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EDITORIAL

Physical Activity, Exercise and Sports and Covid-19: What Really Matters

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The ability to move is essential for an independent life. It is widely recognized that those who spend the most time either lying down or sitting and are physically inactive tend to have limited autonomy, poor health-related quality of life, and adverse prognosis of outcomes.[1]

This editorial will briefly discuss the relationship between COVID-19 and physical movement. To better address this important relationship and for sake of clarity, some concepts should be defined first. In sport and exercise sciences, "physical activity" is defined as any bodily movement produced by skeletal muscles that results in energy expenditure, "exercise" is a subset of physical activity that is planned, structured, and repetitive[2] and "sport" is an activity that most often, but not always, involves exercise and predetermined rules and participation in games or races or competitions.

In this context, Araújo & Scharhag[3] proposed four criteria for defining an individual as "an active athlete": 1) to be training in sports to improve performance/results, 2) to be actively participating in sports competitions, 3) to be formally registered in a local, regional or national sports federation and 4) to have sport training and competition as his/her way of living or focus of interest, devoting several hours in all or most of the days to these activities, exceeding the time allocated to other professional or leisure activities. As a simple example, in a road marathon race, the few runners placed in the very first rows at starting line are the actual athletes, whereas the hundreds or thousands of runners behind these athletes may better be called exercisers. Of note, competitive sports are primarily related to entertainment and business rather than to health.

On the other hand, an important term in sports science is "physical fitness" that can be defined as the ability to perform different forms of physical activities as expected for an individual's age group, sex and physical dimensions, that favor health maintenance, survival and functionality.[4] Physical fitness can be divided into aerobic and non-aerobic components (strength/power muscle strength, flexibility, balance and body composition).[4] It is worthy to mention that although regular exercises and cardiorespiratory or aerobic physical fitness are well related, in terms of outcomes such as cardiovascular and all-cause mortalities, aerobic and non-aerobic physical fitness are much more influential than the amount or pattern of regular physical activity and/or exercise.[5,6] Having these preliminary concepts and thoughts in mind, it is time to address how physical activity, exercise and sports have been impacted by the COVID-19 pandemic.

Starting from the beginning of 2020, COVID-19 has had a tremendous impact on the life of most individuals in the world. The number of infected cases and deaths has continuously increased in almost all countries, which will sadly continue until a combination of sanitary measures and wide vaccination and immunization coverage rates are achieved. Of course, in this prolonged and dramatic public health crisis, the "stay at home" and "keep physical distancing" messages have been widely advocated, and the opportunities to outdoor physical activities, exercises and sports have been substantially diminished or even completely abolished. Consequently, a physical inactivity pandemic has been generated, with potential adverse effects to physical and mental health, as well as to physical fitness on large scale. To minimize the undesirable effects of the restrictions to all forms of outdoor movement, the WHO and other institutional documents and papers have been published,[7,8] providing and encouraging various alternatives of "at home" exercises. This has become

Keywords

COVID-19; Exercise; Sports; Exercise Movement Techniques; Pandemics; Betacoronavirus; Physical Activity.

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particularly important for chronic diseases patients, the elderly, and for debilitated patients for whom there are also interesting proposals based on mobile technology.[9,10]

Despite these recommendations, for different reasons, many individuals have been exercising or practicing sports in the gyms, pools, courts, tracks, fields, clinics or hospitals, or simply outdoors. To minimize the risks for COVID-19 infection and transmission, these individuals should follow strict and proper medical advice, including the following recommendations: 1) to stay two meters away from other people; 2) to limit face-to-face contact with other people who are not from the same household, even if asymptomatic for COVID-19; 3) to wear a properly fitted mask, if viable, all the time, even considering that it might impair ventilation and, perhaps, physical performance;[11] it is also a clear sign to the others that you are being respectful and careful regarding COVID-19 (even if you had already been infected or vaccinated); 4) to wear sunglasses whenever possible; 5) if meeting other exercise or sports partners, to avoid touching others' cell phones, shaking hands, kissing and hugging, and sharing sports gadgets/bottles/clothes; 6) when returning home, to strictly follow recommendations for sanitation of hands, face, clothes and materials; 6) to keep in mind that, the COVID-19 pandemic is not likely the right time to achieve an individual's best mark or a personal record in exercise or sports; 7) in case of any abnormal signs of symptoms, related or not to COVID-19, avoid exercising or sports practice and seriously consider seeking medical advice.

Last but not least, it is worth to comment on the issue on returning to physical activity after being diagnosed with COVID-19. There are several documents and opinion papers on this issue, ranging from very liberal[12] to very conservative recommendations.[13] In a real-world perspective, fortunately, most individuals (especially the younger ones) infected by COVID-19 will have a relatively benign and short-living disease. Nevertheless, and very sadly, a relatively small percentage of COVID-19 infected people will have severe complications that may lead to long-term sequelae or death. However, it should be clearly stated that even this small percentage meant almost two million of deaths in the beginning of 2021.

There are some potential late cardiac complications of COVID-19 that should be considered in returning to exercise after COVID-19, including myocarditis, due to the risk of malignant arrhythmias and sudden death. Notwithstanding, two recent elegant studies showed that myocarditis is, indeed, a rare finding in the course of COVID-19. Halushka et al.[14] identified only four cases in 277 autopsies performed in COVID-19 patients and Linschoten et al.[15] analyzed data from 3,111 patients from the CAPACITY-COVID registry and found only three cases or 0.1%. So, it does not seem reasonable to recommend mandatory medical consultation, resting 12-lead electrocardiogram or other complementary tests for all the millions of men and women of all ages that had uncomplicated forms of COVID-19 before returning to physical activity and/or exercise, as it was proposed in a recent institutional document.[13]

Taking in account the public health perspective, two important, and somewhat antagonist variables should be balanced: 1) the importance of achieving and maintaining good physical fitness, most likely by regular exercising, and 2) minimizing the chances of COVID-19 late complications when returning to physical activity, exercise and/or sports. Table 1 presents some practical tips for return to exercise and sports after COVID-19.

In conclusion, the relationship of physical activity, exercise and sport to COVID-19 is, frankly, really simple. Until the pandemic is over, population should try to keep themselves safe and healthy and, ideally, as aerobic/non-aerobic physically fit as possible. To achieve this, the major exercise components, that is, frequency, time, intensity, and type should be tailored to individual health status and possibilities. Simple "take-home" key messages are presented in Table 2. To end, it is appropriate to wish all a good, pleasant and safe physical activity and/or exercise and/or sports.

Table 1 – Post-Covid-19: Returning to Exercise & Sports

Practical and important tips:
Return to exercise after a couple of weeks free of symptoms;

- The more severe the COVID-19, the slower and later the return to the usual volume / intensity of exercise and/or sports; Listen and read the body response;
- To observe resting and exercising heart rate and perception of exercise intensity, for example, by using a 0 to 10 BORG scale
- Symptoms (particular attention with more intense and prolonged fatigue than usual)
  - Alert for "worsening" or new abnormal symptoms/signs
  - When in doubt, seek qualified medical advice

Table 2 – Physical Activity, Exercise and Sport & Covid-19: Key Messages

- Sanitary measures are essential to control COVID-19 infection and transmission, and should be followed by all exercisers and athletes, even by those who had already been diagnosed with COVID-19;
- For the vast majority of cases of COVID-19, no relevant cardiac sequelae should be presumed or expected;
- For those who were asymptomatic or had mild COVID-19 symptoms, after one to two weeks without symptoms, the gradual return to the usual pattern of physical activity, exercise or sports seems to be safe and does not require medical evaluation, especially in a context of high demands on health services;
- More complex cases (more severe or prolonged) of COVID-19, and athletes competing in sport modalities with high aerobic demands could benefit from qualified medical advice;
- The concept that it is more dangerous and harmful to your health to be sedentary than to exercise or play sports regularly remains extremely valid.

References

1. Myers J, Kokkinos P, Arena R, LaMonte MJ. The impact of moving more, physical activity, and cardiorespiratory fitness: Why we should strive to measure and improve fitness. Prog Cardiovasc Dis. 2020;620(20):30183-3.
2. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. 1985;100(2):126-31.
3. Araujo CG, Scharhag J. Athlete: a working definition for medical and health sciences research. Scand J Med Sci Sports. 2016;26(1):4-7.
4. Araujo CG. Componentes aeróbico e não-aeróbicos da aptidão física: fatores de risco para mortalidade por todas as causas. Revista Factores de Risco. 2015;35:36-42.
5. de Lannoy L, Sui X, Blair SN, Ross R. All-cause mortality risk among active and inactive adults matched for cardiorespiratory fitness. Eur J Prev Cardiol. 2019;26(5):554-6.
6. Brito LB, Ricardo DR, Araujo DS, Ramos PS, Myers J, Araujo CG. Ability to sit and rise from the floor as a predictor of all-cause mortality. Eur J Prev Cardiol. 2014;21(7):892-8.
7. Moulson N, Bewick D, Selway T, Harris J, Suskin N, Oh P, et al. Cardiac Rehabilitation During the COVID-19 Era: Guidance on Implementing Virtual Care. Can J Cardiol. 2020;36(8):1317-21.
8. WHO. #HealthyAtHome - Physical activity 2020 [Available from: https://www.who.int/news-room/campaigns/connecting-the-world-to-combat-coronavirus/healthyathome/healthyathome---physical-activity.
9. Araujo CG, De Souza e Silva CG. A multiprofessional face-to-face and remote real-time hybrid exercise-based cardiac rehabilitation: an innovative proposal during COVID-19 pandemic. Can J Cardiol. 2021 Jan 13;S0828-282X(00007-6)
10. Meinhart F, Stutz T, Sareban M, Kulnik ST, Niebauer J. Mobile Technologies to Promote Physical Activity during Cardiac Rehabilitation: A Scoping Review. Sensors (Basel). 2020;21(1):65
11. Haraf RH, Faghy MA, Carlin B, Josephson RA. The Physiological Impact of Masking Is Insignificant and Should Not Preclude Routine Use During Daily Activities, Exercise, and Rehabilitation. J Cardiopulm Rehabil Prev. 2021;41(1):1-5.
12. McKinney J, Connelly KA, Dorian P, Fournier A, Goodman JM, Grubic N, et al. COVID-19 - Myocarditis and Return-to-play: Reflections and Recommendations from a Canadian Working Group. Can J Cardiol. 2020 Nov 26;S0828-282X(20)311089.
13. Colombo CS, Leitão MB, Avanza Jr. AC, Borges SF, Silveira AD. Posicionamento sobre avaliação pré-participação após a COVID-19: orientações para retorno à prática de exercícios físicos e esportes 2020 [Available from: https://www.portal.cardiol.br/post/posicionamento-sobre-avaliacao-pre-participacao-cardiologica-apos-a-covid-19.
14. Halushka MK, Vander Heide RS. Myocarditis is rare in COVID-19 autopsies: cardiovascular findings across 277 postmortem examinations. Cardiovasc Pathol. 2021;50:107300.
15. Linschoten M, Peters S, van Smeden M, Jewbali LS, Schaap J, Siebelink HM, et al. Cardiac complications in patients hospitalised with COVID-19. Eur Heart J Acute Cardiovasc Care. 2020;9(8):817-23.

International Journal of Cardiovascular Sciences. 2021; 34(2):116-121

ORIGINAL ARTICLE

Atrial Fibrillation and Use of Rivaroxaban: Performance of the Prothrombin Time / INR as a Function of Time After Blood Collection

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Abstract

Background: Traditionally, the most effective therapy in the prevention of stroke in patients with atrial fibrillation (AF) has been oral anticoagulation with vitamin K inhibitors, particularly warfarin, whose disadvantages and adverse effects have led to their replacement by "direct oral anticoagulants", as factor X inhibitor.

Objectives: This study aimed to conduct a brief approach on atrial fibrillation (AF) and use of Rivaroxaban, and to comparatively evaluate the prothrombin time / International Normalized Ratio (PT/INR) in patients with AF in use of this oral anticoagulant, depending on the time elapsed between the last administration of the drug and the time of blood sample venipuncture.

Methods: We evaluated 34 patients with AF in use of Rivaroxaban by using PT / INR, distributed into a subgroup with blood collection time ≤ 12 hours (n = 7) and > 12 hours after the last drug intake (n = 27). Mann-Whitney test was used to compare the groups and $p < 0.05$ was considered significant.

Results: An analysis as a function of time between the Rivaroxaban intake and blood collection, revealed that PT / INR suffers the greatest effect up to 12 hours after ingestion of the drug, dropping to levels close to normal in subsequent hours before the next dose.

Conclusion: We concluded that, in contrast to warfarin, the knowledge of the time interval between drug intake and blood collection from patients taking Rivaroxaban is essential to properly interpret a laboratory test to assess hemostasis, particularly PT and its derivatives. (Int J Cardiovasc Sci. 2021; 34(2):116-121)

Keywords: Atrial Fibrillation; Stroke; Embolism; Rivaroxaban; Warfarin; Anticoagulants; Hemorrhage/ physiopathology; Risk Assessment; Blood Coagulation.

Introduction

Patients with atrial fibrillation (AF) present hemostatic system changes that contribute to an approximately 5-fold increased risk of stroke. According to the American Heart Association,[1] the American College of Chest Physicians,[2] and the European Society of Cardiology (ESC),[3] recommendations for antithrombotic therapy of patients with non-valvular and chronic AF should be based fundamentally on the measurement of the prothrombotic risk factors of each patient, which determines the existence of different levels of risk among the different groups of patients. Traditionally, the most effective therapy in preventing stroke in patients with AF has been oral anticoagulation with vitamin K inhibitors - also referred to as vitamin K antagonists (VKAs), especially warfarin. Although this drug has an established efficacy, a number of genetic and environmental factors - such as

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diet and concomitant drug use - influence the patient's response to the drug, leading to a high risk of bleeding and, thus, requiring frequent monitoring of coagulation.[4] For this purpose, the International Normalized Ratio (INR), obtained using the International Sensitivity Index (ISI), has been used based on the comparison of the sensitivity of commercial thromboplastin in relation to WHO standard thromboplastin.

A number of drawbacks related to the need for strict laboratory monitoring and the occurrence of hemorrhages in the course of warfarin anticoagulation have limited its use in clinical practice and encouraged its replacement with newly developed oral anticoagulants including direct thrombin inhibitors and factor Xa inhibitors,[4] called direct oral anticoagulants (DOACs).[5]

Four DOACs (dabigatran, rivaroxaban, apixaban and edoxaban) have become available for treatment of patients with AF. These drugs have a comparable efficacy to warfarin in large randomized controlled trials and were found to pose a lower risk of intracranial bleeding.[6] DOACs have been widely used for primary or secondary prevention of stroke in patients with AF, while AF ablation under uninterrupted warfarin or rivaroxaban has shown a safety profile.[7,8,9] However, there is no evidence of benefit of DOACs in patients with mechanical prosthetic valves or moderate/severe mitral stenosis.[6]

Direct thrombin inhibitors and factor Xa inhibitors do not usually require laboratory monitoring. However, standardized and well-established tests for laboratory evaluation of the anticoagulant effect of these drugs are not yet available when needed in special occasions in the usual medical practice.[10] Therefore, greater knowledge of this topic should be sought by evaluating hemostatic tests that are routinely used in clinical laboratories, such as prothrombin time (PT), in patients using direct oral anticoagulants since such hemostatic parameters may be extensive and variably affected by these drugs.[11]

Specific tests include the anti-factor Xa chromogenic assay, which has been suggested for laboratory evaluation of Rivaroxaban and is able to estimate the plasma concentration of the drug when specific calibrators are used. This method has been shown to be adequate for determination of Rivaroxaban over a wide concentration range, but it is not routinely available in most laboratories.[12,13]

In order to provide a small contribution in this context, the present study aimed to comparatively evaluate PT/INR in patients with AF using Rivaroxaban, according to the time elapsed between the last administration of the drug and the time of collection of blood samples from the patient.

Methods

We evaluated 34 patients with AF using Rivaroxaban (daily dose of Xarelto® 20mg) divided into subgroups with collection time ≤ 12 hours (n = 7) and collection time > 12 hours after the last administration of the drug (n = 27). The use of the drug was investigated and confirmed by patient self-report. The project was approved by the Research Ethics Committee of the *Universidade Federal de Minas Gerais* and the Lifecenter Hospital Ethics Committee. Individuals selected as participants were informed of the research objectives and signed a free and informed consent form prior to blood collection. For each participant, a standardized clinical record was also filled out, whose data were essential for the analysis of results of the present study. The sample size of both the study group and its subgroups was defined for convenience.

Blood samples of control individuals (not in use of anticoagulant or other medication with effects on the hemostatic system), who were selected by the medical team or by the researchers, were used in parallel to test the quality of the reagents for PT, and whose results were found within normal limits according to the manufacturer.

Samples of 10 mL of venous blood (5 mL in 3.2% sodium citrate and 5 mL without additive) were collected using the Vacutainer® System (Becton-Dickinson) tubes in the morning, requiring a 12-hour fast. Blood samples were rapidly centrifuged at 3500 rpm for 15 minutes in room temperature. Plasma samples were transferred to plastic tubes and then centrifuged again under the same conditions to ensure platelet-poor plasma (<10 000/mm^3). The serum and plasma obtained were divided into several aliquots and stored at -80°C until use.

Regarding the inclusion criteria, the case group of the present study included patients with paroxysmal, persistent, or permanent non-valvular AF at medium or high risk of thromboembolic events (CHADS2 ≥ 2), men and women, aged over 18 years and with a history of AF of any duration, documented by electrocardiogram and/or 2-dimensional doppler echocardiogram within 12 months prior, and for whom anticoagulation was indicated. Patients with transient AF secondary to other reversible disorders (thyrotoxicosis, cardiac or

thoracic surgery, pneumonia, and severe anemia), situations in which chronic anticoagulation therapy was interrupted during the duration of the present study; any contraindication for anticoagulant agents; conditions associated with elevated risk of bleeding (intracranial, intraocular, spinal, retroperitoneal, and intra-articular bleeding, gastrointestinal bleeding, or active ulcer within the previous year; recent severe trauma, major surgery and active infective endocarditis), uncontrolled hypertension (blood pressure greater than 170/100 mmHg), or acquired or hereditary hemorrhagic disorders; association of antiplatelet therapy or fibrinolytic therapy with the use of oral anticoagulants; amiodarone therapy, verapamil, quinidine, ketoconazole, ritonavir, corticosteroids, anti-inflammatories, heparin, fondaparinux, or hormone replacement therapy; hepatic, malignant, autoimmune, thyroid, and infectious diseases; severe renal insufficiency (creatinine clearance less than 30 mL/min); alcoholism; pregnancy, or any relevant laboratory or clinical change detected by the physician investigation were excluded.

PT and INR

PT was measured by automated coagulometric method, using the Destiny Max™ diagnostic system, and TriniCLOT PT Excel S, ISI = 1.2 (TCoag, Wicklow, Ireland) as a reagent.

Biochemical Characterization of Participants

In order to verify the presence and absence of comorbidities that could influence the results of PT/INR, a biochemical characterization of participants was undertaken, including determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatinine, triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and C-reactive protein (CRP).

Statistical Analysis

Statistical analyses were performed by the MiniTab program (version 17). Graphs were plotted (scatter plotting) using GraphPad Prism®, version 6.07. Because PT and INR did not show a normal distribution, the results were presented as median and interquartile range. The Mann-Whitney test was used to compare the groups, and $p < 0.05$ was considered significant.

Results

Regarding the biochemical characterization of patients performed to rule out comorbidities that could influence the results of PT and its derived parameters, no significant changes were detected, that is, hepatic, renal and lipid profiles were within the reference limits.

The comparison of the parameters of PT and INR in patients with AF using Rivaroxaban as a function of the time elapsed between the last dose of the drug and the moment of blood collection is presented in Table 1.

PT, in seconds, was considerably higher in patients whose Rivaroxaban administration was ≤12h, suggesting that the test was sensitive to detect anticoagulant effect. As a consequence, prothrombin activity, evaluated in terms of median and interquartile range was lower in this group. INR also appeared to be different between subgroups and was significantly higher in the ≤12h subgroup, although this ratio is not considered a reliable mode of expression in the context of Rivaroxaban use. The results are shown in Figure 1 and Figure 2.

Discussion

Markers of hepatic and renal function and lipid profile were evaluated in the present study, whose results were normal. This fact is important since it reflects that the results of PT/INR were not changed by liver or kidney diseases. Thus, changes detected in PT/INR are very likely to be attributed to the use of Rivaroxaban, since the patients also did not present other characteristics that could compromise the results, such as weight extremes, for example.

Table 1 - Prothrombin time in patients with atrial fibrillation using rivaroxaban, according to the time between administration of the drug and blood collection

	Time ≤12h (n = 7)	Time >12h (n = 27)	*p value**
PT (s)	32.9 (15.5)	20.5 (7.4)	0.003
INR	2.69 (0.45)	1.54 (0.56)	0.003

**p <0.05. Mann Whitney Test. Values presented as median and interquartile range. PT(s): prothrombin time (seconds). INR: International Normalized Ratio.*

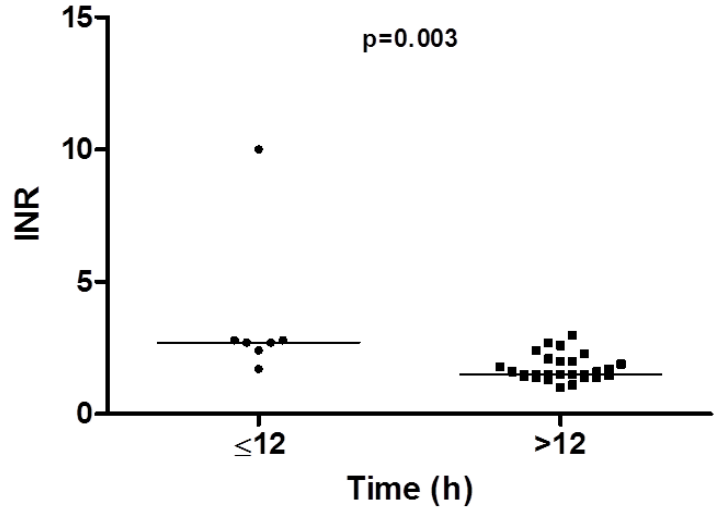

Figure 1 – Prothrombin time of patients in use of Rivaroxaban, as a function of time of blood collection after administration of the drug. The horizontal lines represent the median of each subgroup (Mann Whitney Test).
INR: International Normalized Ratio.

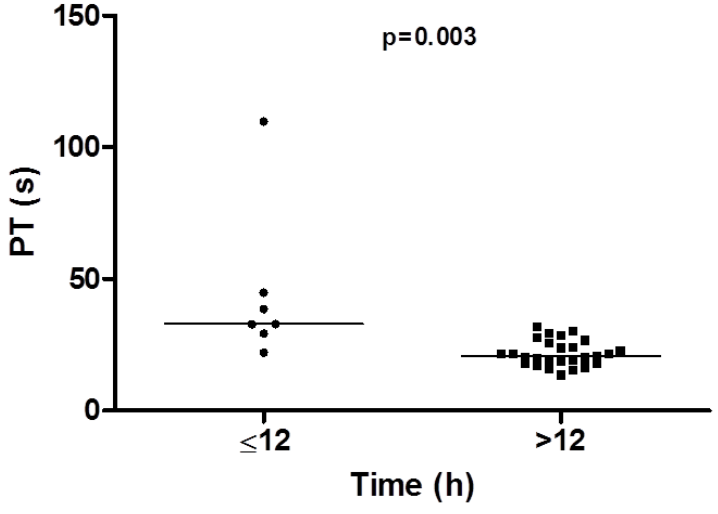

Figure 2 – INR of patients in use of Rivaroxaban, as a function of time of blood collection, after administration of the drug. The horizontal lines represent the median of each subgroup (Mann Whitney Test).
PT(s): prothrombin time (seconds).

The levels of C-reactive protein (CRP) were also evaluated, which were not different between the subgroups, indicating no differences in the inflammatory status for the participants of this study. Regarding the use of other drugs, such as statins or antihypertensives, the limited sample size prevented appropriate analyses.

The prolongation of PT observed in the present study in patients using Rivaroxaban, in relation to those found for healthy non-medicated individuals is in line with previous studies reported in the literature, such as that by Douxfils et al.[9] According to these studies, PT can be used as a screening test to assess bleeding risk, although more specific tests using calibrators should be applied to confirm the plasma concentration of Rivaroxaban. In this regard, ISTH basically defines 2 types of tests for the evaluation of direct oral anticoagulants: simple semiquantitative tests available in most laboratories, and quantitative tests capable of accurately reporting levels of the drug. The effect of Rivaroxaban has been investigated on both types of tests in order to provide evidence to support the standardization of the laboratory evaluation of this drug. It is emphasized that the use of Rivaroxaban does not require frequent monitoring or periodic dose adjustment, however, the need for standardized sensitive and safe laboratory tests (qualitative and quantitative) is unquestionable, which allows the evaluation of the degree of anticoagulation or determination of optimal dose in specific situations.[14] These include, for example, evaluation of special populations of patients, such as those with hepatic or renal insufficiency, weight in extreme ranges, imminent surgery, and cases of therapeutic failure or suspected non-adherence to treatment.[10] In these circumstances, it is necessary to know the performance of the tests available in the investigation of the hemostatic status of the individual.

Although a very limited number of samples were assayed, our findings indicate that at the plasma peak of the drug (around 2 to 3 hours), PT is quite sensitive to evaluate the effect of Rivaroxaban.

However, close to the administration of the subsequent dose (after 24 hours), this test is no longer significantly prolonged as a function of the reduced anticoagulant plasma levels. It should be noted that the result of PT is largely influenced by the quality of thromboplastin employed, whose variability is not corrected when expressing the result in INR. The development of a validated sensitivity index for Rivaroxaban and the consequent use of an INR specific for this anticoagulant have already been proposed to minimize the variability among the reagents.[15]

In summary, the results of PT and its derivatives in the present study suggest a sensitivity of the test to detect the anticoagulant effect up to 12 hours after drug ingestion, dropping to values close to normal in the subsequent hours before the next dose. However, for a greater test reliability it would be necessary to develop a thromboplastin sensitivity index to be used, validated for Rivaroxaban.

Conclusion

In agreement with Douxfils et al.[14] and Samama et al.[16], it is concluded that, in contrast to warfarin, knowledge of the time interval between drug administration and blood collection in patients using Rivaroxaban is essential to interpret a laboratory test that evaluates hemostasis, particularly PT and its derivatives. Therefore, it is essential to be aware that the results of PT will be affected as a function of the time of blood collection after taking the drug.

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Author Contributions

Conception and design of the research: Duarte RCF, Moreira PSS, Ferreira CN, Carvalho MG. Acquisition of data: Duarte RCF, Moreira PSS, Figueiredo EL, Sternick E, Silveira FR, Mota APL. Analysis and interpretation of the data: Duarte RCF, Moreira PSS, Ferreira CN, Alves LCV, Carvalho MG. Statistical analysis: Reis, EA, Duarte RCF, Moreira PSS. Obtaining financing: Carvalho MG, Reis HJ. Writing of the manuscript: Duarte RCF, Moreira PSS. Critical revision of the manuscript for intellectual content: Carvalho MG, Reis HJ.

Potential Conflict of Interest

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

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Study Association

This article is part of the thesis of master submitted by Priscila Samara Sérgio Moreira, from *Universidade Federal de Minas Gerais – Faculdade de Farmácia*.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the *Universidade Federal de Minas Gerais* (COEP-UFMG) under the protocol number CAAE 12603413.0.0000.5149. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

References

1. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland Jr. JC, et al. ACC/AHA Task Force Members, 2014 AHA/ACC/HRS Guideline for the Management of Patients with Atrial Fibrillation: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. 2014; 64(21):2246-80.
2. You JJ, Singer DE, Howard PA, Lane DA, Eckman MH, Fang MC, et al. American College of Chest Physicians. Antithrombotic Therapy for Atrial Fibrillation: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2):e531S-575S.
3. Camm AJ, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, et al. ESC Committee for Practice Guidelines (CPG), 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation. Developed with the special contribution of the European Heart Rhythm Association. Eur Heart. J. 2012;33(21):2719-47.
4. Al-Khatib SM, Alexander JH, Lopes RD, Mahaffey KW, Patel MR, Granger CB. Promise of Factor Xa Inhibition in Atrial Fibrillation. Curr Cardiol Rep. 2012;14(1):70-8.
5. Barnes GD, Ageno W, Ansell J, Kaatz S. Subcommittee on the Control of Anticoagulation of the International Society on Thrombosis and Haemostasis, Recommendation on the nomenclature for oral anticoagulants: communication from the SSC of the ISTH. J Thromb Haemost. 2015;13(6):1154-6.
6. Ferrari F, da Silveira AD, Martins VM, Franzoni LT, Zimerman LI, Stein R. Direct-Acting Oral Anticoagulants in Atrial Fibrillation: What's New in the Literature. Cardiol Rev. 2020 Apr 23. ID mdl 32332235
7. Silva MA, Futuro GMC, Merçon ES, Vasconcelos D, Agrizzi RS, Elias Neto J, Kuniyoshi R. Safety of Catheter Ablation of Atrial Fibrillation Under Uninterrupted Rivaroxaban Use. Arq Bras Cardiol. 2020;114(3):435-42.
8. Kohsaka S, Katada J, Saito K, Jenkins A, Li B, Mardekian J, Terayama Y. Safety and effectiveness of non-vitamin K oral anticoagulants versus warfarin in real-world patients with non-valvular atrial fibrillation: a retrospective analysis of contemporary Japanese administrative claims data. Open Heart. 2020;7(1):e001232.
9. Salmasi S, Loewen PS, Tandun R, Andrade JG, De Vera MA. Adherence to oral anticoagulants among patients with atrial fibrillation: a systematic review and meta-analysis of observational studies. BMJ Open. 2020;10 (4):e034778.
10. Favaloro EJ, Lippi G. The new oral anticoagulants and the future of haemostasis laboratory testing. Biochem Med. 2012;22(3):329-41.
11. Tripodi A. The Laboratory and the New Oral Anticoagulants. Clin Chem. 2013; 59 (2): 353–362.
12. Douxfils J, Mani H, Minet V, Devalet B, Chatelain B, Dogné JM, et al. Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations. Biomed. Res Int. 2015(Special issue):345138.
13. Sarode R. Direct oral anticoagulant monitoring: what laboratory tests are available to guide us? Hematology Am Soc Hematol Educ Program. 2019; 2019 (1):194-7.
14. Douxfils J, Mullier F, Loosen C, Chatelain C, Chatelain B, Dogné JM. Assessment of the impact of rivaroxaban on coagulation assays: laboratory recommendations for the monitoring of rivaroxaban and review of the literature. Thromb Res. 2012;130(6):956-66.
15. Tripodi A, Chantarangukul V, Guinet V, Samama MM. The International Normalized Ratio calibrated for rivaroxaban has the potential to normalize prothrombin time results for rivaroxaban-treated patients: results of an in vitro study. J Thromb Haemost. 2011;9(1):226-8.
16. Samama MM, Contant G, Spiro TE, Perzborn E, Le Flem L, Guinet C, et al. Laboratory assessment of rivaroxaban: a review, Thromb. J. 2013;11(1):11.

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EDITORIAL

Rivaroxaban: is it Really Need to Monitor its Anticoagulant Effect in Clinical Practice?

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Editorial referring to the article: Atrial Fibrillation and use of rivaroxaban: performance of the prothrombin time/ INR as a function of time after blood collection

Oral anticoagulation (OAC) has been the cornerstone for the treatment of atrial fibrillation (AF) patients. The vitamin K antagonist warfarin has been considered the drug of choice in stroke prevention with proven efficacy for more than 60 years. The great inter-patient and intra-patient dose variability and need for routine International Normalized Ratio (INR), are among the main disadvantages of warfarin.[1] The direct-acting oral anticoagulants (DOACs), including direct thrombin inhibitors (dabigatran) and factors Xa inhibitors (apixaban, edoxaban, and rivaroxaban), are currently the therapy of choice for preventing thromboembolic events in patients with atrial fibrillation. Unlike warfarin and other vitamin K antagonists, the DOACs are administered in fixed doses and do not require routine laboratory monitoring.[2]

Rivaroxaban is an oral inhibitor of free and clot-associated factor Xa through reversible, competitive interactions with its active site. Bioavailability following oral administration is dose-dependent, and it is highly bound to plasma proteins.[3] Plasma levels peak 2 to 4 h following oral administration, partially excreted by the kidneys, and has a half-life depending on the dose and age. Factor Xa inhibitors variably affect prothrombin time (PT), with concentration-dependent PT prolongation.[4] The sensitivity of the different assays varies widely, depending on the thromboplastin reagent, and it is recommended to check the sensitivity of PT in each institution. At therapeutic doses, rivaroxaban has a relatively weak effect on PT, but there is a more pronounced effect on supratherapeutic doses.

However, in some clinical situations, monitoring the anticoagulant effect of these drugs may be important, as in cases with increased risk of bleeding, preoperative state, breakthrough thrombosis, suspected overdose, or drug interactions, and in certain populations, including those with extremes in body weight, in the elderly and patients with renal insufficiency who are exposed to a risk of drug accumulation.[5] In addition, in patients with acute ischemic stroke, the evaluation of the anticoagulant effect is extremely important before thrombolytic therapy.

In 2014, Cuker et al.,[6] published a systematic review and summarized evidence regarding laboratory measurement of the anticoagulant activity of dabigatran, rivaroxaban, and apixaban.[6] Generally, rivaroxaban prolonged PT in a concentration-dependent, and assay results vary markedly with different thromboplastin reagents. A normal PT does not rule out the presence of clinically significant below or within on-therapy rivaroxaban concentrations; however, a prolonged PT qualitatively indicates the drug's presence. The APTT (activated partial thromboplastin time) is not suitable for measuring rivaroxaban due to the nonlinear relationship with rivaroxaban concentration, poor sensitivity, and significant variability between reagents. Inter-assay variability was reduced by the use of an international sensitivity index (ISI) specific for rivaroxaban, but not by conversion to an INR used for monitoring VKA therapy.[7]

In this issue of the International Journal of Cardiovascular Sciences, Duarte et al.,[8] evaluated 34 patients with AF in use of Rivaroxaban by using PT/INR, distributed into a subgroup with blood collection time of 12 h (n=7) and > 12 h after the last drug intake (n=27) .[8] PT, in seconds, was considerably higher in patients whose Rivaroxaban administration was ≤12 h, suggesting that the test was sensitive to detect the anticoagulant effect. As a consequence, prothrombin activity, evaluated in terms of the median and interquartile range was lower

Keywords

Rivaroxaban/therapeutic use; Stroke; Anticoagulants; Pulmonary Embolism; Atrial Fibrillation; Thrombosis.

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in this group. INR also appeared to be different between subgroups and was significantly higher in the ≤12 h subgroup, although this ratio is not considered a reliable mode of expression in the context of Rivaroxaban use. In conclusion, the authors suggested that, in contrast to warfarin, knowledge of the time interval between drug administration and blood collection in patients using Rivaroxaban is essential to interpret a laboratory test that evaluates hemostasis, particularly PT and its derivatives.

In 2018, Woodruff et al.,[9] investigated the association between prothrombin times and bleeding in hospitalized patients receiving rivaroxaban.[9] In their single-center retrospective cohort study, adult patients who had the PT measured within 24 h of rivaroxaban administration were identified and a multivariate logistic regression model was used to quantify the association between PT and bleeding events. In total, 199 patients were identified, of which 41 experienced a bleeding event; patients with a PT ≥ 30 s were more likely to have experienced a bleeding event, than those with a PT < 30 s. This study, however, does not report the exact time after the dose that the PT was measured, so it is difficult to know if like is being compared to like (in terms of time after dose) and therefore, it is again difficult to draw any firm conclusions from this data.

The results of the present study should be analyzed carefully, due to the very small number of patients and the inability to draw an exact conclusion. The question that should be answered is if it is really needed to monitor the DOACs anticoagulant effect in clinical practice. What is the clinical relevance of this approach in daily practice?

The truth is, not many quality real-world studies that assessed DOAC plasma concentrations and outcomes have been published to date.

In order to answer these questions, Rottenstreich et al. have shared their experience of DOAC drug level monitoring in clinical patient management. They aimed to describe the real-life utilization of DOAC levels in practice and its effect on clinical management.[10] They reviewed the data between 2013 and 2017 at their institute in Israel, with 212 patients undergoing 292 DOAC measurements. From the requests made, 82.5% were for selected clinical circumstances, e.g., bleeding or breakthrough thrombosis. The majority of patients (71.9%) had concentrations in the expected range. Where concentrations were higher than expected, multivariate analysis revealed that older age, lower glomerular filtration rate, and lower body mass are significant determinants for these measurements. The authors concluded that whilst no benefit of routine monitoring was observed, drug level measurement has an important role in selected circumstances. Future studies are warranted to establish associations between drug levels and outcomes, and better delineate the role of DOAC monitoring.

In the current scenario, the role of monitoring the anticoagulant effect of DOACs is not yet well-defined, but we know that it can be useful in specific situations, where the risk of bleeding is extremely high or when there is an indication for thrombolytic therapy. However, there is still a lack of scientific evidence on which methods and how the DOACs' coagulant effect should be monitored. Robust scientific studies with a larger number of participants are needed to define when and how to monitor anticoagulation.

References

1. Patel JP, Byrne RA, Patel RK, Arya R. Progress in the monitoring of direct oral anticoagulant therapy. Br J Haematol. 2019;184(6):912-24.
2. Gosselin RC, Adcock DM, Bates SM, Douxfils J, Favaloro EJ, Gouin-Thibault I, et al. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost. 2018;118(3):437-50.
3. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet. 2014;53(1):1-16.
4. Conway SE, Hwang AY, Ponte CD, Gums JG. Laboratory and Clinical Monitoring of Direct Acting Oral Anticoagulants: What Clinicians Need to Know. Pharmacotherapy. 2017;37(2):236-48.
5. Samama MM, Contant G, Spiro TE, Perzborn E, Flem LL, Guinet C, et al. Evaluation of the prothrombin time for measuring rivaroxaban plasma concentrations using calibrators and controls: results of a multicenter field trial. Clin Appl Thromb Hemost. 2012;18(2):150-8.
6. Cuker A, Siegal DM, Crowther MA, Garcia DA. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. J Am Coll Cardiol. 2014;64(11):1128-39.
7. Ofek F, Bar Chaim S, Kronenfeld N, Ziv-Baran T, Berkovitch M. International Normalized Ratio Is Significantly Elevated With Rivaroxaban and Apixaban Drug Therapies: A Retrospective Study. Clin Ther. 2017;39(5):1003-10.
8. Duarte RC, Moreira P, Ferreira C, Figueiredo E, Sternick E, Silveira F et al. Atrial Fibrillation and use of rivaroxaban: performance of the prothrombin time/ INR as a function of time after blood collection. Int J Cardiovasc Sci. 2021; 34(2):116-121.
9. Woodruff AE, Wovkulich MM, Mogle BT, Hassan AK. Association between prothrombin time and bleeding in hospitalized patients receiving rivaroxaban. Am J Health Syst Pharm. 2018;75(22):1783-9.
10. Rottenstreich A, Zacks N, Kleinstern G, Raccah BH, Roth B, Da'as N, et al. Direct-acting oral anticoagulant drug level monitoring in clinical patient management. J Thromb Thrombolysis. 2018;45(4):543-9.

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ORIGINAL ARTICLE

Maternal Intake of Flaxseed During Lactation and Exercise Training Protect Against Salt Overload-Induced Aortic Remodeling in Adult Offspring

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Abstract

Background: High dietary sodium intake can induce endothelial stiffness even without changes in blood pressure.

Objectives: To evaluate the effects of exercise training and chronic intake of sodium chloride solution on aortic morphology of male offspring of rat dams who consumed flaxseed during lactation.

Methods: Female rats were fed with a control diet or a flaxseed diet during lactation. At weaning, two male offspring of each rat dam were allocated into eight groups for 180 days: four groups received a control diet e four received a flaxseed diet, with /without exercise and with/without NaCl solution supply. Aorta was collected for histomorphometric analysis. The one-way analysis of variance was used and P value < 0.05 was considered statistically significant.

Results: The chronic use of 1% NaCl solution led to changes in aortic histoarchitecture in the control group: increase in aortic intima-media thickness (10,4%, p<0.0001) and reduced number of elastic lamellae (-8,1%, p<0.0001). Groups of offspring of mother that consumed flaxseed during lactation, the chronic use of 1% NaCl alone did not lead to an increase in the aortic intima-media thickness. Exercise training of adult offspring increased aortic intima-media thickness (13.3%, p<0.0001), with preservation of elastic components and aortic flexibility.

Conclusion: Chronic salt overload caused adverse effects on the aorta of rats, and maternal consumption of the flaxseed diet during lactation protected against aortic remodeling. (Int J Cardiovasc Sci. 2021; 34(2):124-133)

Keywords: Metabolism/physiology; Fatty Acids,Omega-3; Rats; Exercise; Lactation; Flaxseed Diet.

Introduction

There is evidence of the association of high sodium intake with the development of chronic non-communicable diseases (NCDs).[1] High dietary sodium intake can adversely affect various organs and target tissues, such as the vasculature and heart, and some studies in rodents have demonstrated impaired endothelial function in chronic sodium intake, even without changes in blood pressure.[2–5]

High serum sodium increases endothelial stiffness and impairs endothelium-dependent vasodilation, and small changes in serum sodium concentration can induce endothelial stiffness in a matter of minutes. Sodium reduces the size of endothelial cells and their surface area, volume, cytoskeleton, deformability and flexibility. In addition, sodium also reduces nitric oxide (NO) endothelial synthase (eNOS) and NO production and increases transforming growth factor β (TGF-β), thus increasing arterial stiffness.[6]

Although sodium intake required to maintain homeostasis in adults is extremely low (<500 mg),[2] the amount consumed by the world population is nearly 3,950 mg sodium / day.[7] Reduction of salt intake has been identified as one of the most cost-effective interventions to reduce the load of NCDs, with the potential to save

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millions of lives every year.[1] Along with proper eating habits, regular exercise training has been considered one of the main mechanisms to protect against the onset and progression of predisposing risk factors to cardiovascular diseases and other NCDs.[8,9]

Studies have shown that diets containing food with functional properties, such as the flaxseed, during pregnancy and lactation can induce metabolic programming in the offspring due to its interference in the hormonal system [10] and the ability of omega-3, present in this oleaginous, crossing the placenta and incorporating itself into the cellular membrane, altering its properties.[11] Regarding vascular health, some studies using flaxseed during pregnancy, lactation and post-weaning, reported cardioprotective effect, showing decreased aortic thickness.[12–14]

Flaxseed is the main plant source of n-3 fatty acid and its use has become popular due to its health benefits. It is also a source of soluble and insoluble fiber, lignan and protein, and contains 41% lipids, of which 50-55% are alpha-linolenic acid (n-3) and 15-18% linoleic acid (n-6).[15,16]

The study aimed to evaluate the effects of exercise training and chronic sodium intake on the aorta morphology of male offspring of rat dams fed a flaxseed flour diet during lactation.

Material and Methods

Animals

The animal protocol was approved by the Animal Ethics Committee of the Fluminense Federal University (Protocol Number CEA 882/2016), and the procedures were in accordance with the guidelines for experimentation with animals (NIH Publication Nº. 85-23, revised 1996). Female wistar rats (3 months old) from the Centre of Laboratory Animals of Fluminense Federal University were housed under a controlled temperature (21 ± 1ºC), humidity (60 ± 10%) and 12 h light / dark cycle, with free access to water and food.

Experimental Design

Initially, 24 female rats were mated with 12 males (2:1 ratio) and fed a commercial rodent chow (Nuvilab®, Nuvital Ltda, Paraná, Brazil). During pregnancy, all rats were kept in individual cages. After the offspring was born, for the lactation period, the rat dams were divided into two groups (n=12 per group), following the order that the female rats gave birth: the first rat to give birth was allocated into one group, the second was allocated to the other, successively. A control group (CG) was fed a control diet and filtered water, and the flaxseed group (FG) received the diet added with 25% brown flaxseed flour, and filtered water. Diets were manufactured and stored as pellets at 4 °C in agreement with the American Institute of Nutrition (AIN-93G) recommendations for rodent diets[17] (Table1). A concentration of 25% of flaxseed flour in the diet (25g/ 100g diet) would meet the recommended fiber intake. The addition of oil was not necessary, as the seed itself is a source of oil.

At weaning, two male offspring from each mother started to receive commercial rodent chow with a normal lipid and protein content (Nuvilab®, Nuvital

Table 1 - Nutritional composition of the experimental diets given to the rat dams during lactation

	Diets	
Nutrients (g/kg)	**Control**	**Flaxseed Flour**
Casein (≥85% of protein)	190	141
Corn starch	539.5	458.5
Sucrose	100	100
Soybean oil	70	0
Flaxseed	0	250
Fiber (cellulose)	50	0
Vitamin mix (AIN-93G)	10	10
Mineral mix (AIN-93)	35	35
L-Cystine	3	3
Choline	2.5	2.5
Tert-butylhydroquinone	0.014	0.014
Total	1000	1000
Carbohydrate (% of total kcal)	**64**	**59**
Protein (% of total kcal)	**19**	**19**
Fat (% of total kcal)	**17**	**22**
Energy (Kcal/kg)	**3950**	**4009**

AIN: American Institute of Nutrition, Casein: mineral and vitamin mix, L-cystine and choline bitartrate: Pragsoluções®; cornstarch and fiber: FARMOS®; soybean: Liza®; commercial sucrose: União®; flaxseed flour: Mãe terra®. Formulated based on the AIN-93G recommendations for rodent diets

Ltda, Paraná, Brazil), containing 22% protein (main protein sources are meat, fish, soy and amino acids), 66% carbohydrate and 11% lipid and allocated into one of the eight experimental groups with six animals each one (n=6) until they were 180 days old:

- Control group (CG), offspring of rat dams who received control diet during lactation;
- Exercise CG (ECG), offspring of rat dams who received control diet during lactation and underwent exercise training;
- Sodium chloride CG (NaClCG), offspring of rat dams who received control diet during lactation and were hydrated with 1% NaCl solution;
- Exercise sodium chloride CG (ENaClCG), offspring of rat dams who received control diet during lactation and were hydrated with 1% NaCl solution and underwent exercise training;
- Flaxseed group (FG), offspring of rat dams who received flaxseed flour diet during lactation;
- Exercise FG (EFG), offspring of rat dams who received flaxseed flour diet during lactation and underwent exercise training;
- Sodium chloride FG (NaClFG), offspring of rat dams who received flaxseed flour diet during lactation and were hydrated with 1% NaCl solution; and
- Exercise sodium chloride FG (ENaClFG), offspring of rat dams who received flaxseed flour diet during lactation and were hydrated with 1% NaCl solution and underwent exercise training.

The number of six animals per group followed the 3 Rs principle, which aims to reduce the number of animals used in the experiment. According to Damy et al.,[18] the reduced number of animals in biomedical research does not affect the detection of biological effects.

Body Mass, Feed and Sodium Intake, Water and 1% NaCl Solution Intake

For analysis of body mass gain throughout the study, body mass of the groups was measured once a week on a digital scale (precision 0.01g) (Filizola®). Food intake of the groups was measured three times a week. For feed intake calculation, individual intake was obtained by subtracting the remaining feed from the amount offered. Water and 1% NaCl solution intake were measured three times a week with the aid of a graduated test tube with 0.5mL precision. The amount supplied and the amount remained were quantified individually to determine water and NaCl intake. Sodium intake calculation was made adding the sodium intake from the 1% NaCl solution with the sodium contained in the commercial rat chow given to the animals (Nuvilab®, Nuvital Ltda, Paraná, Brazil) (2,700 mg Na / kg of rat chow).

Exercise Training

At 90 days of life, exercise training was performed on a motorized treadmill (AvsProjetos®, Brazil) for 12 weeks, 5 times a week for 60 min each session, in individual lanes. Exercise training was started at a speed of 0.3 km/h in the first session, increasing progressively to a final speed of 1.1 km / h, according to each animal's performance. This exercise intensity corresponded to 70% to 80% of the maximum VO_2.[19]

Blood Pressure Measurement

When the animals were three months of age, systolic blood pressure (SBP) was measured (in mmHg) once a month until six months of age, using the non-invasive method of tail-cuff plethysmography (tail plethysmograph V1.10-Insight, Brazil).

After preconditioning in a chamber, the animals were pre-heated in the chamber to 35±2°C for five minutes. The official SBP of each animal was calculated by averaging three consecutive stable measurements (with a difference of about one minute among them).

Histomorphometric Analysis of the Aorta

At the end of the experimental protocol (180 days), after a 6-hour fasting period, the animals were anesthetized with a solution containing 80mg of ketamine (10% ketamine hydrochloride, Syntec Tecnologia Farmacêutica Aplicada à Medicina Veterinária, Brazil) and 10mg of xylazine (2% xylazine hydrochloride, Syntec Tecnologia Farmacêutica Aplicada à Medicina Veterinária, Brazil); a thoracotomy was performed and the heart and the aorta were removed. The artery was dissected from the aortic arch, and the aorta was sectioned transversely with transverse cuts in the distal end of the sections. Later, the pieces of aorta were fixed in buffered formalin at 10% (Millonnig formalin) for 24 hours, and processed with a standard technique of paraffin inclusion, as described by Pereira et al.[25] Subsequently, the paraffin blocks containing the pieces of aorta were cut using a CUT 4050 microtome (Microtec®), in sections of 5μm, stained with hematoxylin and eosin, and Weigert's Resorcin-Fuchsin for later evaluation.

The measurements were made using the ImageJ® software, with digital images obtained by the cellSens program with an optronics digital video camera and BX51 Olympus microscope. The images were captured at 4x magnification for measurement of the area, at 40x for analysis of thickness, and at 20x for quantification (%) of elastic lamellae and elastic fiber. All images were digitalized in *.tiff* format. The aortic wall area was measured by the difference between the external and internal areas of the arterial wall. For intima-media thickness, four different regions of the same diametrically opposite cut were analyzed for better precision. The number of lamellae was quantified in four different regions of the same cut, and the aortic elastin content (%) was determined by analysis of Weigert's Resorcin-Fuchsin slides using the ImageJ® software with a 20x magnification using the plugin Color Segmentation.

Statistical Analysis

Data were presented as mean ± standard deviation. Data were tested for normality and homogeneity of variances (Kolmogorov-Smirnov test) and the differences among groups were tested, when appropriated, with one-way analysis of variance (ANOVA), followed by a Holm-Sidak post hoc test or non-parametric Kruskal-Wallis test, followed by Dunn's post hoc test. Variables that did not present normal distribution were presented as median and interquartile range (box plot graphs). Regarding body mass and blood pressure, the two-way ANOVA test was performed, followed by the Bonferroni post hoc test. *P* value < 0.05 was considered statistically significant (GraphPad Prism v. 6.01 for Windows, GraphPad Software, San Diego, CA, USA).

Results

Body Mass, Food Intake, Water and 1% NaCl Solution Intake

At weaning (21 days of age), no difference was found in body mass between the groups, however, there was a tendency of the offspring of the dams of the flaxseed group to be smaller than control offspring (-16.7%, p=0.0686). Regarding body mass gain, it was noted that the ENaCLFG showed smaller body mass than the CG (-14%, p<0.05) and the ECG (-14.9%, p<0.05) from the 12th week and the 16th week, respectively, to the end of the study period. The ENaClFG also showed lower body mass than the NaClCG between weeks 18 and 21 (-10.3%, p<0.05) (Figure 1). Daily feed intake was not different between the groups (p=0.1330) (Table 2). During the study period, the animals receiving 1% NaCl solution had a higher fluid intake than animals receiving water (p<0.0001) (Table 2). Regarding daily sodium intake, the animals of the groups NaClCG, ENaClCG, NaClFG and ENaClFG consumed a higher amount of sodium than their respective groups that consumed water (+935.5%, +845.3%, +833.4, +795.2%, respectively, p<0.0001) (Table 2).

Systolic Blood Pressure

Chronic consumption of 1% NaCl solution did not increase blood pressure in the study groups, and neither exercise training nor the flaxseed diet affected blood pressure during the lactation period (p=0.5016) (Figure 2).

Histomorphometric Analysis of the Aorta

At the end of the study, no difference was found in aortic wall area (p=0.9364) or the aortic lumen area between the groups (p=0.8817) (Figures 3 and 4). However, the chronic use of 1% NaCl solution and exercise training increased the aortic intima-media thickness in the CG (10.4% and 13.3%, respectively, p<0,0001) either alone or combined (+ 17.7%, p <0.0001). In male offspring of female rats who consumed flaxseed during lactation, the chronic use of 1% NaCl alone did not increase the aortic intima-media thickness (which was not different from the CG and the FG). Nevertheless, exercise training increased the aortic intima-media thickness in the EFG compared to the CG and FG (12.8% and 9.7%, respectively, p <0.0001), and the use of the 1% NaCl solution combined with exercise training also increased aortic intima-media thickness when compared to CG and FG (+ 17.8% and 14.5%, respectively, p <0.0001) (Figures: 3 and 4). Regarding the elastic component of the arterial wall, the chronic use of 1% NaCl solution by the CG caused a reduction in the number of elastic lamellae (-8.1%, p <0.0001), the FG showed a lower number of elastic lamellae than all CGs (p<0,0001), despite that, no difference amongst the groups were observed regarding the amount of elastin in the aortic intima-media (p=0.1629, Figures 3 and 4).

Discussion

The present study showed that maternal intake of a flaxseed diet during lactation associated with exercise

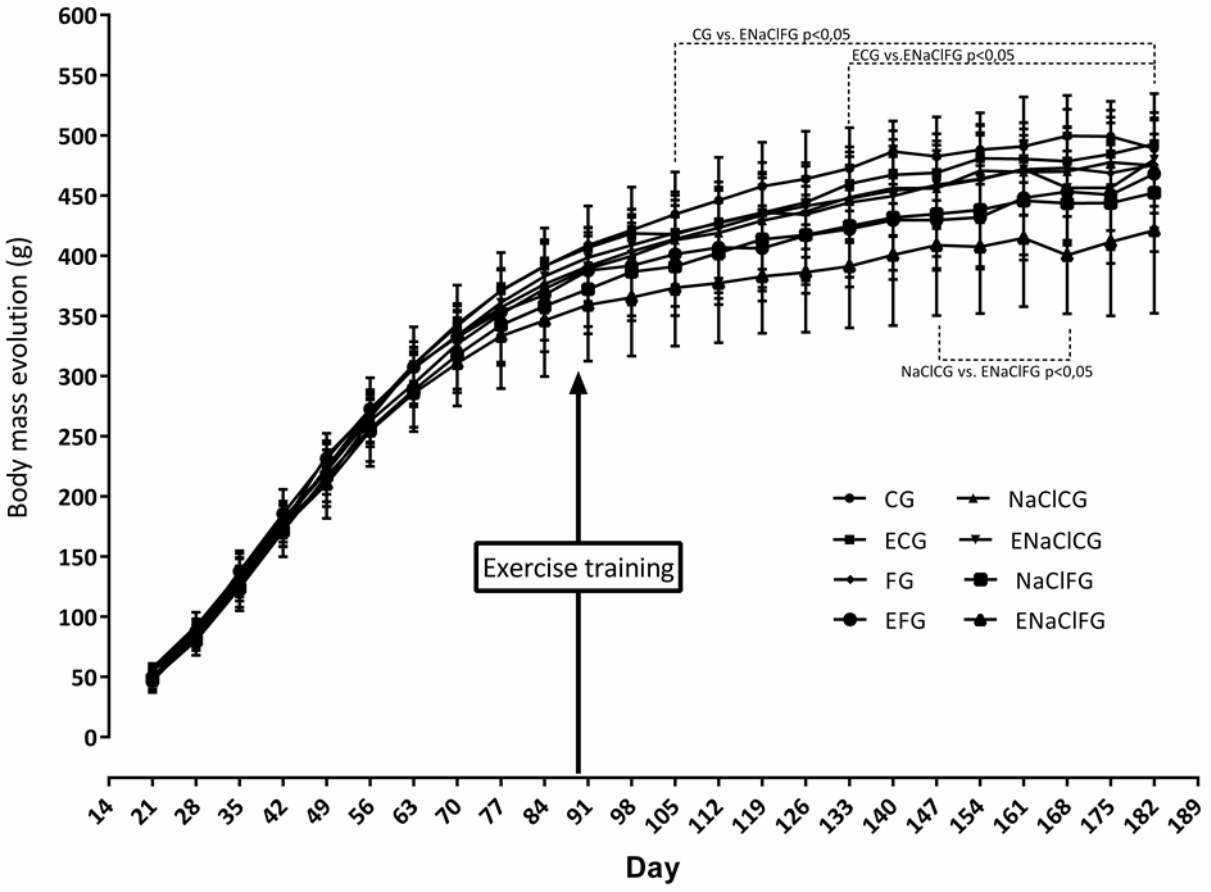

Figure 1 – Body mass evolution throughout the study. The data were presented as mean ± SD, with significance level of p≤0.05 (two-way ANOVA, Bonferroni post-hoc test). CG: control group; NaClCG: Sodium Chloride control group; ECG: Exercise training control group; ENaClCG: Sodium Chloride exercise training control group; FG: Flaxseed group; NaClFG: Sodium Chloride flaxseed group; EFG: Exercise training flaxseed group; ENaClFG: Sodium Chloride exercise training flaxseed group.

Table 2 - Consumption of feed, water, 1% sodium chloride (NaCl) solution and sodium (Na)

Parameters / Groups	CG	NaClCG	ECG	ENaClCG	FG	NaClFG	EFG	ENaClFG	p-value
Diet (g/day/animal)	21.5±3.4	22.6±1.4	23.7±0.9	23.2±1.6	22.7±2.0	22.3±1.6	22.5±1.5	20.5±1.5	0.1330
Water or 1% NaCl solution (mL/day/animal)	31.7±5.8	54.0±2.6*	35.3±2.0†	54.3±6.4*,‡	33.5±4.2†,§	51.2±6.9*,‡,//	33.2±2.9†,§,¶	48.9±3.9*,‡,//,¶,#	<0.0001
Sodium (Na)(mg/day/animal)	58.0±9.3	601.3±30.0*	64.1±2.5†	606.3±69.0*,‡	61.4±5.4†,§	573.1±72.7*,‡,//	60.8±4.3†,§,¶	545.1±40.7*,‡,//,#	<0.0001

*Data were presented as mean ± SD, with significance level of p<0.05 (one-way ANOVA, Holm-Sidak post-hoc test). * ≠ CG; † ≠ NaClCG; ‡ ≠ ECG; § ≠ ENaClCG; // ≠ FG; ¶ ≠ NaClFG; # ≠ EFG; CG: control group; NaClCG: sodium chloride control group; ECG: exercise training control group; ENaClCG: sodium chloride + exercise training control group; FG: flaxseed group; NaClFG: sodium chloride flaxseed group; EFG: exercise training + flaxseed group; ENaClFG: sodium chloride + exercise training + flaxseed group*

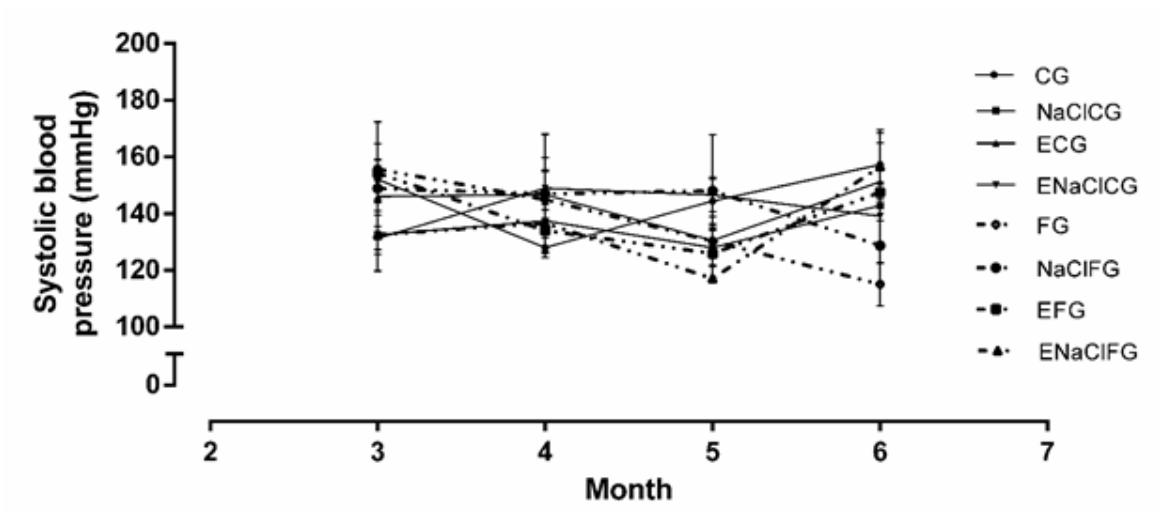

Figure 2 – Monthly systolic blood pressure of study groups. The data were presented as mean ± SD, with significance level of p≤0.05 (two-way ANOVA, Bonferroni post-hoc test). CG: control group; NaClCG: Sodium Chloride control group; ECG: Exercise training control group; ENaClCG: Sodium Chloride exercise training control group; FG: Flaxseed group; NaClFG: Sodium Chloride flaxseed group; EFG: Exercise training flaxseed group; ENaClFG: Sodium Chloride exercise training flaxseed group.

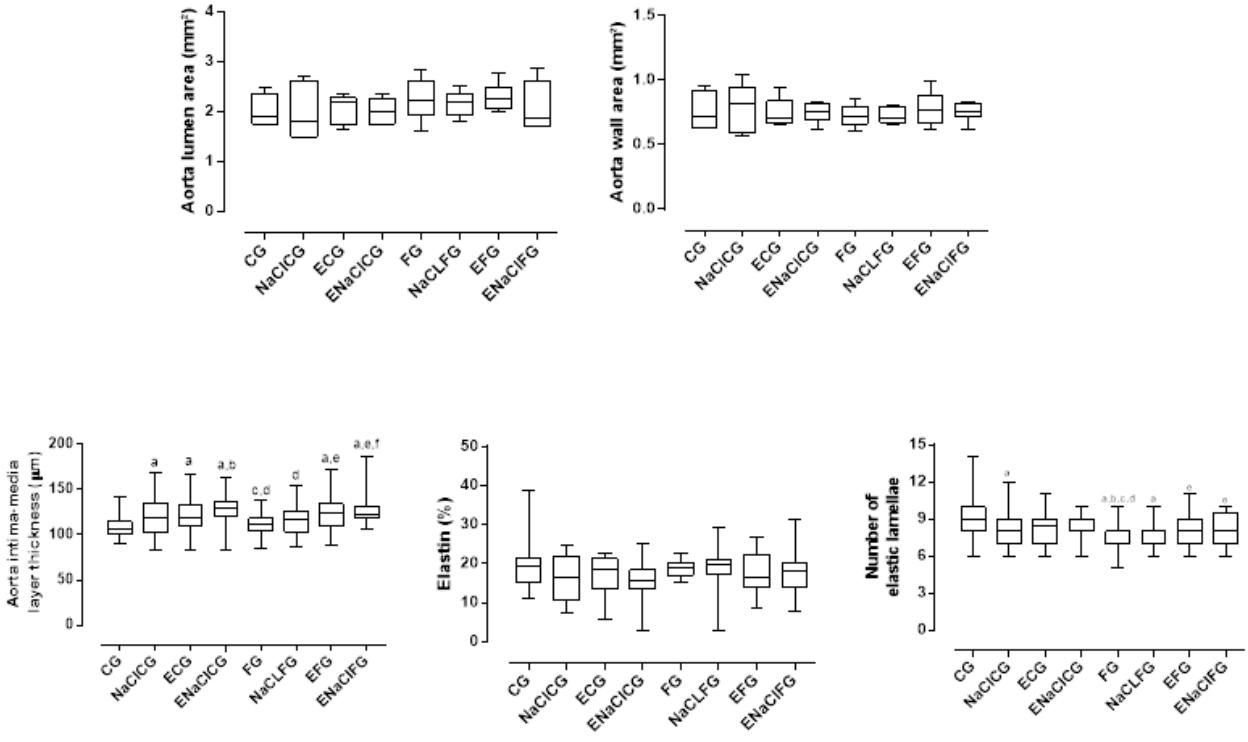

Figure 3 – Aorta histomorphometry of the groups studied at 180 days of age. The data were presented as median and interquartile (Boxplot), with significance level of p<0.05 (one-way ANOVA, Holm-Sidak post-hoc test and Kruskal-Wallis test, Dunn's post-hoc test). A) Aorta wall area, B) Aorta lumen area, C) Aorta intima-media layer thickness, D) Elastic lamellae and E) Elastin. a ≠ CG; b ≠ NaClCG; c ≠ ECG; d ≠ ENaClCG; e ≠ FG; f ≠ NaClFG. CG: Control group; NaClCG: Sodium Chloride control group; ECG: Exercise training control group; ENaClCG: Sodium Chloride exercise training control group; FG: Flaxseed group; NaClFG: Sodium Chloride flaxseed group; EFG: Exercise training flaxseed group; ENaClFG: Sodium Chloride exercise training flaxseed group.

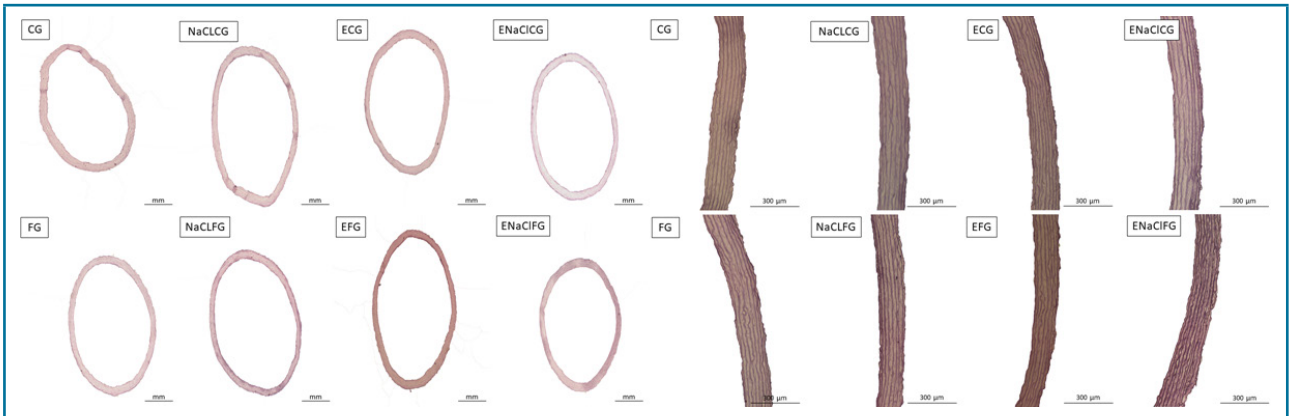

Figure 4 – Microscopic images of the aorta tissue stained with Weigert's Resorcin-Fuchsin in 6- month-old (180 days) male offspring (4 x and 20 x). CG: Control group; NaClCG: Sodium Chloride control group; ECG: Exercise training control group; ENaClCG: Sodium Chloride exercise training control group; FG: Flaxseed group; NaClFG: Sodium Chloride flaxseed group; EFG: Exercise training flaxseed group; ENaClFG: Sodium Chloride exercise training flaxseed group.

training of the offspring resulted in structural changes of the aorta of the offspring in adulthood, regardless of sodium intake.

In a review study on the effects of flaxseed flour intake during pregnancy and lactation on the offspring's body mass at 21 days,[11] it was observed that the consumption of flaxseed flour in a concentration of 25% (25g flour/100g of feed) in the diet did not lead to changes in the animals' body mass at weaning, showing the same body mass as the animals of control rat dams.[21–24] In our study, although no statistically difference was found, a trend to lower body mass in animals of rat dams that consumed flaxseed diet during lactation was found. Other studies [25–27] reported lighter body mass of male offspring of rat dams who consumed flaxseed diet when compared to offspring of rat dams who consumed a control diet. The lower fat mass of these animals is probably the responsible component for the lower body mass at weaning, since animals did not show changes in other compartments of the body.[27] The flaxseed phytoestrogen, secoisolariciresinol *diglucoside* (SDG), can be responsible for this effect. The SDG antiobesity effect may be due to the suppression of genes involved in the synthesis of fatty acids and triglycerides, through the sterol regulatory element binding protein-1c (SREBP-1c) activity. It is well known that estrogen directly inhibits fat deposition by reducing lipoprotein lipase (LPL) activity, an enzyme that regulates adipocyte lipid reabsorption;[28] thus, the lower body mass in the flaxseed group can also be explained, somewhat, by the estrogenic action of the SDG in adipocytes.[11,27]

Flaxseed flour intake by mother during lactation, the practice of regular exercise training after three months of age and consumption of 1% NaCl solution did not lead to changes in feed intake during study time and, hence, the offspring's body mass was similar in most groups throughout the study. Only from the 12th week, the ENaClFG had lower body mass compared to some of the groups. Despite not significantly different, feed intake in this group decreased by 9.4% compared to the other groups, which, in addition to the combination – maternal flaxseed intake during lactation, chronic 1% NaCl solution intake and exercise training – may have contributed to the lower body mass.

Concerning water and 1 % NaCl solution intake, the animals given only saline solution drank more fluid than those given only water, resulting in a higher sodium intake in the former groups. Small increases of 1-2% in the effective osmotic pressure of plasma result in stimulation of thirst in mammals. It has been shown in both human subjects and other mammals, that plasma osmolality is increased experimentally in response to increased concentrations of solutes, such as NaCl, which does not readily pass across cell membranes. Consequently, thirst is stimulated to avoid possible dehydration,[29] which may have occurred in our animals.

Although salt intake in the diet is recognized as a contributing factor to the pathogenesis of hypertension, recent evidence suggests that salt intake in the diet may increase the risk of adverse cardiovascular events regardless of blood pressure.[30,31] This is in accordance with our results, showing that the offspring that received

1% NaCl solution, both of control rat dams and mothers who received the flaxseed diet, showed no increase in blood pressure throughout the study. Similar findings were obtained by offering oral NaCl at 1% [32] or a diet with 1.3% NaCl,[33] which did not result in an increase in systolic blood pressure in normotensive rats. Few studies have investigated the effects of maternal intake of flaxseed flour during lactation on blood pressure in adult offspring. The intake of a high-fat diet added with flaxseed flour during pregnancy and lactation did not lead to changes in systolic blood pressure in male offspring of diabetic rats at 100 days of life,[34] corroborating our results; however, another study reported that the intake of flaxseed flour during pregnancy and lactation led to a blood pressure decrease in the offspring of healthy rat dams.[35] In addition, our study showed that exercise training did not lead to differences in blood pressure between sedentary and trained animals. Studies with sedentary animals and trained animals also presented similar blood pressure levels between groups,[36–38] yet another study showed that chronic exercise training (treadmill) led to a decrease in blood pressure in normotensive rats.[39]

Several lines of evidence suggest that excessive dietary salt intake (NaCl) negatively affects cardiovascular function, regardless of changes in blood pressure. First, population studies have indicated that normotensive humans with a higher level of salt intake are at increased risk for an adverse cardiovascular event. Second, evidence in humans and animal models indicates that excessive dietary salt intake promotes endothelial and microvascular dysfunction.[30] Endothelium has multiple and important roles in physiological and pathophysiological events,[40] and changes in the structure of large arteries, including abnormalities in endothelial function, arterial elasticity, structure and arterial wall thickness, can trigger the onset of cardiovascular disease.[41] Thereby, assessment of large arteries, such as aorta, of lumen area, elasticity changes and intima-media thickness have become important in the investigation of atherosclerosis, since they are indicators of endothelial damage.[42,43]

In this study, the aorta wall area and the aortic lumen area were similar between groups, showing that the interventions did not affect these results. In relation to flaxseed, a study using a diet with 25% of flaxseed flour during pregnancy and lactation of diabetic rats showed no difference in the aorta lumen area of male offspring at 100 days of life.[13] In our study, chronic administration of 1% NaCl solution to offspring of mothers who received control diet during lactation led to an increase in aortic intima-media thickness, and to a smaller number of elastic lamellae and, although not significant, the aorta elastin amount was also lower than the control group (-17,2%). These results were not found in the offspring of mothers who consumed flaxseed during lactation, once the aortic intima-media thickness of the FG was similar to CG; also, chronic salt overload did not increase aortic intima-media thickness, suggesting a protective effect of flaxseed. Sodium's deleterious effects on endothelial function of offspring of the CG may be associated with the action of reactive oxygen species, such as superoxide, resulting in reduced nitric oxide bioavailability. Cell culture studies support that high sodium exposure stiffens endothelial cells and damages the glycocalyx.[2,44] Besides that, changes in elastin deposition in the arteries lead to elasticity loss and consequent increased stiffness, promoting aortic remodeling by thickening of the intima-media layer.[45] Concerning the use of flaxseed during lactation on aortic morphology, different from our results, in a study where flaxseed was given to healthy rats from lactation to adult life, intima-media thickness was smaller than the group that received the control diet.[12] Also, studies in which flaxseed diet was given to diabetic mothers during pregnancy and lactation reported that male and female offspring as adults showed smaller aortic intima-media thickness than the offspring of healthy mothers.[13,14]

Flaxseed is a rich vegetable source of n-3 fatty acid, which is known for its ability of preventing aortic remodeling due to its capability to be transferred by the placental route and incorporated into the cell membrane modifying its properties. Omega−3 fatty acids are also important anti-inflammatory agents able to decrease endothelial activation and generation of inflammatory cytokines, preventing cardiovascular diseases.[11]

It was observed that exercise training (treadmill), as observed with the chronic use of NaCl, increased aortic intima-media thickness, both in the offspring of control group and in the flaxseed group when compared to their respective sedentary groups. Coura et al.,[46] using swimming as exercise training (5 times/week, 30 minutes/day, for eight weeks), also showed that training increased aortic intima-media thickness of elderly Wistar rats. Despite the increase, Mulvany et al.[47] reported that resistance arteries suffer an internal eutrophic remodeling, with no changes in the tissue constituent materials, which is noticed in our results related to the number of elastic lamellae and elastin amount that were similar or greater than the respective sedentary group.

Conclusion

In summary, despite unaltered blood pressure, chronic salt overload caused adverse effects on the aorta of rats, with decreased aortic elasticity. This was prevented by the consumption of a flaxseed diet during lactation, suggesting a protective effect of flaxseed. Exercise training alone increased aortic intima-media thickness but did not affect its elastic components.

Author Contributions

Conception and design of the research: Vicente GC, Correia-Santos AM and Boaventura GT. Acquisition of data: Silva-Couto S, Castro CLC, Barreto VLM, Martins JEC and Lenzi Q. Analysis and interpretation of the data: Correia-Santos AM, Vicente GC, Chagas MA and Boaventura GT. Writing of the manuscript: Silva-Couto S, Correia-Santos AM, Vicente GC and Castro CLC. Critical revision of the manuscript for intellectual content: Silva-Coutro S, Correia-Santos AM, Vicente GC, and Boaventura GT.

Potential Conflict of Interest

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

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Study Association

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Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee on Animal Experiments of the *Universidade Federal Fluminense* under the protocol number 882/2016.

References

1. Oliveira MM, Malta DC, Santos MAS, Oliveira TP, Nilson EAF, Claro RM. Self-reported high salt intake in adults: data from the National Health Survey, Brazil, 2013. Epidemiol Serv Saúde. 2015;24(2):249-56.
2. Farquhar WB, Edwards DG, Jurkovitz CT, Weintraub WS. Dietary sodium and health: more than just blood pressure. J Am Coll Cardiol. 2015;65(10):1042-50.
3. Zhu J, Huang T, Lombard JH. Effect of high-salt diet on vascular relaxation and oxidative stress in mesenteric resistance arteries. J Vasc Res. 2007;44(5):382-90.
4. DuPont JJ, Greaney JL, Wenner MM, Lennon-Edwards S, Sanders PW, Farguhar WB, et al. High dietary sodium intake impairs endothelium-dependent dilation in healthy salt-resistant humans. J Hypertens. 2013;31(3):530-6.
5. Greaney JL, DuPont JJ, Lennon-Edwards SL, Sanders PW, Edwards DG, Farquhar WB. Dietary sodium loading impairs microvascular function independent of blood pressure in humans: role of oxidative stress. J Physiol. 2012;590(21):5519-28.
6. Imaizumi Y, Eguchi K, Murakami T, Arakawa K, Tsuchihashi T, Kario K. High salt intake is independently associated with hypertensive target organ damage. J Clin Hypertens (Greenwich). 2016;18(4):315-21.
7. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, et al. Global sodium consumption and death from cardiovascular causes. N Engl J Med. 2014;371(7):624-34.
8. Coelho CF, Burini RC. Physical activity to prevent and treat non-communicable chronic diseases and functional disability. Rev Nutr. 2009;22(6):937-46.
9. Rique ABR, Soares EA, Meirelles CM. Nutrition and exercise on cardiovascular disease prevention and control. Rev Bras Med Esporte. 2002;8(6):244-54.
10. Corrêa LBNS, Cardozo LFM, Ribeiro ICA, Boaventura GT, Chagas MA. Influence of prolonged flaxseed (Linum usitatissimum) consumption over epididymis and testicle histoarchitecture of Wistar rats. Pesq Vet Bras. 2017;37(6):650-6.
11. Correia-Santos AM, Vicente GC, Boaventura GT. Functional implications of maternal intake of flaxseed and its by-products during pregnancy and lactation on offspring: Review of animal studies. Nutr Food Sci. 2017;47(4):477-89.
12. Cardozo LFMF, Vicente GC, Brant LHC, Mafra D, Chagas MA, Boaventura GT. Prolonged flaxseed flour intake decreased the thickness of the aorta and modulates some modifiable risk factors related to cardiovascular disease in rats. Nutr Hosp. 2014;29(2):376-81.
13. Vicente GC, Correia-Santos AM, Suzuki A, Velarde LGC, Chagas MA, Boaventura GT. Maternal exposure to diets containing flaxseed flour or flaxseed oil during pregnancy and lactation protects the aortic remodeling in adult male offspring of diabetic rat dams. J Sci Food Agric. 2015;95(14):2973-80.
14. Vicente GC, Correia-Santos AM, Suzuki A, Chagas MA, Boaventura GT. Maternal use of a diet rich omega-3 from flaxseed improves aortic remodeling but not the biochemical parameters of female offspring of diabetic rats. Eur J Lipid Sci Technol. 2014;117(3):292-9.
15. Kajla P, Sharma A, Sood DR. Flaxseed-a potential functional food source. J Food Sci Technol. 2015;52(4):1857-71.
16. Khalesi S, Irwin C, Schubert M. Flaxseed consumption may reduce blood pressure: a systematic review and meta-analysis of controlled trials. J Nutr. 2015;145(4):758-65.
17. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993;123(11):1939-51.
18. Damy SB, Camargo RS, Chammas R, Figueiredo LFP. The fundamentals of experiments with animals – applications in experimental surgery. Rev Assoc Med Bras. 2010;56(1):103-11.

19. Felix JVC, Michelini LC. Training-induced pressure fall in spontaneously hypertensive rats is associated with reduced angiotensinogen mRNA expression within the nucleus tractus solitarii. Hypertens. 2007;50(4):780-5.

20. Pereira LM, Vianna GM, Mandarim-de-Lacerda CA. Morphology and stereology of the myocardium in hypertensive rats. Correlation with the time of nitric oxide synthesis inhibition. Arq Bras Cardiol. 1998;70(6):397-402.

21. Brant LHC, Cardozo LFMF, Velarde LGC, Boaventura GT. Impact of flaxseed intake upon metabolic syndrome indicators in female Wistar rats. Acta Cir Bras. 2012;27(8):537-43.

22. Costa CAS, Silva PCA, Ribeiro DC, Pereira ADD, Santos ADSD, Maia LDA, et al. Body adiposity and bone parameters of male rats from mothers fed diet containing flaxseed flour during lactation. J Dev Orig Health Dis. 2016;7(3):314-9.

23. Soares LL, Cardozo LFMF, Troina AA, Ramos CFR, Guzmán-Silva MA, Boaventura GT. Influence of flaxseed during lactation on the reproductive system of female Wistar rats. Nutr Hosp. 2010;25(3):437-42.

24. Leite CDFC, Vicente GC, Suzuki A, Pereira AAP, Boaventura GT, Santos RM, et al. Effects of flaxseed on rat milk creamatocrit and its contribution to offspring body growth. J Pediatr (Rio J). 2012;88(1):74-8.

25. Cardozo LFMF, Soares LL, Chagas MA, Boaventura GT. Maternal consumption of flaxseed during lactation affects weight and hemoglobin level of offspring in rats. J Pediatr (Rio J). 2010;86(2):126-30.

26. Fernandes FS, Souza AS, Carmo MGT, Boaventura GT. Maternal intake of flaxseed-based diet (Linum usitatissimum) on hippocampus fatty acid profile: implications for growth, locomotor activity and spatial memory. Nutrition. 2011;27(10):1040-7.

27. Troina AA, Figueiredo MS, Moura EG, Boaventura GT, Soares LL, Cardozo LFMF, et al. Maternal flaxseed diet during lactation alters milk composition and programs the offspring body composition, lipid profile and sexual function. Food Chem Toxicol. 2010;48(2):697-703.

28. Hamosh M, Hamosh P. The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue. J Clin Invest. 1975;55(5):1132-5.

29. McKinley MJ, Johnson AK. The Physiological regulation of thirst and fluid intake. News Physiol Sci. 2004 Feb;19(1):1-6.

30. Simmonds SS, Lay J, Stocker SD. Dietary salt intake exaggerates sympathetic reflexes and increases blood pressure variability in normotensive rats. Hypertension. 2014;64(3):583-9.

31. Washino S, Hosohata K, Jin D, Takai S, Miyagawa T. Early urinary biomarkers of renal tubular damage by a high-salt intake independent of blood pressure in normotensive rats. Clin Exp Pharmacol Physiol. 2018;45(3):261-8.

32. Oliveira EM, Krieger JE. Chronic β-adrenoceptor stimulation and cardiac hypertrophy with no induction of circulating renin. Eur J Pharmacol. 2005;520(1):135-41.

33. Ferreira DN, Katayama IA, Oliveira IB, Rosa KT, Furukawa LNS, Coelho MS, et al. Salt-induced cardiac hypertrophy and interstitial fibrosis are due to a blood pressure-independent mechanism in Wistar rats. J Nutr. 2010;140(10):1742-51.

34. Vicente GC, Correia-Santos AM, Suzuki A, Anjos JS, Velarde LGS, Chagas MA, et al. Perinatal consumption of flaxseed oil and flaxseed flour has beneficial effects on cardiac fibrosis of male offspring from rat dams with experimental diabetes. Food Res Int. 2016 May;83:162-8.

35. Meneses JA, Trugilho LA, Lima SA, Freitas ACF, Melo HS, Ferreira MR, et al. The influence of a diet based on flaxseed, an omega-3 source, during different developmental periods, on the blood pressure of rats submitted to stress. J Matern Fetal Neonatal Med. 2019;32(9):1516-22.

36. Brum PC, Silva GJJD, Moreira ED, Ida F, Negrão CE, Krieger EM. Exercise training increases baroreceptor gain sensitivity in normal and hypertensive rats. Hypertension. 2000;36(6):1018-22.

37. Kuru O, Şentürk Ü, Demir N, Yeşilkaya A, Ergüler G, Erkılıç M. Effect of exercise on blood pressure in rats with chronic NOS inhibition. Eur J Appl Physiol. 2002;87(2):134-40.

38. Niederhoffer N, Kieffer P, Desplanches D, Lartaud-Idjouadiene I, Sornay MH, Atkinson J. Physical exercise, aortic blood pressure, and aortic wall elasticity and composition in rats. Hypertension. 2000;35(4):919-24.

39. Hsu YC, Chen HI, Kuo YM, Yu L, Huang TY, Chen SJ, et al. Chronic treadmill running in normotensive rats resets the resting blood pressure to lower levels by upregulating the hypothalamic GABAergic system. J Hypertens. 2011;29(12):2339-48.

40. Carvalho MHC, Nigro D, Lemos VS, Tostes RCA, Fortes ZB. Arterial hypertension: endothelium and multiples functions. Rev Bras Hipertens. 2001;8(1):76-88.

41. Pizzi O, Brandão AA, Magalhães MEC, Pozzan R, Brandão AP. Pulse wave velocity – methodology and prognostic implications in hypertension. Rev Bras Hipertens. 2006;13(1):59-62.

42. McCloskey K, Vuillermin P, Ponsonby AL, Cheung M, Skilton MR, Burgner D. Aortic intima-media thickness measured by trans-abdominal ultrasound as an early life marker of subclinical atherosclerosis. Acta Paediatr. 2014;103(2):124-30.

43. Visentin S, Grumolato F, Nardelli GB, Di Camillo B, Grisan E, Cosmi E. Early origins of adult disease: low birth weight and vascular remodeling. Atherosclerosis. 2014;237(2):391-9.

44. Oberleithner H, Peters W, Kusche-Vihrog K, Korte S, Schillers H, Kliche K, et al. Salt overload damages the glycocalyx sodium barrier of vascular endothelium. Pflugers Arch. 2011;462(4):519-28.

45. Kornet L, Lambregts J, Hoeks AP, Reneman RS. Differences in near-wall shear rate in the carotid artery within subjects are associated with different intima-media thicknesses. Arterioscler Thromb Vasc Biol. 1998;18(12):1877-84.

46. Coura MAS, Pacheco ME, Simões HG, Moraes JFVN, Campbell CSG. Morphoquantitative study of the aorta's wall in aerobically trained elderly wistar rats. Motricidade. 2012;8(4):71-9.

47. Mulvany MJ, Baumbach GL, Aalkjaer C, Heagerty AM, Korsgaard N, Schiffrin EL, et al. Vascular remodeling. Hypertension. 1996;28(3):505-6.

International Journal of Cardiovascular Sciences. 2021; 34(2):134-135

EDITORIAL

Can the Impact of the Excessive Use of Salt and its Chronic Consequences be Atenuated by Functional Foods?

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Editorial referring to the article: Maternal Intake of Flaxseed During Lactation and Exercise Training Protect Against Salt Overload-Induced Aortic Remodeling in Adult Offspring

Significant evidence has been showing the strong impact that food has on health. Among the substances that have a great influence on food choices and preferences is sodium (salt),[1] which has been associated with obesity and chronic diseases, such as diabetes mellitus, high blood pressure and cardiovascular diseases.[2,3]

According to National Academies of Sciences, Engineering and Medicine (2019),[4] a sodium intake below 2.3 g/day is recommended for adults to reduce the risk of chronic diseases, such as high blood pressure. Despite great educational efforts on the part of government and medical authorities, populations still maintain a higher than recommended sodium intake,[5] which has led to the development of public health strategies to reduce this problem.

Obesity and excessive adiposity are also important risk factors for the development of high blood pressure. In addition, genetic factors, inflammatory processes, oxidative stress, vascular and cardiac smooth muscle dysfunction and, especially, endothelial dysfunction are also strongly associated with the development of this chronic disease.[6]

Endothelial dysfunction is characterized by a list of changes in the endothelium that promote a reduction in vasodilation, pro-inflammatory state, detachment and apoptosis of endothelial cells and the development of atherosclerosis. Structural and inflammatory cells produce inflammatory mediators, such as certain lipids, free radicals, cytokines, chemokines and growth factors. However, the number of these mediators is high during the development of some diseases, such as obesity and high blood pressure, reaching the circulation and leading to systemic inflammation and, via the vasculature potentiating endothelial dysfunction,[7] contributing to the aortic and/or cardiac remodeling.

Endothelial integrity depends not only on the extent of the injury, but also on the endogenous repair capacity. More recently, it has become clear that circulating endothelial progenitor cells are an important alternative mechanism for maintenance and repair of the endothelium. The mobilization of these cells is partly NO-dependent and may be impaired in patients with cardiovascular risk factors. Likewise, factors that have been shown to lead to improved endothelial function and NO bioavailability, such as exercise and the use of statins, have also been shown to have a powerful positive effect on the mobilization of endothelial progenitor cells.[8]

However, in addition to the practice of physical activity and the use of medications, it is postulated that different dietary interventions and approaches are necessary to minimize or prevent the chronic process characteristic of high blood pressure, as well as to prevent it. In addition to reducing sodium consumption, the beneficial use of functional and nutraceutical foods stands out as adjunctive therapies to pharmacotherapy for the treatment of this disease and its consequences.[9]

Studies have shown that flaxseed, a functional food that has a high content of fibers and essential fatty acids such as omega-3, has antihypertensive, anti-diabetic, anti-inflammatory and anticancer effects, mainly attributed to the activity of Secoisolariciresinol Diglicoside (SDG). The experimental study conducted by Silva-Couto et al.,[10] points out that it is possible to mitigate the impact caused on health by the large consumption of salt, attributed to nutritional intervention with flaxseed. We invite you to read this article, where the reader can glimpse the interesting results about the use of this functional food in the prevention of aortic remodeling related to excessive use of sodium. Good reading!

Keywords

Sodium Chloride; Sodium; Flax; Funcional Food; Obesity; Diabetes Mellitus; Hypertension.

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References

1. Duffy VB, Hayes JE, Sullivan BS, Faghri P. Surveying food and beverage liking: a tool for epidemiological studies to connect chemosensation with health outcomes. Ann N Y Acad Sci. 2009; 1170:558–68.
2. Meneton P, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. Physiol Rev. 2005; 85(2):679–715
3. Moosavian SP, Haghighatdoost F, Surkan PJ, Azadbakht L. Salt and obesity: a systematic review and meta-analysis of observational studies. Int J Food Sci Nutr. 2017; 68(3):265–77
4. National Academies of Sciences, Engineering, and Medicine 2019. Dietary Reference Intakes for Sodium and Potassium. Washington, DC: The National Academies Press.[Cited in 2019 Oct 21] Available at https://doi.org/10.17226/25353.
5. Thout SR, Santos JA, McKenzie B, Trieu K, Johnson C, McLean R, et al. The Science of Salt: Updating the evidence on global estimates of salt intake. J Clin Hypertens (Greenwich). 2019; 21(6):710-721. doi: 10.1111/jch.13546.
6. Butler MG. Pediatric hypertension: genetics of hypertension current status. Le Journal medical libanais. Leban Med J. 2010; 58(3): 175–8.
7. Szucs B, Szucs C, Petrekanits M, Varga JT. Molecular Characteristics and Treatment of Endothelial Dysfunction in Patients with COPD: A Review Article. Int J Mol Sci. 2019; 20(18):4329. doi: 10.3390/ijms20184329.
8. Giribela CRG, Gengo R, Hong V, Consolim-Colombo FM. Função e disfunção endotelial: da fisiopatologia às perspectivas de uso em pesquisa e na prática clínica Rev Bras Hipertens. 2011; 18(1):27-32.
9. Ghaffari S, Roshanravan N. The role of nutraceuticals in prevention and treatment of hypertension: An updated review of the literature. Food Res Int. 2020; 128:108749. doi: 10.1016/j.foodres.2019.108749.
10. Couto SS, Correia-Santos AM, Vicente GC, Castro CLC, Barreto VLM, Martins JEC,et al. Maternal Intake of Flaxseed During Lactation and Exercise Training Protect Against Salt Overload-Induced Aortic Remodeling in Adult Offspring. Int J Cardiovasc Sci. 2021; 34(2):124-133.

International Journal of Cardiovascular Sciences. 2021; 34(2):136-146

ORIGINAL ARTICLE

Correlation between Epicardial Fat Thickness and Clinical and Anthropometric Variables in an Elderly Population

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Abstract

Background: Epicardial fat (EF) thickness is a marker of visceral adiposity and consequently considered an important predictive marker of cardiovascular and metabolic risk.

Objective: To describe echocardiographic features of the heart in an elderly population and to study the correlation between EF thickness and clinical and anthropometric variables.

Methods: A sample of 34 individuals (25 women) aged between 65 and 92 years, who attended a private institution in the central region of Continental Portugal, was analyzed. A standardized sociodemographic questionnaire was applied, and anthropometric assessment, echocardiography and blood pressure measurement were performed in all subjects. A correlational analysis of EF thickness with anthropometric and clinical parameters was performed. The association between variables was tested by Pearson's correlation and point-biserial correlation. A value of $p < 0.05$ was defined as statistically significant.

Results: EF thickness was higher in males (6.0 ± 1.4 mm vs 5.2 ± 0.9 mm in females), and ranged from 4 to 9 mm. There were statistically significant correlations between EF thickness and weight ($r = 0.4$; $p = 0.02$), body surface area ($r = 0.4$; $p = 0.02$), lean mass ($r = 0.4$; $p = 0.03$), calf circumference ($r = 0.5$; $p = 0.01$) and left ventricular end-diastolic diameter ($r = 0.3$; $p = 0.04$).

Conclusion: EF thickness was higher in males and was significantly correlated with anthropometric parameters of adiposity and left ventricular end-diastolic diameter. (Int J Cardiovasc Sci. 2021; 34(2):136-146)

Keywords: Epicardial Fat; Echocardiography/methods; Anthropometry; Aging; Blood Pressure; Diabetes Mellitus; Hypertension; Risk Factors; Adiposity.

Introduction

Cardiac aging is characterized by slow and progressive changes in cardiac structure and function. With advancing age, although systolic ventricular function is relatively preserved, the same is not true for diastolic function.[1] With aging, there are also changes in cardiac valves, mainly in the left valves,[1] mainly calcification of the leaflets of the aortic and mitral valves.[2] Although age-related changes of the right valves are less frequent and milder, there is evidence of small fibroelastic nodules in the tricuspid valve, while the pulmonary valve remains almost unchanged.[3,4]

Epicardial fat (EF) is a component of the visceral fat located between the surface of the myocardium and the visceral layer of the pericardium.[5-13] It can be deposited throughout the entire myocardium, but it is predominant in the atrioventricular and interventricular sulci, extending to the apex and around the coronary arteries.[6,8-9,14-16] The EF shares its embryological origin with the intra-abdominal visceral fat,[10,14] and the microcirculation with the myocardium and coronary arteries. It can be a potential cause of local inflammation and has direct effects on coronary atherosclerosis through the release of anti- and pro-inflammatory substances, with correlations with the severity of metabolic syndrome and

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heart diseases, especially coronary artery disease.[5-10,15-23] Although EF has been considered by some authors as an important predictive marker of cardiovascular and metabolic risk,[8,16] its association with other risk factors in elderly populations, makes its interpretation as an independent risk marker difficult.[8]

The association of EF thickness with age and gender has been controversial in the published studies. While some authors have reported that EF thickness tends to increase with age,[9,19,22,24-28] others have not found statistically significant correlations between these two variables.[7,11-12, 29,30] Regarding gender, some authors [11,12,19,31] have reported higher EF thickness in males, while others[7,25-32] found it to be higher in females. Regarding anthropometric parameters, EF thickness seems to correlate with weight,[25-31,33] height,[31] abdominal circumference,[10-13,19,22,25,28,29,33-34] and fat mass,[11,12] and and regarding the correlation correlation between EF thickness and body mass index (BMI) the results have been contradictory.[2,9-12,22,25-26,28,29,31,33-34] The association of EF thickness with obesity has been widely described, [11,16-17,31,35-37] and significant changes in EF thickness in severely obese individuals undergoing weight loss programs or interventions have been reported.[30,37,38] Although the relationship between EF thickness and some clinical conditions such as diabetes and dyslipidemia is not in the literature, its correlation with hypertension seems to be more evident.[11, 24, 30,33-34]

In addition, several studies have found correlations between EF thickness and some echocardiographic parameters, such as left ventricular mass index (LVMI),[24,29] left ventricular hypertrophy (LVH),[14,18,26] and left ventricular diastolic dysfunction.[7,26,36]

Thus, the interest in the study of the EF thickness through echocardiography has been growing, since it reflects the intra-abdominal visceral fat regardless of obesity degree and age (differently from anthropometry), and has been proposed as a marker of visceral adiposity.[10,11,13-15,39] Apparently, some clinical and demographic characteristics of the populations[8] have an influence on determining the EF thickness limits; even so, while Natale et al.,[24] have suggested an upper limit of 7 mm in a healthy population, Bertaso et al.,[8] have suggested that a thicknesses greater than 5 mm may already be considered abnormal in a low- risk population.

The aim of this study was to present echocardiographic features of the heart, with emphasis on EF characterization, and its correlations with clinical, anthropometric and other echocardiographic variables in an old adult population.

Methods

Study Design, Sample and Ethical aspects

An observational cross-sectional study was carried out with participants of the AGA@4life. The aim of the AGA@4life project is to evaluate the effect of a multidisciplinary and personalized intervention on promoting an active and healthy aging. This preliminary analysis, focused on the data obtained in the initial characterization of the studied population, aimed to identify characteristics of the heart, particularly the EF profile and its correlation with other clinical, anthropometric, and functional variables in adults. The sample was recruited by convenience at a private institution of social solidarity in the central region of Continental Portugal (ADIC, Vilarinho, Portugal). We included participants over the age of 65, of both genders, physically independent and without previous history of cerebrovascular or cardiac disease. Thus, the sample was composed of 34 volunteer participants (9 men and 25 women), aged between 65 and 92 years.

The study complied with the recommendations of the Declaration of Helsinki and was approved by the Ethics Committee of the Polytechnic Institute of Coimbra. Anonymity and confidentiality of the data were guaranteed. The study was conducted for strictly scientific purposes, and there are no conflicts of interest to declare. All participants gave their informed consent to participate in the study.

General Procedure

Eligible elderly adults were invited to participate in the study in January 2018. During the months of February and March 2018, a diagnostic evaluation of each participant was carried out using a structured questionnaire designed for the study. Data on comorbidities, current medication use, nutritional profile, daily activity profile, cardiovascular risk profile and history of falls were collected. Blood and urine samples were collected for laboratory analysis and several diagnostic methods for the detection of multisystemic diseases were applied, whose results will not be discussed in the present study. An echocardiography, and anthropometric and brachial blood pressure (BP) measurements were performed.

Echocardiographic evaluation

Analysis of echocardiographic variables was made by a single and experienced operator, using a Vivid 7 echocardiography device with an echocardiography device (GE Medical, Milwaukee, WI, USA) with a 1.7-3.2 MHz multi-frequency linear probe. All echocardiographic studies were performed according to the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[40,41] An M-mode and two-dimensional echocardiography was performed, with measurement of heart chamber dimensions, left ventricular (LV) wall thickness, and aortic root diameter. The EF thickness was measured at end-systole perpendicularly to the right ventricular free wall in parasternal view (long- and short-axis), in three consecutive cardiac cycles, and defined as the echo-free space between the myocardial surface and the visceral layer of the pericardium,[5-7,11-15,32] as previously described by Iacobellis et al.,[11-15] (Figure 1).

Anthropometry

Height was measured in centimeters, with the participant standing barefoot, with the back straight, against the stadiometer, feet together and head in the Frankfurt position (lower orbital arch aligned horizontally with the ear). Weight was determined in kilograms, and lean and fat masses, expressed as percent, were calculated by bioelectrical impedance analysis using the InBody 230 equipment. Body surface area (BSA, m^2) was calculated using the Dubois & Dubois formula, and the BMI using the Quetelet index. Abdominal circumference was measured with the individual without clothes, abdomen relaxed, arms hanging freely, palms facing inwards, head upright and feet together. For practical purposes and to reduce the margin of error, the measurement was taken at the end of the respiratory cycle, with the tape snug to the skin but not compressing it. Two measurements were taken, and the mean of these two measurements was used for analysis. Waist circumference measured during expiration, at the midway between the last costal arch and the iliac crest. Hip circumference was measured at the level of the maximum prominence of the buttocks in a horizontal plane. Calf circumference was measured in duplicate, at the point of maximal circumference of the lower right leg, and the mean of these two measurements was used in the analysis.

Blood Pressure measurement

Blood pressure measurements were automatically obtained using the Mobil-o-Graph equipment (IEM,

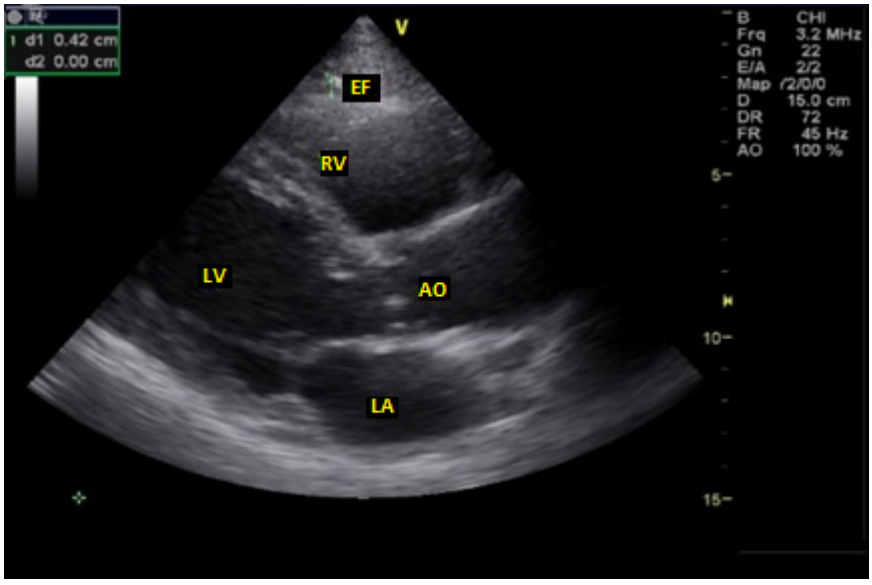

Figure 1 – Measurement of epicardial fat by transthoracic echocardiography in the long-axis parasternal view.
EF: epicardial fat; RV: right ventricle; LV: left ventricle; AO: aorta; LA: left atrium

Stolberg, Germany). Subjects were instructed to refrain from tobacco and caffeine for at least 2 hours prior to the study. Blood pressure was measured at the brachial artery, in sitting position, after a resting period of 5 minutes, with the arm supported at heart level and without constrictive clothing. Three measurements were taken, and the mean was considered for analysis.

Statistical Analysis

The statistical analysis was performed using the Statistical Program for Social Sciences – SPSS, version 24 (IBM, Armonk, NY).

The categorical variables were reported as frequency and percentages, and the $\chi 2$ test or the Fisher Exact test were used when appropriate. The Shapiro-Wilk test was used to confirm the normal distribution of all continuous variables, which were presented as mean and standard deviation.

To determine the degree of association between the variables, Pearson's correlation coefficient and point-biserial correlation coefficient (when one of the variables was categorical) were used. The correlation coefficients were interpreted according to the literature, that is, as trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$) and almost perfect ($r > 0.9$).[42] A value of $p < 0.05$ was defined as a criterion of statistical significance for a 95 % confidence interval.

Results

Mean age of the sample was 81.9 ± 7.8 years, mean weight 65.8 ± 11.6 kg, and mean BMI was 28.2 ± 4.1 kg/m^2. The results obtained from the questionnaire showed that 17.6% of the individuals had a family history of cardiovascular disease, 23.5% were diabetic and 55.9% had dyslipidemia. Echocardiography revealed both structural and functional cardiac changes, which is in accordance with the fact that the sample was composed of a very elderly population – 88% of individuals had aortic fibrosis (...), 73% had mitral fibrosis, and 29% had tricuspid valve regurgitation (four with pulmonary artery systolic pressure greater than 35 mmHg). No significant changes were detected in the pulmonary valve. Other less frequent changes were changes in the left ventricular (LV) segmental contractility, basal septal hypertrophy (BSH), calcification of the mitral valve annulus and ascending aorta dilatation. General characteristics of the study sample are presented in Table 1.

Mean heart rate was 68.7 ± 11.6 bpm and the mean values of SBP and DBP were 148.8 ± 29.5 mmHg and 78.7 ± 15.1 mmHg, respectively. Table 2 shows the structural variables calculated by echocardiography.

Tables 3 and 4 refer to the correlation between EF and age, anthropometric parameters, and clinical variables.

Table 3 shows positive moderate/strong correlations between EF thickness and calc circumference ($r = 0.5$; $p = 0.01$) , and moderate correlation between EF thickness and weight ($r = 0.4$; $p = 0.02$), BSA ($r = 0.4$; $p = 0.02$) and lean mass ($r = 0.4$; $p = 0.03$) (Figure 2). No other statistically significant correlations were found.

As can be seen from Table 4, no statistically significant correlations were found between EF and previous history of cardiovascular disease or any of the clinical variables studied.

Table 5 correlates EF with LVM (corrected for BSA), left atrial and right atrial areas, LV shortening fraction and the LV end-diastolic diameter.

Table 5 shows a positive, but a low to moderate correlation ($r = 0.3$; $p = 0.04$) between EF thickness and LV end-diastolic diameter (Figure 3). For the remaining echocardiographic variables evaluated, no statistically significant correlations were found.

Figure 4 shows the measurement of the EF in two extreme cases, representative of the sample (highest EF value - panel A; lowest EF value - panel B). The participant with the highest EF value was male, 89 years, body weight of 95.5 kg, BSA of 2.03 m^2, BMI of 34.7 kg/m^2, waist/hip ratio of 1.08, CC of 36.5 cm, lean mass of 33% and fat mass of 37%, presenting with hypertension, dyslipidemia and diabetes. On the other hand, the participant with the lowest EF value was an 85-year-old man, body weight of 63.5 kg, BSA of 1.68 m^2, BMI of 24.2 kg/m^2, waist/hip ratio of 0.89, CC of 34.5 cm, lean mass of 26% and fat mass of 16%, dyslipidemic. This comparative analysis suggests the tendency of a positive correlation of EF with body weight and a more unfavorable clinical profile.

Discussion and Conclusions

During the aging process, there is a decrease in lean mass, increase in fat mass and a redistribution of adipose tissue to the trunk and viscera.[43] EF is a component of visceral fat, distributed around the heart, located between the myocardial surface and the visceral layer of the pericardium.[5-13] Iacobellis et al.,[13] found a wide variation in EF thickness (1-23 mm), which probably reflects not only a high variation in the distribution of visceral fat,[14]

Table 1 – General characteristics of the sample

Variables	Women			Men			Total		
	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD
Age (years)	65	92	82.9 ± 7.4	66	90	79.2 ± 8.7	65	92	81.9 ± 7.8
Weight (kg)	37.1	86.2	63.5 ± 10.7	53.8	95.5	72.1 ± 12.2	37.1	95.5	65.8 ± 11.6
Height (cm)	137	160	149.0 ± 6.6	149	172	164.1 ± 7.3	137	172	153.0 ± 9.5
BSA (m^2)	1.2	1.8	1.6 ± 0.2	1.5	2	1.8 ± 0.2	1.2	2	1.6 ± 0.2
BMI (Kg/m^2)	19.8	37.3	28.8 ± 4.4	24.2	34.7	26.9 ± 3.4	19.8	37.3	28.2 ± 4.1
AC (cm)	84	122	102.3 ± 8.8	79	115	100.4 ± 10.1	79	122	101.7 ± 9.0
LM (%)	13.3	24.7	18.8 ± 2.9	24	33.1	28.1 ± 3.2	13.3	33.1	21.5 ± 5.2
FM (%)	10.1	44.4	27.5 ± 8.4	6.8	37	21.8 ± 8.7	6.8	44.4	25.9 ± 8.7
CC (cm)	28	45.5	34.0 ± 3.8	31	36.5	33.9 ± 1.8	28	45.5	34.0 ± 3.3
HR (bpm)	53	102	70.2 ± 11.1	44	90	64.7 ± 12.6	44	102	68.7 ± 11.6
SBP (mmHg) (mm(mmHg)	92	238	147.5 ± 31.2	125	200	152.4 ± 25.7	92	238	148.8 ± 29.5
DBP (mmHg)	56	135	79.9 ± 15.3	46	90	75.4 ± 14.8	46	135	78.7 ± 15.1

SD: Standard deviation, BSA: Body surface area, BMI: Body mass index, AC: Arm circumference,, LM: Lean mass, FM: Fat mass, CC: calf circumference, HR: Heart rate, SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 2 – Structural variables calculated by echocardiography

	Women			Men			Total		
Variables	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD
LVESD (mm)	26	46	33.9 ± 3.8	35	48	38.6 ± 4.1	26	48	35.2 ± 4.4
LVEDD (mm)	41	63	52.3 ± 5.1	55	64	59.2 ± 3.5	41	64	54.2 ± 5.6
PWd (mm)	6.7	8.2	7.4 ± 0.4	7.2	8.7	7.7 ± 0.5	6.7	8.7	7.5 ± 0.4
IVSd (mm)	6.7	9	7.5 ± 0.6	7.2	8.7	7.7 ± 0.6	6.7	9	7.5 ± 0.6
AOR_D (mm)	22	35	29.5 ± 2.8	29	40	34.9 ± 3.7	22	40	30.9 ± 3.8
LA_D (mm)	27	53	36.8 ± 5.9	32	53	38.9 ± 6.2	27	53	37.4 ± 5.9
LVSF (%)	28	41	34.9 ± 3.3	24	38	34.8 ± 4.5	24	41	34.9 ± 3.6
LVM/BSA (g/m^2)	60	128	86.0 ± 15.8	82	120	99.7 ± 12.9	60	128	89.6 ± 16.1
EF (mm)	4	8	5.2 ± 0.9	4	9	6.0 ± 1.4	4	9	5.4 ± 1.1

SD: Standard deviation, LVESD: Left ventricular end-systolic diameter, LVEDD: Left ventricular end-diastolic diameter, PWd: Left ventricular posterior wall end-diastolic thickness, IVSd: Interventricular septum end-diastolic thickness, AOR_D: Aortic root diameter, LA_D: Left atrial diameter, LVSF: Left ventricular shortening fraction, LVM/BSA: Left ventricular mass corrected for body surface area, EF: Epicardial fat.

Table 3 – Correlation between epicardial fat, age and anthropometric parameters

Variables	Age (anos)	Weight (Kg)	Height (cm)	BSA (m2)	BMI (Kg/m2)	AC (cm)	LM (%)	FM (%)	CC (cm)
Pearson's correlation (r)	- 0.1	0.4	0.3	0.4	0.3	0.2	0.4	0.2	0.5
Significance (*p*)	0.7	0.02	0.1	0.02	0.2	0.2	0.03	0.2	0.01

BSA: Body surface area, BMI: Body mass index, AC: Abdominal circumference, LM: Lean mass, FM: Fat mass, CC: calf circumference

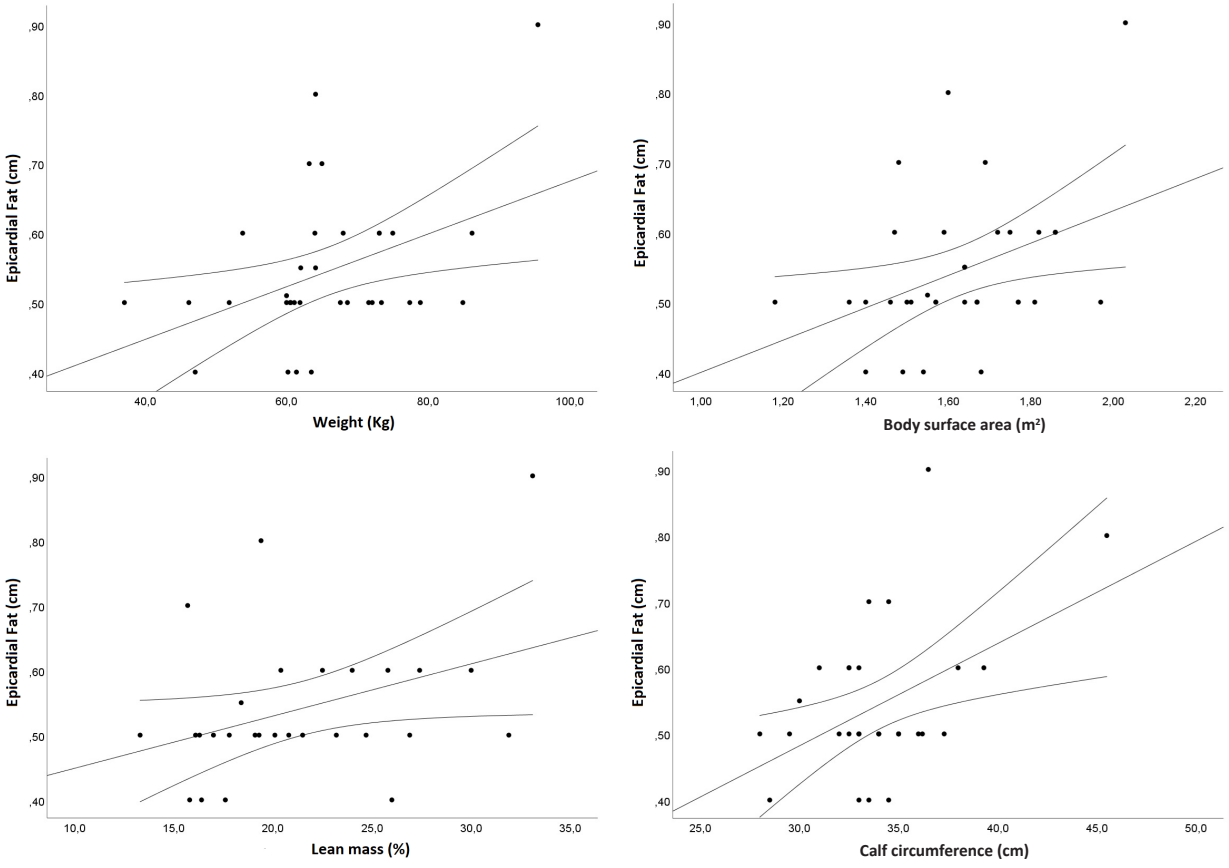

Figure 2 – Scatter plot of the correlation of epicardial fat thickness with weight (upper left panel), body surface area (upper right panel), lean mass (lower left panel) and calf circumference (lower right panel)

Table 4 – Correlation between epicardial fat thickness, history of cardiovascular disease and clinical variables

Variables	Cardiovascular history	Hypertension	Dyslipidemia	Diabetes	SBP (mmHg)	DBP (mmHg)
Point-biserial correlation coefficient (rpbiss)	- 0.2	- 0.1	- 0.1	- 0.3	0.2	0.1
Significance (*p*)	0.2	0.8	0.5	0.2	0.4	0.5

SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 5 – Correlation between epicardial fat thickness and structural echocardiographic parameters

Variables	LVM/BSA (g/m²)	LA_A (cm²)	RA_A (cm²)	LVSF (%)	LVEDD (mm)
Pearson's correlation (r)	0.2	- 0.2	- 0.04	0.2	0.3
Significance (*p*)	0.2	0.3	0.8	0.3	0.04

LVM/BSA: Left ventricular mass corrected for body surface area, LA_A : Left atrial area, RA_A: Right atrial area, LVSF: Left ventricular shortening fraction, LVEDD: Left ventricular end-diastolic diameter

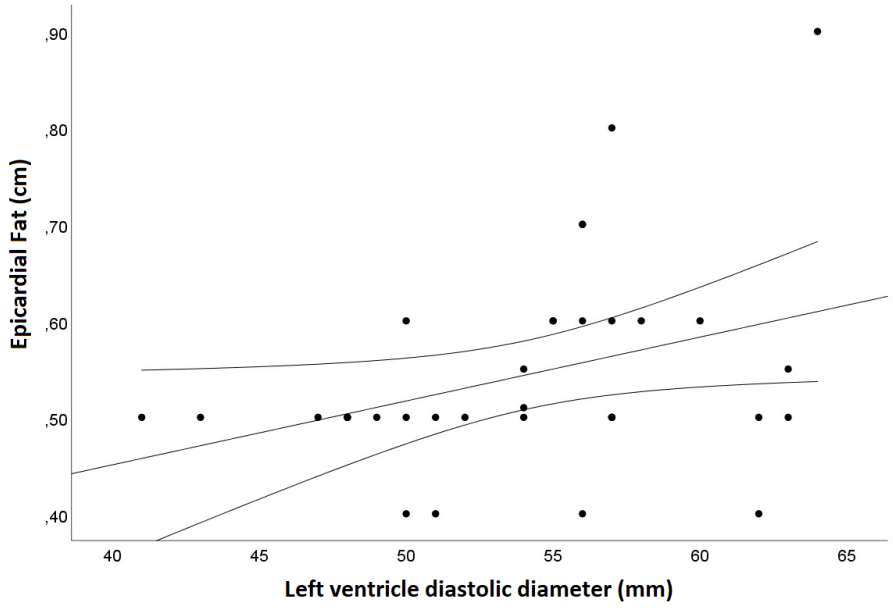

Figure 3 – Scatter plots correlating epicardial fat thickness with left ventricular diastolic diameter

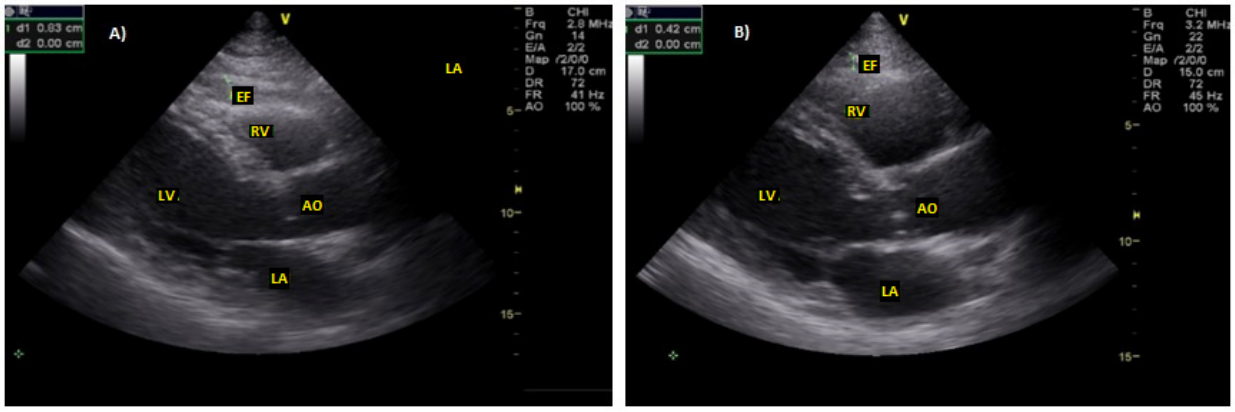

Figure 4 – Representation of epicardial fat assessment in two study participants. Panel A) participant with a high epicardial fat thickness; Panel B) participant with a low epicardial fat thickness. Legend: EF: epicardial fat; RV: right ventricle; LV: left ventricle; AO: aorta; LA: left atrium

but also in the clinical and demographic characteristics of the studied populations.[8]

In our study, mean EF thickness was 5.4 ± 1.1 mm, with higher values in males (6.0 ± 1.4 mm vs 5.2 ± 0.9 mm). While Iacobellis et al.,[11,12] Calabuig et al.,[19] and Alexopoulos et al.,[31] (these using computerized tomography) found similar results, different findings have been reported by other groups.[7,25-32] The correlation between EF and age has not been consensual also; some studies have shown a correlation between these variables,[9,19,22,24,25,27,28,31] in contrast to others.[7,11-12,29-30]

The association between EF and obesity has been widely described, since there is a predisposition for increased EF in obese individuals compared to non-obese individuals.[13,18,19,27,30,36,37] However, in our study, no statistically significant correlations were found between EF and BMI, possibly because our sample was not overweight (63.5 ± 10.7 kg for women and 72.1 ± 12.2 kg for men). These are in agreement with Bertaso et al. [8] and Magliano et al.,[9] but in contrast to others[10-12,22,25,26,29] Also, other studies reported statistically significant correlations for the male gender only[28] (r = 0.329 and $p = 0.041$ in men vs r = 0.105 and $p = 0.362$ in women). Such discrepancy in results may be due to the characteristics and age of the studied populations.

In our study, we did not find a correlation between EF and dyslipidemia or diabetes. Similar results were published by Kim et al.,[26] for dyslipidemia ($p = 0.738$), and Mazzoccoli et al.,[27] ($p = 0.098$) and Alexopoulos et al.,[31] ($p = 0.226$ using CT) for diabetes, but contradictory results were observed by Mazzoccoli et al.,[27] ($p = 0.01$) and Alexopoulos et al.,[31] ($p < 0.05$) for dyslipidemia and Iacobellis et al.,[44] for type 1 diabetes. Moreover, Iacobellis et al.,[44] reported that type 1 diabetes is associated with excess EF regardless of BMI, since EF thickness was significantly higher in diabetic individuals compared to non-diabetics (7.2 ± 2.1 mm vs 4.9 ± 2.5 mm; $p < 0.01$). Similar results were observed by Kim et al.,[26] ($p = 0.022$) and Li et al.,[45]

With aging, calcium deposition and loss of elastic fibers in the middle layer of the arteries are observed, leading to a decrease in their distensibility.[46] In the aortic artery, this fact stimulates the development of hypertension, diastolic dysfunction and heart failure.[47-49] Although in our study 35.3% of the individuals had isolated systolic hypertension, no statistically significant correlations were found between EF and hypertension. However, in the study by Mazzoccoli et al.,[27] Kim et al.,[26] and Alexopoulos et al.,[31] EF thickness was significantly higher in hypertensive individuals. Also, Natale et al.,[24] in a study performed on 459 hypertensive individuals, found an increase in SBP ($p = 0.01$) and a reduction in DBP ($p = 0.01$) when the EF thickness was greater than 7 mm. Thus, they reported the existence of statistically significant positive correlations (r = 0.35; $p < 0.0001$) between EF thickness and SBP, and negative correlations between EF thickness and DBP (r = - 0.13; $p < 0.01$). However, Iacobellis et al.,[29] found statistically significant correlations only for DBP (r = 0.589; $p = 0.02$), and Wang et al., [34] (using CT) did not obtain statistically significant results neither with SBP nor with DBP.

Regarding the anthropometric parameters, in our study, we found statistically significant correlations between EF and weight, BSA, lean mass and CC. Shetty et al.[25] (r = 0.346; $p < 0.001$) and Willens et al.,[30] (r = 0.51; $p = 0.011$) obtained similar results, but only for weight. Using CT, Nakazato et al.,[33] Wang et al.,[34] and Alexopoulos et al.,[31] obtained statistically significant correlations between EF and weight, BMI, height, and abdominal circumference. Other studies refer the existence of statistically significant correlations between EF thickness and abdominal circumference [10-13,19,22,25,28,29] and between EF thickness and fat mass.[11,12]

Among the echocardiographic variables evaluated in our study, statistically significant results were found between EF thickness and LV end-diastolic diameter only. Iacobellis et al.,[29] also obtained statistically significant results for the correlation between EF thickness and LVMI (r = 0.755; $p = 0.01$), although Natale et al.,[24] referred that this correlation is only significant for values of EF thickness higher than 7 mm. Thus, apparently, the increase of the EF thickness seems to be associated with LVH,[14,18] and Kim et al.,[26] even reported that, among hypertensive individuals, EF was higher in those with LVH (7.5 ± 2.3 mm vs 6.6 ± 1.9 mm; $p = 0.034$).

Study Limitations

The small sample size and the fact that the studied population was older when compared with previous studies may have influenced the results. Although the use of more sophisticated methods for statistical analysis was limited by the small sample size, this is one of the few studies that evaluated EF and its relationship with clinical and anthropometric characteristics in old adults. Since the results were obtained from a cross-sectional analysis, no information regarding the variation in the EF content in the heart with ageing was available.

Conclusion and Future Perspectives

The results obtained demonstrate that EF is a common finding in the old adult, especially in males, and correlates with anthropometric parameters and LV end-diastolic diameter. Additional studies may give important contributions to a better understanding of the relationship between aging and EF. A cohort study, with a follow-up of an extended cohort during the aging process, will be important to evaluate the course of EF deposition throughout life, and determine the risks associated with it. Besides, a future challenge will be to understand if multidisciplinary and tailored interventions directed at optimizing functionality and promoting quality of life, including actions such as individualized physical exercise, and nutritional and therapeutic counseling, can be effective strategies to reduce EF and the associated risks.

Author Contributions

Conception and design of the research: Pereira T and Castanheira J. Acquisition of data: Pereira T, Castanheira J, Nunes C. Analysis and interpretation of the data: Pereira T, Castanheira J, Nunes C. Statistical analysis: Pereira T, Castanheira J, Nunes C. Obtaining financing : Pereira T. Writing of the manuscript: Nunes C, Castanheira J. Critical revision of the manuscript for intellectual content : Pereira T, Castanheira J.

Potential Conflict of Interest

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

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Study Association

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the *Instituto Politécnico de Coimbra* under the protocol number 8/2018. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

References

1. Xu B, Daimon M. Cardiac aging phenomenon and its clinical features by echocardiography. J Echocardiogr. 2016;14(4):139-45.
2. Sahasakul Y, Edwards WD, Naessens JM, Tajik AJ. Age-related changes in aortic and mitral valve thickness: implications for two-dimensional echocardiography based on an autopsy study of 200 normal human hearts. Am J Cardiol. 1988;62(7):424-30.
3. Keller KM, Howlett SE. Sex differences in the biology and pathology of the aging heart. Can J Cardiol. 2016;32(9):1065-73.
4. Martins Jr LM. The elderly and heart: valves. Ponto de Vista. 2016;18(1):58-9.
5. Nagy E, Jermendy AL, Merkely B, Maurovich-Horvat P. Clinical importance of epicardial adipose tissue. Arch Med Sci. 2017;13(4):864-74.
6. Talman AH, Psaltis PJ, Cameron JD, Meredith IT, Seneviratne SK, Wong DT. Epicardial adipose tissue: far more than a fat depot. Cardiovasc Diagn Ther. 2014;4(6):416-29.
7. Meenakshi K, Rajendran M, Srikumar S, Chidambaram S. Epicardial fat thickness: a surrogate marker of coronary artery disease - assessment by echocardiography. Indian Heart J. 2016;68(3):336-41.
8. Bertaso AG, Bertol D, Duncan BB, Foppa M. Epicardial fat: definition, measurements and systematic review of main outcomes. Arq Bras Cardiol. 2013;101(1):18-28.
9. Silva CM, Nascimento CAS, Castelli RC, Saraiva RC, Pustilnic A. Reference values for epicardial fat diameter measured by echocardiography in a normal population. Rev Bras Ecocardiogr Imagem Cardiovasc. 2011;24(1):24-9.
10. Sharda M, Nigam H, Meena SR, Soni A, Singh A, Sharma N. Correlation and comparison of epicardial adipose tissue with sagittal abdominal diameter and other anthropometric and biochemical variables of metabolic syndrome. J Assoc Physicians India. 2017;65(5):34-40.
11. Iacobellis G, Ribaudo MC, Assael F, Vecci E, Tiberti C, Zappaterreno A, et al. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. J Clin Endocrinol Metab. 2003;88(11):5163-8.
12. Iacobellis G, Assael F, Ribaudo MC, Zappaterreno A, Alessi G, Di Mario U, et al. Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction. Obes Res. 2003;11(2):304-10.
13. Iacobellis G, Willens HJ, Barbaro G, Sharma AM. Threshold values of high-risk echocardiographic epicardial fat thickness. Obesity (Silver Spring). 2008;16(4):887-92.
14. Iacobellis G, Willens HJ. Echocardiographic epicardial fat: a review of research and clinical applications. J Am Soc Echocardiogr. 2009;22(12):1311-9.
15. Iacobellis G, Corradi D, Sharma AM. Epicardial adipose tissue: anatomic, biomolecular and clinical relationships with the heart. Nat Clin Pract Cardiovasc Med. 2005;2(10):536-43.
16. Silaghi AC, Pais R, Valea A, Mironiuc A, Silaghi H. Epicardial adipose tissue and relationship with coronary artery disease. Cent Eur J Med. 2011;6(3):251-62.
17. Matloch Z, Kotulák T, Haluzík M. The role of epicardial adipose tissue in heart disease. Physiol Res. 2015;65(1):23-32.
18. Silaghi A, Piercecchi-Marti MD, Grino M, Leonetti G, Alessi MC, Clement K, et al. Epicardial adipose tissue extent: relationship with age, body fat distribution, and coronaropathy. Obesity (Silver Spring). 2008;16(11):2424-30.
19. Calabuig Á, Barba J, Guembe MJ, Díez J, Berjón J, Martínez-Vila E, et al. Epicardial adipose tissue in the general middle-aged population and its association with metabolic syndrome. Rev Esp Cardiol (Engl Ed). 2017;70(4):254-60.
20. Iacobellis G. Local and systemic effects of the multifaceted epicardial adipose tissue depot. Nat Rev Endocrinol. 2015;11(6):363-71.
21. Iacobells G, Malavazos AE, Corsi MM. Epicardial fat: from the biomolecular aspects to the clinical practice. Int J Biochem Cell Biol. 2011;43(12):1651-4.
22. Jeong JW, Jeong MH, Yun KH, Oh SK, Park EM, Kim YK, et al. Echocardiographic epicardial fat thickness and coronary artery disease. Circ J. 2007;71(4):536-9.
23. Mookadam F, Goel R, Alharthi MS, Jiamsripong P, Cha S. Epicardial fat and its association with cardiovascular risk: a cross-sectional observational study. Heart Views. 2010;11(3):103-8.
24. Natale F, Tedesco MA, Mocerino R, Simone V, Di Marco GM, Aronne L, et al. Visceral adiposity and arterial stiffness: echocardiographic epicardial fat thickness reflects, better than waist circumference, carotid arterial stiffness in a large population of hypertensives. Eur J Echocardiogr. 2009;10(4):549-55.
25. Shetty R, Vivek G, Naha K, Nayak K, Goyal A, Dias LS. Correlation of epicardial fat and anthropometric measurements in Asian-Indians: a community based study. Avicenna J Med. 2012;2(4):89-93.
26. Kim SA, Kim MN, Shim WJ, Park SM. Epicardial adipose tissue is related to cardiac function in elderly women, but not in men. Nutr Metab Cardiovasc Dis. 2017;27(1):41-7.
27. Mazzoccoli G, Dagostino MP, Greco A. Age-related changes of epicardial fat thickness. Biomed Prev Nutr. 2012;2(1):38-41.
28. Karadag B, Ozulu B, Ozturk FY, Oztekin E, Sener N, Altuntas Y. Comparison of epicardial adipose tissue (EAT) thickness and anthropometric measurements in metabolic syndrome (MS) cases above and under the age of 65. Arch Gerontol Geriatr. 2011;52(2):e79-84.
29. Iacobellis G, Ribaudo MC, Zappaterreno A, Iannucci CV, Leonetti F. Relation between epicardial adipose tissue and left ventricular mass. Am J Cardiol. 2004;94(8):1084-7.
30. Willens HJ, Byers P, Chirinos JA, Labrador E, Hare JM, de Marchena E. Effects of weight loss after bariatric surgery on epicardial fat measured using echocardiography. Am J Cardiol. 2007;99(9):1242-5.
31. Alexopoulos N, McLean DS, Janik M, Arepalli CD, Stillman AE, Raggi P. Epicardial adipose tissue and coronary artery plaque characteristics. Atherosclerosis. 2010;210(1):150-4.
32. Shim IK, Cho KI, Kim HS, Heo JH, Cha TJ. Impact of gender on the association of epicardial fat thickness, obesity, and circadian blood pressure pattern in hypertensive patients. J Diabetes Res. 2015;2015:924539.
33. Nakazato R, Rajani R, Cheng VY, Shmilovich H, Nakanishi R, Otaki Y, et al. Weight change modulates epicardial fat burden: a 4-year serial study with non-contrast computed tomography. Atherosclerosis. 2012;220(1):139-44.
34. Wang CP, Hsu HL, Hung WC, Yu TH, Chen YH, Chiu CA, et al. Increased epicardial adipose tissue (EAT) volume in type 2 diabetes mellitus and association with metabolic syndrome and severity of coronary atherosclerosis. Clin Endocrinol (Oxf). 2009;70(6):876-82.
35. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, et al. Human epicardial adipose tissue is a source of inflammatory mediators. Circulation. 2003;108(20):2460-6.
36. Iacobellis G, Leonetti F, Singh N, Sharma AM. Relationship of epicardial adipose tissue with atrial dimensions and diastolic function in morbidly obese subjects. Int J Cardiol. 2007;115(2):272-3.
37. Iacobellis G, Singh N, Wharton S, Sharma AM. Substantial changes in epicardial fat thickness after weight loss in severely obese subjects. Obesity (Silver Spring). 2008;16(7):1693-7.
38. Kim MK, Tomita T, Kim MJ, Sasai H, Maeda S, Tanaka K. Aerobic exercise training reduces epicardial fat in obese men. J Appl Physiol. 2009;106(1):5-11.

39. Singh N, Singh H, Khanijoun HK, Iacobellis G. Echocardiographic assessment of epicardial adipose tissue -a marker of visceral adiposity. Mcgill J Med. 2007;10(1):26-30.

40. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015;28(1):1-39.

41. Mitchell C, Rahko PS, Blauwet LA, Ogunyankin KO, Palma RA, Velasquez EJ, et al. Guidelines for performing a comprehensive transthoracic echocardiographic examination in adults: recommendations from the American Society of Echocardiography. J Am Soc Echocardiogr. 2019;32(1):1-64.

42. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc. 2009;41(1):3-13.

43. Dey DK, Rothenberg E, Sundh V, Bosaeus I, Steen B. Height and body weight in the elderly. I. A 25-year longitudinal study of a population aged 70 to 95 years. Eur J Clin Nutr. 1999;53(12):905-14.

44. Iacobellis G, Diaz S, Mendez A, Goldberg R. Increased epicardial fat and plasma leptin in type 1 diabetes independently of obesity. Nutr Metab Cardiovasc Dis. 2014;24(7):725-9.

45. Li Y, Liu B, Li Y, Jing X, Deng S, Yan Y, et al. Epicardial fat tissue in patients with diabetes mellitus: a systematic review and meta-analysis. Cardiovasc Diabetol. 2019;18(1):3.

46. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo Jr JL, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. JAMA. 2003;289(19):2560-72.

47. Wajngarten M. O coração no idoso. Jornal Diagnósticos em Cardiologia. 2010;13(43):1-9.

48. Cheitlin MD. Cardiovascular physiology- changes with aging. Am J Geriatr Cardiol. 2003;12(1):9-13.

49. Hall JE. Tratado de fisiologia médica. 12a ed. Rio de Janeiro: Elsevier; 2011.

EDITORIAL

Epicardial Fat Thickness: a Promising Cardiovascular Risk Factor that Requires in-Depth Studies

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Editorial referring to the article: Correlation between epicardial fat thickness and clinical and anthropometric variables in an elderly population

This issue of the International Journal of Cardiovascular Sciences presents a paper by Castanheira et al.[1] that addresses epicardial fat (EF) thickness in an elderly Portuguese population. They describe the mean values of EF thickness and its correlation with anthropometric, echocardiographic, and clinical variables in these individuals.

EF is the visceral adipose tissue located between the myocardium and the visceral pericardium, and commonly found in the atrioventricular and interventricular grooves. EF has several physiological roles, including local effects on the heart, in a paracrine manner.[2-4] EF can be measured non-invasively by echocardiography, and the measurement of EF thickness by 2D echocardiography has been proposed as a surrogate for visceral fat, since it is easier to perform as compared with direct measures such as computed tomography and magnetic resonance imaging (MRI). In addition, a significant correlation between EF and visceral fat measurements by MRI was demonstrated.[5]

Visceral fat accumulation is associated with metabolic syndrome, insulin resistance, impaired glucose tolerance, diabetes mellitus, polycystic ovarian syndrome, and cardiovascular disease.[6] Moreover, visceral fat is an independent predictor of mortality in males.[7] Therefore, the non-invasive, cheap and easy assessment of visceral fat, allowed by the EF thickness measurement, is highly interesting. Moreover, increased EF thickness can be directly associated with cardiac disease, such as coronary artery disease (CAD), due to the secretion of proinflammatory adipokines.[3,8]

Keywords

Pericardium; Adipose Tissue; Portugal/epidemiology; Aged; Anthropometry; Metabolic Syndrome; Echocardiography/methods.

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To test if EF thickness can be a surrogate for visceral fat and consequently, a useful tool for cardiovascular risk stratification, it is important to demonstrate that EF thickness correlates with anthropomorphic measurements, metabolic syndrome, cardiovascular disease and cardiovascular prognosis. EF thickness was described to be greater in patients with metabolic syndrome, independent of sex,[9,10] to be associated with cardiovascular risk burden,[11] CAD,[12-14] and carotid artery disease.[15] However, EF thickness measurement has limitations, including the fact that it is a linear (instead of volumetric) measure, operator dependency, and poor acoustic window. In addition, issues like the association of EF thickness with diabetes mellitus, dyslipidemia, cardiovascular events, and reference values in the elderly population, remain to be properly addressed.

The study of EF thickness in an elderly population is very important as this population has a higher cardiovascular risk. In the paper by Castanheira et al.,[1] the authors included 34 (25 women, 9 men) very old individuals (mean age 82 ± 8 years) without a previous history of cerebrovascular or cardiac disease. The mean EF thickness was 5.4 ± 1.1 mm, and the authors found a correlation between EF thickness and calf circumference, body weight, body surface area, lean mass, and left ventricular end-diastolic diameter. However, despite the importance of the results, conclusions are limited by the small number of participants, especially men. Potential correlations found by the authors between EF thickness and body mass index (r=0.3) and diabetes mellitus (r=-0.3) may have reached statistical significance if a larger population had been used. Therefore, the study lacked power to evaluate the proposed correlations between EF and clinical and echocardiographic characteristics in an elderly population.

There are still many issues to be addressed in longitudinal studies with larger samples before the EF thickness can be incorporated into clinical practice as a new cardiovascular risk factor. We need to understand whether the EF thickness is capable of predicting cardiovascular outcomes independent of traditional risk factors, if its changes over time correlate with a worse prognosis, and finally, whether EF accumulation can be reversed with control of risk factors.

References

1. Castanheira J, Nunes C, Pereira T. Correlation between epicardial fat thickness and clinical and anthropometric variables in an elderly population. Int J Cardiovasc Sci. 2021; 34(2):136-146.
2. Iacobellis G, Willens HJ. Echocardiographic epicardial fat: a review of research and clinical applications. J Am Soc Echocardiogr. 2009;22(12):1311-9.
3. Bertaso AG, Bertol D, Duncan BB, Foppa M. Epicardial fat: definition, measurements and systematic review of main outcomes. Arq Bras Cardiol. 2013;101(1):e18-e28.
4. Iacobellis G, Barbaro G. The double role of epicardial adipose tissue as pro- and anti-inflammatory organ. Horm Metab Res. 2008;40(7):442-5.
5. Iacobellis G, Assael F, Ribaudo MC, Zappaterreno A, Alessi G, Di MU, et al. Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction. Obes Res .2003;11(2):304-10.
6. Villasante Fricke AC, Iacobellis G. Epicardial Adipose Tissue: Clinical Biomarker of Cardio-Metabolic Risk. Int J Mol Sci. 2019;20(23):5989.
7. Kuk JL, Katzmarzyk PT, Nichaman MZ, Church TS, Blair SN, Ross R. Visceral fat is an independent predictor of all-cause mortality in men. Obesity (Silver Spring) 2006;14(2):336-41.
8. Ito T, Nasu K, Terashima M, Ehara M, Kinoshita Y, Ito T, et al. The impact of epicardial fat volume on coronary plaque vulnerability: insight from optical coherence tomography analysis. Eur Heart J Cardiovasc Imaging. 2012;13(5):408-15.
9. Pierdomenico SD, Pierdomenico AM, Cuccurullo F, Iacobellis G. Meta-analysis of the relation of echocardiographic epicardial adipose tissue thickness and the metabolic syndrome. Am J Cardiol. 2013;111(1):73-8.
10. Iacobellis G, Willens HJ, Barbaro G, Sharma AM. Threshold values of high-risk echocardiographic epicardial fat thickness. Obesity (Silver Spring). 2008;16(4):887-92.
11. Mahabadi AA, Massaro JM, Rosito GA, Levy D, Murabito JM, Wolf PA, et al. Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: the Framingham Heart Study. Eur Heart J. 2009;30(7):850-6.
12. Berg G, Miksztowicz V, Morales C, Barchuk M. Epicardial Adipose Tissue in Cardiovascular Disease. Adv Exp Med Biol. 2019;1127:131-43.
13. Jeong JW, Jeong MH, Yun KH, Oh SK, Park EM, Kim YK, et al. Echocardiographic epicardial fat thickness and coronary artery disease. Circ J. 2007;71(4):536-9.
14. Mustelier JV, Rego JO, Gonzalez AG, Sarmiento JC, Riveron BV. Echocardiographic parameters of epicardial fat deposition and its relation to coronary artery disease. Arq Bras Cardiol. 2011;97(2):122-9.
15. Magliano CAS, Nascimento CAS, Innocenzi AM, Pustilnic A, Castelli RC, Saraiva RM. Epicardial fat measured by echocardiograhy correlates to carotid atheromathosis. Rev Bras Ecocardiogr Imagem Cardiovasc. 2011;24(2):16-22.

ORIGINAL ARTICLE

Short-Term Effects of a Resistance Training Program Using Elastic Tubing in Patients with Heart Disease

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Abstract

Background: Resistance training is effective in cardiac rehabilitation; however, it is conventionally performed using free weights or machines, which can pose logistic challenges to patients with restricted mobility. For its ease of access and cost-effectiveness, elastic tubing is a particularly appealing alternative, but it remains underutilized for this purpose.

Objective: To evaluate muscle strength, functional capacity, aerobic capacity, and quality of life in patients with heart disease in phase II of cardiovascular rehabilitation after a resistance training intervention based solely on elastic tubing.

Methods: Thirteen patients with heart disease (age 63.33±10.80 years) trained with elastic tubing twice weekly for 6 weeks, with progressive load increase every 15 days. The following muscle groups were evaluated and trained: shoulder abductors and flexors, elbow flexors, and knee flexors and extensors. Muscle strength was evaluated using a dynamometer; functional capacity, with a 6-minute walk test and cardiopulmonary exercise test; and quality of life, using the SF-36 questionnaire. Data normality was assessed using the Shapiro–Wilk test. The paired Student's t-test was used for comparisons before and after training, at a significance level of <5%.

Results: There were significant differences in muscle strength (except for elbow flexion) and functional capacity (485.5 ± 123.3 vs 578.7 ± 110.5; p=0.0399) after the intervention. No statistical differences were found in cardiorespiratory fitness or quality of life.

Conclusions: Short-term resistance training with elastic tubing improved peripheral muscle strength and functional capacity in patients with heart disease, and should be encouraged for this population. (Int J Cardiovasc Sci. 2021; 34(2):149-156)

Keywords: Resistance Training; Exercise; Cardiovascular Diseases; Muscle Strength Dynamometer; Functional Residual Capacity; Cardiac Rehabilitation; Elastic Tubing.

Introduction

Approximately 7.3 million deaths per year occur worldwide as a result of cardiovascular disease (CVD), a number that is expected to exceed 23.6 million by 2030.[1] In Brazil, 300,000 people die each year from CVD, including stroke, heart failure, and heart attack or sudden cardiac death, representing 820 deaths per day, 30 deaths per hour, or one death every 2 minutes.[2,3]

Heart diseases result in physical deconditioning and skeletal muscle changes, such as atrophy and decreased strength, that interfere with functional capacity and

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quality of life.[4,5] Therefore, it is of paramount importance that individuals with heart disease be included in a cardiac rehabilitation program (CRP).[5]

A CRP consists of four phases, with the first beginning in hospital, and is an ongoing process which aims to maintain well-being and facilitate resumption of social and professional activities.[6,7] Phase 2 of cardiac rehabilitation begins after hospital discharge or a few days after a cardiovascular event. At this stage, resistance training can be initiated to increase fatigue resistance and muscle strength, with a view to return to work or recreational activities.[8,9]

In clinical practice, resistance training to improve muscle strength is conventionally performed by means of free weights or weight machines, however, these require extensive physical space and are usually located at outside facilities, such as gyms and rehabilitation centers, which poses an additional challenge to patients with restricted mobility.[4] On the other hand, elastic tubes are practical, low-cost, portable, safe devices which can be used for resistance training, with the added advantage of increasing tension linearly from the start of the contraction until the end of the movement, which tends to be less damaging to the joints compared to free-weight or weight machine training.[10,11]

Training with elastic tubing has been associated with increased muscle strength in older adults with heart and lung diseases,[4,12] and has been shown to promote cardiorespiratory improvement in patients with chronic obstructive pulmonary disease.[13] However, whether this exercise modality can have a positive impact on cardiorespiratory capacity and quality of life in patients with heart disease remains unclear.[12] We hypothesized that this alternative method of strength training would yield short-term gains in muscle strength, functional ability, aerobic capacity, and quality of life of these patients.

Thus, the objective of this study was to analyze the effects of a strength training program based on elastic tubing on functional capacity, maximum aerobic capacity, muscle strength, and quality of life in individuals with CVD in phase 2 of cardiovascular rehabilitation.

Methods

Case Series

This was a clinical trial conducted at a school of Physical Therapy. Male and female patients aged 45–79 years, with known heart disease (coronary artery disease, status post myocardial revascularization procedures, or acute myocardial infarction) who had been in phase 2 of cardiac rehabilitation for at least 3 months were recruited.

For participation, patients were required to be hemodynamically stable (mean arterial pressure 90–120 mmHg and no arrhythmia of any type), with no changes in medication for a minimum of 30 days. All patients were instructed not to perform resistance training with weights, dumbbells, or any device other than the elastic tubing. Patients with neurological, musculoskeletal, or articular disorders (such as rheumatic disorders), unstable heart disease as detected by electrocardiography, or lung diseases which might prevent them from completing the training protocol were excluded.

To avoid outcome bias, assessments and training sessions were conducted by different therapists. The following variables were evaluated: muscle strength, functional capacity, maximum aerobic capacity, and quality of life before and after 6 weeks of resistance training with elastic tubing.

All participants were given information regarding study procedures and provided written informed consent before enrollment. The study protocol was approved by the institutional Research Ethics Committee (CAAE: 44443915.5.0000.5515) and was registered on the ClinicalTrials.gov platform under identifier code NCT03580538.

Evaluation of Muscle Strength

Muscle strength was evaluated at the start of the intervention protocol and after 6 weeks, using a digital force gauge (Force Gauge®, model FG-100kg, United States), and all results were expressed in newtons (N). The evaluation was performed using a steel cord coated with rigid plastic attached to a dynamometer, with one end attached to a fixed bar and the other end to the distal portion of the dominant limb of the patient.[14]

The following muscle groups were evaluated: shoulder abductors and flexors, elbow flexors, and knee extensors and flexors. The measurement was carried out in triplicate for each proposed movement, and the highest value of the three repetitions was recorded. During evaluation, the patient was required to maintain the maximum isometric contraction achieved for a period of 6 seconds, after which time they were allowed to relax their muscles for 1 minute before a new measurement was performed. Strength of the upper limbs was

measured with the patient in the standing position, and that of the lower limbs, with the patient sitting in the same chair used for resistance training.[14]

Evaluation of Functional Capacity

Functional capacity was evaluated by the 6-minute walk test (6MWT), conducted as per American Thoracic Society recommendations.[15] The test was performed in a gymnasium and was repeated twice, with an interval of 30 minutes between attempts. The highest value was taken into account for analysis. During the test, standardized verbal performance incentives were given every minute. Blood pressure, pulse oximetry, subjective perception of exertion, and heart and respiratory rate were measured throughout.

Evaluation of Maximal Aerobic Capacity

Cardiopulmonary exercise testing (CPET) was performed by a cardiologist. All tests were performed on a treadmill (Inbrasport ATL 2000). The modified Bruce protocol[16] was used, and the test performed until voluntary exhaustion. No patient exhibited any electrocardiographic alterations that might prevent completion of the test.

Cardiovascular parameters were monitored continuously: heart rate (Polar S810i, Finland), arterial oxygen saturation (Mindray PM 50, Brazil), and subjective perception of exertion (modified Borg scale).[17] The ventilatory variables were obtained through a VO2000 gas analyzer (Medical Graphics, USA), calibrated before each test according to the manufacturer's instructions. The average air flow, obtained every 10 seconds (Aerograph®, Michigan, USA), was used for all tests.

Peak oxygen consumption (VO_{2peak}) was calculates as the highest average maximum oxygen consumption (VO_2) of the final 30 seconds of evaluation, when at least two of the following criteria were met: 1) heart rate >90% of maximum predicted for the age of each patient (220 − age); 2) respiratory quotient (RQ) >1.10; 3) variation in VO_2 between the final two stages of evaluation less than 2.1 $ml.kg^{-1}.min^{-1}$. The velocity corresponding to VO_{2peak} (vVO_{2peak}) was recorded as the maximum intensity achieved during the test. If the patient reached exhaustion before the completion of the stage, the vVO_{2peak} was adjusted according to the equation proposed by Kuipers et al.,[18]

Evaluation of Quality of Life

Quality of life was assessed with the SF-36 generic questionnaire, validated for use in Brazil. The SF-36 consists of eight dimensions, scored from 0 to 100. For each dimension, values greater than 50 denote perception of good quality of life.[19]

Cardiovascular Parameters

Before and after each training session, the following cardiovascular parameters were recorded: systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR).

Blood pressure was measured in the dominant arm, with the participant in the seated position, using an aneroid sphygmomanometer (Premium®, China) and stethoscope (3M™ Littmann®, USA), following the latest Brazilian hypertension guidelines.[20] HR was monitored using a Polar® monitor (Polar, USA).

Resistance Training

Training sessions (duration 60 min each) took place in a climate-controlled environment, twice weekly over six weeks, for a total of 12 sessions. For safety, training heart rate reserve was calculated individually by the Karvonen formula.[21,22] During training, patients could not exceed this heart rate. The Borg scale was also used to control exercise intensity.[23]

Each training session was preceded by measurement of heart rate and blood pressure, followed by stretching of the upper and lower limbs. During the training period, patients performed only resistance training, not aerobic training. The resistance exercises were performed using Lemgruber© brand latex tubing (references: 200, 201, 202, 203, and 204), as well as an appropriate chair (see below).

Each reference number denotes different internal and external diameters, with higher numbers indicating higher resistance when the tube is taut; i.e., lower reference numbers imply a lower load for the patient, while higher references imply a higher load. The resistance is increased when more elastic tubing is added in subsequent sessions.

The muscle groups selected for training, on the basis of a previous study, were the shoulder abductors and flexors, elbow flexors, and knee extensors and flexors. The length of elastic tubing was determined according to the individual distance from the upper or lower limb to the hook on the chair. The upper-limb and lower-

limb movements were performed with the patient in the sitting position, with the exception of knee flexion, in which the patient stood in front of the chair used in the dynamometer test. As the elastic tubes should be fixed during training, chairs were constructed especially for this purpose; each chair was 72 cm high and 52 cm wide, and had points of attachment for the elastic tubes depending on the muscle group being exercised. For this purpose, one end of the elastic tube is fixed to the segment of the body that performs the arc of movement and the other is attached to the chair support. All movements were performed alternately and bilaterally throughout the permitted range of motion of each joint, with a 2-minute interval between series.[13]

The training protocol and increase in resistance (table 1), given according to the reference number of the elastic tubing, was evaluated individually based on the Borg rating of perceived exertion (13, slightly tiring).[22]

Statistical Analysis

Sample size calculation was based on the peripheral muscle strength of knee extension in a previous study by Ramos et al.,[14] with a standard deviation of 36.62, difference to be detected of 45.1, significance level of 5%, and test power of 80%. The sample size was calculated as 10 subjects per group.

The Shapiro–Wilk test was used to verify the assumption of normality. As all data were considered normal, Student's *t*-test for paired data was used for comparisons before and after resistance training, with results expressed as mean ± standard deviation. A significance level of <0.05 was established. All analyses were carried out in GraphPad Prism®.

Results

Due to the difficulty of adherence and the musculoskeletal limitations of some patients presented, only 13 subjects with heart disease were evaluated. One patient left the study due to hospitalization. Maximum aerobic capacity data (Table 3) is only available for eight patients, due to failure to capture data during one CPET at the final time point of evaluation. Data on functional capacity were excluded from one patient who experienced ankle pain.

The sample consisted of 12 patients with heart disease, 8 men and 4 women, with a mean age of 63.33±10.80 years. The mean weight, height, and BMI were 77.35±13.95 kg, 1.60±0.07 m, and 29.92±4.28 kg/m^2 respectively. The most commonly used drugs were antihypertensives (n=6), antiplatelet agents (n=6), and antilipemics (n=8). The most prevalent comorbidities were hypertension, diabetes, coronary heart disease, and a history of myocardial revascularization .

Figure 1 shows the differences in peripheral muscular strength between the baseline and week 6 time points. Significant differences were found after resistance training with elastic tubing. In the lower limbs, significant increases in strength were observed during both knee extension and flexion, while in the upper limbs, a significant difference was noted only in shoulder abduction.

Significant differences in 6MWT were also found between the baseline and 6-week time points. These analyses are described in Table 2.

Table 3 shows the maximum aerobic capacity values of the evaluated patients. Analysis of VO_{2peak} (in mL·kg/min and L) and vVO_{2peak} did not demonstrate a significant increase after 6 weeks of training with elastic tubing.

Table 1 - Incremental change in resistance over the course of the training protocol.

Time points	Variables		
	Repetitions	Reference UL	Reference LL
1st and 2nd week	2x15	200	201
3nd and 4th week	2x12	200+201 to 201+203	201+202 to 202+204
5th and 6th week	3x10	200+202 to 203+204	201+202 +203+204 to all references

UL: upper limbs; LL: lower limbs.

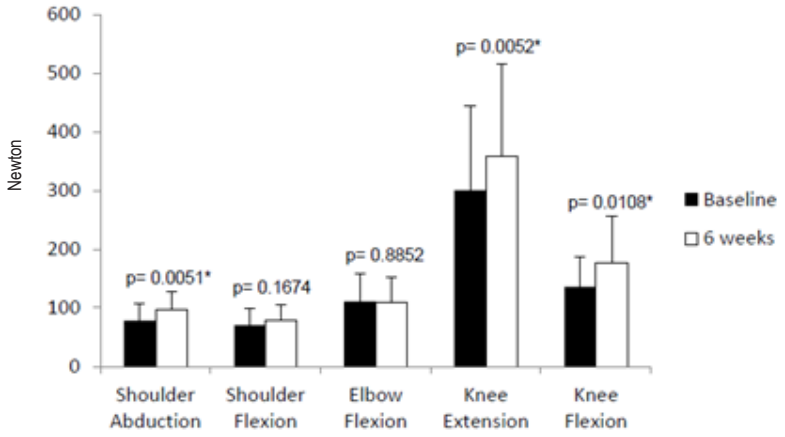

Figure 1 – Peripheral muscle strength presented as mean and standard deviation of the evaluated patients before and after 6 weeks of resistance training with elastic tubing. *Significant difference between the baseline moment and after 6 weeks of training. Shapiro-Wilk test followed by Student t test.

Table 2 - Functional capacity through the 6-minute walk test of the evaluated patients.

Variables	Moments (n= 11)		p value
	Baseline	6 weeks	
Distance traveled (m)	485.50±123.30	578.70±110.50	0.0399
Distance Predicted (m)	531.10±126.30	531.10±126.30	1.0000

M – meters. The results are presented as mean ± standard deviation. Shapiro-Wilk test followed by Student t test.

The distribution of SF-36 quality of life scores is shown in Table 4. There was no significant difference in any of the eight dimensions of the questionnaire after 6 weeks of training with elastic tubing as compared to baseline.

Discussion

The present study showed that 6 weeks of resistance training with elastic tubing had a positive influence on muscle strength of the lower limbs and shoulder abductors, as well as on functional capacity, in patients with heart disease. However, there was no significant difference in maximum aerobic capacity or quality of life.

A gain in muscle strength is an expected result of resistance training,[24] and is of great clinical relevance, as it is related to improved ability to perform activities of daily living and work in patients with heart disease. Moreover, in the specific population of patients with CVD, improved muscle strength is associated with less overload on the cardiovascular system .[14] One previous study of resistance training with elastic tubing demonstrated this effect of increasing muscle strength and increasing functional capacity in patients with heart disease.[4] The authors compared the use of a resistance training program based on elastic tubing and conventional weight training. The intensity of resistance with elastic tubing was varied by changing the elastic tube length in relation to the body segment of the patient under evaluation, which differs from the present study, where resistance was increased by using different diameters of elastic tubing; the studies also followed different training schedules.[25]

Table 3 - Aerobic power of the evaluated patients.

Variables	Moments (n=8)		p-value
	Baseline	6 weeks	
VO_{2peak} (mL·kg/min)	12.19±2.92	14.70±5.12	0.1200
VO_{2peak} (L)	0.94±0.32	1.17±0.59	0.1186
vVO_{2peak} (km/h)	3.12±0.58	3.54±0.66	0.0533

VO_{2peak}: peak oxygen consumption, vVO_{2peak}: exercise velocity corresponding to peak oxygen consumption; mL·kg/min: milliliters/kilograms/minute; L: liters; km/h: kilometers/hour. Results presented as mean ± standard deviation. Shapiro-Wilk test followed by Student's t-test.

Table 4 - Quality of life of the evaluated patients as assessed by SF-36 domains.

Dimensions	Moments (n=10)		p-value
	Baseline	6 weeks	
Functional capacity	76.00±14.10	74.50± 4.80	0.3898
Physical functioning	41.67±50.00	52.50±47.8	1.0000
Pain	63.33±22.88	56.60±22.69	0.4407
General health	58.28±22.57	56.80±13.31	0.9702
Vitality	63.89±14.31	59.50±19.5	0.7759
Role functioning, social	78.33±25.59	88.00±17.35	0.2228
Role functioning, emotional	55.51±47.16	46.20±45.00	0.6250
Mental health	72.89±13.38	68.70±20.18	0.9174

Results expressed as mean ± standard deviation. Shapiro–Wilk test followed by Student's t-test.

In relation to the initial resistance of the elastic tubing, Turban et al.,[4] assessed the one-repetition maximum (1RM), unlike in our study, where increasing resistance was measured according to perceived exertion on the Borg scale. Turban et al.,[4] reported improvement in functional capacity and increased muscle strength and noted that this training modality may be a tool with which to begin cardiac rehabilitation, not excluding the use of conventional equipment, but as an adjunct.

Ramos et al.,[14] compared resistance training performed with elastic tubing versus training carried out with a conventional weight machine in patients with COPD. The elastic resistance protocol used individualized repetitions according to the fatigue resistance test; series started with two repetitions and progressed to seven at the end of the intervention, while the conventional training protocol consisted of three sets of 10 repetitions. Both training programs provided benefits in muscle strength and quality of life, however, greater gains in functional capacity were found in the elastic tubing group.[14]

Some important differences in protocol between the aforementioned study of COPD patients and the present study must be mentioned; for example, those authors used 8 weeks of training, while the present

study used 6 weeks. Another difference concerns the way in which resistance and the number of repetitions were increased. In the present study, a gradual increase in resistance and series and a decrease in number of repetitions were implemented, so that the training was designed to increase muscular strength and not just fatigue resistance, as in the study of COPD patients.[14]

Regarding functional capacity, our participants exhibited a significant improvement after 6 weeks of resistance training. A recent meta-analysis reported similar behavior: conventional resistance with weight machines was associated with a significant gain in functional capacity as soon as 4 weeks after the start of training.[26] Ramos et al.,[14] observed greater effects on functional capacity in training with elastic tubing when compared to conventional training, which demonstrates an advantage of this resource.

This gain in functional capacity observed in these studies may be explained by an increase in type I muscle fiber area and in oxidative enzyme activity in the skeletal musculature, both of which are known to be promoted by resistance training.[27]

The aerobic capacity of these patients did not demonstrate any significant change. This finding may be related to the lack of aerobic activity during the study training protocol. It is known that both the practice of aerobic exercise in isolation and aerobic exercises combined with resistance training promote improvement in VO_{2peak} [14] however, there are no studies to provide evidence of the influence of resistance training with elastic tubing alone on aerobic capacity in patients with heart disease. Other studies have shown similar values when evaluating initial aerobic capacity of similar patient populations; however, in their protocols, aerobic training or combined aerobic and resistance training were performed over a prolonged period (11 months on average), before and after intervention. This indicates that aerobic capacity tends to increase significantly with long-term training.[28]

Analysis of quality of life in our patients did not show significant change in any of the eight SF-36 dimensions. However, it should be noted that, at the start of training, the patients already had a good perception of quality of life (i.e., values above 50 points) in seven of the eight dimensions. The exception was the physical limitation domain, which scored below 50 points on average at baseline and improved after the intervention, although the difference was not significant. The quality of life dimension is more related to functional capacity than muscle strength, as observed in a study by Nogueira et al., which showed a direct correlation between functional capacity and quality of life.[29]

The intervention proposed herein – resistance training with the use of elastic tubing – facilitates adaptation of the neuromuscular system to changes in intensity throughout the range of motion of the exercise, since the elastic tube generates resistance not only in the plane of movement, thus favoring constant modulation of the muscle strength used over the range of motion, but also promoting joint safety as compared to conventional exercise. This characteristic was shown to be beneficial and effective as an alternative to a conventional resistance training program within the context of a CRP.[30]

Limitations of this study include the small sample size and the use of a subjective variable (Borg rating of perceived exertion) as the determinant of training progression. We suggest that future studies consider other measures, such as the 1RM. In addition, future studies to measure the force exerted by elastic tubes of different diameters, which could be readily accomplished using Hooke's law, may also contribute to the literature.

Conclusions

Short-term resistance training with elastic tubing was able to promote improvement in peripheral muscle strength and functional capacity in patients with heart disease. However, no changes were observed in maximal aerobic capacity or quality of life.

Potential Conflict of Interest

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

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Study Association

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

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the *Universidade do Oeste Paulista* under the protocol number 44443915.5.0000.5515. All the procedures in

this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

Author Contributions

Conception and design of the reseach: JPLN Silva. Writing of the manuscript: JPLN Silva. Acquisition of data: TIS Ferreira, GC Cvalleri, BP Galindo, NT Silva, BSA Silva. Writing of the manuscript: MMA Cruz, MR Leite. Analysis and interpretation of the data: APCF Freire, EMC Ramos. Critical revision of the manuscript for intelectual content: LCM Vanderlei, FL Pacagnelli.

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References

1. World Health Organization. (WHO) World Stroke Organization. Global atlas on cardiovascular disease prevention and control: policies, strategies, and interventions. Geneva; 2011.
2. Brasil.Ministério da Saúde. Secretaria de Vigilância à Saúde. Plano de ações estratégicas para o enfrentamento das doenças crônicas não transmissíveis no Brasil, 2011-2022. Rio de Janeiro, DF: OMS; 2011.
3. Ribeiro AG, Cotta RMM, Ribeiro SMR. The promotion of health and integrated preventionof risk factors for cardiovascular diseases. Ciênc. Saúde Coletiva. 2012;17(1):7-17.
4. Turban C, Culas C, Deley G. Effects of a short-term resistance program using elastic bands or weight machines in cardiac rehabilitation. Sci Sports. 2014;29(3):143-9.
5. Adams J, Cline M, Reed M, Masters A, Ehlke K, Hartman J. Importance of resistance training for patients after a cardiac event. Proc (Bayl Univ Med Cent). 2006;19(3):246-8.
6. Muela HCS, Bassan R, Serra SM. Evaluation of the functional benefits of a cardiac rehabilitation program. Rev Bras Cardiol. 2011;24(4):241-50.
7. Guimarães FAB, Gardenghi G, Silva FMF. Reabilitação cardíaca, tratamento e prevenção: revisão bibliográfica. Rev Mov. 2015;8(1):50-9.
8. Smith SC Jr, Benjamin EJ, Bonow RO, Braun LT, Creager MA, Franklin BA, et al. AHA/ACCF secondary prevention and risk reduction therapy for patients with coronary and other atherosclerotic vascular disease: 2011 update: a guideline from the American Heart Association and American College of Cardiology Foundation. Circulation. 2015;131(15):e408.
9. Hillis LD, Smith PK, Anderson JL, Bittl JA, Bridges CR, Byrne JG, et al. 2011 ACCF/AHA Guideline for Coronary Artery Bypass Graft Surgery: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation. 2011;124(23):2610-42.
10. Melchiorri G, Rainoldi A. Muscle fatigue induced by two different resistances: Elastic tubing versus weight machines. J Electromyogr Kinesiol. 2011;21(6):954-9.
11. Thomas M, Müller T, Busse MW. Quantification of tension in Thera-Band and Cando tubing at different strains and starting lengths. J Sports Med Phys Fitness. 2005;45(2):188-98.
12. Gonçalves ACCR, Pastre CM, Camargo Filho JCS, Vanderlei LCM. Resistance exercise in heart disease: systematic review. Fisioter Mov. 2012;25(1):195-205.
13. Ricci-Vitor AL, Vanderlei LCM , Pastre CM, Ramos D, Ramos EMC, Ferreira Filho C, et al. Elastic tubing resistance training and autonomic modulation in subjects with chronic obstructive pulmonary disease. BioMed Res Int. 2018;2018:9573630.
14. Ramos EM, Toledo-Arruda AC, Fosco LC, Bonfim R, Bertolini GN, Guarnier FA, et al. The effects of elastic tubing-based resistance training compared with conventional resistance training in patients with moderate chronic obstructive pulmonary disease: a randomized clinical trial. Clin Rehabil. 2014;28(11):1096-106.
15. Brooks D, Solway S, Gibbons WJ. ATS statement on six-minute walk test. Am J Respir Crit Care Med. 2003;167(9):1287.
16. Meneghelo RS, Araújo CGS, Stein R, Mastrocolla LE, Albuquerque PF, Serra SM. III Diretrizes da Sociedade Brasileira de Cardiologia sobre teste ergométrico. Arq Bras Cardiol. 2010;95(5):1-26.
17. Kendrick KR, Baxi SC, Smith RM. Usefulness of the modified 0-10 Borg scale in assessing the degree of dyspnea in patients with COPD and asthma. J Emerg Nurs. 2000;26(3):216-22.
18. Kuipers H, Verstappen FTJ, Keizer HA, Guerten P, Van Kranenburg G. Variability of anaerobic performance in the laboratory and its physiologic correlates. Int J Sports Med. 1985;6(4):197-201.
19. Ciconelli RM, Ferraz MB, Santos W, Meinão I, Quaresma MR. Tradução para a língua portuguesa e validação do questionário genérico de avaliação de qualidade de vida SF-36 (Brasil SF-36). Rev Bras Reumatol. 1999;39(3):143-50.
20. Tavares A, Brandão AA, Sanjuliani AF, Nogueira AR, Machado CA, Poli-de-Figueiredo, et al., Sociedade Brasileira de Cardiologia. VI Diretrizes Brasileiras de Hipertensão Arterial. Arq Bras Cardiol. 2010;95(1 supl 1):1-51.
21. Karvonen M, Kentala E, Mustata O. The effects of training on heart rate. Ann Med Exp Biol Fenn. 1957;35(3):307-15.
22. Fardy PS, Franklin BA, Porcari JP, Verrill DE. Técnicas de treinamento em reabilitação cardíaca. São Paulo: Manole; 2001. 149p.
23. Borg GAV. Psychophysical bases of perceived exertion. Med Sci Sport Exerc. 1982;14(5):377-81.
24. Granacher U, Lesinski M, Büsch D, Muehlbauer T, Prieske O, Puta C, et al. Effects of resistance training in youth athletes on muscular fitness and athletic performance: a conceptual model for long-term athlete development. Front Physiol. 2016 9 May;7:164.
25. Persch LN, Ugrinowitsch C, Pereira G, Rodacki AL. Strength training improves fall-related gait kinematics in the elderly: a randomized controlled trial. Clin Biomech. 2009;24(10):819-25.
26. American College of Sports Medicine. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. Med Sci Sports Exerc. 2009;41(3):687-708.
27. Simões RP, Mendes RG, Castello V, Machado HG, Almeida LB, Baldissera V, et al. Heart-rate variability and blood-lactate threshold interaction during progressive resistance exercise in healthy older men. J Strengh Cond Res. 2010;24(5):1313-20.
28. Marzolini S, Mertens DJ, Oh PI, Plyley MJ. Self reported compliance to home-based resistance training in cardiac patients. Eur J Cardiovasc Prev Rehabil. 2010;17(1):35-41.
29. Nogueira IDB, Servantes DM, Nogueira PAMS, Pelcerman A, Salvetti XM, Salles F, et al. Correlation between quality of life and functional capacity in heart failure. Arq Bras Cardiol. 2010;95(2):238-43.
30. Guzun R, Aguilaniu B, Wuyam B, Mezin P, Koechlin-Ramonatxo C, Auffray C, et al. Effects of training at mild exercise intensities on quadriceps muscle energy metabolism in patients with chronic obstructive pulmonary disease. Acta Physiol. 2012;205(2):236-46.

EDITORIAL

Home-Based Resistance Training in Heart Diseases: Don't Stop the Music, your Muscles are still Listening

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Editorial referring to the article: Short-Term Effects of a Resistance Training Program Using Elastic Tubing in Patients with Heart Disease

Cardiovascular diseases (CVDs) are a well-known cause of death and physical incapacity worldwide. Heart failure (HF) is a common disease resulting from multiple etiologies that contribute to decreasing physical capacity and quality of life (QOL).[1,2] Not long ago physical exercises were discouraged in CVDs, but evidence-based exercise programs augment QOL, reduce mortality and hospital readmission in HF[2]. Strength training is an important component of a cardiac rehabilitation program due to its close relationship with improvements in functional capacity reverberating in activities of daily living and QOL.[1]

Unfortunately, supervised-rehabilitation programs in fitness centers are not widely available for the majority of patients with heart disease. Nevertheless, home-based rehabilitation programs could be more accessible to most patients, because of its relatively low cost and feasibility.[2] Especially during COVID-19 pandemic lockdown, many opportunities to exercise were suspended, including cardiac rehabilitation services and community health programs.[3] Also, social distancing increased sedentarism numbers and several position statements have encouraged people to stay active at home, trying to reverse or counterbalance the additional impact of social distance on physical inactivity.[3,4] In a practical perspective, resistance training is conventionally performed through free weights or weight machines that usually require extensive physical space and outside facilities (i.e., gym or rehabilitation centers). Thus, resistance exercises, in particular, are not easily adopted at home and could be omitted from home-based cardiac rehabilitation. To underscore, a question that must be made: How patients remain strong at home?

In the current issue of the *International Journal of Cardiovascular Sciences*, Silva and colleagues[5] demonstrated that short-term resistance training with elastic tubing improved peripheral muscle strength and functional capacity in adults (45-79 yrs.) with heart disease in phase II of cardiovascular rehabilitation. Resistance training using elastic tubing is a low-cost and practical tool to increase neuromuscular activation in specific rehabilitation settings with a minimal risk of injury.[6] It was demonstrated that resistance training with elastic tubing promoted similar positive effects on peripheral muscle strength and functional capacity in the elderly compared to conventional resistance training using weight machines.[7] In patients with chronic obstructive pulmonary disease (COPD), resistance training using elastic tubing had a greater effect on functional exercise capacity compared to traditional resistance training. Regarding muscle strength and quality of life improvements, elastic tubing was equal to traditional resistance training in COPD.[8] When performed at home, resistance training with elastic tubing program improved strength after twelve weeks in older adults.[9] Lastly, elastic tubing-based resistance training showed great self-efficacy and adherence to home-based rehabilitation.[10]

In conclusion, resistance training with elastic tubing seems to be a feasible low-cost and practical alternative to improve or maintain the peripheral muscle strength in heart disease, becoming a potential strategy to be part of a home-based rehabilitation program to counterbalance the home isolation effect on physical capacity and clinical outcomes.

Keywords

Cardiovascular Diseases/complications; COVID-19; Cardiac Rehabilitaion; Betacoronavirus; Heart Failure; Resistance Training; Social Distance; Music.

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References

1. Oka RK, DeMarco T, Haskell WL, Botvinick E, Dae MW, Bolen K, Chatterjee K. Impact of a home-based walking and resistance training program on quality of life in patients with heart failure. Am J Cardiol. 2000; 85: 365–369.
2. Kraal JJ, Van den Akker-van Marle ME, Abu-Hanna A, et al. Clinical and cost-effectiveness of home-based cardiac rehabilitation compared to conventional, centre- based cardiac rehabilitation: Results of the FIT@ Home study. Eur J Prev Cardiol 2017; 24: 1260–1273
3. Peçanha T, Goessler KF, Roschel H, Gualano B. Social isolation during the COVID-19 pandemic can increase physical inactivity and the global burden of cardiovascular disease. Am. J. Physiol. Heart Circ. Physiol. 2020; 318: 1441–1446.
4. Rodrigues GD, Alves Junior ED, Soares PPS. Stay active, stay at home and stay safe: the risk of falls in older adults in the COVID-19 quarantine. Geriatr Gerontol Aging.2020; 14(3):216-217.
5. Silva JPLN, Ferreira TIS, Cavalleri GC, Cruz MMA, Galindo BP, Silva NT, Silva BSA, et al. Short-Term Effects of a Resistance Training Program Using Elastic Tubing in Patients with Heart Disease. Int J Cardiovasc Sci. 2021; 34(2):149-156.
6. Melchiorri G, Rainoldi A. Muscle fatigue induced by two different resistances: Elastic tubing versus weight machines. J Electromyogr Kinesiol. 2011; 21(6): 954-9.
7. Lima FF, Camillo CA, Gobbo LA, Trevisan IB, Nascimento W, Silva BSA, et al. Resistance training using low-cost elastic tubing is equally effective to conventional weight machines in middle-aged to older healthy adults: a quasi-randomized controlled clinical trial. J Sports Sci Med. 2018;17(1):153–60.
8. Ramos EM, de Toledo-Arruda AC, Fosco LC, et al. The effects of elastic tubing-based resistance training compared with conventional resistance training in patients with moderate chronic obstructive pulmonary disease: a randomized clinical trial. Clin Rehabil 2014; 28: 1096–1106.
9. Mikesky A, Topp R, Wigglesworth J, Harsha J, Edwards J. Efficacy of a home-based resistance training program for older adults using elastic tubing. Eur J Appl Physiol. 1994; 69(10): 316-320.
10. Millen JA, Bray SR. Self-efficacy and adherence to exercise during and as a follow-up to cardiac rehabilitation. Journal of Applied Social Psychology. 2008; 38: 2072–2087.

ORIGINAL ARTICLE

Elderly Mortality from Cerebrovascular Disease in Alagoas, 2000-2016: Spatial-Temporal Analysis

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Abstract

Background: Cerebrovascular diseases (CVDs) are the second leading cause of death in Brazil.

Objective: This study aimed to describe the epidemiological profile and to analyze the spatiotemporal dynamics of mortality from cerebrovascular disease in the elderly in Alagoas from 2000-2016.

Methods: This is a multilevel ecological study of all deaths from CVD in individuals aged 60 years or older. Data were collected from the Mortality Information System. The variables were submitted to descriptive analysis, trend analysis by Joinpoint Regression method and spatial analysis with Global Moran's and local statistics; 95% confidence interval and significance of 5% were considered in the analysis.

Results: There were 21,440 deaths in the study period, 50.4% (n=10,797) male, 40.5% (n=8,670) aged ≥ 80 years, 44.5% (n=9,465) of "brown" race, 30.1% (n=6,448) married and 36.5% (n=7,828) with less than four years of schooling. Female and male mortality rates were 460.24/100,000 and 602.23 / 100,000, respectively. An annual decreasing trend of -1.4% ($p<0.001$) in overall and male mortality was observed from 2007 on. The highest mortality rates were concentrated in the eastern region of Alagoas (Moran's I =0.766288; p=0.01). Twenty-two municipalities were in quadrant Q1 of Moran's scattering diagram and considered priorities.

Conclusion: Death from CVD in Alagoas occurred equally in men and women in the study period, mostly in individuals of mixed race, married, and with low education attainment. The highest rates were observed in the eastern region of the state . The results highlight the need for public policies aimed at healthy aging in the state. (Int J Cardiovasc Sci. 2021; 34(2):159-167)

Keywords: Cerebrovascular Disorders; Cardiovascular Diseases; Respiratory Tract Diseases; Neoplasms; Epidemiology; Diabetes Mellitus; Mortality; Ecological Studies; Ethnic Inequality; Social Class.

Introduction

The accelerated aging of the Brazilian population has led to an increase in the rates of chronic non-communicable diseases (CNCDs), composed of cardiovascular diseases, chronic respiratory disease, diabetes mellitus and cancer. Among the cardiovascular diseases, ischemic heart disease (IHD) and cerebrovascular diseases (CVDs) are the two main causes of premature death in the world.[1]

CVDs are pathological conditions in which an area of the brain is transiently or permanently affected by ischemia or bleeding.[2] The World Health Organization (WHO) estimates that in 2016, stroke and IHD were responsible for 15.2 million deaths worldwide.[1]

In Brazil, CVDs occupy the second position in number of deaths, just behind IHDs,[3] moving from the eighth to the fourth position in the ranking of morbidities related to years of life lost (YLL) from 1990 to 2016. This expansion of CVD can be related to changes in lifestyle linked to urbanization and globalization processes, since the 1960s.[4]

Brazil is a country of continental dimensions, divided into regions with their own socioeconomic characteristics

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and epidemiological transition.[3] The increase pattern of CVDs was first evidenced in the states of the south and southeast Brazil and later in the states of the north and northeast regions of the country. Therefore, efforts to prevent cerebrovascular events have become earlier in the states of the south and southeast regions and only more recently in the northern and northeastern regions of Brazil.[4]

Given the above, this study aimed to describe the epidemiological characteristics and analyze the spatiotemporal dynamics of CVD mortality in the elderly population of the state of Alagoas, Brazil, from 2000 to 2016.

Methods

Study Design, Population and Period

This was a multilevel ecological study of all deaths from CVDs in residents aged 60 years or older in all cities of the state of Alagoas, Brazil, from 2000 to 2016.

Study Location

The study was carried out in Alagoas, the second smallest state on the northeast region of Brazil. The estimated population of Alagoas is 3.3 million (2018 census data), distributed into 102 municipalities.[5] In the social context, the state has the lowest Human Development Index in Brazil (HDI 0.631) and 96.1% of the municipalities have high or very high social vulnerability (6.7). In addition, 8.9% of the population is elderly, with an aging rate of 30.4%.[6]

Study Variables

In this study, the following sociodemographic variables were analyzed: sex (male and female), age group (60-69, 70-79, > 80 above, and unknown), marital status (single, married, widowed, separated, others, or unknown), schooling (none, 1-3 years, 4-7 years, 8-11 years, > 12 years, or unknown) and color/race (white, black, yellow, mixed race, indigenous, or unknown). The mortality rates were analyzed according to the International Classification of Diseases (ICD-10), categories I60 to I69: I60 - Subarachnoid hemorrhage, I61 - Intracerebral hemorrhage, I62 - Other non-traumatic intracranial hemorrhages, I63- Cerebral infarction, I64 - Stroke not specified as hemorrhagic or ischemic, I65 - Occlusion / stenosis of pre-cerebral arteries that do not result in cerebral infarction, I66 - Occlusion / stenosis of cerebral arteries that do not result in cerebral infarction, I67 - Other cerebrovascular disorders, I68 - Cerebrovascular disorders classified in elsewhere diseases and I69 - Sequelae of cerebrovascular diseases.

Data Source

Mortality data were collected from the Mortality Information System (Ministry of Health).7 Mortality rates were calculated using the following equations:

a) Annual mortality rate: (number of deaths from CVDs in individuals aged 60 or over in the city and year / total of individuals aged 60 or over living in the city and year) x 100,000;

b) Average mortality rate (2000-2016): (average number of deaths from CVDs in individuals aged 60 or over in the city / total of individuals aged 60 or over living in the city in the middle of the period) x 100,000. Data were collected from the Brazilian Institute of Geography and Statistics.

Statistical Analysis

Data analysis was performed in three stages. The first stage consisted of sociodemographic description of the studied population (relative and absolute frequencies). The second stage was characterized by the analysis of mortality trends by identification of inflection points of the regression model (Joinpoint Regression Model). This model analyzes whether a line with multiple segments is more adequate to explain the time course of a data set compared with a straight line or a line with fewer segments.[8] The model allows the calculation of the annual percentage change (APC) and the trend over the interval (AAPC, Average Annual Percent Change), as well as the classification of the trend as increasing, decreasing or stationary.[8]

The third stage was based on spatial modeling and identification of areas with the highest risk of deaths of elderly people due to CVD. Initially, municipal rates were subjected to smoothing by the local empirical Bayesian model to reduce the random fluctuation caused by rare events, small populations and underreporting of events.[9,10] Soon after, global Moran's I was used to identify the global spatial dependence (spatial autocorrelation). The overall Moran's index varies between -1 and +1, and the closer to 1, the greater the spatial dependence of mortality from CVD in the elderly. Once the global

autocorrelation was recognized, Moran's local statistics (LISA, Local Index of Spatial Association) was used to detect the areas with the highest risk of occurrence of the event. Using LISA, the municipalities were positioned in the quadrants of the Moran scattering diagram: Q1 - high / high (positive values and negative average values) and Q4 - low / high (negative values and positive average values). The municipalities located in the Q1 quadrant were considered priority.[9,10] Then, thematic maps were made to present the results. The 95% confidence interval (95% CI) and significance level of 5% were considered in the statistical analysis.

The statistical analysis were made using the Joinpoint regression program version 4.5.0.1 (National Cancer Institute, Bethesda, MD, USA), Terra View (Version 4.2.2, Brazilian Space Research Institute - INPE, São José dos Campos, SP, Brazil) and QGIS (version 2.14.11, Open Source Geospatial Foundation- OSGeo, Beaverton, OR, USA).

Ethical Aspects

Since the study was based on analysis of secondary public data set and did not require identification of the individuals (which was also impossible), so ethics approval for the study was waived .

Results

In the period from 2000 to 2016, 21,440 deaths of elderly people due to CVD were recorded in Alagoas, with a slight predominance of men (50.4%; n=10,797). A total of n=8,670 (40.4%) of the individuals were aged 80 years or over, 44.2% (n=9,465) were brown color/race and 40.8% (n=4,407) were married. Considering educational attainment, 36.5% (n=7,828) of the population had less than 4 years of study. There was also a high proportion of "unknown" fields in the variables color/race (31.3%, n=6,708), marital status (31.0%, n=6,713) and education (54.7%, n=11,719) (Table 1).

The average mortality rate was 523.6/100,000 considering both sexes, 602.2/100,000 for men and 460.2/100,000 for women. In the temporal modeling, there was a trend of growth in the general (APC 3.9%; p<0.001), male (APC 4.1%; p<0.001) and female (APC 3.6%; p<0.001) rates between 2000 and 2007. In the following period (2007-2016), the trend was decreasing in general rate (APC -1.4%; p<0.001) and rate in male patients (APC -1.4%; p<0.001). Considering the total period, a stationary pattern was observed in the three rates (Figure 1).

Based on the ICD-10 categories, "unspecified stroke" was the most prevalent cause of deaths 364.17/100,000 (ICD I64), followed by "sequelae of cerebrovascular diseases" 73.69/100,000 (ICD I69). "Subarachnoid hemorrhage" (ICD I60) was the only category with predominance of female sex (4.12/100,000 in women and 2.39/100,000 in men). There were no deaths from "occlusion / stenosis of pre-cerebral arteries that do not result in cerebral infarction" (ICD I65), "occlusion / stenosis of cerebral arteries that do not result in cerebral infarction" (ICD I66) or "cerebrovascular disorders classified in elsewhere diseases"(ICD I68) reported (Table 2).

Spatial modeling showed a heterogeneous distribution of elderly mortality from CVDs, both in crude and smoothed rates (Moran's index 0.169815; p=0.02 and Moran's index 0.766288; p=0.01, respectively), with concentration in the eastern region of the state. In the gross rate, when comparing spatial patterns, it was observed that all municipalities with a rate lower than 300/100,000 were displaced to the strata with higher rates. The strata with rates between 401 and 500/100,000 and higher than 600/100,000 showed an increase in the number of municipalities, from 25 to 41 and from 11 to 12, respectively (Figure 2).

The highest crude rates were observed in the municipalities of Água Branca (909.70/100,000), Flexeiras (736.07/100,000) and Passo de Camaragibe (711.91/100,000). After approach to smoothing, the highest rates were observed in Água Branca (658.42/100,000), São Luís do Quitunde (657.09/100,000) and Barra de Santo Antônio (640.11/100,000). The state capital, Maceió, recorded a gross rate of 636.61/100,000 and a smoothed rate of 626.30/100,000 (Figure 2).

In the local Moran's I cluster map, 22 municipalities were located in the Q1 quadrant of the scatter plot, and therefore, considered priorities. Together, these municipalities accounted for 50.3% (n=10789) of all deaths in the state, with an average gross rate of 587.16/100,000 and an average smoothed rate of 587.19/100,000 (Figure 2).

Discussion

Despite the reduction in mortality rates from CVDs in recent years,[11] Brazil is still in a prominent position on the world stage. Part of this is due to socioeconomic, demographic and epidemiological disparities between Brazilian's geographic regions, especially when comparing the north-northeast with the southeast-south axis.[3,4]

Table 1 – Sociodemographic characterization of deaths from cerebrovascular diseases among elderly people by sex; Alagoas, Brazil, 2000-2016

Variables	Male n= 10,797 (50.4%)		Female n= 10,643 (49.6%)		Total Deaths n=21,440 (100.0%)	
	n	%	n	%	n	%
Age group						
60-69	2,982	27.62	2383	22.39	5365	25.02
70-79	3,814	35.32	3590	33.73	7403	34.53
80 or over	4,001	37.06	4670	43.88	8670	40.45
Color/race						
White	2,007	18.60	2235	21.00	4242	19.78
Black	510	4.72	437	4.11	947	4.42
Yellow	22	0.20	34	0.32	56	0.26
Brown	4,847	44.89	4618	43.39	9465	44.15
Indigenous	7	0.06	15	0.14	22	0.10
Unknown	3,404	31.53	3304	31.04	6708	31.29
Marital status						
Single	1,386	12.84	1810	17.01	3196	14.91
Married	4,407	40.82	2041	19.18	6448	30.07
Widowed	1,427	13.22	3238	30.42	4665	21.76
Divorced	147	1.36	111	1.04	258	1.20
Other	106	0.98	54	0.51	160	0.75
Unknown	3,324	30.78	3389	31.84	6713	31.31
Schooling						
None	2,644	24.49	3094	29.07	5738	26.76
1-3 years	1,209	11.20	881	8.28	2090	9.75
4-7 years	697	6.45	569	5.34	1266	5.90
8-11 years	260	2.41	201	1.89	461	2.16
12 years or more	93	0.86	73	0.69	166	0.77
Unknown	5894	54.59	5825	54.73	11719	54.66

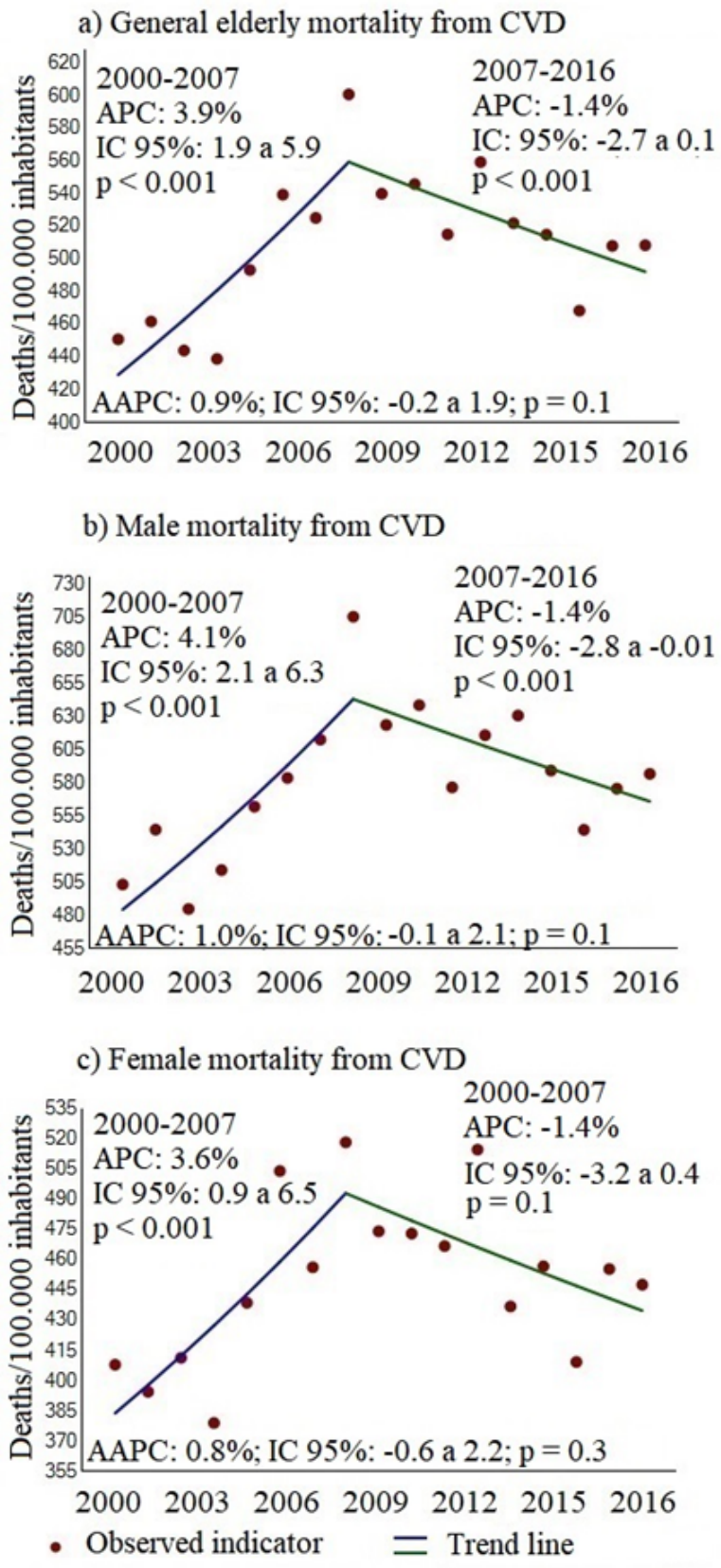

Figure 1 – Time course of the mortality rate from cerebrovascular disease in the elderly population in Alagoas, Brazil, 2000-2016

Table 2 – Mortality rate in elderly people with cerebrovascular disease, according to sex and categories of the International Classification of Diseases (ICD-10), Alagoas, Brazil, 2000-2016

ICD-10 categories	Male			Female			Both sexes		
	n	%	Rate*	n	%	Rate*	n	%	Rate*
I60 - Subarachnoid hemorrhage	43	31.16	2.39	95	68.84	4.12	138	0.64	3.37
I61 - Intracerebral hemorrhage	698	49.89	38.93	701	50.11	30.46	1399	6.53	34.17
I62 - Other non-traumatic intracranial hemorrhages	17	44.74	0.94	21	55.26	0.91	38	0.18	0.92
I63 - Cerebral infarction	81	60.45	4.51	53	39.55	2.30	134	0.63	3.27
I64 - Stroke not specified as hemorrhagic or ischemic	7502	50.32	418.44	7408	49.68	321.89	14910	69.55	364.17
I67- Other cerebrovascular disorders	849	47.11	47.35	953	52.89	41.41	1802	8.41	44.01
I69- Sequelae of cerebrovascular diseases	1605	53.20	89.52	1412	46.80	61.35	3017	14.07	73.69

*No deaths from ICDs I65, I66 and I68 recorded; *deaths per 100,000 inhabitants*

The mortality profile observed in Alagoas is similar to other states in Brazil.[12] Age and sex reflected two important processes in demographic dynamics in Brazil: first, population aging and increased life expectancy are accompanied by increased risk of arterial hypertension and atrial fibrillation, key factors in the development of CVD;[13] second, the feminization of aging, characterized by the predominance of women in the older age groups, especially above 80 years old.[1] It is estimated that 56.6% of the elderly population in the state of Alagoas, in 2019, were female.[5]

Among the factors associated with the development of CVDs, social vulnerability has gained a prominent role. Ribeiro et al.,[1] pointed out that low levels of education increase the risk of stroke by 1.2 times in the Brazilian population. Low education can hinder both the search for health services, delaying the accurate diagnosis and initial therapeutic measures, and post-surgical care as well.[1] These factors directly affect the patient's prognosis and long-term survival.[3]

In addition, we evaluated the influence of marital status on mortality from CVDs in the population of Alagoas. Some authors have reported that marriage appears to be a protective factor against morbidities.[16] Liu et al.,[16] in a multicenter study involving 12,118 participants, demonstrated that marriage can provide important behavioral, biological and psychosocial resources in the prevention and treatment of related morbidities. In addition, men who live alone are more likely to death,[17] which is explained, in part, by the greater delay in arriving at specialized services and late initiation of thrombolytic therapy.[16]

Another epidemiological factor was race/color. Lotufo and Bensenor[18] stated that in Brazil, black individuals have 40% higher risk for CVDs than white individuals. A study conducted in the state of Bahia showed that 35.0% of deaths occurred in the brown or black population.[19] These values differ from those found in the state of Paraná, in which 86.0% of registered deaths due to CVD occurred in the white race/color population.[20] However, it is necessary to point out the influence of colonization on the formation of the Brazilian people (African colonization in the case of Bahia and European colonization in Paraná).[20] Also, the definition of race/color has a subjective trait, and can be done by prior self-declaration of the deceased, by family members or by third parties, representing a criterion of low agreement.[18]

Although the epidemiological characteristics discussed above have influenced the tendency of a stationary mortality from CVD, other factors must be analyzed. The decreasing mortality trend observed since 2007 may be related to the strengthening of primary care programs in the state of Alagoas, including the consolidation of the Family Health Strategy (FHS), which covered only 14 municipalities in 1994 and expanded to all the 102 municipalities of Alagoas State in 2013.[21] A report by the National Health Surveillance System of Brazil states the rate of hospitalization for stroke by patients older than 40 years in Alagoas decreased in the period from 2005 to 2007. This corroborates the results found in our study

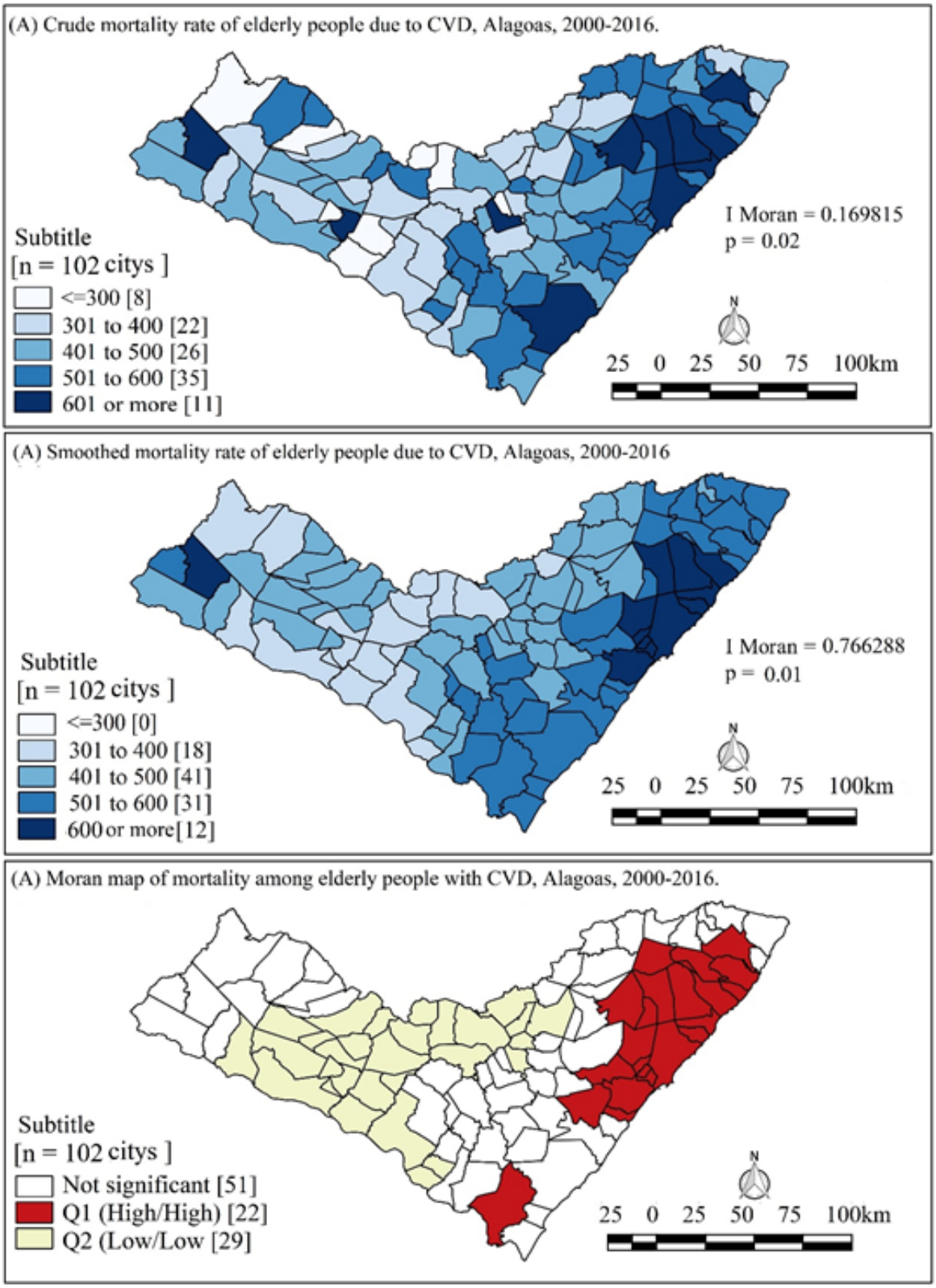

Figure 2 – Spatial distribution of the mortality rate from cerebrovascular disease in the elderly population, Alagoas, Brazil, 2000-2016

and shows how a well-developed strategy could reduce the number of deaths from CVD in the state.[21,22]

It is also important to mention the heterogeneous spatial distribution of deaths, with the highest concentration in the eastern region of the state. This fact is possibly related to the centralization of specialized health services in the capital Maceió, including the stroke unit of the state general hospital (*Hospital Geral do Estado*), in operation since 2015.[23] Such services mainly assist the municipalities located within the first and the second health regions of Alagoas, and the entire first macroregion, equivalent to the eastern territory of the state.[24] The high concentration of healthcare centers around the capital provides a greater number of diagnosed cases and deaths registered in that region.[25]

This study has some limitations that need to be considered: i) use of secondary data from information systems; ii) it is possible that there was an underreporting of deaths from CVDs in the most interior and least assisted regions of the state, influencing on the spatial distribution of cases found in this study; iii) lack of sociodemographic data of some of the patients, due to error in registration or filling out of the death certificate.

Conclusion

The study showed that death from CVDs in Alagoas, occurs equally in men and women, and mostly in individuals of mixed race, with low education attainment, and married. There was a downward trend of overall mortality and mortality among men from 2007 onwards. The highest mortality rates were observed in the eastern region of the state.

The results of this study can contribute to the development of plans and strategies to reduce CVD deaths and promote healthy aging.

Author Contributions

Conception and design of the research: Souza CDF, Cunha, EJO, Silva Junior LCF. Acquisition of data: Souza CDF, Cunha, EJO, Silva Junior LCF. Analysis and interpretation of the data: Souza CDF, Duarte ALF, Cunha, EJO, Silva Junior LCF. Statistical analysis: Souza CDF. Obtaining financing: Souza CDF.Writing of the manuscript: Souza CDF, Duarte ALF, Cunha, EJO, Silva Junior LCF. Critical revision of the manuscript for intellectual content: Souza CDF, Cunha, EJO, Silva Junior LCF.

Potential Conflict of Interest

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

Sources of Funding

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Study Association

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

Ethics Approval and Consent to Participate

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. World Health Organization (WHO). Global Health Estimates 2016: Disease burden by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva: WHO; 2018.
2. Engelhardt E. Apoplexy, cerebrovascular disease, and stroke: Historical Evolution of terms and definition. Dement Neuropsychol 2017; 11(4):449-53.
3. Lotufo PA, Goulart AC, Passos VMA, Satake FM, Souza MFM, França EB, Ribeiro ALP, Bensenõr IJM. Doença cerebrovascular no Brasil de 1990 a 2015: Global Burden of Disease 2015. Rev Bras Epidemiol 2017; 20(1):129-41.
4. Collaboratos, Gbd 2016 Brazil. Burden of disease in Brazil, 1990–2016: a systematic subnational analysis for the Global Burden of Disease Study 2016. Lancet 2018; 392(1): 760-75.
5. Instituto Brasileiro de Geografia e Estatística (IBGE). Resultado dos Dados Preliminares do Censo – 2010; 2019. Disponível em https://cidades.ibge.gov.br/brasil/al/panorama.
6. Costa MA, Marguti BO. Atlas da vulnerabilidade social nos municípios brasileiros. Brasília: IPEA; 2015.
7. Brasil. Ministério da Saúde. Sistema de informações sobre mortalidade, 2019. Disponível em http://datasus.saude.gov.br/. Acesso em 10 de out, 2019.
8. Kim HJ, Fay MP, Feuer EJ, Midthune DN. Permutation tests for joinpoint regression with applications to cancer rates. Stat Med 2000; 19(3):335-51.
9. Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB. Bayesian Data Analysis: Texts in Statistical Science. 3ª Edição. Crc Press; 2013.
10. Souza WV, Barcellos CC, Brito AM, Carvalho MS, Cruz OG, Albuquerque MFM, Alves KR, Lapa TM. Aplicação de modelo bayesiano empírico na análise espacial da ocorrência de hanseníase. Rev Saude Publica 2001; 35(5):474-80.
11. Nuñez-Gonzales S, Duplat A, Simancas D. Mortalidad por enfermedades cerebrovasculares en Ecuador 2001-2015: Estudio de tendencias, aplicación del modelo de regresión joinpoint. Rev Ecuatoriana de Neurologia 2018; 27(1):16-22.

12. Bacurau AGM, Ferraz RO, Donalisio MR, Francisco PMSB. Mortalidade por doenças cerebrovasculares em idosos e a vacinação contra a influenza: Estado de São Paulo, Brasil, 1980-2012. Cad Saude Publica 2019; 35(2):117-35.

13. Katan M, Luft A. Global Burden of Stroke. Semin Neurol 2018; 38(2):208-11.

14. Cordonnier C, Sprigg N, Sandset EC, Pavlovic A, Sunnerhagen KS, Caso V, Christensen H. Stroke in women - from evidence to inequalities. Nat Rev Neurol 2017; 13(9):521-32.

15. Ribeiro IJS, Cardoso JP, Freire IV, Carvalho MF, Pereira R. Determinants of Stroke in Brazil: A Cross-Sectional Multivariate Approach from the National Health Survey. J Stroke Cerebrovasc Dis 2018; 27(6):1616-23.

16. Liu Q, Wang X, Wang Y, Wang C, Zhao X, Liu L, Li Z, Meng X, Guo L, Wang Y. Association between marriage and outcomes in patients with acute ischemic stroke. J Neurol 2018; 265(4):942-48.

17. Ng TP, Jin A, Feng L, Nyunt MSZ, Chow KY, Feng L, Fong NP; Mortality of older persons living alone: Singapore longitudinal ageing studies. BMC Geriatr 2015; 15(126):2-9.

18. Lotufo PA, Bensenor IJM. Raça e Mortalidade Cerebrovascular no Brasil. Rev Saude Publica 2013; 47(6):1201-04.

19. Souza-Junior EVS, Jesus MAS, Bezerra CLS, Rosa RS, Boery EN, Boery RNSO. Taxa de mortalidade por infarto cerebral na macrorregião sudeste do Estado da Bahia, Brasil. Revista Eletrônica Enfermaria Actual en Costa Rica. 2018;34

20. Furukawa TS, Mathias TAF, Marcon SS. Mortalidade por doenças cerebrovasculares por residência e local de ocorrência do óbito: Paraná, Brasil, 2007. Cad Saude Publica 2011; 27(2):327-334.

21. Silva MAP, Menezes RCE, Oliveira, MAA, Longo-Silva G, Asakura L. Atenção Básica em Alagoas: expansão da Estratégia Saúde da Família, do Nasf e da componente alimentação/nutrição. Saúde debate 2014; 38(103):720-32.

22. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde - Sistema nacional de vigilância em saúde: relatório de situação: Alagoas. 5ª ed. Brasília; 2011.

23. Secretaria de Estado da Saúde - Alagoas. Unidade de AVC do HGE atende mais de 1.100 alagoanos; 2018. Disponível em: http://www.saude.al.gov.br/2018/10/30/unidade-de-avc-do-hge-leva-servicos-especializados-a-mais-de-1-100-alagoanos/

24. Superintendência de Planejamento e Participação Social - Alagoas. Plano Diretor de Regionalização da Saúde de Alagoas. Maceió: Secretaria de Estado da Saúde; 2011.

25. Albuquerque MV, Viana ALA, Lima LD, Ferreira MP, Fusaro ER, Iozzi FL. Desigualdades regionais na saúde: mudanças observadas no Brasil de 2000 a 2016. Cien Saude Colet 2017; 22(4):1055-1064.

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EDITORIAL

Mortality in the Elderly Due to Cerebrovascular Disease

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Editorial referring to the article: Elderly Mortality from Cerebrovascular Disease in Alagoas, 2000-2016: Spatial-Temporal Analysis

Cardiovascular Diseases (CVD) are the main cause of death, hospitalization and outpatient care worldwide, including in developing countries like Brazil. More than 75% of deaths from these causes occur in low- or middle-income countries, and about 80% of deaths are due to acute myocardial infarction (AMI) and stroke.[1] Vilela, PB et al.[2] observed that mortality from cerebrovascular disease in Brazil is influenced by socioeconomic factors.

Although the aging process is not necessarily related to diseases and disabilities, elderly people have a high prevalence of chronic non-communicable diseases (NCDs). In the period from 2010 to 2050, the number of strokes is expected to more than double, with most of the increase among the elderly (≥75 years of age) and minority groups.[3] Greater attention is needed to promote optimal cardiovascular health and healthy aging throughout life.

Stroke patients > 85 years of age constitute 17% of all patients with this condition and, in this age group, this condition is more prevalent in women than in men.[4] Risk factors for stroke may be different in older adults. A multi-ethnic population-based NOMAS cohort noted that the effect of the risk of physical inactivity was modified by age, and there was a significant risk only in stroke patients and age > 80 years.[5]

The article published by Silva Junior LCF et al.,[6] in this issue, describes the epidemiological profile and analyze the spatiotemporal dynamics of mortality from cerebrovascular diseases in elderly people from Alagoas in the period 2000-2016. Data were collected from the Mortality Information System. This study showed that deaths from CVD, in Alagoas, were the same between genders, and more evident in brown individuals, with low education, married and in the eastern region of the state. There was a downward trend in general mortality and mortality in males as of 2007. The need for public policies aimed at healthy aging in the state became evident.

Global rates of total CVD have significant implications for clinical practice and public health policy development. In 2021 the American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee published the Heart Disease and Stroke Statistics — 2021 Update: a Report from the American Heart Association,[7] showing that age-standardized rates of DALYs (disability-adjusted life-year) and deaths due to cerebrovascular diseases were substantially higher in men than in women, but the prevalence was higher in women, suggesting the possibility of a higher risk of death and disability in men, but better survival in women. In 2019, total CVD DALYs were higher in men than in women before the age range of 80 to 84. After that range, the pattern is reversed. The gender differences in DALYs are most striking between the ages of 30 and 60 years (older men) and age > 80 years (older women). The excess of deaths from CVD in women between the ages of 80 and 84 should focus attention on mortality from specific causes at older ages and have implications for secondary prevention strategies.

High mortality rates are currently being observed because of the pandemic of severe respiratory syndrome caused by the Sars-CoV-2 virus (COVID-19), and much of that additional disease burden may be cardiovascular disease due to the effects of viral infection, but also of the unintended consequence of socially distant behaviors, such as changes in healthcare provision.[8] In the face of a viral pandemic, we must further emphasize global commitments to the health of the population by reducing suffering and death from cardiovascular disease.

Keywords

Cerebrovascular Disorders; Cardiovascular Diseases; Aged; Strove; Mortality; Epidemiology.

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References

1. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980- 2016: a systematic analysis for the Global Burden of Disease Study . Lancet. 2017;390(10100):1151-210.
2. Villela PB, Klein CH, de Oliveira GMM. Socioeconomic factors and mortality due to cerebrovascular and hypertensive disease in Brazil. Rev Port Cardiol. 2019 Mar;38(3):205-12.
3. Howard G, Goff DC. Population shifts and the future of stroke: forecasts of the future burden of stroke. Ann N Y Acad Sci. 2012; 1268:14–20.
4. Dehlendorff C, Andersen KK, Olsen TS. Sex disparities in stroke: women have more severe strokes but better survival than men. J Am Heart Assoc. 2015;4:e001967.
5. Willey JZ, Moon YP, Sacco RL, Greenlee H, Diaz KM, Wright CB, Elkind MS, Cheung YK. Physical inactivity is a strong risk factor for stroke in the oldest old: findings from a multi-ethnic population (the Northern Manhattan Study). Int J Stroke. 2017; 12(2):197–200.
6. Silva Junior LCF, Cunha EJOD, Souza CDF, Duarte AWF. Elderly Mortality from Cerebrovascular Disease in Alagoas, 2000-2016: Spatial -Temporal Analysis. Int J Cardiovasc Sci. 2021; 34(2):159-167.
7. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al.; on behalf of the American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2021 update: a report from the American Heart Association. Circulation. 202127 Jan;143:e00–e00.
8. Wadhera RK, Shen C, Gondi S, Chen S, Kazi DS, Yeh RW. Cardiovascular Deaths During the COVID-19 Pandemic in the United States. J Am Coll Cardiol. 2021;77(2):159-69.

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ORIGINAL ARTICLE

Cardiovascular Risk Factors in Patients with Chronic Kidney Disease Under Conservative Treatment

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Abstract

Background: Cardiovascular disease (CVD) is commonly associated with chronic kidney disease (CKD). These diseases have a significant impact on life expectancy. Individuals with CKD are more likely to die from CVD than to progress to end-stage kidney disease.

Objective: To assess cardiovascular risk factors of patients with CKD under conservative treatment.

Methods: This was an observational, cross-sectional study. Socioeconomic, anthropometric, biochemical, and physical inactivity data were assessed, and 10-year risk for CVD were estimated using the Framingham Score in patients with CKD under conservative treatment. For statistical analysis, the Student's t-test and Pearson's chi-square test were performed.

Results: A total of 172 individuals were evaluated, 57% of whom were male, with an average age of 68.85 ± 11.41 years. The prevalence of hypertension and diabetes were 87.2% and 53.5%, respectively; 62.2% were physically inactive; 9.9% of men were smokers and 12.8% consumed alcohol. According to BMI, 82.4% of adults <60 years old and 60.6% of those older than 60 years were overweight. High waist circumference and a high waist-hip ratio were highly prevalent in females (91.9% and 83.8%, respectively) and males (64.3% and 39.8%, respectively); 92.4% had a high body fat percentage and 73.3% high uric acid levels. According to the Framingham score, 57% have a medium or high risk of developing CVD in 10 years.

Conclusion: There was a high prevalence of cardiovascular risk factors in the population studied. The assessment of cardiovascular risk factors in patients with CKD makes it possible to guide the conduct of health professionals to prevent mortality from cardiovascular causes. (Int J Cardiovasc Sci. 2021; 34(2):170-178)

Keywords: Renal Insufficiency, Chronic; Risk Factors; Cardiovascular Diseases/complications; Adults; Hypertension; Diabetes Mellitus; Anthropometry; Abdominal Circumference.

Introduction

Chronic kidney disease (CKD) is defined as having an abnormal kidney structure or function for three months or longer, which can be aggravated by the progression of disease and lead to irreversible kidney failure.[1] It is a growing public health problem, with increasing morbidity and mortality in Brazil and worldwide.[2,3]

Cardiovascular disease (CVD) is commonly associated with CKD. When combined, these conditions have a significant impact on life expectancy, since individuals with CKD are more likely to die from CVD than to progress to end-stage renal disease.[4] An important maker of this association is glomerular filtration rate (GFR), since GFR reduces the percentage of mortality and cardiovascular events increase[5] .

The main cause of kidney disease is diabetic nephropathy and hypertensive nephrosclerosis, both accelerated by smoking and dyslipidemia; thus, the higher risk of CVD among CKD patients is secondary to accumulation of risk factors.[6] In addition, increased waist circumference (WC) and overweight further increases this risk, as these factors are associated with a greater likelihood of cardiovascular events.[7-9]

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In addition to anthropometric measurements, it is important to check some biochemical parameters, such as the lipid profile,[10] uric acid levels,[11] glycated hemoglobin (HbA1C) and blood glucose values, to prevent the progression of CKD and the risk of developing CVD.[12]

The Framingham Risk Score is a tool widely used for calculation of cardiovascular risk and estimation of the risk of developing CVD in ten years. It has been applied for risk stratification, making it possible to target specific groups of patients who will benefit from drug therapy as a form of primary intervention in CVD prevention.[13,14]

The aim of this study was to assess cardiovascular risk factors in patients with CKD under conservative treatment in a secondary care hospital.

Methods

This was an observational, cross-sectional study on the cardiovascular risk factors in CKD patients under conservative treatment. The inclusion criteria were patients with stages 3, 4 or 5 CKD,[1] aged over 20 years, attending (i.e., not the first consultation) a secondary care center for chronic diseases in Juiz de Fora, Brazil. The exclusion criteria were presence of metabolic diseases, such as cancer, AIDS, chronic obstructive pulmonary disease, among others; amputation of a limb; use of a wheelchair; and use of a pacemaker.

For sample calculation, the population covered by the service (805,722),[15] the prevalence of CKD stages 3 to 5 (10.6%),[16] confidence limits of 5% and confidence interval of 95% were considered, yielding a sample of 146 individuals. This sample was calculated using the Epi Info software. Considering a loss of 15%, we chose to evaluate at least 169 individuals.

Socioeconomic data were collected through questionnaires, and nutritional status were evaluated by anthropometric measurements, bioelectrical impedance analysis, and biochemical tests. For physical activity level, those who reported at least 150 minutes of physical activity weekly were considered as physically active.[17]

Height was measured using a portable stadiometer, and weight was measured using a Tanita BC-553 Ironman® body composition monitor. Then, body mass index (BMI) was calculated and classified according to the World Health Organization (WHO)[9] criteria for adults and the elderly.[18]

Body fat percentage (BF%) was measured using the Fresenius Medical Care body composition monitor, and classified according to Lohman and Champaign, 1992.[19]

WC was measured as the smallest circumference between the lowest rib and the iliac crest and classified according to the WHO criteria.[9] Hip circumference was measured at the widest area of the hips at the greatest protuberance of the buttocks; the waist-hip ratio (WHR) was then calculated and classified according to the WHO.[9]

Regarding biochemical tests, fasting glycemia, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides (TG), uric acid and HbA1C were evaluated, which were classified according to the reference values.[20] For this, patients were asked to bring, on the day of the anthropometric assessment, the last tests performed within the last 90 days.

Blood pressure was measured using the auscultatory method, with an aneroid sphygmomanometer, properly calibrated. The values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were classified according to the 7th Brazilian Guideline for Hypertension.[21]

Glomerular filtration rate (GFR) was estimated by the CKD-EPI formula,[22] and classified according to the KDIGO (2012) guidelines.[1]

The Framingham score was used to estimate the cardiovascular risk; the sum of scores was performed based on the variables: sex, age, smoking, diabetes mellitus, HDL-c, TC, SBP, DBP and then classified as low, medium and high risk of developing cardiovascular disease in the next ten years.[23]

Statistical analysis

First, an exploratory analysis was carried out to verify the integrity (coherence) of the data. Quantitative variables were analyzed for the presence of outliers and the Kolmogorov-Smirnov test was used to verify the normality of data distribution.

Descriptive analysis of the sample was carried out by sex. Continuous variables were expressed as mean ± standard deviation and compared using the unpaired Student's t-test. The categorical variables, on the other hand, were described as absolute and relative frequencies, and compared, by sex and the Framingham score classification, using the Pearson's chi-square test.

The SPSS version 20.0 software was used, and the level of significance adopted was 5%.

The study was approved by the research ethics committee of Juiz de Fora Federal University Teaching Hospital (submission number: 1.147.858).

Results

The sample consisted of 172 participants (57% male), with mean age of 68.85 ± 11.41 years, 80.2% were elderly.

Table 1 shows the general characteristics of the sample, including age, GFR, and anthropometric, biochemical and blood pressure data stratified by sex. Mean values of BMI, BF%, HDL-c, LDL-c and TC were higher in women, whereas WHR was higher in men. In both sexes, high mean values (above recommendations) were found for BMI, uric acid, fasting glucose and HbA1C for diabetics. Mean values of lipid parameters were within or close to the reference values for both sexes, as well as fasting blood glucose, HbA1C for non-diabetics, and SBP and DBP values.

The frequency of cardiovascular risk factors by sex is shown in Table 2. The frequency of diabetes mellitus (DM) and arterial hypertension was high in both men and women, and 50% of patients had both diseases. Most patients (51.5%) had CKD stage 3B, and most participants were physically inactive (62.2%). Smoking habit was more prevalent in males (15.3%) than in females (2.7%).

Table 1 – General characteristics of chronic kidney disease patients under conservative treatment stratified by sex, Juiz de Fora, Brazil

Characteristics	Female	Male	p[£]
Age (years)	67.88 ± 12.84	69.59 ± 10.20	0.347
GFR (ml/min/1.73m²)	32.64±11.42	34.60±11.58	0.271
BMI (kg/m²) Adults <60 years	31.33±7.12	30.57±6.59	0.756
BMI (kg/m²) Adults ≥60 years	29.85±5.16	27.78±4.89	0.019
Waist circumference (cm)	95.84±12.90	99.01±12.95	0.113
WHR	0.90±0.07	0.96±0.08	<0.001
BF%	41.01±9.79	30.92±9.27	<0.001
Uric acid (mg/dL)	7.85±5.33	7.22±1.54	0.363
HDL-c (mg/dL)	50.30±18.02	42.24±12.14	0.003
LDL-c (mg/dL)	109.61 ± 45.83	91.13 ± 31.47	0.008
Total Cholesterol (mg/dL)	195.61±55.97	169.76±42.03	0.003
Triglycerides (mg/dL)	179.63±104.11	176.4±98.32	0.867
Fasting Glucose Diabetics (mg/dL)	147.55±89.48	138.78±61.00	0.592
Fasting Glucose Non-diabetics (mg/dL)	102.31±28.95	98.81±15.32	0.527
HbA1c Diabetics (%)	7.67±1.77	7.80±1.97	0.753
HbA1c Non-diabetics (%)	5.82±1.09	5.88±1.57	0.894
SBP Hypertensive (mmHg)	135.06±20.34	136.81±24.39	0.642
SBP Non-hypertensive	136.87±17.10	133.57±11.50	0.593
DBP Hypertensive (mmHg)	76.70±14.30	76.50±12.31	0.925
DBP Non-hypertensive	78.75±6.40	78.57±10.27	0.965

GFR: glomerular filtration rate; BMI: body mass index; WHR: waist-hip ratio; BF: body fat; HDL-c: high-density lipoprotein; LDL-c: low-density lipoprotein; HbA1c: glycated hemoglobin; SBP: systolic blood pressure; DBP: diastolic blood pressure
[£] Student's t-test

Table 2 – Cardiovascular risk factors, by gender, of patients with chronic kidney disease (n=172) under conservative treatment, Juiz de Fora, Brazil

Variable		Female n(%)	Male n (%)	Total	p value €
Sample	< 60 years	20 (27.0)	14 (14.3)	34 (19.8)	0.052
	≥ 60 years	54 (73.0)	84 (85.7)	138 (80.2)	
Physical activity	> 150 minutes/ week	28 (37.8)	37 (37.8)	65 (37.8)	1.000
	< 150 minutes/ week	46 (62.2)	61 (62.2)	107 (62.2)	
Smoking	Yes	2 (2.7)	15 (15.3	17 (9.9)	0.008
	No	72 (97.3)	83 (84.7)	155 (90.1)	
Use of alcohol	Yes	6 (8.1)	16 (16.3)	22 (12.8)	
	No	68 (91.9)	82 (83.7)	150 (87.2)	0.166
SAH	Present	66 (89.2)	84 (85.7)	150 (87.2)	0.646
	Absent	8 (10.8)	14 (14.3)	22 (12.8)	
DM	Present	38 (51.4)	54 (55.1)	92 (53.5)	0.646
	Absent	36 (48.6)	44 (44.9)	80 (46.5)	
SAH and DM	Present	36 (48.6)	50 (51.0)	86 (50.0)	0.878
	Absent	38 (51.4)	48 (49.0)	86 (50.0)	
CKD Stage	3A	9 (12.2)	17 (17.5)	26 (15.2)	
	3B	39 (52.7)	49 (50.5)	88 (51.5)	0.788
	4	21 (28.4)	26 (26.8)	47 (27.5)	
	5	5 (6.8)	5 (5.2)	10 (5.8)	
BMI Adults (Kg/m²)	< 25	3 (15.0)	3 (21.4)	6 (17.6)	0.672
	≥25	17 (85.0)	11 (78.6)	28 (82.4)	
BMI Elderly (Kg/m²)	<27	16 (29.6)	38 (45.8)	54 (39.4)	0.074
	≥27	38 (70.4)	45 (54.2)	83 (60.6)	
WC (cm)	♀ < 80; ♂< 94	6 (8.1)	35 (35.7)	41 (23.8)	< 0.001
	♀ ≥ 80; ♂ ≥ 94	68 (91.9)	63 (64.3)	131 (76.2)	
WHR (cm)	♀ < 0.85; ♂ < 1.0	12 (16.2)	59 (60.2)	71 (41.3)	< 0.001
	♀ ≥ 0.85; ♂ ≥ 1.0	62 (83.8)	39 (39.8)	101 (58.7)	
Body fat (%)	♀ < 27; ♂ < 17	6 (8.1)	7 (7.3)	13 (7.6)	1.000
	♀ ≥27; ♂ ≥17	68 (91.9)	89 (92.7)	157 (92.4)	
Uric Acid (mg/dL)	♀ < 6; ♂ < 7	14 (18.9)	32 (32.7)	46 (26.7)	0.056
	♀ ≥ 6; ♂ ≥ 7	60 (81.1)	66 (67.3)	126 (73.3)	
HDL-c (mg/dL)	> 60	43 (58.1)	43 (43.9)	86 (50.0)	0.090
	<40	31 (41.9)	55 (56.1)	86 (50.0)	
LDL-c (mg/dL)	<160	66 (89.2)	94 (95.9)	160 (93)	0.129
	≥160	8 (10.8)	4 (4.1)	12 (7.0)	

Cont. Table 2 – Cardiovascular risk factors, by gender, of patients with chronic kidney disease (n=172) under conservative treatment, Juiz de Fora, Brazil

Variable		Female n(%)	Male n (%)	Total	p value €
TC (mg/dL)	<240	62 (83.8)	94 (95.4)	156 (90.7)	0.008
	≥240	12 (16.2)	4 (4.1)	16 (9.3)	
TG (mg/dL)	<200	56 (75.7)	74 (75.5)	130 (75.6)	0.790
	≥200	18 (24.3)	24 (24.5)	42 (24.4)	
Glucose (mg/dL)	DM < 126; No DM <100	47 (63.5)	58 (59.2)	105 (61)	0.636
	DM ≥ 126; No DM ≥100)	27 (36.5)	40 (40.8)	67 (39)	
Hb1Ac (%)	DM < 7; No DM <5.3	17 (23.0)	30 (30.6)	47 (27.3)	0.302
	DM ≥ 7; No DM >5.3	57 (77.0)	68 (69.4)	125 (72.7)	
Framingham score CVD risk	Low (< 10%)	30 (40.5)	44 (44.9)	74 (43.0)	0.267
	Moderate (10-20%)	31 (41.9)	30 (30,6)	61 (35.5)	
	High (> 20%)	13 (17.6)	24 (24.5)	37 (21.5)	

♀: woman; ♂: man; SAH: systemic arterial hypertension; DM: diabetes mellitus; CKD: chronic kidney disease; BMI: body mass index; WC: waist circumference; WHR: waist-hip ratio; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein; TC: total cholesterol, TG: triglycerides; Hb1Ac: glycated hemoglobin; CVD: cardiovascular disease.
€ Pearson's Chi-square test.

Regarding the BMI classification, 82.4% of all the sample and 60.6% of the elderly were overweight. Elevated WC and WHR were highly prevalent among women (91.9% and 83.8%, respectively), and significantly higher than men (64.3% and 39.8%, respectively). Furthermore, a high BF% was found in 92.4% of the sample. Regarding biochemical tests, the high prevalence of high uric acid levels stood out, both in women and men (81.1% and 67.3%, respectively).

As for the risk of CVD, according to the Framingham score, 43% of the sample was at low risk, followed by 35.5% at medium risk and 21.5% at high risk of developing CVD in ten years.

The factors associated with cardiovascular risk, according to the Framingham score are shown in Table 3. It was verified that individuals with an income above the minimum wage and adults with a high BMI had a higher risk of developing CVD.

Discussion

The main cause of death in patients in stage 1 CKD is CVD,[24] and the associated risks include traditional ones, such as old age, male gender, arterial hypertension, elevated LDL-c, reduced HDL-c, DM, smoking, sedentary lifestyle, and non-traditional ones such as decreased GRF, anemia, type of CKD, among others.[25]

Most of the sample was composed of male adults, older than 60 years. This finding can be justified by the fact that older adults are at relatively higher risk for developing CKD, due to physiological decrease in GFR and kidney injury secondary to aging and associated chronic disease, such as DM and hypertension.[26] Moreover, according to Bregman,[12] male sex is a non-modifiable risk factor for the progress of CKD.

Low income and low education, as observed in our study group (median per capita income of R$880.00 and incomplete elementary school in 66.3% of participants), are determining factors for the occurrence of CKD and must be analyzed for screening and monitoring of the disease.[9,27]

In our study, the patients with CKD had a high prevalence of arterial hypertension (87.2%) and DM (53.5%), and 50% of them had both diseases concomitantly, which is a risk factor for CVD. Similar findings were

Table 3- Factors associated with cardiovascular risk, according to the Framingham score

Variable		Framingham score classification			p value€
		Low n (%)	Medium n (%)	High n (%)	
Sample	Adults < 60 years old	14 (41.2)	13 (38.2)	7 (20.6)	0.931
	Adults ≥ 60 years old	60 (43.5)	48 (34.8)	30 (21.7)	
Income	Less than minimum wage	50 (47.6)	40 (38.1)	15 (14.3)	0.015
	More than minimum wage	24 (35.8)	21 (31.3)	22 (32.8)	
Education	Incomplete elementary school	57 (44.5)	47 (36.7)	24 (18.8)	0.444
	Complete elementary school	17 (39.5)	14 (32.6)	12 (27.9)	
Physical activity	Yes (> 150 minutes/week)	27 (41.5)	20 (30.8)	18 (27.7)	0.278
	No (< 150 minutes/week)	47 (43.9)	41 (38.3)	19 (17.8)	
CKD stages	3 A	11 (42.3)	9 (34.6)	6 (23.1)	0.990
	3 B	39 (44.3)	32 (36.4)	17 (19.3)	
	4	19 (40.4)	17 (36.2)	11 (23.4)	
	5	4 (40.0)	3 (30.0)	3 (30.0)	
BMI Adults < 60 years old (Kg/m²)	Appropriate	5 (83.3)	0 (0.0)	1 (16.7)	0.050
	High	9 (32.1)	13 (46.4)	6 (21.4)	
BMI Adults ≥ 60 years old (Kg/m²)	Appropriate	26 (48.1)	15 (27.8)	13 (24.1)	0.353
	High	34 (41.0)	33 (39.8)	16 (19.3)	
WC (cm)	♀ < 80; ♂ < 94	24 (58.5)	10 (24.4)	7 (17.1)	0.068
	♀ ≥ 80; ♂ ≥ 94	50 (38.2)	51 (38.9)	30 (22.9)	
WHR	♀ < 0.85; ♂ < 1.0	37 (52.1)	19 (26.8)	15 (21.1)	0.086
	♀ ≥ 0.85; ♂ ≥ 1.0	37 (36.6)	42 (41.6)	22 (21.8)	
BF (%)	♀ < 27; ♂ < 17	6 (8.1)	2 (3.3)	5 (14.3)	0.146
	♀ ≥27; ♂ ≥17	68 (91.9)	59 (96.7)	30 (85.7)	

CKD: chronic kidney disease; BMI: body mass index; WC: waist circumference; WHC: waist-hip ratio; BF: body fat; € Pearson's Chi-square test.

reported in a survey conducted by Pinho et al.,[28] in a general medical clinic in São Paulo, in which 75.2% of CKD patients were hypertensive and 49.5% diabetic.[28] Our population (both hypertensive and non-hypertensive individuals) had high mean SBP and DBP values according to the reference values. This is a positive finding, since the control of hypertension is relevant to delay the progression of CKD and possibly the development of CVD.[29]

Regarding smoking habit, it was more prevalent among men than women, as reported in a study carried out in São Paulo, Brazil, in which 36.4% of men were smokers. Smoking is one of the most important factors for acute myocardial infarction.[29]

The high prevalence of physical inactivity (62.2%) is worrying, since physical inactivity, added to other traditional cardiovascular risk factors, promotes accelerated atherosclerosis and early mortality.[30] A high prevalence of physical inactivity (74%) was also identified in patients with CKD under conservative treatment by Fortes et al.[31]

Overweight in patients with CKD can increase renal plasma flow and intraglomerular pressure, which, in turn, increases cardiac output.[31,32] Increased central adiposity demarcated by high WHR is associated with metabolic complications.[9] In the present study, there was a prevalence of overweight of 82.4% in adults (<60 years old)

and of 60.6% in the elderly (60 years old), and WHR values were higher in females, in agreement with a study carried out in Brusque, a city in Santa Catarina State, Brazil.[33]

BMI was high in both sexes and in all age groups and correlated with cardiovascular complications. However, BMI is considered a controversial marker, since it does not discriminate between the different components of the body (lean and fat mass) and does not describe the fat distribution (visceral fat and subcutaneous fat).[8] For this reason, the use of WC is recommended, which has been suggested as a direct predictor of all-cause mortality from CVD.[7] High values of WC was found in most patients (76.2%), with higher prevalence in women than men ($p<0.001$), similar to previously reported data.[34]

Alarming prevalence of increased BF% was found in men (92.7%) and women (91.9%). This fact draws attention, since excess body fat is associated with cardiovascular risk factors, such as metabolic syndrome, DM, hypertension, hypercholesterolemia and atherosclerosis.[34]

Dyslipidemia is common in CKD and its presence can contribute to increase cardiovascular risk,[35] since elevated TG and reduced HDL-c are independent predictors of cardiometabolic episodes[36]. Regarding elevated LDL-c, data from the National Health and Nutrition Examination Survey (NHANES 1999-2006) demonstrated a 46% prevalence of this lipid alteration in adults with stages 1-2 CKD and 80% with CKD stages 3 and 4.[37] Despite these findings, in the present study, the values of serum lipid parameters were within or close to the recommended values, similar to the results found in the study by Fortes et al.[31]

Regarding uric acid levels, these were above the recommended levels. It is known that hyperuricemia is highly prevalent in CKD; however, it is still unclear whether uric acid is merely a marker of comorbidities and kidney damage or whether it is a factor for cardiovascular outcomes.[38] According to a cohort study by Wan-Chun Liu , which evaluated 3,749 patients with stages 3-5 CKD for three years, hyperuricemia is a risk factor for cardiovascular events in these patients.

The values found for fasting blood glucose and HbA1C were higher than recommended. According to Hage,[4] the lack of glycemic control is associated with the progression of CKD and cardiovascular mortality. To prevent these outcomes, it is recommended to keep HbA1C at levels below 7.0% and postprandial glycemia below 140mg/dL40 for diabetics, and from 3.6 to 5.3% and less than 100mg/dL20, respectively, for non-diabetics.

In relation to GFR, it was shown that for each decrease of 10mL/min/1.73m in the GFR, there is a 10% increase in the relative risk of death or non-fatal cardiovascular complication.[39] In the present study, patients were mostly in stage 3B, indicating that they should continue with nutritional monitoring and undergo treatment to prevent the progress to CKD stage 5 or even develop cardiovascular complications.

Regarding the 10-year cardiovascular risk estimated using the Framingham score, 43% of the sample was at low risk, 35.5% at medium risk and 21.5% at high risk. These results are similar to those reported by Cesarino.[39] These findings can be used by health professionals to improve treatment adherence and develop strategies to the reduce risk and morbidity and mortality of CVD.

A limitation of the study is the fact that it is a cross-sectional study, which makes it impossible to infer that patients at cardiovascular risk will in fact develop CVD. However, despite its limitations, the relevance of the study lies in its originality and importance of the theme.

Assessing cardiovascular risk factors in patients with CKD is of paramount importance, since this condition is commonly concomitant with many factors associated with an increased risk of cardiovascular diseases, such as older age, comorbidities like DM and hypertension, and overweight. Thus, a multidisciplinary approach is needed, to assist in the prevention and control of cardiovascular mortality in this population. since Adherence to healthy eating patterns is a protective factor for renal function, helping to prevent the development of chronic diseases, thereby improving the prognosis and favoring a better quality of life.

Conclusions

There was a high prevalence of cardiovascular risk factors in the population studied, such as physical inactivity, presence of comorbidities (hypertension and DM), increased BMI, WC, WHR and BF%, and elevated uric acid and high HbA1C levels. Nevertheless, 57% of the sample were at medium or high risk of developing CVD in 10 years, according to the Framingham score. Individuals with a per capita income above the minimum wage and high BMI had a higher 10-year risk of developing CVD.

Assessing cardiovascular risk factors in patients with CKD is important and allows guiding the conduct of health professionals to prevent mortality from cardiovascular causes.

Author contributions

Conception and design of the research: Pereira PML, Bastos MG, Candido APC. Acquisition of data: Oliveira CFM, Pereira PML, Soares IT, Monteiro MG. Analysis and interpretation of the data: Oliveira CFM, Pereira PML, Soares IT, Candido APC. Statistical analysis: Oliveira CFM, Pereira PML, Candido APC. Writing of the manuscript: Oliveira CFM, Pereira PML. Critical revision of the manuscript for intellectual content: Pereira PML, Soares IT, Monteiro MG, Bastos MG, Candido APC.

Potential Conflict of Interest

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

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Study Association

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the *Hospital Universitário da Universidade Federal de Juiz de Fora* under the protocol number 1384.797. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

References

1. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. KidneyIntSuppl. 2013; 3(1): 5-14.
2. Bastos, R.M.R. Prevalência da doença renal crônica nos estágios 3,4 e 5 em adultos. Rev Assoc Med Bras.2009; 55(1):40-4.
3. Sociedade Brasileira de Nefrologia. Censo de diálise [serial online] SBN; 2012[cited 2018 jan. 29]. 1:[1 screens].Available from: URL: https://sbn.org.br
4. Hage FG. The scope of coronary heart disease in patient swith chronic kidney disease. J Am Coll Cardiol. 2009; 53(23):2129-40.
5. Van Der Velde M, Astor BC , K Matsushita , Gansevoort RT, Levey AS , Jong PE et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney Int. 2011;79(12):1341-52.
6. Martin, C. L. A doença renal como fator de risco cardiovascular. Arquivos brasileiros de cardiologia. 2005;85(6):432-6.
7. Postorino, M. et al. Abdominal obesity and all-cause and cardiovascular mortality in end-stage renal disease. J Am Coll Cardiol. 2009;53(15):1265-72.
8. Gohda T, Gotoh H, Tanimoto M, Sato M, Io H, Kaneko K, et al. Relationship between abdominal fatac cumulation and insulin resistance in hemodialysis patients. Hypertens Res 2008; 31(1):83-8.
9. WHO, World Health Organization. Obesity: Preventing and managing the global epidemic – Report of a WHO consultation on obesity. Geneva; 2000.
10. Postorino M, Marino C, Tripepi G, Zoccali C. Abdominal obesity modifies the risk of hypertriglyceridemia for all-cause and cardiovascular mortality in hemodialysis patients. International Society of Nephrology. 2011;79(7):765–72.
11. Liu WC, Kuo MC , Chang JM , Hwang SJ , Chen HC, Chiu YW et al. Association of Hyperuricemiawith Renal Outcomes, Cardiovascular Disease, and Mortality. The American Society of Nephrology. 2012;7(4):541-8.
12. Bregman R. Prevenção da progressão da doença renal crônica. J. Bras. Nefrol. 2004;26(3 suppl. 1):11- 4.
13. Framingham Heart Study. A Project of National Heart, Lung and Blood Instituteand Boston University[serial online] 2018 [cited 2018 Set 28];1:[6 screens].Available from: URL: https://www.framinghamheartstudy.org/fhs-risk-functions/hard-coronary-heart-disease-10-year-risk/
14. Lloyd Jones DM; Wison PW, Larson MG, Beiser A, Leipe EP, D' Agostino RB, et al. Framingham risk score and prediction of life time risk for coronary heart disease. Am J Cardiol. 2004;94(1):20-4.
15. Instituto Brasileiro de Geografia e Estatística (IBGE). Estimativas da população, 2015. [Citado em 2017 jan 10]. Available from: URL: https://www.ibge.gov.br/estatisticas/sociais/populacao/9103-estimativas-de-populacao.html?edicao=17283&t=downloads
16. Hill NR, Fatoba ST,Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS,et al. Global Prevalence of Chronic Kidney Disease. – A Systematic Review and Meta-Analysis. PLoS One. 2016;11(7):1-18.
17. Pescatello L.S, Arena R, Riebe D, Thompson P.D ACSM's guidelines for exercise testing and prescription. 9.ed. Baltimore: Lippincott Williams & Wilkins; 2014. p.2-18.
18. Lipschitz, D.A. Screening for nutritional status in the elderly. Prim Care. 1994;21(1):55-67.
19. Lohman T.G, Champaign, I.L. Advances in body composition assessment. Champaign, IL: Human Kinetics Publishers, 1992.
20. Nemer, A.G.A.; Neves, F.J.; Ferreira, J.E.S.editors; Manual de solicitação e interpretação de exames laboratoriais, Rio de Janeiro: Revinter, 2010.
21. MVB Malachias, FL Plavnik, CA Machado, D Malta, LCN Scala, S Fuchs. Arquivos Brasileiros de Cardiologia. 7° Diretriz Brasileira de hipertensão arterial [serial online] Sociedade Brasileira de Cardiologia; 2016.117(3):3-83.
22. Levey, AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150(9):604-12.
23. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Estratégia para o cuidado da pessoa com doença crônica hipertensão arterial sistêmica [serial online]. Brasília, 2013 (Cadernos de Atenção Básica). [cited 2018 Out 24];37:[130 screens].Available from: URL: http://bvsms.saude.gov.br/bvs/publicacoes/estrategias_cuidado_pessoa_doenca_cronica.pdf

24. Sarnak M.J, Levey AS, Schoolwerth A.C, Coresh J, Culleton B, Hamm L.L, et al. Kidney disease as a risk factor for development of cardiovascular disease a statement from the American Heart Association Councilson Kideney in Cardiovascular Disease, High Blood Pressures Research, Clinical Cardiology, and Epidemiology and Prevention. Hypertension. 2003; 42(5):1050-65.

25. Ernesto L. S, Mark, L.L, Johannes, F.E.M. Chronic Kidney Disease Effects on the Cardiovascular System. Circulation. 2007; 116(1):85-97.

26. K/doqi clinical practice guidelines for chronic kidney disease: evaluation, classification And stratification [editorial]. Am J Kidney Dis. 2002;39(2 Suppl 1):S1-266.

27. Bastos, MG, Kirsztajn, GM. Doença renal crônica: importância do diagnóstico precoce, encaminhamento imediato e abordagem interdisciplinar estruturada para melhora do desfecho em pacientes ainda não submetidos à diálise. J Bras Nefrol, 2011;33(1):93-108.

28. Bruce MA, Beech MB, Sims M, Brown TN, Wyatt SB, Taylor HA, et al. Social environmental stressors, psychological factors and kidney disease. J Investig Med, 2009;57(4):583-9.

29. Pinho NA, Silva GV, Pierin MG. Prevalência e fatores associados à doença renal crônica em pacientes internados em um hospital universitário na cidade de São Paulo, SP, Brasil. J Bras Nefrol. 2015; 37(1): 91-7.

30. James M.T, Hemmelgarn B.R, Tonelli M. Early recognition and prevention of chronic kidney disease. Lancet 2010; 375(9722):1296-309.

31. Fortes A. A, Lima, G.M.H, Santana, G.T, Barbosa, R.L, Luiz, N.G.A, Pereira, C.M.E, et al. Razão TG/HDL-c, indicadores antropométricos e bioquímicos de risco cardiovascular no renal crônico em tratamento conservador. Nutr clin diet hosp. 2017; 37(4):10-6.

32. Junior G.B.S, Bentes, A.S.N, Daher, E. F, Matos, S. M.A. Obesidade e doença renal. J Bras Nefrol. 2017; 39(1):65-9.

33. Rosini N, Machado, M. J, Xavier, H. T. Study of the prevalence and multiplicity of cardiovascular risk factors in hypertensive individuals from the city of Brusque, SC, Brazil. ArqBrasCardiol.2006; 86(3):219-22.

34. Elsayed E.F, Tighiouart H, Weiner DE, Griffith J, Salem D, Levey AS, et al. Waist-to-hip ratio and body mass index as risk factors for cardiovascular events in CKD. Am J Kidney Dis. 2008; 52:49-57.

35. Akpan E.E, Ekrikpo, U. E, Effa, E. E, Udo, A. I. A, Kadiri, S. Assessment of dyslipidemia in pre-dialysis patients in south-west Nigeria. NigerMed J. 2014; 55(3):214-9.

36. Salazar M.R, Carbajal H.A, Espeche W.G, Leiva Sisnieguez C.E, Balbín E, Dulbecco C.A et al. Relation Among the Plasma Triglyceride/High-Density Lipoprotein Cholesterol Concentration Ratio, Insulin Resistance, and Associated Cardio-Metabolic Risk Factors in Men and Women. Am J Cardiol. 2012; 109(12):1749 –53.

37. Snyder JJ, Foley, N.R, Collins, J. A. Prevalence of CKD in the United States: a sensitivity analysisus ing the National Health and Nutrition Examination Survey (NHANES) 1999-2004. Am J KidneyDis. 2009; 53(2):218-28.

38. Lordsleem A.Gueiros, A.P.S, Gueiros, J.E.B, Filho,B.M, Victor,E.G. Cardiac evaluation of patients with chronic kidney disease: what lessons?. J Bras Nefrol. 2012; 34(1):8-15.

39. Cesarino, B.C, Borges, P.P, Ribeiro, M.H.C.R, Ribeiro, F.D, Kusumota, L. Avaliação do risco cardiovascular de pacientes renais crônicos segundo critérios de Framingham. Acta Paul Enferm. 2013; 26(1):101-7.

ORIGINAL ARTICLE

The Association between Tp-e interval, Tp-e/QT , and Tp-e/QTc Ratios and Coronary Artery Disease Spectrum and Syntax Score

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Abstract

Background: Coronary artery disease (CAD) causes electrical heterogeneity on ventricular myocardium and ventricular arrhythmia due to myocardial ischemia linked to ventricular repolarization abnormalities.

Objective: Our aim is to investigate the impact of increased level of CAD spectrum and severity on ventricular repolarization via Tp-e interval, Tp-e/QT and Tp-e/QTc ratios.

Methods: 127 patients with normal coronary artery (group 1), 129 patients with stable CAD (group 2) and 121 patients with acute coronary syndrome (group 3) were enrolled. Tp-e interval, Tp-e/QT and Tp-e/QTc ratios were evaluated as well as baseline demographic and clinical parameters. Kruskal-Wallis one-way ANOVA test was used for comparing quantitative variables with abnormal distribution while One-Way ANOVA test was used for comparing the means between groups with normal distribution. Tukey HSD and Welch tests were used for subgroups analyses with normal distribution. Spearman analysis was used to evaluate the correlation between clinical variables and repolarization markers. A p-value < 0.05 was considered statistically significant.

Results: Tp-e interval [66(50-83), 71(59-82) and 76(64-86); group 1,2 and 3 respectively, p<0.001], Tp-e/QT (0.170.02, 0.180.01 and 0,190.01; group 1,2 and 3 respectively, p<0.001) and Tp-e/QTc (0.150.02, 0.160.02 and 0.170.02; group 1,2 and 3 respectively, p<0.001) ratios were found to be associated with increased level of CAD spectrum. Syntax score was positively correlated with Tp-e interval (r=0.514, p<0.001), Tp-e/QT (r=0.407, p<0.001), and Tp-e/QTc ratios (r=0.240, p<0.001).

Conclusion: Prolonged Tp-e interval and increased Tp-e/QT and Tp-e/QTc ratios were detected in the presence of CAD and especially in patients with acute ischemic syndromes. (Int J Cardiovasc Sci. 2021; 34(2):179-187)

Keywords: Cardiovascular Diseases; Coronary Artery Disease; Arrhythmias Cardiac; Electrocardiography;/ methods; Anthropometry; Score Sintax.

Introduction

Atherosclerotic cardiovascular diseases are still major underlying reasons for all-cause morbidity and mortality worldwide.[1] While several factors have been described to explain the possible etiologic bases for mortality in the presence of coronary artery disease (CAD), ventricular arrhythmia is one the most important causes of catastrophic outcomes due to myocardial ischemia.[2] Coronary atherosclerosis can cause electrical heterogeneity in ventricular myocardium and ventricular repolarization abnormalities linked to ventricular arrhythmia. These clinical presentations are seen more commonly in the presence of acute ischemia.[3] While obstructive atherosclerosis damages ventricular myocardium, acute ischemia renders myocardium more sensitive to arrhythmia.

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Electrocardiography (ECG) is often used to detect the electrical instability of the heart. QT intervals and T waves show ventricular depolarization and repolarization on the ECG surface, while abnormalities of the mentioned parameters are important to predict arrhythmias in patients with CAD. The QT interval is described as a continuation from the beginning of the QRS complex to the end of the T wave. Higher QT and corrected QT (QTc) intervals are found to be related to ventricular arrhythmia and mortality, as well as to increased QT dispersion (QTd), which is the difference between the longest and the shortest QT interval.[4-6] The Tp-e interval, which is between the peak and the end of the T wave, is an index of transmural dispersion of repolarization. A prolonged Tp-e interval is related to serious arrhythmias and sudden cardiac death.[7-11] Although QT and Tp-e interval can be affected by heart rates and body weights, the Tp-e/QT ratio is a novel marker to reflect ventricular repolarization and is not affected by changes in heart rates.[12] The present study aimed to investigate the impact of the CAD spectrum on ventricular electrical activity detected by Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios.

Methods

Study population

This cross-sectional study was conducted at two tertiary centers from May 2018 to July 2018. One hundred twenty-seven patients with normal coronary arteries (NCA-group 1), one hundred twenty-nine patients with stable coronary artery disease (SCAD-group 2), and one hundred twenty patients with acute coronary syndrome (ACS-group 3) were prospectively and consecutively enrolled in this study.

Patients who had previously undergone coronary angiography and patients with known CAD, severe valvular heart disease, atrial fibrillation, bundle branch block, or evidence of any other intraventricular conduction defect, previous pacemaker implantation, ECGs without clearly analyzable QT interval, electrolyte abnormalities, type I and III antiarrhythmic usages, and end-stage hepatic failure were excluded from the study.

At the beginning of the study, demographic and anthropometric measurements were recorded after performing a detailed cardiovascular and systemic examination. Biochemical analyses including serum creatinine, total cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglyceride (TG), serum electrolyte levels, and complete blood count were assessed. A 12-lead surface ECG was performed on all subjects before performing coronary angiography. The study was approved by the Ethics Committee of Istanbul Yeni Yuzyil University (Date: 28.05.2018, issue no: 019). Detailed, written, informed consent was obtained from each subject. The study was conducted in accordance with the Declaration of Helsinki.

Electrocardiography

A 12-lead surface ECG (Nihon Kohden Corporation, Cardiofax M Model ECG-1250, Tokyo, Japan) was performed in the supine position, with a 50 mm/s paper speed and a voltage of 20 mm/s, before performing coronary angiography. While the ECG was performed according to the routine polyclinic evaluation for patients from groups 1 and 2, it was performed in the emergency clinic on patients with ACS. Patients presenting a U wave and biphasic or negative T wave on surface ECG were excluded. Measurements were performed by two different cardiologists who were blinded to the patients' data. Parameters were obtained by using a software after x400% magnification. The QT interval was measured from the beginning of the QRS complex to the end of the T wave. The QTc interval was calculated by using Bazett's formula (QTc interval= QT/$\sqrt{RR\ interval}$).[13] While QTd was measured as the difference between the longest and the shortest QT intervals, QTc dispersion (QTcd) was measured as the difference between the longest and the shortest QTc intervals. Tp-e is measured from the peak of the T wave to the end of the T wave. In accordance with previous studies, the Tp-e was performed from the precordial leads as the preferred lead for measurements of Tp-e intervals in a descending order of V5, V4, V6, and an average value of at least three readings was calculated for each lead and measurement.[14] Finally, Tp-e/QT and Tp-e/QTc ratios were obtained from these measurements (Figure 1).

CAD categories and severity

Three hundred seventy-six patients were divided into three groups as follows: group 1: patients with NCA, group 2: patients with SCAD, and group 3: patients with ACS according to recent guidelines. Coronary angiography was performed for each patient after hospital admission with chest pain and evidence of ischemia in exercise stress testing in groups 1 and 2. Patients with normal coronary arteries were defined

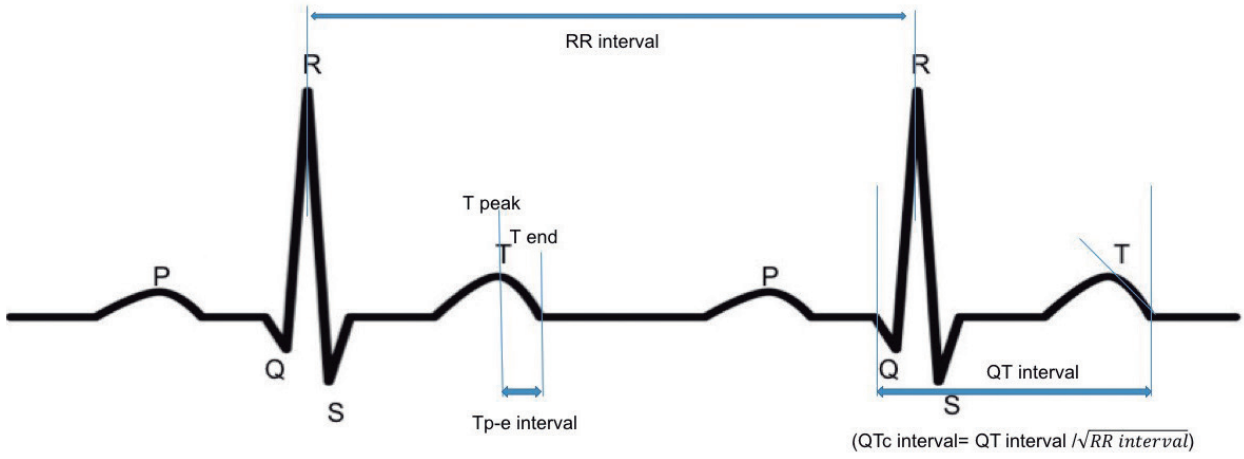

Figure 1 – An image of measurement of electrocardiographic variables. How should we measure electrocardiographic variables?

as group 1. Patients with coronary slow flow were excluded from the study. SCAD is that of a disease causing exercise or stress linked to chest symptoms because of the coronary artery disease with ≥50% stenosis in the left main coronary artery and/or ≥70% stenosis in one or more of the major coronary arteries.[15] These patients were defined as group 2. ACS patients were defined according to the aforementioned criteria. The diagnostic criteria for ST-elevation myocardial infarction (STEMI) were as follows: (a) typical chest pain for more than 20 minutes and (b) ST-segment elevation in at least two contiguous leads with the following cut-off points: ≥0.2mV in men ≥40 years old; ≥0.25mV in men <40 years old; or ≥0.15mV in women in leads V2–V3; and/or ≥0.1mV in the other leads. When indicated, posterior (V7–V9) and right (V3R–V4R) derivations were also obtained. A cut-off point was set at 0.05mV for V7-9 (≥0.1mV in men <40 years old) and ≥0.05mV for V_3R and V_4R (≥0.1mV in men <30 years old). Patients without persistent (>20 min) ST-segment elevation with acute chest pain and detection of a rise and/or fall of cardiac troponin values with at least one value of above the 99th percentile in the upper reference limit was defined as non-ST elevation myocardial infarction (NSTEMI). Both NSTEMI and STEMI patients formed group 3.

The anatomical-based Syntax score was used to evaluate coronary artery disease severity. Briefly, coronary arteries were evaluated as 16 separate segments and segments having 50% or more luminal stenosis and ≥1.5mm diameter were assessed. Every segment has a pre-specified corresponding weight factor as well as other determining factors, such as calcification and lesion length, which were assessed and taken into account in the Syntax score. The Syntax score calculator (www.syntaxscore.com) was used to obtain each patient's score.

Statistical analysis

Statistical analysis was made using the computer software Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, New York, USA). Pearson chi-square analysis was performed for categorical variables and Bonferoni method was used for subgroups. Fitness to normal distribution was analyzed with the Kolmogorov-Simirnov test. Data were expressed as "mean±standard deviation (SD)" for normal distribution, "median (1st and 3rd quartiles)" for abnormal distribution and "n (%)" for categorical variables. Kruskal-Wallis one-way ANOVA test was used for comparing quantitative variables without normal distribution while One-Way ANOVA test was used for comparing the means between groups with normal distribution. Tukey HSD and Welch tests were used for subgroup analyses with normal distribution. Spearman analysis was used to evaluate the correlation between clinical variables and repolarization markers. A p-value < 0.05 was considered statistically significant.

Results

One hundred twenty-seven patients with NCA (group 1), one hundred twenty-nine patients with SCAD (group 2), and one hundred twenty patients with ACS, including seventy-seven NSTEMI and forty-three STEMI patients (group 3) were enrolled in this study. Demographic parameters for each group were demonstrated in Table 1. There were no statistically significant differences in age, gender, smoking status, body mass index, hemoglobin, creatinine, LDL cholesterol, hypertension, diabetes mellitus, peripheral arterial disease, and use of medication, such as betablocker, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, and statin between three groups. Total cholesterol levels were significantly lower in patients with NCA than other groups and HDL cholesterol levels were significantly higher in group 1. In subgroup analyses, a statistically significant difference was only observed in HDL levels between groups 1 and 3. TG levels were lower in group 1. In subgroup analyses, it was demonstrated that

Table 1 – Baseline clinical and demographic parameters of study population

	NCA group (n=127)	SCAD group (n=129)	ACS group (n=120)	p
Age	59 (50-71)	58 (52-67)	57 (48-65)	0.101
Gender (female), n (%)	36.2 (46)	27.1 (35)	30.0 (36)	0.277
Smoking % (n)	45.7 (58)	41.9 (54)	42.5 (51)	0.807
BMI (kg/m^2)	28 (25-32)	28 (26-29)	28 (27-31)	0.354
Hemoglobin (g/dl)	13.85±1.73	13.6±1.60	13.72±2.03	0.486
Creatinine (mg/dl)	0.80 (0.70-1.00)	0.81 (0.70-1.00)	0.84 (0.70-1.00)	0.206
Total cholesterol (mg/dl)	175 (156-205)	192 (164-223)	188 (164-218)	0.043
LDL cholesterol (mg/dl)	109 (89-128)	114 (89-142)	116 (89-141)	0.228
HDL cholesterol (mg/dl)	44 (38-52)	41 (35-51)	40 (35-46)*	0.011
Triglyceride (mg/dl)	129 (100-165)	146 (106-210) †	162 (120-227) †	<0.001
HT % (n)	63.8 (81)	58.1 (75)	56.7 (68)	0.481
HL % (n)	18.9 (24)	34.9† (45)	40.8† (49)	0.001
DM % (n)	28.3 (36)	39.5 (51)	32.5 (39)	0.159
PAD % (n)	7.9 (10)	2.3 (3)	9.2 (11)	0.061
Medication				
BB % (n)	41.7 (53)	38.0 (49)	40.8 (49)	0.816
ACEI % (n)	34.6 (44)	39.5 (51)	26.7 (32)	0.097
ARB % (n)	15.7 (20)	8.5 (11)	8.3 (10)	0.099
CCB % (n)	22.8 (29)	32.6 (42)	26.7 (32)	0.213
Statin % (n)	17.3 (22)	21.7 (28)	11.7 (14)	0.108
EF (%)	60 (55-65)	60 (50-60)	55 (45-60) *	0.001
Syntax score	0 (0-0)	7 (4-17)†	16 (9-23) †,‡	<0.001

*** significantly decreased compared to NCA group, † significantly increased compared to NCA group, ‡ significantly increased compared to SCAD group**

(ACEI: angiotensin converting enzyme inhibitor, ACS: acute coronary syndrome, ARB: angiotensin receptor blocker, BB: beta blocker, BMI: body mass index, CCB: calcium channel blocker, DM: diabetes mellitus, EF: ejection fraction, HDL: high density lipoprotein, HL: hyperlipidemia, HT: hypertension, LDL: low density lipoprotein, NCA: normal coronary artery, PAD: peripheral artery disease, SCAD: stable coronary artery disease)

TG levels were lower with a statistically significant difference in group 1 when compared to groups 2 and 3. The prevalence of hyperlipidemia was found to be lower in patients with NCA. Ejection fraction (EF) values were lower in patients with ACS. According to subgroup analyses, statistical significant differences were only found between groups 1 and 3, with lower EF values in group 3 (p<0.001).

Electrocardiographic parameters for each group were demonstrated in Table 2. No significant differences were identified in the heart rates among the groups. QRS duration was shorter in group 1 than in the others. However, there was no difference in QRS duration between groups 2 and 3, while groups 2 and 3 presented a statistically significant longer duration time than did group 1. The QT interval, QTc interval, QTd, QTcd, and Tp-e interval (Figure 2) were prolonged in patients with ACS and shortened in patients with NCA. However, in subgroup analyses, the QTc interval was not found to be different between groups 2 and 3, and QTd was found to be different, with a statistical significance observed only between groups 1 and 3. Tp-e/QT ratio and Tp-e/QTc ratio (Figure 2) were lowest in group 1 and highest in group 3.

Spearman correlation analyses revealed that there was a negative correlation between LVEF and the Tp-e interval (r=-0.103, p=0.045), as well as a negative correlation between LVEF and the Tp-e/QT ratio (r=-0.106, p=0.040) (Figure 3). The syntax score was strongly and positively correlated with the Tp-e interval (r=0.514, p<0.001), Tp-e/QT ratio (r=0.407, p<0.001), and Tp-e/QTc ratio (r=0.240, p<0.001) (Figure 4).

Table 2 – Comparison of electrocardiographic parameters of the study population

	NCA group (n=127)	SCAD group (n=129)	ACS group (n=120)	p
Heart rate (beat/min)	79 (68-86)	79 (69-87)	80 (71-88)	0.374
QRS duration (ms)	84±8.8	89±11.1*	87±9.1*	<0.001
QT interval (ms)	381 (372-392)	390 (381-404)*	396 (385-410)*,†	<0.001
QTc interval (ms)	432 (406-464)	446 (412-484)*	450 (430-481)*	<0.001
QT dispersion (ms)	35 (32-37)	35 (32-37)	36 (34-38)*	0.026
QTc dispersion (ms)	37.5 (34.5-39.5)	38.0 (34.9-40.0)*	39.25 (37.0-41.7)*,†	<0.001
Tp-e interval (ms)	66 (61-71)	71 (66-75)*	76 (73-80)*,†	<0.001
Tp-e/QT ratio	0.17±0.02	0.18±0.01*	0.19±0.01*,†	<0.001
Tp-e/QTc ratio	0.150.02	0.16±0.02*	0.17±0.02*,†	<0.001

*significantly increased compared to NCA group, † significantly increased compared to SCAD group
ACS: acute coronary syndrome, NCA: normal coronary artery, SCAD: stable coronary artery disease

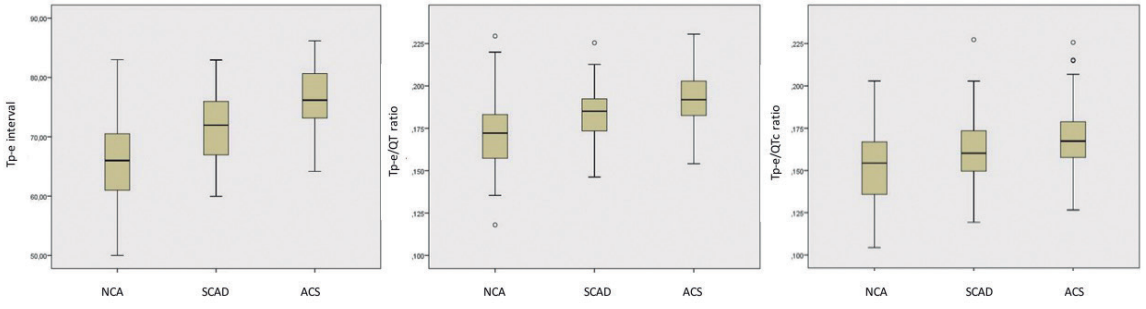

Figure 2 – Comparison of Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios between groups. Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios increase, together with a parallel increase in the severity of coronary artery disease, from normal coronary arteries to an acute coronary syndrome.

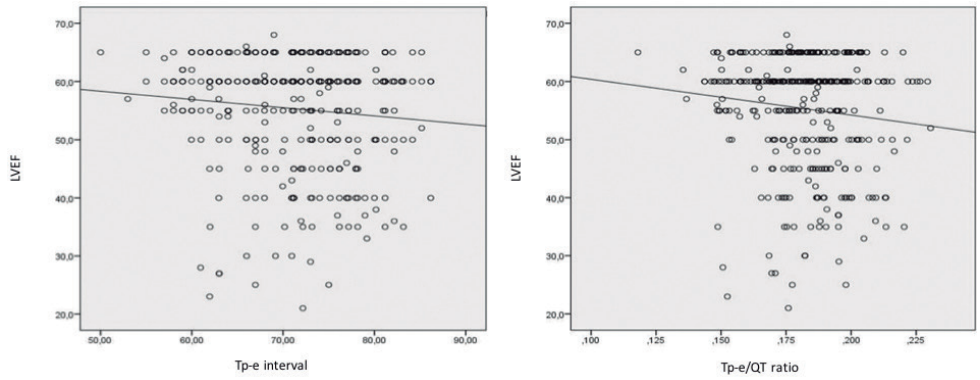

Figure 3 – Correlation between left ventricular ejection fraction and Tp-e interval and Tp-e/QT ratio. Tp-e interval and Tp-e/QT ratio were found to be negatively correlated with the left ventricle ejection fraction.

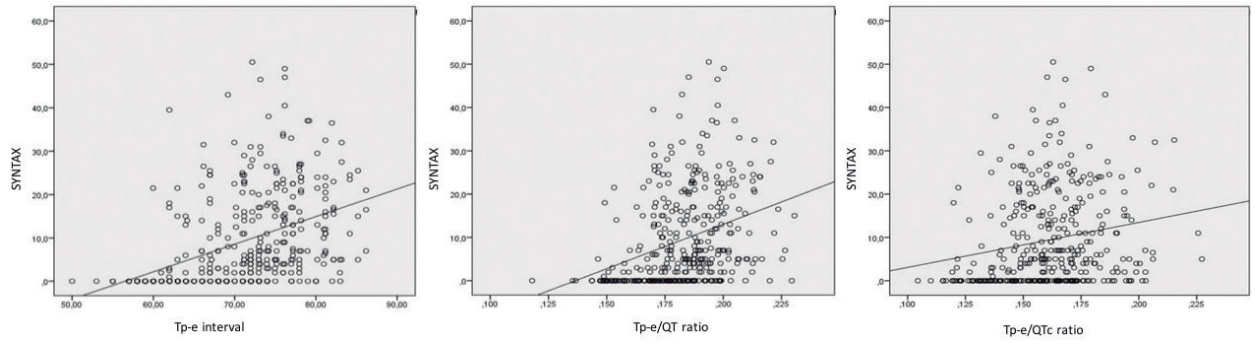

Figure 4 – Correlation between Syntax score and Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios. Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios were positively correlated with the severity of coronary artery disease, calculated by the Syntax score.

Discussion

In the present study, the increase in the coronary artery disease spectrum from normal coronary arteries to acute coronary syndrome is accompanied by a parallel prolongation in the Tp-e interval and elongation in the Tp-e/QT and Tp-e/QTc ratios. Additionally, progressive coronary atherosclerosis and unstable disease lead to ventricular repolarization, which seems to be correlated with an increase in the QT interval, QTc interval, QTd, and QTcd. This study also found a negative correlation between LV systolic function and total ventricular dispersion. Coronary artery disease severity is also found to be positively correlated with ventricular repolarization abnormalities if one calculates by Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios.

Obstructive coronary atherosclerosis causes electrical impairment in the ventricle myocardium due to the imbalance in arterial supply and demand. The size of the ischemic area and the presence of previous scar formation are the main determining factors of myocardial heterogeneity and the formation of arrhythmia. Arrhythmia is related to significant obstructive CAD, leading to ischemic episodes without acute or old myocardial infarction (MI). These pathological pathways result in myocardial electrical imbalance, which is reflected in abnormal ventricular repolarization. While previous studies demonstrated that the incidence of arrhythmia was higher in CAD patients, it was more commonly seen in patients with acute ischemia due to the increased risk of transient ischemic attack with or without previous scar formation. In light of foregoing data, this could be the reason for increased abnormal ventricular repolarization parameters in CAD patients, such as that seen in

groups 2 and 3, than in the control group with normal coronary artery patients. In a study by Pascale et al.,[16] 252 patients who were admitted to the hospital due to ventricular arrhythmia linked to CAD were evaluated, in which acute MI was found to be the most frequent mechanism of arrhythmia. About half of the patients with an acute MI presented a previous MI scar without transient ischemia, whereas the other half presented transient ischemic areas. Patients with obstructive CAD without ACS had a relatively lower risk of arrhythmia than did patients with MI.[16] This supports our results of increased arrhythmia-related ECG findings in group 3 patients as compared to other group patients. Moreover, decreased EF represents the other facilitative factor of ventricular arrhythmia when in the presence of coronary atherosclerosis.[16] Our study also discovered that the EF was significantly lower in patients with ACS. This may well be one of the reasons for prolonged Tp-e intervals and increased Tp-e/QT and Tp-e/QTc ratios due to ischemia-related electrical heterogeneity on the myocardium. Unsurprisingly, these elongated variables could possibly be the predictor of ventricular arrhythmia. Our correlation analyses demonstrated a negative correlation between LVEF and Tp-e and Tp-e/QT ratios. This also supports the negative relationship between LV systolic function and ventricular dispersion of repolarization. In addition to these, patients with left ventricular systolic dysfunction in group 1 may be the reason of abnormal repolarization in the same group when compared to the normal population. Patients with ischemia evidence on exercise stress testing and normal coronary arteries (group 1) may also have impaired ventricular repolarization because of undetected microvascular dysfunction or coronary vasospasm.

According to these data, increased coronary atherosclerosis causes the impairment of left ventricular repolarization. This abnormal repolarization is seen as more distinct in patients with acute coronary events. The syntax score is that of a score showing coronary artery disease severity and is related to adverse cardiac events, such as cardiac arrhythmia. Increased

Tp-e interval and Tp-e/QT and Tp-e/QTc ratios are indicators of abnormal ventricular repolarization and can be related to CAD severity. In this same light, a strong correlation was found between a higher syntax score and an increased Tp-e interval and Tp-e/QT and Tp-e/QTc ratios.

Dyslipidemia is one of the major risk factors for cardiovascular atherosclerotic diseases. While elevated serum lipid levels are associated with coronary artery disease, they play key roles in atherosclerotic disease spectrum changes from a stable to an unstable disease, such as ACS. In a previous study, it was demonstrated that the presence of CAD and a poorer prognosis of the disease due to acute complication, such as an acute ischemic syndrome, proved to be related to high serum lipid levels.[17] The present study revealed that serum lipid levels, such as total cholesterol and triglyceride, were higher in patients with CAD, especially in ACS patients. Lower HDL levels were also demonstrated in patients with CAD, unveiling another risk factor of coronary atherosclerosis. Although hyperlipidemia did prove to be higher in patients with CAD, serum LDL cholesterol levels exhibited no difference between both groups. This could be explained by the treatment of patients with statin therapy.

Higher QT and QTc intervals are related to an increased risk of mortality due to the occurrence of the early ??? after depolarization. An abnormal depolarization, occurring during phases 2 and/or 3 of the action potential, makes ventricular myocardium sensitive to arrhythmia because of the development of functional reentry.[4,18] Ventricular arrhythmias linked to higher QT and QTc intervals commonly occur in the presence of obstructive coronary atherosclerosis, especially during acute ischemia. One prior study demonstrated that there was a strong positive association between a higher QTc interval and mortality in 3,837 patients with post-myocardial infarction.[19] Moreover, increased QTd, which indicates the dispersion of ventricular repolarization, is commonly related to serious arrhythmia and sudden death.[20] Several studies demonstrated that QTd was a more sensitive marker to detect ventricular arrhythmia, when compared to the QT interval.[21] While QTd tends to increase more in patients with acute MI than in a normal population,[22] Tikiz et al.,[23] revealed that the severity of the localized ischemia was more important than the extent of coronary atherosclerosis.[23] To support these findings, in our study, the QT interval, QTc interval, QTd, and QTcd proved to be increased in patients with obstructive coronary atherosclerosis, and this increase was more distinct in acute coronary syndrome patients. This result is due to the fact that acute ischemia causes ventricular electrical heterogeneity and instability, which often results in ventricular arrhythmia.

Increased dispersion of repolarization is more commonly associated with the heterogeneity of repolarization as compared to the total duration of

repolarization. This degenerated repolarization has proven to be associated with the increased risk of ventricular arrhythmia and sudden cardiac death in various cardiac disorders, especially in patients with CAD.[9-11,14] Coronary atherosclerosis-related clinical conditions are still the most important reasons for cardiac death worldwide. Myocardial ischemia-related ventricular arrhythmias, due to damaged repolarization, are commonly seen in the presence of obstructive CAD. The Tp-e interval, which reflects a transmural dispersion of ventricular repolarization, is related to the increased risk of ventricular arrhythmia and sudden cardiac death in CAD patients.[14] In one study, it was demonstrated that the Tp-e interval was independently associated with cardiac death in patients with obstructive coronary atherosclerosis, while the QTc interval remained normal.[24] The Tp-e interval also proved to be a strong predictor of mortality in STEMI and NSTEMI patients.[11] This can be explained by the increased risk of myocardial injury in the presence of ventricular ischemia, the risk of which is more explicit during acute coronary syndrome. The present study demonstrated that the prolongation of the Tp-e interval was related to CAD, and this prolongation was quite apparent in patients with ACS. This increase could be the predictor of mortality due to the increased risk of ventricular arrhythmia. Tp-e/QT and Tp-e/QTc ratios are novel markers that are more accurate predictors of the dispersion of ventricular repolarization and ventricular arrhythmias than the QT, QTc, and Tp-e intervals. These are also independent predictors of alterations in heart rate and a significant association with ventricular arrhythmia was demonstrated in many clinical conditions.[12] While the higher Tp-e/QT ratio was associated with CAD, in one study, it was demonstrated that the Tp-e/QT ratio was linked to malignant ventricular arrhythmia in patients with STEMI.[25] In the present study, Tp-e/QT and Tp-e/QTc ratios proved to be associated with the CAD spectrum as well as with QT, QTc, and Tp-e intervals. Unsurprisingly, a more distinct increase in ACS patients seemed to be correlated with the increased risk of ventricular arrhythmia and mortality. However, large-scale studies are needed for future investigations.

Study limitation

The main limitations of the study are the small sample size of the study and the lack of patient follow-up visits concerning ventricular arrhythmia with 24-hour rhythm monitoring. The other limitation was the lack of data about the information on the actual arrhythmic burden of those patients. Thus, any inference about the actual significance of the modest difference observed in the repolarization parameters as regards the differences in the arrhythmogenic risk among the three study groups remains uncertain. A heterogeneous population of the study was also another limitation, especially considering the fact that NSTEMI and STEMI were evaluated in the same group as ACS patients.

Conclusions

The presence of myocardial ischemia is one the most important reasons for abnormal ventricular repolarization, which can be reflected on a surface ECG by the Tp-e interval, as well as the Tp-e/QT, and Tp-e/QTc ratios. However, the relationship between the CAD spectrum and repolarization markers has not yet been fully investigated. Unsurprisingly, in the present study, a prolonged Tp-e interval and increased Tp-e/QT and Tp-e/QTc ratios were detected in the presence of CAD as well as in patients with unstable coronary atherosclerosis. These prolongations were seen more distinctly in patients with acute ischemic syndromes. A negative correlation was found between the LV systolic function and the dispersion of the ventricle's myocardium, and a strong and positive correlation was also identified between CAD severity and prolonged Tp-e interval and increased Tp-e/QT and Tp-e/QTc ratios.

Author contributions

Conception and design of the research: Kahraman S, Dogan A, Kurtoglu N, Erturk M. Acquisition of data: Demirci G, Guler A. Analysis and interpretation of the data; Kahraman S, Dogan A, Demirci G, Guler A, Kalkan AK, Uzun F, Kurtoglu N, Erturk M, Kalkan ME. Statistical analysis: Kahraman S, Dogan A. Writing of the manuscript: Kahraman S, Dogan A, Kalkan AK, Uzun F, Kurtoglu N, Erturk M, Kalkan ME. Critical revision of the manuscript for intellectual content: Kahraman S, Dogan A, Demirci G, Guler A, Kalkan AK, Uzun F, Kurtoglu N, Erturk M, Kalkan ME.

Potential Conflict of Interest

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

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Study Association

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Yeni Yüzyıl University under the protocol number 019/2018. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Executive summary: heart disease and stroke statistics—2016 update: a report from the American Heart Association. Circulation. 2016;133(4):447-54.
2. Wit AL, Janse MJ. Experimental models of ventricular tachycardia and fibrillation caused by ischemia and infarction. Circulation. 1992;85(1 Suppl):32–42.
3. Zhao X, Xie Z, Chu Y, Yang L, Xu W, Yang X, et al. Association between Tp-e/QT ratio and prognosis in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction. Clin Cardiol. 2012;35(9):559-64.
4. Zhang Y, Post WS, Blasco-Colmenares E, Dalal D, Tomaselli GF, Guallar E. Electrocardiographic QT interval and mortality: a meta-analysis. Epidemiology. 2011;22(5):660-70.
5. Beinart R, Zhang Y, Lima JA, Bluemke DA, Soliman EZ, Heckbert SR, et al. The QT interval is associated with incident cardiovascular events (the MESA study). J Am College Cardiol. 2014;64(20):2111–9.
6. Zareba W, Moss AJ, le Cessie S. Dispersion of ventricular repolarization and arrhythmic cardiac death in coronary artery disease. Am J Cardiol. 1994;74(6):550–3.
7. Kors JA, Ritsema van Eck HJ, van Herpen G. The meaning of the Tp-Te interval and its diagnostic value. J Electrocardiol. 2008;41(6):575-80.
8. Antzelevitch C, Sicouri S, Di Diego JM, Burashnikov A, Viskin S, Shimizu W, et al. Does Tpeak-Tend provide an index of transmural dispersion of repolarization? Heart Rhythm. 2007;4(8):1114-6.
9. Castro Hevia J, Antzelevitch C, Tornes Barzaga F, Dorantes Sanchez M, Dorticos Balea F, Zayas Molina R, et al. Tpeak-Tend and Tpeak-Tend dispersion as risk factors for ventricular tachycardia/ ventricular fibrillation in patients with the Brugada syndrome. J Am Coll Cardiol. 2006;47(9):1828-34.
10. Smetana P, Schmidt A, Zabel M, Hnatkova K, Franz M, Huber K, et al. Assessment of repolarization heterogeneity for prediction of mortality in cardiovascular disease: peak to the end of the T wave interval and nondipolar repolarization components. J Electrocardiol. 2011;44(3):301-8.
11. Erikssen G, Liestol K, Gullestad L, Haugaa KH, Bendz B, Amlie JP. The terminal part of the QT interval (T peak to T end): a predictor of mortality after acute myocardial infarction. Ann Noninvasive Electrocardiol. 2012;17(2):85-94.
12. Gupta P, Patel C, Patel H, Narayanaswamy S, Malhotra B, Green JT, et al. T(p-e)/QT ratio as an index of arrhythmogenesis. J Electrocardiol. 2008;41(6):567–74.
13. Goldenberg I, Moss AJ, Zareba W. QT interval: how to measure it and what is "normal". J Cardiovasc Electrophysiol. 2006;17(3):333-6.
14. Haarmark C, Hansen PR, Vedel-Larsen E, Pedersen SH, Graff C, Andersen MP, et al. The prognostic value of the Tpeak-Tend interval in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction. J Electrocardiol. 2009;42(6):555-60.
15. Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. Eur Heart J. 2013;34(38):2949-3003.
16. Pascale P, Schlaepfer J, Oddo M, Schaller MD, Vogt P, Fromer M. Ventricular arrhythmia in coronary artery disease: limits of a risk stratification strategy on the ejection fraction alone and impact on infarct localization. Europace. 2009;11(12):1639-46.
17. Pekkanen J, Linn S, Heiss G, Suchindran CM, Leon A, Rifkind BM, et al. Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. N Eng J Med. 1990;322(24):1700-7.
18. Antzelevitch C, Shimizu W, Yan GX, Sicouri S. Cellular basis for QT dispersion. J Electrocardiol. 1998;30(Suppl):168-75.
19. Peters RW, Byington RP, Barker A, Yusuf S. Prognostic value of prolonged ventricular repolarization following myocardial infarction: the BHAT experience. J Clin Epidemiol. 1990;43(2):167–72.
20. Dilaveris PE, Gialafos EJ, Sideris SK, Theopistou AM, Andrikopoulos GK, Kyriakidis M, et al. Simple electrocardiographic markers for the prediction of paroxysmal idiopathic atrial fibrillation. Am Heart J. 1998;135(5 Pt 1):733-8.
21. Kautzner J, Malik M. QT interval dispersion and its clinical utility. Pacing Clin Electrophysiol. 1997;20(10 Pt 2):2625-40.
22. Glancy JM, Garrat CJ, de Bono DP. Dynamics of QT dispersion during myocardial infarction and ischemia. Int J Cardiol. 1996;57(1):55-60.
23. Tikiz H, Terzi T, Balbay Y, Demir AD, Soylu M, Keles T, et al. QT dispersion in single coronary artery disease: is there a relation between QT dispersion and diseased coronary artery or lesion localization? Angiology. 2001;52(1):43–51.
24. Panikkath R, Reinier K, Uy-Evanado A, Teodorescu C, Hattenhauer J, Mariani R, et al. Prolonged Tpeak-Tend interval on the resting ECG is associated with increased risk of sudden cardiac death. Circ Arrhythm Electrophysiol. 2011;4(4):441–7.
25. Shu J, Li H, Yan G, Cui C. Tp-e/QT ratio as a predictive index of sudden cardiac death in patients with ST-segment elevation myocardial infarction. J Xi_an Jiaotong Univ Med Sci. 2010;31:441-3.

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ORIGINAL ARTICLE

The Effect of Psychotherapy on Anxiety, Depression, and Quality of Life of Patients with Heart Failure: A Randomized Clinical Trial

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Abstract

Background: Patients with heart failure often experience depression, anxiety, and impacts on quality of life. Psychotherapeutic interventions have been used for chronic conditions, including cardiovascular diseases, showing improvement in mental health. However, no studies have assessed the effects of a psychological intervention using psychoanalysis on patients with heart failure.

Objective: To assess the effect of short-term psychotherapy on depressive symptoms, anxiety, and quality of life of patients seen at a specialty clinic.

Method: A randomized clinical trial with scales to assess outcomes before and after psychotherapy, under the perspective of psychoanalysis, with 12 weekly sessions. The Beck Depression Inventory, the Beck Anxiety Inventory, and the Minnesota Living with Heart Failure Questionnaire were used. The mean initial and final scores for quality of life, anxiety, and depression were compared using Student's t-test for independent samples when distribution was normal, or Mann-Whitney test when distribution was non-normal. A bivariate p-value < 0.05 was considered statistically significant for all analyses.

Results: This study involved 32 patients, 50% were female, mean age was 64.3±11.6, and most participants were New York Heart Association (NYHA) functional class I (56.3%). For anxiety (p = 0.196), there was no statistically significant difference between groups. For quality of life and depression, there was a statistical difference (p = 0.009 and 0.035, respectively), with a medium effect (Cohen's d = 0.593) on quality of life.

Conclusion: Short-term psychotherapy in outpatients with heart failure showed an impact on depression and quality of life but did not improve anxiety. (Int J Cardiovasc Sci. 2021; 34(2):188-196)

Keywords: Heart Failure; Anxiety; Depression; Quality of Life; Psychotherapy; Mental Health; Psychoanalytic Therapy.

Introduction

According to the World Health Organization,[1] billions of people will die worldwide from chronic noncommunicable diseases, especially cardiovascular conditions. Patients with heart disease are an object of interest in the field of mental health because of the impacts on the subjects' lives; therefore, mind and heart are closely interconnected.[2]

Heart failure (HF) is a complex and progressive clinical syndrome[3] in which the heart is unable to pump efficiently to supply the body's needs, seeking compensatory hemodynamic pathways. Thus, common symptoms are dyspnea, fatigue, tiredness, exercise intolerance, weight gain, loss of appetite, nocturia, and oliguria.[4] The main risk factors for hypertension are hypercholesterolemia, coronary artery disease, diabetes mellitus, smoking, and obesity.[5]

HF is little recognized both by the population and the political power, and affects 1% to 2% of the population worldwide, with an estimated growth of 25% until 2030.[6]

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In the United States and Europe, it is the leading cause of hospitalization for older individuals, with a very significant socioeconomic impact, being accountable for 68% of hospitalization expenses.[6] In Brazil, cardiovascular diseases are the main cause of death, affecting 20% of people over 30 years old.[7] It is noteworthy that 50% of all hospitalized patients are readmitted within 90 days, which is one of the main risk factors for death.[8]

I Brazilian Registry of Heart Failure (BREATHE)[8] described high in-hospital mortality related to low rates of evidence-based therapy. Poor adherence to treatment is one of the main factors for decompensation. Moreover, depression is prevalent in hospitalized patients, with an average ranging from 15% to 36%.[4] This association makes adherence to treatment more difficult, increases hospitalizations, and elevates morbidity and mortality.[9] The coexistence between depressive and anxiety symptoms directly impacts quality of life.[10] Thus, it is essential that health professionals detect and treat such conditions early, in addition to developing new studies to define effective interventions.[11]

In this regard, psychologists identify important obstacles, such as difficulty in understanding and accepting the disease and treatment, defense mechanisms, impasses or family conflicts, psychopathological conditions, among others. This reality was observed during psychological consultations at a specialty clinic in Niterói, Rio de Janeiro, Brazil, especially concerning the patients' physical, psychological, and social fragility, with reports of personal and family issues, depressive symptoms, anxiety, and impaired quality of life.

To our knowledge, no studies have assessed the effects of short-term psychotherapy on depression, anxiety, and quality of life of patients with HF. Therefore, this is a pioneer study that aims to assess the effects of short-term psychotherapy, under the perspective of psychoanalysis, on depression, anxiety, and quality of life of outpatients with HF.

Method

Study design

A randomized clinical trial (RCT) was conducted with two distinct parallel groups. This RCT aimed to test a psychological intervention consisting of short-term psychoanalytic listening in patients with HF seen at a specialty clinic.

Participants

Patients over 18 years old and classified as any New York Heart Association (NYHA) functional class were included. Psychotherapy patients who started using psychotropic drugs during intervention with severe neurological sequelae or severe psychiatric disorders were excluded. Participants were invited, by telephone or personal contact, to participate in the study at a specialty HF clinic, where all data were collected.

Intervention

Sociodemographic data were collected, informed consent was obtained, and then the scales were administered. Control group (CG) patients followed routine clinic visits, without psychotherapy; meanwhile, intervention group (IG) patients were scheduled to start psychotherapy with the study psychologist. The psychologist introduced participants to a short-term psychological follow-up proposal based on a psychoanalytical approach aiming to improve depressive and anxiety symptoms and, consequently, their quality of life. This semistructured, flexible treatment plan tailored to each patient's personal demands (Treatment Plan - Annex 1) could involve, whenever possible, family members. Altogether, 12 weekly sessions were held.

Each session lasted 45 minutes, with duration monitored by a watch. The environment was maintained at a pleasant temperature (average of 23ºC), bright, with little outside noise, and included a table and two chairs (or three when there was a family member). Statements were not recorded. Session notes were taken later by the researcher.

The patients' statements showed the degree of understanding of the disease, adherence to treatment, social and family support, psychiatric history, and issues highlighted by them. The follow-up sessions addressed topics such as quality of life, lifestyle, personal, professional, religious, social, and family choices , sexuality, coping with the disease, and life itself. Patients were encouraged to talk about themselves and about aspects related to the disease, in addition to being encouraged to be assertive, to perform self-care, and to improve quality of life. Psychoanalysis, as a theoretical framework, was based on free association considering unconscious contents, insights, defense mechanisms, bonds, traumas, among other concepts, all considered in the proposed treatment plan.

Outcomes

To assess anxiety, depression, and quality of life, the Beck Anxiety Inventory (BAI), the Beck Depression Inventory (BDI), and the Minnesota Living with Heart Failure Questionnaire (MLHFQ) were used, respectively.[12-16]

Sample size

The sample size was calculated with WinPepi 11.46, based on the sample calculation of two studies that used depression as an outcome based on BDI. The first study[17] was conducted by a research group named "Heart failure: from the molecule to the population," which includes the main researcher and served as a pilot for this study because of the same patient profile. Mean score was 31.5±3.53 before intervention and 50.5±13.4 after intervention. The second study[18] found the following: before intervention, scores were 29.6±10.2 for CG and 30.7±10.2 for IG; after intervention, scores were 16.0±10.6 for CG and 11.2±10.7 for IG. We considered a sample of 16 patients in each group, with a difference of 17 points, 20% of losses, a significance level of 5%, and a power of 80%.

Randomization

Randomization was performed using a simple sequence, at www.randomization.com, considering 20% of losses from an initial list with the names of patients who met the inclusion criteria.

Statistical methods

Data were organized by preparing and synthesizing a database with Microsoft Excel 2010. The data collected from the study instruments were tabulated and analyzed by SPSS, version 20.0. Categorical variables were expressed by frequency and percentage distributions and compared between groups using chi-square test and Fisher's exact test.

Continuous variables were expressed by mean and standard deviation or median and interquartile range according to the behavior of variables (normality) as identified by Kolmogorov-Smirnov test. Comparison of continuous variables between groups was performed by Student's t-test for independent samples or Mann-Whitney test.

Mean initial and final scores for quality of life, anxiety, and depression were also compared with Student's t-test for independent samples when distribution was normal, or Mann-Whitney test when distribution was non-normal. A bivariate p-value < 0.05 was considered statistically significant for all analyses.

The effect size was measured, which made it possible to add information to the concept of statistical significance. The effect size was calculated using Cohen's d.

Ethical aspects

This study was approved by the Research Ethics Committee of *Hospital Universitário Antônio Pedro* (REC/HUAP), under Certificate of Presentation for Ethical Consideration (CAAE - *Certificado de Apresentação para Apreciação Ética*) no. 57827916.3.0000.5243.

All participants were informed about the objectives, risks, and benefits of the study, and their doubts were clarified. Then they completed the informed consent form and received a copy, as provided in Resolution no. 466/2012 of the Brazilian National Health Council (*Conselho Nacional de Saúde*).[19] This study ensured the confidentiality and privacy of the data collected from all participants.

Results

Figure 1 represents the flowchart of selection of participants since recruitment, as recommended by the Consolidated Standards of Reporting Trials (CONSORT) 2010.[20]

Table 1 shows sociodemographic and clinical characteristics of the 32 participants, which included 16 women (50%). There was no statistically significant difference when comparing the groups ($p > 0.05$).

Table 2 shows the results of the initial interview regarding patients' self-perception, the areas they considered most affected by HF, their expectations, and their role in treatment.

Table 3 shows a comparison between the initial and final scores of IG and CG. There was a statistically significant difference between the groups in terms of quality of life and depression before and after intervention ($p < 0.05$). Psychotherapy proved to have a positive effect on these outcomes. Cohen's d[21] was used for the outcome quality of life (0.593), which refers to the mean effect of the intervention performed. It was not possible to calculate the effect of the outcome depression; this calculation is only possible using mean and standard deviation, but depression was calculated using median.

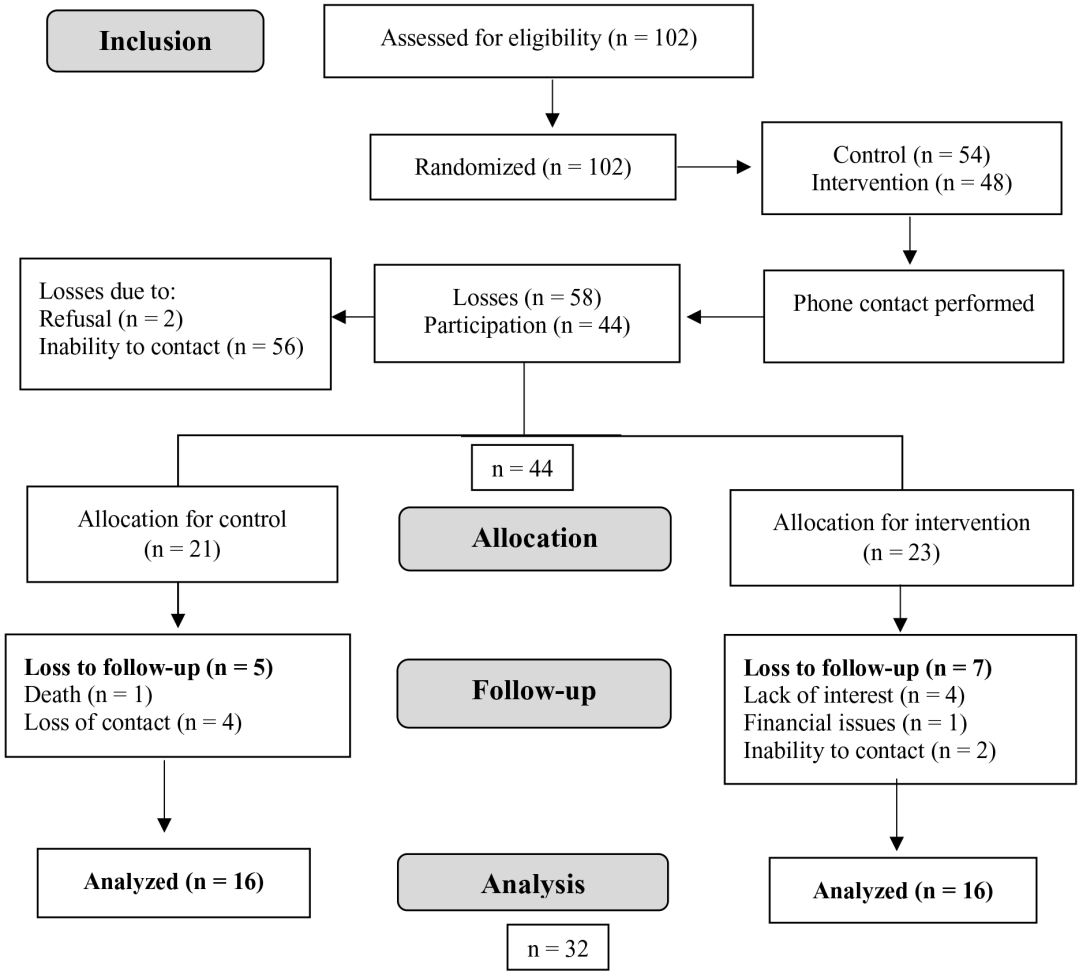

Figure 1 – Flowchart of selection of participants. Niterói, RJ, Brazil, 2019.
Source: created by the author based on CONSORT 2010.[20].

Discussion

This study tested a short-term psychological intervention under the perspective of psychoanalysis and assessed its effect on the symptoms of anxiety, depression, and quality of life of patients with HF seen at a specialty clinic. The results demonstrated a positive effect of the proposed intervention for quality of life (p = 0.009; Cohen's d = 0.593) and depression (p = 0.035) in patients with HF.

No study was found in the scientific literature assessing the effects of psychotherapy under the perspective of psychoanalysis in relation to depression, anxiety, and quality of life of patients with HF. Morais et al.,[10] examined and correlated symptoms of depression and anxiety with quality of life in HF, not involving any type of intervention. They concluded that depression was the variable that had the strongest correlation with quality of life. Furthermore, they highlighted the lack of studies assessing outpatients. Quality of life was moderately affected, especially in the physical dimension, which is consistent with the present study. Therefore, the innovative feature of this study, which consisted of a short-term psychological intervention conducted at a clinic that specializes in HF, was evidenced, and the results demonstrated its positive effect on depression and quality of life of patients.

Although there was no statistical significance between groups in the results for anxiety, a slight improvement in the severity of these symptoms was observed in IG. In other words, patients with moderate and severe anxiety scores had them reduced to mild, minimal, or moderate. Lundgren

Table 1- Sociodemographic and clinical characteristics of the trial sample (n = 32). Niterói, RJ, Brazil, 2019

Characteristics	Control (n = 16)	Intervention (n = 16)	Total (n = 32)	p-value
Age, Years†	63.3±12.7	65.3±10.6	64.3±11.6	0.644§
Sex, Male*	8 (50.0)	8 (50.0)	16 (50.0)	0.638‡
Education, Elementary school*	13 (81.3)	14 (87.5)	27 (84.4)	0.499‡
Marital status, Married*	6 (37.5)	7 (43.8)	13 (40.6)	0.869‡
Occupation, Retired*	5 (31.3)	8 (50.0)	13 (40.6)	0.085‡
Hometown, São Gonçalo*	7 (43.8)	10 (62.5)	17 (53.1)	0.581‡
Income, US$200 - US$500*	7 (43.8)	7 (43.8)	14 (43.8)	0.817‡
Living alone, No*	11 (68.8)	14 (87.5)	25 (43.8)	0.197‡
Caregiver, No*	11 (68.8)	12 (75.0)	23 (71.9)	0.500‡
Hospitalization in the past year, No*	12 (75.0)	12 (75.0)	24 (75.0)	0.657‡
History of psychotropic drug use, No*	13 (81.3)	11 (68.8)	24 (75.0)	0.240‡
Children, Yes*	14 (87.5)	14 (87.5)	28 (87.5)	0.700‡
Functional class, NYHA I*	6 (37.5)	12 (75.0)	18 (56.3)	0.100‡
Arterial hypertension, Yes*	13 (81.3)	12 (75.0)	25 (78.1)	0.500‡
Diabetes mellitus, Yes*	4 (25.0)	5 (31.3)	9 (28.1)	0.500‡
Smoking, No*	13 (81.3)	13 (81.3)	26 (81.3)	0.673‡
Alcoholism, No*	14 (87.5)	14 (87.5)	28 (87.5)	0.700‡
Independence, Yes*	10 (62.5)	12 (75.0)	22 (68.8)	0.720‡

**n (%); †mean ± standard deviation; §Student's t-test; ‡chi-square test.*
NYHA: New York Heart Association functional class.

et al.,[22] also concluded in their study of 64 participants that there was no difference in intervention between groups from the internet-based cognitive behavioral therapy (CBT) for depression. However, they found an intragroup difference in this outcome, describing an improvement in symptoms.

Depressive symptoms can be confused with HF symptoms such as fatigue, changes in sleep, and appetite. Also, periods of sadness do not necessarily represent a depressive condition. For this reason, medical assessment with proper use of screening instruments is essential.[23] It is worth mentioning that this patient profile tends to have experienced several losses intrinsic to their discourse, including health, profession, role in the family, in society, and in the marriage, leisure, and self-esteem. Within this context of vulnerability, patients face frequent and evolutionary limitations that may generate sadness or even depression.

Studies have shown that depression increases the number of readmissions,[9] as self-care is greatly impaired. Clinical outcomes are impacted by this comorbidity. A survey[24] showed that depressive symptoms and social isolation influenced the prognosis and survival (75% in up to 1 year) of patients waitlisted for heart transplant, and individuals without this condition had a survival of up to 8 years after transplant. The authors concluded on the importance of well-defined screening and intervention in patients at risk.

The Brazilian Guideline on Heart Failure[3] and other studies emphasize the importance of psychological monitoring to patients with HF, especially in relation to depression. However, there are still few studies, especially RCTs, on psychotherapy in HF. Some research using CBT is described in the scientific literature, but with limitations in terms of sample, intervention time, and risk of bias.[25] Studies on cardiology and psychoanalysis were found in the literature, but not specific studies using the psychoanalytic approach in an HF outpatient setting. Therefore, this is a pioneer study using this approach in this patient profile with these outcomes.

Table 2 - Affected areas, self-perception, expectations, and role in treatment (n = 32). Niterói, RJ, Brazil, 2019

Variables	Control (n = 16)	Intervention (n = 16)	p-value
Affected areas			
Emotions‖	6 (37.5)	6 (37.5)	0.642
Marriage†	2 (12.5)	3 (18.8)	0.500
Family‖	4 (25.0)	6 (37.5)	0.352
Work‖	5 (31.3)	6 (37.5)	0.500
Friends†	3 (18.8)	3 (18.8)	0.673
Religion†	0	2 (12.5)	0.242
None†	2 (12.5)	2 (12.5)	0.700
Self-perception‖			0.548
Healthy	4 (25.0)	6 (37.5)	
Sick without significant limitations	4 (25.0)	5 (31.3)	
Sick with significant limitations	8 (50.0)	5 (31.3)	
Expectations†			0.584
Healing	6 (37.5)	7 (43.8)	
Self-care and better quality of life	9 (56.3)	9 (56.3)	
None	1 (6.3)	0	
Role in treatment†			0.500
Engaged and following all guidelines	13 (81.3)	12 (75.0)	
Somewhat engaged and not always following all guidelines	3 (18.8)	4 (25.0)	
Never following all guidelines	0	0	

†Fisher's exact test; ‖chi-square test.

Table 3 - Comparison between the control group and intervention group scores (n = 32). Niterói, RJ, Brazil, 2019

Scores	Control Initial (n = 16)	Intervention Initial (n = 16)	p-value Initial	Control Final (n = 16)	Intervention Final (n = 16)	p-value Final	Cohen's d
Anxiety†	8.5 (4.00-26.50)	13.5 (3.75-20.50)	0.956	11.00 (5.00-23.00)	8.00 (3.00-14.00)	0.196	-
Depression†	19.5 (9.5-29.00)	12.5 (6.5-19.25)	0.102	11.50 (5-23.75)	8.25 (2.25-17.00)	0.035	-
Quality of life‖	46.5±24.0	37.4±22.4	0.276	48.6±27.8	25.3±18.3	0.009	0.593

†Mann-Whitney test; ‖Student's t-test.

Physical and emotional symptoms have significant impacts on quality of life.[10] A Brazilian study that used the same outcomes and the same scales concluded that quality of life was moderately affected, with the physical dimension being the most impacted,[10] as shown in the present study.

Another study[26] described the effects of a repetitive monitoring and education program on quality of life of outpatients with HF. The results were positive both for the total scores and for each dimension. The long-term program included a multidisciplinary team (nurses, psychologists, nutritionists, social workers, and pharmacists) and

supported the results, despite differences in intervention duration and technique.

Bordoni et al.,[27] described anxiety as a neglected symptom, which increases hospitalization rates, and a gap in the scientific literature. It is often related to a high expectation or even to worry or fear, commonly described by patients with HF. Lack of social and/or family support and situations of socioeconomic vulnerability may aggravate these symptoms. Studies have described the relationship of this condition with the absence of social support and inability to deal with stressful situations.[26] Such studies suggested that stress is a risk factor for acute myocardial infarction and recommended interventions focusing on prevention. Generalized anxiety disorder, for instance, may increase the risk of cardiovascular events, therefore being predictive of hospital complications and deaths.[11]

Mourning experiences were described by participants and reported by Knebel & Marin[28] in their study, which assessed psychosocial factors and their psychological management[27]. Losing beloved ones was related to symptoms of depression and anxiety, in addition to loss of vitality, productivity, and sexuality.

A small sample (n = 32), only one professional to work with all participants, and irregular attendance by some of them (financial difficulties, personal and/or medical appointments, discouragement, health problems, etc.) were some of the limitations of this study. Another limitation was the extreme difficulty in contacting patients, as despite seeking constant updating, the team found incorrect or unavailable phone numbers. Using registered letters was an ineffective alternative to contact patients.

Investigating patients with HF promoted reflections and considerations for future studies, especially those using a qualitative approach. The patients' statements portrayed their vulnerability, as well as individual and collective physical and psychological suffering. Thus, future investigations should describe and analyze the content of the participants' complaints; the helpless state of patients with HF; mourning; the experience of the study psychologist at the specialty clinic; and patients' perception of the psychotherapeutic process.

We hope that the experience described in this paper can be used in other HF outpatient settings. Short-term psychotherapy using psychoanalytic listening proved to be effective in improving quality of life and depression. The semistructured, flexible treatment plan made it possible to approach patients, who were able to express their anxieties, fears, and concerns, as well to devise better ways to face illness and their personal issues. An alternative method for a high number of users is group therapy, which was not tested this time but is a future research opportunity. Moreover, other researchers are encouraged to expand this study by investigating the benefits of this process to hospital readmissions and assessing gratitude and resilience in this patient profile.

Conclusion

This study tested a short-term psychological intervention using the perspective of psychoanalysis and assessed its effect on anxiety, depression, and quality of life of patients with HF seen at a specialty clinic. The results demonstrated a positive effect of the proposed intervention on quality of life and depression in this patient profile.

Author contributions

Conception and design of the research, Writing of the manuscript, Analysis and interpretation of the data:Rocha, ICAO. Acquisition of data: Figueiredo, LS, Cruz, DCS, Freitas, RVM, Oliveira, SX. Critical revision of the manuscript for intellectual content, Supervision / as the major investigador: Cavalcanti, ACD, Mesquita, ET. Analysis and interpretation of the data: Pereira, JMV.

Potential Conflict of Interest

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

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Study Association

This article is part of the thesis of master submitted by Isaura Cristina Azambuja de Oliveira Rocha, from *Universidade Federal Fluminense*.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the *Hospital Universitário Antônio Pedro* (CEP/HUAP) under the protocol number CAAE: 57827916.3.0000.5243. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

References

1. World Health Organization. (WHO). Time to deliver: report of the WHO independent high-level commission on noncommunicable diseases. Geneva: 2018.
2. Junqueira L, Pina M. A psicologia cardíaca e a intervenção mente-coração: novos paradigmas para a prevenção das enfermidades cardíacas e o tratamento psicológico de pacientes cardíacos. Psychiatry on line Brasil. 2017 Nov;22. [Acesso 22 jun 2019]. Disponível em: https://www.polbr.med.br/ano17/art0317.php.
3. Rohde LE, Montera MW, Bocchi EA, Clausell N, Albuquerque DC, Rassi S. Comitê Coordenador da Diretriz de Insuficiência Cardíaca. Diretriz Brasileira de Insuficiência Cardíaca Crônica e Aguda. Arq Bras Cardiol. 2018;111(3):436-539.
4. Bocchi EA, Braga FGM, Ferreira SMA, Rohde LEP, Oliveira WA, Almeida DR, et al. III Diretriz Brasileira de Insuficiência Cardíaca Crônica. Arq Bras Cardiol. 2009;93(1 supl.1):1-71.
5. Fonseca C, Brás D, Araújo I, Ceia F. Heart failure in numbers: Estimates for the 21st century in Portugal. Rev Port Cardiol. 2018;37(2):97-104.
6. Fonseca C, Brito D, Cernadas R, Ferreira J, Franco F, Rodrigues T, et al. For the improvement of Heart Failure treatment in Portugal - Consensus statement. Rev Port Cardiol. 2017;36(1):1-8.
7. Mansur AP, Favarato D. Trends in mortality rate from cardiovascular disease in Brazil, 1980-2012. Arq Bras Cardiol. 2016;107(1):20-5.
8. Albuquerque D, Souza Neto JD, Bacal F, Rohde LEP, Bernardez-Pereira S, Berwanger O, et al. I Brazilian registry of heart failure - clinical aspects, care quality and hospitalization outcomes. Arq Bras Cardiol. 2015;104(6):433-42.
9. Karataş MB, Şahan E, Özcan KS, Çanga Y, Güngör B, Onuk T, et al. Anxiety, depression, and general psychological distress in patients with coronary slow flow. Arq Bras Cardiol. 2015;105(4):362-70.
10. Morais ER, Carvalho CS, Euqueres L, Viana FP, Fantinati AMM, Rassi S. Quality of life and symptoms of depression and anxiety in patients with chronic heart failure. Estudos Vida Saúde. 2018;45:71-9.
11. Dekker RL, Lennie TA, Doering LV, Chung ML, Wu JR, Moser DK. Coexisting anxiety and depressive in patients with heart failure. Eur J Cardiovasc Nurs. 2015;3(2):168-76.
12. Cunha JA. Psicodiagnóstico-V. 5. ed. Porto Alegre: Artmed. 2007; p.237-41.
13. Goreinstein C, Andrade L. Validation of a portuguese version of the Beck depression inventory and the state trait anxiety inventory in brazilian subjects. Braz J Med Biol Res. 1996;29(4):453-7.
14. Rector TS, Kubo S, Cohn J. Patient's self-assessment of their congestive heart failure. Part 2: content, reliability and validity of a new measure, The Minnesota living with heart failure questionnaire. Heart Fail. 1987;198-219.
15. Carvalho VO, Guimarães GV, Carrara D, Bacal F, Bocchi EA. Validation of the portuguese version of the Minnesota living with heart failure questionnaire. Arq Bras Cardiol. 2009;93(1):39-44.
16. Santos JJ, Plewka JE, Brofman PR. Quality of life and clinical indicators in heart failure: a multivariate analysis. Arq Bras Cardiol. 2009;93(2):159-66.
17. Guerra TRB. Impacto prognóstico dos biomarcadores serotonina e fator diferenciador neutrófilo cerebral (BDNF) em pacientes com insuficiência cardíaca sistólica [tese]. Niterói: Universidade Federal Fluminense; 2014.
18. Freedland KE, Carney RM, Rich MW, Steinmeyer BC, Rubin EH. Cognitive behavior therapy for depression and self-care in heart failure patients: a randomized clinical trial. JAMA Intern Med. 2015;175(11):1773-82.
19. Brasil. Resolução nº 466, de 12 de dezembro de 2012. Aprova normas regulamentadoras de pesquisas envolvendo seres humanos. Diário Oficial da União 13 jun 2013; Seção1: 59.
20. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ. 2010 Mar 23;340:c332.
21. Espírito Santo H, Daniel F. Calculating and reporting effect sizes on scientific papers (1): $p < 0.05$ limitations in the analysis of mean differences of two groups. Port J Behav Social Res. 2015;1(1):3-16.
22. Lundgren JG, Dahlström O, Andersson G, Jaarsma T, Köhler AK, Johansson P. The effect of guided web-based cognitive behavioral therapy on patients with depressive symptoms and heart failure: a pilot randomized controlled trial. J Med Internet Res. 2016;18(8):e194.
23. Cameron OG, Ehrmann D, Pitt B. Depression, anxiety, anger and heart failure. In: Riba M, Wulsin L, Rubenfire M. Psychiatry and heart disease: the mind, brain and heart. New Jersey: John Wiley & Sons. 2012; p.34-8.
24. Spaderna H, Zittermann A, Reichenspurner H, Ziegler C, Smits J, Weidner G. Role of depression and social isolation at time of waitlisting for survival 8 years after heart transplantation. J Am Heart Assoc. 2017;6(12):1-11.
25. Jeyanantham K, Kotecha D, Thanki D, Dekker R, Lane DA. Effects of cognitive behavioural therapy for depression in heart failure patients: a systematic review and meta-analysis. Heart Fail Rev. 2017;22(6):731-41.
26. Cruz FD. A avaliação dos componentes da qualidade de vida na insuficiência cardíaca. [tese]. São Paulo: Universidade de São Paulo; 2010.
27. Bordoni B, Marelli F, Morabito B, Sacconi B. Depression and anxiety in patients with chronic heart failure. Future Cardiol. 2018;14(2):115-9.
28. Knebel IL, Marin AH. Psychosocial factors associated with heart disease and clinical psychological management: perception of psychologists and patients. Rev SBPH. 2018;21(1):1-20.

Treatment Plan		
Sessions	**Description of activities**	**Aspects addressed and assessed by the psychologist (concepts of psychology and psychoanalysis)**
1st	- Explain the psychotherapeutic process thoroughly, stating the importance of following the treatment plan. - Identify together with patients three priority aspects for treatment.	- Initial demands - Diagnosis = traumatic event (intense event that individuals are unable to react properly and that causes pathogenic effects on psychic organization). - Representations of the disease and fantasies (subjects represent, somewhat distorted by defensive processes, the fulfillment of a wish) - Assessment of bonds (affective bonds) and affection - Psychoeducation - Psychic ambivalences and conflicts - Defense mechanisms (aims to reduce or eliminate any danger to the biopsychological individual) - Conscious vs. unconscious - Free association method (connection between two or more psychic elements spontaneously) - Insights
2nd to 8th	- Address issues related to the disease, self-care, personal needs, life expectations, personal and family history, self-perception, bonds, beliefs, religiousness, etc. - Clarify patients' doubts with the help of the multidisciplinary team, if necessary. - Invite family members, if possible, for assistance and guidance, especially on how to help/facilitate/encourage patients to have better adherence to treatment and better coping with the disease. - Assess the possibility of adjustments in lifestyle as well as encourage changes in habits, offering support and outlining coping strategies (including social, family, and religious support, social, leisure, and/or physical activities, public spaces, specialty clinics, etc.). - Discuss the progress of the process with patients and make adjustments, if necessary. - Discuss the possibility of changing the priority aspects of treatment. - Provide feedback to patients. - Address changes in personal choices. - Address the impacts and limitations of the disease and think of possibilities or alternatives for everyday life, be it work, social interaction, or family environment.	
9th	- Address treatment closure and aspects related to the termination of this bond, as well as highlight the gains obtained during therapy.	
10th and 11th	- Address some possible issues.	
12th	- End the process, reinforcing all topics covered, and hand over the discharge guidance sheet. - Deliver referral to patients who need to continue treatment. - Administer a final questionnaire. - New administration of the Beck Anxiety/Depression Inventory (BAI and BDI) and the Minnesota Living with Heart Failure Questionnaire (MLHFQ) by a psychologist or a nursing student.	

Annex 1 – *Semistructured treatment plan*
Source: created by the author. Niterói, RJ, Brazil, 2019.

REVIEW ARTICLE

Nutrition and Cardiovascular Diseases: Programming and Reprogramming

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Abstract

The Developmental Origin of Health and Disease (DOHaD) is an area of science dedicated to studying the processes by which insults during critical periods of mammals development leading to physiological changes resultig in diseases throughout life. Studies point to a complex interaction between nutritional status in early life and cardiovascular system homeostasis in which maternal malnutrition during gestation and/or lactation, as well as early weaning, are associated with development of cardiovascular diseases in adulthood. In this context, epigenetic changes, such as DNA methylation, histone acetylation, and change in microRNA expression have been considered molecular bases of cellular plasticity, which can also be gender-dependent. Experimental studies have demonstrated that interventions encompassing the consumption of functional food/bioactive compounds, as well as energetic and nutrients adjustments on the diet, may attenuate or even prevent consequences associated with plasticity of development, improving cardiovascular health. This review aimed to gather and discuss the findings within this context, published over the last ten years.

Introduction

Cardiovascular diseases (CVDs) are the main cause of death worldwide and belongs to the group of chronic non-communicable diseases (NCDs). According to the World Health Organization (WHO), approximately 17.9 million people died of CVD in 2016, nearly 31% of the total number of deaths in the world.[1] In Brazil, where the scenario is similar, these diseases are responsible for the highest mortality rates in the same year.[2] This health public problem results in high costs with treatments and hospitalizations by Brazilian Unified Health System (SUS in Portuguese), in addition to indirect economic impact caused by reduction on productivity and sick absence.[3]

For years, it was believed that CVDs were determined by genetic factors and lifestyle. However, evidences has shown that, in the majority of cases, CVDs in adulthood may be related to the process of cellular plasticity due to nutritional insults during pregnancy and/or lactation.[4] Experimental studies have proven that mother's nutritional status during the critical periods of development, such as gestation and lactation, as well as early weaning, may favor NCDs programming throughout life.[5] These studies encompass a new area of science called *Developmental Origin of Health and Disease* (DOHaD) introduced by David Barker, which investigate the programming of diseases with fetal origins.[5-7]

Currently, it is well-known that the programming of the cardiovascular system can also occur directly in critical stages of life.[4] Recent studies have illustrated that the differentiation and proliferation of cardiac cells are not entirely complete at birth, continuing in the immediate post-natal period. DNA synthesis of these cells still takes place during the first two weeks of life in rodents and, in human beings, in the first 20 years, making the mammals' hearts vulnerable to insults during intrauterine development as well as during lactation period.[8, 9] Moreover, embryonic and immediate post-natal development periods are stages of life characterized by rapid growth and organs and

Keywords

Cardiovascular Diseases; Nutrition; Pregnancy; Lactation; Functional Food; Epigenomics.

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systems maturation. Thus, the lack or excess of nutrients during these periods favors adaptations that look for guarantee of survival in an adverse environment.[10] The direct impact upon gene expression, stimulating or inhibiting factors that control the cell cycle, mitosis rates, maturity, and cell death alter homeostasis, characterizing the adaptive process of many species.[7] In this context, epigenetic changes, such as DNA methylation, histone acetylation, and change in the microRNA expression have been considered molecular bases of cell plasticity.[11]

Diets that are inadequate in macro and micronutrients have been identified as an important stimulator of epigenetic processes, especially DNA methylation.[12] Studies in animal models have demonstrated that maternal malnutrition during the critical periods of development affects the DNA methylation of specific genes, changing the phenotype of the offspring and contributing to the programming of diseases, including cardiometabolic diseases.[7,13] By contrast, nutritional interventions in these same periods could favor the reprogramming of diseases through similar mechanisms.[6,14] interventions with bioactive compounds from functional foods may attenuate or even reprogram the physiological changes related to cell plasticity (Figure 1).[15]

Although evidence shows that the sex is an important variable, for various reasons this variable is ignored in many studies conducted primarily with individuals of the male sex. The literature points out differences in gender, regarding the progression, response, and treatment of CVDs, in such a way that studies conducted only with men can lead to incorrect diagnoses and inappropriate therapies.[16] Thus, it is important to consider that disease development may be influenced by sex, as well as be passed down through generations.[18] This review aimed to gather the findings about programming and reprogramming of CVDs associated with malnutrition during gestation and/or lactation, as well as about interventions with functional foods/bioactive compounds, addressing whenever possible, sexual dimorfism and physiologic changes passed down through generations. Since epidemiological studies often fail to answer many questions related to developmental plasticity, this review focused in rodents studies.

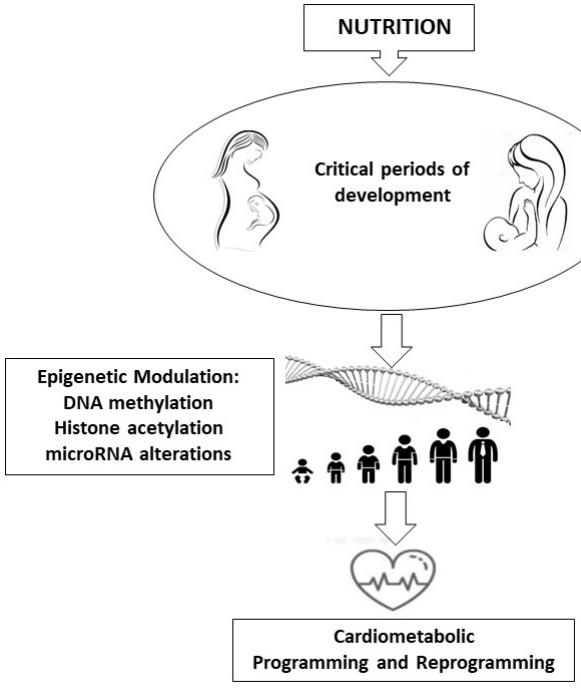

Figure 1 – Schematic representation of cause-and-effect relationship associated with cardiometabolic programming/reprogramming due to nutrition during pregnancy and lactation (Ordovás & Smith, 2010; Mathias et al., 2014).

Methods

To conduct this review, a bibliography study was carried out in January 2020 by 05 (five) separate researchers, with a subsequent group discussion. The studies were selected by accessing the Pubmed/MEDLINE and Scielo databases. The terms used in this research sought to gather studies involving cardiometabolic programming/development plasticity, including different animal models (rodents) of maternal malnutrition during gestation and/or lactation, including early weaning, as well as studies involving interventions with functional foods and/or isolated bioactive compounds. This study searched for articles that presented outcomes associated with the risk of development and CVDs, as well as those which had been published over the last ten years. Studies in languages other than Portuguese or English, through the reading of titles and abstracts, were excluded. Finally, some articles were also excluded upon reading the full text.

Results

Figure 2 illustrates the literature selection process. In total, 848 results were found, of which 799 articles were excluded due to the criteria mentioned above. Therefore, 48 articles were selected as they presented a direct connection to the object of this study.

Maternal malnutrition: lack or excess of macronutrients

The study including the Dutch Famine, which occurred during World War II, represents a historical milestone for studies involving the origin of diseases related to malnutrition in the early life. This work demonstrated that maternal malnutrition during gestation disrupts the metabolism of the descendants in the long run, resulting in hyperglycemia, a greater incidence of CVD, blood clotting disorders, as well as an increase in the capacity of response to stress and obesity.[19]

According to the WHO,[20] malnutrition refers to the deficiencies, excesses, or imbalances in the energy and/or nutrient intake. The inadequate consumption of macronutrients during the critical periods of development has been related to cardiometabolic diseases in one's offspring.[21] Malnutrition during the intrauterine life associated with maternal food restrictions contributes to a worse performance and a premature phenotype of cardiac aging in adult offspring. Similarly, nutritional

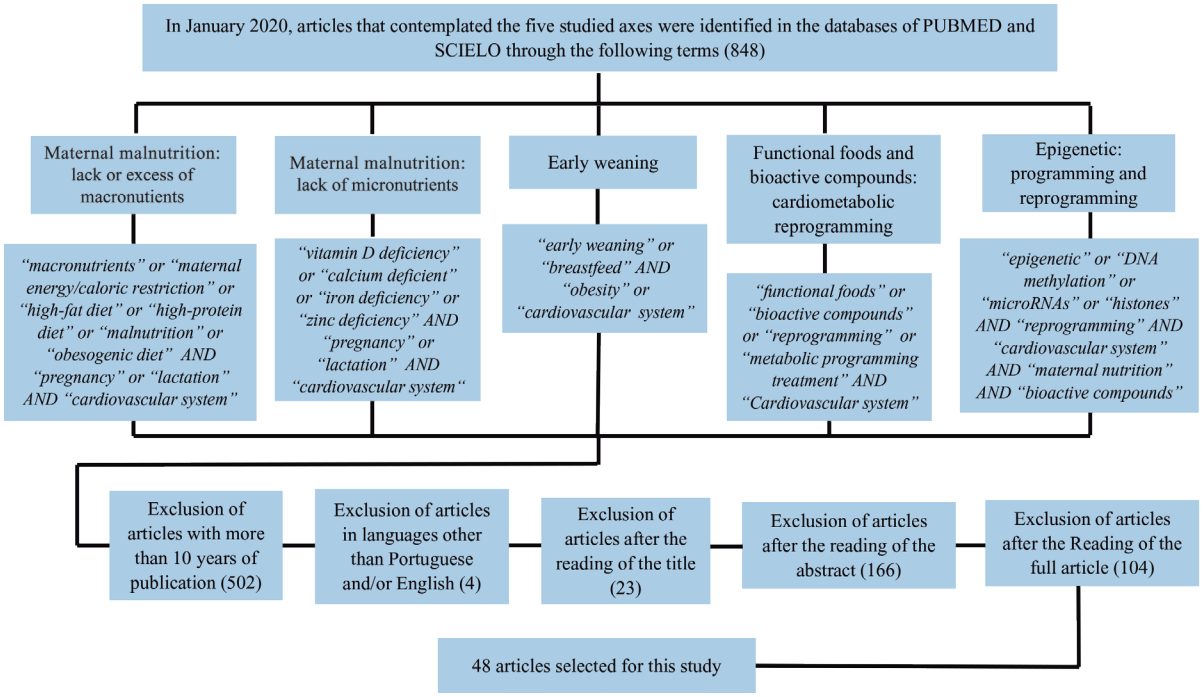

Figure 2 – Flowchart of study selection process.

excesses in the early life, associated with maternal obesity can induce the involvement of the descendants' cardiac function over the long term. These exposures alter one's cardiac metabolism and the microRNAs involved in each development stage of the heart and seem to play a crucial role in the unfavorable programming of CVDs.[22]

Thus, diets that are restrictive in macronutrients and/or obesogenic diets, characterized by the excess of calories, sugars, and lipids, can lead to malnutrition and, if consumed by the mother during gestation and/or lactation, can be equally capable of programming the offspring for cardiometabolic diseases.[23] Maternal malnutrition (F0 generation) programs its direct descendants (F1 generation), and it is capable of programming its subsequent generations (F2, F3, for example).[24] Table 1 shows studies, including animal models, which evaluate the consequences, over the long term, of the excess or restriction of macronutrients during these critical periods.

Maternal malnutrition: lack of micronutrients

The same way as macronutrients, the micronutrients are also essential to biological activity.[35] Their deficiencies affect many pregnant women and are considered a growing health problem that encompasses nearly 2 billion people worldwide. This condition is also known as hidden hunger, and includes deficiencies of iron, calcium, iodine, folate, and zinc, as well as other lesser-known micronutrients, such as selenium, chromium, and vanadium.[36,37]

The consumption of micronutrients varies widely during gestation and among populations. Women from underdeveloped countries are more exposed to malnutrition during gestation, and the nutritional demands of this period can accentuate the deficiencies of these nutrients, with severe consequences for the health of the mother and the baby. It is currently estimated that 15-20% of these women suffer from lack of iron, 15% from lack of vitamin A, and 25% from lack of zinc.[35] Micronutrient supplements diminish the risk of low birth weight, small size for the gestational age, and stillbirths due to malnutrition, thus justifying this intervention during pre-natal follow-up. The positive effects of a proper maternal diet regarding micronutrients can persist into childhood, but data is scarce concerning possible benefits in the long run.[35] Table 2 summarizes the main findings resulting from the need for micronutrients in animal models during critical periods of development.

Early weaning

Breast milk is the ideal food for a newborn's nutrition. In general, human milk is 87% water, 7% lactose, 3.8% fat and 1% protein; however, its composition varies during lactation, adapting to the changes needed for the child's growth.[46,47] Supplementation with infant formula is recommended only for the cases in which exclusive breastfeeding is impossible, unsustainable, or inadequate. In most cases, cow's milk or soy milk is used as a base, complemented with ingredients produced by genetic engineering, which most closely resembles breast milk. Even though the industry's intention is to imitate human milk as closely as possible, the use of infant formulas should only be considered after having depleted all alternative possibilities of breastfeeding.[47,48]

Adequate breastfeeding is essential for the survival, health, and growth of children; it preserves the mother's health and contributes to the development of human capital. Strong evidence of this can be found in studies with humans that show the existence of a proportionally inverse relationship between the duration of breastfeeding and the risk of obesity, hypertension, dyslipidemia, and type II diabetes mellitus in adulthood.[49] Based on this, since 1990, international agencies specialized in health adopted the Innocenti Declaration, recommending exclusive breastfeeding until six months of age.[50] However, the WHO analyzed the duration of breastfeeding in 108 countries and found that only 32% of the children received only breast milk in the first semester of life. One of the determining factors of early weaning seems to be the socioeconomic aspect.[51] Some studies discuss that in high-income countries, the socioeconomic status seems to be positively associated with the duration of breastfeeding. Others believe that breastfeeding became less common in high-income countries during the 20th century and that similar standards have been observed in women with a high educational level, from urban environments, and who have a higher purchasing power in middle and low-income countries.[50,51] Although breastfeeding is cited as the reason for women to leave the workplace, evidence suggests that the majority remain in their jobs and choose to use breast milk substitutes or interrupt breastfeeding.[50]

Table 3 presents studies including animal models that evaluate the consequences of early weaning over the long term.

Table 1 – Studies in animal models, including maternal malnutrition resulting from a lack or excess macronutrients and its outcome in offspring

Reference	Model of malnutrition (F0 generation)	Species	Post-natal moment of evaluation, generation, and evaluated sex	Main outcomes in the offspring
24	Maternal energy restriction: gestation	Wistar Rats	16 weeks (F1, F2, and F3 generation) Male	↑ Blood pressure ↓ Vasodilator response to acetylcholine Damage to the production of nitric oxide in the F1, F2, and F3 generations
25	Protein restriction: gestation	Wistar Rats	70 days (F1 generation) Male	Induction of cardiac fibrosis markers Damage to myocardial function ↓ Ejection and shortening fractions ↑ Ventricular diameter
26	Protein restriction: gestation /lactation	Wistar Rats	90 days (F1 generation) Male	↑ Cardiovascular sympathetic tone ↑ Blood pressure
27	Protein restriction: gestation /lactation	Wistar Rats	70-90 days (F1 generation) Male	Disruption of glutamatergic signaling in the nucleus of the solitary tract ↑ Blood pressure
28	Protein restriction: gestation	Albino Rats	24 weeks (F1 generation) Male	Positive regulation of nitric oxide synthase Cell apoptosis ↓ Number of cardiomyocytes ↑ Interstitial Fibrosis
29	Hyperlipidic Maternal Diet: gestation /lactation	Sprague Dawley Rats	60 days (F1 generation) Male	↑ Blood pressure ↓ Cardiac baroreflex function ↑ Response to angiotensin II and pro-inflammatory cytokines through the positive regulation of the cerebral renin-angiotensin system Oxidative and inflammatory stress
30	Hyperlipidic Maternal Diet: gestation /lactation	Sprague Dawley Rats	Neonatal (F1 generation) Male and Female	Damage in the glycolytic and respiratory capacity ↑ Lipid peroxidation and mitochondrial dysfunction Cardiac dysfunction
31	Hyperlipidic Maternal Diet: gestation	Sprague Dawley Rats	3 months (F1 generation) Male and Female	Gender-dependent cardiac hypertrophy ↑ Susceptibility of the heart to the ischemia/reperfusion lesion in male rats ↓ Connection of the glucocorticoid receptors to their response elements in the angiotensin II receptor ↓ Glucocorticoid receptors in the heart of male rats
32	Pre-gestational Maternal Obesity: gestation/lactation	Mice C57BL/6	3, 5, 8, and 12 weeks (F1 generation) Male	Cardiac hypertrophy Cardiac dysfunction Dominance of the sympathetic activity in the heart
33	Obesogenic Maternal Diet: lactation	Mice C57BL/6	8 weeks (F1 generation) Male	Hypertension Cardiac dysfunction Hypertrophy ↑ Cardiomyocyte area Cardiac remodeling
34	Obesogenic Maternal Diet: gestation/ lactation	Sprague Dawley Rats	12 weeks (F1 generation) Male	Modulation of the angiogenetic activity in endothelial cells ↓ Vasorelaxant response Poor revascularization ↑ Tissue fibrosis

Table 2 – Studies in animal models including the maternal malnutrition resulting from a lack of micronutrients and its outcome in offspring.

Reference	Model of malnutrition (F0 generation)	Species	Post-natal moment of evaluation and evaluated sex (F1 generation)	Main outcome in offspring
38	Maternal diet deficient in Iron: 2 weeks after mating and in the perinatal period	Sprague Dawley Rats	6 months Male and Female	↓ Production of nitric oxide
39	Maternal diet deficient in Zinc: gestation/lactation	Wistar Rats	60 days Male	↑ Blood pressure Vascular dysfunction Damage to the renal function
40	Maternal diet deficient in Zinc: gestation/lactation	Wistar Rats	6 and 81 days Male and Female	↓ Nitric oxide synthase in the aorta ↑ Oxidative stress ↑ Deposition of collagen in the tunica media ↓ Activity of endothelial nitric oxide synthase ↑ Systolic blood pressure in male rats
41	Maternal diet deficient in Zinc: gestation/lactation	Wistar Rats	81 days Male and Female	Activation of apoptotic and inflammatory processes ↓ Growth factor expression TGF-β1 ↓ Activity of nitric oxide synthase in the cardiac tissue
42	Maternal diet deficient in vitamin D: 4 weeks before mating and during gestation/lactation	Sprague Dawley Rats	3 and 30 days Male and Female	↑ Volume of left ventricle Hyperplasia and hypertrophy of cardiomyocytes ↑ Proportion of mononuclear cardiomyocytes
43	Maternal diet deficient in vitamin D: 6 weeks before mating and during gestation/lactation	Sprague Dawley Rats	8 weeks Male and Female	↑ Systolic blood pressure ↓ Vascular complacency
44	Maternal diet deficient in vitamin D: 10 weeks before mating and during gestation	Sprague Dawley Rats	12 weeks Male and Female	↑ Systolic blood pressure Involvement of endothelium relaxation
45	Maternal diet deficient in Calcium: gestation/lactation	Wistar Rats	180 days Male and Female	↑ Serum glucose and insulin in male rats ↑ HOMA-IR Index in male rats ↓ Systolic blood pressure in female rats ↓ Heart rate in female rats

Functional foods and bioactive compounds: cardiometabolic reprogramming

Specialists highlight a balanced, healthy, and varied diet as the best way to prevent chronic diseases. These recommendations are based on existing associations between the consumption of foods, such as fruits, vegetables, and whole grains, and the prevention of CNCD. In fact, epidemiological studies show an inverse association between the prevalence of these diseases and the consumption of these foods. As a result, the so-called "functional foods" emerged, which provide benefits to the health of those who eat them.[56]

Brazilian law sets forth the legal definition of functional foods: "foods or ingredients that, in addition to their basic nutritional functions, when consumed as part of the usual diet, produce metabolic effects and/or physiological and/or benefits to health, which should be safe for consumption without medical prescription".[57] These foods contain

Table 3 – Studies in animal models including early weaning and its outcomes in offspring.

Reference	Protocol for early weaning (F0 generation)	Species	Post-natal moment of evaluation and evaluated sex (F1 generation)	Main outcome in offspring
52	Physical barrier: Bandages on the mother on post-natal day 18	Wistar Rats	21 and 180 days Male	Metabolic syndrome ↑ adiposity dyslipidemia resistence to insulin resistence to leptin
53	Physical barrier: Bandages on the mother or pharmacological barrier: administration of bromocriptine (0.5 mg/kg ip twice daily) on post-natal day 18	Wistar Rats	90, 120, 150, and 180 days Male	Hyperphagia, ↑ visceral fat and bone mass ↑ plasmatic levels of leptin and vitamin D
54	Maternal separation: 3h/day of the post-natal days 3 to 12	Sprague Dawley Rats	55-66 days Male and Female	↑ cardiac response to hypoxia Instability of the cardiorespiratory response
55	Physical barrier: Bandages on the mother or pharmacological barrier: administration of bromocriptine (0.5 mg/kg ip twice daily) on post-natal day 18	Wistar Rats	180 days Female	Weight gain ↑ adiposity hyperleptinemia ↓ estrogen

components, called "bioactive compounds", defined as "nutrients or non-nutrients normally eaten as a component of some other food, producing metabolic or physiological action in the body."[58]

Studies point out that diets rich in bioactive compounds can reduce the risk of CVD. For instance, anthocyanins present in red fruits; resveratrol, phenolic compound found in grapes; catechins, found in large quantities in green tea; phytosterols, present in vegetable oils; isoflavones, found in soy beans; Omega-3 fatty acids observed in linseed, chia, and fish; probiotics present in dairy products; prebiotics found in vegetables, fruits, and whole grain, oleaginous, and leguminous cereals; among others.[56,59-61] Evidence still suggests that intestinal dysbiosis plays an important role in the pathogenesis of these diseases, in which, bioactive compounds may act in the modulation of the microbiota in order to favor a healthy bacterial population.[61]

The literature proposes that these compounds can unleash epigenetic changes that have accumulated throughout life, involved in the pathogenesis of diseases related to age.[61] Based on DOHaD theory, it is reasonable to assume that the early life could also constitute a temporal window to begin dietetic interventions focused on the prevention of diseases. The knowledge about the consequences of the early exposure to bioactive compounds is stiil limited. Table 4 presents experimental studies that investigated whether or not these compounds would be able to prevent both the development of cardiometabolic diseases associated with malnutrition in critical periods, as well as attenuate the injuries caused by it.

Epigenetics: programming and reprogramming

As the epigenetic changes are modulated by environmental exposure, epigenetics is considered the interface between genetics and the environment. Thus, these changes have been highlighted as molecular mechanisms that are subjacent to the process that associates malnutrition in critical periods of development and cardiometabolic programming. In addition, given the capacity of response of the epigenetic markers to

Table 4- Studies including the effects of bioactive compounds in the prevention or reprogramming of cardiometabolic diseases programmed in the offspring in different animal models of malnutrition in the beginning of life.

Reference	Model of malnutrition (F0 generation)	Species	Functional food/ bioactive compound	Intervention: F0 or F1 generation Post-natal moment of evaluation and evaluated sex	Main outcomes in offspring
62	Overnutrition: Reduction in the litter during lactation	Wistar Rats	Yerba mate tea (*Ilex paraguariensis*)	F1: treatment by gavage with yerba mate solution (1g/kg/2mL of water) between post-natal days 150 and 180 Male	Reversion of hypothalamic resistance to insulin Normalization of enzyme activity in the liver ↓ Lipid peroxidation ↓ Level of steatosis and of hepatic triglycerides
63, 64	Early weaning: physical barrier	Wistar Rats	Calcium carbonate	F1: diet supplemented with calcium carbonate (10g/kg of feed) between post-natal days 120 and 180 Male	Mitigated hyperphagia ↓ Overweight and adiposity ↓ Inflammatory state ↓ Resistance to vitamin D in adipocytes
65	Early weaning: physical barrier	Wistar Rats	Resveratrol	F1: treatment by gavage with resveratrol solution (30mg/kg in a 0.5% p/v solution of methylcellulose) between post-natal days 150 and 180 Male	↓ Overweight ↓ Feed intake ↓ Hypertrophia of adipocytes ↓ Serum leptin ↓ Glycemia ↓ Resistence to leptin and insulin ↑ Adiponectine
66	Hyperlipidic maternal diet: gestation/lactation	Wistar Rats	Grape seed extract rich in Procyanidin	F0: treatment by gavage with the extract (25mg/kg): gestation/lactation F1: evaluated on post-natal day 30 Male	↑ White adipose tissue ↑ Monocyte chemoattractant protein-1 (MCP-1) ↓ Plasmatic levels of glycerol
67	Hyperlipidic maternal diet: lactation	Wistar Rats	Grape skin extract (*Vitis vinífera L.*)	F0: treatment by gavage with the extract (200mg/kg): lactation F1: evaluated on post-natal days 90 and 150 Male	Reversion of resistance to insulin ↓ Systolic blood pressure ↓ Adiposity ↓ Plasmatic triglycerides ↓ Glycemia ↓ Oxidative stress
68	Maternal diabetes induced by diet or administration of streptozocine (35mg/kg)	Wistar Rats	Linseed flour and oil	F0: feed supplemented with linseed flour (25%) or linseed oil (7%): gestation/ lactation F1: evaluated on post-natal day 100 Male	Unaltered biochemical parameters Hindered aortic remodeling

69	Maternal hyperlipidic diet: gestation/ lactation	Sprague Dawley	Prebiotic: Long chain inulin or Probiotic: *Lactobacillus casei*	F0: treatment with Inulin (5% p/p) or *Lactobacillus casei* (2×108 UFC/day), concomitant to hyperlipidic diet F1: evaluated after 16 weeks Male	Hindered increase in blood pressure ↑ Beneficial bacteria
70	Maternal diet with high fructose content (60%): gestation/lactation	Sprague Dawley	Resveratrol	F1: diet similar to maternal + resveratrol (50 mg/L) until post-natal day 90 Male	↓ Blood pressure ↓ Renal oxidative stress Positive modulation of the intestinal microbiota
71	Supplementation of fructose in the mother's water (100 g/L): gestation	Wistar Rats	Melinjo seed extract (*Gnetum gnemon*)	F0: diet supplemented with 0.1% Melinjo: lactation F1: evaluated in day 17 after weaning Female	↑ Protein kinase expression activated by phosphorylated adenosine monophosphato (AMPK) and of endothelial nitric oxide synthase in the kidneys ↓ Systolic blood pressure
72	Maternal hypoprotein diet: gestation	Wistar Rats	Green tea extract	F0: diet supplemented with 0.12% or 0.24% of green tea extract: lactation F1: evaluated after weaning Female	Higher dose of green tea extract: ↓ Infiltration of macrophages and areas of fibrosis in the cardiac tissue Restored the serum concentration of insulin
73	Maternal hypoprotein diet: gestation.	Wistar Rats	Adzuki beans *(Vigna angularis)*	F0: received a normoprotein diet supplemented with Adzuki beans: lactation F1: evaluated at 3 and 23 weeks of age Male	↓ Body weight ↓ Hepatic levels of triglycerides ↑ Phosphorylation of AMPK (liver and skeletal muscle) and Acetyl coenzyme Carboxylase (skeletal muscle).

food factors, one can speak of "epigenetic foods", a type of functional food containing bioactive compounds capable of modulating the microRNA expression, DNA methylation, or histone modifications.[74]

Epigenetic modifications have been observed in cardiac pathologies. Analyses of newborns mice or healthy and heart failure (HF) adult mice cardiomyocytes DNA, showed an important role in DNA methylation in the modulation of many aspects of the cardiac biology, including in the development of the disease.[75] By contrast, the role of histones in the cardiovascular biology, as well as the role of histone deacetylase (HDAC) enzymes, as therapeutic targets of heart diseases, has been well defined.[76] Treatment using an *in vivo* HDAC inhibitor attenuated the cardiac hypertrophy and fibrosis[77] in hearts of rodents exposed to hypertrophic stimuli. Moreover, the microRNAs can regulate multiple cell functions involved in atherosclerosis, such as oxidative stress, cholesterol metabolism, and endothelial dysfunction.[78]

Analyses of DNA methylation in the entire genome of siblings of the same sex in the cohort of the Dutch Famine study revealed a pattern of differentially methylated regions, associated with malnutrition.[79] Experimental studies that analyzed offsprings of mothers submitted to protein restrictions during gestation observed a reduction in the supply of methyl groups stemming from glycine, hypomethylation of the hepatic receptor of the glucocorticoids, altered histone methylation, and an increase in the DNA methylation in hepatocytes.[21] Consistent with these findings, the mother's energy restriction during gestation led to DNA hypomethylation in the hepatic tissue of the offspring, resulting in an increase in the gene expression involved in the oxidation of fatty acids and the reduction of genes involved in the lipid

synthesis.[21] By contrast, the excessive consumption of carbohydrates by the mother during gestation changes the DNA methylation, leading to changes in the gene expression responsible for adipogenesis in the white adipose tissue, in turn programming obesity in the offspring.[21] Finally, Strakovsky et al.[80] showed that, upon modifying the histone acetylation, a maternal hyperlipidic diet contributed to changing the expression of antioxidant enzymes in newborns.

Many micronutrients are essential for a wide range of metabolic processes, including methylation and transamination reactions.[81] These processes involve many enzymes with methyltransferase activity, in addition to co-factors, such as choline, methionine, zinc, and vitamins B6, B12, and B9, acting as methyl donors. B9 is a donor of one-carbon for DNA methylation and synthesis; its role is crucial during the early post-natal development, when rapid growth and cell proliferation take place. The B12 deficiency can result in global hypomethylation, since, together with the B9, it is involved in methionine synthesis and S-adenosil methionine, necessary donors for the maintenance of DNA methylation patterns.[81,82] Due to its role in DNA methylation, zinc can exert a key influence upon the epigenome. Its deficiency during the intrauterine and post-natal life can contribute to change the methylation processes, which can lead to the development of chronic diseases and increase cardiovascular risks.[82]

There is evidence that sustains the notion that breast milk influences the DNA methylation and that this food contains microRNAs involved in the regulation of the gene expression at the post-transcriptional level. This study raised the hypothesis that the microbioma mediates the effects of this compound in this process, given that breastfeeding can modulate the composition of the intestinal microbiota and that this influences the DNA methylation.[83] One study, which compared rats submitted to early weaning, who were fed a formula rich in carbohydrates, and rats fed only with breast milk, showed lower levels of mRNA of the Nyp gene (which codifies the Y neuropeptide – orexigenic peptide) and of histone acetylation, as well as higher levels of mRNA of the Pomc gene (anorexigenic peptide) in the rats that received breast milk, possibly associated with high levels of histone acetylation in this group. Both of the genes are involved in many physiological processes, mainly energy homeostasis.[83]

As regards the bioactive compounds, *in vitro* and *in vivo* studies have demonstrated that these perform their protector effects in the chronic diseases by means of different mechanisms involving nutrigenomics. Anthocyanins have been associated with histone modifications, DNA methylation, and microRNA expression. Other bioactive compounds, such as isoflavones, curcumin, and resveratrol can regulate the activity of HDAC enzymes and histone acetyltransferase (HAT) and, consequently, modulate the histone acetylation. The benefits to one's health are attributed to these epigenetic mechanisms.[15,61]

DNA methylation has also been associated with the catechins present in green tea. The epigallocatechin-3-gallate (EGCG) can inhibit the DNMT enzyme (DNA methyltransferase) through indirect mechanisms or through the reduction of its expression, in addition to modulating epigenetic processes at the level of histone modifications. Moreover, studies have also suggested that butyrate, a short chain fatty acid formed in the colon through prebiotic fermentation by bacteria, seems to inhibit the HDAC activity, corroborating with a possible interaction between the epigenome and the microbioma.[15,61]

Discussion

The DOHaD theory search to fill in the blanks in the knowledge of how nutritional experiences in crucial stages of early life can impact one's health in the long term.[6, 10] Both, programming and reprogramming of diseases, seem to occur through epigenetic mechanisms that show how the nutritional environment, whether adequate or not, can affect the functioning of genes, influencing the phenotype[15] However, epidemiological studies in involving human beings present some limitations that make a definitive conclusion difficult, not only due to the long interval of time between the cause (malnutrition in early life) and the effect (CVD in adulthood), but also due to the difficulty to obtain precise nutritional data in both qualitative and quantitative terms. These questions highlight the relevance of studies including animal models, especially small animals, such as rodents, which have a short lifecycle, which in turn makes the control of nutritional aspects feasible.[7,84] Even including distinct species, with different body sizes in the maturity rate and in other life history aspects, the literature suggests that the use of animal models

in studies involving development plasticity are appropriate for the study of similar effects in humans, though the strength of this association can vary with the study's design,[85] corroborating the relevance of studies with a translational approach.

Studies have demonstrated that, in addition to the adaption of energy and micro-macronutrients in one's diet, bioactive compounds also modulate effectors of the cardioprotector genes. Nevertheless, one major challenge includes a better definition of the regulator adaptive aspects of the cardiac epigenome, considering various feeding patterns in a complete meal. Trials that evaluate the conventional consumption of foods are different from those that involve pharmacos and present innumerous variables, including not only the adhesion to the diet, but also the lifestyle, the nutrient-nutrient, pharmaco-nutrient, or comorbidity-nutrient interactions. The challenge in terms of food components, concentrations, processing, solubility in water/lipids and stability, bioconversion, metabolytes, interactions, and time of ingestion, focused on the variety of epigenetic agents, requires a detailed evaluation in the context of cardiovascular health. Circulating epigenetic markers do not always reflect epigenetic effects of diet at the tissue/cell level in healthy individuals and patients in different stages of the disease. Tools involving sequencing and bioinformatics, for example, can minimize the limitations of these studies in defining the cause-effect relationships and the composition of ingredients, as well as the level of ideal intake. Despite the scarcity of beneficial results from clinical trials, approaches based on health teams can be useful for the comprehension of the interindividual response to a cardioprotector diet in the presence of common genetic variants and a similar microenvironment, as well as its point of intersection with the epigenome, to personalize the results.[86]

Final considerations

The development of public policies that favor the adequate nutrition of mothers in gestational and breastfeeding periods, as well as exclusive breastfeeding up to six months of age, seem to constitute important strategies to reduce the incidence of cardiometabolic diseases in the population, diminishing direct and indirect expenses in health care.

Although it is challenging to obtain consistent findings in clinical studies with bioactive compounds, the introduction of functional foods in one's diet can constitute an additional strategy to mitigate the deleterious effects observed in the long run, resulting from malnutrition in early life. Epigenetic mechanisms, which can be gender dependent, represent the key to developmental plasticity and are involved in both programming and reprogramming of the cardiovascular system.

Author contributions

Conception and design of the research: Marques EB, Scaramello CVB. Acquisition of data: Marques EB, de Souza KP, Alvim-Silva T, Martins ILF, Pedro SS, Scaramello CVB. Analysis and interpretation of the data: Marques EB, de Souza KP, Alvim-Silva T, Martins ILF, Pedro SS, Scaramello CVB. Obtaining financing: Scaramello CVB. Writing of the manuscript: Marques EB, de Souza KP, Alvim-Silva T, Martins ILF, Pedro SS, Scaramello CVB. Critical revision of the manuscript for intellectual content: Marques EB, de Souza KP, Alvim-Silva T, Scaramello CVB.

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References

1. WHO (2016). World Health Organization. Cardiovascular Diseases (CVDs). [Cited in 2020 Jan 15] Available from:<http://www.who.int/mediacentre/factsheets/fs317/en/index.html>.
2. GBD 2016 Brazil Collaborators. Burden of disease in Brazil, 1990-2016: a systematic subnational analysis for the Global Burden of Disease Study 2016. Lancet. 2018; 392(10149):760-75.
3. Siqueira ASE, Siqueira Filho AG, Land MGP. Análise do Impacto Econômico das Doenças Cardiovasculares nos Últimos Cinco Anos no Brasil. Arq Bras Cardiol. 2017; 109(1):39-46.
4. Crispi F, Crovetto F, Gratacos E. Intrauterine growth restriction and later cardiovascular function. Early Hum Dev. 2018;126:23-27.
5. Barker DJ, Eriksson JG, Forsén T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002;31(6):1235-39.
6. Mathias PCF, Elmhiri G, Oliveira JC, Delayre-Orthez C, Barella LF, Tófolo LP, et al. Maternal diet, bioactive molecules, and exercising as reprogramming tools of metabolic programming. Eur J Nutr. 2014;53:711-22.
7. Langley-Evans SC. Nutrition in early life and the programming of adult disease: a review. J Hum Nutr Diet. 2015;28 (Suppl 1):1-14.
8. Senyo SE, Lee RT, Kühn B. Cardiac regeneration based on mechanisms of cardiomyocyte proliferation and differentiation. Stem Cell Res. 2014;13(3 Pt B):532-41.
9. Zebrowski DC, Jensen CH, Becker R, Ferrazzi F, Baun C, Hvidsten S, et al. Cardiac injury of the newborn mammalian heart accelerates cardiomyocyte terminal differentiation. Sci Rep. 2017;7(1):8362.
10. Reynolds CM, Gray C, Li M, Segovia SA, Vickers MH. Early Life Nutrition and Energy Balance Disorders in Offspring in Later Life. Nutrients. 2015; 7(9):8090-111.
11. Wells JC. The thrifty phenotype: An adaptation in growth or metabolism? Am J Hum Biol. 2011;23(1):65-75.
12. Tiffon C. The Impact of Nutrition and Environmental Epigenetics on Human Health and Disease. Int J Mol Sci. 2018;19(11).pii: E3425.
13. Ordovás JM, Smith CE. Epigenetics and cardiovascular disease. Nat Rev Cardiol. 2010;7(9):510-9.
14. Vickers MH. Developmental programming of the metabolic syndrome-critical windows for intervention. World J Diabetes. 2011;2(9):137-48.
15. Silva LBAR, Pinheiro-Castro N, Novaes GM, Pascoal GFL, Ong TP. Bioactive food compounds, epigenetics and chronic disease prevention: Focus on early-life interventions with polyphenols. Food Res Int. 2019;125:108646.
16. Baggio G, Corsini A, Floreani A, Giannini S, Zagonel V. Gender medicine: a task for the third millennium. Clin Chem Lab Med. 2013;51(4):713-27.
17. Woodman AG, Noble RMN, Panahi S, Gragasin FS, Bourque SL. Perinatal iron deficiency combined with a high salt diet in adulthood causes sex-dependent vascular dysfunction in rats. J Physiol. 2019; 597(18):4715-28.
18. Ponzio BF, Carvalho MH, Fortes ZB, Franco MC. Implications of maternal nutrient restriction in transgenerational programming of hypertension and endothelial dysfunction across F1-F3 offspring. Life Sci. 2012; 90(15-16):571-7.
19. Thurner S, Klimek P, Szell M, Duftschmid G, Endel G, Kautzky-Willer A, et al. Quantification of excess risk for diabetes for those born in times of hunger, in an entire population of a nation, across a century. Proc Natl Acad Sci USA. 2013;110(12):4703-7.
20. World Health Organization.(WHO).2018 Malnutrition. [Cited in 2020 Jan 26] Available from: https://www.who.int/news-room/fact-sheets/detail/malnutrition>. Acesso em 26 de janeiro de 2020.
21. Kereliuk SM, Brawerman GM, Dolinsky VW. Maternal Macronutrient Consumption and the Developmental Origins of Metabolic Disease in the Offspring. Int J Mol Sci. 2017;18(7), pii: E1451.
22. Siddeek B, Mauduit C, Yzydorczyk C, Benahmed M, Simeoni U. At the heart of programming: the role of micro-RNAs. J Dev Orig Health Dis. 2018;9(6):615-31.
23. Heidari-Beni M. Early life Nutrition and Non-Communicable Disease. Adv Exp Med Biol. 2019;1121:33-40.
24. Ponzio BF, Carvalho MH, Fortes ZB, Franco MC. Implications of maternal nutrient restriction in transgenerational programming of hypertension and endothelial dysfunction across F1-F3 offspring. Life Sci. 2012; 90(15-16):571-7.
25. Menendez-Castro C, Toka O, Fahlbusch F, Cordasic N, Wachtveitl R, Hilgers KF, et al. Impaired myocardial performance in a normotensive rat model of intrauterine growth restriction. International Pediatric Research Foundation. 2014;75(6):697-706.
26. Barros MA, De Brito Alves JL, Nogueira VO, Wanderley AG, Costa-Silva JH. Maternal low-protein diet induces changes in the cardiovascular autonomic modulation in male rat offspring. Nutr Metab Cardiovasc Dis. 2015;25(1):123-30.
27. Alves DS, Barbosa DFS, Nogueira VO, Tourneur Y, Fontes DAF, Brito-Alves JL, et al. Maternal protein restriction affects cardiovascular, but not respiratory response to L-glutamate microinjection into the NTS of conscious rats. Nutr Neurosci. 2019:1-12.
28. Amer MG, Mohamed NM, Shaalan AAM. Gestational protein restriction: study of the probable effects on cardiac muscle structure and function in adult rats. Histol Histopathol. 2017;32(12):1293-303.
29. Zhang YP, Yan-Li Huo YL, Fang ZQ, Wang XF, Li JD, Wang HP et. al. Maternal high-fat diet acts on the brain to induce baroreflex dysfunction and sensitization of angiotensin II-induced hypertension in adult offspring. Am J Physiol Heart Circ Physiol. 2018;314(5):1061-69.
30. Mdaki KS, Larsen TD, Wachal AL, Schimelpfenig MD, Weaver LJ, Dooyema SDR et al. Maternal high-fat diet impairs cardiac function in offspring of diabetic pregnancy through metabolic stress and mitochondrial dysfunction. Am J Physiol Heart Circ Physiol. 2016;310(6):681-92.
31. Xue Q, Chen P, Li X, Zhang G, Patterson AJ, Luo J. Maternal High-Fat Diet Causes a Sex-Dependent Increase in AGTR2 Expression and Cardiac Dysfunction in Adult Male Rat Offspring. Biol Reprod. 2015;93(2):49.
32. Blackmore HL, Niu Y, Fernandez-Twinn DS, Tarry-Adkins JL, Giussani DA, Ozanne SE. Maternal Diet-induced Obesity Programs Cardiovascular Dysfunction in Adult Male Mouse Offspring Independent of Current Body Weight. Endocrinology. 2014;155(10):3970-80.
33. Loche E, Blackmore HL, Carpenter AA, Beeson JH, Pinnock A, Ashmore TJ, et al. Maternal diet-induced obesity programmes cardiac dysfunction in male mice independently of post-weaning diet. Cardiovasc Res. 2018;114(10):1372-84.
34. Leu S, Wu KLH, Lee WC, Tain YL, Chan JYH. The impact of maternal fructose exposure on angiogenic activity of endothelial progenitor cells and blood flow recovery after critical limb ischemia in rat offspring. Int J Mol Sci. 2019; 20(10):1-20.
35. Gernand AD, Schulze KJ, Stewart CP, West KP, Christian P. Micronutrient deficiencies in pregnancy worldwide: health effects and prevention. Nat Rev Endocrinol. 2016;12(5):274-89.
36. Aguirre S, Veiras LC, Sánchez R, Alcalde DLC, Elesgaray R, Costa MA, et al. Fetal Programming of Hypertension Induced by Moderate Zinc Restriction during Prenatal Life and Lactation: Early Morphological and Functional Alterations in Cardiovascular System in Both Sexes. Rev Argent Cardiol. 2011;79:322-28.
37. Amoroso L. The Second International Conference on Nutrition: implications for hidden hunger. World Rev Nutr Diet. 2016; 115:142–52.
38. Woodman AG, Noble RMN, Panahi S, Gragasin FS, Bourque SL. Perinatal iron deficiency combined with a high salt diet in adulthood causes sex-dependent vascular dysfunction in rats. J Physiol. 2019; 597(18):4715-28.

39. Tomat A, Elesgaray R, Zago V, Fasoli H, Fellet A, Balaszczuk AM, et al. Exposure to zinc deficiency in fetal and postnatal life determines nitric oxide system activity and arterial blood pressure levels in adult rats. British Journal of Nutrition. 2010;104:382-9.

40. Abregú FMG, Gobetto MN, Juriol LV, Caniffi C, Elesgaray R, Tomat AL, et al. Developmental programming of vascular dysfunction by prenatal and postnatal zinc deficiency in male and female rats. Journal of Nutritional Biochemistry. 2018;56:89-98.

41. Juriol LV, Gobetto MN, Abregú FMG, Dasso ME, Pineda G, Güttlein L, et al. Cardiac changes in apoptosis, inflammation, oxidative stress, and nitric oxide system induced by prenatal and postnatal zinc deficiency in male and female rats. Eur J Nutr. 2018;57:569-83.

42. Gezmish O, Tare M, Parkington HC, Morley R, Porrello ER, Bubb KJ, et al. Maternal Vitamin D Deficiency Leads to Cardiac Hypertrophy in Rat Offspring. Reproductive Sciences. 2010;17(2):168-76.

43. Tare M, Emmett SJ, Coleman HA, Skordilis C, Eyles DW, Morley R, et al. Vitamin D insufficiency is associated with impaired vascular endothelial and smooth muscle function and hypertension in young rats. J Physiol. 2011; 589(Pt 19):4777-86.

44. Meems LMG, Mahmud H, Buikema H, Tost J, Michel S, Takens J, et al. Parental vitamin D deficiency during pregnancy is associated with increased blood pressure in offspring via Panx1 hypermethylation. Am J Physiol Heart Circ Physiol. 2016;311: H1459-H1469.

45. Takaya J, Yamanouchi S, Kino J, Tanabe Y, Kaneko K. A Calcium-Deficient Diet in Dams during Gestation Increases Insulin Resistance in Male Offspring. Nutrients. 2018;10(11):pii: E1745.

46. Boquien CY. Human Milk: An Ideal Food for Nutrition of Preterm Newborn. Front Pediatr. 2018;6:295.

47. Martin CR, Ling P, Blackburn GL. Review of Infant Feeding: Key Features of Breast Milk and Infant Formula. Nutrients. 2016;8:279.

48. Ballard A, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am. 2013;60(1):49-74.

49. Kelishadi R, Farajian S. The protective effects of breastfeeding on chronic non-communicable diseases in adulthood: A review of evidence. Adv Biomed Res. 2014;3:3.

50. Rollins NC, Bhandari N, Hajeebhoy N, Horton S, Lutter CK, Martines JC, et al. Lancet Breastfeeding Series Group. Why invest, and what it will take to improve breastfeeding practices? Lancet. 2016;387(10017):491-504.

51. Maciel BLL, Moraes ML, Soares AM, Cruz IFS, de Andrade MIR, Filho JQ, et al. Infant feeding practices and determinant variables for early complementary feeding in the first 8 months of life: results from the Brazilian MAL-ED cohort site. Public Health Nutr. 2018; 21(13):2462-70.

52. Lima NS, Moura EG, Passos MC, Neto JF, Reis AM, Oliveira E et al. Early weaning causes undernutrition for a short period and programmes some metabolic syndrome components and leptin resistance in adult rat offspring. Br J Nutr. 2011;105(9):1405-13.

53. Maia LA, Lisboa PC, Oliveira E, Lima NS, Lima ICB, Lopes RT, et al. Bone metabolism in obese rats programmed by early weaning. Metabolism. 2014; 63(3):352-64.

54. Soliz J, Tam R, Kinkead R. Neonatal Maternal Separation Augments Carotid Body Response to Hypoxia in Adult Males but Not Female Rats. Front Physiol. 2016;7:43.

55. Pietrobon CB, Bertasso IM, Silva BS, Peixoto-Silva N, Oliveira E, Moura EG, et al. Body Adiposity and Endocrine Profile of Female Wistar Rats of Distinct Ages that were Early Weaned. Horm Metab Res. 2020; 52(1):58-66.

56. Mulero J, Abellán J, Zafrilla P, Amores D, Hernández Sánchez P. Bioactive substances with preventive effect in cardiovascular diseases. Nutr Hosp. 2015; 32(4):1462-7.

57. Brasil.Ministério da Saúde. ANVISA - Agência Nacional de Vigilância Sanitária. Aprova o regulamento técnico que estabelece as diretrizes básicas para análise e comprovação de propriedades funcionais e ou de saúde alegadas em rotulagem de alimentos. Resolução n. 18, de 3 de dezembro de 1999. [Citado em 26 jan 2020] Disponível em: < http://portal.anvisa.gov.br/documents/33916/388845/RESOLUCAO_18_1999.pdf/d2c5f6d0-f87f-4bb6-a65f-8e63d3dedc61>.

58. Brasil.Ministério da Saúde. ANVISA - Agência Nacional de Vigilância Sanitária. Aprova o Regulamento Técnico de Substâncias Bioativas e Probióticos Isolados com Alegação de Propriedades Funcional e ou de Saúde. Resolução RDC nº 2, de 07 de janeiro de 2002. [Citado em 26 jan 2020]. Disponível em: <http://portal.anvisa.gov.br/documents/10181/2718376/RDC_02_2002_COMP.pdf/68a25113-35e2-4327-a75f-ae22e714ca7c>.

59. Hunter PM, Hegele RA. Functional foods and dietary supplements for the management of dyslipidaemia. Nat Rev Endocrinol. 2017;13(5):278-88.

60. Plumb J, Pigat S, Bompola F, Cushen M, Pinchen H, Nørby E, et al. eBASIS (Bioactive Substances in Food Information Systems) and Bioactive Intakes: Major Updates of the Bioactive Compound Composition and Beneficial Bioeffects Database and the Development of a Probabilistic Model to Assess Intakes in Europe. Nutrients. 2017; 9(4).pii: E320.

61. Vahid F, Zand H, Nosrat-Mirshekarlou E, Najafi R, Hekmatdoost A. The role dietary of bioactive compounds on the regulation of histone acetylases and deacetylases: a review. Gene. 2015;562(1):8-15.

62. Conceição EP, Kaezer AR, Peixoto-Silva N, Felzenszwalb I, de Oliveira E, Moura EG, et al. Effects of Ilex paraguariensis (yerba mate) on the hypothalamic signaling of insulin and leptin and liver dysfunction in adult rats overfed during lactation. J Dev Orig Health Dis. 2017;8(1):123-32.

63. Nobre JL, Lisboa PC, Lima Nda S, Franco JG, Nogueira Neto JF, de Moura EG, et al. Calcium supplementation prevents obesity, hyperleptinaemia and hyperglycaemia in adult rats programmed by early weaning. Br J Nutr. 2012; 107(7):979-88.

64. Nobre JL, Lisboa PC, Peixoto-Silva N, Quitete FT, Carvalho JC, de Moura EG, et al. Role of vitamin D in adipose tissue in obese rats programmed by early weaning and post diet calcium. Mol. Nutr. Food Res. 2016;60:810–22.

65. Franco JG, Lisboa PC, da Silva Lima N, Peixoto-Silva N, Maia LA, Oliveira E, et al. Resveratrol prevents hyperleptinemia and central leptin resistance in adult rats programmed by early weaning. Horm Metab Res. 2014; 46(10):728-35.

66. Del Bas JM, Crescenti A, Arola-Arnal A, Oms-Oliu G, Arola L, Caimari A. Grape seed procyanidin supplementation to rats fed a high-fat diet during pregnancy and lactation increases the body fat content and modulates the inflammatory response and the adipose tissue metabolism of the male offspring in youth. Int J Obes (Lond). 2015;39(1):7-15.

67. Resende AC, Emiliano AF, Cordeiro VS, de Bem GF, de Cavalho LC, de Oliveira PR, et al. Grape skin extract protects against programmed changes in the adult rat offspring caused by maternal high-fat diet during lactation. J Nutr Biochem. 2013; 24(12):2119-26.

68. Vicente GC, Correia-Santos AM, Suzuki A, Velarde LG, Chagas MA, Boaventura GT. Maternal exposure to diets containing flaxseed flour or flaxseed oil during pregnancy and lactation protects the aortic remodeling in adult male offspring of diabetic rat dams. J Sci Food Agric. 2015;95(14):2973-80.

69. Hsu CN, Hou CY, Chan JYH, Lee CT, Tain YL. Hypertension Programmed by Perinatal High-Fat Diet: Effect of Maternal Gut Microbiota-Targeted Therapy. Nutrients. 2019;11(12).pii: E2908.

70. Tain YL, Lee WC, Wu KLH, Leu S, Chan JYH. Resveratrol Prevents the Development of Hypertension Programmed by Maternal Plus Post-Weaning High-Fructose Consumption through Modulation of Oxidative Stress, Nutrient-Sensing Signals, and Gut Microbiota. Mol Nutr Food Res. 2018:e1800066.

71. Uson-Lopez RA, Kataoka S, Mukai Y, Sato S, Kurasaki M. Melinjo (Gnetum gnemon) Seed Extract Consumption during Lactation Improved Vasodilation and Attenuated the Development of Hypertension in Female Offspring of Fructose-Fed Pregnant Rats. Birth Defects Res. 2018;110(1):27-34.

72. Matsumoto E, Kataoka S, Mukai Y, Sato M, Sato S. Green tea extract intake during lactation modified cardiac macrophage infiltration and AMP-activated protein kinase phosphorylation in weanling rats from undernourished mother during gestation and lactation. J Dev Orig Health Dis. 2017;8(2):178-87.

73. Mukai Y, Sun Y, Sato S. Azuki bean polyphenols intake during lactation upregulate AMPK in male rat offspring exposed to fetal malnutrition. Nutrition. 2013;29(1):291-7.

74. Milagro FI, Mansego ML, De Miguel C, Martínez JA. Dietary factors, epigenetic modifications and obesity outcomes: progresses and perspectives. Mol Aspects Med. 2013;34(4):782-812.

75. Gilsbach R, Preissl S, Grüning BA, Schnick T, Burger L, Benes V, et al. Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. Nat Commun. 2014; 5:5288.

76. Tingare A, Thienpont B, Roderick HL. Epigenetics in the heart: the role of histone modifications in cardiac remodeling. Biochem Soc Trans. 2013; 41(3):789-96.

77. Iyer A, Fenning A, Lim J, Le GT, Reid RC, Halili MA, et al. Antifibrotic activity of an inhibitor of histone deacetylases in DOCA-salt hypertensive rats. Br J Pharmacol. 2010;159(7):1408-17.

78. Schober A, Weber C. Mechanisms of MicroRNAs in Atherosclerosis. Annu Rev Pathol. 2016;11:583-616.

79. Tobi EW, Goeman JJ, Monajemi R, Gu H, Putter H, Zhang Y, et al. DNA methylation signatures link prenatal famine exposure to growth and metabolism. Version 2. Nat Commun. 2014;5:5592.

80. Strakovsky RS, Zhang X, Zhou D, Pan Y-X. The regulation of hepatic Pon1 by a maternal high-fat diet is gender specific and may occur through promoter histone modifications in neonatal rats. J Nutr Biochem. 2014;25:170–6.

81. Skjærven KH, Jakt LM, Fernandes JMO, Dahl JA, Adam AC, Klughammer J, et al. Parental micronutrient deficiency distorts liver DNA methylation and expression of lipid genes associated with a fatty-liver-like phenotype in offspring. Sci Rep. 2018;8(1):3055.

82. Indrio F, Martini S, Francavilla R, Corvaglia L, Cristofori F, Mastrolia SA, et al. Epigenetic Matters: The Link between Early Nutrition, Microbiome, and Long-term Health Development. Front Pediatr. 2017;5:178.

83. Hartwig FP, Loret de Mola C, Davies NM, Victora CG, Relton CL. Breastfeeding effects on DNA methylation in the offspring: A systematic literature review. PLoS One. 2017;12(3):e0173070.

84. Grandjean AC. Dietary intake data collection: challenges and limitations. Nutr Rev. 2012;70 (Suppl 2):S101-S104.

85. Thayer ZM, Wilson MA, Kim AW, Jaeggi AV. Impact of prenatal stress on offspring glucocorticoid levels: A phylogenetic meta-analysis across 14 vertebrate species. Sci Rep. 2018;8(1):4942.

86. Lionetti V, Tuana BS, Casieri V, Parikh M, Pierce GN. Importance of functional food compounds in cardioprotection through action on the epigenome. Eur Heart J. 2019;40(7):575-82.

REVIEW ARTICLE

Effects of Chloroquine and Hydroxychloroquine on the Cardiovascular System - Limitations for Use in the Treatment of COVID-19

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Abstract

Chloroquine (CQ) and Hydroxychloroquine (HCQ) are antimalarial drugs, with anti-inflammatory properties that justify their use in the treatment of systemic lupus erythematosus and rheumatic diseases. A pandemic caused by the new coronavirus led the entire world's scientific community to look for drugs already available on the market, capable of exercising beneficial actions in the fight against the disease. Preliminary studies in patients, as well as in vitro studies, suggested possible therapeutic effects associated with the use of HCQ and CQ in the treatment of COVID-19. Despite controversies over the effects of these drugs in combating the "cytokine storm" associated with COVID and the dismal of results in different clinical trials in Brazil, their use has been encouraged and several ongoing investigative studies are underway. In addition to the possible beneficial effects on the prognosis of patients with SARS-CoV-2, such drugs include varied effects on the cardiovascular system, ranging from positive developments related to their vasodilator properties to potential negative effects, such as cardiotoxicity. This work presents the main effects exerted by these drugs on the cardiovascular system, in order to contribute to a scientific discussion about the repurposing of these drugs in the context of COVID-19.

Introduction

COVID-19, a disease caused by the coronavirus (SARS-CoV-2), was discovered in Wuhan, China, and spread rapidly throughout the world (World Health Organization - WHO).[1] Recent reports have highlighted the possible benefits of chloroquine (CQ) and hydroxychloroquine (HCQ) use in COVID-19 treatment.[2] Chloroquine emerged in the 1940s and is clinically used in malaria treatment due to its effectiveness, low cost, safety, and easy manufacture. CQ was synthesized for the first time in 1934, and although its antimalarial properties have been identified, its development was blocked due to the high toxicity observed for this class of drugs. Subsequently, CQ was resynthesized, presenting less toxicity and reported to be superior to sontoquine and quinacrine in malaria treatment.[3] HCQ, clinically introduced in 1955, is an analogue of CQ, also used on malaria treatment, sharing the same mechanism of action. Its clinical indications include skin diseases, sarcoidosis, extra-intestinal amebiasis, chronic Q fever, rheumatoid, and autoimmune diseases.[4,5]

Its antimalarial actions are associated with lysosomal activity and autophagy signaling pathways.[6] CQ and HQ bind preferentially to phospholipids, accumulating in lysosomes which promote changes in pH and direct inhibition of lysosomal enzymes. These effects lead to the impairment of intracellular degradation processes, along with the accumulation of pathological metabolic products (especially phospholipids and glycogen). Histologically, these seem to be vacuolar granule cell mutations and ultrastructural as lamellar membrane inclusion bodies and as "curvilinear bodies" in the cytoplasm.[7]

HCQ has some benefits, such as a reduced incidence of kidney injury and a lower risk of developing serious comorbidities, including venous thromboembolism and pregnancy complications.[8-10] It is considered an essential drug for the treatment of systemic lupus erythematosus (SLE), reducing the impact of the disease and improving

Keywords

Cardiovascular System; Chloroquine/toxicity; Hydroxychloroquine/toxicity; Betacoronavirus; COVID-19; Azithromycin/ therapeutic use.

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patient survival. On the other hand, it has a potential retinotoxic effect, a factor which can limit the dose to be used.[11] A previous report indicated that HCQ has less toxic potential than CQ due to the hydroxyl group, which limits the HCQ's ability to cross the blood-retinal barrier.[12]

Investment in the development of malaria treatments has led to a decrease in cases, reducing morbidity and mortality of the disease. WHO estimates that between 2000 and 2010, the incidence of malaria was reduced, and this is due to vector control, improvements in the health system, effective treatments, an increase in notifications, and cases of surveillance.[13] In addition to their antimalarial effects, CQ and HCQ have immunomodulatory actions that are recommended for the treatment of autoimmune diseases. Moreover, it has recently been shown to be effective in reducing cardiovascular risk factors, including hyperlipidemia and hyperglycemia.[14,15]

The adverse effects triggered by the long-term administration of CQ and HCQ were first described in 1948.[16-18] The first publications reported specific toxic effects, such as retinopathy,[19] neuromyopathy,[20,21] cardiomyopathy,[22] and third-degree atrioventricular block.[23] The main reported cardiovascular effects are: vasodilation, hypotension, hypokinesia, and cardiac arrhythmias.[24] The toxic effect is usually dose-dependent. HCQ is melanotropic, and it bioaccumulates in tissues with a high melanin content, such as skin, ciliary bodies, and retinal pigment epithelium.[25] Therefore, allergic patients, psoriasis, porphyria, and alcoholism are more susceptible to cutaneous side effects. HCQ has a toxic potential in pediatric patients,[26] but one study indicated no potential cardiotoxic fetuses after HCQ use during pregnancy.[27]

CQ and HCQ are administered orally, with bioavailability between 70-80%, a long half-life time (30-60 days) and a large volume of distribution (116-285 L/kg).[28] Like all aminoquinolines, metabolites are eliminated through the kidney and liver, thus their excretion decreases in patients with renal or hepatic dysfunction, placing them at high risk for developing toxic effects.[25] Even after treatment is discontinued, they remain detectable in the urine for years.[29]

In general, CQ and HCQ can be considered safe drugs, and side effects are usually mild and transient. However, the therapeutic index is narrow and CQ intoxication has been associated with cardiovascular disorders which can be fatal.[30] Therefore, the use of CQ and HCQ should be restricted and self-medication poses potential risk to patients.

In vitro antiviral activity of CQ has been described since the late 1960s,[31-33] and its role on inhibition of SARS-CoV-1 replication has been shown in Vero E6 cell cultures.[34] In animal models, CQ has presented important effects in a variety of viruses, including human coronavirus OC43,[34] enterovirus EV-A71,[35] Zika virus,[36] and influenza A H5N1.[37] However, CQ was unable to prevent influenza infection in a randomized, double-blind, placebo-controlled clinical trial,[38] as well as having no effects in dengue-infected patients in a randomized clinical trial in Vietnam.[39] CQ was also active in *ex vivo*, but not in *in vivo* studies against ebolavirus,[40,41] Nipah virus,[42] and influenza virus in different animal models.[43] Regarding chikungunya virus (CHIKV), CQ showed promising *in vitro* antiviral activity[44,45] but increased replication of the alphavirus in several animal models.[46,47] These effects are most likely associated with its immunomodulatory and anti-inflammatory properties *in vivo*.[48-50] In a non-human primate model of CHIKV infection, CQ treatment has shown to aggravate acute fever and delay the cellular immune response, leading to incomplete viral clearance.[47] To date, it is important to emphasize that CQ has not been effective in any human acute viral infection.[39,45,47,51,52]

Effects of CQ and HCQ on the Cardiovascular System

The effects of CQ and HCQ on the cardiovascular system are diverse. In the literature, positive effects are reported in patients who used HCQ for SLE treatment, such as a reduction in the incidence of acute myocardial infarction, coronary artery disease, and peripheral arterial disease. These effects are associated with a potential antiplatelet, antithrombotic, and antihypertensive activity of HCQ.[53] In addition, reports indicate that HCQ has a potential to reduce the diabetes development, low-density lipoprotein (LDL) levels, as well as atherosclerosis progression which can contribute to the decrease in cardiovascular events.[54]

However, despite its anti-inflammatory effects and potential antithrombotic action, the literature indicates cardiotoxicity cases with the use of CQ and HCQ. At the molecular level, CQ and HCQ are amphipathic cationic drugs with the capacity to bind to myocyte phospholipids and accumulate in lysosomes, thereby inhibiting their enzymatic activity. This process impairs intracellular degradation, allowing the accumulation of toxic metabolic products.[55] A study conducted by Chatre et al.[56] demonstrated that cardiac disorders associated with HCQ and CQ treatment lead to ventricular

arrhythmias and irreversible damage, which can lead to death. Cardiac side effects are less reported than effects such as retinopathy, but in some cases, they can be severe and irreversible.

Among the main reported effects, conduction disorders and cardiomyopathy, usually with hypertrophy and congestive heart failure, are highlighted. As the clinical characteristics of cardiotoxicity are nonspecific, the identification and monitoring of potentially affected patients is extremely important. Diagnostic confirmation requires histological examination of myocardium associated with electron microscopy. Reports in the literature indicate cases of hypertrophic cardiomyopathy,[57-64] restrictive cardiomyopathy,[65] biventricular dilation,[66-67] and left chamber dilation.[66] The development of cardiomyopathy is recurrent in patients who used CQ and HCQ, and in some cases it is reversed after treatment discontinuation.[60-63,67-69]

Some previous risk factors can increase the incidence of cardiomyopathy, The most important of which are: age, sex, time of use, high doses, pre-existence of heart disease, and renal failure.[69] The HCQ use is associated with diffuse ventricular myocardial thickening, the main secondary myocardial alteration being related to its use.[18,70] In addition, patients with severe heart failure, a worsening of exercise capacity, dyspnea, and angina can also be observed.[68]

In the literature, it is described that the CQ and HCQ use can present long-term effects, even after the diagnosis and immediate suspension of treatment, with clinical and histological characteristics for years.[18,61] Thus, cardiomyopathy is a relevant adverse effect associated with CQ and HCQ treatment. Early diagnosis and periodic cardiological follow-up of the patients are essential for preventing cardiotoxicity and, consequently, the evolution of heart failure.

In addition to morphofunctional changes in cardiomyocytes, conduction disorders have also been reported. Conduction disorders are reported more frequently after chronic treatment with HCQ and CQ, as seen in patients on long-term treatment for SLE, in which cumulative doses are high. However, it is important to note that patients affected by severe form of COVID-19 often have other comorbidities and/or use pro-arrhythmic drugs such as azithromycin, factors that increase the risk of conduction disorders.[71] HCQ and CQ block I_{Kr} potassium channels in cardiomyocytes, which can result in conduction disorders such as: prolongation of the QT interval, atrioventricular blocks, enlargement of the QRS complex, depression of the ST segment, and inversion of the T wave,[72,73] which can result in conduction disturbances, including QT prolongation, atrioventricular block, expansion of the complex QRS, depression of the ST segment, and T-wave inversion.[74] Some reports of HCQ intoxication have been described in the literature, such as the occurrence of sudden ventricular tachycardia after the use of high doses of the drug.[75] As well as quinidine, an antiarrhythmic drug with a chemically similar structure,[76] CQ and HCQ have the potential to cause changes in the QT interval, a risk factor for the development of ventricular tachyarrhythmia.[77-80] Long QT syndrome is an electrophysiological disorder characterized by an increase in the QT interval and abnormalities in the T wave, whose clinical consequences can be dizziness, syncope, and sudden death. [81]

In the literature, cases of syncope and *torsade de pointes* secondary to HCQ-induced cardiotoxicity have been described.[79,82] A study developed by van den Broek et al.[83] demonstrated that CQ treatment was able to generate significant prolongation of the corrected QT interval (QTc) in 23% of the patients with COVID-19 (n = 95). In a cohort study conducted with 201 patients undergoing treatment for COVID-19 with HCQ (95%) and CQ (5%), with or without azithromycin, a high incidence of QTc interval prolongation was observed when compared to the baseline; 8.9% of the patients had a QTc interval above 500ms, of whom 3.5% were indicated for suspension of therapy and 1% were submitted to lidocaine use in order to reverse the condition.[84] A case of *torsade de pointes* was also reported by Szekely et al.[85] in a patient hospitalized with COVID-19, whose QTc interval was excessively prolonged (627 ms; baseline of 462 ms) after the introduction of HCQ therapy. In COVID-19 patients treated with the HCQ and azithromycin association, *torsade de pointes* has been reported.[86,87] To avoid electrophysiological complications, cardiological follow-up during treatment with HCQ or CQ is recommended, with emphasis on electrocardiographic monitoring of the QTc interval.

The occurrence of syncope associated with CQ use may also originate in cases of atrioventricular block or bundle branch block.[88] A retrospective study conducted with 103 patients undergoing treatment for SLE has shown that 18 cases of conduction disorder were identified; 5 cases presented third degree atrioventricular block, of which 4 were using CQ in the treatment protocol.[89] In addition, cases of right and left bundle branch secondary

to CQ use have also been reported (90,91). The treatment of third-degree atrioventricular block usually requires a pacemaker implant to reverse the condition.[75,88] McGhie et al.[75] observed 453 patients treated with HCQ and CQ drugs, and found, through electrocardiographic exams, that conduction disorders are more prevalent than structural changes, with right branch block being the most common among electrophysiological disorders, followed by bradycardia and first-degree atrioventricular block.

Pulmonary hypertension (PH) is a hemodynamic condition caused by an increase in mean pulmonary arterial pressure, caused by pulmonary dysfunctions or cardiovascular changes.[92] In a model of PH induced in rats, CQ exerted a pulmonary vasodilator effect. The authors suggest that this effect may be related to direct or indirect blockage of voltage-operated calcium channels, store-operated calcium channels, and receptor-operated calcium channels on pulmonary artery smooth muscle cells. The potential therapeutic of CQ in PH is probably associated with the combination of its vasodilator, antiproliferative, and autophagy inhibitory effects.[93]

CQ and HCQ were able to prevent right ventricular hypertrophy and vascular remodeling, as well as improve contractility and cardiac output parameters in PH experimental model. In this model, it was observed that CQ and HCQ treatment inhibited monocrotaline-induced autophagy, preventing the p62 expression, a key protein in autophagy modulation.[94] In addition, CQ has a therapeutic potential in the management of hereditary pulmonary hypertension, since it has the capacity to increase the expression of type II bone morphogenetic protein receptor (BMPR -II) on the cell surface.[95] On the other hand, despite the vasodilatory effects, the cardiotoxicity caused by CQ and HCQ may predispose PH development due to pulmonary circulation overload, as a result of congestion triggered by flow disorders through the left atrium and ventricle.[96]

In a systematic review by Chatre et al.,[56] 86 studies involving patients undergoing CQ or HCQ treatment were evaluated, in which 3.9% developed PH. In addition to this pathology, other important changes were observed, such as: conduction disorder (85%), ventricular hypertrophy (22%), hypokinesia (9.4%), and heart failure (26.8%). Therefore, it was possible to observe that PH, even if present, did not present such a significant prevalence in patients who used the drug. Table 1 shows a compilation of studies that describe electrophysiological and morphofunctional cardiovascular changes in patients after chronic treatment with CQ and HCQ.

Drug Interactions of Clinical Importance Associated with CQ and HCQ Use

Patients with rheumatoid arthritis and SLE are treated chronically with CQ or HCQ. The continuous treatment exposes patients to the development of significant side effects, as well as contributes to an incidence of adverse reactions caused by drug interactions. CQ and HCQ interact with several drugs of clinical relevance, mainly through microsomal enzymes belonging to the cytochrome P450 family (CYP), culminating in the impairment of hepatorenal clearance caused by these therapies.[97] Drug interactions are of great clinical importance, and for this reason, they should receive particular attention. CQ and HCQ are substrates of CYP2D6, CYP3A4, CYP2C8, and CYP1A1, and may change the plasma levels of many drugs and vice versa.[98]

CQ and HCQ interact with clinically important drugs, such as antibiotics, aspirin, paracetamol, cholestyramine, proton pump inhibitors, H2 receptor antagonists, imipramine, methotrexate, cyclosporine, caffeine, metoprolol, among others. Analgesics, often used as acetylsalicylic acid and paracetamol, need more attention. CQ both increases the maximum plasma concentrations of paracetamol[99] and affects its clearance.[100] *In vitro*, HCQ inhibits the activity of plasmatic esterases responsible for aspirin degradation, thereby contributing to increase their circulating levels.[101]

The use of CQ and HCQ associated with drugs used in the treatment of cardiovascular diseases requires adequate monitoring in order to minimize the possible cardiotoxic effects inherent to the combination therapy. CQ can increase the plasma digoxin concentration up to 4-fold and precipitates clinical manifestations, such as arrhythmia and cardiotoxicity.[102] Additionally, metoprolol can present changes on its plasmatic concentration when used concomitantly with HCQ. This interaction occurs through competition for the same metabolizing enzyme of both drugs, CYP2D6, resulting in an increase of concentration and bioavailability of metoprolol.[103]

Due to the pandemic caused by COVID-19, several clinical studies have been conducted in order to characterize a safe and effective therapy for the treatment of this disease. Some already published studies emphasize that HCQ associated with azithromycin presents potentialized effects, exerting promising actions in the COVID-19 treatment.[104,105] In addition, a study by Fantini J et al.[106], using a dynamic molecular simulation technique, demonstrated a synergistic antiviral effect

Table 1 – Electrophysiological and morphofunctional cardiac alterations in patients after chronic treatment with chloroquine (CQ) and hydroxychloroquine (HCQ)

Reference	Therapeutic Indication	Electrophysiological alteration	Morphofunctional alteration	Intervention
Nord et al., 2004	SLE	Atrial flutter	LV and RV dilation; LVPW hypokinesia; (EF: 20%)	HCQ suspension; radiofrequency ablation; cardioverter implantation.
Nord et al., 2004	SLE	VPC	LA e LV dilation; global hypokinesia; SAH and PH (EF: 23%)	HCQ suspension
Lenfant et al., 2020	SCLE	CD	cardiomyopathy (LV hypertrophy)	CQ suspension
Cervera et al., 2001	SLE	Complete heart block	Restrictive cardiomyopathy (EF: 36%)	Pacemaker implantation; CQ suspension
Gentille et al., 2011	SLE	Complete heart block	-	Pacemaker implantation; CQ suspension
Lee et al., 2010	RA	Sinus arrest; junctional rhythm	Hypertrophic cardiomyopathy; (EF: 44%)	Pacemaker implantation; HCQ suspension
Chatre et al., 2016	SLE	-	Cardiomyopathy; (LV hypertrophy)	HCQ suspension
Yogasundaral et al., 2018	PR	Bifascicular block	Cardiomyopathy; (EF: 60-67%)	CQ suspension
Baguet et al., 1999	SLE	Heart block; PR interval prolongation	Cardiomyopathy; (LV hypertrophy) (EF: 45%)	Pacemaker implantation; CQ suspension
Naqvi et al., 2005	SLE	-	Cardiomyopathy; (LV and RV hypertrophy) (EF: 60%)	Pacemaker implantation; CQ suspension
Dogar et al., 2017	RA	-	(EF: 55%)	HCQ suspension
Cotroneo et al., 2007	SLE, RA	Incomplete RBBB	Cardiomyopathy; (LV hypertrophy) (EF: 40%)	HCQ suspension
Reffelmann et al., 2015	RA	Complete RBBB; sinus arrest; junctional rhythm	Cardiomyopathy; (LV hypertrophy); PH	CQ suspension; Pacemaker implantation
Abid et al., 2020	SLE	Complete heart block	-	Pacemaker implantation; HCQ suspension
Cubero et al., 1993	SLE	Complete heart block	Atrial dilation; (LV and RV hypertrophy) (EF: 42%)	-
Reuss-Borst et al., 1999	RA	Complete heart block	-	Pacemaker implantation
Reuss-Borst et al., 1999	SLE	Complete heart block	-	Pacemaker implantation
Saussine et al., 2009	SLE	Complete heart block	-	Pacemaker implantation; CQ suspension
Saussine et al., 2009	SLE	LAHB; LPHB; Complete heart block	hypokinesia	Pacemaker implantation
Costedoat-Chalumeau et al., 2007	SLE	Complete heart block	LV and RV enlargement; (EF: 24%);	Heart transplantation; CQ suspension
Aslanger et al., 2008	HPul	Complete heart block	-	Pacemaker implantation
Chen et al., 2006	SLE	TdP; PR interval prolongation with VPC	-	HCQ suspension

Abbreviations – CD: conduction disorder; EF: ejection fraction; HPul: pulmonary hemosiderosis; LA: left atrium; LAHB: left anterior hemiblock; LPHB: left posterior hemiblock; LV: left ventricle; LVPW: left ventricle posterior wall; PH: pulmonary hypertension; PR: palindromic rheumatism; RA: rheumatoid arthritis; RBBB: right bundle branch block; RV: right ventricle; SAH: systemic arterial hypertension; SCLE: subacute cutaneous lupus erythematosus; SLE: systemic lupus erythematosus; TdP: torsades de pointes; VPC: ventricular premature contraction.

of HCQ combined with azithromycin in the COVID-19 treatment. However, both treatments are able to promote prolongation of QT interval, which trigger refractory ventricular arrhythmia, even when used alone.[79,107] Chorin et al.[86] reported cases of *torsade de pointes* in patients with COVID-19 who were treated with the HCQ and azithromycin association. In addition, they observed a prolongation of the QTc interval above 500 ms in 23% of the cases (n = 211). The French Pharmacovigilance Database reported that 14% of the adverse effects linked to the association of HCQ with azithromycin corresponded to blockage in the cardiac conduction system (n = 120).[87] Although CQ and HCQ show *in vitro* antiviral activity against COVID-19 in some studies, there is no robust evidence to demonstrate the clinical benefit of combined HCQ and azithromycin therapy in reducing the mortality of hospitalized patients with a severe form of COVID-19. Moreover, the deleterious effects on the cardiovascular system are notorious and should not be neglected.[65,108]

Other important drug interactions are associated with the widespread use of CQ and HCQ with immunosuppressive drugs in the treatment of rheumatoid arthritis and SLE, such as methotrexate and cyclosporine. CQ and HCQ impair the methotrexate absorption by pH variation, thereby reducing its oral bioavailability, which contributes to sub-therapeutic effects of methotrexate.[109,110] CQ and HCQ may also increase cyclosporine plasma levels, enhancing the risk of nephrotoxicity. Thus, cyclosporine use should be monitored during combination therapy in order to avoid potentially toxic effects.[111,112] CQ is also able to reduce the bioavailability of some classes of antibiotics, such as penicillin and quinolones, limiting their therapeutic effects.[113,114]

On the other hand, drugs that raise gastric pH can reduce the CQ and HCQ bioavailability, such as proton pump inhibitors and H2 receptor antagonists, restricting their therapeutic effects.[115] Since a range of drug interactions involving the long-term use of CQ and HCQ and these drugs have a low therapeutic index, it is extremely important to monitor patients who continuously use these drugs to ensure both clinical efficacy and safety.

Effects of Chloroquine and Hydroxychloroquine on other Systems

Retinopathy is an adverse effect widely described as a consequence of CQ and HCQ use. However, the mechanism involved in toxicity associated with retinopathy is not fully understood.[116] Retinal toxicity can cause irreversible visual loss. The result of the analysis of 2,361 patients using HCQ revealed an overall prevalence of 7.5% of toxicity in patients treated for more than 5 years and 20% in those treated for more than 20 years.[117] The main identified risk factors were doses of HCQ above 5mg/kg or CQ above 2.3mg/kg, duration of use for more than 5 years, previous renal failure, use of tamoxifen, and macular disease.[116,118]

Ponticelli and Moroni[119] observed that 10% of patients treated with HCQ developed corneal deposits which were dose-dependent, transient and reversible. However, in most cases, the retinopathy was irreversible. It is important that patients treated with HCQ and CQ receive a warning of the risk of toxicity as well as a periodic evaluation. The most common visual symptoms include reading and sight difficulties, photophobia, and visual blur.

The multifocal electroretinography (mfERG) provides objective documentation of visual functions, and exerts an important role in screening and evaluation in order to manage the discontinuation of treatment. However, further studies are needed to define the relationship between the time of physiological evolution and structural abnormalities. In addition, the sensitivity and specificity of mfERG can be assessed when compared to automated visual fields, fundus autofluorescence imaging, and optical coherence tomography.[120] A recent study showed that retinopathy progresses for 3 years even after HCQ treatment suspension. In this same study, they have observed more severe changes in patients who received doses above 11mg/kg/day. Thus, it is extremely important to periodically evaluate patients who use these drugs in order to diagnose the early stages of development of retinopathy and provide a detailed analysis of risk and progression of visual loss in these patients.[25]

Undesirable dermatological events include such pathologies as psoriasis, alopecia, itching, skin pigmentation and mucous membranes, photosensitivity, and skin rashes.[121] Another adverse effect reported is myopathy, the main symptoms of which are muscle weakness, increased levels of muscle enzymes, electromyographic changes, and histological lesions.[122]

Chloroquine and Hydroxychloroquine in the COVID-19 Treatment: Impact on the Cardiovascular System

Diseases that affect the cardiovascular system represent a risk factor for patients with COVID-19, as well as diabetes, pulmonary disorders, and obesity.

Therefore, such individuals are more susceptible to developing the severe form of the disease.[123-125] On its surface, SARS-CoV-2 expresses proteins that interact with its receptor, ECA2.[126] Tissues that widely express this receptor are more vulnerable to viral invasion, such as lungs, cardiovascular system, intestine, kidneys, central nervous system, and adipose tissue.[127,128]

Patients with COVID-19 have shown a higher incidence of acute heart failure,[123,129] and critically ill patients are more susceptible to present cardiovascular damage.[123,130,131] Mehta et al.[132] have observed that patients with COVID-19 have a cytokine production profile similar to secondary hemophagocytic lymphohistiocytosis (sHLH), which is associated with poor prognosis. This phenomenon has been described as "cytokine storm", characterized by an unregulated response of type 1 and type 2 auxiliary T cells.[123] This "cytokine storm" is marked by exacerbated increase in interleukins (IL), such as IL-1, IL-6, and IL-7, granulocyte colony stimulating factor (G-CSF), protein 10 induced by γ-interferon (CXCL10/IP-10), monocyte chemotactic protein 1 (MCP-1), inflammatory macrophage protein 1-α (MIP-1α), and tumor necrosis factor-α (TNF-α). Mortality predictors from a recent retrospective, multicenter study of 150 confirmed cases of COVID-19 in Wuhan, China, included elevated ferritin (mean 1297.6 ng/mL in non-survivors vs. 614.0 ng/mL in survivors; $p < 0.001$) and IL-6 ($p < 0.0001$), suggesting that mortality may be associated with hyperinflammation promoted by the virus.[131]

Cardiac injury is related to direct damage caused by viral replication and cytokine storm. In addition, respiratory dysfunction and hypoxia generated by COVID-19 can also cause damage in cardiomyocytes.[123,133,134] Clerkin et al.[135] reported that 7% of infected patients developed myocardial injury, diagnosed through echocardiographic examination or electrocardiogram, and presented elevated troponin I levels. Considering that the cardiovascular system is directly affected by SARS-CoV-2 infection, therapy with drugs that have a cardiotoxic potential becomes even more contraindicated.

Although the *in vitro* antiviral effects of CQ have been demonstrated by reducing the viral replication of SARS-CoV,[34,136] inhibiting HIV replication,[50] in addition to in vivo antiviral effects reported in an H1N1 animal model,[37] clinical studies demonstrate controversial data regarding its effects on reducing viral loads in humans. Engchanil et al.[51] reported that CQ was not effective in improving clinical, immunological, and virological parameters in HIV-infected pediatric patients, and tended to cause an increase in undesirable gastrointestinal events. In a double-blind randomized study with 307 hospitalized adults with dengue fever, it was observed that CQ was not able to reduce viremia,[39] and actually increased the incidence of adverse gastrointestinal effects. A study performed by Borges et al.[137] showed that CQ apparently reduced symptoms related to the disease, but was not able to decrease the time infection caused by dengue virus. CQ treatment in Chikungunya infection was evaluated in a double-blind, placebo-controlled study developed by Lamballerie et al.,[45] which indicated that CQ did not reduce viral loads and in fact increased the incidence of arthralgia when compared to the placebo arm. The work performed by Gautret et al.,[104] reported in March 2020, demonstrated that HCQ exerts beneficial effects in COVID-19 treatment. However, the study showed several limitations that were clarified by Toumi and Aballea,[138] such as a reduced number of enrolled patients, outcome measures, and a lack of homogeneity in both the control and treated groups. These questions compromise comparability between arms and lead to a deficiency in the control of the occurrence of type I statistical error and other inconsistencies in the protocol study.

Due to the widespread use of HCQ in hospitalized patients with COVID-19 without robust evidence to support its use, several clinical trials have been performed to confirm or refute its efficacy and safety in these patients. A randomized, controlled, multicenter clinical study conducted in China, evaluated 150 hospitalized patients with moderate and severe COVID-19. HCQ treatment did not promote additional beneficial effects in eliminating viral loads when compared to standard treatments, in addition to causing significant adverse events.[139] An observational study by Geleris et al.,[140] evaluated 1,446 patients with COVID-19 and found that HCQ treatment neither reduced nor increased the risk of intubation or death in the evaluated patients. Another observational study evaluated the HCQ effectiveness in 181 patients admitted with COVID-19 pneumonia who needed oxygenotherapy. In this study, the use of HCQ was not indicated for these patients, since treatment did not reduce the length of hospital stay nor the mortality rate. In addition, 10% of patients treated with HCQ have presented significant electrocardiographic changes and discontinued treatment.[141] Finally, a recent clinical study conducted with 1,438 hospitalized patients diagnosed with COVID-19, has shown that HCQ therapy is not associated with a reduction in mortality by COVID-19.[52]

Given the unproven efficacy, it is possible to promote cardiotoxicity in isolated or combined therapy with other drugs commonly used in the treatment of COVID-19, in addition to the fact that patients infected with COVID-19 may have direct damage in their cardiovascular system. It is important to evaluate critically and ethically if the CQ and HCQ use is necessary as a therapeutic approach to SARS-CoV-2 infection,[142] considering all the risks associated with their use (figure 1).

Conclusion

Chloroquine and hydroxychloroquine represent drugs with a potential benefit in the treatment of several pathologies, presenting important anti-inflammatory and immunomodulatory actions, with their pharmacological effects evidenced in the chronic treatment of autoimmune diseases. However, their side effects should not be overlooked, especially ophthalmic and cardiovascular effects, which can lead to vision loss and cardiotoxicity. This article describes relevant negative impacts of these drugs on the cardiovascular system. The performance of several clinical trials with CQ and HCQ in COVID-19 patients leads to an ineffectiveness of these drugs. The absence of efficacy, in addition to potential deleterious effects on the cardiovascular system, suggest that this pharmacological approach should be used with caution, especially due to the large number of patients with COVID-19 who have pre-existing cardiovascular disorders.

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

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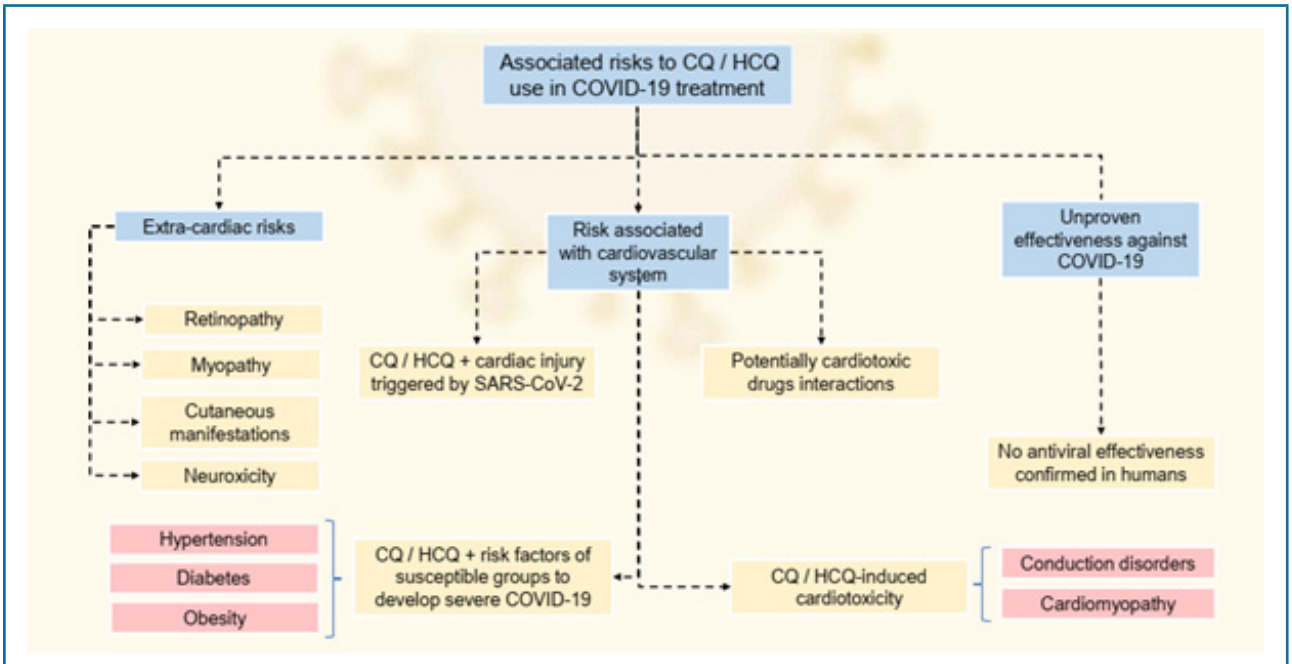

Figure 1 – Associated risks with CQ and HCQ use in the treatment of patients with COVID-19.

References

1. World Health Organization. Coronavirus disease (COVID-19) pandemic. [acesso em 01 de junho de 2020]. Disponível em: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
2. Touret F, Lamballerie X. Of chloroquine and COVID-19. Antiviral Res. 2020 May;177:104762.
3. Slater AFG. Chloroquine: mechanism of drug action and resistance in plasmodium falciparum. Pharmacol Ther. 1993;57(2-3):203-35.
4. Costedoat-Chalumeau N, Leroux G, Amoura Z, Piette JC. Hydroxychloroquine dans le traitement du lupus : le renouveau. Rev Med Interne. 2008;29(9):735-7.
5. Ruiz-Irastorza G, Ramos-Casals M, Brito-Zeron P, Khamashta MA. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: A systematic review. Ann Rheum Dis. 2010;69(1):20-8.
6. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020;16(3):155-66.
7. Gérard JM, Stoupel N, Collier A, Flament-Durand J. Morphologic study of a neuromyopathy caused by prolonged chloroquine treatment. Eur Neurol. 1973;9(6):363-79.
8. Alarcón GS, McGwin G, Bertoli AM, Fessler BJ, Calvo-Alén J, Bastian HM, et al. Effect of hydroxychloroquine on the survival of patients with systemic lupus erythematosus: Data from LUMINA, a multiethnic US cohort (LUMINA L). Ann Rheum Dis. 2007;66(9):1168-72.
9. Petri M. Use of hydroxychloroquine to prevent thrombosis in systemic lupus erythematosus and in antiphospholipid antibody-positive patients. Curr Rheumatol Rep. 2011;13(1):77-80.
10. Pons-Estel GJ, Alarcón GS, Hachuel L, Boggio G, Wojdyla D, Pascual-Ramos V, et al. Anti-malarials exert a protective effect while mestizo patients are at increased risk of developing SLE renal disease: Data from a Latin-American cohort. Rheumatol (Oxford). 2012;51(7):1293-8.
11. Jorge A, Ung C, Young LH, Melles RB, Choi HK. Hydroxychloroquine retinopathy- implications of research advances for rheumatology care. Nat Rev Rheumatol. 2018;14(12):693-703.
12. Raines MF, Bhargava SK, Rosen ES. The blood-retinal barrier in chloroquine retinopathy. Investig Ophthalmol Vis Sci. 1989; 30(8):1726-31.
13. Cotter C, Sturrock HJW, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: New strategies for new challenges. Lancet. 2013;382(9895):900-11.
14. Wasko MCM, Hubert HB, Lingala VB, Elliott JR, Luggen ME, Fries JF, et al. Risk of diabetes in patients with rheumatoid arthritis taking hydroxychloroquine. JAMA. 2007;298(20):2368-9.
15. Schneider JG, Finck BN, Ren J, Standley KN, Takagi M, Maclean KH, et al. ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. Cell Metab. 2006;4(5):377-89.
16. Nelson AA, Fitahugh OG. Chloroquine (SN-7618) pathologic changes observed in rats which for 2 years had been fed various proportions. Arch Pathol. 1948;45(4):454-62.
17. Alving AS, Eichelberger L. Studies on the chronic toxicity of chloroquine (SN-7618). J Clin Invest. 1948;27(3 Pt1):60-5.
18. Tönnesmann E, Kandolf R, Lewalter T. Chloroquine cardiomyopathy-a review of the literature. Immunopharmacol Immunotoxicol. 2013;35(3):434-42.
19. Cambiaggi A. Unusual ocular lesions in a case of systemic lupus erythematosus. AMA Arch Ophthalmol. 1957;57(3):451-3.
20. Loftus LR. Peripheral neuropathy following chloroquine therapy. Can Med Assoc J. 1963;89(18):917-20.
21. Whisnant JP, Espinosa RE, Kierland RR, Lambert EH. Chloroquine neuromyopathy. Proc Staff Meet Mayo Clin. 1963 Nov 6;38:501-13.
22. Hughes JT, Esiri M, Oxbury JM, Whitty CW. Chloroquine myopathy. Q J Med. 1971;40(157):85-93.
23. Edwards AC, Meredith TJ, Sowton E. Complete heart block due to chronic chloroquine toxicity managed with permanent pacemaker. Br Med J. 1978;1(6120):1109-10.
24. Ben-Zvi I, Kivity S, Langevitz P, Shoenfeld Y. Hydroxychloroquine: from malaria to autoimmunity. Clin Rev Allergy Immunol. 2012;42(2):145-53.
25. Ding HJ, Denniston AK, Rao VK, Gordon C. Hydroxychloroquine-related retinal toxicity. Rheumatology (Oxford). 2016;55(6):957-67.
26. Smith ER, Klein-Schwartz W. Are 1-2 dangerous? Chloroquine and hydroxychloroquine exposure in toddlers. J Emerg Med. 2005;28(4):437-43.
27. Friedman D, Lovig L, Halushka M, Clancy RM, Izmirly PM, Buyon JP. No histologic evidence of foetal cardiotoxicity following exposure to maternal hydroxychloroquine. Clin Exp Rheumatol. 2017;35(5):857-85.
28. Tett S, Cutler D, Day R, Brown K. Bioavailability of hydroxychloroquine tablets in healthy volunteers. Br J Clin Pharmacol. 1989;27(6):771-9.
29. Rubin M, Zvaifler N, Bernstein H MA,Mansour A. Chloroquine toxicity. In: Brodie BB, Gillette JR. Drug and enzymes: Proceedings of the Second International Pharmacological Meeting. Oxford: Pergamon Press;1965. v.4. p:467-87
30. Frisk-Holmberg M, Bergqvist Y, Englund U. Chloroquine intoxication. Br J Clin Pharmacol. 1983;15(4):502-3.
31. Inglot AD. Comparison of the antiviral activity in vitro of some non-steroidal antiinflammatory drugs. J Gen Virol. 1969;4(2):203-14.
32. Miller DK, Lenard J. Antihistaminics, local anesthetics, and other amines as antiviral agents. Proc Natl Acad Sci U S A. 1981;78(6):3605-9.
33. Shimizu Y, Yamamoto S, Homma NI. M, Ishida N. Effect of chloroquine on the growth of animal viruses. Arch Gesamte Virusforsch. 1972;36(1):93-104.
34. Keyaerts E, Vijgen L, Maes P, Neyts J, Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem Biophys Res Commun. 2004;323(1):264-8.
35. Tan CYQ, Gonfrier G, Ninove L, Zandotti C, Dubot-Pérès A, Lamballerie X, et al. Screening and detection of human enterovirus 71 infection by a real-time RT-PCR assay in Marseille, France, 2009-2011. Clin Microbiol Infect. 2012;18(4):E77-80.
36. Li W, Hulswit RJG, Widjaja I, Raj VS, McBride R, Peng W, et al. Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein. Proc Natl Acad Sci U S A. 2017;114(40):E8508-17.
37. Yan Y, Zou Z, Sun Y, Li X, Xu KF, Wei Y, et al. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. Cell Res. 2013;23(2):300-2.
38. Paton NI, Goodall RL, Dunn DT, Franzen S, Collaco-Moraes Y, Gazzard BG, et al. Effects of hydroxychloroquine on immune activation and disease progression among HIV-infected patients not receiving antiretroviral therapy: a randomized controlled trial. JAMA. 2012;308(4):353-61.
39. Tricou V, Minh NN, Van TP, Lee SJ, Farrar J, Wills B, et al. A randomized controlled trial of chloroquine for the treatment of dengue in vietnamese adults. PLoS Negl Trop Dis. 2010;4(8):e785.
40. Dowall SD, Bosworth A, Watson R, Bewley K, Taylor I, Rayner E, et al. Chloroquine inhibited Ebola virus replication in vitro but failed to protect against infection and disease in the in vivo guinea pig model. J Gen Virol. 2015;96(12):3484-92.
41. Falzarano D, Safronetz D, Prescott J, Marzi A, Feldmann F, Feldmann H. Lack of protection against Ebola virus from chloroquine in mice and hamsters. Emerg Infect Dis. 2015;21(6):1065-7.

42. Pallister J, Middleton D, Crameri G, Yamada M, Klein R, Hancock TJ, et al. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. J Virol. 2009;83(22):11979-82.

43. Vigerust DJ, McCullers JA. Chloroquine is effective against influenza A virus in vitro but not in vivo. Influenza Other Respi Viruses. 2007;1(5–6):189-92.

44. Coombs K, Mann E, Edwards J, Brown DT. Effects of chloroquine and cytochalasin B on the infection of cells by Sindbis virus and vesicular stomatitis virus. J Virol. 1981;37(3):1060-5.

45. De Lamballerie X, Boisson V, Reynier JC, Enault S, Charrel RN, Flahault A, et al. On chikungunya acute infection and chloroquine treatment. Vector-Borne Zoonotic Dis. 2008;8(6):837-9.

46. Maheshwari RK, Srikantan V, Bhartiya D. Chloroquine enhances replication of Semliki Forest virus and encephalomyocarditis virus in mice. J Virol. 1991;65(2):992-5.

47. Roques P, Thiberville SD, Dupuis-Maguiraga L, Lum FM, Labadie K, Martinon F, et al. Paradoxical effect of chloroquine treatment in enhancing chikungunya virus infection. Viruses. 2018;10(5):268.

48. Connolly KM, Stecher VJ, Danis E, Pruden DJ, LaBrie T. Alteration of interleukin-1 production and the acute phase response following medication of adjuvant arthritic rats with cyclosporin-A or methotrexate. Int J Immunopharmacol. 1988;10(6):717-28.

49. Katz SJ, Russell AS. Re-evaluation of antimalarials in treating rheumatic diseases: Re-appreciation and insights into new mechanisms of action. Curr Opin Rheumatol. 2011;23(3):278-81.

50. Savarino A, Lucia MB, Rastrelli E, Rutella S, Golotta C, Morra E, et al. Anti-HIV effects of chloroquine: inhibition of viral particle glycosylation and synergism with protease inhibitors. J Acquir Immune Defic Syndr. 2004;35(3):223-32.

51. Engchanil C, Kosalaraksa P, Lumbiganon P, Lulitanond V, Pongjunyakul P, Thuennadee R, et al. Therapeutic potential of chloroquine added to zidovudine plus didanosine for HIV-1 infected children. J Med Assoc Thai. 2006;89(8):1229-36.

52. Mehra MR, Desai SS, Ruschitzka F, Patel AN. Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet. 2020;6736(20):31180-6.

53. Sharma TS, Wasko MCM, Tang X, Vedamurthy D, Yan X, Cote J, et al. Hydroxychloroquine use is associated with decreased incident cardiovascular events in rheumatoid arthritis patients. J Am Heart Assoc. 2016;5(1):e002867.

54. Sun L, Liu M, Li R, Zhao Q, Liu J, Yang Y, et al. Hydroxychloroquine, a promising choice for coronary artery disease? Med Hypotheses. 2016 Aug;93:5-7.

55. Page RL, O'bryant CL, Cheng D, Dow TJ, Ky B, Stein CM, et al. Drugs that may cause or exacerbate heart failure: a scientific statement from the American Heart Association. Circulation. 2016;134(6):e32-69.

56. Chatre C, Roubille F, Vernhet H, Jorgensen C, Pers YM. Cardiac complications attributed to chloroquine and hydroxychloroquine: a systematic review of the literature. Drug Saf. 2018;41(10):919-31.

57. Lenfant T, Dion J, Maisonobe T, Costedoat-Chalumeau N. A rare cause of impaired general condition: muscular and cardiac toxicity of antimalarials. Rev Med Interne. 2020;41(5):335-8.

58. Lee JH, Chung WB, Kang JH, Kim HW, Kim JJ, Kim JH, et al. A case of chloroquine-induced cardiomyopathy that presented as sick sinus syndrome. Korean Circ J. 2010;40(11):604-8.

59. Chatre C, Filippi N, Roubille F, Pers YM. Heart involvement in a woman treated with hydroxychloroquine for systemic lupus erythematosus revealing fabry disease. J Rheumatol. 2016;43(5):997-8.

60. Baguet JP, Tremel F, Fabre M. Chloroquine cardiomyopathy with conduction disorders. Heart. 1999;81(2):221-3.

61. Naqvi TZ, Luthringer D, Marchevsky A, Saouf R, Gul K, Buchbinder NA. Chloroquine-induced cardiomyopathy-echocardiographic features. J Am Soc Echocardiogr. 2005;18(4):383-7.

62. Cotroneo J, Sleik KM, Rodriguez ER, Klein AL. Hydroxychloroquine-induced restrictive cardiomyopathy: correlation between clinical, echocardiographic and pathologic findings. Eur J Echocardiogr. 2007;8(4):247-51.

63. Reffelmann T, Naami A, Spuentrup E, Kühl HP. Contrast-enhanced magnetic resonance imaging of a patient with chloroquine-induced cardiomyopathy confirmed by endomyocardial biopsy. Circulation. 2006;114(8):357-9.

64. Cubero II, Lopez-Espinosa MTP, Richardson AC. Enantiospecific synthesis fromD-fructose of (2S,5R)- and (2R,5R)-2-methyl-1,6-dioxaspiro[4.5]decane [the odor bouquet minor components of Paravespula Vulgaris (L.)]. J Chem Ecol. 1993;19(6):1265-83.

65. Cervara A, Espinosa G, Font J, Ingelmo M. Cardiac toxicity secondary to long term treatment with chloroquine. Ann Rheum Dis. 2001;60(3):301.

66. Nord JE, Shah PK, Rinaldi RZ, Weisman MH. Hydroxychloroquine cardiotoxicity in systemic lupus erythematosus: a report of 2 cases and review of the literature. Semin Arthritis Rheum. 2004;33(5):336-51.

67. Costedoat-Chalumeau N, Hulot JS, Amoura Z, Delcourt A, Maisonobe T, Dorent R, et al. Cardiomyopathy related to antimalarial therapy with illustrative case report. Cardiology. 2007;107(2):73-80.

68. Yogasundaram H, Hung W, Paterson ID, Sergi C, Oudit GY. Chloroquine-induced cardiomyopathy: a reversible cause of heart failure. ESC Hear Fail. 2018;5(3):372-5.

69. Dogar MU, Shah NN, Ishtiaq S, Shah PN, Shah P, Mathew S, et al. Hydroxychloroquine-induced restrictive cardiomyopathy: a case report. Postgrad Med J. 2018;94(1109):185-6.

70. Joyce E, Fabre A, Mahon N. Hydroxychloroquine cardiotoxicity presenting as a rapidly evolving biventricular cardiomyopathy: key diagnostic features and literature review. Eur Heart J Acute Cardiovasc Care. 2013;2(1):77-83.

71. Jankelson L, Karam G, Becker ML, Chinitz LA, Tsai M-C. QT prolongation, torsades de pointes and sudden death with short courses of chloroquine or hydroxychloroquine as used in COVID-19: a systematic review. Heart Rhythm. 2020;17(9):1472-9.

72. Capel RA, Herring N, Kalla M, Yavari A, Mirams GR, Douglas G, et al. Hydroxychloroquine reduces heart rate by modulating the hyperpolarization-activated current If: Novel electrophysiological insights and therapeutic potential. Heart Rhythm. 2015;12(10):2186-94.

73. Traebert M, Dumotier B, Meister L, Hoffmann P, Dominguez-Estevez M, Suter W. Inhibition of hERG K+ currents by antimalarial drugs in stably transfected HEK293 cells. Eur J Pharmacol. 2004;484(1):41-8.

74. Sanghvi LM, Mathurm BBS. Electrocardiogram after chloroquine and emetine. Circulation. 1965;32(2):281-9.

75. McGhie TK, Harvey P, Su J, Anderson N, Tomlinson G, Touma Z. Electrocardiogram abnormalities related to anti-malarials in systemic lupus erythematosus. Clin Exp Rheumatol. 2018;36(4):545-51.

76. Queyriaux B, Carlioz R, Perrier E, Micaelli P, Gressard A, Deroche J, et al. Les effets cardiovasculaires liés à l'utilisation de la chloroquine. Ann Cardiol Angeiol (Paris). 2001;50(5):285-92.

77. Rey LD, Berneck A, Gonçalves L, Silva MB, Skare TL, Silva JA. ECG QT Interval prolongation in rheumatic patients using antimalarial drugs. Rev Bras Reumatol. 2003;43(5):275-8.

78. Newton-Cheh C, Lin AE, Baggish AL, Wang H. Case records of the Massachusetts General Hospital. Case 11-2011: a 47-year-old man with systemic lupus erythematosus and heart failure. N Engl J Med. 2011;364(15):1450-60.

79. Chen CY, Wang FL, Lin CC. Chronic hydroxychloroquine use associated with QT prolongation and refractory ventricular arrhythmia. Clin Toxicol. 2006;44(2):173-5.

80. Stas P, Faes D, Noyens P. Conduction disorder and QT prolongation secondary to long-term treatment with chloroquine. Int J Cardiol. 2008;127(2):e80-2.

81. Shah SR, Park K, Alweis R. Long QT syndrome: a comprehensive review of the literature and current evidence. Curr Probl Cardiol. 2019;44(3):92-106.

82. O'Laughlin JP, Mehta PH, Wong BC. Life threatening severe QTc prolongation in patient with systemic lupus erythematosus due to hydroxychloroquine. Case Rep Cardiol. 2016 Jul 12;2016:4626279.

83. Broek MPH, Möhlmann JE, Abeln BGS, Liebregts M, Dijk VF, Garde EMW. Chloroquine-induced QTc prolongation in COVID-19 patients. Neth Heart J. 2020;28(7):406-9.

84. Saleh M, Gabriels J, Chang D, Kim BS, Mansoor A, Mahmood E, et al. The Effect of chloroquine, hydroxychloroquine and azithromycin on the corrected QT interval in patients with SARS-CoV-2 infection. Circ Arrhythm Electrophysiol. 2020;13(6).e008662.

85. Szekely Y, Lichter Y, Shrkihe BA, Bruck H, Oster HS, Viskin S. Chloroquine-induced torsade de pointes in a patient with coronavirus disease 2019. Heart Rhythm. 2020;17(9):1452-5.

86. Chorin E, Wadhwani L, Magnani S, Dai M, Shulman E, Nadeau-Routhier C, et al. QT interval prolongation and torsade de pointes in patients with COVID-19 treated with hydroxychloroquine/azithromycin. Heart Rhythm. 2020;17(9):1425-33.

87. Rosenberg ES, Dufort EM, Udo T, Wilberschied LA, Kumar J, Tesoriero J, et al. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York state. JAMA. 2020;323(24):2493-502.

88. Guedira N, Hajjaj-Hassouni N, Srairi JE, El Hassani S, Fellat R, Benomar M. Third-degree atrioventricular block in a patient under chloroquine therapy. Rev Rhum Engl Ed. 1998;65(1):58-62.

89. Godeau P, Guillevin L, Fechner J, Bletry O, Herreman G. Les troubles de conduction au cours du lupus érythémateux. Fréquence et incidence dans une population de 112 patients. Ann Med Interne (Paris). 1981;132(4):234-40.

90. Fourcade L, Camus O, Roche N, Chenilleau MC, Gil JM, Massoure PL. Bloc de branche gauche douloureux d'effort associé à la chimioprophylaxie antipaludique par chloroquine. Med Sante Trop. 2014;24(3):320-2.

91. Ogola ES, Muita AK, Adala H. Chloroquine related complete heart block with blindness: case report. East Afr Med J. 1992;69(1):50-2.

92. Dodson MW, Brown LM, Elliott CG. Pulmonary arterial hypertension. Heart Fail Clin. 2018;14(3):255-69.

93. Wu K, Zhang Q, Wu X, Lu W, Tang H, Liang Z, et al. Chloroquine is a potent pulmonary vasodilator that attenuates hypoxia-induced pulmonary hypertension. Br J Pharmacol. 2017;174(22):4155-72.

94. Long L, Yang X, Southwood M, Lu J, Marciniak SJ, Dunmore BJ, et al. Chloroquine prevents progression of experimental pulmonary hypertension via inhibition of autophagy and lysosomal bone morphogenetic protein type II receptor degradation. Circ Res. 2013;112(8):1159-70.

95. Dunmore BJ, Drake KM, Upton PD, Toshner MR, Aldred MA, Morrell NW. The lysosomal inhibitor, chloroquine, increases cell surface BMPR-II levels and restores BMP9 signalling in endothelial cells harbouring BMPR-II mutations. Hum Mol Genet. 2013;22(18):3667-79.

96. Opitz CF, Hoeper MM, Gibbs JSR, Kaemmerer H, Pepke-Zaba J, Coghlan JG, et al. Pre-capillary, combined, and post-capillary pulmonary hypertension: a pathophysiological continuum. J Am Coll Cardiol. 2016;68(4):368-78.

97. Rainsford KD, Parke AL, Clifford-Rashotte M, Kean WF. Therapy and pharmacological properties of hydroxychloroquine and chloroquine in treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. Inflammopharmacology. 2015;23(5):231-69.

98. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020;16(3):155-66.

99. Adjepon-Yamoah K, Woolhouse N, Prescott LF. The effect of chloroquine on paracetamol disposition and kinetics. Br J Clin Pharmacol. 1986;21(3):322-4.

100. Raina RK, Bano G, Amla V, Kapoor V, Gupta KL. The effect of aspirin, paracetamol and analgin on pharmacokinetics of chloroquine. Indian J Physiol Pharmacol. 1993;37(3):229-31.

101. Gupta JD, Gruca M, Ablett W. Effect of other drugs and chemicals on the degradation of aspirin in vitro: possible extrapolation to in vivo metabolism of aspirin. Eur J Drug Metab Pharmacokinet. 1978;4(2):103-8.

102. Leden I. Digoxin-hydroxychloroquine interaction ? Acta Med Scand. 1982;211(5):411-2.

103. Somer M, Kallio J, Pesonen U, Pyykkö K, Huupponen R, Scheinin M. Influence of hydroxychloroquine on the bioavailability of oral metoprolol. Br J Clin Pharmacol. 2000;49(6):549-54.

104. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020;56(1):105949.

105. Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amrane S, et al. Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of 1061 cases in Marseille, France. Travel Med Infect Dis. 2020 May-June;35:101738.

106. Fantini J, Chahinian H, Yahi N. Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: what molecular dynamics studies of virus-host interactions reveal. Int J Antimicrob Agents. 2020;56(2):106020.

107. Vicente J, Zusterzeel R, Johannesen L, Ochoa-Jimenez R, Mason JW, Sanabria C, et al. Assessment of multi-ion channel block in a phase I randomized study design: results of the CiPA phase I ECG biomarker validation study. Clin Pharmacol Ther. 2019;105(4):943-53.

108. Molina JM, Delaugerre C, Le Goff J, Mela-Lima B, Ponscarme D, Goldwirt L, et al. No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. Med Mal Infect. 2020;50(4):384.

109. Furst DE. Pharmacokinetics of hydroxychloroquine and chloroquine during treatment of rheumatic diseases. Lupus. 1996;5(Suppl 1):S11-5.

110. Bannwarth B, Péhourcq F, Schaeverbeke T, Dehais J. Clinical pharmacokinetics of low-dose pulse methotrexate in rheumatoid arthritis. Clin Pharmacokinet. 1996;30(3):194-210.

111. Nampoory MR, Nessim J, Gupta RK, Johny KV. Drug interaction of chloroquine with ciclosporin. Nephron. 1992;62(1):108-9.

112. Borne BE, Landewé RB, Goei The HS, Rietveld JH, Zwinderman AH, Bruyn GA, et al. Combination therapy in recent onset rheumatoid arthritis: a randomized double blind trial of the addition of low dose cyclosporine to patients treated with low dose chloroquine. J Rheumatol. 1998;25(8):1493-8.

113. Ali HM. Reduced ampicillin bioavailability following oral coadministration with chloroquine. J Antimicrob Chemother. 1985;15(6):781-4.

114. Ilo CE, Ezejiofor NA, Agbakoba N, Brown SA, Maduagwuna CA, Agbasi PU, et al. Effect of chloroquine on the urinary excretion of ciprofloxacin. Am J Ther. 2008;15(5):419-22.

115. Namazi MR. The potential negative impact of proton pump inhibitors on the immunopharmacologic effects of chloroquine and hydroxychloroquine. Lupus. 2009;18(2):105-5.

116. Marmor MF, Kellner U, Lai TYY, Melles RB, Mieler WF, American Academy of Ophthalmology. Recommendations on screening for chloroquine and hydroxychloroquine retinopathy (2016 revision). Ophthalmology. 2016;123(6):1386-94.

117. Melles RB, Marmor MF. The risk of toxic retinopathy in patients on long-term hydroxychloroquine therapy. JAMA Ophthalmol. 2014;132(12):1453-60.

118. Yusuf IH, Sharma S, Luqmani R, Downes SM. Hydroxychloroquine retinopathy. Eye (Lond). 2017;31(6):828-45.

119. Ponticelli C, Moroni G. Hydroxychloroquine in systemic lupus erythematosus (SLE). Expert Opin Drug Saf. 2017;16(3):411-9.

120. Tsang AC, Ahmadi Pirshahid S, Virgili G, Gottlieb CC, Hamilton J, Coupland SG. Hydroxychloroquine and chloroquine retinopathy: a systematic review evaluating the multifocal electroretinogram as a screening test. Ophthalmology. 2015;122(6):1239-51.e4.

121. Soria A, Barbaud A, Assier H, Avenel-Audran M, Tétart F, Raison-Peyron N, et al. Cutaneous adverse drug reactions with antimalarials and allergological skin tests. Dermatology. 2015;231(4):353-9.

122. Le Quintrec JS, Le Quintrec JL. Drug-induced myopathies. Baillieres Clin Rheumatol. 1991;5(1):21-38.

123. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.

124. Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, et al. Risk factors of critical & mortal COVID-19 cases: a systematic literature review and meta-analysis. J Infect. 2020;81(2):e16-25.

125. Sanchis-Gomar F, Lavie CJ, Mehra MR, Henry BM, Lippi G. Obesity and outcomes in COVID-19: when an epidemic and pandemic collide. Mayo Clin Proc. 2020;95(7):1445-53.

126. Boopathi S, Poma AB, Kolandaivel P. Novel 2019 Coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. J Biomol Struct Dyn. 2020 Apr 30. [Epub ahead of print].

127. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res. 2020;126(10):1456-74.

128. South AM, Diz DI, Chappell MC. COVID-19, ACE2, and the cardiovascular consequences. Am J Physiol Heart Circ Physiol. 2020;318(5):H1084-90.

129. Lei J, Li J, Li X, Qi X. CT imaging of the 2019 novel coronavirus (2019-nCoV) pneumonia. Radiology. 2020;295(1):18.

130. Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, et al. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clin Res Cardiol. 2020;109(5):531-8.

131. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020;46(5):846-8.

132. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.

133. Wong CK, Lam CWK, Wu AKL, Ip WK, Lee NLS, Chan IHS, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol. 2004;136(1):95-103.

134. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol. 2020;17(5):259-60.

135. Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, et al. (COVID-19) and cardiovascular disease. Circulation. 2020;141(20):1648-55.

136. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread.Virol J.2005;2:1-10.

137. Borges MC, Castro LA, Fonseca BAL. Chloroquine use improves dengue-related symptoms. Mem Inst Oswaldo Cruz. 2013;108(5):596-9.

138.Toumi M, Aballea S. Commentary on "Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open label non-randomized clinical trial" by Gautret et al. J Mark Access Health Policy. 2020;8(1):1758390.

139. Tang W, Cao Z, Han M, Wang Z, Chen J, Sun W, et al. Hydroxychloroquine in patients with mainly mild to moderate coronavirus disease 2019: open label, randomised controlled trial. BMJ. 2020;369:m1849.

140. Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, et al. Observational study of hydroxychloroquine in hospitalized patients with COVID-19. N Engl J Med. 2020;382(25):2411-8.

141. Mahévas M, Tran VT, Roumier M, Chabrol A, Paule R, Guillaud C, et al. Clinical efficacy of hydroxychloroquine in patients with covid-19 pneumonia who require oxygen: observational comparative study using routine care data. BMJ. 2020;369:m2328.

142. Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, Brito M, et al. Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: a randomized clinical trial. JAMA Netw Open. 2020;3(4):e208857.

BRIEF COMMUNICATION

Acute Effect of Resistance Exercise on Mucociliary Clearance in Active Smokers

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Abstract

Background: Nasal mucociliary clearance (NMC) responds to autonomic activity through exercise. However, there is a gap in the literature on how NMC responds to resistance exercise.

Objective: To evaluate the acute effects of resistance tube exercise on NMC and the autonomic nervous system in smokers.

Methods: Clinical trial was performed with 18 individuals. Personal, anthropometric, and smoking history data were collected, and a pulmonary function test was performed by spirometry. The fatigue resistance test was performed in order to obtain the number of repetitions for the prescription of exercise. Heart rate variability was captured using a monitor. Subsequently, the exhaled carbon monoxide (exCO) was measured and a saccharin transit test (STT) was performed for NMC.

Results: The non-smoking group presented a significant decrease of 4.0±3.2 minutes in STT after P1 (p=0.021). Regarding HRV, the smoking group presented a significant decrease of mean RR (-90.3±53.0; p=0.011), SDNN (-560.0±1333.2; p=0.008), RMSSD (-13.6±10.5; p=0.011), LFms² (-567.3±836.1; p=0.008), HFms² (-223.8±231.8; p=0.008), SD1 (-9.7±7.4; p=0.011) and SD2 (-20.7±17.0; p=0.008), and an increase of mean HR (10.2±5.9; p=0.011) after P2. In the non-smoking group, a significant decrease was observed in the mean RR (-67.1±70.7; p=0.038), SDNN (-16.8±15.0; p=0.015), RMSSD (-12.3±14.7; p=0.011), LFms² (-831.2±1347.5; p=0.015), SD1 (-8.7±10.4; p=0.011), and SD2 (-22.0±19.1; p=0.015), while an increase in HR (7.1±7.3; p=0.028) was found after P1.

Conclusions: The intensity of the resistance exercise applied to the patient was not enough to promote changes in smokers. By contrast, in non-smokers, the same intensity of exercise was effective in promoting alterations in the NMC and autonomic activity. (Int J Cardiovasc Sci. 2021; 34(2):223-230)

Keywords: Smoking; Mucociliary Clearance; Autonomic Nervous System; Resistance Training.

Introduction

Nasal mucociliary clearance (NMC) is the interaction between the cilia and the mucus layer of the respiratory system, and it is influenced by cigarette smoking and exercise.[1-6]

Chronic exposure to cigarette smoking induces oxidative stress and leads to changes in the respiratory epithelium, such as a decline in the frequency of the ciliary beat and increasing mucus hypersecretion, increasing the vulnerability to respiratory infections. Previous studies showed that NMC time is longer for smokers when compared to that for non-smokers due to the activation of nicotinic receptors[1-3] and autonomic nervous system dysfunction.[7] In addition to harmful agents of cigarette smoking, NMC responds through physical exercise.[5-9]

The impact of exercise induces an increase in the autonomic nervous system activity, a release of adrenergic mediators, and an increase in the ventilation and respiratory rate, which causes an increase in the NMC.[10] However, concerning the type and duration of physical exercise on mucociliary transport, studies in

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the literature have presented contradictory evidence in different populations.

Leite et al.,[8] conducted a study with the effect of 12 weeks of aerobic training on autonomic modulation and NMC in subjects with COPD, reaching the conclusion of an increase in parasympathetic activity, a reduction in sympathetic activity in autonomic modulation, but no significant influence in NMC. Silva et al.,[9] evaluated the effects of 12 weeks of resistance training and reported significant improvement in NMC in COPD.

In response to the acute effect, the study of Ramos et al.,[5] reported an increase in NMC after the stimulus of aerobic exercise, and this response was correlated with sympathetic activity in smokers. However, this behavior is not yet known in resistance exercise in smokers.

Resistance training is generally carried out on expensive machines and devices. An alternative method is the use of such devices as the elastic tube, a portable and inexpensive instrument that has shown results for muscle activation, strength gain, and quality of life, equivalent to conventional training,[9-12] but still with no NMC results in smokers. Therefore, the goal of the present study was to evaluate the acute effects of resistance tube exercise on the NMC and autonomic nervous system in smokers and to observe the responses found in isolated and smoking-associated exercise.

Methods

A clinical trial was conducted with 30 to 50-year-old smokers and non-smokers, regardless of gender, recruited through media and advertisements in newspapers. Individuals with pre-existing chronic diseases that prevent physical exercise, who have undergone surgery or suffered nasal trauma, who report a history of alcoholism and/or illicit drug use, who present respiratory infections, who are in the process of quitting smoking, who practice regular physical exercise at least 20 minutes a day, three days a week for six months, and who did not complete all of the study protocols were excluded from this study.

Thirty-five individuals were recruited for this study, 25 in the smoking group and 10 in the non-smoking group. However, after applying the exclusion criteria, 9 smokers were included (11 were over 50 years of age and 5 did not attend all of the evaluation days) and 9 non-smokers (1 did not attend all of the evaluation days).

The protocol was performed on three non-consecutive days (48-h interval between days), in the mornings, in rooms in which the temperature (22.4±2.0 ºC) and relative humidity (51.4±7.9%) were controlled through a thermometer-hygrometer (Incoterm, model 766.02.0.00). Each day, subjects were asked to eat a light meal two hours before the evaluations and abstain from alcohol, caffeine, smoking, and vigorous exercise for 12 hours before the evaluations. Individuals were previously informed about the research objectives and procedures and, after agreement, signed an informed consent form. The project was approved by the Institutional Review Board (CAAE: 56.405.316.1.0000.5-402).

On the first day of the protocol, personal data, anthropometric (weight, height, and body mass index (BMI)) and smoking history (years of smoking, number of cigarettes/day and packs/year), as well as the level of nicotine addiction assessed by the Fagerstrom test[12] were collected. A pulmonary function test was then performed by spirometry, with normality values relative to the Brazilian population, using a portable MIR – Spirobank spirometer version 3.6.[13,14] Finally, fatigue resistance test was performed in order to obtain the number of repetitions for the individual prescription of the resistance exercise session.

The fatigue resistance test consists of pushing the individual to fatigue within 40–75 seconds after the beginning of each movement execution (knee extension and flexion, shoulder abduction and flexion, and elbow flexion). The initial load used to perform the test was determined by the therapist's perception. When the maximum time of 75 seconds is exceeded, the load was increased, whereas if the fatigue occurred before 40 seconds, the load was reduced.[15]

A maximum of five attempts were made for each movement, where, in each attempt, the individual should perform as many repetitions as he/she can, as quickly as possible, and maintain the pace of movement from the beginning of the test. The test was interrupted by: fatigue, significant reductions in the amplitude or speed of movement, and muscle compensations.

Elastic tubing was used for both groups (smoking and non-smoking). Different tubes with progressive resistance were used in such a way that the higher the reference number, the higher the resistance of the tube (#200, #201, #202, #203, and #204, Lemgruber©, Brazil).[16]

The number of repetitions achieved in these 40 seconds was used as a reference to calculate the number of repetitions proportional to 20 seconds. From the

definition of the number of repetitions, the training was performed in 2 sets of 20 seconds each, with an interval of 2 minutes between them. The abduction and shoulder flexion and elbow flexion movements were performed in an orthostatic position (Figure 1A, B, and C). The knee extension was performed sitting on a chair in the patterns of 72 cm in height and 52 cm in width (Figure 1E). Knee flexion, however, was performed in an orthostatic position, in front of the same chair (Figure 1D). One end of the tube was attached to an iron bar and the other end to the member to be worked.[11]

The smoking group performed two distinct protocols: (1) a resistance tube exercise session performed in 2 sets of 20 seconds each (P1) and (2) a resistance tube exercise session performed in 2 sets of 20 seconds, each immediately followed by smoking two cigarettes for 20 minutes (P2). The non-smoking group performed only the P1 protocol.

On exercise days (second and third day), participants initially remained at rest, in a sitting position, for 20 minutes for the collection of baseline heart rate variability (HRV). After, the exhaled carbon monoxide (exCO) was collected and a saccharin transit test (STT) was performed in order to collect the nasal MC at rest. These proceedings were repeated immediately after finishing protocols P1 and P2 (effect acute - less than 5 minutes).

Beat-to-beat HRV was captured using a heart rate monitor (Polar S810i, Finland).[17] A total of 256 consecutive RR intervals were selected at the time the participant reported the STT saccharin taste. In other words, 128 beats before and 128 beats from the STT on were filtered at the beginning (baseline) and at the end (acute effect) of the day of each protocol.[5] The selected piece was subjected to digital (and complemented by manual) filtering by the Polar Precision Performance SW software (version 4.01.029) for the elimination of premature ectopic beats and artifacts, and only a series with more than 95% of sinus rhythm were included in the study. The Kubios software (Biosignal and Medical Image Analysis Group, Department of Physics, University of Kuopio, Kuopio, Finland)[18] was used to calculate the HRV indexes.

The following indexes in the time domain were analyzed: mean interval between adjacent normal heartbeats (RR intervals), the square root of the sum of successive differences between RR intervals (RMSSD, expressed in ms - parasympathetic activity), and the SD of all normal RR intervals (SDNN, expressed in ms - global variability). In the frequency domain, low (LF, 0.04 - 015 Hz, global variability) and high (HF, 0.15 - 0.40 Hz, parasympathetic activity) frequency spectral components were analyzed in normalized units (un), squared milliseconds (ms2), and the ratio between

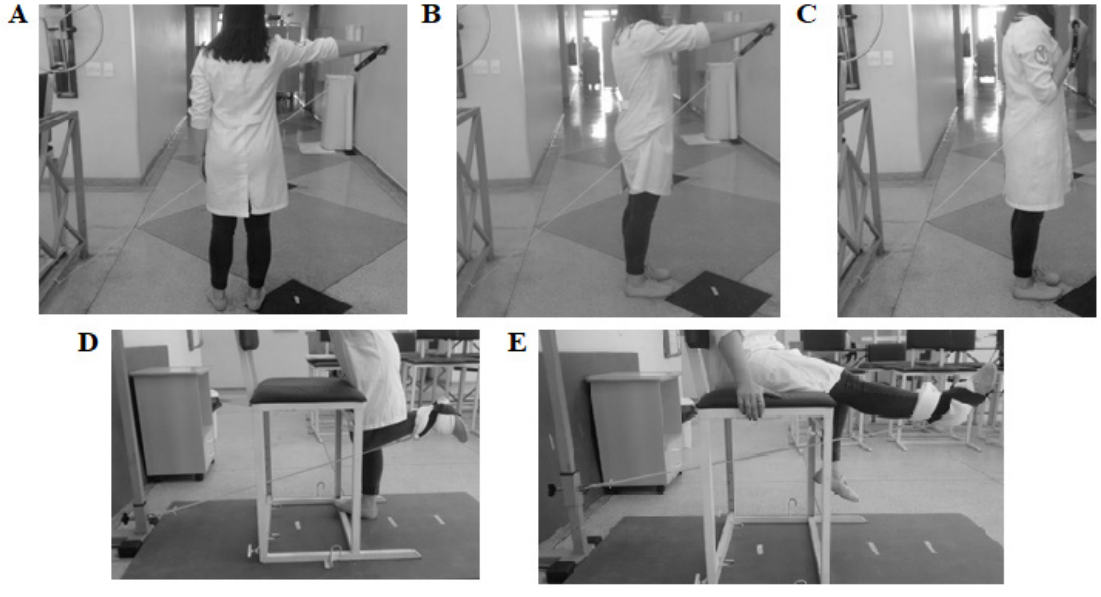

Figure 1 – Elastic tubing resistance exercise for abduction (A), shoulder flexion (B), elbow flexion (C), knee flexion (D) and extension (E).

these components (LF/HF ratio).[17,19,20] The frequency domain analysis was calculated using the Fast Fourier Transform algorithm.[21]

The Poincaré plot is a diagram in which each RR interval is plotted as a function of the next interval. Its analysis was performed by adjusting the figure ellipse formed by the attractor, from which the following indexes were calculated: SD1 (standard deviation of instantaneous beat-to-beat variability - vagal modulation) and SD2 (standard deviation of long-term continuous RR interval variability).[17,19,20]

exCO was collected by the monoximeter (Micro Medical Ltd., Rochester, Kent, United Kingdom), and data were expressed as parts per million (ppm) and carboxyhemoglobin (HbCO).

STT was performed according to previous studies.[1-6,8,9] Approximately 250 micrograms of granulated sodium saccharin were applied, two centimeters inside the right nostril, and the time it has taken for the participant to identify a taste in the mouth was recorded before and after of the day of each protocol. When the taste was not identified within 60 minutes, the test was stopped.

Data analysis was performed using the statistical software SPSS 22.0. Data normality assumption was tested using the Shapiro-Wilk test, and the results were described as mean and standard deviation, except for variables with non-normal distribution, which were described as median and interquartile range (25-75). For intergroup analysis, the delta (Δ) was performed, that is, the final result was divided by the baseline moment and the values obtained were compared between the groups, using the Student's t-test for independent samples or the Mann Whitney test, depending on the normality of the data.

For the intragroup analysis, either the Student's t-test for dependent samples or the Wilcoxon test was used, depending on the normality of the data. The significance level used was $p<0.05$.

Results

The smoking group consisted of nine subjects, 41.4±6.7 years of age, BMI of 24.6±4.5 kg/m², FEV_1/FVC ratio of 97.1±7.5%, smoking on average 16.3±5.0 cigarettes/day for 23.0±8.6 years (19.3±9.1 packs/year), with a moderate dependence level (5.3±1.8 points). The non-smoker group consisted of nine subjects, 37.6±8.4 years of age, BMI of 27.2±4.9 kg/m², and FEV_1/FVC ratio of 105.0±9.4%.

No significant difference was observed between the groups regarding the above characteristics and regarding the number of repetitions per series for each movement performed during resistance training with elastic tubes (Table 1).

Table 2 presents the STT, exCO, environment control, and HRV data.

Only the non-smoking group presented a significant average decrease of 4.0±3.2 minutes in STT after P1 (p=0.021). The smoking group presented a significant decrease in exCO (-2.3±3.0 ppm; p=0.016) and HbCO (-0.4±0.5%; p=0.016) in P1 and a significant increase in exCO (8.0±5.7 ppm; p=0.008) and HbCO (2.4±3.5 %; p=0.008) in P2.

Regarding HRV, the smoking group presented a significant decrease in mean RR (-90.3±53.0; p=0.011), SDNN (-560.0±1333.2; p=0.008), RMSSD (-13.6±10.5; p=0.011), LFms² (-567.3±836.1 p=0.008), HFms² (-223.8±231.8; p=0.008), SD1 (-9.7±7.4; p=0.011), and SD2 (-20.7±17.0; p=0.008), as well as an increase in mean HR (10.2±5.9; p=0.011) only after P2. In the non-smoking group, a significant decrease was found in mean RR (-67.1±70.7; p=0.038), SDNN (-16.8±15.0; p=0.015), RMSSD (-12.3±14.7; p=0.011), LFms² (-831.2±1347.5; p=0.015), SD1 (-8.7±10.4; p=0.011), and SD2 (-22.0±19.1; p=0.015), as well as an increase in HR (7.1±7.3; p=0.028) after the P1 protocol.

In P1, a difference between the two groups was observed for the following variables: STT (p=0.049), Mean HR (p=0.015), and SD2 (p=0.017). No significant difference in the temperature and relative humidity data were found in the intragroup and intergroup evaluation .

Discussion

In the present study, it was observed that the time of NMC and HRV of smokers did not change in response to isolated resistance tube exercises. However, after smoking-associated exercise, the same group showed significant changes in HRV, which may be explained by immediate smoking. In contrast, non-smokers showed a significant decrease in STT, suggesting an acceleration of NMC and changes in HRV.

Under basal conditions, smokers' STT was reduced if compared to the group of non-smokers, a result which proved to be similar to that reported in the literature.[1,3] After the acute act of smoking, a decrease in STT was observed due to the acute response of NMC defense against the toxic

Table 1 – Baseline characteristics of the study sample. Data expressed as mean and standard deviation

Anthropometric variables	Smoking (n=9)	Non-Smoking (n=9)	p-value
Sex (F/M)	6/3	7/2	1
Age (years)	41.4±6.7	37.6±8.4	0.293
Weight (kg)	68.7±16.0	76.2±20.7	0.399
Height (m)	1.7±0.1	1.7±0.1	1
BMI (kg/m²)	24.6±4.5	27.2±4.9	0.161
Smoking history			
Cigarettes/day	16.3±5.0	NA	
Years of smoking	23.0±8.6	NA	
Packs/year	19.3±9.1	NA	
Fagerstrom	5.3±1.8	NA	
Spirometric variables			
FVC (% pred)	99.0±15.1	91.0±8.8	0.188
FEV_1 (% pred)	96.0±15.4	94.0±8.7	0.739
FEV_1/FVC (%)	97.1±7.5	105.0±9.4	0.066
$FEF_{25-75\%}$ (% pred)	100.0±30.3	113.2±32.7	0.387
Number of repetitions per set			
shoulder abduction	25.6±6.4	24.4±5.1	0.690
shoulder flexion	27.3±5.0	25.7±6.1	0.534
elbow flexion	28.0±3.8	28.6±5.5	0.806
knee extension	31.9±6.8	31.8±5.7	0.970
knee flexion	30.3±5.6	29.2±4.4	0.644

***Significant difference (p<0.05). [a]Student's t-test for independent samples.**
F/M: female/male, BMI: Body mass index (weight/height2), FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, FEV_1/FVC ratio, $FEF_{25-75\%}$: forced expiratory flow between 25% and 75%, % pred: percentage of predicted and NA: not applicable.

components of cigarettes and by the nicotine action that promotes sympathetic activation and, consequently, the acceleration of a ciliary beat.[3,5,6]

For isolated exercise, there was a decrease in STT in non-smokers. The mechanism by which exercise improves mucociliary transportability time is related to the stimulation of the autonomic nervous system.[5,6] Increased exercise-induced ventilation stimulates the respiratory center, increasing vagal activity, which, in turn, increases mucus secretion. Moreover, by exercising, plasma catecholamine levels are elevated and stimulate sympathetic activity, accelerating the ciliary beat[10,22] in aerobic[5] or resistance exercises.[9]

One of the hypotheses that isolation exercise had no effect on smokers' NMC may be related to a decrease in STT at baseline. Previous studies have shown that the chronic effect of smoking causes changes in HRV, with a predominantly sympathetic tone.[23,24] NMC is a complex system where the sympathovagal balance plays an important role in its functioning.[5,6]

Regarding the HRV outcomes, it was observed that the smoking group showed changes in the parasympathetic system expressed by the RMSSD, HF, and SD1 indexes, in addition to other indices, such as SDNN, LF, and SD2 only in the protocol associated with smoking. A previous study corroborates the results and concluded that smoking has

Table 2. Nasal mucociliary clearance, exhaled carbon monoxide, and heart rate variability at P1 and P2. Data expressed as mean and standard deviation or median and median and interquartile range of 25-75%

Variables	Smoking (n=9)						Non-smoking (n=9)					
	P1 (Exercise)			P2 (Exercise + Cigarette)			P1 (Exercise)			P1 (Exercise)- Δ after-before		
	Before	After	p-value	Before	After	p-value	Before	After	p-value	Smoking (n=9)	Non-smoking (n=9)	p-value
STT (min)	3.1(2.1;9.3)	1.8(1.2;7.3)	0.594‡	6.4(0.8;10.2)	4.2(1.6;10.7)	0.173†	8.4 (4.7;11.4)	3.6 (2.4;5.4)	0.021‡*	-0.1±4.4	-4,0±3,2	0.049§*
exCO (ppm)	5.0 (2,0;7,5)	2.0(0.0;5.0)	0.016‡*	4.0(1.0;11.0)	13.0(4.0;21.0)	0.008‡*	1.0 (0.0;3.0)	0.0 (0.0;1.0)	0.066‡	-2.3±3.0	-1,0±1,6	0.222//
HbCO (%)	0.8 (0.3;1.2)	0.3(0.0;0.8)	0.016‡*	0.6(0.2;1.8)	2.1(0.6;5.5)	0.008‡*	0.2 (0.0;0.5)	0.0 (0.0;0.2)	0.066‡	-0.4±0.5	-0,2±0,3	0.222//
T (ºC)	21.5(21.2;23.5)	21.9(21.3;22.7)	0.262†	21.1(20.6;23.0)	21.8(20.8;24.0)	0.078†	23.4 (21.5;24.0)	22.5 (21.7;24.0)	0.429†	-0.1±0.7	-0.2±0.6	0.489//
RH (%)	58.0 (51.5;58.5)	56.0(52.0;58.5)	0.136‡	51.0(45.0;53.5)	51.0(45.5;54.5)	0.306†	50.0 (45.0;56.5)	49.0 (44.5;56.5)	0.458‡	-0.7±1.7	-0.2±1.1	0.730//
Mean RR	773.0(708.0;792.5)	739.0(702.5;844.0)	0.953‡	803.0(712.0;818.5)	684.0(629.0;729.5)	0.011‡*	781.0(704.5;844.5)	746.0(635.0;786.0)	0.038‡*	-3.6±81.2	-67.1±70.7	0.222//
Mean HR	78.0 (75.5;85.0)	81.0(71.5;85.5)	0.553‡	75.0(73.5;85.0)	88.0(82.0;95.5)	0.011‡*	77.0(71.5;85.0)	80.0(76.5;95.0)	0.028‡*	1.6±8.8	7.1±7.3	0.165§
SDNN	37.0 (31.4;46.9)	34.7(25.2;54.1)	0.859‡	30.1(26.8;58.7)	17.2(12.0;20.5)	0.008‡*	44.8(34.9;64.5)	31.6(21.6;41.4)	0.015‡*	0.8±12.3	-16.8±15.0	0.015§*
RMSSD	26.5 (21.9;41.5)	23.5(15.3;47.5)	0.859‡	26.5(17.1;39.1)	12.0(6.9;18.3)	0.011‡*	39.0(22.0;52.8)	19.3(16.6;37.8)	0.011‡*	2.2±17.3	-12.3±14.7	0.297//
LF (ms^2)	857.0 (575.0;1686.0)	770.0(279.5;1945.0)	0.678‡	497.0(340.0;1869.0)	207.0(92.0;285.0)	0.008‡*	960.0(426.5;1651.5)	515.0(237.5;856.5)	0.015‡*	-399.3±1853.9	-831.2±1347.5	0.387//
LF (nu)	73.5 (64.1;86.5)	80.8(46.3;86.7)	0.515‡	79.5(54.4;88.3)	77.0(65.7;85.6)	0.441‡	72.4(52.2;82.4)	80.9(40.7;87.4)	0.953‡	-4.6±21.7	1.6±14.2	0.482§
HF (ms^2)	261.0 (156.5;571.0)	225.0(95.0;836.0)	0.859‡	263.0(99.0;539.5)	51.0(25.5;157.5)	0.008‡*	367.0(242.5;566.0)	169.0(54.5;805.0)	0.110‡	142.3±503.8	-169.9±418.2	0.172§
HF (nu)	26.4 (13.5;35.8)	19.2(13.3;53.6)	0.515‡	20.5(11.7;45.6)	22.9(14.4;34.3)	0.441‡	27.6(17.2;17.6)	19.0(12.5;58.6)	1‡	4.6±21.7	-1.7±14.1	0.470§
LF/HF	2.8 (1.8;6.8)	4.2(0.9;6.6)	0.953‡	3.9(1.3;7.6)	3.4(1.9;6.0)	0.821†	2.6(1.1;4.7)	4.2(0.7;7.0)	0.594‡	0.1±5.9	0.9±4.0	0.736§
SD1	18.8 (15.5;29.4)	16.7(10.8;33.7)	0.859‡	18.8(12.1;27.7)	8.5(4.9;13.0)	0.011‡*	27.6(15.6;37.4)	13.7(11.8;26.8)	0.011‡*	1.6±12.3	-8.7±10.4	0.297//
SD2	51.0 (40.4;60.0)	46.3(34.0;71.8)	0.859‡	38.(34.3;63.3)	22.5(15.5;26.9)	0.008‡*	57.1(46.5;84.5)	36.0(28.1;54.0)	0.015‡*	0.2±16.1	-22.0±19.1	0.017§*
SD1/SD2	2.3 (2.0;3.2)	2.6(1.8;3.6)	0.739†	2.4(1.8;3.2)	2.5(1.9;3.0)	0.977†	2.8(1.9;3.0)	2.3(1.9;3.1)	0.743†	0.2±1.3	-0.1±0.5	0.664§

**Significant difference (p<0.05), †Student's t-test for dependent samples, ‡Wilcoxon test, §Student's t-test for independent samples, // Mann-Whitney test, STT: saccharin transit test, exCO: exhaled carbon monoxide, ppm: parts per million, T: Temperature, RH: relative humidity: nu: normalized units, RR: interval between consecutive heart beats, HR: heart rate, SDNN: standard deviation of the average of all normal RR intervals, RMSSD: square root of the mean of the squared differences between the adjacent normal RR intervals, LF: low frequency, HF: high frequency, SD1: standard deviation of instantaneous beat-to-beat variability, SD2: long-term standard deviation of continuous RR intervals.*

been associated with an increase in the sympathetic nervous system and a decrease in the parasympathetic nervous system.[25]

The sample was calculated based on the study conducted by Habesoglu et al.,[26] in which an index of STT was selected. The difference to be detected was 17.19, and the SD was 12.41. The significance level for the sample calculation was 5%, with a test power of 80% and a 2-tailed hypothesis test. The value obtained from the sample calculation was 9 subjects per group.

The duration and intensity of light to moderate resistance tube exercise may have been another associated and limiting factor that was unable to increase sympathetic stimulation and, consequently, decrease transportability time in smokers. Other limiting factors of the study were the lack of heart rate variability analysis in isolated smoke to confirm the autonomic responses found. Thus, further research on the effects of resistance exercise at different intensities and compared to aerobic exercise are suggested in order to identify the effectiveness of this exercise modality, using elastic tubes, which are portable and inexpensive devices that provided the same effectiveness as conventional training with high-cost machines and devices in smokers' respiratory health.

Conclusion

In summary, the intensity of resistance exercise applied in the present study was not enough to promote alterations in the NMC or in the autonomic nervous system of smokers. However, in non-smokers, the same intensity of exercise was effective in causing NMC acceleration and changes in the autonomic nervous system.

Author contributions

Conception and design of the research: Masuda AMM, Trevisan IB, Gouveia TS, Tacao GY, Ramos EMC, Ramos D. Acquisition of data: Masuda AMM, Trevisan IB, Gouveia TS, Tacao GY, Ramos EMC, Ramos D. Analysis and interpretation of the data: Masuda AMM, Trevisan IB, Gouveia TS, Tacao GY, Ramos EMC, Ramos D. Writing of the manuscript: Masuda AMM, Trevisan IB, Ramos EMC, Ramos D. Critical revision of the manuscript for intellectual content: Masuda AMM, Trevisan IB, Ramos EMC, Ramos D.

Potential Conflict of Interest

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

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Study Association

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References

1. Xavier RF, Ramos D, Ito JT, Rodrigues FM, Bertolini GN, Macchione M, et al. Effects of cigarette smoking intensity on the mucociliary clearance of active smokers. Respiration. 2013;86(6):479-85.
2. Ramos EM, Toledo AC, Xavier RF, Fosco LC, Vieira RP, Ramos D, et al. Reversibility of impaired nasal mucociliary clearance in smokers following a smoking cessation programme. Respirology. 2011;16(5):849-55.
3. Proença M, Xavier R, Ramos D, Cavalheri V, Pitta F, Ramos EM. Immediate and short term effects of smoking on nasal mucociliary clearance in smokers. Rev Port Pneumol. 2011;17(4):172-6.
4. Dulger S, Akdeniz O, Solmaz F, Dikiş OS, Yildiz T. Evaluation of nasal mucociliary clearance using saccharin test in smokers: A prospective study. Clin Respir J. 2018;12(4):1706–10.
5. Ramos EM, Vanderlei LC, Ito JT, Lima FF, Rodrigues FM, Manzano BM, et al. Acute mucociliary clearance response to aerobic exercise. Respir Care. 2015;60(11):1575-84.
6. Proença M, Pitta F, Kovelis D, Mantoani LC, Furlanetto KC, Zabatiero J, et al. Mucociliary clearance and its relation with the level of physical activity in daily life in healthy smokers and nonsmokers. Rev Port Pneumol. 2012;18(5):233-8.
7. Manzano BM, Vanderlei LCM, Ramos EMC, Ramos D. Smoking implications on cardiac autonomic control. Arq Ciênc Saúde. 2010 Apr-Jun; 17(2): 97-101
8. Leite MR, Ramos EM, Kalva-Filho CA, Freire AP, de Alencar Silva BS, Nicolino J, et al. Effects of 12 weeks of aerobic training on autonomic modulation, mucociliary clearance, and aerobic parameters in patients with COPD. Int J Chron Obstruct Pulmon Dis. 2015;10:2549-57.
9. Silva BS, Ramos D, Bertolini GN, Freire AP, Leite MR, Camillo CA, et al. Ramos. Resistance exercise training improves mucociliary clearance in subjects with COPD: A randomized clinical trial. Pulmonol. 2019;25(6):340-7.

10. Wolff RK, Dolovich MB, Obminski G, Newhouse MT. Effects of exercise and eucapnic hyperventilation on bronchial clearance in man. J Appl Physiol. 1977;43(1):46-50.

11. Ramos EM, Toledo-Arruda AC, Fosco LC, Bonfim R, Bertolini GN, Guarnier FA, et al. The effects of elastic tubing-based resistance training compared with conventional resistance training in patients with moderate chronic obstructive pulmonary disease: a randomized clinical trial. Clin Rehabil. 2014;28(11):1096-106.

12. Meneses-Gaya IC, Zuardi AW, Loureiro SR, Crippa JAS. As propriedades psicométricas do teste de fagerström para dependência de nicotina. J Bras Pneumol. 2009;35(1):73-82.

13. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardization of spirometry. Eur Respir J. 2005;26(2):319-38.

14. Duarte AA, Pereira CA, Rodrigues SC. Validation of new Brazilian predicted values for forced spirometry in Caucasians and comparison with predicted values obtained using other reference equations. J Bras Pneumol. 2007;33(5):527-35.

15. Lopes JS, Micheletti JK, Machado AF, Souto LR, Lima HP, Vanderlei FM, et al. Test-retest reliability of knee extensors endurance test with elastic resistance. PLoS One. 2018 Aug 31;13(8):e0203259.

16. Lima FF, Camillo CA, Reis EA, Job AE, Silva BS, Topalovic M, et al. Mechanical properties, safety and resistance values of Lemgruber((R)) elastic tubing. Braz J Phys Ther. 2018;23(1):41-7.

17. Vanderlei LC, Silva RA, Pastre CM, Azevedo FM, Godoy MF. Comparison of the Polar S810i monitor and the ECG for the analysis of heart rate variability in the time and frequency domains. Braz J Med Biol Res. 2008;41(10):854-9.

18. Tarvainen MP, Niskanen J-P, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV – A software for advanced heartrate variability analysis. Berlin: Springer: In: 4th European Conference os the International Federation for Medical and Biological Engineering. Berlin:Springer; 2008. p.1022-5.

19. Vanderlei LC, Pastre CM, Hoshi RA, Carvalho TD, Godoy MF. Noções básicas de variabilidade da frequência cardíaca e sua aplicabilidade clínica. Rev Bras Cir Cardiovasc. 2009;24(2):205-17.

20. Ferreira MT, Messias M, Vanderlei LC, Pastre CM. Caracterização do comportamento caótico da variabilidade da frequência cardíaca (VFC) em jovens saudáveis. TEMA Tend Mat Apl Comput. 2010;11(2):141-50.

21. Godoy MF, Takakura IT, Correa PR. Relevância da análise do comportamento dinâmico não linear (Teoria do Caos) como elemento prognóstico de morbidade e mortalidade em pacientes submetidos à cirurgia de revascularização miocárdica. Arq Ciênc Saúde. 2005; 12(4):167-71.

22. Mendonca GV, Pereira FD, Fernhall B. Effects of cigarette smoking on cardiac autonomic function during dynamic exercise. J Sports Sci. 2011;29(9):879-86.

23. Santos AP, Ramos D, Oliveira GM, Santos AA, Freire AP, Ito JT, et al. Influence of Smoking Consumption and Nicotine Dependence Degree in Cardiac Autonomic Modulation. Arquivos Brasileiros de Cardiologia. 2016;106(6):510-8.

24. Middlekauff HR, Park J, Moheimani RS. Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. J Am Coll Cardiol. 2014;64(16):1740-50.

25. Sumartiningsih S, Lin HF, Lin JC. Cigarette Smoking Blunts Exercise-Induced Heart Rate Response among Young Adult Male Smokers. Int J Environ Res Public Health. 2019;16(6):1032.

26. Habesoglu M, Demir K, Yumusakhuylu AC,Yilmaz AS, Oysu C. Does Passive Smoking Have an Effect on Nasal Mucociliary Clearance? Otolaryngol Head Neck Surg. 2014; 147(1): 151-6.

VIEWPOINT

Research and Publication in Brazil: Where we are and Where we Head to

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In a report by Clarivate Analytics for the Brazilian Coordination for the Improvement of Higher-Level-Education Personnel (CAPES) about the Brazilian research productivity between 2013 and 2018, Brazil ranked 13th (250 680 publications) among countries with the highest research productivity, corresponding to 11% and 16% of the first ranked countries, United States and China, respectively.[1,2] In that period, the publications in Brazil increased by 30%, twice the global mean,[2] with over 50 000 articles published in 2018 only.

A good example of that increase has been described in the study analyzing the number of papers published in *Nature* and *Science* from the University of São Paulo, University of Campinas, and the Federal University of Rio de Janeiro, from 1980 onwards. The publication counts were categorized by decade from 1980 to 2010 and for 2017. The authors have reported that those institutions together published 0.08 papers, on average, in each edition of those two journals. The total number of papers from those universities increased by 2200% from 1980 to 2017, with a higher representativity in the past decade (from 7 papers in 2010 to 23 in 2017). It is worth noting the increase in partnership with international institutions for the publication of scientific papers.[3]

It is important to emphasize that the reputation of teaching and research institutions is directly related to the volume, quality and global influence of their publications. It is worth noting that research depends on the availability of local resources and occasional opportunities for international collaboration.[2]

Keywords

Databases, Bibliographics; Scientific Publications; Brazil; Bibliometrics; Portals for Scientific Journals.

A supplement published by Nature in 2016 assessed a group of 68 journals, which represented less than 1% of the journals of the *Journal Citation Reports* but accounted for 30% of the citations in natural sciences from several countries. Count, fractional count, and weighted fractional count of articles were used for that assessment. The authors showed differences between countries of the same region, such as Chile and Brazil.[4] The papers published by Chile showed a significant international participation via collaborative co-authorship and funding of high-cost projects, with an efficiency 15 times higher than that of Brazilian publications. In 2013, 717 Chilean articles were published, at the cost of 2 billion dollars, as compared to 670 Brazilian studies, at the cost of 30 billion dollars.[4] However, the way the journals were selected as well as the metrics proposed in the supplement were harshly criticized. One of the reasons is the lack of representativeness, in that analysis, of the most influential journals of the 20th century, such as the *New England Journal of Medicine, JAMA, BMJ, American Journal of Botany, Journal of Zoology, American Journal of Physical Anthropology,* and *Journal of Paleontology*. The warning for the cost and efficiency of the publications with international collaboration was considerable.[5]

However, the percentage of Brazilian publications among the *Top 1%* of the most cited articles worldwide, those with a mean citation impact of 4.0 and over, more than doubled from 2011 to 2016, reaching the world mean between 2015 and 2016. When expanding that metric to the Top 10%, only 6.4% of the Brazilian publications were contemplated, far below the world mean.[1,2] Approximately one third of the Brazilian publications among the Top 10% counted with the collaborative partnership of researchers who networked in 205 countries and obtained the highest number of citations, especially those in collaboration with emerging countries, such as China and India.[2]

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It is important noting that the number of Brazilian periodicals comprising the database of international publications in the 2011-2016 period increased by 400%, which might have influenced the difference in the metrics previously cited.[5]

Regarding the proportion of collaborations in Health Sciences and Biological Sciences, the United States was Brazil's main partner (40% to 50%), followed by European countries and Argentina.[2] Papers published in partnership in Health Sciences have the highest citation impact, ranging from 4.13 (Spain) to 15.51 (Russia).[2]

The report by Clarivate Analytics has shown that the citations of Brazilian publications were below the world mean (0.88 %) in 2017, despite the 15% increase in the number of citations in the past 20 years. The Brazilian fields with citation impact close to the world mean were environment, ecology, psychiatry, psychology, and mathematics. Regarding health sciences, the highest citation impact was observed in oncology, infectious diseases, neurology, odontology, surgery, oral medicine, and cardiovascular sciences, which were outshined only by internal medicine, whose citation impact was 0.87, as compared to 0.76 of the other Brazilian publications.[1,2]

It is worth noting that the quality assessment of scientific productivity by using citation frequency has been harshly criticized. This metrics has limitations for the individual assessment of the researcher, the productivity of specific fields, and the comparability between journals of different coverages. This metrics is influenced by some factors, such as biases against the language of origin and the gender of authors, as well as the number of researchers involved in the scientific production.[6]

There is great discussion about the bias associated with the authorship proportion of men and women, either as first, last, or corresponding authors. A study carried out between 1980 and 2017 with data from 55 085 articles from the *Journal of the American College of Cardiology, Circulation*, and *European Heart Journal* showed that female authors accounted for 33.1% of all authors but represented only 26.7% of first authors and 19.7% of senior authors. In addition, women corresponded to only 5% of the 100 most prolific authors in cardiovascular research in those journals. The authors concluded that the female representation in published cardiology research has increased over the past 4 decades, but women continue to be underrepresented when considering the total number of female researchers.[7]

The study analyzing citations from 2379 articles from the top 20 impact cardiology journals from the Entrez database on PubMed, in 2017, has reported that less than one third of the publications of single authors had a female authorship. Of the publications with multiple authors, only 15% of the corresponding authors were women. However, in articles with female researchers as senior authors, more women were coauthors as compared to those with male senior authors. That study has risen the hypothesis that senior authorship would be a way to identify cardiology research leadership, reinforcing the gender bias in cardiovascular science publications.[8]

It is worth highlighting the most studied and most cited fields in cardiovascular research. The study assessing the publications in 47 topics associated to the field of cardiovascular disease has listed 5 topic clusters with more than 400 publications each between 2014 and 2018. The cluster topic 'percutaneous coronary intervention and myocardial infarction' accounted for the greatest number of publications, followed by 'atrial fibrillation and catheter ablation', and 'cholesterol, lipids and atherosclerosis'. All those topics had weighted citation index for the field higher than 1, in addition to collaboration of foreign authors in one third of the articles.[9]

Regarding the impact of Brazilian papers in patent generation, 16 patents issued in the cardiovascular field have cited Brazilian papers on coronary artery bypass grafting, atherosclerotic plaque vulnerability and inflammation, statin therapy, and pulmonary hypertension.[9] Most of those papers and patents cited originated from the São Paulo state, and the University of São Paulo accounted for 20% of the Brazilian production, followed by the University of Campinas and the Federal University of Rio de Janeiro.[2,10]

In Brazil, public universities were the leading institutions regarding research, citation impact, and relationship with industry. All 15 most prolific organizations, 13 universities and 2 specialized research institutes, are public.[2]

The increase in the Brazilian intellectual production has been attributed to the annual investment goal of 2% of the Gross Domestic Product (GDP) as part of the Brazilian Strategy in Science, Technology, and Innovation 2016-2019 (ENCTI 2016-19), which has focused on innovation to boost socioeconomic development. That annual investment goal, however, has not been met because the universities received only 60% of that value (1.2% of the GDP) as a result of the world economic crisis.[1,2]

On March 29, 2019, the Brazilian government announced the preventive blockade of R$ 30 billion (US$ 7.5 billion) from the annual budget, including 2.2 billion from the budget of the Ministry of Science, Technology, and Innovations, which would be used to honor the ENCTI 2016-19. This hindered research financing in Brazil. Those sums were allocated as contingency funds and could only be spent in case of fiscal recovery or new income sources.[11]

With the Budget Law Project 2020, part of the resources destined to promote research, including those to provide for CAPES research grants, has been recovered. The mo*bilization of the scientific community was fundamental for scientific research and publications to gain additional momentum,[12] although investments in research fell beneath the value suggested by the ENCTI 2016-19.*

Additional initiatives, such as the maintenance of cardiovascular journals, promote the dissemination of Brazilian research. The *Arquivos Brazileiros de Cardiologia* (*ABC Cardiol*) and the *International Journal of Cardiovascular Sciences* (*IJCS*), supported by the Brazilian Society of Cardiology, are examples of private funding for the dissemination of knowledge in the field.

The *ABC Cardiol* is currently the cardiology publication with the highest impact in Brazil and Latin America. It is a 71-year-old journal, indexed in the major journal databases. The *IJCS*, created in 2015, replaced the *Revista da SOCERJ* and the *Revista Brasileira de Cardiologia*, created in 1998 and 2010, respectively. The *IJCS* was indexed in SciELO and DOAJ in 2017 and awaits assessment for indexation in Scopus and Web of Science.[13,14]

Approximately 65% of the original articles published in the *ABC Cardiol* are contributions from postgraduation programs. Thus, the major cardiology publications are in accordance with the mission of the SBC, which aims at increasing and spreading knowledge about the cardiovascular science, representing cardiologists and promoting their development, in addition to enhancing cardiovascular health in Brazil. The creation of population-based registries of cardiovascular disease is part of SBC attributions.[15] Those population-based registries can contribute with information on several epidemiological aspects as well as with daily clinical practice, providing statistical data with potential impact on the improvement of routine medical practice. The use of population-based registries to assess the outcomes and cost-effectiveness of already implemented conducts has the advantage of analyzing interventions in the 'real world' in clinical practice. They have acquired substantial relevance in medical publications in recent decades and have been the object of research of the SBC in past years.[16,17]

Population-based registries and pragmatic clinical trials, designed to assess efficacy in real clinical practice, have less selection bias.[16] The recent registry-based randomized controlled trials are defined as pragmatic clinical trials that use registries as a platform for data collection, randomization, and follow-up.[18-20] Usually, the use of registries by the SBC has allowed the improvement of the knowledge about the Brazilian cardiovascular health and has provided actions to implement the best clinical practices.[21-25] The SBC has taken initiatives regarding national research in partnership with international medical societies, such as the project "Best Practices in Cardiology - Brazil (BPC-Brazil)" with the American Heart Association (AHA). In the future, registries for quality control will be able to provide additional information to be conveyed into actions to improve cardiovascular care.[17]

Despite the substantial efforts of researchers and the highly qualified centers in research fields, the major obstacle to creating population-based registries in Brazil is that of a financial nature. The planning and execution of a research project require a model of promotion and achievement of resources. Gliklich *et al*.[17] have suggested that, in case of registries of national or regional relevance, the costs can be subsidized by provider organizations (*e.g*., hospitals, clinics, government agencies, and foundations that support research), professional associations, medical specialty societies, and the pharmaceutical and medical device industries. The support of the private sector to fund cardiovascular health research in Brazil would be of great help. Although the SBC is not an institution primarily dedicated to research, it feels responsible, with the essential support of the public and private sectors, for proposing, promoting, exhorting, and disseminating effective actions to develop cardiovascular research in Brazil.

Aiming at valuing cardiovascular research, the SBC discloses the best studies performed in Brazilian postgraduation programs. The 'SBC Award for Best Theses – 2020 Edition' will be granted to the best dissertations and theses of postgraduation programs (professional and academic master's degree and doctorate degree) defended in 2019. The theses and dissertations will be selected from the Brazilian System of Postgraduation in Cardiology in Medicine I from CAPES programs.[26]

The recent challenge to human health represented by the COVID-19 pandemic, the increase in chronic diseases, and the population ageing will accelerate translational

research, which will need to rapidly benefit patients via transfer of knowledge generated in laboratory benches.

In addition, the COVID-19 pandemic has emphasized the importance of collaboration of international research groups to solve an emerging global problem that causes many health losses and has severe socioeconomic implications.

Several molecular and genetic tools have been developed in recent years and will be able to accelerate the development of new drugs, such as vaccines. It is worth noting that the interdisciplinary activity will be strengthened, playing a fundamental role in the solution of common problems targeting a sustainable future for mankind.

The use of artificial intelligence, the incorporation of large databases with emphasis on big data, and the Precision Medicine will provide an immeasurable advance.

The integration of the world funding sources, with the participation of the private and public sectors, will allow the advance on understanding the pathophysiology of cardiovascular diseases and on multidisciplinary solutions for their treatment.

The population is increasingly aware of their food and their leisure activities. This is a unique opportunity for research focused on changes in lifestyle, such as the effects of food processing and practice of physical exercises on cardiovascular health.

In conclusion, despite the advance of medical research in Brazil in past decades, there are considerable gaps of knowledge to be filled regarding Brazilian cardiovascular health. The challenges in the coming years are significant, but the Brazilian cardiovascular research is being rapidly and proficiently organized, with a high potential of response to the Brazilian needs.

Author contributions

Conception and design of the research: Oliveira GMM, Lopes MACQ, Brasil D. Acquisition of data: Oliveira GMM, Lopes MACQ, Brasil D. Analysis and interpretation of the data: Oliveira GMM, Lopes MACQ, Brasil D. Writing of the manuscript: Oliveira GMM, Lopes MACQ, Brasil D. Critical revision of the manuscript for intellectual content: Oliveira GMM, Lopes MACQ, Brasil D.

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

References

1. Cross D, Thomson S, Sinclair A. Research in Brazil: a report for CAPES by Clarivate Analytics [Internet]. 2017 [citado 30 maio 2020]. Disponível em: http://portal.andes.org.br/imprensa/noticias/imp-ult-992337666.pdf
2. A Pesquisa no Brasil: Promovendo a excelência. Análise preparada para a CAPES pelo Grupo Web of Science [Internet]. 2019 [citado 30 maio 2020]. Disponível em: https://propp.ufms.br/files/2019/09/Pesquisa-no-Brasil.pdf
3. Carli GJ, Pereira TC. Sharp rise in premier papers from Brazilian universities. Nature. 2018;556(7701):309.
4. NATURE INDEX. 2016 [Internet]. 2016 [citado 30 maio 2020]. Disponível em: https://www.natureindex.com/annual-tables/2016/institution/all/all
5. Silva V. Scientometrics: Nature Index and Brazilian science. An Acad Bras Ciênc. 2016;88(3):1597-9.
6. Opthof T. Differences in citation frequency of clinical and basic science papers in cardiovascular research. Med Biol Eng Comput. 2011;49(6):613-21.
7. Ouyang D, Sing D, Shah S, Hu J, Duvernoy C, Harrington RA, et al. Sex disparities in authorship order of cardiology scientific publications. Circ Cardiovasc Qual Outcomes. 2018;11(12):e005040.
8. Ouyang D, Harrington RA, Rodriguez F. Association between female corresponding authors and female co-authors in top contemporary cardiovascular medicine journals. Circulation. 2019;139(8):1127-9.
9. Krauskopf E. Cardiovascular disease: The Brazilian research contribution. Braz J Cardiovasc Surg. 2019;34(5):VI-IX.
10. Pontes P. Brazilian scientific production vs. innovation and technology. Braz J Otorhinolaryngol. 2015;81(4):343-4.
11. Escobar H. We can't take another hit like this': Brazilian scientists lament big budget freeze; 2019. Science [Internet]. [citado 30 maio 2020]. Disponível em: https://www.sciencemag.org/news/2019/04/we-cant-take-another-hit-brazilian-scientists-lament-big-budget-freeze
12. Mazza M. PLOA 2020 é aprovado pelo Congresso Nacional com pequenos acréscimos na Ciência; 2019. Jornal da Ciência [Internet]. [citado 30 maio 2020]. Disponível em: http://www.jornaldaciencia.org.br/ploa-2020-e-aprovado-pelo-congresso-nacional-com-acrescimos-na-ciencia/
13. Evora PRB, Moreira LFP. The representativeness of the Arquivos Brasileiros de Cardiologia for Brazilian Cardiology Science. Arq Bras Cardiol. 2015;104(2):94-6.
14. Moreira LFP. Role of the International Journal of Cardiovascular Sciences in the Increase of Brazilian publications in Cardiology and Cardiovascular Sciences. Arq. Bras. Cardiol. 2016;107(1):1.
15. Rochitte CE, Quadros AS, Sousa AGMR, Ladeia AMT, Brandão AA, Lorenzo A, et al. Arquivos Brasileiros de Cardiologia (ABC Cardiol) and the new classification Qualis of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Arq Bras Cardiol. 2019;113(3):333-4.

16. Barnish MS, Turner S. The value of pragmatic and observational studies in health care and public health. Pragmat Obs Res. 2017;8:49-55.

17. Agency for Healthcare Research and Quality [Internet]. Effective Health Care Program; 2020. [citado 30 maio 2020]. Disponível em: https://effectivehealthcare.ahrq.gov/products/registries-guide-3rd-edition/research.

18. Gilmartin-Thomas JFM, Liew D, Hopper I. Observational studies and their utility for practice. Aust Prescr. 2018;41(3):82-5.

19. Li G, Sajobi TT, Menon BK, Korngut L, Lowerison M, James M, et al. Registry-based randomized controlled trials- what are the advantages, challenges, and areas for future research? J Clin Epidemiol. 2016 Dec;80:16-24.

20. Bartlett VL, Dhruva SS, Shah ND, Ryan P, Ross JS. Feasibility of using real-world data to replicate clinical trial evidence. JAMA Netw Open. 2019;2(10):e1912869.

21. Wang R, Neuenschwander FC, Lima Filho A, Moreira CM, Santos ES, Reis HJL, et al. Use of evidence-based interventions in acute coronary syndrome - Subanalysis of the ACCEPT registry. Arq Bras Cardiol. 2014;102(4):319-26.

22. Piva e Mattos LA, Berwanger O, Santos ES, Reis HJL, Romano ER, Petriz JLF, et al. Clinical outcomes at 30 days in the Brazilian Registry of Acute Coronary Syndromes (ACCEPT). Arq Bras Cardiol. 2013;100(1):6-13.

23. Berwanger O, Piva e Mattos LA, Martin JFV, Lopes RD, Figueiredo EL, Magnoni D, et al. Evidence-based therapy prescription in high-cardiovascular risk patients: the REACT study. Arq Bras Cardiol. 2013;100(3):212-20.

24. Jardim PCBV, Souza WKSB, Lopes RD, Brandão AA, Malachias MVB, Gomes MM, et al. I RBH - First Brazilian Hypertension Registry. Arq Bras Cardiol. 2016;107(2):93-8.

25. Albuquerque DC, Souza Neto JD, Bacal F, Rohde LEP, Bernardez-Pereira S, Berwanger O, et al. I Brazilian Registry of Heart Failure - Clinical Aspects, Care Quality and Hospitalization Outcomes. Arq Bras Cardiol. 2015;104(6):433-42.

26. Sociedade Brasileira de Cardiologia. Prêmio Tese da SBC. Edição 2020 [Internet]. 2020 [citado 30 maio 2020]. Disponível em: http://publicacoes.cardiol.br/portal/portal-publicacoes/portugues/premio-tese/Edital_Premio_Teses_SBC.pdf

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CASE REPORT

Acute Myocardial Infarction as the Initial Clinical Manifestation of Pernicious Anemia

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Introduction

Despite its lower prevalence in young individuals, acute myocardial infarction (AMI) causes significant morbidity and socioeconomic impact when occurs in this group. In 2010, considering only the hospitals accredited by the Brazilian Unified Health System, there were 8,368 hospitalizations and 2,556 deaths from ischemic heart diseases among patients younger than 40 years.[1] Although hospitalization rates are lower at this age range, the importance of this theme lies mainly in the absolute numbers of cases.

Acute coronary syndrome (ACS) in young patients has characteristics that differ from older patients. Although coronary atherosclerotic disease is a common cause, the prevalence of AMI without obstruction in the coronary arteries is higher among the young.[2] Among the causes of AMI without atheromatous disease, thromboembolism is a differential diagnosis, with hypercoagulability correlated with formation of coronary embolism. Thus, it is important to identify alternative etiologies in the approach of AMI in young patients since modifiable factors can be diagnosed and treated. In this article, we present a case in which an autoimmune disease may be the cause of ACS in a young patient with few attributable risk factors for cardiovascular disease.

Clinical case

Male patient, 29 years old, non-smoker, non-diabetic, with hypertension controlled with monotherapy, with vitiligo and compensated hyperthyroidism, treated with propylthiouracil. The patient was admitted to the emergency department with angina-like, exertional chest pain that began more than 24 hours during the first medical visit. Electrocardiography revealed signs of transmural myocardial infarction of lateral wall in progression. The patient showed an important reduction of pain intensity, but was still symptomatic, and received anti-ischemic treatment (beta-blocker, intravenous nitrate), antithrombotic treatment (acetylsalicylic acid, ticagrelor, unfractionated heparin), and referred for emergency coronary angiography, which showed thrombotic occlusion of the left marginal artery (Figure 1), and no evidence of atheromatous plaques in the other coronary arteries.

Percutaneous coronary intervention with stent implantation was performed, with concomitant administration of glycoprotein iib/iiia inhibitors due to the high thrombus load, with restoration of epicardial blood flow (TIMI 3). Echocardiography of control revealed hypokinesia of the inferolateral wall, with mildly abnormal ejection fraction (47%). The patient remained asymptomatic after the procedure; tests for thrombophilia were performed, with normal results. Renal function and thyroid function were preserved, and the patient had macrocytic anemia (Hb 10.0 g/dL / VCM 117fl). The patient was discharged with optimized therapy for secondary prevention and a plan of outpatient follow-up for investigation of anemia.

After 30 days, the patient was referred from a health care center presenting with the skin pale and complete blood count compatible with severe anemia (hemoglobin of 5.6 g/dL), with macrocytosis (MCV 123fl), without hemodynamic instability or signs of bleeding. Upper endoscopy was performed and showed a pattern of atrophic gastritis, which was later confirmed by biopsy

Keywords

Myocardial Infarction/economics; Anemia,Pericious; Homocysteine; Mortality & Morbidity; Percutaneous Coronary Intervention/economics.

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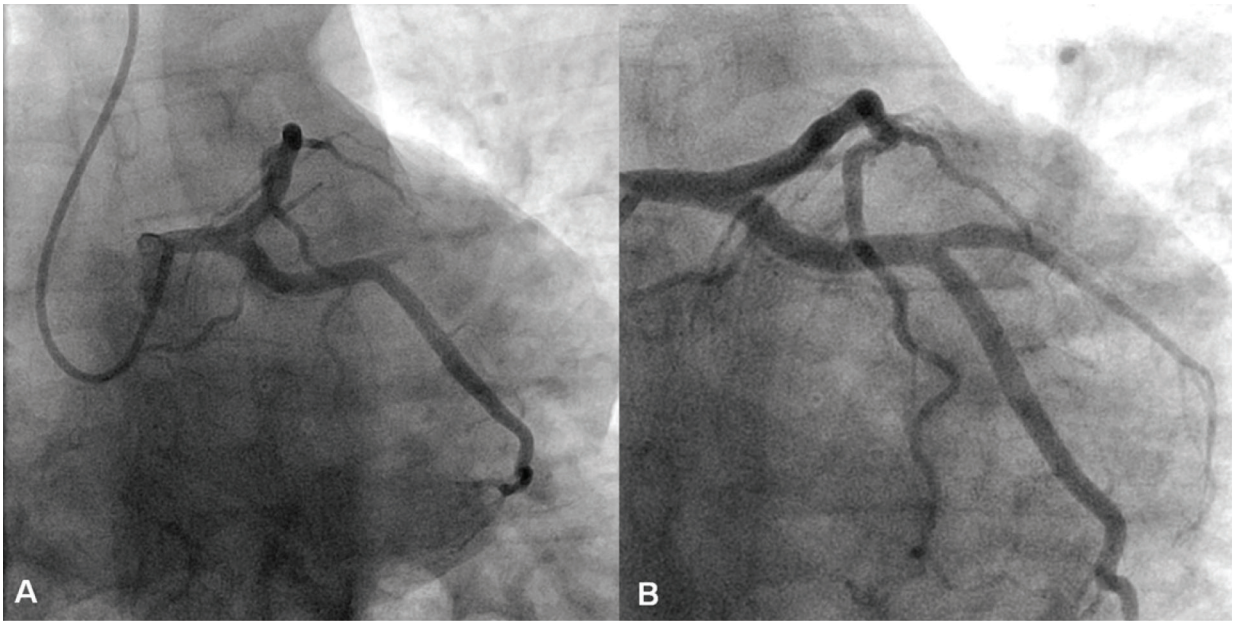

Figure 1 - A) Thrombotic occlusion in the left marginal artery; B) Post-percutaneous coronary intervention with stent implantation.

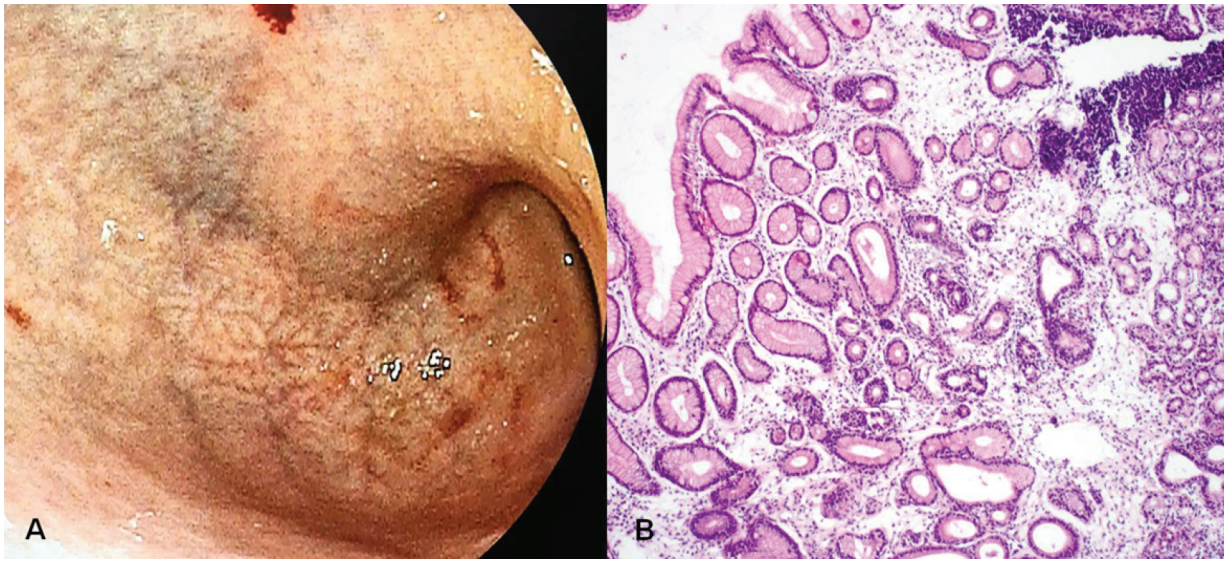

Figure 2 - A) Upper gastrointestinal endoscopy showing atrophic gastritis in gastric body; B) Histological slice (HE 100x) showing complete loss of oxyntic glands and lymphocytic infiltrate in the gastric body mucosa

(Figure 2). Blood tests showed undetectable levels of vitamin B12 (< 83 pg/mL) and increased homocysteine levels by approximately 10 times the upper reference level (155.85 mmol/L). These results led to the diagnosis of pernicious anemia, and the therapy with intramuscular injection of vitamin B12 was started as recommended. After one month of therapy, patient was asymptomatic, the levels of homocysteine decreased to 8.44 mmol/L, with normalization of complete blood count (hemoglobin 14.3g/dL and MCV 82.8fl).

Discussion

Pernicious anemia is the most common cause of vitamin B12 deficiency.[3] It is an autoimmune disease associated with atrophic gastritis characterized by synthesis of antibodies against parietal cells of the stomach, inhibiting the synthesis of intrinsic factor, which is an essential binder for the absorption of cobalamin in the terminal ileum. In the intracellular milieu, vitamin B12 is the cofactor for conversion of homocysteine to methionine and tetrahydrofolate, the active form of folic acid. Thus, in case of cobalamin deficiency, the synthesis of cellular DNA is impaired, resulting in megaloblastic anemia and accumulation of homocysteine in the plasma.[4]

Hyperhomocysteinemia has been shown as a risk factor for cardiovascular disease, leading to atheroma plaque formation, and arterial or venous thrombosis, involving vessels of all sizes. The association between high homocysteine levels and thrombosis may be related with platelet dysfunction, thrombin generation and decreased fibrinolysis. Regarding atherosclerotic coronary disease, hyperhomocysteinemia has been associated with increased oxidative stress and proliferation of the vascular smooth muscle, resulting in endothelial injury and atheromatous plaque formation.[5] Individuals with plasma homocysteine levels 12% above the upper normal limit (15 mmol/L) have a three times greater risk of developing an AMI compared with those with homocysteine levels below 10 mmoL/L, even after correction for other risk factors.[6] An increase by 5 mmoL/L in homocysteine levels increases the relative risk for myocardial ischemia by 1.7 times.[7]

A possible relationship between hyperhomocysteinemia and coronary artery disease was initially suggested in 1976.[8] However, hyperhomocysteinemia has not been established as an isolated risk factor for cardiovascular disease, considering that, so far, current evidence is not sufficient to support or reject this association. In some groups of patients, however, determination of serum levels of this amino acid may be recommended, particularly in those with few traditional risk factors, patients with premature atherosclerosis and young patients with diagnosis of AMI or stroke.

Two thirds of the cases of hyperhomocysteinemia are related with low vitamin B12 concentrarions.[9] Thus, conditions that lead to low plasma cobalamin levels, such as pernicious anemia, may have close relationship with atherosclerotic or thrombotic vascular disease. Considering such association, the investigation of pernicious anemia as differential diagnosis of coronary disease in young patients.

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Author contributions

Conception and design of the research: Silva LMA, Barros Jr, AXS. Acquisition of data: Silva LMA, Barros Jr, AXS, Galina JOT. Analysis and interpretation of the data: Silva LMA, Andrade PB. Writing of the manuscript: Silva LMA, Andrade PBA. Critical revision of the manuscript for intellectual content: Rodrigues A, Bienert IRC, Andrade PBA.

Potential Conflict of Interest

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

Sources of Funding

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Study Association

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

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the FAMEMA under the protocol number 08051019.9.0000.5413. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

References

1. Brasil. Ministério da Saúde. DATASUS: banco de dados. [Internet]. {Acesso em 2019 jan 13]. Disponível em: <http://tabnet.datasus.gov.br/cgi/deftohtm.exe?idb2011/d29.def>.
2. Agewall S, Beltrame JF, Reynolds HR, Niessner A, Rosano G, Caforio, ALP, et al. ESC working group position paper on myocardial infarction with non-obstructive coronary arteries. Eur Heart J. 2017;38(3):143-53.
3. Toh BH, van Driel, IR, Gleeson, PA. Pernicious anemia. N Engl J Med. 1997; 337(20):1441-8.
4. Antony AC. Anemias megaloblásticas. In: Goldman L, Shafer AI (orgs). Cecil Medicina. 24 ed. Rio de Janeiro: Atheneu;2014. p.1230-40
5. Kuller LH, Evans RW. Homocysteine, vitamins, and cardiovascular disease. Circulation. 1998;98(3):196-9.
6. Arruda VR, von Zuben PM, Chiaparini LC, Annichino-Bizzacchi JM, Costa FF. The mutation Ala677-->Val in the methylene tetrahydrofolate reductase gene: a risk factor for arterial disease and venous thrombosis. Thromb Haemost. 1997; 77(5):818-21.
7. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med. 1997;337(4):230-6.
8. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham, I. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med. 1991;324(17):1149-55.
9. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, et al. A prospective study of plasma homocyst(e)ine and risk factor of myocardial infarction in US Phisicians. JAMA. 1992;268(7):877-81.

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CASE REPORT

Double-lumen Aortic Arch: Persistence of the Fifth Aortic Arch?

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Abstract

Double-lumen aortic arch is a rare congenital anomaly related to persistence of the fifth aortic arch. It may be found alone or in association with other anatomical changes of the heart. We report a case of double-lumen aortic arch associated with coarctation of the aorta and patent ductus arteriosus in a child with a congenital malformation known as the VACTERL association (vertebral defects, imperforate anus, cardiopathy, tracheoesophageal fistula, renal abnormalities and limb anomalies).

Introduction

Double-lumen aortic arch is a rare, usually underdiagnosed congenital anomaly, possibly secondary to the persistence of the fifth embryonic aortic arch or the presence of dorsal collateral arteries that connect the fourth and sixth aortic arch.[1-3] The authors report a case of double-lumen aortic arch associated with coarctation of the aorta and ductus arteriosus in a child with congenital malformation characterized by presence of vertebral defects, imperforate anus, heart disease, tracheoesophageal fistula, renal anomalies and limb abnormalities, known as the VACTERL association, which was accurately diagnosed by echocardiography and confirmed by computed tomography.

Keywords

Aorta,Thoracic/ abnormalities; Vascular Ring; Echocardiography/ methods; Vascular Malformation/ diagnostic imaging.

Case Report

Seven-year-old child with VACTERL association, with previous history of surgical treatment for ductus arteriosus and repair of aortic coarctation in the first year of life. During hospitalization for reconstruction of intestinal transit, an echocardiography revealed dextrocardia, bicuspid aortic valve, significant ascending aortic enlargement (z-score +5.04) and transverse aorta with two lumens, compatible with double-lumen aortic arch. Both anterior and posterior aortic lumens were unobstructed, with laminar flow, measuring about 14.6 mm and 6.5 mm, respectively. The cephalic veins emerged from the anterior lumen, and the posterior lumen originated proximal to the brachiocephalic artery and ended in the descending aorta (Figures 1a and b, 2a, video 1). The echocardiographic findings were confirmed by chest computed tomography, which also showed the presence of two brachiocephalic trunks (Figure 2b).

Discussion

Double-lumen aortic arch, an exceptionally rare congenital anomaly,[4] was first described in humans by Van Praagh and Van Praagh[5] in 1969, and persistence of the fifth aortic arch was proposed as the cause of this condition. Descriptions of persistence of the fifth arch in the literature have been limited to case reports. Brown in 1913 and Huntington in 1919 reported the first hypotheses for the development of this persistent arch.[4]

However, Gupta et al.,[2] studying developing human embryos, identified remnants of an unambiguous fifth arch artery in only one embryo and showed that dorsal collateral canals connecting the fourth and sixth arches are much more common and may best explain the formation of a double aortic lumen. According to these authors, a persistent fifth aortic arch is defined as an

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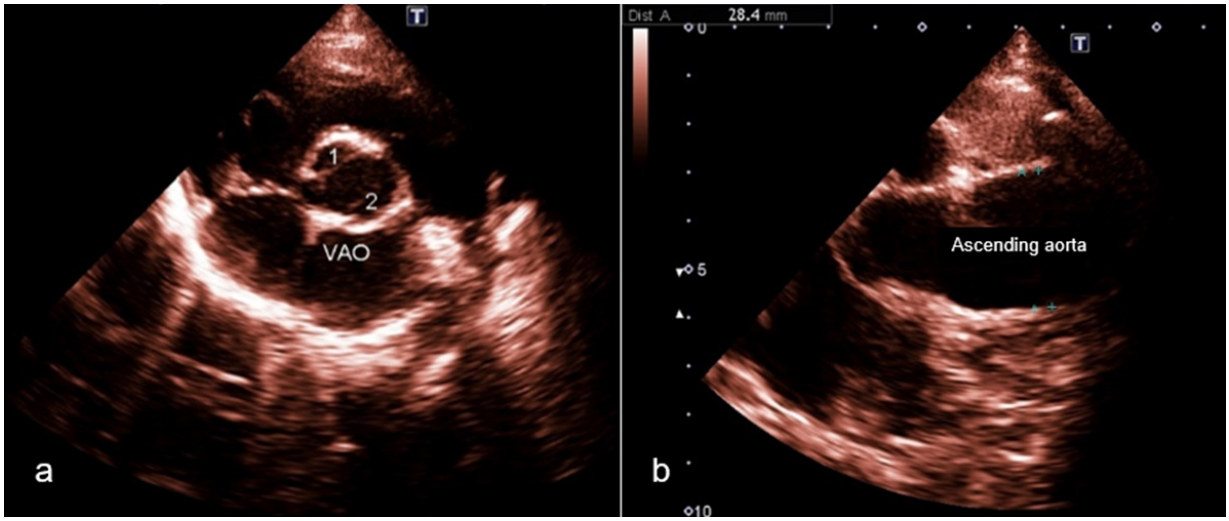

Figure 1 - Two-dimensional mode echocardiogram: (a) Bicuspid aortic valve; (b) Significant enlargement of the ascending aorta.
VAo: aortic valve.

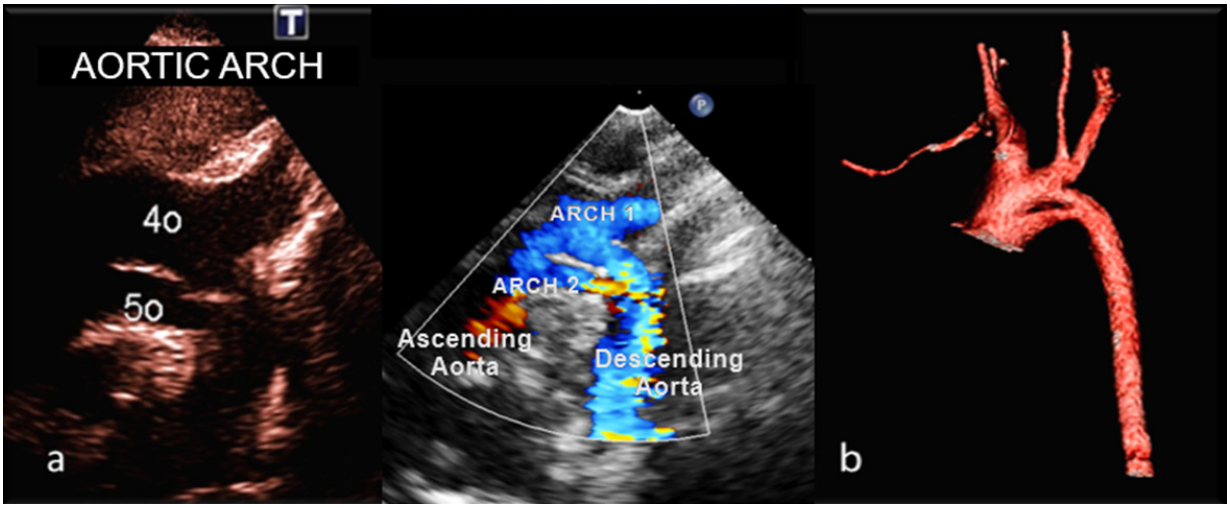

Figure 2 - Double-lumen aortic arch: (a) two-dimensional echocardiography in the suprasternal view.

extrapericardial arch that arises from the ascending aorta proximal to the brachiocephalic artery, runs a serpentine course and terminates in the dorsal aorta or in the sixth aortic arch of the pulmonary artery.[2]

Double-lumen aortic arch can be classified into:

I. Double lumen with both lumens patent;

II. Atresia or interruption of one (upper) lumen and patency of the other;

III. Connection between the lower lumen and the pulmonary artery.[6,7]

Although frequently associated with several cardiac defects, such as coarctation or interruption of the aorta, patent ductus arteriosus, tetralogy of Fallot, atrioventricular septal defect, truncus arteriosus, pulmonary and tricuspid atresia, this anomaly can also be found alone.[3,8]

The clinical presentation of double-lumen aortic arch depends on the type of connection and presence of associated anomalies. Type I, as reported here, is the most common form, usually of no clinical significance. Types II and III have positive hemodynamic consequences, by providing an alternative systemic arch in case of coarctation or aortic arch interruption (type II), and by acting as a systemic-pulmonary shunt when associated with pulmonary or tricuspid atresia (type III).[3,6-8]

In the present case, both arches ran on the same side of the trachea and, unlike the classical double aortic arch, which involves both trachea and esophagus, and does not result in a vascular ring.[6] Thus, it may correspond to a non-classical double arch possibly secondary to the presence of collaterals between the fourth and sixth artery arches, as described by Gupta et.al. Differential diagnosis should be made with patent ductus arteriosus and aortopulmonary window, especially in type III.[1,3,6,7]

Echocardiography allows the detection of two lumens of the aortic arch running in parallel and helps in the identification of coexisting anomalies. Computed tomography and magnetic resonance imaging help define the anatomy of the aortic arch and its variants, confirm the diagnosis of this anomaly and detect possible coexisting abnormalities such as pulmonary or cardiovascular diseases.[6]

Conclusion

Type I aortic double lumen is an occasional finding with no significant hemodynamic consequences. We described a case of double-lumen aortic arch, possibly caused by factors related to a non-classical double arch, including formation of dorsal collaterals, rather than a persistence of the fifth aortic arch. Detection of this anomaly is important for familiarization with the various presentations of the aortic arch.

Author contributions

Conception and design of the research: Monteze NM, Guimaraes AFM. Acquisition of data: Monteze NM, Guimaraes AFM. Analysis and interpretation of the data: Monteze NM, Guimaraes AFM, Araujo FDR. Writing of the manuscript: Monteze NM. Critical revision of the manuscript for intellectual content: Monteze NM, Guimaraes AFM, Araujo FDR.

Potential Conflict of Interest

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Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Gerlis LM, Ho SY, Anderson RH. Da Costa P. Persistent 5th aortic arch - a great pretender: three new covert cases. Int J Cardiol. 1989;23(2):239-47.
2. Gupta SK, Bamforth SD, Anderson RH. How frequent is the fifth arch artery? Cardiol Young. 2015;25(4):628-46.
3. Linhares RR, Silva CES. Monaco CG, Ferreira LDC, Gil MA, Ortiz J, et al. Double lumen aortic arch or persistence of fifth aortic arch? – Report of a Case with No Associated Cardiac Defects and Literature Review. Echocardiography. 2011;28(7):E143-5.
4. Naimo PS, Vasquez-Alvarez Mdel, d'Udekem Y, Jones B, Konstantinov IE, et al. Double-Lumen Aortic Arch: Persistence of the Fifth Aortic Arch Ann Thorac Surg. 2016;101(5):e155–6.
5. Van Praagh R, Van Praagh S. Persistent fifth arterial arch in man. Congenital double-lumen aortic arch. Am J Cardiol. 1969;24(2):279-82.
6. Al Akhfash AA, Al Mutairi MB, Al Habshan FM. Persistent fifth aortic arch diagnosed by echocardiography and confirmed by angiography: Case report and literature review. J Saudi Heart Assoc. 2009;21(4):245-8.

7. Priya S, Sharma A. TWIN ARCH: A rare case of type III Persistent 5th aortic .[Internet] [Cited in 2018 Dec 10] Available from:arch. URL: http://www.eurorad.org/case.php?id=14252

8. Khan S, Nihill MR. Clinical presentation of persistent 5th aortic arch: 3 new cases. Tex Heart Inst J. 2006;33(3):361-4.

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Potential Impact of the New American High Blood Pressure Guidelines on Hypertension Prevalence in a Primary Health Care Unit in Rio de Janeiro – the LapARC Study

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