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Original Article

MODERATE TRAINING REDUCES EPICARDIAL FAT, AND HYPERTRIGLYCERIDEMIA AFFECTS CARDIAC PLEXUS IN APOCIII-TGN MICE

TREINAMENTO MODERADO REDUZ GORDURA EPICÁRDICA E HIPERTRIGLICERIDEMIA AFETA PLEXO CARDÍACO EM CAMUNDONGOS APOCIII-TGN

Nilton Rodrigues Teixeira Junior, Bruno Jun Komagome, Diogo Rodrigues Jimenes; Cristiany Schultz; Carmem Patrícia Barbosa, and **Jairo Augusto Berti**

State University of Maringá, Maringa-PR, Brazil.

RESUMO

A hipertrigliceridemia está diretamente relacionada ao aumento global de doenças cardiovasculares e metabólicas. Tal condição clínica pode ser investigada por meio de modelos animais com alteração gênica e proteica da apolipoproteína CIII (apoCIII). O plexo cardíaco e o tecido adiposo epicárdico (TAE) são importantes estruturas cardíacas com papéis vitais no processamento nervoso do coração e na disponibilização de suporte e energia para a sua contração. Este estudo analisou o plexo cardíaco e o TAE de camundongos transgênicos (CIII) e não-transgênicos (NTG), submetidos a um treinamento físico em esteira, (3 dias na semana por 40 minutos a 60% da velocidade pico) durante 8 semanas. Os animais CIII apresentaram hipertrigliceridemia e hipercolesterolemia. No entanto, a superexpressão da apoCIII não modificou a glicemia, a massa corporal e cardíaca, e a área dos adipócitos do TAE. Todavia, a área neuronal dos animais CIII foi significativamente menor (NTG Sed 193±38 μm^2 vs. CIII Sed 161±11 μm^2) do que a dos animais não transgênicos. Com o treinamento, a área dos adipócitos do TAE foi menor, de modo independente da expressão da apoCIII (NTG Sed 380±60 μm^2; CIII Sed 358±58 μm^2; NTG Ex 305±52 μm^2; CIII Ex 307±57 μm^2), mas não houve alteração na área neuronal. Conclui-se que os grupos treinados presentaram menor área nos neurônios do plexo cardíaco, enquanto os CIII tiveram redução deste parâmetro

Palavras-chave: Dislipidemia. Neurônios cardíacos. Tecido adiposo epicárdico. Exercício físico. Apolipoproteína CIII.

ABSTRACT

Hypertriglyceridemia is directly related to the global increase in cardiovascular and metabolic diseases. This clinical condition can be investigated using animal models with gene and protein alterations in apolipoprotein CIII (apoCIII). The cardiac plexus and epicardial adipose tissue (EAT) are important cardiac structures with vital roles in the nervous processing of the heart and in providing support and energy for its contraction. This study analyzed the cardiac plexus and the TAE of transgenic (CIII) and non-transgenic (NTG) mice submitted to treadmill exercise training (3 days a week for 40 minutes at 60% of peak speed) for 8 weeks. The CIII animals showed hypertriglyceridemia and hypercholesterolemia. However, overexpression of apoCIII did not modify glycemia, body and heart mass, and the area of adipocytes in the TAE. However, the neuronal area of the CIII animals was significantly smaller (NTG Sed 193±38 µm2 vs. CIII Sed 161±11 µm2) than that of the non-transgenic animals. With training, the area of TAE adipocytes was smaller, independently of apoCIII expression (NTG Sed 380±60 µm2; CIII Sed 358±58 µm2; NTG Ex 305±52 µm2; CIII Ex 307±57 µm2), but there was no change in neuronal area. It can be concluded that the training groups have a smaller, while the CIII groups have a smaller neuronal area in the cardiac plexus.

Keywords: Dyslipidemia. Cardiac neurons. Epicardial adipose tissue. Physical exercise. Apolipoprotein CIII.

Introduction

Hypertriglyceridemia is characterized by an increase in triglycerides (TG) in the blood and is associated with many diseases that rank among the leading causes of global mortality[1]. Its origin is complex and multifaceted, as it can be linked to primary factors (such as genetic alterations)[2] and secondary factors (related to lifestyle and behavior: poor diet, alcoholism, stress, smoking, and physical inactivity). Thus, the multiplicity in etiology highlights the need for personalized initial approaches that facilitate its diagnosis, treatment, and prevention[3].

The study of hypertriglyceridemia and lipid metabolism has been enabled through various laboratory models, among which the use of genetically modified mice that overexpress or lack certain genes stands out. One such experimental model uses transgenic mice that

overexpress human apolipoprotein CIII (apoCIII), due to the gene and protein modulation it performs on fat metabolism regulation[4]. ApoCIII is synthesized predominantly in the liver (and to a lesser extent in the intestine) and is found in large proportions in triglyceride-rich lipoproteins (chylomicrons, VLDL, and IDL). The gene and protein overexpression of this apolipoprotein in mice results in hypertriglyceridemia, mainly by delaying TG removal from lipoproteins, decreasing their affinity for lipoprotein lipase (LPL), and reducing their uptake. Thus, elevated levels of apoCIII can also be associated with disorders such as hypercholesterolemia and increased free fatty acids in the blood[5-8].

Hypertriglyceridemia is recognized as a serious risk factor for the development of metabolic (such as type II diabetes mellitus, hepatic steatosis, and metabolic syndrome) and cardiovascular diseases (such as ischemic heart diseases and atherosclerosis)[9]. The emergence of cardiovascular diseases is related to the fact that, under such conditions, various functional impairments occur not only in the cardiac muscle tissue but also in other important heart structures[10-12].

Among these structures, the cardiac plexus stands out, playing an essential role in heart function. Composed of a vast set of neuronal ganglia located in the atrial region, near the major base vessels, it is also known as the little brain or intrinsic cardiac nervous system. Its neurons are responsible for processing and transmitting sympathetic and parasympathetic nerve impulses, thus contributing to the autonomic regulation of the nodal system and, consequently, to cardiac efficiency[13-15]. Additionally, studies have indicated that morphological changes in the cardiac plexus can compromise its function, and its dysfunction can be associated with severe conditions, such as myocardial infarction[16-17].

Another important heart structure considered essential to its function and potentially related to hypertriglyceridemia is the epicardial adipose tissue (EAT), located between the myocardium and visceral pericardium. This tissue has elastic and compressibility properties that provide mechanical protection to the coronary vessels and represents a support structure for the cardiac plexus[18]. Furthermore, EAT can supply energy to the heart due to its ability to absorb and release free fatty acids, thus being considered a lipid modulation factor. Therefore, EAT is also understood as an endocrine organ, releasing adipokines that, in addition to functioning as inflammatory mediators, can regulate vascular tone[19].

Given the detrimental role of hypertriglyceridemia on cardiac health, physical training has been pointed out as a non-pharmacological tool for preventing and treating comorbidities associated with this condition. Solid evidence has highlighted the benefits of exercise for improving all bodily systems and is therefore considered fundamental in treating various diseases, including cardiovascular ones[20-22]. Several studies have pointed out the ability of physical training to improve adipose tissue function and combat metabolic diseases[23], protect the neurons of the cardiac plexus from the degenerative effects of aging[24-25], and improve endothelial function by reducing plasma TG concentration[26-27].

In this context, considering the relevance of hypertriglyceridemia in the development of severe diseases and the numerous benefits of physical training for cardiac health, this study aimed to deepen the understanding of the effects of moderate aerobic training and hypertriglyceridemia on the morphology of the cardiac plexus and EAT in transgenic mice for human apoCIII.

Methods

Sample

This study is a continuation of a previously published study by our research group[28]. All procedures were approved by the Internal Biosafety Committee - CTNBio nº 819/2013 and the Animal Ethics Committee (CEUA nº 7925010719) of the State University of Maringá.

Initially, 34 male mice aged 15 months of the C57Bl/6 strain were used. Of these, 18 animals represented the primary hypertriglyceridemia model through the overexpression of human apoCIII gene and protein (CIII group). The remaining 16 animals represented the non-transgenic control group (NTG group), as they had basal apoCIII levels and did not overexpress human apoCIII[6]. All animals were bred and maintained in the sectoral animal facility of the Department of Physiological Sciences at the State University of Maringá (DFS-UEM), with a 12-hour light-dark cycle, temperature at 23 ± 1°C, and free access to water and food (Nuvilab® balanced diet).

The differentiation between the groups was made through genotyping[29], which consisted of measuring triglyceridemia on the 60th postnatal day. Thus, animals with TG levels less than 100 mg/dL were considered non-transgenic (NTG), and those with TG levels above 300 mg/dL were classified as transgenic (CIII).

After confirming the genotypes, the NTG and CIII animals were subdivided into trained and sedentary groups: NTG Sed, NTG Ex, CIII Sed, CIII Ex (n=8). However, due to training adherence issues, the trained groups had a sample loss, resulting in 6 animals in the NTG Ex group and 5 in the CIII Ex group.

Initially, 1 capillary (~50µL) of tail blood was collected from all animals to obtain initial plasma concentrations of TG and cholesterol (CHOL) using colorimetric kits. Additionally, a drop of blood was separated for initial blood glucose measurement using an ACCU-CHEK® glucometer. It is worth noting that total body mass, water, and food intake of all animals were measured throughout the experimental period.

Treadmill Adaptation

All animals underwent adaptation on a treadmill (Insight® model ET2000)[30]. The protocol consisted of a daily exercise session with a fixed and light load (16 cm/s) and progressive duration, increased daily over a week. Thus, it started with 10 minutes and ended with 20 minutes. After the last day of adaptation, the animals had a minimum rest of 24 hours before the first exercise test, which aimed to assess the aerobic capacity of the animals.

Maximum Effort Test

The effort test was also performed on all animals, on a treadmill specific for rodents (Panlab® model LE8700CTS Treadmill, Barcelona - Spain), coupled to a gas exchange analyzer (Harvard Apparatus®, model LE405 Gas Analyser). A protocol was adopted that started with light intensity (10 cm/s) for 5 minutes, increasing by 9 cm/s every 3 minutes until the animal reached fatigue[31]. To avoid increasing the stress load on the animal, the effort test was performed without external stimuli and was terminated when the animal reached fatigue. Fatigue was defined as the moment when the animal could no longer sustain the test speed (the animal supported its hind limbs on the fixed platform while the forelimbs remained on the treadmill).

The maximum speed reached in the test (V_{peak}) characterized the aerobic capacity of the animals and was used to determine the training prescription speed. Aiming for moderate-intensity training, 60% of V_{peak} was prescribed. Considering that in the effort test the animals presented homogeneous performance, reaching Vp_{eak} of 64 cm/s, the prescribed training speed was ~38 cm/s. It is noteworthy that this same effort test was repeated on all animals at the end of the 8-week proposed protocol.

Continuous Moderate-Intensity Aerobic Training

For the trained groups (NTG Ex and CIII Ex), training was conducted on alternate days, 3 times a week, always after 5 PM (due to the nocturnal habits of the animals used in this experimental model). Each session had a total duration of 44 minutes, subdivided into 2 minutes of warm-up (16 cm/s), 40 minutes of training (38 cm/s), and 2 minutes of cool-down (16 cm/s). All training sessions were conducted on a treadmill adapted for small rodents (Inbrasport® model ATL 32X23), with electronic speed control. The only exclusion criterion from the study was the withdrawal of animals from more than five training sessions.

To ensure the best performance and safety of the animals during training, a small plastic ball was placed at the bottom of each individual lane. Thus, upon reaching the end region of the treadmill, the animals touched the ball and were encouraged to return to the beginning of the treadmill.

Euthanasia and Tissue Collection

At the end of the last effort test, the animals rested for 24 hours with free access to water and food. Subsequently, after a 12-hour fasting period, they were subjected to a new tail blood collection (100 µL) for biochemical assays of TG, CHOL, and glucose. Then, the animals were individually anesthetized in a euthanasia chamber filled with a flow of Isoflurane anesthetic gas (Isoforine®), where they remained for approximately 1 minute to ensure a state of unconsciousness and absence of pain perception. Afterward, the animals were exsanguinated (via retro-orbital; using a heparinized capillary for total blood collection) and thoracotomized for heart extraction.

Heart Collection

The surgical removal of the heart was performed by making an incision over the great vessels at the base of the organ. Immediately, the heart was perfused with Phosphate Buffered Saline (PBS 0.1 M; pH 7.4) solution to completely remove the blood lodged in the heart chambers and was weighed on an analytical balance (Shimadzu®). The perfusion was performed by injecting PBS into the myocardial tissue (left ventricle) using a syringe (BD®; 10 ml) and needle (BD®; 25x7). Subsequently, the heart was fixed in 4% paraformaldehyde for 48 hours and stored in 70% alcohol until paraffin embedding[32].

Embedding and Sectioning

Initially, the heart underwent histological processing to prepare it for semi-serial sections of 5 µm thickness using a microtome (LEICA RM 2145®). The sections were made in the transverse direction of the organ, from apex to base, and only sections above the atrioventricular septum were used, i.e., sections from the base of the atria to the blood vessels of the cardiac hilum[33-34].

Three histological slides with 5 sections from each animal were mounted (totaling 15 sections/animal). All slides were stained with hematoxylin-eosin (HE). With the aid of a histological analysis software (Image Pro-Plus®) and microphotographs taken at a 20x objective, the location of the cardiac plexus was described. Additionally, the main morphological characteristics of the plexus were described, and morphometric analyses of both the cardiac plexus (by measuring 100 neurons from each animal) and the epicardial adipose tissue were performed.

Statistical Analysis

All data were subjected to the Shapiro-Wilk test to check data normality. To compare the effect of the independent variables (training and hypertriglyceridemia), two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for comparison between groups was used. Normally distributed data were presented as mean ± standard deviation.

Results

Body Mass, Cardiac Mass, Triglycerides, Cholesterol, and Glycemia

The CIII animals showed elevated plasma levels of TG and CHOL compared to NTG animals, confirming their genotypes, as reported in a recent publication from our research group[28]. The CIII Ex group exhibited reduced levels of these parameters compared to the CIII Sed group (Table 1). However, no statistically significant differences were found in body mass, cardiac mass, or glycemia among the groups.

Table 1 – Body mass, heart mass, triglyceride, total cholesterol, and plasma glucose levels in NTG sed, NTG Ex, CIII Sed, and CIII Ex groups

	Data per group (Mean ± SD)				**Source of variation**		
	NTG Sed	**NTG Ex**	**CIII Sed**	**CIII Ex**	**Dyslipidemia**	**Exercise**	**Interaction**
Body mass (g)	25,9 ± 1,1	26,5 ± 2,1	26,1 ± 0.3	25,2 ±2,3	ns	ns	ns
Heart mass (g)	0,17 ± 0,02	0,17 ± 0,01	0,18 ± 0,04	0,15 ± 0,01	ns	ns	ns
Triglyceride (mg/dL)	67 ± 14	61 ± 10	562 ± 163	362 ± 58	<0.0001	<0.05	<0.05
Total Cholesterol (mg/dL)	164 ± 44,1[a]	174 ± 42[a]	248 ± 35	166 ± 57[a]	<0.05	<0.05	<0.05
Glycemia (mg/dL)	87,8 ± 9,3	85 ± 11	90 ± 13	96 ± 10	ns	ns	ns

Note: Mean ± standard deviation. ANOVA Two-way and Tukey's post hoc test. a = p<0.05 in Tukey's post hoc test vs. CIII Sed.
Source: Authors adapted[28].

Location and Morphological Characteristics of the Cardiac Plexus

The ganglia that comprise the cardiac plexus were located between the myocardium and the adipose tissue near the base of the heart, where the roots of the major vessels are found. In terms of morphological characteristics, the cardiac plexus ganglia varied in shape and size. Hematoxylin and eosin (HE) staining revealed oval-shaped neurons with decentralized nuclei (Figure 1).

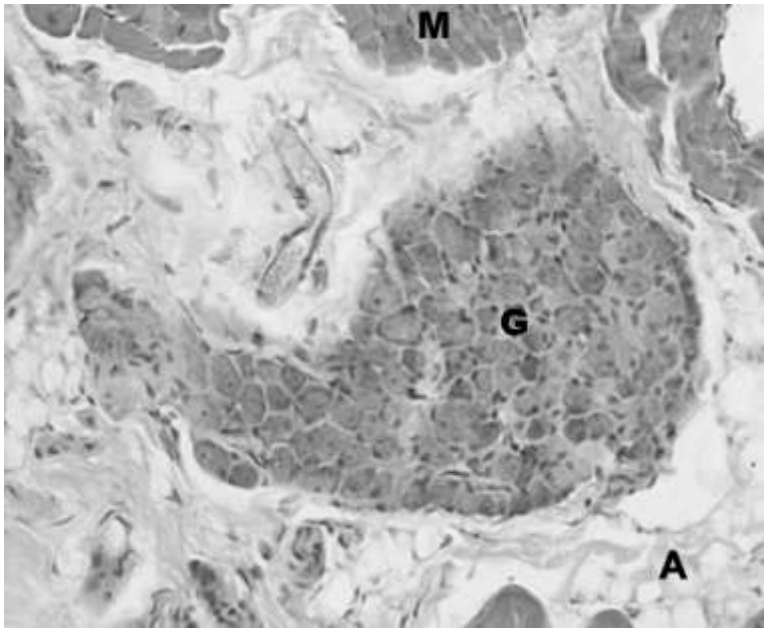

Figure 1. Overview of Cardiac Plexus Morphology
Note: Photomicrograph obtained with a 20x objective lens and HE staining. Cross-sectional view of the basal cardiac region near the major vessel emergence, highlighting a cardiac plexus ganglion. **A** (adipose tissue), **G** (ganglion), **M** (myocardium).
Source: Authors

Morphometric Analysis of the Cardiac Plexus

ANOVA results showed that all CIII animals had a significantly smaller neuronal area compared to NTG animals (p=0.01): NTG Sed 193±38 µm², CIII Sed 161±11 µm², NTG Ex 194±45 µm², and CIII Ex 160±19 µm². However, physical training did not alter this parameter (Figure 2).

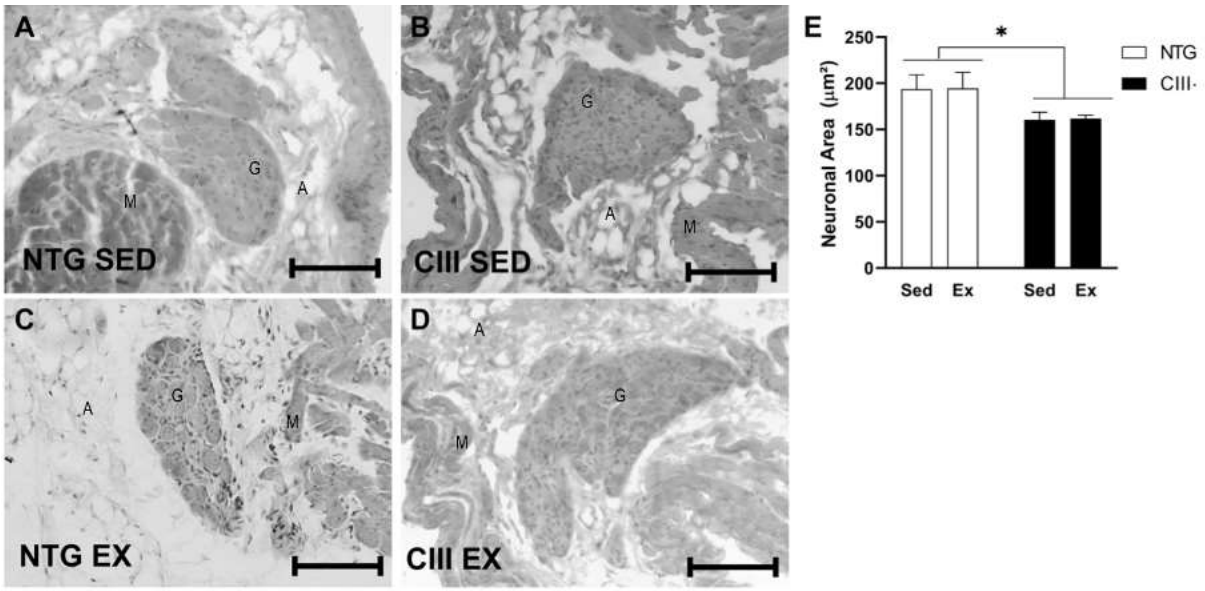

Figure 2. Photomicrographs of the Cardiac Plexus in Mice
Note: A: NTG Sed group (n=8); **B:** CIII Sed group (n=8); **C:** NTG Ex group (n=6); **D:** CIII Ex group (n=5). **E** = Mean ± standard deviation. ANOVA Two-way with Tukey's post hoc test. (*) $p<0.05$ for apoCIII overexpression in ANOVA test. **A** (adipose tissue), **G** (ganglion), **M** (myocardium).
Source: Authors

Morphometric Analysis of Epicardial Adipose Tissue

Histological analysis of epicardial adipose tissue (EAT) showed that hypertriglyceridemia did not affect the adipocyte area in any of the groups (NTG Sed 380±60 µm²; CIII Sed 358±58 µm²; NTG Ex 305±52 µm²; CIII Ex 307±57 µm²). However, ANOVA identified that trained groups had a smaller adipocyte area in EAT, regardless of apoCIII overexpression (p=0.03).

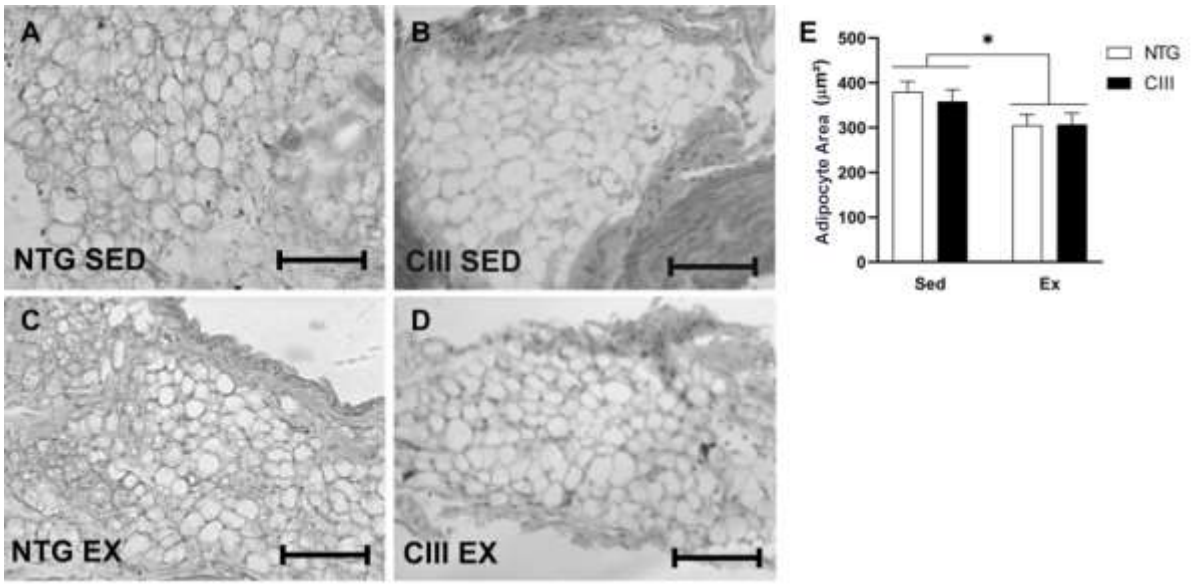

Figure 3. Photomicrographs of Epicardial Adipose Tissue in Mice
Note: A = NTG Sed group (n=8); **B** = CIII Sed group (n=8); **C** = NTG Ex group (n=6); **D** = CIII Ex group (n=5). **E** = Mean ± standard deviation. ANOVA Two-way with Tukey's post hoc test. (*) $p<0.05$ for exercise variable in ANOVA test.
Source: Authors

Discussion

This study is innovative in relating the morphology of the cardiac plexus and epicardial adipose tissue (EAT) in transgenic mice for human apolipoprotein CIII, using a moderate-intensity continuous training (MICT) method. To enhance understanding of a potential exercise effect on these variables, biochemical analyses of lipid and glucose metabolism, as well as morphological and morphometric analyses of the cardiac plexus ganglion neurons and EAT, were conducted, as EAT is a significant component in the metabolic and pathophysiological regulation of the heart.

This study showed that apoCIII overexpression did not alter glycemia, body mass, or cardiac mass in the animals. Although training can increase food intake[28], all groups had free access to water and food, and all animals were of the same age at the time of euthanasia. Thus, this result is consistent with the literature[14], which indicates that CIII mice have cardiac mass equivalent to their NTG controls.

In a previous study by our research group[28], biochemical analyses confirmed the genotype of this animal model. Besides identifying a state of hypertriglyceridemia caused by human apoCIII overexpression in CIII animals, hypercholesterolemia (total cholesterol > 200 mg/dL) was also found. Furthermore, with the application of MICT, the CIII group exhibited TG and COL levels about 30% lower than the sedentary control group. This effect suggests a

physiological adaptation of CIII animals to training due to the hydrolysis of TG and beta-oxidation of fatty acids present in VLDL and chylomicrons[35].

The morphological analysis of the cardiac plexus and its location in these animals showed that our findings align with the current literature, as ganglia were observed in emergence of the major vessels at the heart's base, between the muscular and adipose tissues. Additionally, the neurons were oval-shaped with decentralized nuclei and occasionally multinucleated[14,16,36].

Furthermore, an isolated effect of apoCIII overexpression was observed, as the morphometric analysis of cardiac plexus neurons showed that the neuronal area in CIII animals was significantly smaller, regardless of physical training. Contrary to our findings, a study14 with CIII mice aged eight and 12 months found that only older mice had an altered (larger) neuronal area, highlighting the effect of aging independent of apoCIII overexpression. This contradiction suggests that the physiological mechanisms involving neuronal area modification due to hypertriglyceridemia remain controversial and require further elucidation. However, evidence suggests that not only aging but also metabolic issues[14] can be directly related to these mechanisms. In agreement with this hypothesis, other animal models with metabolic alterations (such as rats with laboratory-induced diabetes mellitus) showed reduced neuronal area associated with remodelling of the cardiac plexus[33].

Evaluating the effect of training on cardiac plexus morphology, the data obtained in this study showed that eight weeks of MICT did not significantly alter the neuronal area in any of the trained groups, regardless of apoCIII overexpression. On the other hand, a study[26] evaluating the cardiac plexus of rats under physical training conditions observed that MICT (applied five times a week for 10 months) reduced neuronal area. According to the authors, training-induced adaptation was beneficial and protected the cardiac plexus from degenerative effects of aging, as smaller neurons have a lower excitatory threshold than larger neurons, thus having greater excitability.

From the above, it is observed that the relationship between the cardiac plexus and physical training is still not fully conclusive. This is because, although some modulatory factors of this important cardiac structure are well described (such as age and heart diseases)[16-18], the mechanisms explaining the modulation of the plexus through exercise still need more in-depth investigation. Additionally, MICT requires further attention as it differs from other training modalities; although there is evidence that it can promote morphological changes in the plexus, the involved mechanisms and the relationship between volume and intensity necessary to induce such adaptations remain unclear.

The morphometry of epicardial adipose tissue (EAT) was preserved and did not show significant differences due to apoCIII overexpression. Thus, although this experimental model is related to obesity predisposition[37], EAT remained conserved even under hypertriglyceridemia and hypercholesterolemia conditions. In a previous study[16] by our research group using sedentary animals, CIII mice had a larger area of EAT adipocytes compared to NTG animals. Additionally, the area of these adipocytes was greater with aging (comparing eight- and 12-month-old animals). This difference in findings may be due to the modulatory effect of aging on EAT, as the animals in this study were 15 months old, i.e., middle-aged[38]. Thus, it is possible that there is indeed an as-yet-unnamed mechanism correlating the morphology of this tissue to aging observed in this animal model.

Conversely, physical training reduced the area of EAT adipocytes. This effect is consistent with current literature, as exercise can effectively cause remodelling of adipose tissue[39]. Moreover, a previous study[28] from our group on visceral adipose tissue (perigonadal)

showed that MICT could reduce the area of such fat. It is worth noting that this effect has also been described in other animal models, such as rats on different types of diets[40].

The main limitation of this study was the establishment of the experimental model itself, due to its complexity and difficulty in replicating the apoCIII colony, as the mice were crossed among themselves. Additionally, in accordance with the exclusion criteria for the training protocol, animals that did not complete at least 80% of the protocol were excluded from the final analysis. Thus, due to the challenges faced with training, the sample size was reduced from 10 animals to 5 in the CIII Ex group and 6 animals in the NTG Ex group.

Conclusion

It can be concluded that hypertriglyceridemia induced by apoCIII overexpression did not alter glycemia, body and cardiac masses, or the area of adipocytes in epicardial adipose tissue in the animals. However, CIII animals had smaller ganglionic neuronal areas in the cardiac plexus. Additionally, continuous moderate-intensity aerobic training did not alter the morphometry of cardiac plexus neurons, even though it reduced TG and blood COL. Furthermore, it reduced the area of EAT adipocytes, regardless of the dyslipidemia caused by apoCIII overexpression. Therefore, more studies are needed to elucidate the mechanisms that modify cardiac plexus neurons due to physical training, as well as the relationship between different training modalities, volumes, and intensities in these experimental conditions.

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References

1. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. Br J Sports Med. 2020;54:1451-62. DOI: 10.1136/bjsports-2020-102955.
2. Carson V, Lee E-Y, Hewitt L, Jennings C, Hunter S, Kuzik N, et al. Systematic review of the relationships between physical activity and health indicators in the early years (0-4 years). BMC Public Health. 2017;17:854. DOI: 10.1186/s12889-017-4860-0
3. Poitras VJ, Gray CE, Borghese MM, Carson V, Chaput J-P, Janssen I, et al. Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. Appl Physiol Nutr Metab. 2016;41. DOI: 10.1139/apnm-2015-0663.
4. Chen W, Hammond-Bennett A, Hypnar A, Mason S. Health-related physical fitness and physical activity in elementary school students. BMC Public Health. 2018;18:195. DOI: 10.1186/s12889-018-5107-4
5. Gomes TN, Katzmarzyk PT, Hedeker D, Fogelholm M, Standage M, Onywera V, et al. Correlates of compliance with recommended levels of physical activity in children. Sci Rep. 2017;7:16507. DOI: 10.1038/s41598-017-16525-9.
6. Henriksson P, Leppänen MH, Henriksson H, Delisle Nyström C, Ortega FB, Rautiainen S, et al. Physical fitness in relation to later body composition in pre-school children. J Sci Med Sport. 2019;22:574-9. DOI: 10.1016/j.jsams.2018.11.024.
7. Reisberg K, Riso E, Jürimäe J. Associations between physical activity, body composition, and physical fitness in the transition from preschool to school. Scand J Med Sci Sports. 2020;30:2251-63. DOI: 10.1111/sms.13784.
8. Migueles JH, Delisle Nyström C, Dumuid D, Mora-Gonzalez J, Cadenas-Sanchez C, Ekelund U, et al. Longitudinal associations of movement behaviours with body composition and physical fitness from 4

to 9 years of age: structural equation and mediation analysis with compositional data. Int J Behav Nutr Phys Act. 2023;20:11. DOI: 10.1186/s12966-023-01417-1.
9. Biddle SJ, Asare M. Physical activity and mental health in children and adolescents: a review of reviews. Br J Sports Med. 2011;45:886-95. DOI: 10.1136/bjsports-2011-090185.
10. Lees C, Hopkins J. Effect of aerobic exercise on cognition, academic achievement, and psychosocial function in children: a systematic review of randomized control trials. Prev Chronic Dis. 2013;10. DOI: 10.5888/pcd10.130010. DOI: 10.5888/pcd10.130010.
11. Martin A, Saunders DH, Shenkin SD, Sproule J, Kirk A. Lifestyle intervention for improving school achievement in overweight or obese children and adolescents. Cochrane Database Syst Rev. 2014. DOI: 10.1002/14651858.CD009728.pub4..
12. Sallis JF. Age-related decline in physical activity: a synthesis of human and animal studies. Med Sci Sports Exerc. 2000;32:1598-600. DOI: 10.1097/00005768-200009000-00012.
13. Singh A, Uijtdewilligen L, Twisk JW, van Mechelen W, Chinapaw MJ. Physical activity and performance at school: a systematic review of the literature including a methodological quality assessment. Arch Pediatr Adolesc Med. 2012;166:49-55. DOI: 10.1001/archpediatrics.2011.716.
14. Van Dijk ML, De Groot RH, Savelberg HH, Van Acker F, Kirschner PA. The association between objectively measured physical activity and academic achievement in Dutch adolescents: findings from the GOALS study. J Sport Exerc Psychol. 2014;36:460-73. DOI: 10.1123/jsep.2014-0014.
15. Gibson CA, Smith BK, DuBose KD, Greene JL, Sullivan DK, Washburn RA, et al. Physical activity across the curriculum: year one process evaluation results. Int J Behav Nutr Phys Act. 2008;5:1-11. DOI: 10.1186/1479-5868-5-36.
16. Whitt-Glover MC, Ham SA, Yancey AK. Instant Recess®: a practical tool for increasing physical activity during the school day. Prog Community Health Partnersh Res Educ Action. 2011;5:289-97. DOI: 10.1353/cpr.2011.0031.
17. Watson A, Timperio A, Brown H, Best K, Hesketh KD. Effect of classroom-based physical activity interventions on academic and physical activity outcomes: a systematic review and meta-analysis. Int J Behav Nutr Phys Act. 2017;14:1-24. DOI: 10.1186/s12966-017-0569-9.
18. Taras H. Physical activity and student performance at school. J Sch Health. 2005;75:214-18. DOI: 10.1111/j.1746-1561.2005.00026.x.
19. Trudeau F, Shephard RJ. Physical education, school physical activity, school sports and academic performance. Int J Behav Nutr Phys Act. 2008;5:1-12. DOI: 10.1186/1479-5868-5-10.
20. Gába A, Baďura P, Vorlíček M, Suchomel A, Rubín L. The Czech Republic's 2022 Report Card on Physical Activity for Children and Youth: A rationale and comprehensive analysis. J Exerc Sci Fit. 2022;20:340-8. DOI: 10.1016/j.jesf.2022.08.002.
21. Hu D, Zhou S, Crowley-McHattan ZJ, Liu Z. Factors that influence participation in physical activity in school-aged children and adolescents: a systematic review from the social ecological model perspective. Int J Environ Res Public Health. 2021;18:3147. DOI: 10.3390/ijerph18063147.
22. Petersen TL, Møller LB, Brønd JC, Jepsen R, Grøntved A. Association between parent and child physical activity: a systematic review. Int J Behav Nutr Phys Act. 2020;17:1-16. DOI: 10.1186/s12966-020-00966-z.
23. Trost SG, Loprinzi PD. Parental influences on physical activity behavior in children and adolescents: a brief review. Am J Lifestyle Med. 2011;5:171-81. DOI:10.1177/1559827610387236.
24. Bauman AE, Reis RS, Sallis JF, Wells JC, Loos RJ, Martin BW, et al. Correlates of physical activity: why are some people physically active and others not? The Lancet. 2012;380:258-71. DOI: 10.1016/S0140-6736(12)60735-1.
25. Pugliese J, Tinsley B. Parental socialization of child and adolescent physical activity: a meta-analysis. J Fam Psychol. 2007;21:331-43. DOI: 10.1037/0893-3200.21.3.331
26. Faria TO, Mello LG, Pinto GC, Vassallo DV, Lizardo JF. Uma Única Sessão de Exercício Resistido Melhora a Função Endotelial Aórtica em Ratos Hipertensos. Arq Bras Cardiol. 2017;108(3):228-36. DOI: 10.5935/abc.20170023.
27. Silvestre R, Kraemer WJ, Quann EE, Seip RL, Maresh CM, Vingren JL, et al. Effects of exercise at different times on postprandial lipemia and endothelial function. Med Sci Sports Exerc. 2008;40(2):264-74. DOI: 10.1249/mss.0b013e31815c485a.
28. Teixeira Junior N, Jimenes D, Schultz C, Almeida D, Mathias P, Berti J. Moderate-intensity continuous training reduces triglyceridemia and improves oxygen consumption in dyslipidemic apoCIII transgenic mice. Braz J Med Biol Res. 2024;57. DOI: 10.1590/1414-431X2024e13202

29. Salerno AG, Silva TR, Amaral ME, Alberici LC, Bonfleur ML, Patrício PR, et al. Overexpression of apolipoprotein CIII increases and CETP reverses diet-induced obesity in transgenic mice. Int J Obes (Lond). 2007;31(10):1586-95. DOI: 10.1038/sj.ijo.0803646
30. Moreira VM, da Silva Franco CC, Prates KV, Gomes RM, de Moraes AM, Ribeiro TA, et al. Aerobic Exercise Training Attenuates Tumor Growth and Reduces Insulin Secretion in Walker 256 Tumor-Bearing Rats. Front Physiol. 2018;9:465. DOI: 10.3389/fphys.2018.00465.
31. Brooks GA, White TP. Determination of metabolic and heart rate responses of rats to treadmill exercise. J Appl Physiol Respir Environ Exerc Physiol. 1978;45(6):1009-15. DOI: 10.1152/jappl.1978.45.6.1009.
32. Batulevicius D, Pauziene N, Pauza DH. Topographic morphology and age-related analysis of the neuronal number of the rat intracardiac nerve plexus. Ann Anat. 2003;185(5):449-59. DOI: 10.1016/S0940-9602(03)80105-X.
33. Batulevicius D, Frese T, Peschke E, Pauza DH, Batuleviciene V. Remodelling of the intracardiac ganglia in diabetic Goto-Kakizaki rats: an anatomical study. Cardiovasc Diabetol. 2013;12:85. DOI: 10.1186/1475-2840-12-85.
34. Behmer OA, Tolosa EM, Freitas-Neto AG. Manual de técnicas para histologia normal e patológica. São Paulo: Manole; 1976.
35. Wang Y, Xu D. Effects of aerobic exercise on lipids and lipoproteins. Lipids Health Dis. 2017;16(1):132. DOI: 10.1186/s12944-017-0515-5.
36. Aksu T, Gopinathannair R, Gupta D, Pauza DH. Intrinsic cardiac autonomic nervous system: What do clinical electrophysiologists need to know about the "heart brain"? J Cardiovasc Electrophysiol. 2021 Jun;32(6):1737-47. DOI: 10.1111/jce.15058.
37. Raposo HF, Paiva AA, Kato LS, de Oliveira HC. Apolipoprotein CIII overexpression exacerbates diet-induced obesity due to adipose tissue higher exogenous lipid uptake and retention and lower lipolysis rates. Nutr Metab (Lond). 2015;12:61. DOI: 10.1186/s12986-015-0058-6.
38. Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, Smith AL, eds. The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models. 2nd ed. San Diego, CA: Elsevier; 2007.
39. Pedersen BK, Kellis M, Middelbeek RJ, Goodyear LJ. Exercise training remodels inguinal white adipose tissue through adaptations in innervation, vascularization, and the extracellular matrix. Cell Rep. 2023 Apr 25;42(4):112392. DOI: 10.1016/j.celrep.2023.112392.
40. Gollisch KS, Brandauer J, Jessen N, Toyoda T, Nayer A, Hirshman MF, Goodyear LJ. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. Am J Physiol Endocrinol Metab. 2009 Aug;297(2). DOI: 10.1152/ajpendo.90424.2008.

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ORCID
Nilton Rodrigues Teixeira Junior: https://orcid.org/0000-0002-2015-7027
Bruno Jun Komagome: https://orcid.org/0009-0006-4768-5547
Diogo Rodrigues Jimenes: https://orcid.org/0000-0002-1862-9275
Cristiany Schultz: https://orcid.org/ 0000-0003-3725-4621
Carmem Patricia Barbosa: https://orcid.org/0000-0002-8227-5993
Jairo Aubusto Berti: https://orcid.org/0000-0002-4142-6601

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Correspondece address: Nilton Rodrigues Teixeira Junior. Email: nilton98rodrigues@gmail.com