blood pressure measurement. The free end of a catheter was plugged in a stainless steel obturator and inserted subcutaneously to exit from the back of the neck. The animals were placed in separate cages and were allowed to recover for 24 hours before the initiation of the experimental procedures. The rats were monitored for any signs of infection.

Blood pressure and heart rate were continuously recorded in conscious rats before the resistance exercise session to confirm the presence of arterial hypertension. The blood pressure was determined by connecting the arterial catheter to a TSD104A pressure transducer that was coupled to a DA100C amplifier. An acquisition system (MP 100 Biopac Systems, Inc., CA, USA) was used for real-time blood pressure and heart rate recording and for subsequent analysis.

On the day of the experiment, the rats were allowed to adapt to the laboratory environment for 1 hour before their resting hemodynamic measurements were recorded. After the adaptation period, baseline blood pressure levels were measured in conscious animals for 10 minutes before exercising. Subsequently, the animals were randomly divided into two experimental groups: the exercise group (n = 11), in which rats were submitted to a single resistance exercise session; and the control group (n = 11), where the animals were submitted to a single simulation of a resistance exercise session. Ten minutes after exercise training, the animals from both groups were anesthetized with sodium thiopental (50 mg/kg, i.p.) and were euthanized by exsanguination. The thoracic aorta was carefully dissected for the analysis of vascular reactivity and protein expression.

Exercise protocol

Initially, all animals were adapted to the exercise apparatus for 4 – 5 days. For adaptation, the rats were placed on the exercise apparatus without weight in the rest position, and, therefore, the animals did not move, although received tail electrical stimulus. Afterwards, one repetition of the maximal test was performed. The maximum repetition (RM) was determined to be the maximum weight that was lifted by each rat using the exercise apparatus. After 2 days of rest, the animals were submitted to an exercise protocol. The rats performed the resistance exercise according to a model adapted from previous studies.[12-15] Rats that were wearing a canvas jacket were able to regulate the twisting and flexion of their torsos and were fixed by a holder in a standing position on their hindlimbs. An electrical stimulation (20 V for 0.3-second duration and at 3-second intervals) was applied to the rat's tail through a surface electrode. As a result, the animals extended their legs repeatedly, which lifted the weight on the arm of the exercise apparatus. This apparatus was chosen because mimics traditional squat exercises that are performed by humans, and the results obtained in rat skeletal muscles are similar to those observed in humans.[15] The rats were exercised for 20 sets with 15 repetitions per set in the exercise apparatus. The repetitions were performed at 3-second intervals with a 1-minute rest between the sets. The exercise intensity was 50% of one RM. The control group received the same stimulus for the same frequency and duration and at the same intensity and intervals as the exercise group. However, the exercise apparatus was unweighted and in the rest position, and therefore, these animals did not lift a load.

Vascular reactivity measurements

The thoracic aorta was carefully dissected out and cleaned of fat and connective tissue. For the reactivity experiments, the aorta was divided into 3-4 mm cylindrical segments. The functional testing of the aortic rings was performed as previously described.[16] Briefly, 4 mm-long segments of thoracic aorta were mounted in an isolated tissue chamber containing Krebs–Henseleit solution (in mM: 118 NaCl; 4.7 KCl; 23 $NaHCO_3$; 2.5 $CaCl_2$; 1.2 KH_2PO_4; 1.2 $MgSO_4$; 11 glucose and 0.01 EDTA), gassed with 95% O_2 and 5% CO_2, and maintained at a resting tension of 1 g at 37°C. The isometric tension was recorded using an isometric force transducer (TSD125C, CA, USA) that was connected to an acquisition system (MP100 Biopac Systems, Inc., Santa Barbara, CA, USA).

After a 45-min equilibration period, all of the aortic rings were initially exposed twice to 75 mM KCl, the first time to check their functional integrity and the second time to assess the maximal tension that developed. Afterwards, 10 μM ACh was used to test the endothelial integrity of the segments that had been previously contracted with 1 μM PHE. A relaxation response that was equal to or greater than 90% was considered to be demonstrative of functional endothelial integrity. After a 45-min washout, concentration–response curves to PHE were determined. Single curves were generated for each segment. The role of select, local vasoactivators on the PHE-elicited contractile response was investigated. The effects of the following

drugs were evaluated: (1) the nonspecific NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME) (100 μM) and (2) the nonspecific cyclooxygenase (COX) inhibitor indomethacin (10 μM). These drugs were added to the bath 30 min before generating PHE concentration–response curves.

The influence of the endothelium on the response to PHE in the absence or presence of exercise was investigated after its mechanical removal was achieved by rubbing the vessel lumen with a needle. The absence of endothelium was confirmed by the inability of 10 μM ACh to induce relaxation.

In another set of experiments, after a 45-min equilibration period, the aortic rings of control and exercise rats were pre-contracted with 1 μM PHE and concentration-response curves to ACh (0.1 nM – 30 mM) were determined.

Western blot analyses

After performing euthanasia as previously described, the thoracic aorta was obtained. To analyze the endothelial nitric oxide synthase (eNOS) expression, phosphorylated endothelial nitric oxide synthase (p-eNOS) expression and inducible nitric oxide synthase (iNOS) expression, the arteries were rapidly frozen and kept at -80°C. From each homogenized artery, 80 μg of protein were separated by 10% SDS-PAGE. The protein was transferred to nitrocellulose membranes that were incubated with blocking buffer, and then incubated with antibodies for eNOS, eNOS that was phosphorylated on the amino acid serine at position 1177 (p-eNOS1177) (1:250; BD Transduction Laboratories™, Lexington, UK), and iNOS (1:250; BD Transduction Laboratories™, Lexington, UK). After washing, the membranes were incubated with anti-mouse immunoglobulin antibody (1:5,000; StressGen, Victoria, Canada) that was conjugated to horseradish peroxidase. After a thorough washing, the immunocomplexes were detected using an enhanced horseradish peroxidase/luminol chemiluminescence system (ECL Plus, Amersham International, Little Chalfont, UK) and film (Hyperfilm ECL International). The signals on the immunoblot were quantified using the ImageJ computer program, and the same membrane was used to determine α-actin expression with a mouse monoclonal antibody (1:5,000; Sigma, USA).

Data analysis and statistics

The contractile responses were expressed as a percentage of the maximal response that was induced by 75 mM KCl. The relaxation responses to ACh were expressed as the percentage of relaxation of the maximal contractile response. For each concentration-response curve, the maximal effect (E_{max}) and the concentration of agonist that produced 50% of the maximal response (-log EC_{50}) were calculated using a non-linear regression analysis. Thus, the sensitivity (50% of the maximal response) of the agonists was expressed as EC_{50} (-log EC_{50}) and the maximal contractile response to drug was expressed as E_{max}. To compare these variables (EC_{50} and E_{max}) between groups, unpaired Student's *t*-test was used.

To compare the effects of endothelial denudation or L-NAME on the contractile responses to PHE, the results were expressed as the differences in the area under the concentration-response curve (dAUC) for the control and experimental groups.

For protein expression, the data were expressed as the ratio between the signals on the immunoblot that correspond to the protein of interest and to α-actin. The differences were analyzed using unpaired Student's *t*-test. All the results were expressed as mean ± SE (standard error). $P < 0.05$ was considered to be significant. For all analyses, GraphPad Prism Software (Inc., San Diego, CA, USA) was used.

Results

The effect of exercise on aortic reactivity

To investigate the attenuation of aortic reactivity after exercise, the endothelium-dependent relaxation was elicited by the addition of ACh (Figure 1). A single resistance exercise increased the endothelium-dependent relaxation as observed in the concentration-response curve to ACh. Moreover, after exercise, there was an increase in the E_{max} to ACh ($p < 0.05$), however, the EC_{50} was not altered ($p > 0.05$) (Table 1).

Aortic reactivity to PHE was attenuated after exercise (Table 1). In the presence of L-NAME, the decrease in vascular reactivity to PHE after exercise was abolished, and there was a significant increase in vascular response in both groups (Table 1). Figure 2 shows the concentration-response curves to PHE, as well as the percentage of the dAUC after L-NAME or indomethacin administration, and following endothelial damage. Vascular reactivity was increased significantly in both groups after endothelial damage ($p < 0.05$) (Figure 2; Table 1). Under this condition, the percentage change of the dAUC was also greater in the exercised rats, suggesting that there was an important endothelial modulation on the vascular reactivity to PHE (Figure 2). Aortic reactivity to PHE was significantly decreased in both groups in the presence of indomethacin, suggesting that there is an increased COX-mediated vasoconstrictor prostanoid production in hypertensive rats. This result is reinforced by the percentage change of the dAUC, which demonstrated a greater effect in the exercise group (Figure 2, Table 1).

Expression of iNOS, eNOS and p-eNOS

As shown in Figures 3 and 4, the protein expression level of iNOS and eNOS was not altered after acute exercise. However, the level of p-eNOS protein was 38% higher ($p < 0.05$) in the exercised rats as compared to the controls (Figure 5), suggesting that there is increased NO production after a single resistance exercise session.

Discussion

The present study demonstrated that a single resistance exercise session that was conducted at 50% of one RM increases the endothelium-mediated vasodilatation and decreases the vascular responsiveness to PHE. This response was associated with an increase in the level of p-eNOS117, indicating that NO has an important role in the improvement of endothelial function following acute exercise.

Table 1 – EC_{50} and E_{max} values for each protocol

	EC_{50}		E_{max}	
	Ct	Ex	Ct	Ex
ACh	8.9 ± 0.2	9.0 ± 0.1	50 ± 6.8	80 ± 4.6*
E+	6.7 ± 0.09	6.7 ± 0.1	120 ± 4.2	95 ± 7.9*
E–	7.6 ± 0.1	7.7 ± 0.2	159 ± 7.2†	162 ± 7.1†
LN	7.0 ± 0.2	7.0 ± 0.2	149 ± 7.9†	148 ± 5.1†
Indo	6.6 ± 0.3	5.9 ± 0.1	17 ± 6.2†	9.5 ± 2.9*†

*EC_{50}: 50% of the maximum effect of the drug; E_{max}: maximum effect of the drug; Ach: acetylcholine; E+: phenylephrine without damaged endothelium; E–: phenylephrine with damaged endothelium; LN: L-NAME; Indo: indomethacin on the isolated aortic rings in control (Ct, n = 11) and exercise (Ex, n = 11) conditions in spontaneously hypertensive rats; *p < 0.05 vs. E_{max} Ct. †p < 0.05 vs. E_{max} E+ Ct and Ex.*

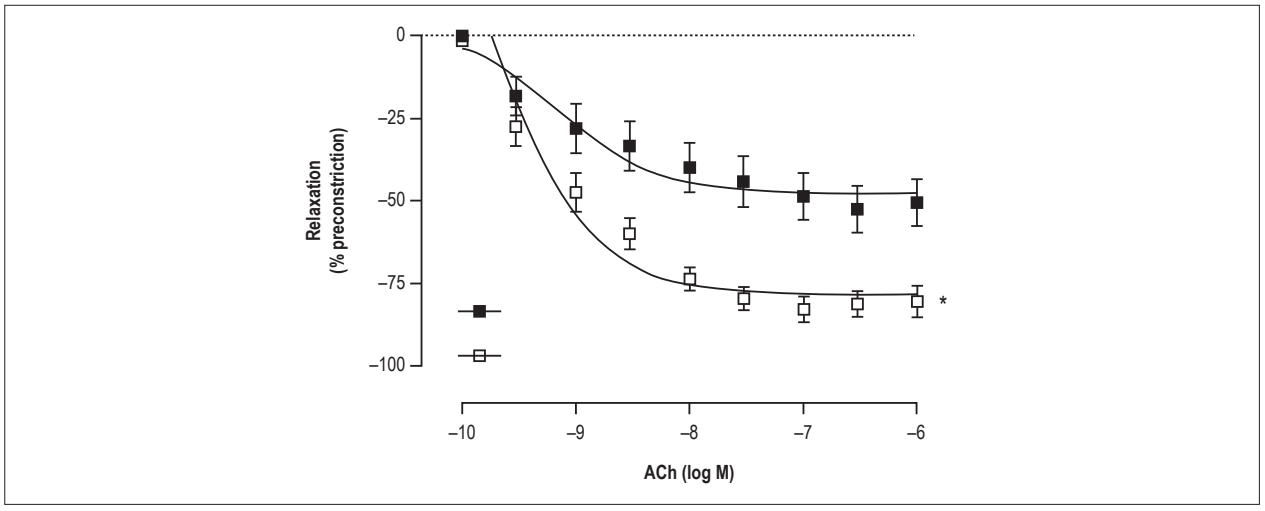

Figure 1 – *Effects of exercise training on concentration-response curve in aortic rings. Concentration-response curve to acetylcholine (ACh) obtained in aortic rings pre-contracted with phenylephrine (PHE) in control (Ct, n = 17) and exercise rats (Ex, n = 15). *p < 0.05 vs. Ct.*

Using a similar exercise protocol, we previously demonstrated that a single resistance exercise session decreases blood pressure in conscious SHRs,[17] reduces responsiveness to PHE and increases endothelium-dependent relaxation[12] in the tail arteries of SHR. These responses appear to be primarily mediated by NO. It has been demonstrated that chronic exercise, as well as acute aerobic exercise, decreases α-adrenergic vascular responsiveness[18-23] and increases endothelium-dependent relaxation in humans and in normotensive and hypertensive animals.[18,24,25] This response is thought to be mediated by NO production and by other vasodilators, such as prostacyclin.[18,24,26] Definitive data on the effects of acute resistance exercise on vascular function are limited. Two previous studies investigated the effects of isometric exercise using a handgrip[27,28] and aimed to evaluate brachial artery function, primarily in patients with endothelial dysfunction. There is no other study about vascular function and acute resistance exercise in conductance vessels.

We initially evaluated the endothelium-dependent vasodilatation that was elicited by ACh on the isolated aortic rings and showed that a single resistance exercise session evoked an increase in this response (Figure 2). These results corroborate the previous findings in normotensive and hypertensive rats after acute dynamic exercise.[18-21] Additionally, Maiorana et al.[29] investigated the response of the brachial artery to ACh in patients with heart failure after 8 weeks of endurance and resistance training and also demonstrated a significant increase in the vasodilatation response to ACh.

We also demonstrated that acute resistance exercise decreases the vasoconstriction response to PHE mediated by increased endothelial NO production (Figure 2). Using similar methods, Howard et al.[20] have demonstrated that a single aerobic exercise session reduced the response to PHE in normotensive rabbits. Additionally, Patil et al.[30] have shown *in vivo* a significant attenuation of the maximal vasoconstriction response to PHE in the iliac arteries of Sprague-Dawley rats after a single running session. This response was abolished with the inhibition of NO synthesis. Similarly, in the present study, the responsiveness to PHE after exercise returned to control levels following L-NAME administration, suggesting that NO production is increased during post-exercise recovery. Rao et al.[22] have also demonstrated a significant reduction in the responsiveness to PHE in the femoral arteries of SHR after acute exercise on a treadmill, which was

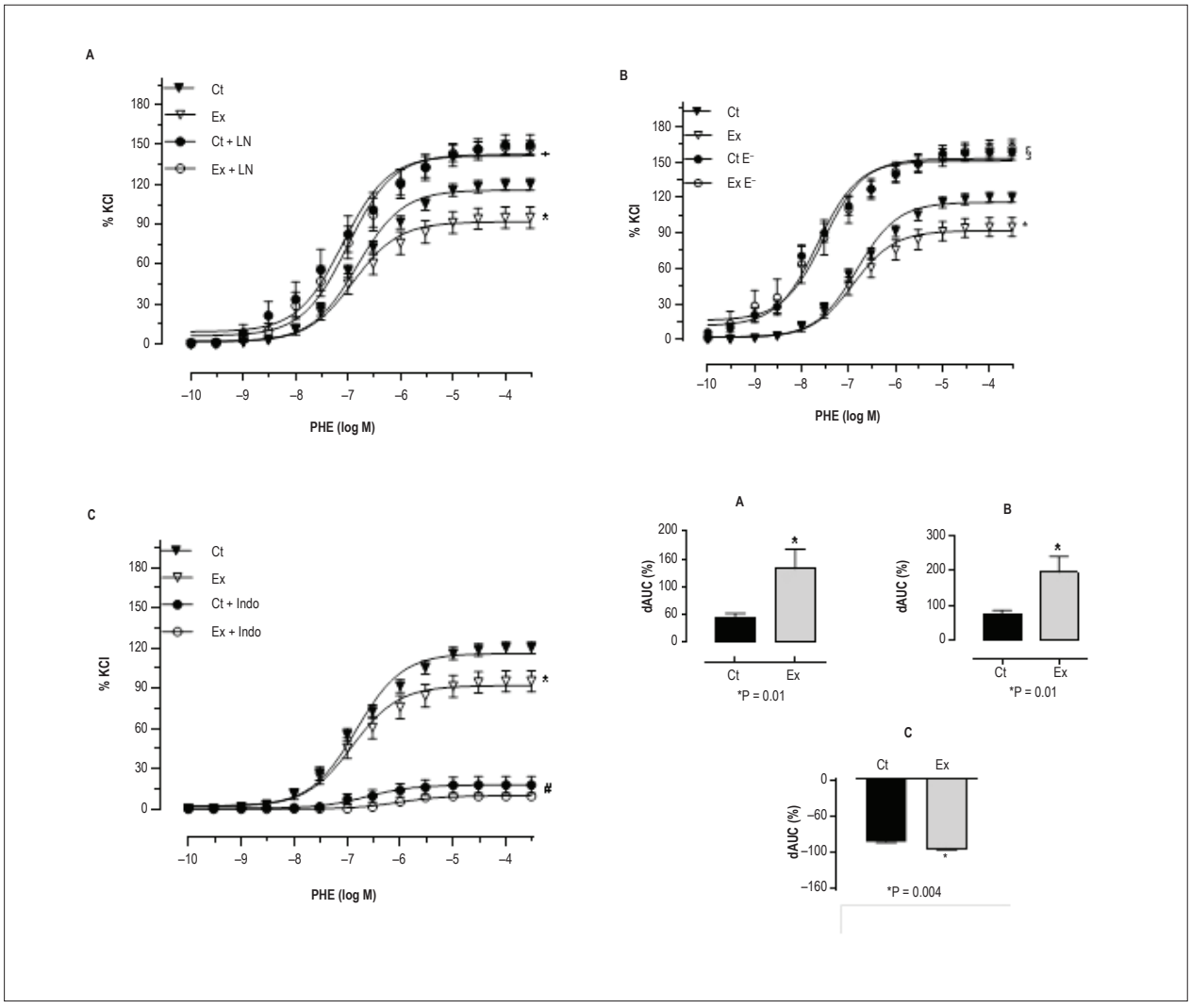

Figure 2 – *Effects of exercise training on concentration-response curve in aortic rings. Concentration-response curve to phenylephrine (PHE) obtained in aortic rings in control (Ct, n = 22) and exercise rats (Ex, n = 22) (A) before and after L-NAME administration (Ct+LN, n = 11; Ex+LN, n = 12); (B) after endothelial damage (Ct E⁻, n = 7; Ex E⁻, n = 6); and (C) after indomethacin administration (Ct+Indo, n = 7; Ex+Indo, n = 7). dAUC, difference in the area under the curve. *p < 0.05 Ex vs. other conditions. +p < 0.05 Ct+LN and Ex+LN vs. other conditions. §p < 0.05 Ct E⁻ and Ex E⁻ vs. other conditions. #p < 0.05 Ct+Indo and Ex+Indo vs. other conditions. The values are expressed as percentage of maximal response to KCl.*

abolished after the inhibition of NO synthesis with L-NAME. In humans, NO synthesis inhibition also abolishes the vasodilatation response after acute exercise.[31] Our results are in agreement with the data obtained from chronic exercise experiments as well. Chen et al.[19] have shown that the reduction of the vascular response to norepinephrine after treadmill training was mediated by NO. Similarly, Chen et al.[18] has demonstrated a reduction in sensitivity to norepinephrine and PHE in the aorta of SHR and Wistar Kyoto rats after treadmill training, due to increased NO production.

Thus, it appears that both acute and chronic exercise elicit a reduction in vascular responsiveness that is primarily mediated by the increase in NO synthesis. Nevertheless, the underlying mechanisms that generate the increased NO production after chronic and acute exercise are different. It has been previously reported that exercise training increases eNOS expression.[26,32] We investigated whether eNOS and iNOS protein expression were increased after a single resistance exercise session. As expected, the expression of these isoforms was not altered after acute exercise because it is unlikely that a single exercise session represents a sufficient stimulus to induce protein expression. Because eNOS activation is dependent on the phosphorylation pattern of well-characterized sites,[33] we hypothesized that eNOS phosphorylation could be the mechanism involved in NO production after acute exercise; therefore, we measured p-eNOS1177 protein levels. The amino acid serine at position 1177 is the primary activating eNOS phosphorylation site, and when it is modulated by the Akt kinase (also known as kinase protein B) and eNOS, it demonstrates an increased sensitivity to baseline Ca^{2+}/calmodulin concentrations.[34]

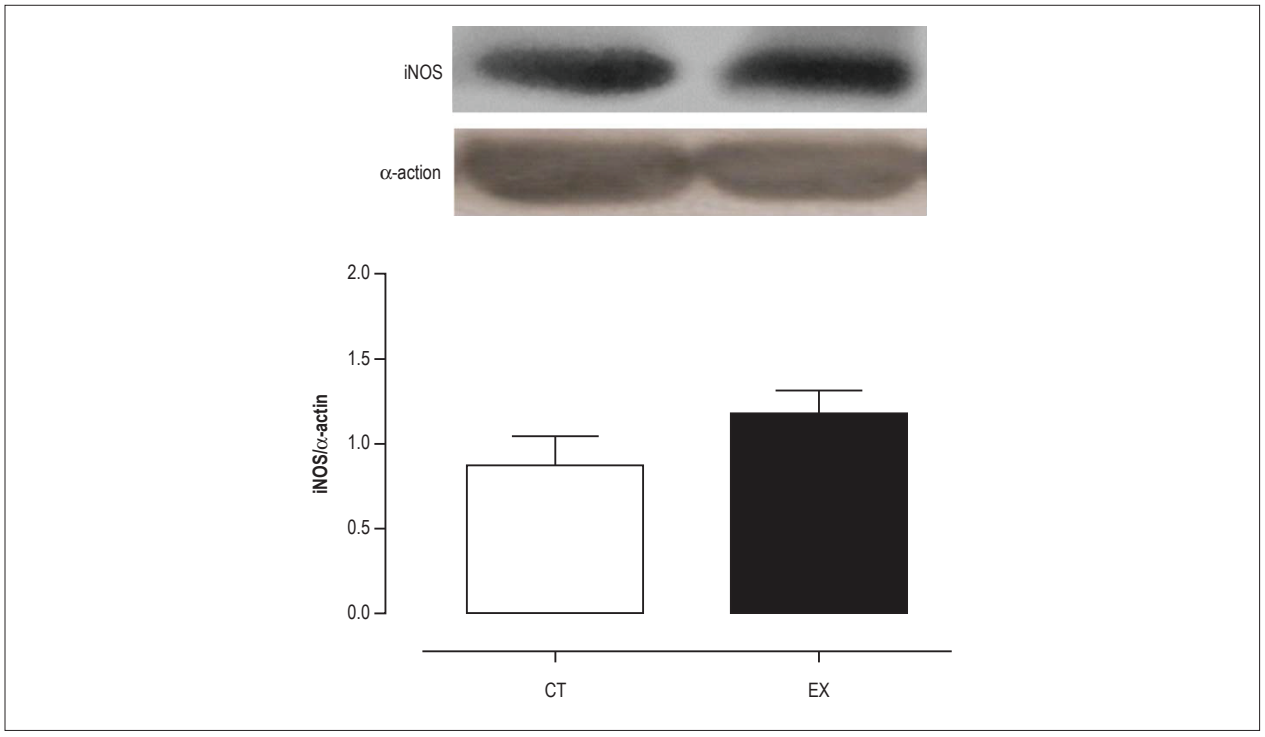

Figure 3 – *iNOS as determined by Western blot analysis in the aorta of control (Ct) and exercise training rats (Ex). Mean ± SEM (n = 7).*

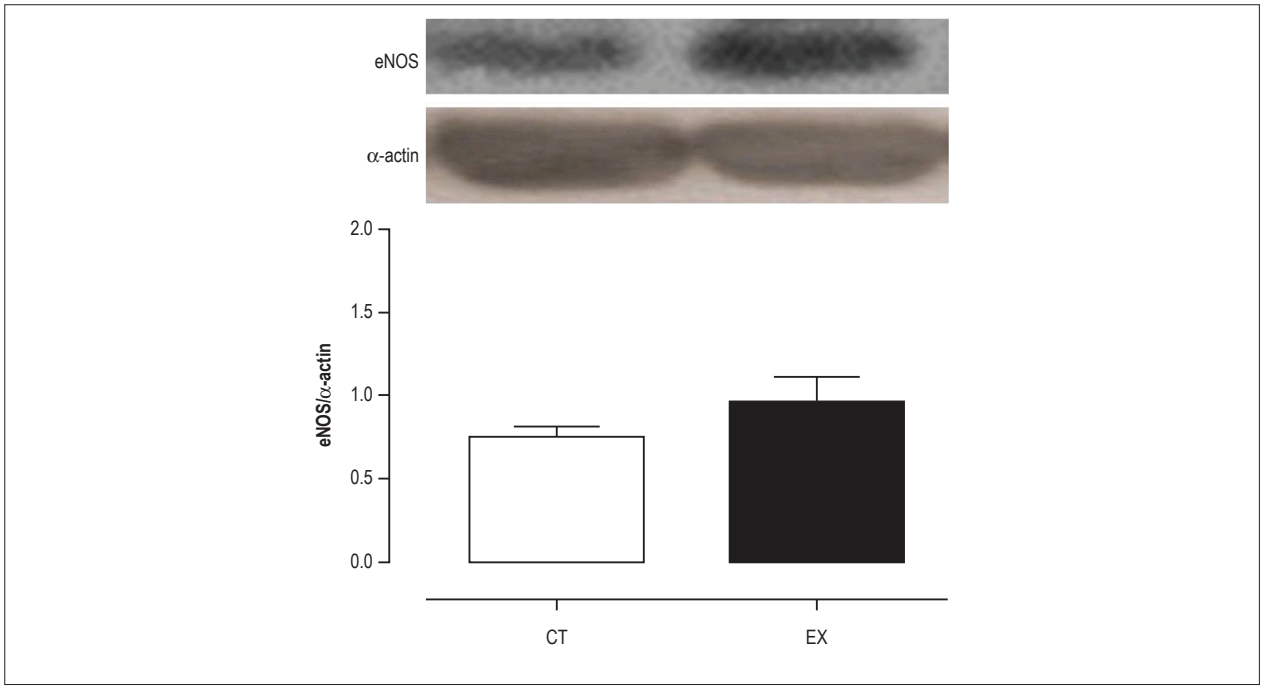

Figure 4 – *Effects of exercise on protein level. eNOS as determined by Western blot analysis in the aorta of control (Ct) and exercise training rats (Ex). Mean ± SEM (n = 7).*

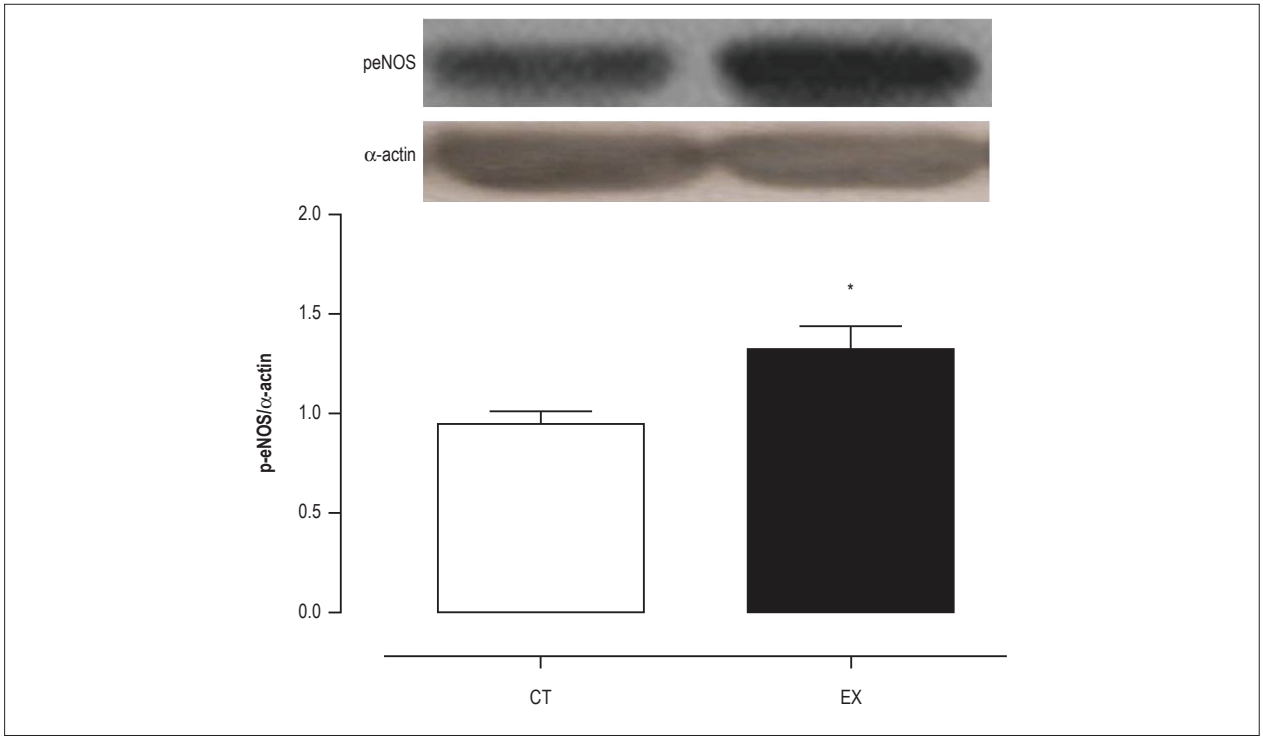

Figure 5 – *Effects of exercise on protein level. eNOS phosphorylation at Ser1177 as determined by Western blot analysis in the aorta of control (Ct) and exercise training rats (Ex). Mean ± SEM (n = 7). *p < 0.05 vs. CT.*

The level of p-eNOS1177 protein was significantly increased after acute resistance exercise when compared to control rats, indicating that eNOS is activated after acute resistance exercise. This finding confirms our hypothesis that the decrease of vasoconstriction and the increase of ACh-stimulated post-exercise vasodilatation were mediated by NO. Some of the factors that are involved in eNOS activation and, consequently, NO synthesis, such as shear stress, hypoxia and catecholamine release, are present during exercise and during the post-exercise recovery. Therefore, after exercise, the eNOS activity could remain increased for an extended time, resulting in a reduction in the vascular reactivity that is mediated by NO.

To investigate the possible role of vasodilator prostanoids in the reduction of vascular responsiveness after exercise, we evaluated the response to PHE in the presence of indomethacin, a COX inhibitor. In contrast to the results obtained in the tail artery in our previous study,[12] the aortic responsiveness was significantly decreased after COX inhibition (Figure 2C) in control and exercised rats. This response may be explained by the increase of the COX-induced vasoconstrictor prostanoid production in hypertensive rats.[35] Moreover, the increased prostanoid synthesis was shown to be due to an increase of COX-2 activity. Our data indicate that there is an important effect provoked by a single resistance exercise session. As observed in Figure 2, the percentage of the area under the curve was greater after the exercise, suggesting that acute resistance exercise decreased the vasoconstrictor prostanoid release in conductance vessels. The endothelial dysfunction present in arterial hypertension evokes an increase in vasoconstrictor prostanoid production,[35-38] and a single resistance exercise session has an important impact on vascular function improvement, because it decreases the vasoconstrictor prostanoid release. Moreover, it is well established that NO can regulate the activity of COX enzymes[39] and the activity of NOS is increased when the COX pathway is inhibited by indomethacin.[40]

Conclusions

This study demonstrated that a single resistance exercise session decreased the vascular response to PHE and increased the endothelium-dependent relaxation mediated by ACh in SHR. This adaptation appears to be mediated by NO, due to the increase in the p-eNOS1117 protein levels. Moreover, the present investigation also showed that acute resistance exercise may decrease the production of vasoconstrictor prostanoids in the aortic rings of SHR. Thus, our findings suggest that the practice of resistance exercise, even in a single session, might have great clinical relevance for hypertension control.

Author contributions

Conception and design of the research: Stefanon I, Vassallo DV, Lizardo JHF; Acquisition of data: Faria TO, Angeli JK, Mello LGM, Pinto GC, Lizardo JHF; Analysis and interpretation of the data: Faria TO, Angeli JK, Mello LGM, Stefanon I, Vassallo DV, Lizardo JHF; Statistical analysis: Faria TO, Angeli JK, Lizardo

JHF; Obtaining financing: Stefanon I, Vassallo DV; Writing of the manuscript: Faria TO, Lizardo JHF; Critical revision of the manuscript for intellectual content: Faria TO, Angeli JK, Mello LGM, Pinto GC, Stefanon I, Vassallo DV, Lizardo JHF.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by CNPq.

Study Association

This article is part of the thesis of Doctoral submitted by Juliana Hott de Fúcio Lizardo, from Universidade Federal do Espírito Santo.

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Effects and Mechanisms of Radiofrequency Ablation of Renal Sympathetic Nerve on Anti-Hypertension in Canine

Wei Chen, Xiaohong Tang, Xiaofang Yang, Chunyan Weng, Kan Yang, Juan Wen, Hong Liu, Yang Wu

Department of Cardiology - the Third Xiangya Hospital - Central South University, Changsha - Hunan

Abstract

Background: Radiofrequency ablation of renal sympathetic nerve (RDN) shows effective BP reduction in hypertensive patients while the specific mechanisms remain unclear.

Objective: We hypothesized that abnormal levels of norepinephrine (NE) and changes in NE-related enzymes and angiotensin-converting enzyme 2 (ACE2), angiotensin (Ang)-(1-7) and Mas receptor mediate the anti-hypertensive effects of RDN.

Methods: Mean values of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) were assessed at baseline and follow-up. Plasma and renal norepinephrine (NE) concentrations were determined using high-performance liquid chromatography with electrochemical detection, and levels of NE-related enzyme and ACE2-Ang(1-7)-Mas were measured using real time PCR, Western blot and immunohistochemistry or Elisa in a hypertensive canine model fed with high-fat diet and treated with RDN. The parameters were also determined in a sham group treated with renal arteriography and a control group fed with normal diet.

Results: RDN decreased SBP, DBP, MAP, plasma and renal NE. Compared with the sham group, renal tyrosine hydroxylase (TH) expression was lower and renalase expression was higher in the RDN group. Compared with the control group, renal TH and catechol-o-methyl transferase (COMT) were higher and renalase was lower in the sham group. Moreover, renal ACE2, Ang-(1-7) and Mas levels of the RDN group were higher than those of the sham group, which were lower than those of the control group.

Conclusion: RDN shows anti-hypertensive effect with reduced NE and activation of ACE2-Ang(1-7)-Mas, indicating that it may contribute to the anti-hypertensive effect of RDN. (Arq Bras Cardiol. 2017; 108(3):237-245)

Keywords: Sympatectomy; Hypertension; Renal Insufficiency; Radio Waves; Dogs.

Introduction

Hypertension is the leading cause of cardiovascular diseases worldwide,[1] resulting in an estimated 7.6 million deaths annually.[2] Globally, 40.8% of the population is affected by hypertension, with an awareness rate of 46.5% and a control rate of 32.5%.[3] The control of hypertension is a challenge due to the side effects, low compliance and limited efficacy of anti-hypertensive drugs.

The anti-hypertensive effects of radiofrequency ablation of renal sympathetic nerve (RDN) were first reported by Henry Krum in 2009.[4] The Symplicity HTN-1[5] and Symplicity HTN-2[6] trials showed profound anti-hypertensive effects during a follow-up period of 36 months. A meta-analysis confirmed the effectiveness of RDN therapy for resistant hypertension,[7] and was found superior to maximal medical therapy in lowering blood pressure (BP).[8] However, the Symplicity HTN-3 study did not show effective BP reduction in resistant hypertensive patients,[9] indicating that only a minority of patients was eligible for RDN.

Since the specific anti-hypertensive mechanisms of RDN are not clear, norepinephrine (NE) concentrations are an index of sympathetic neural activity in humans, which is positively correlated to BP. RDN may decrease NE that contributes to low BP, although the effect of RDN on NE is inconsistent.[10,11] The inconsistent NE levels after RDN may be caused by tyrosine hydroxylase (TH), renalase, catechol-o-methyl transferase (COMT) and norepinephrine transporter (NET) activity, which are the enzymes associated with the synthesis and metabolism of NE. On the other hand, the angiotensin-converting enzyme 2 (ACE2)/angiotensin (Ang)-(1-7)/Mas axis constitutes an alternative to the renin - angiotensin system (RAS) and represents an intrinsic mechanism to induce vaso-protective actions by counter regulating the ACE/AngII/AT1R axis, thus inducing many beneficial effects on cardiovascular diseases (CVDs). It is inversely related to BP[12] and exhibits cardiovascular and renal protection. Therefore, we aim to determine the effect of RDN on renal NE and changes in NE-related enzymes (TH, renalase, COMT and NET). Additionally, we investigated the levels of renal ACE2 - Ang (1-7) - Mas axis after RDN and discussed the potential anti-hypertensive mechanisms of RDN.

Mailing Address: Xiaohong Tang •
The Third Xiangya Hospital of Central South University, Changsha, Hunan 410013, P.R. China, Department of Cardiology
E-mail: xhtang007@126.com
Manuscript received May 12, 2016; revised manuscript August 08, 2016, accepted August 31, 2016.

DOI: 10.5935/abc.20170014

Methods

Animal preparation

All procedures on the use and care of animals was approved by the Ethical Committee of Central South University. Beagle dogs (n=28, 10 to 12 months of age, weighing 11 ~ 12kg) were randomly divided into a hypertensive model group (n = 22) and a control group (n = 6). Throughout the study, dogs were fed high-fat diet (lard 0.3 ~ 0.4kg/day was added to 250g/day regular diet) in the model group and regular diet (250g/day including 23% protein, 11% fat, 4.9% fiber, water 10%, 1-3% calcium, 0.8% phosphorus, 0.29% methionine, vitamin A 11000IU / kg, vitamin D3 1000 IU / kg and vitamin E 500 IU / kg) in the control group. After 3 months of high-fat diet, 20 dogs achieved an approximate 50% increase in body weight, and the fat intake was reduced to a maintenance level. This model of canine obesity following a high-fat diet closely mimics the cardiovascular, renal, hormonal, and metabolic changes observed in obese human subjects. The hypertensive group was divided into a surgery group (n = 10) and a sham surgery group (n = 10). Three dogs were excluded for the following reasons: retroperitoneal hematoma caused by femoral artery puncture (n=1) and death due to anesthesia (n=2). The surgery group (n=9) was treated with radiofrequency ablation of the renal sympathetic nerve, and the sham surgery (n=8) and control groups (n=6) were treated with renal arteriography. Six months after RDN, we sacrificed the beagle dogs under deep anesthesia by intramuscular injection of pentobarbital sodium (30-35mg/kg).

Radiofrequency ablation of the renal sympathetic nerve

Surgery was performed at room temperature, with prior fasting for 24 hours, and after anesthetizing dogs with an intramuscular injection of sodium pentobarbital (30-35mg / kg). After successful anesthesia, the dogs were placed in the supine position on the operating table, followed by routine disinfection of the right femoral artery. A catheter was inserted through the femoral artery to monitor BP and renal arteriography. The radiofrequency ablation catheter was inserted through the femoral artery into the renal artery and connected to a radiofrequency ablation device (IBI, St. Jude Medical, Inc., St. Paul, MN, USA). Three to four ablation sites were selected from each site and a spiral shape local ablation was performed (5F IBI radiofrequency ablation catheter; St. Jude Medical). Each spot was ablated for 120 sec, with a power limit of 8 W, until the tumor temperature reached 55°C. Renal arteriography was performed immediately after the surgery, pressure was applied at the puncture point for 15 - 30 min, bandaged and fixed.

Analytic methods

Body weight was determined by electronic scales. SBP, DBP and MAP were measured by BP-2010E (Softron, China), a tail arterial blood pressure measuring instrument. Plasma concentration and renal tissue levels of NE were measured by high-performance liquid chromatography with electrochemical detection (HPLC). Renal ACE2 and Mas mRNA levels were measured by real time PCR. Renal Ang (1-7) (Cusabio, China) was estimated by ELISA. TH, renalase, COMT, NET, ACE2 and Mas protein expression levels in renal tissue were measured by Western blot and immunohistochemistry.

HPLC

NE in acidified release medium, perfusate, and superperfusate samples was identified and quantified by HPLC. The system consists of a Varian Pro-Star solvent delivery system and a model 9090 autosampler (Varian, Walnut Creek, CA), coupled to a C18 column and an ESA Coulochem II detector. Separations were performed isocratically using a filtered and degassed mobile phase, consisting of 12% methanol, 0.1 M sodium phosphate, 0.2 mM sodium octyl sulfate, and 0.1 mM EDTA, adjusted to pH 2.8 with phosphoric acid. The high-pressure liquid chromatography system is coupled to a computer, where the chromatograms were recorded and analyzed using the Varian Star workstation software.

Western blot

Frozen tissues were lysed with cell lysis buffer containing protease inhibitor. The protein concentration of each specimen was measured based on the Bradford method utilizing the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin (BSA) as the standard. After the protein denaturing procedure with loading buffer, each sample (50 μg) was resolved on 8-12% SDS-polyacrylamide gel electrophoresis (PAGE) gel (Bio-Rad Laboratories) at room temperature and transferred onto a polyvinylidene fluoride membrane at 4°C. After blocking in 5% non-fatty milk for 1 hour at room temperature, it was incubated overnight at 4°C with polyclonal rabbit anti-TH antibody (1:500 Abcam, USA), polyclonal goat anti-renalase antibody (1:500, biorbyt, USA), polyclonal goat anti-COMT antibody (1:500, LifeSpan BioSciences, USA), polyclonal rabbit anti-NET antibody (1:500, Abcam, USA), polyclonal goat anti- ACE2 antibody (1:200, Santa Cruz, USA) and polyclonal rabbit anti-Mas antibody (1:200, Santa Cruz, USA) with β-actin (1 : 1000, Abcam, USA) as the positive control. The HRP-conjugated rabbit anti-goat (1 : 2000) or goat anti-rabbit (1 : 3000) secondary antibody was added and the membranes were then incubated for 1 hour at room temperature. After washing, signals were visualized by luminol reagents (Bio-Rad Laboratories) and the densitometry of each blot was analyzed with the latest version of Scion Image 4.0.3.2.

Immunohistochemistry

The staining procedure was performed on paraffin-embedded renal tissue sections (5 μm). Antigen was retrieved from all the sections by boiling with sodium citrate buffer (pH 6) and incubating with polyclonal rabbit anti-TH antibody (1:150 Abcam, USA), polyclonal goat anti-renalase antibody (1:150, biorbyt, USA), polyclonal goat

anti-COMT antibody (1:150, LifeSpan BioSciences, USA), polyclonal rabbit anti-NET antibody (1:150, Abcam, USA), polyclonal goat anti- ACE2 antibody (1:100, Santa Cruz, USA) and polyclonal rabbit anti-Mas antibody (1:100, Santa Cruz, USA) overnight at 4°C. After staining, all specimens were dehydrated and sealed for microscopic observation. Protein occurrence and distribution in antibody-stained tissue sections were observed using the Nikon eclipse E400 microscope and Digital HyperHAD Color Video Camera (Sony) using the Easy Image Analysis software (NIS-Elements BR v3.0) for evaluation of immunostaining.

Statistical analyses

Results were expressed as means ± standard error of the means, and all data passed a normality test. Comparisons between the hypertensive model group and control groups were done using the unpaired Student's *t*-test assuming unequal variance, whereas within 3 groups analysis was performed using one-way ANOVA with a Neuman-Keuls post hoc analysis. Paired Student's *t*-test was used to compare before and after establishing the hypertensive model, and before and after RDN. Linear correlation was used to evaluate the association between SBP and level of the above mentioned factors. All data were analyzed by SPSS 22.0. $P < 0.05$ was considered significant.

Results

Canine model of hypertension and response to RDN

After 3 months on high-fat diet, there was a marked increase in body weight, HR, SBP, DBP and MAP of the hypertensive group (*$p < 0.05$ vs. baseline, # $p < 0.05$ vs. control group). SBP, DBP and MAP of hypertensive group increased by approximately 28±10mmHg, 17±8mmHg and 21±8mmHg, respectively, along with the target weight gain of 45.2%. Moreover, plasma NE in the hypertensive group was remarkably increased after 3 months of high-fat diet (*$p < 0.05$ vs. baseline, # $p < 0.05$ vs. control group). (Figure 1). SBP, DBP and MAP of the surgery group dramatically decreased by approximately 24±9mmHg, 13±6mmHg and 16±7mmHg 6 months after surgery, respectively. When compared with the sham surgery group, SBP, DBP and MAP of the surgery group also declined significantly. (Figure 2A) Six months after RDN, plasma NE of the surgery group was significantly reduced (*$p < 0.05$ vs. pre-surgery of surgery group, # $p < 0.05$ vs. sham surgery group). In addition, renal NE in the surgery group was also lower than the in the sham surgery group ($p < 0.05$). (Figure 2B)

Levels of renal TH, renalase, COMT and NET response to RDN

Six months after surgery, renal TH protein expression in the surgery group was lower than in the sham surgery group ($p < 0.05$). TH immunohistochemical staining (brown) was located in the cytoplasm of renal tubules in beagle dogs (Figure 3A). Kidney level of renalase in the surgery group was significantly higher than in the sham surgery group ($p < 0.05$). Immunohistochemical results showed that renalase protein was expressed in the cytoplasm of renal tubular epithelial cells (Figure 3B). The renal COMT protein expression in the sham surgery group was lower than in the control group ($p < 0.05$). In the immunohistochemical study, COMT was located in the cytoplasm of renal tubules in beagle dogs (Figure 3C). NET was located in the cytoplasm of renal tubules in beagle dogs. However, there was no statistical difference of the renal NET among the 3 groups (Figure 3D).

Levels of renal ACE2, Ang-(1-7) and Mas response to RDN

Renal ACE2 mRNA and protein expression in the surgery group were significantly higher than in the sham surgery group ($p < 0.05$). Immunohistochemical staining (brown) of ACE2 was located in the cytoplasm and membrane of renal tubules in beagle dogs. Six months after surgery, values with positive area density in the surgery group were significantly stronger than in the sham group ($p < 0.05$) (Figure 4A). Similar to ACE2, renal tissue Ang-(1-7) concentration in the sham surgery group was the lowest, dramatically lower than in the surgery group and control group ($p < 0.05$) (Figure 4B). And the level of renal Mas was also higher in the surgery group and control group than in the sham surgery group. Immunohistochemical staining (brown) of Mas was located in the renal glomeruli and proximal tubule cell cytoplasm and cell membrane in beagle dogs (Figure 4C).

Discussion

In our study, obesity-related hypertension, induced by high-fat diet, was associated with increase in body weight, HR, SBP, DBP and MAP, and this was in agreement with the results of previous studies.[13] RDN effectively reduced blood pressure for SBP, DBP and MAP by approximately 24±9mmHg, 13±6mmHg and 16±7mmHg in the surgery group after 6 months of surgery, respectively. SBP, DBP and MAP of the surgery group also declined significantly when compared with the sham surgery group, which was similar to findings of previous clinical studies[5,6] and animal experiment[14]. The Symplicity HTN-1 trial enrolled 153 resistant hypertensive patients, of whom 111 agreed to the 36-month follow-up. SBP (-32.0mmHg) and DBP(-14.4mmHg) decreased significantly.[5] The Symplicity HTN-2 trial randomized 106 subjects with resistant hypertension, whose SBP and DBP were reduced by 33 mmHg and 14 mmHg at 36 months, respectively.[6] However, the Symplicity HTN-3 study did not show effective BP reduction in resistant hypertensive patients,[9] indicating that only a minority of patients was eligible for RDN.

NE is an important indicator of sympathetic neural activity, and is elevated in diseases with high sympathetic activity. Tiroch et al.[10] found that RDN resulted in a marked decrease of the NE spillover rate, while Machino et al.[11] observed no significant difference after RDN of systemic NE in spontaneously hypertensive rats (SHR). We observed that plasma NE increased significantly in an obesity-related hypertensive canine model, and decreased nearly 44% with RDN. Furthermore, RDN was effective in reducing renal NE concentration, consistent with the results of Rimoldi et al.[15]

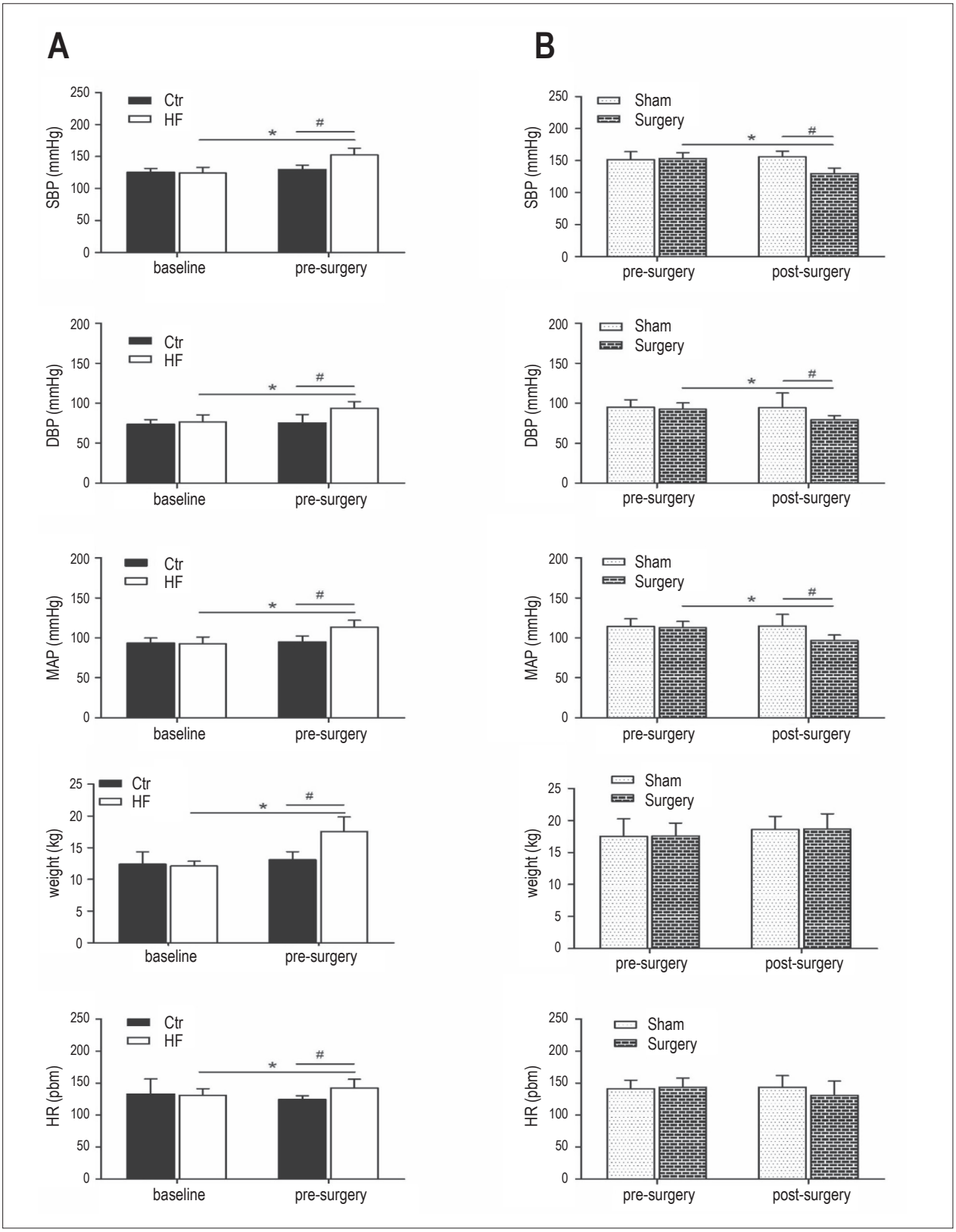

Figure 1 – *Effects of high-fat diet on body weight, HR and BP (SBP, DBP and MAP) of beagle dogs (A). Body weight, HR and BP (SBP, DBP and MAP) responses to RDN (B). Values are mean ± SEM.*p < 0.05 versus baseline, # p < 0.05 versus control group in figure 2A. *p < 0.05 versus pre-surgery of surgery group, # p < 0.05 versus sham surgery group in figure 2B. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressure; HR: Heart rate.*

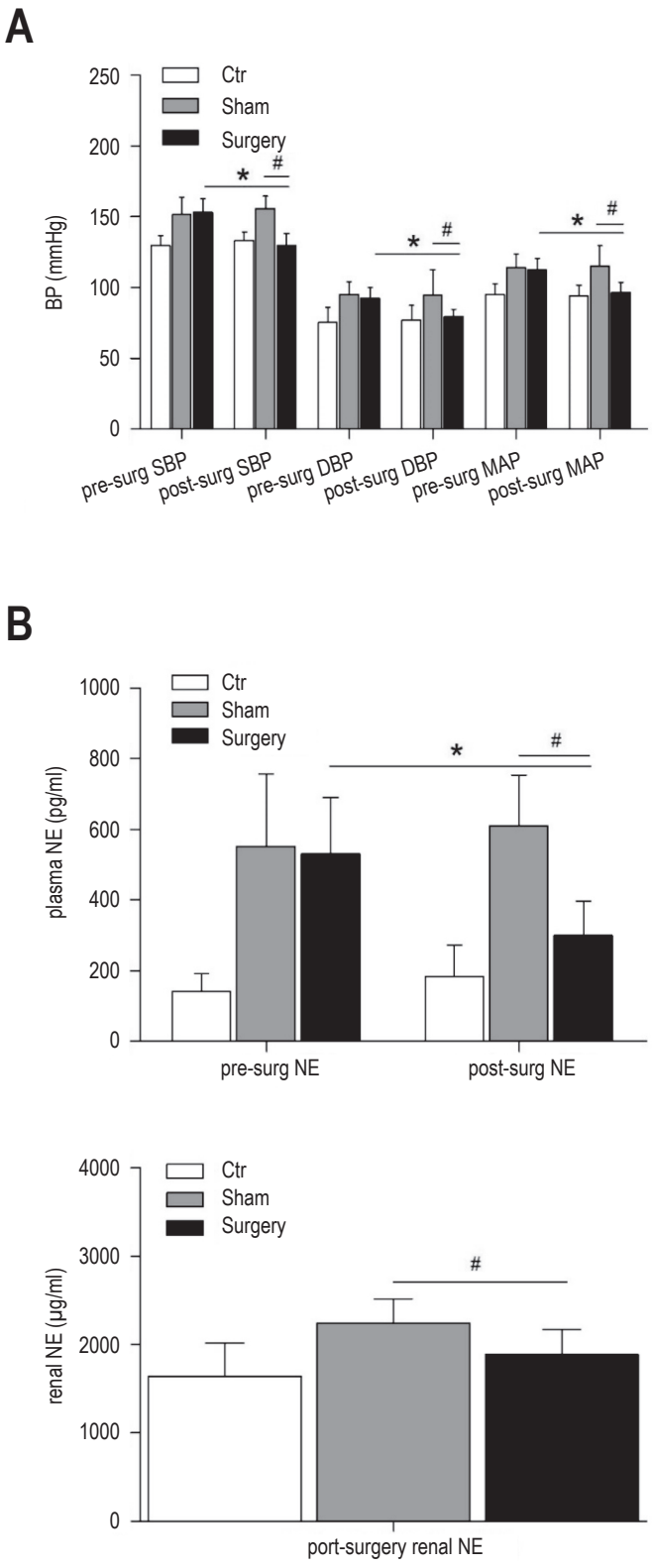

Figure 2 - *BP (SBP, DBP and MAP)(A) and NE (plasma and renal) (B) in response to RDN. Values are mean ± SEM.*p < 0.05 versus pre-surgery of surgery group, # p < 0.05 versus sham surgery group in figure 2. BP: blood pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean arterial pressur; NE: norepinephrine.*

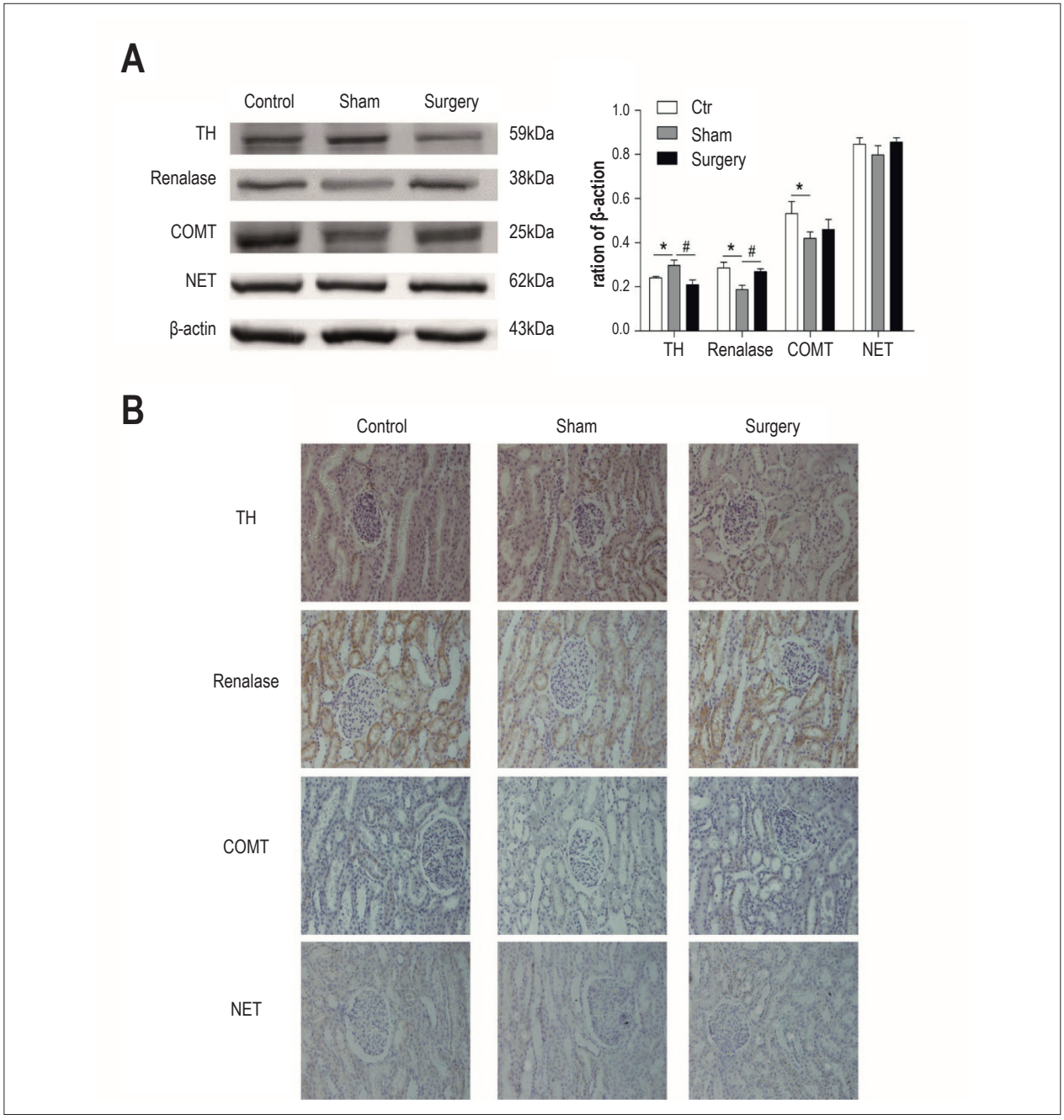

Figure 3 – *Effects of RDN on renal TH, renalase, COMT and NET protein expression of beagle dogs. Values are mean±SEM. * p < 0.05 versus control group, # p < 0.05 versus sham surgery group. TH: tyrosine hydroxylase ; COMT: catechol-o-methyl transferase; NET: norepinephrine transporter.*

As reported, RDN was associated with additional benefits[16] and improvement of the cardiac and renal functions.[17,18] It was also proposed as a promising treatment in diseases with sympathetic overactivation.[19,20] In this study, we determined the antihypertensive value of RDN using an obesity-related hypertensive canine model with high NE levels.

Renal TH level was also increased in obesity-related hypertension in this study. In addition, RDN decreased the renal TH protein expression in the hypertensive model, which is substantially similar to previous studies. Down-regulation of TH in the adrenal medulla of SHR was accompanied by a potent decrease of NE and SBP,[21] suggesting that RDN may influence NE concentrations by affecting the renal TH level. On the other hand, renalase was remarkably lower in the obesity-related hypertensive canine model in this study, suggesting an inverse relationship with hypertension similar to findings of previous studies.[22] Desir GV showed that recombinant renalase *in vitro* or *in*

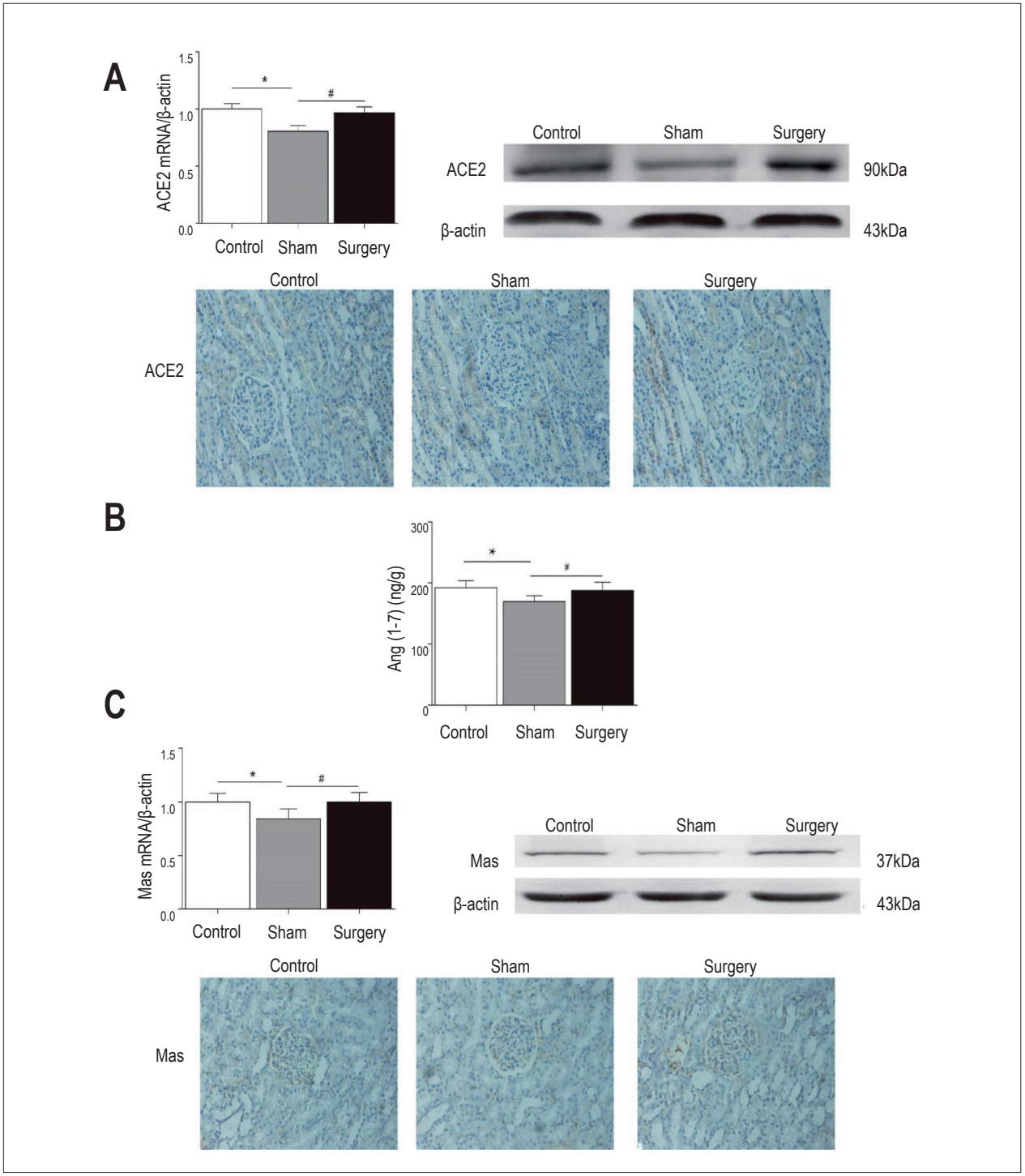

Figure 4 – *Effects of RDN on renal ACE2 mRNA and protein expression (A), Ang-(1-7) concentration (B) and Mas mRNA and protein expression(C) of beagle dogs. Values are mean±SEM. * p < 0.05 versus control group, # p < 0.05 versus sham surgery group. ACE2 mRNA: angiotensin-converting enzyme 2.*

vivo lowers blood pressure by degrading plasma adrenaline, with its antihypertensive effect directly related to its enzymatic activity.[23] SHR plasma and renal renalase levels were profoundly increased after RDN compared with the baseline, sham and control groups, with MAP significantly decreased,[24] which is consistent with this study, suggesting that RDN may lower the NE concentrations by elevating the renal renalase expression. COMT and NET are major enzymes involved in degrading catecholamines, which is inversely related to hypertension. As shown in our study, renal COMT level profoundly lowered

obesity-related hypertension compared with the control group, while RDN was ineffective against the expression of renal COMT and NET. As they are mainly expressed in nerve endings, it suggested that renal COMT and NET may have had no significant change after RDN.

The ACE2-Ang-(1-7)-Mas axis is involved in hypertension. Transgenic mice overexpressing growth hormone showed increased SBP, a high degree of both cardiac and renal fibrosis and a markedly decreased level of ACE2-Ang-(1-7)-Mas, and Ang-(1-7) administration reduced SBP.[25] Activation of the ACE2-Ang-(1-7)-Mas pathway reduces oxygen-glucose deprivation-induced tissue swelling, ROS production, and cell death in mouse brain associated with angiotensin II overproduction.[26] Consistent with the previous study, in addition to the reduction of ACE2, we also observed that renal Ang-(1-7) concentration and Mas mRNA and protein expression decreased in obesity-related hypertension. We first found that RDN increased renal ACE2-Ang-(1-7)-Mas axis in an obesity-related hypertensive canine model.

RDN shows anti-hypertensive effect with reduced NE and activation of ACE2-Ang-(1-7)-Mas, indicating that this may contribute to the anti-hypertensive effect of RDN. However, the relationship between these two pathways was not clear in this study. Ang-(1-7) elicits a facilitatory presynaptic effect on peripheral noradrenergic neurotransmission,[27,28] and is inhibitory at the central nervous system through the Mas receptor.[29,30] It is suggested that ACE2-Ang(1-7)-Mas may decrease the concentration of NE to achieve the anti-hypertensive effect, however, this needs to be confirmed by further studies.

Nevertheless, this study has limitations as follows. Firstly, the number of dogs is small, and this may lead to a bias result. Secondly, changes of NE, NE-related enzymes and ACE2-Ang-(1-7)-Mas were detected during the procedure, while the relationship between these changes was unclear. The variation of renal TH, renalase and ACE2-Ang-(1-7)-Mas may have affected the level of NE to contribute to the anti-hypertensive effect of RDN. These limitations should be addressed in further studies to clarify the possible mechanisms of RDN, thus contributing to develop and improve this new treatment method.

Conclusions

The initial question that motivated our study was to determine whether NE and ACE2-Ang-(1-7)-Mas would prove to participate in the antihypertensive effect of RDN. Our study confirmed that RDN shows an antihypertensive effect with reduced plasma and renal NE, which may be related to the decrease of TH and increase of renalase in the kidney. Furthermore, RDN activates the ACE2-Ang-(1-7)-Mas pathway and this may contribute to the antihypertensive effect of RDN. Although the application of RDN is not clear because of its varying effectiveness, our data suggested that it may be an excellent choice in obesity-related hypertension patients with high levels of NE and over-activation of the renin-angiotensin system.

Author contributions

Conception and design of the research: Chen W, Tang X, Yang K. Acquisition of data: Chen W, Yang X. Analysis and interpretation of the data: Chen W, Yang X.

Statistical analysis: Chen W, Yang X. Obtaining financing: Chen W, Tang X, Yang K. Writing of the manuscript: Chen W. Critical revision of the manuscript for intellectual content: Tang X, Weng C, Wen J, Liu H, Wu Y, Yang K. Supervision / as the major investigador: Tang X, Weng C, Wen J, Yang K.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by Hunan Provincial Natural Science Foundation of China (10JJ3048), the Fundamental Research Funds for the Central Universities of Central South University (2015zzts123).

Study Association

This article is part of the thesis of master submitted by Wei Chen, from Central South University, Changsha - Hunan.

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Prognostic Factors in Severe Chagasic Heart Failure

Sandra de Araújo Costa, Salvador Rassi, Elis Marra da Madeira Freitas, Natália da Silva Gutierrez, Fabiana Miranda Boaventura, Larissa Pereira da Costa Sampaio, João Bastista Masson Silva

Universidade Federal de Goiás - (UFG), Goiânia, GO - Brazil

Abstract

Background: Prognostic factors are extensively studied in heart failure; however, their role in severe Chagasic heart failure have not been established.

Objectives: To identify the association of clinical and laboratory factors with the prognosis of severe Chagasic heart failure, as well as the association of these factors with mortality and survival in a 7.5-year follow-up.

Methods: 60 patients with severe Chagasic heart failure were evaluated regarding the following variables: age, blood pressure, ejection fraction, serum sodium, creatinine, 6-minute walk test, non-sustained ventricular tachycardia, QRS width, indexed left atrial volume, and functional class.

Results: 53 (88.3%) patients died during follow-up, and 7 (11.7%) remained alive. Cumulative overall survival probability was approximately 11%. Non-sustained ventricular tachycardia (HR = 2.11; 95% CI: 1.04 – 4.31; p<0.05) and indexed left atrial volume ≥ 72 mL/m² (HR = 3.51; 95% CI: 1.63 – 7.52; p<0.05) were the only variables that remained as independent predictors of mortality.

Conclusions: The presence of non-sustained ventricular tachycardia on Holter and indexed left atrial volume ≥ 72 mL/m² are independent predictors of mortality in severe Chagasic heart failure, with cumulative survival probability of only 11% in 7.5 years. (Arq Bras Cardiol. 2017; 108(3):246-254)

Keywords: Heart Failure / mortality; Prognosis; Chagas Cardiomyopathy; Chagas Disease.

Introduction

Heart failure (HF) is a clinical syndrome in which the heart cannot provide a cardiac output that meets the needs of the peripheral organs and tissues, or does it under conditions of high filling pressures in its chambers.[1]

The American Heart Association (AHA) estimates a HF prevalence of 5.1 million individuals in the United States between 2007 and 2012.[2] In Brazil, the HF prevalence is 2 million patients, and its incidence, 240,000 new cases per year.[3]

Chagas disease is still an important etiology of HF. Approximately 10-12 million people worldwide are infected with *Tripanossoma cruzi*, and 21% to 31% of them will develop cardiomyopathy. This pathology accounts for 15,000 deaths per year and approximately 200,000 new cases. In Brazil, there are 3 million people with Chagas disease.[1]

Knowledge and experience indicate that the prognosis of individuals with HF is poor, and, of all etiologies, Chagasic HF has the worst prognosis.[4]

Studies on the poor prognosis of patients with Chagasic HF have been valued. However, information on mortality predictors in that disease are limited, and knowing those factors enables the treatment in the presence of some unfavorable conditions.[5-7]

Access to those parameters is usually easy, inexpensive and allows identifying the patients at higher mortality risk.

This study was aimed at identifying the association of clinical and laboratory factors with the prognosis of severe Chagasic HF, as well as the association of those factors with mortality rate and survival in a 7.5-year follow-up.

Methods

This is a subset of the "*Estudo Multicêntrico, Randomizado de Terapia Celular em Cardiopatias (EMRTCC) – Cardiopatia Chagásica*", with retrospective analysis of prospectively collected data.[8]

The research was conducted at the Heart Failure Service of the Hospital das Clínicas (HC) of the Goiás Federal University (UFG).

This study's target population was formed by 60 patients of the 234 participants in the EMRTCC, who remained being followed up at the HF outpatient clinic of the HC/UFG.

The EMRTCC study showed that the intracoronary injection of autologous stem-cells conferred no additional benefit over standard therapy to patients with Chagasic cardiomyopathy. Neither the left ventricular function nor the quality of life of those patients improved.[9] The neutral result ensured that the population assessed had no interference of that procedure.

The complete follow-up duration in this study was 7.5 years.

Mailing Address: Sandra de Araújo Costa •
Primeira Avenida, 565. CEP 74605-020, Setor Leste Universitário, Goiânia, GO - Brasil
E-mail: sandracardio@hotmail.com, cardioufg@yahoo.com.br
Manuscript received May 31, 2016, revised manuscript October 25, 2016, accepted October 26, 2016.

DOI: 10.5935/abc.20170027

Analyzed parameters

Systolic blood pressure

Systolic blood pressure (SBP) was measured by using the auscultatory technique standardized by the VI Brazilian Guidelines on Arterial Hypertension, with duly calibrated aneroid sphygmomanometer and stethoscope. Normality was considered as SBP of 120 mm Hg and diastolic blood pressure (DBP) of 80 mmHg.[10]

Age

Age was calculated based on the birth date recorded on the patient's identification document, considering the complete years of life at the time of study selection.

Simpson's left ventricular ejection fraction

Left ventricular ejection fraction (LVEF) was measured by echocardiography with the Simpson's method. All exams were performed by one single examiner in a Toshiba Xario device.

Serum sodium

Ion-selective electrode photometry was used to measure serum sodium concentration.[11] The normal reference value adopted at the local analysis laboratory was 135-144 mEq/L. Serum sodium concentration below the lower limit of normality (< 135 mEq/L) was considered hyponatremia, and above 144 mEq/L, hypernatremia.[11]

Creatinine

Automated Jaffe's reaction was used to measure serum creatinine concentration. The reference values adopted for creatinine were 0.7 - 1.3 mg/dL for men, and 0.6 - 1.2 mg/dL for women.[11]

6-minute walk test

The 6-minute walk test (6MWT) was performed twice, at a minimum 15-minute interval for rest. At the end of the 6MWT, the vital data initially obtained were collected again, and the distance covered by the patient was calculated as the mean of the two tests.[12]

The normal reference values for the 6MWT ranged from 400m to 700m for healthy individuals. So far, the literature has no standardized 6MWT reference value for individuals with heart disease.[13] We adopted the value of ≥ 400m for a satisfactory result, and < 400m for an unsatisfactory result, based on data published in the SOLVD Study.[14]

Non-sustained ventricular tachycardia

Non-sustained ventricular tachycardia (NSVT) was defined as three or more consecutive heartbeats, originating below the atrioventricular node, with heart rate > 100 beats per minute and duration < 30 seconds, identified on 24-hour Holter.[15]

QRS width

The QRS width was obtained on an electrocardiographic tracing in a duly calibrated device. Values ≤ 120ms were considered normal QRS width, while those > 120ms, extended QRS.[16]

Indexed left atrial volume

Indexed left atrial volume (ILAV) was obtained from the left atrial contour in two orthogonal views (apical 2- and 4-chamber views)[17] on echocardiography performed by a single observer in all patients.

Values up to 34 mL/m^2 were considered normal, between 35 and 41 mL/m^2, mild increase, between 42 and 48 mL/m^2, moderate increase, and greater than 48 mL/m^2, significant increase.[17]

Functional class

Functional class was categorized based on the New York Heart Association (NYHA) classification, whose validity and reliability have been well established.[14] The classification was based on the severity of the symptoms reported, and ranged from I to IV.[14]

Statistical analysis

The data were collected and recorded in an electronic spreadsheet and analyzed with the IBM SPSS statistical software, version 21.0.

The categorical variables were expressed as frequency, with absolute numbers and proportions. The association analysis between predicting variables and outcomes was performed with the chi-square test.

The chi-square test was used to compare outcome (death) and the different categories of predicting variables, such as age group, SBP, serum sodium, NSVT and QRS width.

The continuous quantitative variables were expressed as means, medians (non- parametric distribution), standard deviation and confidence interval (CI). Data distribution was analyzed by using the Shapiro Wilks test, considering the sample size smaller than 100 participants. To compare the means of the predicting variables, non-paired Student *t* test or Mann Whitney *U* test was used, depending on data distribution.

All tests were performed considering the 5% significance level, two-tailed probability and 95% CI.

Survival analysis

The survival time was calculated as the interval between the dates of treatment beginning and death. The maximal follow-up duration was 90 months, and those remaining alive after that time were censored. Because the participants underwent different follow-up durations and entered the study at different times, their prognoses were assessed with Kaplan-Meier statistics.

To compare stratified survival curves, hazard ratio (HR) was used as the measure of association between survival variables. Log-Rank (Mantel-Cox) test was used to compare the expected

values of each stratum under the null hypothesis that the risk is the same in all strata, that is, the number of events observed in each category of the variable analyzed, with the number of events (outcomes) expected.

Cox proportional hazards model, a semiparametric model to estimate the proportionality of hazards during the entire follow-up in an adjusted way, was performed to estimate the effect of the predicting variables. The continuous variables whose p-value < 0.20, in their quantitative format, and the categorical dichotomous or polychotomous variables were included in the model. The p-value of the Wald test was used.

Initially, univariate analysis of risk estimation was performed, and only the variables showing association with p < 0.20 were entered in the multivariate model. The model was adjusted step-by-step, with the inclusion of the variable that associated best in the first step, and considering theoretical criteria of previous knowledge.

Results

Baseline characteristics

Table 1 shows the initial characteristics of the 60 participants in this study.

Follow-up

The patients were followed up regularly at the HF outpatient clinic of the HC/UFG.

All patients were assessed at time zero and every 15 days, up to completing 60 days. This period was necessary to optimize medication for HF therapy and clinical stabilization of patients. Then, there was a baseline assessment, in which data were collected for analysis.

The patients were followed up with regular visits at 15 days, 1, 2, 4, 6, 9 and 12 months, and then every 6 months after the 1-year visit, until the end of the 7.5-year follow-up.

Medicamentous treatment

All participants were duly medicated, according to the III Brazilian Guideline on Chronic Heart Failure and patients' tolerance to medications.[1]

Appropriate medicamentous treatment was based on the association of a loop diuretic (furosemide), an angiotensin-converting-enzyme inhibitor (ACEI - enalapril), spironolactone and a beta-blocker (carvedilol). Patients would not receive a beta-blocker in case of intolerance. Digoxin was added when the patient remained symptomatic despite the use of those drugs. An angiotensin-receptor blocker (losartan) was prescribed in case of ACEI intolerance. Amiodarone was used in patients with symptomatic ventricular arrhythmia, documented on ECG or Holter. All patients with atrial fibrillation were anticoagulated, aiming at reaching an international normalized ratio (INR) between 2.0 and 3.0.[1]

The mean doses of ACEI and beta-blocker used were 10 mg/day and 25 mg/day, respectively. We aimed at the best drug treatment for all patients, with maximum tolerated doses of each medication. This process lasted, on average, 60 days.

Characterization of the sample according to the variables analyzed and outcome

Analyzing the clinical variables and comparing with death and non-death, the following three variables were found to be related to the mortality outcome: serum sodium, serum creatinine and ILAV.

Table 1 – Characteristics of the sample according to the variables analyzed

Variables	Mean Median	SD/ 95%CI
Age (years)	52.6 54.0	±9.4 50.2 – 55.0
Systolic blood pressure (mm Hg)	98.4 100.0	±14.2 94.8 – 102.1
LV ejection fraction (%)	27.1 26.5	±5.5 25.3 – 28.9
Serum sodium (mEq/L)	137.3 137.0	±4.2 136.2 – 138.4
Creatinine (mg/dL)	1.2 1.2	±0.3 1.1 – 1.3
6-minute walk test (meters)	433.4 433.5	±139.1 397.5 – 469.4
QRS width (ms)	125.3 120.0	±29.4 117.7 – 132.9
ILAV (mL/m^2)	107.0 102.7	±47.8 94.7 – 119.4

SD: standard deviation; CI: confidence interval; LV: left ventricular; ms: millisecond; ILAV: indexed left atrial volume.

Mean serum sodium concentrations were significantly lower in the patients who died, while mean serum creatinine levels were higher for the same outcome.

Similarly to creatinine, the mean ILAV levels were higher in patients who died.

Survival analysis

Of the 60 participants in this study, 53 (88.3%) died during the entire follow-up (90 months), and 7 (11.7%) were censored (alive by the end of follow-up) (Table 2).

The median follow-up was 24.5 months (±27.3; 95% CI: 28.5 - 42.6) and the cumulative overall survival probability for that follow-up period was approximately 50% (Figure 1). In the median follow-up period (24.5 months), there were 30 deaths, representing 50% of the total sample.

Most deaths were related to cardiovascular diseases, 47 (88.69%) being due to progressive HF, 3 (5.67%) to sudden death, and 1 (1.88%) to acute myocardial infarction. Of the other 2 deaths, 1 (1.88%) was due to non-Hodgkin's lymphoma, and the other (1.88%) to multiple organ failure consequent to sepsis.

Result of the Log-Hank (Mantel-Cox) – Kaplan-Meier test

The Log-Hank (Mantel-Cox) – Kaplan-Meier test was used to compare the survival curve with general mortality for the clinical and laboratory variables.

Regarding survival, the NSVT and ILAV variables showed significance. Patients with ILAV < 72 mL/m^2 had higher survival (35.7%) (Log-Rank, p=0.001), as had those with no NSVT (12.9%) (Log-Rank, p=0.040) (Table 3).

Table 2 – Cumulative overall survival probability (Kaplan-Meier)

Time [months (year)]	Participants at risk	Cumulative survival (%)	Deaths in the time interval	Alive at the beginning of the time interval
0	60	-	-	60
12 (1 year)	42	70	18	42
24 (2 years)	30	50	12	30
36 (3 years)	28	46	2	28
48 (4 years)	24	40	4	24
60 (5 years)	14	23	10	14
72 (6 years)	10	16	4	10
84 (7 years)	8	13	2	8
90 (7.5 years)	7	11	1	7

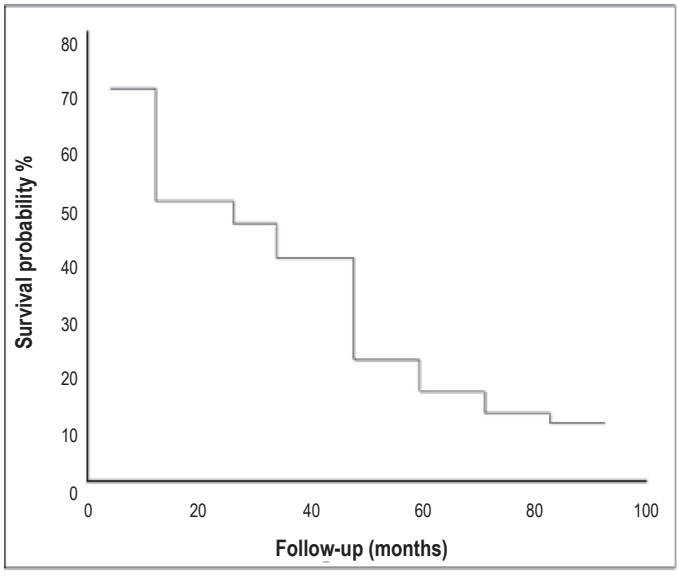

Figure 1 – *Cumulative overall survival curve.*

Table 3 – Comparison of the survival curve and general mortality for the variables analyzed [Log-Hank (Mantel-Cox) – Kaplan-Meier test]

Variables	n	Events	Censored	Survival %	p value
Age (years)					0.666
< 60	47	42	5	10.6	
≥ 60	13	11	2	15.4	
General	60	53	7	11.7	
SBP (mmHg)					0.325
< 120	50	45	5	10.0	
≥120	10	8	2	20.0	
Serum sodium (mEq/L)					0.128
<135	14	13	1	7.1	
135| --- 144	42	37	5	11.9	
≥ 144	4	3	1	25.0	
NSVT					0.040
Yes	29	26	3	10.3	
No	31	27	4	12.9	
QRS width (ms)					0.606
Normal (< 120)	18	16	2	11.1	
Extended (≥120)	42	37	5	11.9	
Functional class (NYHA)					0.066
II	32	26	6	18.8	
III	28	27	1	3.6	
ILAV (mL/m²)					0.001
< 72	14	9	5	35.7	
≥ 72	46	44	2	4.3	
Creatinine (mg/dL)					0.267
> 1.30	16	15	1	6.3	
≤1.30	44	38	6	13.6	
Ejection fraction (%)					0.446
>25%	34	30	4	11.8	
≤ 25%	26	23	3	11.5	

SBP: systolic blood pressure; NSVT: non-sustained ventricular tachycardia; ILAV: indexed left atrial volume; ms: millisecond.

Multivariate analysis - Cox regression

The variables used in Cox regression that remained in the last adjusted model were: NSVT, ILAV, serum sodium, and functional class, but only the first two had significant risk values (Table 4).

This study identified an increased risk for death of 2.11 (1.04 – 4.31) among patients with NSVT, and of 3.51 (1.63 – 7.52) among those with ILAV ≥ 72 mL/m² ($p < 0.05$ for both).

Discussion

Survival in heart failure

In this study, the cumulative overall survival probability of patients with severe Chagasic HF was approximately 11%, resulting from 53 deaths during the 90-month follow-up of a population of 60 patients.

The results found in this study are similar to those by Theodoropoulos et al.,[18] who have assessed 127 patients

Table 4 – Distribution of the variables based on univariate analysis of risk and Cox regression

Variable	Univariate analysis		Multivariate analysis	
	Hazard Ratio 95%CI	Wald coef. (p value)	*Hazard Ratio* 95%CI	Wald coef. (p value)
NSVT	3.0 (1.02 – 8.48)	4.01 (0.045)	3.83 (1.29 – 11.35)	5.84 (0.016)
ILAV	3.4 (1.58 – 7.24)	9.84 (0.002)	3.51 (1.63 – 7.52)	10.77 (0.001)
Sodium	0.9 (0.86 – 1.01)	2.80 (0.095)	0.98 (0.90 – 1.07)	0.22 (0.639)
FC	1.6 (0.96 – 2.86)	3.22 (0.073)	1.34 (0.76 – 2.36)	1.03 (0.311)

NSVT: non-sustained ventricular tachycardia; ILAV: indexed left atrial volume; FC: functional class; CI: confidence interval; coef.: coefficient.

with Chagasic HF and found cumulative survival probabilities of 78%, 59%, 46% and 39% in 1-, 2-, 3- and 4-year follow-ups, respectively (Table 5).

Clinical studies on HF of different etiologies have shown a slightly better probability in the long run. The cumulative overall survival probabilities reported by Rassi et al.[19] were 90.6%, 82.3%, 73.3%, 70.2% and 64.4% after 1, 2, 3, 4 and 5 years of follow-up, respectively. That population had HF of recent symptom onset.[19]

The survival reported by Areosa et al.[5] in a study with patients with severe HF of different etiologies, referred for cardiac transplantation, was 84.5% in the first year, 74.3% in the second year, 68.9% in the third year, 64.8% in the fourth year, and 60.5% in the fifth year.

The patients included in our analysis were properly medicated, and had age groups, functional class, SBP and LVEF similar to those of other studies (Theodoropoulos et al.,[18] Rassi et a.l[19] and Areosa et al.[5]). The present study and that by Theodoropoulos et al.[18] assessed only Chagasic patients, while the other cohorts comprised patients with HF of different etiologies (Table 5), that being their major difference.

Our study follow-up was long (7.5 years). Because Chagasic HF is a severe disease, with high mortality, the survival rate was expected to be low. The comparison of the survival rates reported by Rassi et al.[19] and Areosa et al.[5] and ours evidenced the lowest survival rate of severe Chagasic HF since the first year of follow-up, characterizing the worst prognosis of Chagasic individuals. When comparing our results with those by Theodoropoulos et al.,[18] who recruited only Chagasic patients, the similarity of data is evident.

To our knowledge, ours is the only study following up a population with HF longer than 5 years. Thus, there is no study on a 7.5-year survival that allows the comparison with ours.

Prognostic factors with no statistical significance

The variables SBP, age, LVEF, 6MWT, QRS width, and functional class showed no statistical significance regarding the outcome mortality.

Serum sodium and creatinine concentrations showed statistical significance regarding the outcome mortality on univariate analysis; after adjusting the model in multivariate analysis, however, they lost significance.

Prognostic factors with statistical significance

Indexed left atrial volume

This study used the cut-off point of 72 mL/m^2, similarly to that determined by Rassi et al.,[19] who identified, by using the ROC curve, 70,71 mL/m^2 as the best cut-off point.[20]

An ILAV $\geq$ 72 mL/m^2 was associated with a significant increase in mortality. Individuals with ILAV $\geq$ 72 mL/m^2 had increased risk for death (HR = 3.51; 95% CI: 1.63 - 7.52; p<0.05). Nunes et al.[6] have assessed the prognostic vale of ILAV in a population of 192 patients with Chagasic HF. They have identified a 4.7% increase in the risk for cardiac events for each 1-mL/m^2 increment in ILAV (HR = 1.047; 95% CI: 1.035 - 1.059; p <0.001), ILAV being, thus, considered a strong predictor of adverse results, implicating in worse prognosis and increased risk of death in that population.

Of the 20 echocardiographic parameters studied, ILAV proved to be the only independent predictor of cardiovascular mortality in patients with Chagasic HF.[20,21]

The echocardiogram, by identifying ILAV, adds significant information, and is a widely used non-invasive method that can play an important role in risk stratification, follow-up and treatment of Chagasic dilated cardiomyopathy.[10,22]

Non-sustained ventricular tachycardia on Holter

The NSVT was one of the variables analyzed with Cox regression that showed significant risk (HR = 2.11; 95% CI: 1.04 - 4.31; p < 0.05). Ventricular arrhythmias, such as NSVT, have been reported as extremely frequent in Chagas disease. The episodes of NSVT have been closely related to the ventricular dysfunction degree and its clinical

Table 5 – Comparison of cumulative survival rate in the studies

Follow-up [months (years)]	Costa, S.A. (2016)	Theodoropoulos, T.A. et al.[18]	Areosa, C.M.N. et al.[5]	Rassi, S. et al.[19]
	CS (%)	CS (%)	CS (%)	CS (%)
12 (1 year)	70	78	84.5	90.6
24 (2 years)	50	59	74.3	82.3
36 (3 years)	46	46	68.9	73.3
48 (4 years)	40	39	64.8	70.2
60 (5 years)	23	-	60.5	64.4
72 (6 years)	16	-	-	-
84 (7 years)	13	-	-	-
90 (7.5 years)	11	-	-	-

CS: cumulative survival

repercussions, occurring in approximately 40% of the patients with Chagasic HF.[23]

In our case series, all patients had LVEF <35%, and 48.34% of them had NSVT on 24-hour Holter. Despite the high mortality of this population, only 5.67% of the deaths occurred suddenly. These patients were under optimal medical therapy with amiodarone and beta-blocker, which can partially explain this fact.[23]

Two Argentinian randomized studies, GESICA and EPAMSA, assessing the effect of amiodarone in patients with HF, have included 10% and 20% of Chagasic patients in their cohorts, respectively. They have suggested that amiodarone could reduce total mortality when administered to patients with complex ventricular arrhythmias associated with reduced LVEF (< 35%).[23] However, at the time those studies were conducted, there was no formal indication for the use of beta-blockers in systolic HF.[24]

A sub-analysis of the REMADHE study, assessing the mode of death of patients with Chagasic HF as compared to that of patients with non-Chagasic cardiomyopathy, has shown higher mortality due to progressive HF among Chagasic patients, and that the use of amiodarone in that group was an independent predictor of mortality.[24]

In our case series, no patient had an implantable cardioverter-defibrillator, and 18 (30%) had a pacemaker.

Study limitations

This is a retrospective analysis of data prospectively collected in the EMRTCC study, originating from a single center. Despite the limitations inherent in a retrospective analysis, the parameters prospectively collected met well-defined criteria.

In addition, the population studied met very restrictive inclusion criteria, such as functional class (II and III), LVEF (≤ 35%) and creatinine (≤ 2.5mgd/L), which limited the expression of those variables to the correlation analysis with outcomes.

Another limitation was the small number of patients on beta-blockers, which is due to the low blood pressure of that specific population of patients, the bradycardia inherent in the heart disease, added to the use of amiodarone and digitalis.

Conclusions

In patients with Chagasic HF and important ventricular dysfunction, the presence of NSVT on Holter, as well as an ILAV greater than 72 mL/m^2 on echocardiography, are independent predictors of mortality.

The general prognosis of those patients is poor, with a cumulative survival probability of 11% in 7.5 years.

Author contributions

Conception and design of the research: Costa SA, Rassi S. Acquisition of data: Costa SA. Analysis and interpretation of the data: Costa SA, Rassi S. Statistical analysis: Costa SA, Rassi S.

Obtaining financing: Costa SA. Writing of the manuscript: Costa SA, Freitas EMM, Rassi S. Critical revision of the manuscript for intellectual content: Costa SA, Freitas EMM, Gutierrez NS, Boaventura FM, Silva JBM, Sampaio LPC, Rassi S. Supervision / as the major investigador: Costa SA, Rassi S.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of master submitted by Sandra de Araújo Costa, from Universidade Federal de Goiás.

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Sensitivity, Specificity and Predictive Value of Heart Rate Variability Indices in Type 1 Diabetes Mellitus

Anne Kastelianne França da Silva,[1] Diego Giuliano Destro Christofaro,[1] Aline Fernanda Barbosa Bernardo,[2] Franciele Marques Vanderlei,[1] Luiz Carlos Marques Vanderlei[1]

Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP),[1] Presidente Prudente, SP; Faculdade de Educação e Cultura de Vilhena,[2] Vilhena, RO – Brazil

Abstract

Background: Heart rate variability (HRV) indices may detect autonomic changes with good diagnostic accuracy. Type diabetes mellitus (DM) individuals may have changes in autonomic modulation; however, studies of this nature in this population are still scarce.

Objective: To compare HRV indices between and assess their prognostic value by measurements of sensitivity, specificity and predictive values in young individuals with type 1 DM and healthy volunteers.

Methods: In this cross-sectional study, physical and clinical assessment was performed in 39 young patients with type 1 DM and 43 young healthy controls. For HRV analysis, beat-to-beat heart rate variability was measured in dorsal decubitus, using a Polar S810i heart rate monitor, for 30 minutes. The following indices were calculated: SDNN, RMSSD, PNN50, TINN, RRTri, LF ms², HF ms², LF un, HF un, LF/HF, SD1, SD2, SD1/SD2, and ApEn.

Results: Type 1 DM subjects showed a decrease in sympathetic and parasympathetic activities, and overall variability of autonomic nervous system. The RMSSD, SDNN, PNN50, LF ms², HF ms², RRTri, SD1 and SD2 indices showed greater diagnostic accuracy in discriminating diabetic from healthy individuals.

Conclusion: Type 1 DM individuals have changes in autonomic modulation. The SDNN, RMSSD, PNN50, RRtri, LF ms², HF ms², SD1 and SD2 indices may be alternative tools to discriminate individuals with type 1 DM. (Arq Bras Cardiol. 2017; 108(3):255-262)

Keywords: Heart Rate; Diabetes Mellitus, Type 1, Predictive Value of Tests; Sensitivity and Specificity; Autonomic Nervous System.

Introduction

Type 1 diabetes mellitus (DM), an autoimmune disease that results from the destruction of pancreatic beta cells with consequent insulin deficiency,[1,2] has affected an increasing number of individuals in the world at younger ages.[3] Every year, approximately 15 thousand children are diagnosed with type 1 DM and 3,700 children with type 2 DM.[4]

Type 1 DM patients may have autonomous nervous system (ANS) dysfunction, which may be identified by heart rate variability (HRV) analysis.[5,6] HRV is a simple, accessible, non-invasive method that describes oscillations between consecutive heartbeats (RR intervals, RRIs), which are associated with the effects of ANS on sinus node.[7]

Analysis of HRV has shown that individuals with type 1 DM have reduced overall variability as compared with healthy subjects of different ages.[8-11] Besides, parasympathetic loss with sympathetic override[12] and reduced magnitude and complexity of HRV[13,14] have been reported in these individuals.

HRV has been used to identify autonomic changes and, despite studies showing its efficacy in the clinical practice in different populations, the use of HRV for this purpose is still incipient. In this context, studies have indicated that some HRV indices can detect autonomic changes with relative sensitivity and describe changes in cardiac rhythm with good diagnostic and prognostic value.[15,17]

In middle-aged adults with type 2 DM, Khandoker *et* al.[15] found that the SD1 (standard deviation of the instantaneous beat-to-beat variability) index, extracted from the Poincaré plot, and the SampEn (sample entropy) can identify cardiac autonomic dysfunction with the best diagnostic accuracy. The authors also showed that the HRV may have a practical diagnostic and prognostic marker in this population.

However, in type 1 DM patients, studies of this nature are still scarce, since most of them provides a comparison of HRV between subjects with and without DM, without analyzing the discriminatory power of these indices. Such studies would not only provide new information on the theme, but also determine HRV indices with the best diagnostic

Mailing Address: Anne Kastelianne França da Silva •
Rua Roberto Simonsen, n 305. Postal Code: 19060-900, Centro Educacional, Presidente Prudente, SP – Brazil
E-mail: anne_franca@hotmail.com, annekastelianne@gmail.com
Manuscript received July 26, 2016; revised manuscript July 30, 2016; accepted November 17, 2016.

DOI: 10.5935/abc.20170024

and prognostic value in these individuals. This would allow a better risk stratification, and elaboration of preventive programs and new therapeutic strategies for these patients.

In light of this, the aim of this study was to compare HRV indices and evaluate their sensitivity, specificity and predictive value in young type 1 diabetic patients and healthy volunteers. We hypothesize that changes in autonomic behavior in young subjects with type 1 DM can be identified by HRV analysis and that this is an effective diagnostic and prognostic marker in this population.

Methods

Patients

Patients with diagnosis of type 1DM were recruited from the database of community health centers and by contact with endocrinologists in Presidente Prudente, Brazil, and healthy volunteers were recruited from a public university of the same city. Sample size calculation was performed based on the RMSSD (square root of the mean of the squares of successive differences between normal RRI). Considering a magnitude of the difference of 19.85, standard deviation of 25,30,[18] and alpha and beta risk of 5% and 80% respectively, the sample size calculated was 25 individuals per group.

A total of 88 volunteers of both sexes, aged between 18 and 30 years were recruited and allocated into two groups: type 1 DM group, composed of 43 young type 1 DM patients (20 men and 23 women, mean age of 21.82 ± 5.07 years, time of diagnosis of 11.20 ± 6.01 years), and control group, composed of 45 young healthy volunteers (21 men and 24 women; mean age of 21.35 ± 2.82 years).

Inclusion criteria were age between 18 and 30 years, clinical diagnosis of type 1 DM confirmed by blood test and medical records (for type 1 DM group), and individuals with cardiorespiratory diseases, smoking habit, or alcoholics were excluded. Six volunteers with RRI time series with a sinus beat < 95%[19] were excluded.

All subjects were informed about the objectives and procedures of the study, and those who agreed to participate signed an informed consent form before being included in the study. All procedures were approved by the Ethics Committee of the School of Science and Technology of UNESP, Presidente Prudente campus (report number 417.031).

Data collection

Data were collected in a temperature (21°C-23°C) and humidity (40%-60%) controlled room, in the afternoon period from 13h and18h to minimize the influence of the circadian rhythm.[20] For individual assessments, patients were instructed to abstain from alcohol and autonomic nervous system stimulants, such as coffee, tea and cocoa in the 24 hours before the study day.

All volunteers were assessed using a protocol that included 'identification' – age, sex, time of diagnosis (for DM group) and use of drug therapy, 'physical examination', 'clinical evaluation', and 'autonomic assessment, in this order. Physical and clinical evaluation included the assessment of cardiovascular and body composition parameters, physical activity level and postprandial glycemia.

Physical and clinical assessment

Systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) were measured on the left arm, in patients in sitting position, using a stethoscope (Littman, Saint Paul, USA) and aneroid sphygmomanometer (Welch Allyn - Tycos, New York, USA), according to the VI Brazilian Guidelines for Arterial Hypertension.[21] Heart rate (HR) was determined using the Polar S810i monitor (Polar Electro, Kampele, Finland).

Anthropometric measurements and body fat percentage were determined in all volunteers. Body weight was measured using a digital scale (Welmy R/I 200, Brazil), height was determined using a stadiometer (Sanny, Brazil), and body mass index (BMI) (weight/height^2, kg/m^2) was calculated according to the Brazilian Guidelines for Obesity.[22] Waist circumference (narrowest abdominal perimeter between the lowest ribs and the iliac crest) and hip circumference (widest part of the gluteal region at the level of the great trochanters) were measured with an inelastic tape (Sanny, Brazil), and the waist/hip ratio (WHR) were calculated.[23]

Percentage of body fat was measured by bioelectrical impedance analysis (Maltron BF 906 Body Fat Analyser).[24] The level of physical activity was determined by the short version of the International Physical Activity Questionnaire (IPAQ).[25] For random glucose test, a drop of blood from a finger prick was placed on a One touch ultra test strip (Johnson & Johnson Medical, Brazil) and analyzed by its glucometer. All participants were free to eat, and fasting was not required for the tests.

Autonomic assessment

After initial instructions, a chest strap was placed on the distal third of the sternum, and the Polar S810i HR monitor was placed on the wrist (Polar Electro, Finland). This instrument has been previously validated for detecting beat-to-beat heart rate variability.[26,27] Then, the volunteers were placed on a bed in dorsal decubitus position, and instructed to breath spontaneously remain at rest, yet awake, for 30 minutes, and avoid conversation. After data collection for autonomic modulation analysis, the subjects were allowed to leave the room.

For HRV analysis, beat-to-beat heart rate was recorded during all the experiment. One thousand consecutive RRIs were selected from the highest signal stability period by digital filtering[28] (using the Polar Precision Performance SW software, version 4.01.029 with a moderate filter), complemented by manual filtering to eliminate premature, ectopic beats and artifacts. Only series with more than 95% of sinus beats were included in the study.[19] By a visual analysis, there were no artifacts or ectopic beats that could affect the HRV analysis. For analysis of HRV, time- and frequency-domain linear indices, geometric indices and nonlinear indices were used.

Time-domain analysis[7] was performed by SDNN (standard deviation of normal RR intervals), RMSSD and PNN50 (percentage of adjacent RRIs that differ by more than 50 ms). For frequency-domain analysis,[7] low-frequency (LF: 0.04 – 0.15 Hz) and high-frequency (HF: 0.15 – 0.40 Hz) spectral components (ms^2 and normalized unit) were used, as well as the LF/HF ratio). Spectral analysis was calculated using the fast Fourier Transform algorithm.

The triangular index and TINN (triangular interpolation of RR intervals) were calculated using the density histogram of normal RRIs, which displayed all possible RRIs values in the horizontal axis and their frequencies in the vertical axis. The connection of the midpoints of each column of the histogram generates a triangular figure, from which these indices were extracted. Both triangular index and TINN values express the global ANS condition.[29]

The nonlinear indices used for the analysis were the Poincaré plot and the approximate entropy (ApEn). The Poincaré plot is a time series graphic representation which plots each RRI against its previous interval.[29] The plot was analyzed by using the following indices: SD1, SD2 (long term variability of continuous RRIs) and SD1/SD2 ratio.[7] ApEn describes the RRI complexity; it measures the regularity and the logarithmic probability that the time series patterns remain similar for all comparisons. The greater the ApEn value, the higher the RR series complexity.[30]

All indices were calculated by the HRV analysis software, version 2.0[31] (Kubios, Biosignal Analysis and Medical Image Group, Department of Physics, University of Kuopio, Finland).

Data analysis

First, data normality was tested using the Shapiro-Wilk test. Between-group comparisons were performed by the independent t test (for parametric data) or the Mann-Whitney test (for nonparametric test). Data with Gaussian distribution (height, HR, WHR and body fat percentage) were expressed as mean and standard deviation, whereas data whose normality was not confirmed (age, weight, BMI, SAP, DAP, glycemia and weekly physical activity) were presented as median and interquartile range. Between-group comparisons of HRV data were performed by covariance analysis, adjusted by confounding factors (BMI and random glucose levels).

The HRV cutoff points were defined by the Receiver Operating Characteristic (ROC) curve. The sensitivity, specificity, positive predictive value and negative predictive value for the occurrence of events were also determined. An area under the curve ≥ 0.650 was considered significant.[17]

The level of significance was set at 5%. Statistical analysis was performed using the Statistical Package for the Social Sciences, version 15.0 (SPSS Inc., Chicago, IL, EUA) and MedCalc Software bvba, version 14.10.2 (Ostend, Belgium).

Results

Data of 88 volunteers (43 type 1 DM patients and 45 healthy controls) were assessed. Six volunteers were excluded because of errors in RRIs greater than 5%, and the final sample was composed of 39 young subjects with type 1 DM (19 men and 20 women) and 43 young healthy controls (21men and 22 women).

Table 1 describes general characteristics of both groups. Subjects with type 1 DM had higher body mass, BMI, HR, random glucose levels and % body fat than controls ($p < 0.05$). All diabetic individuals were insulin-dependent, and 15 (38.46%) used additional medications, other than insulin – five (12.82%) used antihypertensive agents, eight (20.51%) for thyroid diseases, three (7.69%) for cholesterol control, five (12.82%) used contraceptive agents, and eight for other conditions, including rhinitis, diabetic polyneuropathy, peripheral neuropathy and epilepsy.

Table 2 shows linear and nonlinear values of HRV of both groups. Diabetic subjects had significant lower values of DNN, RMSSD, PNN50, RRTri, LF ms^2, HF ms^2, SD1 and SD2.

Table 3 shows sensitivity, specificity, ROC curve, positive predictive value and negative predictive value of HRV. The RMSSD, SDNN, LF ms^2, HF ms^2, RRTri, SD1 and SD2 indices showed the best diagnostic accuracy (ROC curve > 0.65).

Table 4 shows sensitivity, specificity, ROC curve and cutoff point for HRV indices that had a ROC curve > 0.65. Among these indices, the SDNN and SD2 showed the best accuracy.

Discussion

Our findings indicate that individuals with type 1 DM have altered HRV, characterized by a reduction in sympathetic and parasympathetic activities, and in overall variability as compared with healthy controls. In addition, the RMSSD, SDNN, PNN50, LF ms^2, HF ms^2, RRTri, SD1 and SD2 indices had the best diagnostic accuracy in discriminating type 1 diabetic patients from healthy individuals.

Also, type 1 DM subjects had higher body mass, BMI, random glucose levels, and percentage of body fat compared with healthy volunteers, whereas the variables age, height, WHR, SAP, DAP and physical activity were not different between the groups. Similar results were reported by Javorka et al.[32] for age, BMI, SAP and DAP, and by Jaiswal et al.[12] for HR and physical activity.

The HRV results indicated a decrease in sympathetic (LF ms^2) and parasympathetic activities (RMSSD, PNN50, HF ms^2), and in overall variability (SDNN, RRtri and SD2) in type 1 DM subjects as compared with healthy controls. These findings are corroborated by Javorka et al.[9], who reported a decrease in SDNN, RMSSD, PNN50, LF ms^2 and HF ms^2 in 17 type 1 DM subjects (22.4 ± 1.0 years). Jaiswal et al.,[12] in a study on more than 350 young individuals (18.8 ± 3.3 years with type 1 DM), observed significantly lower SDNN, RMSSD, HF nu, LF nu and LF/HF ratio in this population than in healthy controls. However, in our study, no differences in SD1/SD2, LF/HF and LF and HF in normalized units were observed between the groups.

Changes in HRV are indicative of abnormal, insufficient adaptation of ANS,[7] which increases the risk of sudden death for heart arrhythmias, and is associated with increased mortality rate for other causes.[33] This indicates that the cardiovascular autonomic dysfunction may be a complicating factor in patients already at risk, as in DM patients.[34]

Other studies demonstrated that some HRV indices have good diagnostic accuracy in some populations.[15-17] In our study, the RMSSD, SDNN, PNN50, LF ms^2, HF ms^2, RRtri, SD1 and

Table 1 – Characteristics of diabetes mellitus and control groups

Variables	Control (43)	Type 1 DM (39)	p value
Age[b] (years)	21.00 (5.00)	21.00 (7.00)	0.534
Body mass[b] (kg)	60.30 (22.80)	68.15 (22.90)	0.013
Height[a] (m)	1.69 (0.09)	1.73 (0.17)	0.461
BMI[b] (Kg/m^2)	22.19 (4.67)	24.19 (5.84)	0.011
WHR[a] (cm)	0.77 (0.06)	0.80 (0.10)	0.102
SAP[b] (mmHg)	110.00 (20.00)	110.00 (10.00)	0.757
DAP[b] (mmHg)	70.00 (10.00)	60.00 (10.00)	0.620
HR[a] (bpm)	70.76 (10.04)	80.00 (16.00)	0.000
Random glycemia[b] (mg/dl)	93.00 (20.00)	162.00 (168.00)	0.000
Body mass[a] (%)	21.86 (7.58)	26.00 (9.60)	0.044
Weekly physical activity[b] (minutes)	320.00 (440.00)	280.00 (510.00)	
Time of diagnosis[a]	---	11.71 (5.99)	---

[a]mean (standard deviation); [b]median (interquartile range). Type 1 DM: type 1 diabetes mellitus; BMI: body mass index; WHR: waist-hip ratio; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; HR: heart rate.

Table 2 – Indices of heart rate variability in diabetes mellitus and control groups adjusted by body mass index and random glucose levels

Index	Controls (n = 43)	Type 1 DM (n = 39)	p value
SDNN	66.97 (22.17)	41.99 (19.65)	0.000
RMSSD	55.59 (21.60)	32.73 (17.43)	0.000
PNN50	33.64 (19.97)	14.79 (15.68)	0.000
TINN	220.81 (85.36)	191.25 (76.14)	0.439
RRTri	16.31 (4.95)	12.62 (9.76)	0.019
LF ms^2	1187.97 (743.46)	556.25 (542.06)	0.001
HF ms^2	1141.65 (899.22)	572.87 (517.38)	0.006
LF un	49.76 (16.72)	54.54 (14.83)	0.452
HF un	50.23 (16.72)	45.45 (14.84)	0.452
LF/HF	1.24 (0.84)	1.65 (1.71)	0.562
SD1	39.01 (15.43)	23.16 (12.33)	0.000
SD2	85.64 (29.36)	54.41 (25.54)	0.000
SD1/SD2	0.46 (0.15)	0.41 (0.12)	0.469
ApEn	1.46 (0.10)	1.44 (0.11)	0.677

Type 1 DMT1: type 1 diabetes mellitus; SDNN: standard deviation of normal RR intervals in a time interval (ms) RMSSD: square root of the mean of the squares of successive differences between normal RR intervals in a time interval (ms); PNN50: percentage of adjacent RRIs that differ by more than 50ms; TINN: triangular interpolation of RR intervals; RRTri: triangular index; LF: low-frequency component; HF: high-frequency component; SD1: standard deviation of the instantaneous RR intervals; SD2: long-term variability of continuous RR intervals; ApEn: approximate entropy.

SD2 indices showed greater sensitivity and specificity to detect autonomic dysfunction in type 1 DM patients and in healthy individuals. Indices with higher discriminatory power were those with significantly lower values in the type 1 DM group than in the control group.

These indices are associated with the analysis of parasympathetic activity (RMSSD, PNN50, HF ms^2 and SD1), sympathetic activity (LF ms^2) and overall ANS behavior (SDNN, RRtri and SD2),[7] suggesting that discrimination of patients with type 1 DM may be related to the reduction in autonomic, global and sympathetic modulation of the heart.

Few studies have evaluated the diagnostic power of HRV in type 1 DM. Ziegler et al.[35] have shown that the HF index showed greater sensitivity to detect early autonomic dysfunction in type 1 and type 2 DM patients classified in the three stages of cardiac autonomic neuropathy. Khandoker et al.[15] found that the SampEn and the SD1/SD2 ratio, obtained from the

Table 3 – Sensitivity, specificity, ROC curve, positive predictive value and negative predictive value for heart rate variability indices

Indices	SEN	SPE	ROC	PPV	NPV
RMSSD	0.66 [0.49 – 0.80]	0.81 [0.66 – 091]	0.79 [0.69 – 0.87]	0.76 [0.58 – 0.89]	0.72 [0.58 – 0.84]
SDNN	0.57 [0.40 – 0.73]	0.88 [0.74 – 0.96]	0.80 [0.70 – 0.88]	0.81 [0.61 – 0.93]	0.70 [0.56 – 0.82]
PNN50	0.71 [0.55-0.85]	0.72 [0.56-0.84]	0.77 [0.66-0.85]	0.70 [0.53-0.83]	0.73 [0.58-0.83]
LF (ms^2)	0.79 [0.63 – 0.90]	0.69 [0.53 – 0.82]	0.75 [0.64 – 0.84]	0.70 [0.54 – 0.83]	0.75 [0.59 – 0.87]
HF (ms^2)	0.82 [0.66 – 0.92]	0.55 [0.39 – 0.70]	0.74 [0.63 – 0.83]	0.62 [0.47 – 0.76]	0.77 [0.58 – 0.90]
LF/HF (ms)	0.84 [0.69 – 0.94]	0.32 [0.19 – 0.48]	0.56 [0.45 – 0.67]	0.53 [0.40 – 0.66]	0.70 [0.45 – 0.88]
LF nu	0.84 [0.69 – 0.94]	0.32 [0.19 – 0.48]	0.56 [0.45 – 0.67]	0.53 [0.40 – 0.66]	0.70 [0.45 – 0.88]
HF nu	0.84 [0.69 – 0.94]	0.32 [0.19 – 0.48]	0.56 [0.45 – 0.67]	0.53 [0.40 – 0.66]	0.70 [0.45 – 0.88]
TINN	0.53 [0.37 – 0.69]	0.79 [0.64 – 0.90]	0.63 [0.52 – 0.74]	0.70 [0.50 – 0.85]	0.65 [0.50 – 0.78]
RRTri	0.69 [0.52 – 0.83]	0.76 [0.61 – 0.88]	0.76 [0.65 – 0.85]	0.73 [0.55 – 0.86]	0.73 [0.58 – 0.85]
SD1	0.66 [0.49 – 0.80]	0.79 [0.64 – 0.90]	0.78 [0.68 – 0.87]	0.74 [0.56 – 0.87]	0.72 [0.57 – 0.84]
SD2	0.61 [0.44 – 0.76]	0.88 [0.74 – 0.96]	0.80 [0.70 – 0.88]	0.82 [0.64 – 0.94]	0.71 [0.57 – 0.83]
SD1/SD2	0.46 [0.30 – 0.62]	0.76 [0.61 – 0.88]	0.58 [0.46 – 0.68]	0.64 [0.44 – 0.81]	0.61 [0.46 – 0.74]
ApEn	0.35 [0.21 – 0.52]	0.86 [0.72 – 0.94]	0.56 [0.44 – 0.67]	0.70 [0.45 – 0.88]	0.59 [0.46 – 0.71]

SEN: sensitivity; SPE: specificity; PPV: positive predictive value; NPP: negative predictive value; SDNN: standard deviation of normal RR intervals in a time interval (ms); RMSSD: square root of the mean of the squares of successive differences between normal RR intervals in a time interval (ms); PNN50: percentage of adjacent RRIs that differ by more than 50 ms; TINN: triangular interpolation of RR intervals; RRTri: triangular index; LF: low-frequency component; HF: high-frequency component; nu: normalized unit; SD1: standard deviation of the instantaneous RR intervals; SD2: long-term variability of continuous RR intervals; ApEn: approximate entropy.

Table 4 – Sensitivity, specificity, ROC curve and cutoff points of heart rate variability indices with ROC curve > 0.65

Indices	SEN	SPE	ROC	Cutoff point
RMSSD	0.66 [0.49 – 0.80]	0.81 [0.66 – 091]	0.79 [0.69 – 0.87]	37.00
SDNN	0.57 [0.40 – 0.73]	0.88 [0.74 – 0.96]	0.80 [0.70 – 0.88]	41.90
PNN50	0.71 [0.55-0.85]	0.72 [0.56-0.84]	0.77 [0.66-0.85]	18.50
LF (ms^2)	0.79 [0.63 – 0.90]	0.69 [0.53 – 0.82]	0.75 [0.64 – 0.84]	711.00
HF (ms^2)	0.82 [0.66 – 0.92]	0.55 [0.39 – 0.70]	0.74 [0.63 – 0.83]	826.00
RRTri	0.69 [0.52 – 0.83]	0.76 [0.61 – 0.88]	0.76 [0.65 – 0.85]	12.66
SD1	0.66 [0.49 – 0.80]	0.79 [0.64 – 0.90]	0.78 [0.68 – 0.87]	26.20
SD2	0.61 [0.44 – 0.76]	0.88 [0.74 – 0.96]	0.80 [0.70 – 0.88]	55.60

SEN: sensitivity; SPE: specificity; RMSSD: square root of the mean of the squares of successive differences between normal RR intervals in a time interval (ms); SDNN: standard deviation of normal RR intervals in a time interval (ms); LF: low-frequency component; HF: high-frequency component; RRTri: triangular index; SD1: standard deviation of the instantaneous RR intervals; SD2: long-term variability of continuous RR intervals.

Poincaré plot, were better discriminators of type 1 or type 2 DM patients with cardiac autonomic neuropathy, with a 100% sensitivity and 75% specificity.

Takase et al.[36] demonstrated that SDANN lower than 30ms had greater sensitivity (72%) and specificity (92%) than SDANN higher than 20 ms (31% sensitivity and 100% specificity) to detect autonomic dysfunction and cardiac events in type 2 DM patients with cardiac autonomic neuropathy.

Nonetheless, in these studies,[35,36] only patients with established cardiac autonomic neuropathy were included, except for the study by Khandoker et al.,[15] that evaluated diabetic individuals, regardless of the diagnosis of neuropathy. In our study, diagnostic accuracy of HRV was analyzed in both groups (DM and control) at the same time, aiming to evaluate the power to discriminate type 1 DM subjects from controls by the presence of changes in cardiac autonomic modulation, providing results that are closer to the clinical practice.

Therefore, a strength of the study is that the capacity of HRV to diagnose possible autonomic changes were assessed in type 1 DM individuals, resulting in a cutoff value that provides evidence to healthcare professionals for changes that may be associated with early cardiac autonomic neuropathy. It is worth mentioning that none of the volunteers had cardiac autonomic neuropathy as a complication of type 1 DM. For this reason, different from previous studies,[15,35,36] we cannot affirm that the cutoff values identified in this study are associated with this

condition, but rather with an ANS depression possibly related to the type 1 DM,[8-14] that should be investigated and treated, to prevent the progression to cardiac autonomic neuropathy. Also, it is worth mentioning that a decrease in HRV is the first sign of autonomic neuropathy and suggested as one of the diagnostic tests in a statement by the American Diabetes Association's position statement.[6]

The validity of a test refers to its capacity in diagnosing or predicting an event, and the values of sensitivity and specificity give the probability of a test to correctly discriminate an individual with a disease from a healthy individual,[37] hence reducing the risk of an erroneous diagnosis. In our study, 8 of the 14 indices tested showed greater sensitivity and specificity in discriminating type 1 DM individuals from those without the disease, and their use as diagnostic tools may be encouraged.

HRV analysis is a fast, safe, non-invasive and financially accessible method, which enables the clinical follow-up of ANS condition. This is essential to reduce and intervene in case of complications to reduce cardiovascular events,[33] sudden death,[38] and loss of quality of life[39] in this population.

One limitation of this study is its cross-sectional design that prevented us to evaluate the autonomic behavior for a longer period and conclude whether the changes in ANS condition, detected in the study, were in their initial stage or not. In addition, the fact the group of diabetic patients had different time of diagnosis, and that this group had greater mean BMI and body fat percentage than the control group may have influenced the results. Longitudinal studies are needed to confirm whether these indices with the best discriminatory power maintain their prognostic capacity in long term.

Conclusion

type 1 DM patients have autonomic changes characterized by reduction in sympathetic and parasympathetic activities and overall variability. The SDNN, RMSSD, PNN50, RRtri, LF ms^2, HF ms^2, SD1 and SD2 indices had the best diagnostic accuracy in discriminating individuals with type 1 DM.

Author contributions

Conception and design of the research, Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Silva AKF, Christofaro DGD, Bernardo AFB, Vanderlei FM, Vanderlei LCM; Acquisition of data: Silva AKF, Bernardo AFB; Statistical analysis: Silva AKF, Christofaro DGD, Vanderlei LCM; Obtaining funding: Silva AKF, Vanderlei LCM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by FAPESP (Case number: 2013 / 19055-00).

Study Association

This article is part of the thesis of master submitted by Anne Kastelianne França da Silva, from Universidade Estadual Paulista – UNESP.

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Genetic Evaluation, Familial Screening and Exercise

Ricardo Stein,[1,2,3,4] Juan Pablo Trujillo,[5,6] Anderson Donelli da Silveira,[1,4] Arsonval Lamounier Júnior,[5,6] Lorenzo Monserrat Iglesias[5,6]

Programa de Pós-Graduação em Cardiologia e Ciências Cardiovasculares da Faculdade de Medicina da Universidade Federal do Rio Grande do Sul[1]; Grupo de Pesquisa em Cardiologia do Exercício do Hospital de Clínicas de Porto Alegre (CardioEx);[2] Serviço de Fisiatria e Reabilitação do Hospital de Clínicas de Porto Alegre - Universidade Federal do Rio Grande do Sul;[3] Vitta Centro de Bem Estar Físico,[4] Porto Alegre, RS - Brasil; Health in Code,[5] La Coruña - Spain; Grupo de Investigación Cardiovascular de La Universidad de La Coruña,[6] La Coruña - Spain

**Ricardo Stein and Juan Pablo Trujillo contributed equally and are the first authors*

Introduction

Regular physical activity practice benefits individuals of all ages, sexes and ethnicities.[1] If on one hand the practice of moderate exercise is considered a healthy activity that favors the cardiovascular system, on the other, high-intensity exercise for a long period can increase the risk for sudden death (SD).[2] Even considering the huge number of individuals exercising daily, SD in that context is rare. However, the prevention of SD can be difficult and it has significant repercussion, mainly among young practitioners of leisure exercise or athletes. From the epidemiological viewpoint, cardiac SD affects 200,000 to 400,000 individuals in the United States of America (USA) annually.[3] In the sports scenario, around 200 athletes per year are estimated to have a fatal event.[4] In Spain, the national registry of SD in athletes reported 180 cases from 1995 to 2007, suggesting an incidence of 15 to 20 cases per year.[5]

For athletes, preparticipation evaluation (PPE) is indicated, and can be effective in preventing cardiac SD in that context.[6] However, that type of screening has great variability between different countries and entities that perform it. In the sports context, genetic evaluation is performed only in specific cases.

This review describes basic aspects of genetic evaluation, as well as the indications for molecular analysis and their correct clinical interpretation, for practitioners of recreational exercise, amateur sportsmen and high-performance athletes.

Sudden death of athletes: What diseases can be involved?

One of the major preoccupations in different sports modalities is to establish the risk of SD for each individual and if that modality can increase that risk. There might be a relationship between the sport modality and the cause of SD, which should be taken into consideration on the occasion of screening and prevention. Recent data estimate that among young North-American athletes (<35 years), the incidence of SD would be between 1 and 3 per 100,000 athletes.[7] However, among athletes older than 35 years, that incidence can be greater, because the risk of SD due to ischemic heart disease increases progressively with age.

Few observational studies are available, most conducted in the USA, Italy, Spain and Denmark.[5,8,9] Such studies agree on the identification of the different causes of SD among athletes aged less than or over 35 years. In the younger age groups, the most frequent causes are cardiomyopathies, channelopathies and coronary artery anomalies. However, in older age groups, the major cause is coronary artery disease (CAD), accounting for more than half of the cases of SD in that scenario.[10] In Spain, according to the *Registro Nacional de Morte Súbita em Deportistas,*[5] the major causes are: unidentified (27%), arrhythmogenic right ventricular cardiomyopathy (ARVC, 14%), hypertrophic cardiomyopathy (HCM, 12%), idiopathic left ventricular hypertrophy (8%), coronary artery anomalies (10%), aortic stenosis (6%) and myocarditis (4%). In Brazil, no epidemiological data on SD of athletes are available.

Thus, for young athletes, screening should focus on identifying inherited heart diseases, such as channelopathies and cardiomyopathies. For older individuals and the general population, however, that assessment should focus on diagnosing CAD.[11]

Clinical Genetic Evaluation of the Sportsman

According to different expert opinions and consensus, genetic evaluation should not be routinely indicated for athletes. Considering that whether electrocardiography should be routinely indicated in PPE is still a matter of discussion, the performance of genetic evaluation should always be very well substantiated for the athlete.

Genetic evaluation is especially indicated on the following two occasions:

a) positive family history of inherited heart disease (cardiomyopathies, channelopathies, aortopathies) or suspicion of that type of disease (syncope episodes, arrhythmias, cardiac arrest/SD). In such cases, the genetic evaluation should be first performed in the individual or in one of the affected relatives. Once detected the mutation causing the disease, the other family members should be assessed;

b) when the athlete's phenotype strongly indicates the presence of an inherited disease (signs, symptoms and/or tests suggesting specific disease or compatibility with a disease).[12]

Conducting a clinical genetic evaluation should always be the first step before performing a genetic test. That investigation

Keywords

Genetics; Genotype; Exercise; Heredity; Sports Medicine; Death, Sudden, Cardiac.

Mailing Address: Ricardo Stein •
HCPA - UFRGS. João Caetano, 20/402, Petrópolis. Postal Code: 90470-260, Porto Alegre, RS - Brazil.
E-mail: rstein@cardiol.br
Manuscript received November 06, 2016; revised manuscript November 17, 2016; accepted November 17, 2016.

DOI: 10.5935/abc.20170015

should include the detailed assessment of family antecedents, as well as a complete physical examination. The family history should include the following: age at symptom onset; triggering activities; diagnosed disease; degree of kinship; and number of affected relatives. Building genealogical trees and family pedigree charts (Figure 1), representing family relationships, allows details on the ancestors; in addition, it is worth noting that the affected family side should always be the one investigated. If inherited heart disease is strongly suspected, but there is no suspected first-degree relative, the study should be extended to one more generation.

Thus, the clinician/cardiologist conducting the evaluations should be aware of the signs and symptoms of that group of diseases, and could even refer suspected cases to experts on family heart diseases and/or cardiovascular genetics. Delaying the diagnosis is not wanted, and physical exercise should be avoided in the period. It is worth emphasizing that the evaluation should not be restricted to the one individual diagnosed, but extended to his entire family.

Genetic Cardiovascular Diseases

Inherited cardiovascular diseases, such as cardiomyopathies (hypertrophic, dilated, arrhythmogenic, restrictive and non-compacted), channelopathies [long QT syndrome (LQTS), Brugada syndrome (BrS) and catecholaminergic polymorphic ventricular tachycardia (CPVT)] and aortopathies (Marfan and Loeys-Dietz syndromes), are a group of entities of high clinical and genetic heterogeneity. Molecular studies performed in different populations have shown that each of those conditions associate with hundreds of different pathogenic mutations.[12] There are mutations in different genes associated with the same phenotype. In some cases, the genes behave similarly or transcribe proteins that are part of the same structure or functional path (sarcomeric proteins, desmosomal junctions, ionic channels). In other cases, the presence of one single mutation can be enough for the disease development. It is worth noting that the clinical variability of the diseases can be explained by epigenetic factors and/or environment interaction. Finally, we emphasize that, the development of next generation sequencing (NGS), providing complete and parallel analysis of different genes, enables the identification of the causal genetic variant or variants of a disease in a faster and less expensive manner.[13]

Cardiomyopathies, Genetics and Sports

The European Society of Cardiology (ESC) defines cardiomyopathy as a myocardial disorder with structural and functional abnormalities, in the absence of CAD, hypertension, valvular disease and congenital heart disease sufficient to cause the observed myocardial abnormality. Some examples of cardiomyopathies are as follows: HCM; dilated cardiomyopathy (DCM); ARVC; restrictive cardiomyopathy; and non-compacted cardiomyopathy (NCCM). Those cardiomyopathies, except for ARVC, share sarcomeric gene mutations. Different pathogenic mutations in sarcomeric genes, such as MYH7 or MYBPC3, can be associated with several cardiomyopathies (Figure 2). In addition, one same mutation can be expressed with a different phenotype in different patients (even in the same family).

Non-sarcomeric genes can produce phenocopies. This is the case of the GLA gene, whose mutation causes Fabry's disease. That gene can be associated with HCM development (around 0.5-1% of the cases of HCM are explained by mutations in GLA gene).

The HCM is an autosomal dominant genetic disease. It is relatively common, with prevalence of 1:500 individuals in the general population. According to North-American data, that disease is the most frequent cause of SD in apparently healthy young individuals, especially athletes.[4] In many cases, SD can occur during or right after exercise (approximately 40% of cases).

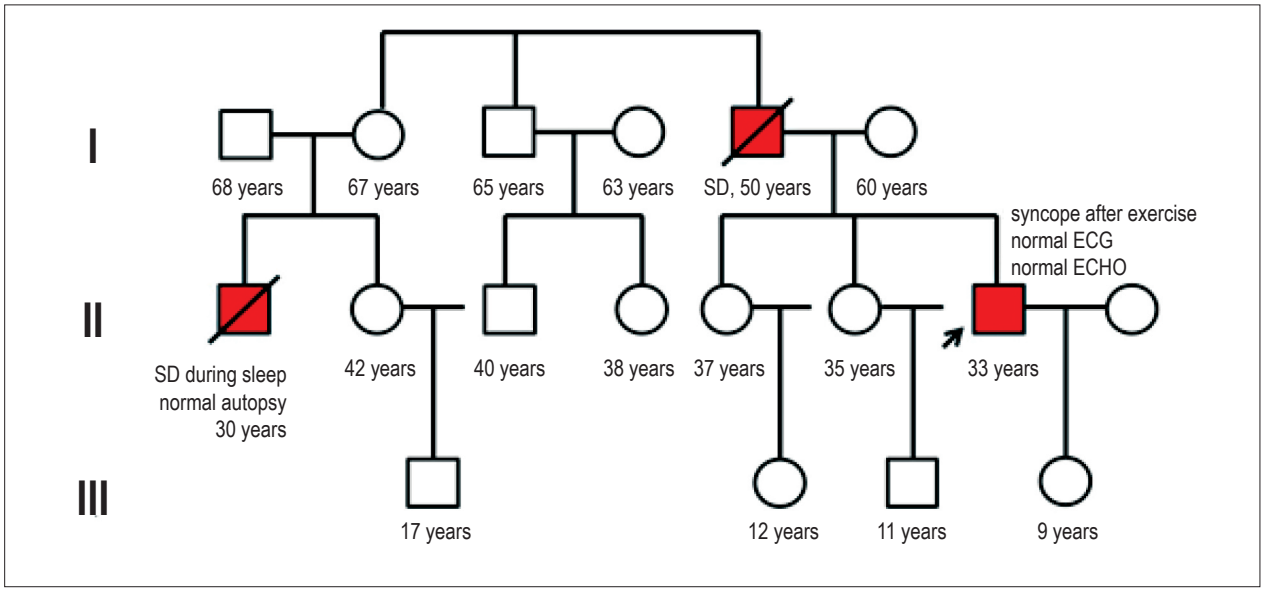

Figure 1 – *Pedigree chart of a family with clinical suspicion of channelopathy. Square: man; circle: woman; oblique bar: deceased; arrow: index-case; red circle or square: affected individual; SD: sudden death; ECHO: echocardiography.*

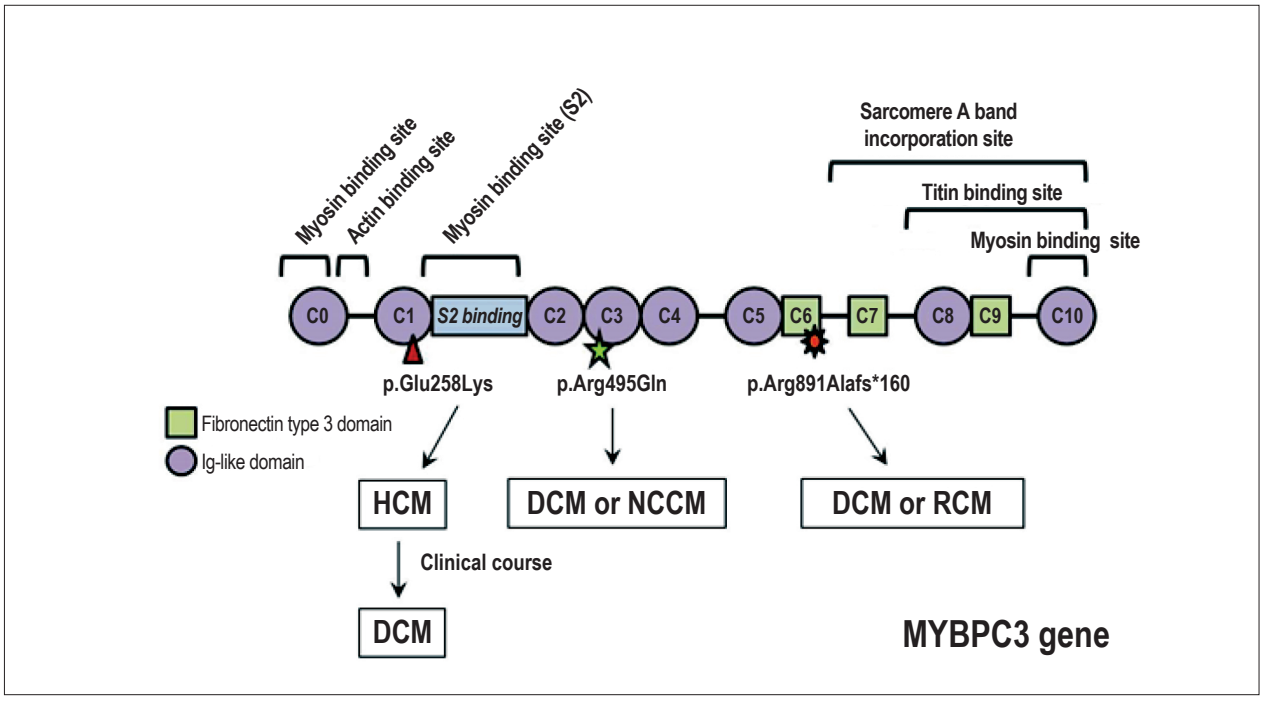

Figure 2 – *Clinical-molecular correlation of three pathogenic mutations in the MYBPC3 gene. One mutation can be associated with different cardiomyopathies. HCM: hypertrophic cardiomyopathy; DCM: dilated cardiomyopathy; NCCM: non-compacted cardiomyopathy; RCM: restrictive cardiomyopathy.*

Despite its catastrophic potential, however, the annual mortality rate in all patients with HCM is lower than 1%.[14] A large number of mutations in different genes associated with HCM has been described. So far, hundreds of mutations in around 20 sarcomeric genes related to HCM have been identified (MYBPC3, MYH7, TNNC1, TNNT2 and TNNI3 are the most frequent genes). In addition, phenocopies of HCM derived from pathologies caused by mutations associated with the glycogen metabolism (PRKAG2, LAMP2) in storage diseases (GAA, GLA) and in mitochondrial genes have been reported.

The ARVC is characterized by ventricular myocardial tissue replacement with fibrous and adipose tissue, which has been associated with ventricular arrhythmias. The clinical diagnosis may be complicated, requiring extensive research for confirmation. From the epidemiological viewpoint, its prevalence ranges from 1:5,000 to 1:2,000 individuals.[15] Most cases have an autosomal dominant pattern of inheritance. In some cases, however, as in Naxos disease, caused by pathogenic mutations in the plakoglobin (JUP) gene, that inheritance is autosomal recessive. ARVC has been associated with mutations in desmosomal (DSC2, DSG2, DSP, JUP, PKP2) and non-desmosomal (LMNA, CNLF, TMEM43 and PLN) genes. In that disease, regular and high-intensity exercise (competitive sports) has been associated with accelerated progression and worsening in animal and human models.[16,17] Competitive sports increases by five times the risk for SD in adolescents and young adults with that disorder.[10]

Channelopathies, Genetics and Sports

Channelopathies are a group of diseases sharing some characteristics, such as genetic and clinical heterogeneity. Most of them are explained by changes in the genes that encode myocardial ionic channels. Some channelopathies are as follows: short-QT syndrome, LQTS, BrS and CPVT.

In LQTS, three most frequent subtypes were identified.[18] In type 1 LQTS, patients can have cardiac events in adrenergic situations (in the sports context, swimming is a classic example). That is why the European and North-American guidelines recommend those individuals refrain from practicing water competitive sports. Mutations in the KCNQ1 gene (slow component of the delayed rectifier potassium channel, Kv7.1) has been associated with the development of that syndrome. In type 2 LQTS, patients can have cardiac events due to auditory stimuli (radio or telephone ring), and puerperal women can be more susceptible to auditory stimuli (newborn crying). Mutations in the KCNH2 gene (rapid component of the delayed rectifier potassium channel, Kv11.1) have been associated with the development of that syndrome. Less prevalent than the other two, type 3 LQTS has a parasympathetic substrate. In that subtype, patients can have cardiac events during resting periods or sleep. Mutations in the SCN5A gene (sodium channel gene, Nav1.5) have been associated with the development of that syndrome and with the BrS.

The arrhythmic events associated with the BrS usually occur during fever episodes, use of some medications, sleep or after exercise.[19] Physical activity might have a pro-arrhythmic effect, associated with either hyperthermia or sympathetic withdrawal and/or increased vagal tonus in athletes after exertion. However, that association is still uncertain and the North-American guidelines do not recommend sports restrictions for those patients.

Finally, CPVT is associated with changes in intracellular calcium release from the sarcoplasmic reticulum. It is often expressed during the first decades of life, manifesting as syncope or SD associated with exercise and/or stressful situations. Therefore, international guidelines recommend strict sports restrictions in such cases.[20] Patients with CPVT can have mutations in the RYR2 (major), CASQ2 and KCNJ2 genes.

Inherited Aorta Diseases, Genetics and Sports

This group of diseases includes a set of inherited connective tissue disorders that predispose to aortic dilations and aneurysms (AA) and/or dissection (AD), with an increased risk for SD during physical activity. Some of those diseases are rare genetic syndromes (Marfan, Loeys-Dietz and vascular type Ehlers-Danlos) and no-syndromic presentations, such as familial thoracic aortic aneurysm disease.

When performing PPE in patients with aortopathies classified as syndromic, the variability or phenotypical overlapping of those diseases should be considered. Identification of some of the following clinical signs in an individual who would apparently have a normal phenotype can contribute to the differential diagnosis:[21] Marfanoid *habitus*; kyphosis/scoliosis; changes in skin elasticity and/or joints; *ectopia lentis*; and craniofacial dysmorphism. In a case report, abdominal AA was detected in an elite athlete of North-American basketball after the late diagnosis of Marfan.[22] In another case, a young weightlifter died suddenly from an AD, and upon autopsy was diagnosed as having "non-Marfan's fibrillinopathy". His echocardiogram was normal, but his mother had died at a young age, also from AD.[23] Other authors[24] have reported a family with three generations affected (Marfan). The diagnosis was established only after AD findings in a 30-year-old weightlifter. Both his father and brother had died on separate occasions following loss of consciousness after weight lifting.

Phenotypic overlapping can occur in those diseases, as seen with the Loeys-Dietz and Marfan's syndromes. Differentiating between them is important to establish the prognosis and regularity of the clinical-cardiological follow-up. In patients with Marfan's syndrome, it is essential to keep watch on the aortic diameter in relation to the body surface,[25] arterial stiffness, and ventricular function.[26] In patients with Loeys-Dietz syndrome, a systematic assessment of aneurysms in multiple arteries is necessary. Referring to an expert in medical genetics or to a specialized center helps in the management of those patients.[25] The genetic exam helps to define borderline or dubious cases (which do not meet the criteria for the clinical diagnosis of that syndrome), in addition to aiding in the differential diagnosis of aortopathies.[27]

In athletes with Marfan's syndrome or other aortopathies, AD or aortic rupture can cause SD. The increase in aortic blood pressure and stress during exercise, in the presence of genetic predisposition, can accelerate aneurysm formation, serving as a trigger for AD/rupture of the aorta or of other arteries. A cohort of individuals admitted with AD to the emergency unit has evidenced that syndromic individuals are at higher risk for recurrence and death as compared to non-syndromic ones.[28] Guidelines recommended that, in general, athletes with increased aortic diameter (> 40 mm in adults) only participate in low-intensity dynamic and static sports (class IA sports).[29] A study has followed up a cohort of 732 individuals with Marfan's syndrome, all of them on pharmacological treatment, for 6 years. The risk for aortic events and SD remained low in those with aortic diameter between 35 and 49 mm. However, an aortic diameter of 50 mm has been described as the cut-off point for indicating prophylactic surgery.[30] Finally, management based on the aortic diameter has been proposed, not only for Marfan's syndrome, but also for other aortopathies, such as familial thoracic aortic aneurysm disease and Ehlers-Danlos syndrome.[31]

Usefulness and Limitations of Genetic Testing in Inherited Heart Diseases

Currently, with the emergence of NGS, diseases with high clinical and genetic heterogeneity can be studied faster and more accurately. This sequencing technology allows the construction of panels that capture the genes involved in each group of diseases (specific genetic panels for cardiomyopathies, channelopathies or aortopathies). In addition, enlarged panels directed to the study of SD with either structural or non-structural heart disease are useful in this context.[13]

In the presence of evident clinical findings that raise the suspicion of a particular disease, diagnostic genetic testing is usually more likely to confirm it (high pretest likelihood). Regarding the performance of genetic tests in primary cardiomyopathies, using a well-designed panel, mutations can be identified in up to 70% of the cases of HCM, for example. Other genes associated and the pretest likelihood of their identification in cardiomyopathies, channelopathies and aortopathies are shown in Tables 1, 2 and 3.

Table 1 – Genes frequently associated with the development of different cardiomyopathies

Cardiomyopathy	Gene (symbol)	Pretest likelihood
HCM	MYBPC3, MYH7, TNNC1, TNNT2, TNNI3, TPM1, ACTC1, MYL2, MYL3, PRKAG2, LAMP2, GLA, GAA, TTR, PTPN11.	70%
DCM	TTN, ACTC1, BAG3, DES, DMD, DSP, FLNC, LMNA, MYBPC3, MYH7, PKP2, PLN, RBM20, TAZ, TNNC1, TNNT2, TNNI3, TPM1.	40 - 50%
ARVC	DSC2, DSG2, DSP, JUP, PKP2, LMNA, FLNC, TMEM43, PLN.	50 - 65%
NCCM	MYBPC3, MYH7, ACTC1, TAZ, LDB3.	40 - 50%

HCM: Hypertrophic cardiomyopathy; DCM: Dilated cardiomyopathy; ARVC: Arrhythmogenic right ventricular cardiomyopathy; NCCM: Non-compacted cardiomyopathy.

Table 2 – Genes frequently associated with the development of different channelopathies

Channelopathy	Gene (symbol)	Pretest likelihood
LQTS	KCNQ1, KCNH2, SCN5A, KCNJ2, KCNE1, KCNE2, CACNA1C.	70%
SQTS	KCNH2, KCNQ1, KCNJ2	Unknown
BrS	SCN5A, SCN10A	30%
TVPC	RYR2, CASQ2, KCNJ2	50 - 60%

LQTS: Long QT syndrome; SQTS: Short QT syndrome; BrS: Brugada syndrome; CPVT: Catecholaminergic polymorphic ventricular tachycardia.

Table 3 – Genes frequently associated with the development of different genetic aortopathies

Genetic aortopathies	Gene (symbol)	Pretest likelihood
Marfan's syndrome	FBN1	~70 - 93%
Loeys-Dietz syndrome	TGFBR2, TGFBR1, SMAD3, TGFB2, TGFB.	~70 - 95%
Vascular-type Ehlers-Danlos syndrome	COL3A1	>95%
Familial thoracic aortic disease	ACTA2, TGFBR2, TGFBR1, MYH11, SMAD3, MYLK, FBN1	~17 - 20%

Clinical Interpretation of the Results of Genetic Studies

Proper interpretation of the results of a genetic test is essential not only to establish the correct diagnosis, but also to properly guide athletes and their families. Therefore, careful assessment of the pathogenesis of a variant (Table 4)[32] is a key aspect. All information available at major databases and publications should be taken into consideration, and that information should be analyzed by a skilled team, ensuring a reliable result.

It is consensus that, in case of uncertainty about the pathogenesis of the mutation (uncertain clinical significance), it should be used for neither disease diagnosis nor familial screening (no clinical predictive value). In addition, some cases are of difficult solution, even after proper identification of the pathogenic variants. International guidelines disagree about athletes who are clinically healthy or not affected (negative phenotype), but carry the pathogenic variant (positive genotype). Considering the early diagnosis in athletes with positive genotype and negative phenotype, the North-American guidelines are much more liberal, and often do not disqualify those athletes for competitive sports. The ESC guideline, however, is much more restrictive.[33]

What to do if an athlete has a positive genetic test?

The presence of a genetic mutation in an athlete does not mean the athlete will develop the disease, but increases his susceptibility to develop it. Sometimes, not all carriers of a mutation develop the disease (incomplete penetrance). Some mutations require additional environmental (sports, hypertension) or genetic factors (presence of other mutations in the same or other genes). In the following situations an athlete can test positive for a genetic study:

- The athlete clearly has a familial heart disease (cardiomyopathy, channelopathy or aortopathy). The presence of a positive genetic test will confirm the diagnosis and help in screening the athlete's family.

- There is a previous diagnostic suspicion that the athlete has a family heart disease. A positive genetic test might help in establishing the definitive diagnosis and identifying whether the mutation is pathogenic, very likely pathogenic, or likely pathogenic.

- The athlete has no clinical manifestation of the disease, but an affected first- or second-degree relative. A positive genetic test in the family's index-case will confirm or discard that variant in the athlete.

What to do if an athlete has a negative genetic test?

Absence of a genetic variant does not rule out the disease in the athlete. In the following situations an athlete can test negative for a genetic study:

- The athlete clearly has a familial heart disease. In the presence of a negative genetic test, other genes that have not been described may be involved. In this case, the percentage of positivity (yield) of currently available genetic studies (in the presence of disease), which vary according to different pathologies (Tables 1, 2 and 3), should be considered.

- There is a previous diagnostic suspicion that the athlete has a family heart disease. A negative genetic test confirms the absence of disease in this individual, although a follow-up is

Table 4 – Clinical significance of the variant according to available information (Modified from *Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology*)[32]

Classification of the variant	Classification criteria	Clinical usefulness
Pathogenic	Not identified in the general population; variant widely described in the literature, with cosegregation demonstrated and strong evidence of genotype-phenotype association. Deleterious functional studies.	- Predictive clinical value. - Widely available clinical information. - Inclusion in familial screening is recommended. - Useful in PGD*.
Very likely pathogenic	Not identified in the general population; likely cosegregation of the variant in at least one family, truncating-type or in frame ins/del mutation in genes described with genotype-phenotype association that explains the patient's disease. Deleterious functional study.	- Predictive clinical value. - Inclusion in familial screening is recommended. - Limitation in PGD (elucidation on expressivity and incomplete penetrance).
Likely pathogenic	Absent truncating-type or in frame ins/del mutation or identified in the general population with very low allele frequency (<0.01%); intronic variant that affects splicing. Genotype-phenotype association documented in at least two individuals.	- No predictive clinical value. - Allows cosegregation study in the family, which might aid in defining the pathogenesis.
Uncertain clinical significance	Variant with contradictory information on its pathogenesis, does not meet the criteria to be included in another category of the classification.	- No predictive clinical value. - Allows cosegregation study in the family, in the investigation context at the attending physician's discretion.
Likely non pathogenic or benign	Allele frequency of the variant in control populations is higher than expected for the pathology. Absence of cosegregation. Missense variant in one gene, where only radical mutations are considered pathogenic. Benign functional study.	- No predictive clinical value. - Inclusion in familial screening not recommended.
Non pathogenic or benign	High frequency in the control population or previously described as benign. Absence of cosegregation. Benign functional study.	- Benign variant. - Should not be included in familial screening.

* PGD: Pre-implantation Genetic Diagnosis.

recommended, at least annually, if there is a borderline change in previous diagnostic tests.

- The athlete has no clinical manifestation of the disease, but an affected first- or second-degree relative. A negative genetic test in the family's index-case will not allow proper familial screening. Thus, predisposition to develop the disease can be neither confirmed nor ruled out. In this case, follow-up is recommended, at least annually, especially if there is a borderline change in previous diagnostic tests.

Conclusion

Genetic studies have become an instrument to help in the diagnostic confirmation of different inherited heart diseases. Physicians, including those of sports and exercise medicine, however, should know very clearly their indications and limitations in clinical practice. In PPE, complete history (individual and family) and a detailed physical exam, in addition to complementary tests, should always precede the application of genetic analysis (Figure 3). There is consensus that genetic testing, at least as a routine process, is not indicated in athletes. Its use is clearly indicated only in two particular cases: a) athletes with suspected or definite diagnosis of a familial disease; b) healthy or non-affected athletes, with a positive family history of an inherited disease, as part of the familial screening.

In the sports context, it is essential to consider that the correct interpretation of the genetic tests will reduce false-positive and false-negative results. This can prevent incorrect interpretation and recommendations, inappropriate disqualifications or unwanted events (SD, for example). We hope that, in the future, when the epidemiological and molecular aspects of these diseases are better known, a better genotype/phenotype correlation by use of genetic studies will be available. Therefore, it is necessary to create and foster multidisciplinary teams dedicated to information management and analysis, aimed at elaborating effective SD prevention programs for individuals who exercise in a recreational way, as well as for amateur and professional athletes.

Author contributions

Writing of the manuscript: Stein R, Trujillo JP, Silveira AD, Lamounier Júnior A. Critical revision of the manuscript for intellectual content: Stein R, Trujillo JP, Silveira AD, Lamounier Júnior A, Iglesias LM.

Potential Conflict of Interest

The authors report a conflict of interest, but this did not influence the writing of the manuscript.

Sources of Funding

There were no external funding sources for this study.

Study Association

This article is part of the thesis of Doctoral submitted by Anderson Donelli da Silveira, from PPG Cardiologia e Ciências Cardiovasculares da UFRGS.

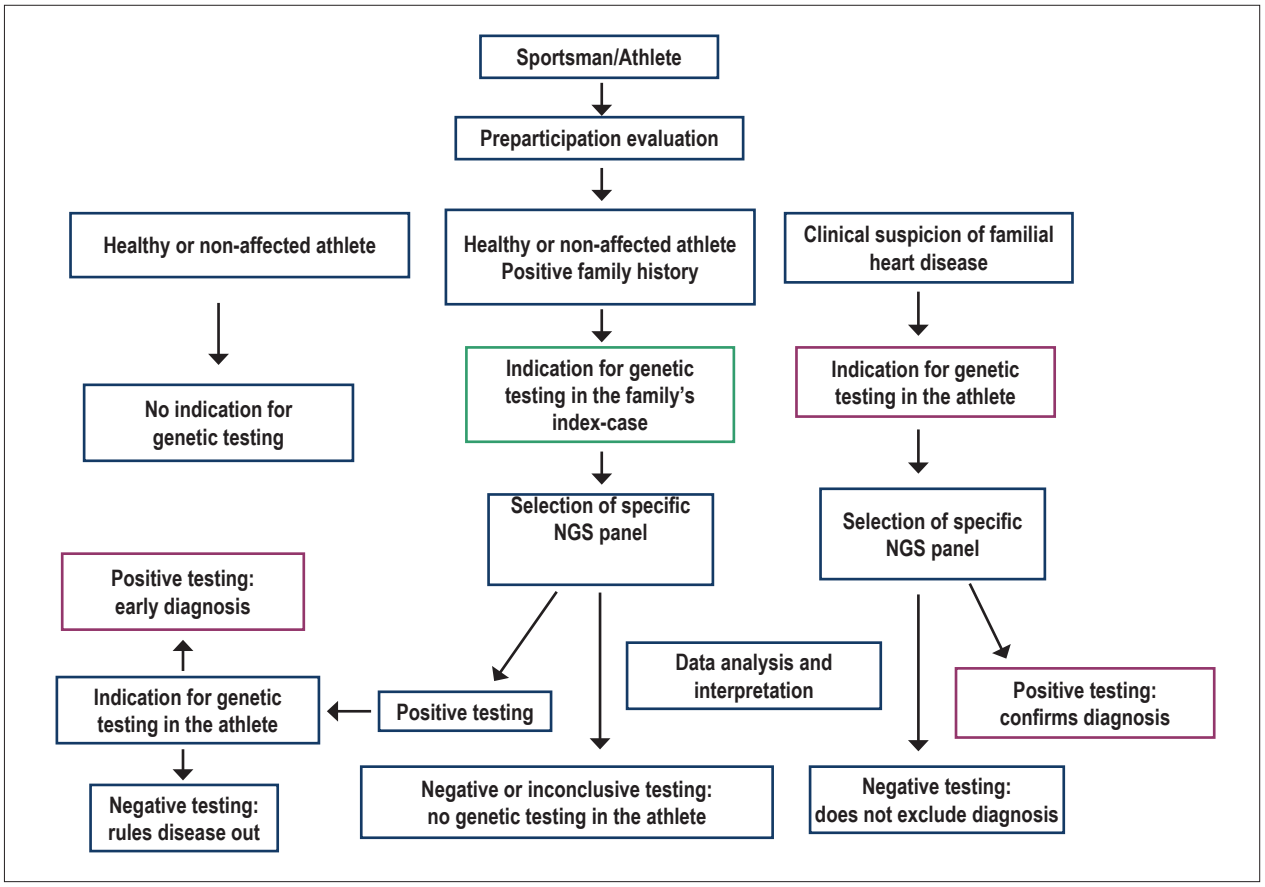

Figure 3 – *Possible clinical scenarios in the context of an athlete's genetic testing.*

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Aerobic Exercise and The Heart: Discussing Doses

Claudio Gil Soares de Araújo[1, 2], Claudia Lucia Barros de Castro[1,2], João Felipe Franca[1], Christina Grüne de Souza e Silva[2]

Clínica de Medicina do Exercício, CLINIMEX;[1] Universidade Federal do Rio de Janeiro (UFRJ),[2] , Rio de Janeiro, RJ - Brazil

The recent and exciting images of the 2016 Olympic and Paralympic Games in Rio will be forever imprinted in our minds. Hundreds of world, Olympic, continental and national records were conquered with a combination of discipline, resilience, competitiveness and overcoming challenges. Overcoming them in every sense – a result of thousands of hours of training that ensure exceptional aerobic performance from athletes with powerful and extremely healthy hearts.

The theme of aerobic exercise and the heart has never been so in evidence. Thus, in the perspective of this Olympic legacy, it is very appropriate to address the relation between aerobic exercise and the heart in the Brazilian Archives of Cardiology. More specifically, it is important to discuss the most suitable posology or dosage of exercise for adults in the context of primary and secondary prevention of cardiovascular diseases. Succinctly, the objective of this viewpoint to discuss and delimit the therapeutic range of the aerobic exercise dose for the heart and make suggestions about how this dose can be individualized according to certain criteria and objectives.

Preliminary considerations

Free animals have been and will always be active, with confinement or sedentary styles considered unnatural. Humankind, as it is known today, was able to set off from central regions and, through its own means of locomotion (exercise) spread across far areas of the planet. Seeking food and water, running from predators, from childhood to old age, have always been linked to exercise. Indeed, a sedentary lifestyle is very recent in the history of humankind.

Before approaching the theme of exercise and the heart, it is important to conceptualize some terms (Chart 1).[1,2] Among several terms, it is worth highlighting the difference between physical activity and exercise. While both involve movement and/or muscle contraction with energy expenditure, in exercise there is movement intention (physical activity) in a structured and repetitive manner, with the objective of maintenance or optimization of physical conditioning and/or health and/or body aesthetics.[1]

Keywords

Cardiovascular Diseases; Exercise; Prevention; Running; Longevity.

Mailing Address: Claudio Gil Soares de Araújo •
Rua Siqueira Campos, 93/101, Copacabana. Postal Code: 22031-070, Rio de Janeiro, RJ - Brazil
E-mail: cgaraujo@iis.com.br
Manuscript received October 18, 2016; revised manuscript November 01, 2016; accepted November 01, 2016.

Regarding the pharmacological dose, it can generally be defined by a therapeutic range with objective minimum and maximum limits. For a given individual, it is also possible to characterize an optimal dose with the best benefit/harm ratio. In the relation between aerobic exercise and the heart, it is also possible that there is something similar; however, to better understand this matter, we will use a few extreme examples.

From one extreme to another

In 1968, the Dallas Bed Rest Study[3] analysed the cardiovascular effects of extreme inactivity. Five healthy young individuals were bedridden for 21 days, and at the end of this period, a reduction of 30% was observed in VO_2max (maximum oxygen consumption), and it took them 60 days of training to recover pre-admission conditions. This 30% loss was greater then the "physiologic" reduction of aging observed in a re-evaluation done 30 years later.[4]

In October of 2016, two fantastic aerobic feats were accomplished. In the United States, Pete Kostelnick ran from San Francisco to New York (± 5,000 km), with an average of 110 km/day at different altitudes (http://www.petesfeetaa.com/).[5] In England, Ben Smith burned over 2.5 million calories to complete 401 marathons (42.195 km) in 401 days (± 17,000 km) (http://www.the401challenge.co.uk/).[6] These two integrate the growing contingent of ultra-runners - > 60,000 Americans in 2013 (https://www.ultrarunning.com/featured/which-state-has-the-most-ultrarunning-finishers-as-percentage-of-population/)[7] – that is, those who participate in running competitions (most of which are off-road) over distances longer than marathons and, in general, more than 50 miles (80 km). These ultra-runners compose an advanced and differentiated group when compared to the more than half a million Americans who complete a marathon every year.

These two paragraphs above illustrate the extremes between the "almost zero" physical activity or exercise (bed rest) and running several hours on consecutive days for long periods of time, without even one day of rest. It objectively shows how exercise dosage is, in fact, very wide and more elastic than any cardiovascular drug, and it exemplifies the cardiac tolerance of certain individuals to enormous doses of aerobic exercise.

Discussing doses: minimum, optimal, and maximum

There is a lot of epidemiological evidence that regular aerobic exercise and high functional capacity and/or aerobic condition (VO_2max) are associated to better health and longevity, while with a sedentary lifestyle, the opposite occurs.[8] Indeed, the VO_2max obtained (ideally through direct measure with the analysis of expired gases) in an incremental and truly

DOI: 10.5935/abc.20170020

Chart 1 – Main concepts and terms relevant to the analysis of aerobic dose recommendations for the heart

Physical fitness	Ability to perform different forms of activities and physical exercise expected for their age group, gender and physical dimensions, favoring health maintenance, survival, and adequate functionally of individuals in their environment. It can be divided into aerobic and non-aerobic components (strength/muscle power, flexibility, balance, and body composition).
Physical activity	any body movement produced by skeletal muscles that results in energy expenditure.
Exercise	structured and repetitive physical activity, whose objective is the maintenance or optimization of physical conditioning and/or body aesthetics and/or health.
Sport	physical exercise of variable energy demand which involve rules and competitions.
Sedentary lifestyle	it is the condition in which there is complete absence of regular physical exercise and frequent physical activity that involves energy expenditure (> 2 to 3 times the rest value), be it of laborious nature, for transportation or leisure.
Exerciser	it is the denomination given to the individual who exercises, that is, practices physical exercise.
Athlete	those who simultaneously meet the following criteria: a) sports training to improve performance; B) actively participate in sporting competitions; c) formally registered in sporting federations; and d) having training and sporting competitions as their main interest focus or way of life.
VO_2 max	Maximum aerobic power or maximum oxygen consumption or, simply, aerobic condition reflects the maximum quantity of oxygen that an individual can consume in one minute of an exercise activity that involves large masses or muscle groups; it can be expressed in $L.min^{-1}$ or, ideally, relativized by body weight and expressed in $mL.kg^{-1}.min^{-1}$.
Aerobic exercise volume (h/week)	it is the number of hours (or fractions of hours) per week of aerobic exercise; it is equivalent to the product (hours/week) of the number of weekly sections by the mean duration of sessions measured in hours.
Exercise intensity	it is expressed as the caloric expenditure in relation to the rest value (1 MET); aerobic intensity (METs) is frequently expressed by adjectives – low (light); medium (moderate); high (vigorous) – according to the % of the individuals' own VO2max. Low intensity is considered up to 30% of VO_2max, medium from 30 to 60 or 70% of the VO_2max (or anaerobic threshold); and high if it is above this value. In simple terms, it is possible to suppose that aerobic intensity is high when it is not possible to maintain normal conversation and/or when this intensity cannot be maintained at the same level for a long period of time.
Aerobic exercise dose	it is the product of the average intensity of exercise sessions by the respective duration in hours throughout (METs-h/week) the week.

maximum exercise test is an excellent health indicator[9] and has recently been suggested as a vital sign.[10]

Even more importantly, VO_2max estimates the prognosis for mortality from all causes better than any other biological marker, including glycidic/lipid profile and inflammatory markers. For example, data from Myers et al.[11] indicate that, for every 1 more MET in the aerobic condition, there is a reduction of 12% in mortality from all causes; therefore, the difference in risk between two middle-age individuals with 50% and 100% of the predicted VO_2max (age and gender) can be up to five times, that is, respectively, something like 1% vs 5% per year. Furthermore, in an recent example from Finland, Laukkanen et al.[12] verified that, in healthy 50-year-old men, who increased their aerobic condition (direct measure) in 1 MET after 11 years of follow-up, the risk of mortality decreased by five times, when compared to those who kept their aerobic condition, and in up to 20 times, when compared to those with VO_2max reduced by 4 or 5 METs in the same period. Thus, having a VO_2max equal to or above the predicted (age/gender) should become a goal in the primary and secondary prevention of cardiovascular diseases.

In the characterization of the exercise dose, it is appropriate to have a unit of measurement. While there are several units proposed in literature, a very convenient one is METs-h/week, which consists of multiplying the number of exercise hours, in a given week, by the mean intensity in METs. Thus, considering that walking fast (6 km/h) and slow running (8 km/h) demand, respectively, 4 and 8 METs, we have that: if the individual completes the dose recommended in most guidelines and walks fast for 30 minutes 5x/week (150 minutes or 2 and a half hours/week) or runs slowly for 25 minutes 3x/week (total of 75 minutes or 1.25 hours/week), the exercise dose will be the same in both examples – 2.5x4 or 1.25x8 – that is, ± 10 METs-h/week.

Two articles from 2015 brought relevant contributions regarding the relation between all-cause mortality and exercise dose, considering, separately, relative volume and intensity of the exercise. Arem et al.[13] identified that a maximum reduction in mortality was obtained with a dose of aerobic exercise corresponding to 3 to 5x the minimum dose recommended in the guidelines, that is, something between 20 and 50 METs-h/week (running 2 to 5 hours per week at 10 km/h) and that even a very high dose of 75-100 METs-h/week (8 to 12 hours of running per week) maintained the benefits of mortality reduction. Yet Gebel et al.[14] showed that, for a given weekly duration of exercise, benefits were greater when, in at least 30% of the duration, aerobic exercise was of high intensity. In fact, these two important facts can be conflated towards the definition of an optimum dose, and also characterize what could be denominated as extreme exerciser; that is, the one that exceeds 100 METs-h/week for long periods of time, which is still scarcely found in literature. We can then initially propose that the therapeutic range of aerobic exercise dose is between approximately 7.5 to 100 METs-h/week.

In our opinion, the issue of aerobic exercise dose should be individualized according to objectives to be reached. The only known way to increase VO_2 max, regardless of the current level, is by incrementing the dose of aerobic exercise. Therefore, if there is a clinical objective of increasing VO_2max to reduce future mortality rate, or for the individual's sport

goal, we can define the optimum dose as the one capable of offering the desired gains within a pre-established period. On the other hand, if the objective is only to maintain an excellent VO_2max (> 120% of the predicted for age and gender), the optimum aerobic exercise dose may be one that is able to ensure the maintenance of this privileged aerobic condition, according to cyclical adjustments recommended by the science of physical training. Chart 2 presents recommendations of aerobic dose and periodicity of re-evaluations, considering a classification founded on the % of the predicted VO_2max obtained through assessment and based on the current exercise pattern.

Exercise exaggeration?

In 1899, Williams & Arnold[15] did a thorough medical evaluation of Boston Marathon runners before the competition and one hour after completion (first place time of completion = 2h54m). They concluded that participation in the marathon did not seem to cause significant damage to the cardiovascular system of those young and healthy individuals. After more than 110 years of this rich description, there is a lot of positive evidence of athletes' and exercisers' cardiac health, such as the observation that athletes who participate in longer sporting competitions[16] and physically active adults tend to live longer,[17] and there is also the fact that deaths are extremely rare in half-marathons

Chart 2 – Aerobic exercise dose recommendations for adults based on: predicted %VO_2max obtained in the evaluation, and current exercise pattern

Predicted %VO2max (adjusted for age and gender) obtained in the evaluation*	Current exercise pattern	Aerobic dose	Recommendation details: practical suggestions**	Periodicity of aerobic re-evaluations***
> 120%	regular	maintain	Evaluate according to clinical or sporting objectives.	Elective
	irregular	adjust	Make it regular (minimum of 3x/week); consider varying modality and/or dose per exercise session; intensity can be high, at least occasionally.	Biannual
100 to 120%	regular	maintain	Evaluate according to clinical and/or sporting objective.	Biannual
	irregular	adjust	Make it regular (minimum of 3x/week); increase % of high intensity in the exercise session.	Annual
80 to 99%	regular	increase	+1 day/week or > total duration or increase of mean intensity of the exercise session.	Annual
	irregular	increase	Make it regular (minimum of 4x/week); increase % high intensity in the exercise session.	Annual
	none ou almost none	start/increase	3-4x/week 20 to 40 min; increase initially duration and then mean intensity; increase mean intensity; stimulate interval training and vary aerobic modalities.	Semester
60 to 79%	regular	increase	+2 days/week or >20% total duration and increase of average intensity; mean intensity; stimulate interval training and vary aerobic modalities.	Semester
	irregular	increase	Make it regular (minimum of 4x/week); simultaneously increase volume and % high intensity.	Semester
	none ou almost none	start/increase	4 to 6x/week 15 to 45 min; increase initially duration and after reaching 150 min/week also increase mean intensity of exercise session; consider varying aerobic modalities.	Semester
< 60%	regular	increase	Make it practically daily or >30% total duration and increase mean intensity stimulate interval training and vary aerobic modalities.	Semester
	irregular	increase	Make it regular (minimum de 4x/week); significantly increase volume and % high intensity.	Semester
	none ou almost none	start/increase	4a7x/week 10 to 50 min (possible to make it 2 daily sessions of 10-15 min); increase initially duration, and after reaching 150 min/week also increase mean intensity of exercise session; consider varying aerobic modalities.	Quarterly or Semester

**preferably performed with maximum exercise test (ideally direct measure); clinical or sporting objectives can determine the need for a re-evaluation at any moment.*
*** if there are clinical or sporting objectives to be reached, the exercise dose should be increased according to the objective to be contemplated; on the other hand, in case of clinical restrictions, it may be convenient to reduce the dose, especially when the exercise is not performed in exercising program sessions with medical supervision.*
**** the suggestion of evaluation periodicity is specific for VO_2max; clinical criteria or other objectives can determine different periodicities.*

and marathons, with a rate under 1/100,000 participants.[18] In this context, it is worth mentioning the case of Alexandre Ribeiro, six-time Ultraman champion, who, at 47 years of age, had accumulated 50,000 hours of aerobic training, in a typical dose of 250 METs-h/week. After extensive cardiologic, functional and image evaluation, the only finding worth commenting on was a discreet increase in the left atrium in the MRI.[19]

On the other hand, there are data from observational studies suggesting that there seems to be an increased risk of developing atrial fibrillation,[20] of having coronary artery calcification, and presenting late enhancement or other abnormalities in cardiac imaging exams.[21] However, to our best current knowledge, the clinical significance of these "abnormal" findings is still unknown.[18] In this context, further studies involving large samples of ultra-runners and/or individuals who have been exercisers for over 40 years may contribute to a better understanding of the real cause-effect relation between exercise dose and benefit or harm and/or unfavorable cardiac outcomes.[21] The fact is that it is currently known that very low doses of aerobic exercise bring benefits, while the maximum limit of the therapeutic dose of aerobic exercise is still unknown. That is, from a medical perspective, there is no evidence that founds a hypothesis of exercise exaggeration.[22]

Contextualizing in terms of public healthcare

Recent global data suggest that around 5 million deaths per year (almost 10% of total non-violent deaths) are caused by sedentary lifestyles. Moreover, it is estimated that annual worldwide costs due to low levels of physical activity/exercise surpass 67 billion dollars.[23] In Brazil, government data indicate that tens of millions of Brazilians – almost half the adult population – do not exercise enough. At another extreme, it is estimated that, including athletes and exercisers, much less than 10,000 individuals do intense aerobic training regularly and for periods longer than 10 hours per week or 100 METs-h/week. That is, for every one "extreme" exerciser, there are approximately 5,000 to 10,000 sedentary individuals. It is then clear that the question of a rare and unlikely theoretical excess of exercise for the heart is not a priority in terms of public healthcare.

Therefore, a priority for cardiologists must be the reduction or elimination of the sedentary lifestyle and stimulation of an increase in the exercise dose of the population in general, thus obtaining an improvement in the aerobic condition, and, consequently, a higher life expectancy with the additional bonus of a wide range of beneficial physiological effects. On the other hand, in the case of those rare "extreme" exercisers, we can suggest that they be monitored by qualified professionals when dealing with health and training issues pertinent to this performance profile, while new evidence is gathered on the results of this "extreme" dose of aerobic exercise and its effect on the heart through the future analysis of a cohort made up of 1,200 ultra-runners.[24]

Author contributions

Conception and design of the research, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Araújo CGS, Castro CLB, Franca JF, Souza e Silva CG

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was partially funded by CNPq and FAPERJ.

Study Association

This study is not associated with any thesis or dissertation work.

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Case 2/2017 – Cor Triatriatum, without Clinical Manifestation, in A 6-Year-Old Girl

Edmar Atik and Gláucia M. P. Tavares
Clínica Dr. Edmar Atik, São Paulo, SP – Brazil

Clinical data: cardiac murmur was routinely identified at 2 years of age, which was characterized, on this occasion, as functional. A recent echocardiogram demonstrated a membranous partition in the left atrium with ample communication between the two cavities, proximal and distal. The child usually performs usual physical activities, just like other children.

Physical examination: eupneic, acyanotic, normal pulses. Weight: 19.6 kg; Height: 116 cm; Blood pressure: 95/60 mmHg; Heart rate: 78 bpm, oxygen saturation: 97%. Aorta not palpated in the suprasternal notch.

In the precordium, non-palpable *ictus cordis* and absence of systolic impulses in the Left Sternal Border (LSB). Normal heart sounds; systolic murmur, +/++/4, rough, medium LSB, variable in intensity, dependent on the position, sharply decreasing in the sitting position. The liver was not palpable.

Complementary examinations

Electrocardiogram showed sinus rhythm and no signs of cavitary overload. The QRS complex had RS morphology in V1, qRs in V6. AP: +50°, AQRS: +80°, AT: +40°.

Chest X-ray showed normal cardiac area (cardiothoracic index: 0.46). The pulmonary vascular net was normal and the medial arch was rectified.

Transthoracic echocardiogram (Figures 1 and 2) showed a membrane in the middle of the left atrium, which was enlarged. The proximal cavity received the four pulmonary veins, and the distal cavity, in communication with the mitral valve, did not have any atrial septal defect. There were two fenestrations in the membrane, the largest being 10 mm and the smaller, 4 mm in diameter. The maximum transmembrane gradient was 9 mmHg, with a mean of 2.5 mmHg. The flow velocity through the pulmonary veins was normal and without turbulence, characterizing the absence of intra-atrial obstruction. The systolic pressure of the pulmonary artery was 30 mmHg. No other defects were found.

Keywords

Cor Triatriatum; Congenital Abnormalities; Echocardiography; Signs and Symptoms.

Mailing Address: Edmar Atik •
Private Office. Rua Dona Adma Jafet, 74, conj.73, Bela Vista.
Postal Code 01308-050, São Paulo, SP – Brazil
E-mail: conatik@incor.usp.br

DOI: 10.5935/abc.20170030

Clinical diagnosis: *cor triatriatum sinister* with no associated defects (type A of the Lam classification) in an asymptomatic child, in the presence of mild intra-atrial flow limitation

Clinical reasoning: the available clinical elements were insufficient to characterize the existence of any congenital heart disease. The systolic murmur present in the LSB, variable and discrete, had functional characteristics, unrelated to any abnormality. Also, the usual complementary exams did not disclose any abnormalities. Thus, the left intra-atrial defect was discovered through a routine echocardiographic finding, motivated by the presence of functional murmur.

Differential diagnosis: other cardiac congenital anomalies can also be routinely diagnosed, without any suggestive elements, such as cardiac defects with mild effects, exteriorized by discrete and non-significant murmurs. Interatrial and interventricular septal defects, obstructive pulmonary and aortic valve lesions, coarctation of the aorta, and obstructions in the right ventricle and even in the left ventricle are examples of this situation.

Conduct: the ideal would be to remove the left intra-atrial membrane by surgical intervention. However, considering that this obstruction does not cause enough hemodynamic disorders to externalize any symptom or sign of a developing clinical problem, we chose the clinical observation until there is some manifestation.

Comments: *Cor triatriatum* is a rare anomaly, in which the atria are divided by a membrane, characterized as *sinister* to the left (present case) and *dexter* to the right.[1,2] Embryology explains the anomaly by the inadequate incorporation of the pulmonary veins in the left atrium, causing the intra-atrial division. On the left side, the pulmonary veins drain into the proximal (posterosuperior) chamber, and the mitral valve and left atrial appendage are located in the distal (anteroinferior) chamber. *Cor triatriatum* is classified, according to Lam (1962), as type A, without associations (as in the present case); A1, in which the ASD occurs in the proximal chamber (50%); A2, in which the ASD occurs in the distal chamber (10%); B, in which the pulmonary veins drain into the coronary sinus (1%); and C, when there is Total Anomalous Pulmonary Venous Drainage (5%). Depending on the degree of the obstruction and associations, this obstructive anomaly can be diagnosed at any age.[1] In the most severe situation, there is marked pulmonary venous drainage obstruction, pulmonary hypertension, and heart failure. Over the course of 50 years, 25 patients with *cor triatriatum* were operated at the Mayo Clinic, whose age ranged from 1 day to 73 years.[1] The first corrective surgery for this

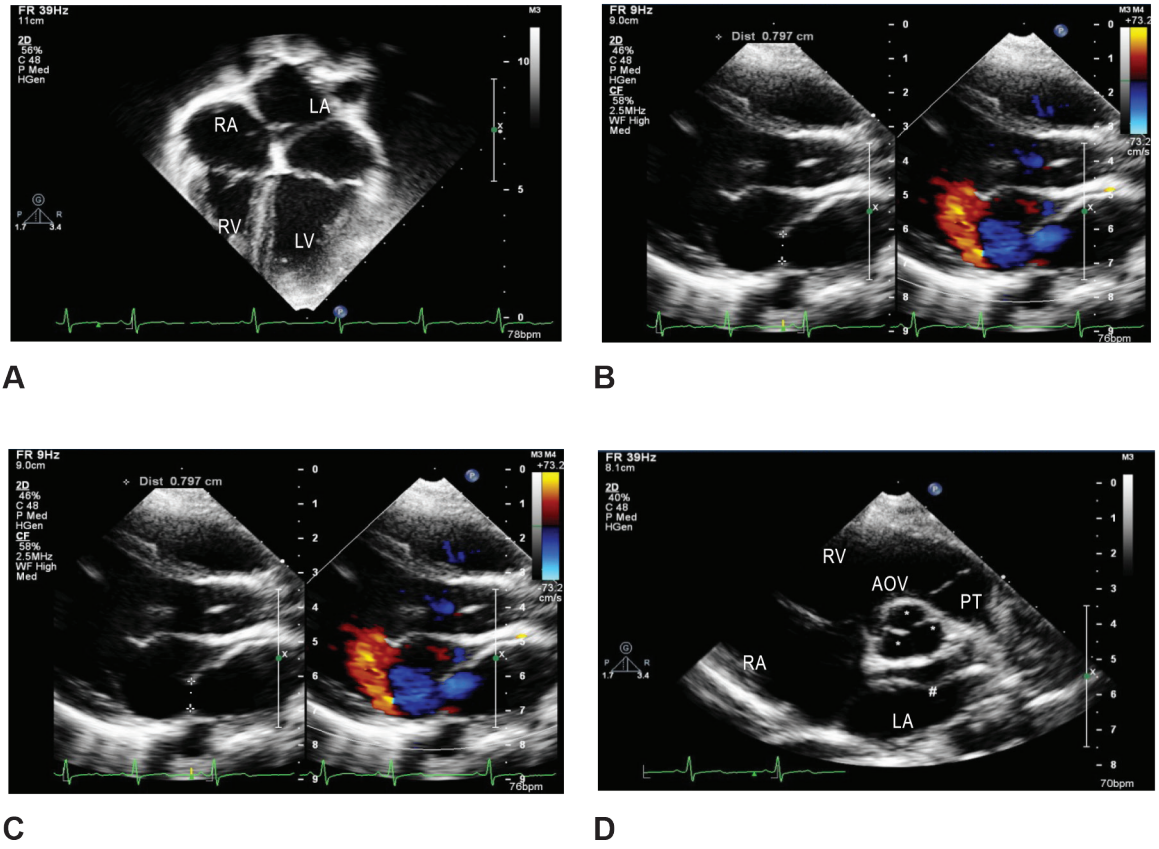

Figure 1 – *Echocardiograms demonstrate cor triatriatum in the 4-chamber apical plane in anatomical position, showing dilated left atrium, with septal defect (A); In the same plane, in black and white and with color mapping, showing the fenestration measurement and the flow passage in colors (B); in the long-axis parasternal plane, showing simultaneously, in black and white and with color mapping, the cor triatriatum membrane in the left atrium and the main fenestration measurement (C); and, in the short-axis parasternal plane, showing the septal defect in the left atrium (D). RA: right atrium; LA: left atrium; RV: right ventricle; LV: left ventricle; MV: mitral valve; AOV: aortic valve; TP: pulmonary trunk.*

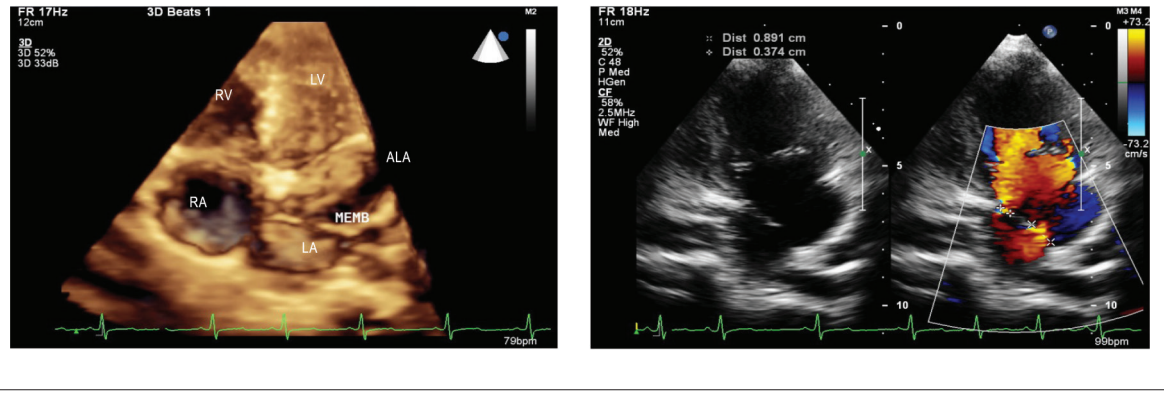

Figure 2 – *Echocardiograms in the 4-chamber apical plane in three-dimensional image acquisition, showing the left atrium divided by a fenestrated membrane and in the 2-chamber apical plane, simultaneously showing, in black and white and with color mapping, the cor triatriatum membrane in the left atrium, showing that there are two fenestrations, their measurements, and the flow passage in colors. RA: right atrium; LA: left atrium; MEMB: cor triatriatum membrane; RV: right ventricle; LV: left ventricle; ALA: accessory left atrium.*

anomaly was performed in 1956 by Lewis and, since then, approximately 250 cases have been surgically repaired. It is concluded that there is great diversity regarding the repercussion and also the age of clinical manifestation, and that it can be identified early in life and even in old age, when problems arise due to disease evolution, such as atrial fibrillation, pulmonary arterial hypertension and right heart failure.[2] There are no reports of percutaneous involvement in this obstructive abnormality in the literature, but this idea is tempting, in view of the fact that this anomaly can be resolutively simpler, in the presence of an even more discrete residual obstruction.

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Miniaturized Transcatheter Leadless Pacemaker in a Patient with Double Mechanical Prosthesis

Marta Pachón, Alberto Puchol, Finn Akerström, Andrés Sánchez-Pérez, Miguel A. Arias

Cardiac Arrhythmia and Electrophysiology Unit - Department of Cardiology - Hospital Virgen de la Salud, Toledo – Espanha

Introduction

Despite technical advances and accumulated experience, complications continue being a concern for patients implanted with permanent pacemakers. Several leadless pacemaker systems have now been developed in order to reduce the rate of complications in patients implanted with conventional transvenous pacemaker.

Case Report

A 75-year-old female patient with a background of systemic arterial hypertension, chronic atrial fibrillation with an episode of peripheral arterial embolism in the right upper limb and rheumatic valve disease, underwent mitral and aortic valve replacement receiving two mechanical valve prosthesis in combination with left atrial appendage occlusion. Six months after surgery, pharmacologic therapy to achieve heart rate control was very difficult and inadequate and the patient was scheduled for permanent pacemaker implantation. In order to avoid lead or pocket complications, the Micra transcatheter leadless pacemaker (Medtronic Inc., Minneapolis, MN, USA) was implanted through the femoral vein using a steerable catheter delivery system with the use of a 23-French introducer. The procedure was performed under uninterrupted acenocoumarol therapy with therapeutic international normalized ratio (INR 2.5). Sedation and local anesthesia was applied and the implant was successful upon initial device positioning at the mid-septum of the right ventricle with no complications. Access site closure was performed using a subcutaneous venous figure-of-8 suture. The pacing capture threshold at implant was 0.38 V measured at 0.24 ms, the R-wave sensing amplitude was 8.8, and the pacing impedance was 730 ohms. There were no complications and the patient was discharged home the next day after chest X-ray showed the device was positioned perfectly (Figure1) and electrical pacing parameters were appropriate. At three months of follow-up the patient has shown no complications and the pacing capture threshold was 0.38 V at 0.24 ms, the R-wave sensing amplitude was 9.2 and the pacing impedance was 680 ohms.

Discussion

In spite of technological advances and the enormous accumulated experience, conventional pacemaker therapy continues to be associated with a great variety of potential complications either in the short and long-term.[1] They are particularly related to the device (hematoma, skin erosion, pocket infection) or as a result from transvenous lead placement (pneumothorax, cardiac perforation, lead dislodgement, venous occlusion, loose connector pin, conductor lead fracture, insulation lead break, infections, tricuspid valve damage, etc.). Early performance and safety data for the Micra transcatheter leadless pacemaker are positive[2,3] and leadless pacemakers represent a promising alternative for many patients, eliminating the main sources of complications associated with conventional transvenous pacemaker implantation.

Conclusion

Patients with mechanical heart valve prosthesis might represent a subgroup of patients for whom this new therapy can bring higher benefits due to the need for lifelong anticoagulation and the serious consequences of permanent transvenous pacemaker system infections.

Author contributions

Conception and design of the research: Pachón M, Arias MA; Acquisition of data and Writing of the manuscript: Pachón M, Puchol A, Akerström F, Sánchez-Pérez A, Arias MA; Analysis and interpretation of the data: Pachón M; Critical revision of the manuscript for intellectual content: Pachón M, Puchol A, Akerström F, Arias MA.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

Keywords

Pacemaker, Artificial/adverse effects; Heart Valve Prosthesis; Arrhythmias, Cardiac/therapy

Mailing Address: Miguel A. Arias •
Avda Barber. Postal Code: 45003, Toledo – Spain
E-mail: maapalomares@secardiologia.es
Manuscript received December 30, 2015; revised manuscript February 19, 2016; accepted May 13, 2016.

DOI: 10.5935/abc.20160192

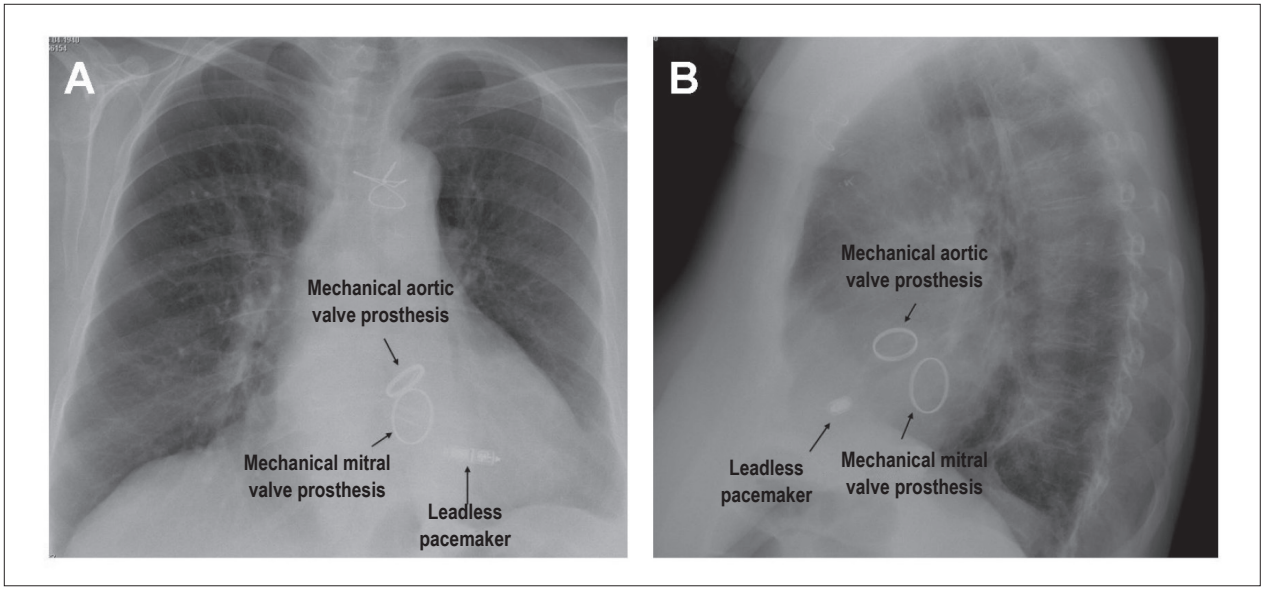

Figure 1 - *Chest X-Ray of the patient (panel A, posteroanterior view; panel B: lateral view) after leadless pacemaker implantation.*

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Patient with Atrial Myxoma and Signs of Obstruction of the Left Ventricular Outflow Tract

Antonio Fernando Diniz Freire, Alexandre Anderson de Sousa Soares, Tatiana de Carvalho Andreucci Torres Leal, Mucio Tavares de Oliveira Junior, Alexandre de Matos Soeiro

Instituto do Coração (InCor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, SP – Brazil

Female patient, 45 years-old, reported dyspnea on routine efforts with 6 months of evolution with dry cough. Previously healthy she refused to take regular medication. According to the physical examination, she had a heart rate of 105 bpm, blood pressure of 90x60mmHg, respiratory frequency 30 incursions per minute, arterial saturation of 88%, slow capillary filling time, rhythmic sounds, diastolic rumble murmur (+++/6) in mitral focus and bibasal pulmonary crepitations. Chest X-Ray showed a discreet widening of the mediastinum. Chest CT was performed to evaluate the mediastinum and pulmonary parenchyma and an intracardiac mass was found. It evolved with hypotension and worsening of pulmonary congestion.

Emergency echocardiogram showed a moving rounded hyperechoic image in the left atrium, measuring 66x36mm with a pedicle adhered to the membrane of the oval fossa dislocating to the left ventricle (LV) during systole causing hemodynamic repercussion (signs of LV outflow tract obstruction). The patient underwent emergency surgery. Retraction of the tumor revealed a cleft in the anterior cusp of the mitral valve and it was closed. In the immediate postoperative, the patient developed cardiogenic shock refractory to vasoactive drugs; an intra-aortic balloon was implanted, but the patient died 30 hours later. Anatomopathological confirmed the diagnosis of a myxoma: 7.5x4.6x3.4cm. In conclusion, the atrial myxoma with obstruction of the outflow tract of the LV is rare, its clinical manifestations can mislead the evaluator, clinical suspicion and the correct use of propaedeutic are essential for early diagnosis and successful clinical treatment.

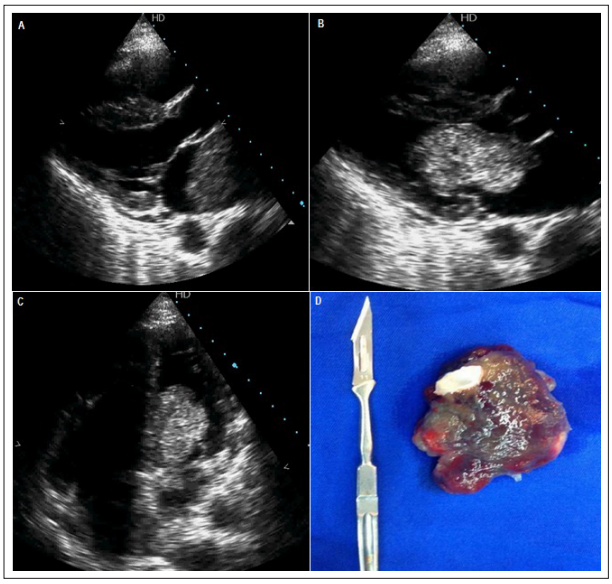


Figure 1 – *A) Long axis parasternal view in diastole showing hyperechoic image in the interior of the left atrium - round, mobile, with approximately 66x36 mm, with pedicle adhered to the fossa ovalis membrane (myxoma). B) Long axis parasternal view in systole showing atrial myxoma dislocated to the interior of the left ventricle obstructing outflow tract. C) 4-chamber apical view in systole showing atrial myxoma dislocated to the interior of the left ventricle. D) Anatomopathological piece of irregular material of blue-greyish hue, with reddish areas, slightly translucent and sparkly, of gelatinous consistency, measuring 7.5 x 4.6 x 3.4 cm: left atrium myxoma with wide hemorrhage areas.*

Author contributions

Conception and design of the research: Freire AFD, Leal TCAT, Oliveira Junior MT, Soeiro AM; Acquisition of data, Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Freire AFD, Soares AAS, Leal TCAT, Oliveira Junior MT, Soeiro AM.

Keywords

Myxoma/surgery; Hypotension; Tomography; Heart Atria/abnormalities,

Mailing Address: Antônio Fernando Diniz Freire •
Rua Martiniano de Carvalho, 807. Postal Code 013210-000, Bela Vista, SP – Brazil
E-mail: antoniofernando85@yahoo.com.br
Manuscript received July 22, 2016, revised manuscript August 31, 2016, accepted on August 31, 2016.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

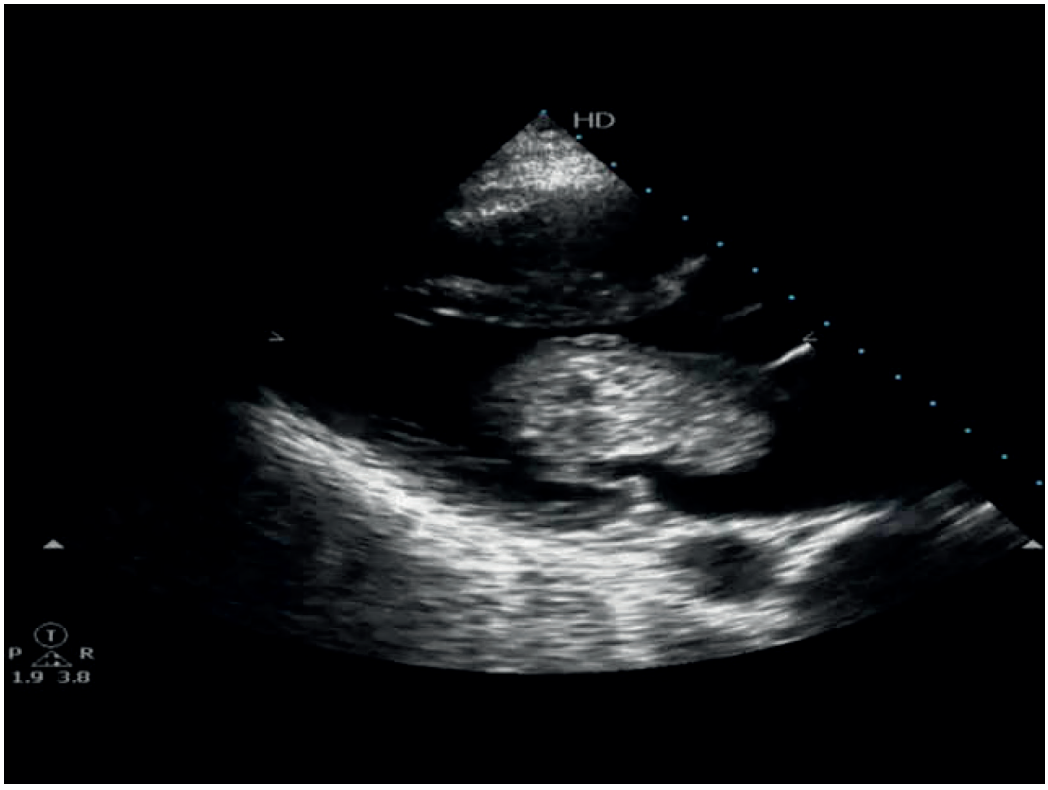
Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

DOI: 10.5935/abc.20170007



Video 1 – *Watch the videos here: http://www.arquivosonline.com.br/2017/english/10801/video_ing.asp*

Prognosis Determinants after Cardioverter-Defibrillators Implantation in Brazil

Arn Migowski e Regina Maria de Aquino Xavier
Instituto Nacional de Cardiologia, Rio de Janeiro, RJ – Brazil

The study by Silva et al.[1] presented the results of a series of cases from a single-center registry of implantable cardiac devices, including 28 implantations of isolated cardioverter defibrillators (ICD) and 14 associated to resynchronization (CRT-ICD), in addition to procedures for generator replacement and handling of electrode cable.[1] ICD and CRT-ICD implantations were considered the strongest predictors of re-admission into the hospital during a six-month period after the surgical procedure, although it is not clear whether there was adjustment by functional class or left ventricular ejection fraction.[1]

We have recently published the results of short, medium, and long term follow-ups of all ICD and CRT-ICD implantations funded by The Brazilian Unified Healthcare System (SUS) in all of Brazil, between 2001 and 2007, including 3,295 ICD implantations in 85 hospitals and 681 CRT-ICD implantations in 50 hospitals.[2]

In our study, when compared to the ICD implantation group, patients who underwent CRT-ICD implantation had a worse mortality relative to the procedure and also a worse survival in the medium and long run.[2] However, in the study by Silva et al.[1], there apparently was no worse short-term prognosis of patients who underwent CRT-ICD with relation to those who underwent ICD implantation, at least in regards to re-admission.[1] In relation to survival time, it would be interesting to consider stratification by device type, since the presented 6-month survival[1] seemed low in relation to our finding,[2] especially considering most implantations were for pacemakers.[1] The percentage of double-chamber ICD would also be interesting information, considering some studies point to an association with poorer results.[2]

We have observed in the national multi-center study that the type of technique used for CRT-ICD implantation affected short-term mortality, and there was an important decrease in survival, approximately four years after the initial implantation in this group, possibly related to complications associated to the need for intervention.[2] It would be interesting to know the magnitude of re-interventions in the short period of follow-up of the single-center registry and the possible impacts of implantation techniques in the outcomes.

The greater representativeness of Chagas heart disease, in the group with complications in the study by Silva et al.[1], may be related to the type of implanted device. In our study, Chagas heart disease patients did not have a worse prognosis when compared to those with ischemic heart disease,[2] similarly to another study recently published in *Arquivos Brasileiros de Cardiologia*.[3]

Keywords

Cardiac Resynchronization; Therapy Devices; Defibrillators, Implantable; Chagas Cardiomyopathy; Survival Analysis; Brazil.

Mailing Address: Arn Migowski •
Rua das Laranjeiras 374, Instituto Nacional de Cardiologia, 5º andar. Postal Code 22240-006, Laranjeiras, RJ – Brazil
E-mail: arnmigowski@yahoo.com.br, arn.santos@inca.gov.br
Manuscript received November 30, 2016, revised manuscript January 26, 2017, accepted January 26, 2017

DOI: 10.5935/abc.20170034

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Reply

The care with patients with implantable electronic cardiac devices (IECD) demands more and more the attention to the different clinical presentations of these patients. Such differences are becoming more prominent due to two main factors: an increase in the population longevity, which has increased comorbidities, and the increasing frequency of patients with cardiomyopathy and ventricular dysfunction.

We believe that the main message of this publication is to prove, through data derived from real clinical practice, that older patients, just as individuals with severe left ventricular dysfunction, are more prone to clinical events, related or not to cardiac disease, that require readmission to the hospital and that, therefore, must be observed more closely in order to be avoided.

Undoubtedly, ICD and CRT incorporation to the list of artificial cardiac stimulation procedures has brought a considerable contingent of patients with severe heart failure, which did not use to be as frequent when the only implanted devices were pacemakers. It is also not likely that this is the reason for the association mentioned in the commentary. With regards specifically to the relation between Chagas disease and mortality, our study was not designed with this objective nor would it have the sample power for that.

To make any comparison between the results of the presently published study and those of the publication of the comment's authors does not seem possible. While our study is a prospective analysis of primary data collected from a population of patients with all types of implantable devices, treated in one single, highly specialized, center, actively monitored by the researchers, the study by Migowski et al. is a retrospective analysis of secondary data obtained from administrative bases of SUS, from a population with cardio-defibrillators implanted at various centers with a varied level of specialization. Furthermore, the follow-up information is clearly incomplete, especially considering the authors of the study themselves have informed they censored all deaths from causes not related to heart disease or treatment.

We believe an attempt to make this kind of comparison would be like trying to compare the wine from a single producer to water from a container whose samples come from different sources.

Katia Regina da Silva
Caio Marcos de Moraes Albertini
Elizabeth Sartori Crevelari
Eduardo Infante Januzzi de Carvalho
Alfredo Inácio Fiorelli
Martino Martinelli Filho
Roberto Costa

Errata

July issue of 2016, vol. 107 (1), pages. 77-80

In the Case Report “Anatomopathological Aspects of Acute Chagas Myocarditis by Oral Transmission”, pages 77-80, by authors Dilma do Socorro Moraes de Souza, Marialva TF Araujo, Paulo da Silva Garcez, Julio Cesar Branco Furtado, Maria Tereza Sanches Figueiredo, Rui M.S. Povoa, please be aware that the correct spelling for Paulo da Silva Garcez is Paulo Roberto Silva Garcez dos Santos.

DOI: 10.5935/abc.20170038