ORIGINAL ARTICLE

DD Genotype and Atherosclerosis in Overweight Menopausal Women

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Abstract

Background: Sex-specific pathology of coronary artery disease (CAD) has not been recognized. Women with obstructive or nonobstructive CAD associated with traditional risk factors have similar events; no studies have explored both populations in association with genetic markers.

Objective: To evaluate the DD genotype in overweight menopausal women and its association with CAD and traditional risk factors.

Method: This cross-sectional study included 356 menopausal women who underwent coronary angiography as CAD assessment. The patients' DNA was extracted and polymorphisms were detected with a single polymerase chain reaction assay. Two groups were formed based on luminal lesions (normal [n = 134] or pathological [n = 222]) with a cutoff value > 30%, considering overweight and age. The chi-square test, Student's t-test, and multivariate logistic regression were performed as appropriate ($p < 0.05$) using the following variables: overweight, diabetes, hypertension, dyslipidemia, smoking status, sedentary lifestyle, and a family history of CAD.

Results: The mean age of the sample was 63 ± 8 years, and the mean BMI was 28 ± 5 kg/m^2. The DD genotype was slightly more prevalent in the pathological group (30.2% vs. 21.6%, p = 0.079), but this significantly changed when BMI > 25 was considered (33% vs. 18%, p = 0.012). In multivariate analysis with two threshold levels (> 50 and > 60 years), diabetes was significantly associated with CAD in both models (p = 0.021 vs. 0.009) but the genotype was only associated with younger age (p = 0.034).

Conclusion: These data support an association between atherosclerosis and the renin–angiotensin system in overweight menopausal women that is dependent on the age at which the ischemic event occurs.

Keywords: Overweight; Menopause; Coronary Artery Disease; Genetic Markers; DD Genotype.

Introduction

Menopause, a unique physiological stage that occurs in middle-aged women, involves important metabolic changes. One of these changes is an increase in low-density lipoprotein levels, which is sometimes associated with age at menopause onset.[1] In addition, lipid profile changes are strongly associated with coronary artery disease (CAD) in these women. This alteration has sometimes been attributed to the effects of hormone replacement on low-density lipoprotein particles.[2] Studies have emphasized that although estrogen therapy increases the levels of high-density lipoprotein and its components,[4] individual variability, which depends on allelic variants of the estrogen receptor gene, is also a factor.[3] A publication

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by the Women's Health Group recommends limiting hormone replacement therapy in menopausal women due to the higher relative risk of CAD in a population that already had a high rate of CAD prior to enrolment.[5]

Recent studies have also investigated the role of adipose tissue, specifically its molecular mediators (eg, adipocytokines) in menopausal women.[6] Of note, obesity is common in menopausal women and increases inflammatory cytokine levels, integrating metabolic and inflammatory responses.[7] One of these concepts is derived from an observed association between the blockade of AT1 receptors and adiponectin expression.[8] Low levels of adiponectin are associated with higher levels of interleukin-6,[9] a molecule that is involved in atherosclerosis.[10] In addition, AT1 receptors in circulating macrophages play a role in angiotensin II-mediated cytokine production.[11]

There is a 287-base pair insertion/deletion polymorphism in the angiotensin-converting enzyme gene in intron 16, resulting in three genotypes; II, ID and DD. The latter is a linkage marker with therapeutic implications in cardiovascular disease.[12]

Clinically, the DD genotype has already been associated with endothelial dysfunction in postmenopausal women,[13] as well as with CAD. Amara et al,[14] found a significant prevalence of the DD genotype in a population with symptomatic CAD (odds ratio [OR] = 6.8, 95% confidence interval [CI]: 4.4–10, $p < 0.001$). The risk was greatly potentiated by several concomitant risk factors (smoking, diabetes, hypertension, dyslipidemia, and family history of CAD).

Considering this scenario, a better diagnostic method for classifying CAD risk is necessary. Based on existing evidence, the DD genotype increases the risk of CAD in younger individuals.[15] Moreover, a metabolic transition threshold is observed during the menopausal age.[16] We suggest that the polymorphism in this special condition, along with related factors, could be involved in atherosclerosis and can be evaluated using coronary angiography.

Materials and Methods

This was a retrospective, observational, analytic cross-sectional cohort single-center study (at the Heart Institute, University of São Paulo). Participants were selected from a database of 1449 patients during 2001 to 2003, (only one investigator was present at upon patient arrival for coronary angiogram and his availability depended on institutional hours). The investigator was available approximately 70% of the time at the hemodynamic laboratory to perform the optional coronary angiogram for the patients. Thus, a mixed sample design was selected to produce a representative sample and to surpass the minimum required sample size, using purposeful random sampling (random cases were selected from the sampling frame, alternating morning and afternoon tests), criterion sampling (only patients with suspected or proven CAD), and convenience sampling (in the chosen setting, groups and/or individuals who are conveniently available and willing to participate) as previously described.[17] From this database we selected 583 female patients, whose mean age was 63 $\pm$ 8 years (Table 1). The sample's clinical characteristics included the

Table 1 – Population characteristics

	General (356)	Normal (134) vs. Pathological (222)		p-value
Age (years)	63±8	61±8	65±8	<0.001
BMI (Kg/m²)	28±5	29±5.8	27±4.9	0.008
DM	128 (36%)	34(26.6%)	94(73.4%)	0.001
HT	288 (81%)	102(35.4%)	186(64.6%)	0.075
DLP	213 (59.8%)	76(35.7%)	137(64.3%)	0.352
SM	87 (24.4%)	32(36.8%)	55 (63.2%)	0.849
CAD hist.	255 (71.6%)	96 (37.6%)	159 (62.4%)	0.997
DD genotype	96 (27%)	29(30.2%)	67(69.8%)	0.079

BMI: Body Mass Index, DM: Diabetes, HT: Hypertension, DLP: Dyslipidemia, SM: smoking,CAD hist: family history of Coronary Artery Disease, first-degree relative <65 years. Continuous variables are expressed as mean and standard deviation, nominal variables are expressed as absolute value and percentage.

following variables: age, menopause (permanent cessation of ovulation) for at least 1 year.

Hypertension was defined as having been prescribed anti-hypertensive medications or blood pressure exceeding 140/90 mmHg. Diabetes was defined as hemoglobin a A1c value > 6.5% or fasting plasma glucose ≥ 126 mg/dl and/or the use of insulin or oral hypoglycemic agents. Dyslipidemia was defined according to laboratory results (total cholesterol ≥ 200 mg/dl, low-density lipoprotein cholesterol ≥ 130 mg/dl, high-density lipoprotein cholesterol < 40 mg/dl, triglycerides ≥ 150 mg/dl and/or hypolipidemic agent use), sedentary lifestyle, and a first-degree family history of CAD (< 65 years). Smoking was defined as daily or occasional self-reported cigarette consumption without having quit in the last year. Weight and height were assessed to determine body mass index (BMI), expressed as kg/m^2 (Table 1). A BMI > 25 Kg/m^2 was considered overweight.

Inclusion criteria

All patients underwent coronary catheterization to determine the presence of CAD, in addition to considering the presence of angina pectoris or the results of one of the following positive noninvasive tests: treadmill, echocardiogram with dobutamine, or cardiac scintigraphy. Some patients were asymptomatic, while others had adverse events such as unstable angina or myocardial infarction without previous coronary angiography. To be included, a patient had to provide all clinical data regarding the study variables (anthropometric measures and risk factors) and written informed consent prior to the coronary angiogram.

Exclusion criteria

Patients were excluded if they had previously undergone coronary angiography due to an ischemic event or if they were admitted to the hospital in an unstable condition.

Genotyping

An 8-mL peripheral blood sample was taken from each patient. The sample was stored in a tube containing ethylenediaminetetraacetic acid, and the DNA was obtained through the saline method. A polymerase chain reaction assay was performed to amplify the selected strain, using two primer kits for the insertion/deletion polymorphism of the angiotensin-converting enzyme gene (including an intronic pair), as described elsewhere. This was considered a *post-hoc* analysis from a previous study.[18]

Twenty coronary segments were examined to determine the presence of atherosclerosis. Epicardial vessels or main branches were divided into three segments (proximal, medial, and distal), except for secondary branches of the right coronary artery, which were divided into proximal and distal portions; this special classification was part of a previous study.[18] Patients were divided into two groups: those with and without lesions (ie, normal vs. pathological angiogram). A cutoff value of > 30% obstruction was established. The coronary angiogram threshold was considered non-significant from the point of view of obstructive angiography, but it established the presence of atherosclerosis. This cutoff value was derived from the Assessing Angiography Project as a lower range in visual assessment.[19] We also divided the population into groups according to BMI > or < 25 kg/m^2 and the presence or absence of CAD.

For logistic analysis, age was dichotomized as > 50 years or > 60 years, since: (1) studies have reported that the mean age of menopause onset ranges from 49–52 years,[20] and (2) the World Health Organization describes those aged ≥ 60 years as older adults.

This study was approved by the institutional ethics and research committee and was conducted in accordance with the most recent Declaration of Helsinki and World Medical Association guidelines. All patients provided written informed consent prior to participation. The study protocol did not interfere with any medical treatment and/or recommendations or other institutional protocols.

Statistical analyses

The sample size was calculated considering two groups for regression analysis and a power of 95%. A minimum of 119 patients were required for each group and a total of 234 cases. We used the Kolmogorov-Smirnov test to assess data normality. Continuous variables were expressed as means and standard deviations and categorical variables as absolute values and percentages. To assess the difference between the groups, we used the chi-square test to determine the frequency of the angiotensin-converting enzyme genotype and alleles and to determine the association between gene polymorphisms and normal and pathological angiograms. The chi-square test

was also used to compare the proportions of classic cardiovascular risk factors between the groups. An unpaired *t*-test was used to determine the differences in continuous variables between the groups at baseline. The OR of a pathological coronary angiogram was determined using dichotomized risk factors, including the DD genotype, as independent predictors. Multivariate logistic regression analysis was performed in three different settings (the whole population and for two age ranges, > 50 and 60 years). P-values < 0.05 were considered significant. All tests were two-tailed. The data were analyzed using SPSS version 23 (SPSS, Chicago Illinois) and G*Power version 3.1.9.7 (Heinrich Heine University, Düsseldorf, Germany).

Results

Ninety-five patients were excluded due to previous bypass surgery or angioplasty, and another 129 were excluded because they were non-menopausal at enrolment. As a result, 356 patients were included in the study (Figure 1). The mean age of those in the pathological groups was significantly older but they had lower BMI values (Table 1). In both groups, non-significant associations were found between a pathological angiogram and hypertension, dyslipidemia, smoking, a family history of CAD, and the DD genotype. However, a pathological angiogram was also associated with older age, diabetes, and BMI (Table 1).

Frequency of alleles and genotypes

The relative frequencies of II, ID, and DD genotypes were 21.3%, 50.4%, and 28.3%, respectively. The allele frequencies were 46.5% and 53.5% for inserted and deleted alleles, respectively. These results were consistent with the Hardy–Weinberg equilibrium.

Body mass index and DD genotype

There was a high prevalence of overweight patients (BMI > 25 kg/m^2) in this population (n = 244, 68.5%), and 61.5% had a pathological angiogram (n=150). Similarly, 64.3% of patients in the BMI ≤ 25 kg/m^2 group had a pathological angiogram, which was a non-significant difference (p = 0.611). There was a higher prevalence of the DD genotype in the pathological group (30.2%) than the normal group (21.6%), but this was also non-significant (Figure 2). However, among menopausal with with a BMI > 25 kg/m^2, DD genotype frequency was significantly higher in the pathological group (34%) than the normal group (19.1%), (Figure 3).

Postmenopausal cardiovascular disease has two changing factors regarding the DD genotype. First,

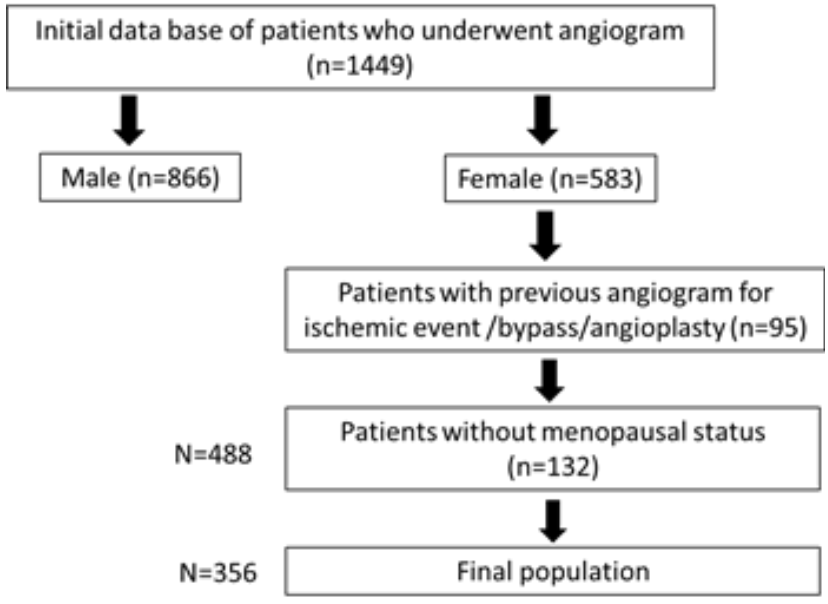

Figure 1 – Flow diagram of the study population

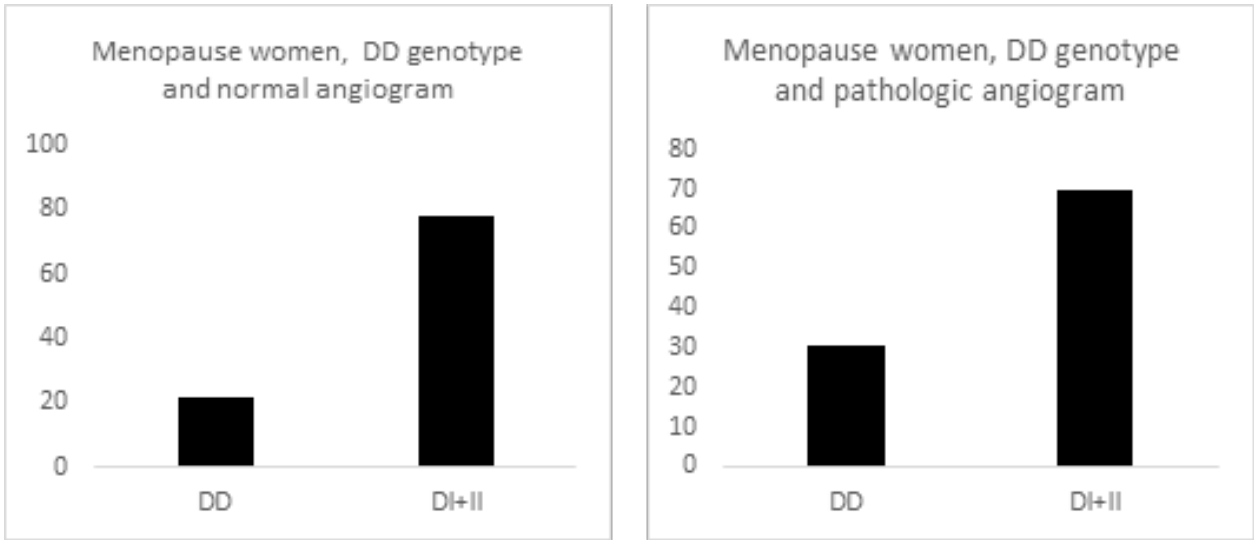

Figure 2 – DD genotype and angiogram status in menopausal women. Values expressed as percentages, p=0.079

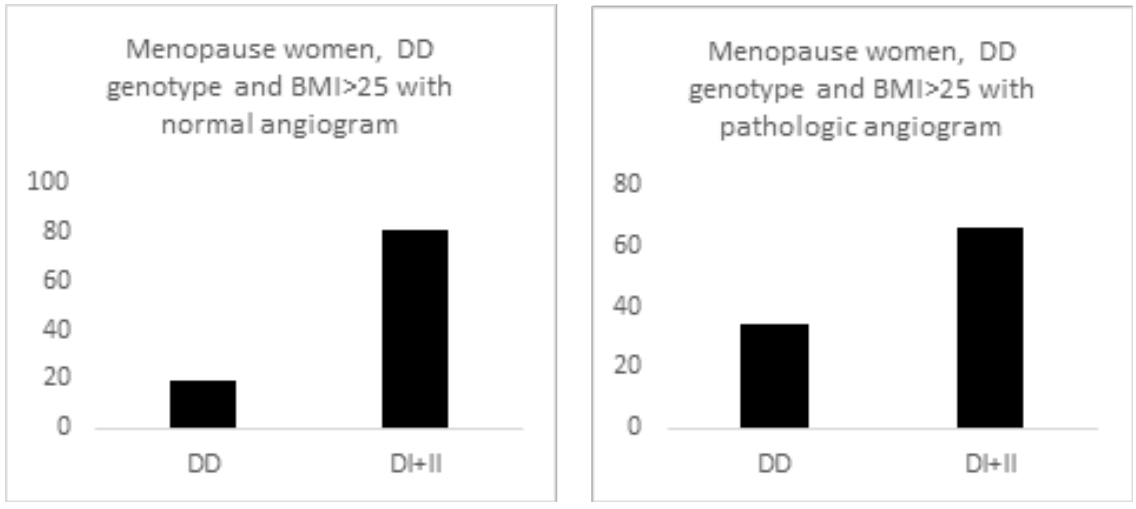

Figure 3 – DD genotype and angiogram status in menopausal women with BMI > 25. Values expressed as percentages, p=0.012

according to univariate analysis, there was an association between a pathological angiogram and age ($p < 0.001$), diabetes ($p = 0.009$), and DD genotype ($p = 0.012$) in postmenopausal women with a BMI > 25 kg/m^2. The remaining variables were not associated with pathology, although they were included in the logistic model (Table 2). In overweight women, diabetes was associated with pathology in both age ranges, but the DD genotype was only associated with the younger age range (Table 2). For women with a lower BMI, pathology was associated with age, diabetes, and hypertension. In a multivariate analysis, only age was significant ($p = 0.037$) (data not shown).

Age was significantly associated with coronary lesions in the overall population, but when divided by age, the association was not observed in the younger group (> 50 years, $p = 0.345$; > 60 years, $p = 0.057$). However, age

Table 2 – The relationship between angiogram lesion and cardiovascular risk factors in menopausal overweight women

*	B	SD	Wald	p-value	Exp(B)	95%CI	
						Inferior	Superior
M50	0.526	0.489	1.157	0.282	1.692	0.649	4.410
DM	0.666	0.288	5.358	0.021	1.946	1.107	3.418
HT	0.023	0.380	0.004	0.951	1.023	0.486	2.156
DLP	0.139	0.283	0.240	0.624	1.149	0.660	2.001
SM	-0.187	0.338	0.308	0.579	0.829	0.428	1.607
Sedentary	-0.545	0.849	0.412	0.521	0.580	0.110	3.061
DD	0.680	0.320	4.517	0.034	1.975	1.054	3.699
**	B	SD	Wald	p-value	Exp(B)	95%CI	
						Inferior	Superior
M60	0.842	0.288	8.531	0.003	2.322	1.319	4.088
DM	0.768	0.296	6.747	0.009	2.157	1.208	3.854
HT	-0.062	0.391	0.025	0.874	0.940	0.437	2.022
DLP	0.148	0.287	0.265	0.607	1.159	0.660	2.035
SM	-0.118	0.344	0.118	0.731	0.888	0.453	1.742
Sedentary	-0.702	0.877	0.641	0.423	0.496	0.089	2.762
DD	0.621	0.326	3.622	0.057	1.861	0.982	3.529

*M50: >50 years, M60: >60 years, HT: Hypertension, DM: Diabetes Mellitus, DLP: Dislipidemia, SM: Smoking. DD: presence of the DD genotype vs. II+ID, B: Beta coefficient, SD: standard deviation, Exp(B): Beta exponential, 95%CI: 95% confidence interval * and ** include tables for BMI>25 depending on age category.*

was associated with pathology in older women when evaluating the overall population. Those > 60 years had a higher risk of CAD (OR: 2.296, 95% CI: 1.296–4.068) and diabetes (OR: 2.141, 95% CI: 1.198–3.824).

There was a weak association between DD genotype and pathology in the overall population (p = 0.071), as well as in women > 60 years. However, there was a significant association between DD genotype and pathology in younger overweight patients. According to the regression analysis, there was a significant association between pathology, BMI < 25, and age (p = 0.037) but not for the remaining variables, including the DD genotype and diabetes (data not shown).

In a complementary analysis in which the traditional cutoff value of 50% luminal obstruction was used to define a pathological angiogram, the prevalence was 68.6%. Regression analysis revealed a significant association between diabetes and vessel obstruction in overweight women (p = 0.002 for > 50 years and p = 0.001 for > 60 years), but there were no significant associations with any of the other variables, including the DD genotype (data not shown).

Discussion

The pathways by which estrogen interacts with the cardiovascular system are not fully understood. Some experimental data on gene expression indicate that 17-B estradiol causes the downregulation of AT1 receptor mRNA.[21] Other studies report that estrogen interferes with neointima formation, attenuating AT1 receptor-mediated activation of extracellular signal-regulated kinases and c-fos expression, thereby inhibiting vascular smooth muscle cell proliferation, an important step in atherosclerosis.[22]

Hypothetically, the more activated renin–angiotensin system in the DD genotype could be minimized with adequate estrogen levels until a follicular age-dependent deficit is observed. At that time, hormone-associated metabolic changes in the lipid profile promote the deposition of cholesterol, and there is a greater availability of low-density lipoprotein particles associated with angiotensin II throughout the LOX-1 receptor.[23] Furthermore, in women on hormone replacement therapy, it has been shown that estrogen has lower levels of monocyte chemoattractant protein-1, which is involved in the progression of atherosclerosis by increasing both the number of macrophages and oxidized lipid accumulation in vessel walls. Other factors could also contribute to the risk of postmenopausal cardiovascular disease, one of which is obesity. In the current COVID-19 pandemic, the treatment and prevention of cardiovascular disease is receiving much attention, principally because it involves pathways that contribute to atherosclerosis.[24] Obesity has also been associated with the polymorphism of angiotensin, and ethnic differences might affect this association. In a meta-analysis of 14 studies, the DD genotype was a risk factor for obesity.[25] Differences were found between the DD and DI + II genotypes, at least among Africans, although three populations were evaluated (Asians, Caucasians, and Africans).

As expected, the authors of the review found an association between classic risk factors, hypertension, and diabetes in the univariate and multivariate analyses. In our multivariate analysis, the independent variables were age and diabetes; the latter was maintained in the three models only among patients with BMI > 25 kg/m^2.

Certain factors must be considered when determining atherosclerosis with a coronary angiogram. Mild luminal irregularities in angiography are associated with a higher disease burden and greater high-risk plaque density than when more accurate methods, such as intravascular ultrasound, are used.[26] Furthermore, nonobstructive coronary artery disease is becoming more common in women, and its risk of major adverse events is similar to obstructive CAD.[27] Thus, studying patients with obstructive lesions as well as those with lesser plaque obstruction is justifiable. Considering the disease as a continuum, it was important to compare patients with some degree of atherosclerotic lesion who required clinical or noninvasive assessment to diagnose coronary ischemia and to use a lower limit than the routine threshold of 50% in traditional angiography.[28] Moreover, through vascular remodeling, plaque can frequently modulate the vascular bed without reducing vessel volume.[29] Thus, atherosclerotic plaque could be underestimated in a routine coronary angiogram, and using the standard threshold of 50% could complicate the prediction of clinical outcomes.

Finally, apart from the expressive association between diabetes and CAD,[30] the incidence of postmenopausal cardiovascular disease appears to be strongly associated with two independent factors when evaluating the DD genotype: younger age (> 50 years) at first ischemic event, followed by weight (BMI > 25 kg/m^2). Both of these results should be explored in the angiotensin system in future studies.

Study limitations

Since this was a cross-sectional study, future outcomes, such as new coronary events (myocardial infarction or stable/unstable angina), cannot be predicted. Additionally the sample could have been larger if routine DNA testing had been performed for every patient who required a coronary angiogram for their first coronary event, which might be possible in other institutions around the world.

Potential clinical value

It is anticipated that genetic information will become increasingly available for postmenopausal patients. It is important to identify examples in which the evidence is sufficiently robust and predictive to allow genetic information to guide clinical decisions and formulate preventive guidelines for CAD.

Conclusion

These data support an association between atherosclerosis and the renin–angiotensin system in a hypoestrogenic environment, which is intensified in overweight women. This association is dependent on the age at which the ischemic event is diagnosed.

Author contributions

Conception and design of the research: Lanz-Luces JR, Costa FA. Acquisition of data: Lanz-Luces JR, Lanz-Souquett JD. Analysis and interpretation of the data: Costa FA, Guzman L, Lanz-Luces JA, Costa LA. Statistical analysis: Costa FA, Lanz-Souquett JD. Obtaining financing: Lanz-Luces JR. Writing of the manuscript:

Lanz-Luces JR, Guzman L, Lanz-Luces JA, Lanz-Souquett JD. Critical revision of the manuscript for intellectual content: Costa FA, Guzman L, Lanz-Luces JA, Costa LA.

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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