

Short-term Aerobic Exercise did Not Change Telomere Length while it Reduces Testosterone Levels and Obesity Indexes in PCOS: A Randomized Controlled Clinical Trial Study.

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Abstract

Background: Physical activity is an effective non-pharmacological treatment for polycystic ovary syndrome (PCOS) and the reproductive outcomes which may have implications on telomere biology.

Objective: To observe the effects of continuous (CAT) and intermittent (IAT) aerobic training on telomere length, inflammatory biomarkers, and its correlation with metabolic, hormonal, and anthropometric parameters of PCOS.

Design and Methods: This randomized controlled clinical trial study included 87 PCOS women randomly stratified according to body mass index (BMI) in the continuous (CAT, n = 28) and intermittent aerobic training (IAT, n = 29) and non-training control group (CG, n = 30). The exercises were carried out on a treadmill, three times per week for 16 weeks. The participants' anthropometric characteristics and biochemical and hormonal concentrations were measured before and after aerobic training or observation period, as the telomere length that was evaluated using quantitative real-time PCR.

Results: Four months of aerobic exercises (CAT or IAT) did not alter telomere length and inflammatory biomarkers in PCOS women. Obesity index as BMI and waist circumference (WC), and inflammatory biomarkers negatively affect telomeres. The hyperandrogenism measured by testosterone levels was reduced after both exercises (CAT, $p \leq 0.001$; IAT, $p = 0.019$). In particular, the CAT reduced WC ($p = 0.045$), hip circumference ($p = .032$), serum cholesterol ($p \leq 0.001$), low-density lipoprotein ($p = 0.030$). Whereas, the IAT decreased WC ($p = 0.014$), waist-to-hip ratio ($p = 0.012$), free androgen index (FAI) ($p = 0.037$). WC ($p = 0.049$) and body fat ($p = 0.015$) increased in the non-training group while total cholesterol was reduced ($p = 0.010$).

Conclusions: Both exercises reduced obesity indices and hyperandrogenism on PCOS women without changes in telomere length or inflammatory biomarkers.

Clinical trial registration: Brazilian Clinical Trials Registry (ReBec; RBR-78qtwy, August 20, 2015), and the International Controlled Randomized Trial Registry (ISRCTN10416750, July 24, 2018), retrospectively registered.

Key Points

- Four months of different type of aerobic exercises, continuous and intermittent, did not change telomere length and inflammatory biomarkers in PCOS.
- Both continuous and intermittent aerobic exercises reduced testosterone levels and waist circumference (WC) in PCOS, and non-training period increased WC and body fat percentage.
- Continuous aerobic exercise decreased hip circumference and improved total cholesterol and low-density lipoprotein, while intermittent exercise decreased waist-to-hip ratio and free androgen index in PCOS.

- Obesity index as BMI and WC and inflammatory biomarkers, CRP and homocysteine, negatively affect telomere length in PCOS.

Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial heterogeneous endocrine disorder where the main characteristic behind this syndrome is the chronic anovulation due to hyperandrogenism, a striking feature in this disease. However, the PCOS clinical expression varies and may include oligo-ovulation or anovulation and/or clinical or biochemical hyperandrogenism and evidence of polycystic ovaries[1]. Infertility and metabolic complications, such as dyslipidemia, hypertension, abnormal glucose metabolism, insulin resistance (IR), and obesity, are often present in PCOS[2]. This variability of phenotypes associated to PCOS depends on the ethnicity and directly interferes on the prevalence of this syndrome, that affect between 5 and 16% of women in reproductive age[2].

Despite the genetic alterations related to PCOS[3], a strong environmental contribution is related to the development of the syndrome or even the worsening of the clinical conditions. The management of obesity with a diet[4] or physical activity[5, 6] has been found to improve PCOS-related symptoms, such as infertility, a matter of concern in PCOS treatment. This suggests an epigenetic component related to the pathogenesis of PCOS that affects gene expression, genomic stability, and telomere attrition[7]. Progressive telomere shortening is associated with loss of the cellular proliferative capacity and premature reproductive aging, leading to chronic anovulation and infertility[8]. Several factors as oxidative stress, inflammation, mitochondrial dysfunction, and hormonal alterations, as observed in PCOS, may accelerate telomere erosion[9]. On the other hand, increased levels of androgens in PCOS may be a protective factor improving telomerase activity [10] thereby not changing [5, 11] or increasing telomere repeats [12]. These conflicting results were recently reported and is being continuously investigated [13].

It is well-known that regular practice of physical activity can improve metabolic complications and hyperandrogenism in women with PCOS, with implications in chronic anovulation and ultimately restoring the fertility. Some studies have proposed that physical training could protect progressive shortening of telomeres, preventing premature aging[5, 14, 15]. Telomere shortening is associated to sedentarism, obesity, cardiometabolic risk factors, and oxidative stress, which leads to development of many human diseases, in addition to a shorter life expectancy[16, 17]. The intensity and interval training may have different effects on telomere biology. The aerobic physical exercise of moderate to high intensity improved metabolic and reproductive outcomes of PCOS women, reducing chronic anovulation, cardiometabolic risk, IR, and obesity-related indexes[18]. Larocca et al. (2010)[14] showed that the telomere length is more preserved in the physically active elderly compared to the inactive ones, and a positive correlation between telomeres and the aerobic capacity was observed.

Previously we reported that progressive resistance training (PRT)[5, 6] had positive effects on hormonal and physical characteristics of women with PCOS, with no effects on telomere length specific related to

PCOS. However, the type of physical exercise and the intensity, have different effects on metabolic rate, hormonal levels, body composition, and reproductive health in women with PCOS [6, 19, 20] that could interfere in telomere biology. The effects of supervised aerobic physical exercise on telomere length and its implication on inflammatory biomarkers, metabolic disturbance, and reproductive outcomes of PCOS was not investigated. Considering the importance of the practice of physical exercise in women with PCOS, we now investigate the effects of two aerobic physical training protocols, continuous (CAT) and intermittent (IAT) on telomere length its correlation with metabolic, hormonal, and anthropometric parameters in women with PCOS.

Participants, Materials And Methods

Study Design and Ethics Statement

This randomized, controlled, three-arm parallel-group study was approved by the Institutional Review Board of the University Hospital (UH), Ribeirão Preto Medical School - University of São Paulo (FMRP-USP) (Protocol number nº 9640/2014, and all participants gave written informed consent. The authors confirm that all ongoing and related trials for this intervention were registered in the Brazilian Clinical Trials Registry (ReBec; RBR-78qtwy) and after the study was also registered in the International Controlled Randomized Controlled Trial Registry (ISRCTN10416750).

Participants

In order to determine the clinical baseline for phenotypic disease, the volunteers were divided into two groups according to their BMI (<30 and ≥ 30 kg/m²) and then stratified into three subgroups within the two groups. The allocation group was placed inside opaque, sealed envelopes, grouped in blocks of 15 and consecutively picked depending on the BMI of the participant at the time of study inclusion. After the run-in period, 110 volunteers were randomly assigned in a 1:1:1 fashion to one of three groups (continuous aerobic training (CAT, n = 28), intermittent aerobic training (IAT, n = 29), and control group (CG), without training (n = 30). Random allocation was conducted by the principal investigator and participants were enrolled and assigned to the intervention groups by research assistants. The intervention groups CAT and IAT trained for 16 weeks. CG were asked to maintain their usual daily physical activity profile. In addition, all volunteers were instructed to maintain their daily diets during the intervention.

The women included in this study were women with PCOS, aged between 18 and 39 years, which did not regularly practice physical exercise before the start of the study, and had a BMI between 18 and 39.9 kg/m². All volunteers were selected at Gynecological Endocrinology Outpatient Clinic of the Human Reproduction Service of the Gynecology and Obstetrics Department of Ribeirão Preto Medical School, University of São Paulo. Exclusion criteria were the presence of systemic diseases, use of drugs that interfere in the hypothalamic-pituitary ovarian axis, congenital adrenal hyperplasia, diabetes, smoking, pregnancy, thyroid diseases, hyperprolactinemia, musculoskeletal disorders, or Cushing's disease. PCOS diagnosis was based on the Rotterdam consensus, established on at least 2 of the following 3 features:

chronic anovulation, hyperandrogenism (clinical or biochemical), and polycystic ovaries on ultrasound[21]. The presence of polycystic ovaries was determined by transvaginal pelvic ultrasonography with a Voluson E8 Expert machine (GE HealthCare, Zipf, Austria).

Clinical and Biochemical measurements

Clinical characteristics as age, diastolic blood pressure, systolic blood pressure and heart rate were evaluated. The concentrations of total testosterone, androstenedione, Follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), sex steroid hormone-binding globulin (SHBG), fasting insulin, and 17-hydroxyprogesterone (17-OHP), prolactin, estradiol, homocysteine and C-reactive protein (CRP) were determined using a chemiluminescence method (Immulin 1000; Immunoassay System; Siemens®, Santa Ana, CA). Fasting blood glucose was determined using the oxidase method (CMD 800X1 / CMD 800iX1, Wiener Lab, São Paulo). Fasting High-density lipoprotein (HDL), total cholesterol, and triglycerides were evaluated using the enzymatic method (CMD 800X1 / CMD 800iX1, Wiener Lab, São Paulo). Low-density lipoprotein (LDL) was measured using the Friedewald formula: $LDL\ cholesterol = total\ cholesterol - (HDL\ cholesterol + triglycerides/5)$. The Free Androgen Index (FAI) was obtained from the following formula: $[total\ testosterone\ (nmol/L)/SHBG\ (nmol/L) \cdot 100]$. The homeostatic model assessment of insulin resistance (HOMA-IR) was evaluated using the formula: $(fasting\ blood\ glucose\ in\ mg/dL \cdot 0.05551) \cdot fasting\ insulin\ \mu UI/mL / 22.5$.

Anthropometry and DXA

Body weight and height were assessed, and body mass index (BMI) was subsequently determined from these measurements. Waist circumference (WC) was measured at the midpoint between the lateral iliac crest and the lowest rib margin at the end of normal expiration. The hip circumference (HC) was measured in the region where the buttocks are largest. The waist-to-hip ratio (WHR) was obtained by dividing WC (cm) by HC (cm). All anthropometric indexes were assessed according to the procedures described by the "International Standards for Anthropometric Assessment" [22]. Body composition was measured using dual energy X-ray absorptiometry (DXA) (Hologic 4500 device QDR Discovery® Series; Hologic, Waltham, MA, USA). Analysis was performed using the 5 Discovery Wi model (SN/84826) software (version 13.0). The regions of interest (ROIs) for assessment of the total body fat (BF) [fat mass (g) plus lean mass including bone mineral content (g)] and the percentage fat (%) (fat mass/total mass × 100) were evaluated and the android and gynoid fat distributions were calculated.

Aerobic physical training protocols

The aerobic physical training protocols were performed according to the regulations of the American College of Sport Medicine (ACSM)[23]. The training protocols were recently published by our research group[20] and are present in the Supplemental Figure S1. Two protocols were applied: the continuous aerobic training (CAT) and the intermittent aerobic training (IAT). All exercises were performed under personal supervision of a physical education professional and the measurements were carried out at baseline and after 16 weeks of training or the observational period for the control group (CG). The exercises were carried out on a treadmill (Embree 570-L and Embree 570-Pro, SC, Brazil), three times per

week (wk) for 16 weeks, lasting equally and progressively from 30 min in the first wk, to 50 min in the last wk. The target intensity training areas followed the ACSM recommendations. Light (50%- 64% HRmax), moderate (64%-77% HRmax) and vigorous (77%-94% HRmax) intensities were considered to calculate the progression of the protocols in order to be applied in the context of the clinical profile of participants.¹⁵ To calculate the intensity of training, the HRmax formula (220-age) was used [23]. The protocols were equalized by volume at each progression. The equivalent result for each week of each protocol was added, and the total volume of IAT was similar to that of CAT. In both CAT and IAT, volunteers underwent training three times per week at nonconsecutive days.

For both protocols, five minutes warmups and five minutes cool downs, between 50% and 60% of the MHR, were included. The exercises were performed at the Department of Gynecology and Obstetrics and Cardiovascular Physiology and Physiotherapy Laboratory at FMRP-USP. Adherence was determined from the supervised participation of the exercise session and the conclusion of the aerobic exercise training protocols. The participants were monitored daily and in case of absence, the session was remarked for another day of the same week. Non-adherence criterion was considered to fail to participate in at least 20% of the proposed protocol training sessions. The amount of exercise / physical activity that the CG performed during the study was self-reported using recall diaries. In addition, all participants were instructed to maintain their daily diets during the intervention or observational period.

Telomere length measurement

Genomic DNA was isolated from peripheral blood leukocytes of all participants before and after the training protocols or the observation period using *MasterPure Complete DNA and RNA Purification Kit* (Epicentre, Illumina Company, USA), according to the manufacturer's instructions. DNA integrity was accessed by agarose gel stained with GelRead (Unisciences, USA), and the concentration determined using the Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, USA). Telomere length was measured by the quantitative polymerase chain reaction, as described previously[24, 25] using the following primer sequences for the telomere: Tel-Fw, 5'-CGGTTTGTGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3' and Tel-Rv, 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'; and for the single gene S-Fw, 5'-CAGCAAGTGGGAAGGTGTAATCC-3' and S-Rv, 5'-CCCATTCTATCATCAACGGGTACAA-3'.

Telomere length was determined by calculating the telomere to single-copy gene ratio using ΔCt [$Ct(\text{telomere})/Ct(\text{single gene})$]. The telomere length was expressed as the relative T/S (Telomere/Single gene) ratio, normalized to the mean of the T/S ratio of the reference sample [$2^{-(\Delta Ct_x - \Delta Ct_r)} = 2^{-\Delta \Delta Ct}$]. The reference sample was also used as the standard curve and validation sample. The samples that were analyzed in triplicate and reaction mix contained: 1.6 ng of genomic DNA, 7.2 pg of each primer (except for the reverse primer of the single gene, which will contain 12 pg) and 16 μL of the *Rotor-Gene SYBR Green PCR Master Mix* (Qiagen Hilden, Germany). The PCR conditions used for the test were: 95° C 5min, 98° C 7s, 60° C 10s (25 cycles). The 100-well discs were handled using the *QIAgility* (Qiagen Hilden, Germany) liquid handling instrument. The single gene reaction was obtained in 35 cycles, for the duration

of 61 minutes and the PCR conditions were: 95°C 5 minutes; 98°C 7 seconds, 58°C 10 seconds (35 cycles).

Statistical analysis

The sample size was calculated based on the previously published data for telomere length in young PCOS women (LI et al. 2014), using mean 0.80 and standard deviation ± 40 , which showed that 30 participants (60 for each group) will be necessary to observe a difference of 0.3 in telomere length between the exercise groups and non-exercise group, 80% of statistical power, and a level of significance of .05. For other outcomes, the sample size was obtained considering a Cohen's d effect size of 0.6, a significance level of 5%, and a test power of 80%.

The analysis of variables studied are presented as mean and standard deviation. To evaluate the effects of physical training (CAT and IAT) or observational period on clinical characteristics, anthropometric indices and telomere content, The Mann-Whitney nonparametric test was used to independently compare the distribution of variables between the PCOS and the control groups. A general linear mixed model (GLMM) analysis of repeated measures was used considering time, group, and group x time interactions, adjusted for the independent variables age, BMI, total testosterone and androstenedione. A residual analysis was performed to verify the adjustments of the statistical models. To compare the groups, orthogonal contrasts were used considering linear models of mixed effects. Also, an analysis of the correlation between the telomere length and quantitative PCOS variables (age, BMI, total testosterone and androstenedione) were assessed using Pearson correlation coefficients. All statistical analyses were performed using SAS® 9.3 software (SAS Institute Inc, University of North Carolina, North Carolina), and $P < .05$ was considered significant.

Results

The flowchart of the study is illustrated in Figure 1. According to the eligibility criteria, 126 participants were recruited. From these 126, 16 were unable to attend the inclusion criteria for PCOS after initial evaluations, thus 110 women with PCOS started the physical training protocols. Of these, 23 did not finish the protocols and 87 participants completed the study: 28 in CAT, 29 in the IAT, and 30 in the CG groups. To attend the protocols and complete the study, the adherence was at least 90% of the training sessions.

The physical, anthropometric and hormonal characteristics of the groups analyzed before and after the training or the observational period, are presented in Table 1. The age, diastolic and systolic blood pressure were not different between the studied groups. To characterize the PCOS, prolactin (CG = 16.6 ng/ml \pm 9.1; CAT = 17.4 ng/ml \pm 12.7; IAT = 16.8 ng/ml \pm 11.7), 17-OHP (CG = 106.0 uUI/ml \pm 38.0; CAT = 98.0 uUI/ml \pm 47.0; IAT = 86.0 uUI/ml \pm 40.0), and TSH (CG = 2.38 ng/dL \pm 1.18; CAT = 1.76 ng/dL \pm 0.67; IAT = 2.64 ng/dL \pm 1.60) were measured. At baseline, the total testosterone level was higher in the CAT group (117 \pm 50 ng/dl) when compared to the CG (86 \pm 37 ng/dl), $p = 0.01$. The other variables analyzed were not different at the beginning of the training protocols. Serum levels of androstenedione, SHBG,

estradiol, FSH, and LH did not change after aerobic physical training protocols (CAT and IAT) and the observational period in CG. The testosterone level decreased after CAT ($p \leq 0.001$) and IAT ($p = 0.019$) and the FAI was reduced only in the IAT group ($p = 0.037$).

Table 1

– Clinical, anthropometric, hormonal and metabolic parameters of women with PCOS before and after the aerobic physical training protocols or observation period.

Variables	CG (n= 30)		CAT (n=28)		IAT (n=29)	
	Before Mean (SD)	After Mean (SD)	Before Mean (SD)	After Mean (SD)	Before Mean (SD)	After Mean (SD)
Age (years)	28.50 (5.76)	—	29.14 (5.26)	—	28.97 (4.32)	—
Height (m)	1.61 (0.07)	—	1.62 (0.06)	—	1.64 (0.07)	—
Weight (Kg)	75.37 (14.33)	76.05 (15.09)	74.4 (16.5)	73.74 (16.78)	77.36 (16.91)	77.00 (16.81)
BMI (Kg/m ²)	29.09 (5.25)	29.33 (5.43)	28.43 (5.62)	28.17 (5.67)	28.67 (4.76)	28.53 (4.82)
WC (cm)	89.52 (12.61)	90.98 (13.14)*	88.12 (13.60)	86.58 (13.12)*	90.54 (11.33)	88.67 (12.43)*
HC (cm)	106.34 (10.15)	107.23 (9.75)	105.88 (9.58)	104.55 (10.27) ^a	107.31 (9.50)	107.17 (10.98)
WHR (cm)	0.84 (0.08)	0.85 (0.07)	0.83 (0.08)	0.82 (0.07)	0.84 (0.06)	0.83 (0.07)*
SBP (mm Hg)	105.07 (11.29)	108.13 (10.42)	104.36 (9.39)	100.39 (9.88)	104.69 (12.12)	103.93 (14.19)
DBP (mm Hg)	71.40 (10.11)	72.00 (8.89)	69.64 (9.85)	68.25 (9.02)	68.52 (9.38)	68.52 (11.38)
Heart Rate (bpm)	74.93 (11.17)	76.90 (10.00)	76.82 (12.44)	71.86 (9.52)*	73.90 (9.98)	73.86 (12.34)
Biochemical parameters						
Testosterone (ng/dL)	86,2 (37)	99.67 (46.40)	116.7 (49.5)	92.7 (37.8)*	107.69 (51.53)	87.8 (54.2)*
Androstenedione (ng/dL)	87.2 (56)	78.63 (45.60)	86.64 (44.61)	82.0 (28)	77.69 (59.75)	74.52 (49.42)

Data are presented as mean and standard deviation (SD). CG. control group; CA, continuous aerobic physical training group; AI, intermittent aerobic physical training group; ng / dL, nanomol per deciliter; nmol / L, nanomol per liter; mg / dL, milligrams per deciliter; pg / mL, picogram per milliliter; uIU / mL, international microunits / milliliter; BMI. body mass index; %. fat percentage; cm. centimeters; WC. waist circumference; HC. hip circumference; WHR. waist hip ratio; DBP, diastolic blood pressure; SBP, systolic blood pressure; BPM, beats per minute; SHBG, sex hormone binding globulin; FAI, free testosterone index; E2, estradiol; LH, luteinizing hormone; FSH, follicle stimulating hormone; HDL, High Density Lipoproteins; LDL, Low Density Lipoproteins; HOMA-IR, homeostatic model assessment; T/S ratio, Telomere/single gene. bold, p <0.05; a = intra-group;

	CG (n= 30)		CAT (n=28)		IAT (n=29)	
SHBG (nmol/L)	50.56 (34.21)	62.26 (45.29)	54.31 (40.89)	58 (61)	47.82 (28.19)	53.49 (31.00)
FAI	7.52 (4.2) ¹	7.90 (5.98)	11.33 (9.58)	10.3 (10)	9.87 (7.20)	7.84 (7.72)*
E2 (pg/mL)	48.12 (23.73)	48.31 (22.07)	55.83 (42.84)	67 (69)	63.44 (55.42)	61.41 (48.73)
LH (μUI/mL)	9.25 (8.40)	6.78 (4.81)	7.79 (4.76)	8.32 (8.56)	7.77 (4.87)	7.63 (4.74)
FSH (μIU/mL)	5.63 (1.99)	5.16 (1.78)	5.66 (2.14)	5.12 (1.83)	5.21 (2.95)	5.59 (2.71)
Total Cholesterol (mg/dL)	188.27 (34.13)	177.53 (24.44)*	184.64 (29.83)	171.45 (28.07)*	178.86 (29.34)	174.17 (26.86)
Triglycerides (mg/dL)	111.77 (55.82)	103.17 (58.87)	151.43 (172.63)	144.35 (139.16)	98.62 (54.49)	106.83 (60.85)
HDL (mg/dL)	50.10 (13.09)	48.47 (12.66)	45.67 (9.33)	44.28 (10.29)	48.78 (10.62)	47.08 (10.27)
LDL (mg/dL)	115.73 (31.55)	108.33 (26.80)	111.71 (23.55)	102.46 (23.14)*	112.31 (23.49)	106.24 (23.19)
Fasting Glycemia (mg/dL)	83.0 (7.0)	81.0 (9.0)	84.0 (12.0)	84.0 (11.0)	82.0 (11.0)	82 (11)
Fasting Insulin (μIU/mL)	12.83 (8.5)	12.45 (9.79)	11.31 (8.09)	11.2 (8.3)	9.52 (7.18)	10.40 (7.03)
HOMA-IR	2.64 (1.77)	2.59 (2.17)	2.45 (1.90)	2.42 (1.97)	2.02 (1.88)	2.20 (1.79)
Homocysteine (μmol/L)	7.62 (1.74)	7.89 (1.64)	8.05 (2.40)	7.66 (1.70)	7.17 (1.80)	7.54 (1.94)
C-reactive protein (mg/dL)	0.51 (0.41)	0.53 (0.54)	0.36 (0.41)	0.43 (0.54)	0.36 (0.32)	0.35 (0.32)
Body composition (DXA)						

Data are presented as mean and standard deviation (SD). CG. control group; CA, continuous aerobic physical training group; AI, intermittent aerobic physical training group; ng / dL, nanomol per deciliter; nmol / L, nanomol per liter; mg / dL, milligrams per deciliter; pg / mL, picogram per milliliter; uIU / mL, international microunits / milliliter; BMI. body mass index; %. fat percentage; cm. centimeters; WC. waist circumference; HC. hip circumference; WHR. waist hip ratio; DBP, diastolic blood pressure; SBP, systolic blood pressure; BPM, beats per minute; SHBG, sex hormone binding globulin; FAI, free testosterone index; E2, estradiol; LH, luteinizing hormone; FSH, follicle stimulating hormone; HDL, High Density Lipoproteins; LDL, Low Density Lipoproteins; HOMA-IR, homeostatic model assessment; T/S ratio, Telomere/single gene. bold, p <0.05; a = intra-group;

	CG (n= 30)		CAT (n=28)		IAT (n=29)	
Body Fat (%)	40.59 (6.26)	41.83 (4.36)*	40.25 (4.67)	39.20 (5.63)	41.97 (3.79)	41.80 (5.53)
Android (%)	42.33 (8.09)	43.90 (3.08)	42.25 (6.30)	40.69 (7.13)	43.37 (4.77)	43.17 (5.60)
Gynoid (%)	43.79 (6.10)	44.93 (4.26)	43.58 (4.97)	42.41 (5.60)	45.23 (4.59)	44.22 (5.54)
Fat Mass_height ² (Kg/m ²)	11.80 (3.45)	12.13 (3.08)	11.43 (3.38)	11.09 (3.49)	11.80 (2.60)	11.67 (2.74)
Lean Mass_height ² (Kg/m ²)	16.80 (2.76)	16.53 (2.53)	16.51 (2.49)	16.64 (2.45)	16.11 (2.39)	16.32 (2.61)
Telomere Length						
T/S ratio	1.40 (0.50)	1.45 (0.46)	1.43 (0.39)	1.45 (0.46)	1.53 (0.46)	1.54 (0.51)
Data are presented as mean and standard deviation (SD). CG. control group; CA, continuous aerobic physical training group; AI, intermittent aerobic physical training group; ng / dL, nanomol per deciliter; nmol / L, nanomol per liter; mg / dL, milligrams per deciliter; pg / mL, picogram per milliliter; uIU / mL, international microunits / milliliter; BMI. body mass index; %. fat percentage; cm. centimeters; WC. waist circumference; HC. hip circumference; WHR. waist hip ratio; DBP, diastolic blood pressure; SBP, systolic blood pressure; BPM, beats per minute; SHBG, sex hormone binding globulin; FAI, free testosterone index; E2, estradiol; LH, luteinizing hormone; FSH, follicle stimulating hormone; HDL, High Density Lipoproteins; LDL, Low Density Lipoproteins; HOMA-IR, homeostatic model assessment; T/S ratio, Telomere/single gene. bold, p <0.05; a = intra-group;						

After the aerobic physical exercises or no training periods, no differences were observed in the anthropometric indices BMI and weight, or in the metabolic parameters such as HDL, triglycerides, fasting glycemia and insulin and HOMA-IR. The lipidic profile as total cholesterol ($p \leq 0.001$) and LDL ($p = 0.030$) were reduced after CAT. The anthropometric evaluation showed that WC and HC were reduced in the CAT group ($p = 0.045$ and $p = 0.032$, respectively), as well as the heart rate ($p = 0.016$), and the WC and WHR decreased in the IAT group ($p = 0.014$ and $p = 0.012$, respectively). In the control group without training (CG), it was observed an increase in WC ($p = 0.049$) and body fat percentage ($p = 0.015$) and a reduction in total cholesterol ($p = 0.010$). No differences were observed in other anthropometric or DXA variables evaluated.

Regarding the effects of CAT and IAT or non-training on telomere length, we observed a great variability in the subjects in both training and non-training control groups (Figure 1), however in the adjusted model the telomere length did not change after CAT ($p=0.6912$), IAT ($p=0.9099$) or CG ($p=0.6028$) (Table 1). Regardless of continuous and intermittent aerobic training, four months of aerobic exercise seems not change telomere length (Figure 3A). Indeed, the inflammatory biomarkers' homocysteine and c-reactive protein were not altered after the aerobic training neither in the control non-training groups ($p>0.05$). In the general linear mixed models, the confoundable variables, such as age ($p = 0.4204$), BMI ($p = 0.2983$), total testosterone ($p = 0.7115$), androstenedione ($p = 0.8993$), did not interfere in telomere length in these

groups, even the independent variables group ($p = 0.2552$) and time ($p = 0.4098$). The correlation analysis showed that the anthropometric variables age ($r = -0.1633$, $p = 0.0324$), BMI ($r = -0.1784$, $p = 0.0192$) and WC ($r = -0.1613$, $p = 0.0334$) (Table 2, Figure 3B and 3C, respectively) had a negative correlation with telomere length, as well as the inflammatory biomarkers CRP ($r = -0.2161$, $p = 0.0041$) and homocysteine ($r = -0.1635$, $p = 0.0311$). The hyperandrogenism, including total testosterone and androstenedione was not correlated with telomere length in women with PCOS (Table 2).

Table 2
Pearson correlation analysis between telomere length and quantitative variables of PCOS women regardless of group.

	Telomere	
	R	P value
Age	-0.1633	0.0324
BMI	-0.1770	0.0194
WC	-0.1613	0.0334
Testosterone	0.1027	0.1773
Androstenedione	0.0090	0.9057
CRP	-0.2161	0.0041
Homocysteine	-0.1635	0.0311
T/S ratio, Telomere/single gene; BMI, Body mass index; CRP, C-reactive Protein, in bold $p < .05$.		

Discussion

The importance of physical training in the treatment of PCOS needs to be clarified. In the present study we did not observe differences in telomere length after the CAT and IAT training protocols, with no changes in the inflammatory biomarkers' homocysteine and C-reactive protein. Despite no differences in telomere length and inflammation, the CAT protocol reduced the hormonal level of total testosterone, total cholesterol, and LDL, while also improving anthropometric indexes, decreasing WC and HC. The IAT, on the other hand, showed a decrease in total testosterone and FAI, and a reduction in WC and WHR. The non-training CG's total cholesterol was reduced; however, the WC and body fat percentage were increased after non-intervention observational period. Considering the evaluation of structured aerobic physical training protocols in PCOS women, most studies are transversal and unsupervised, and the effects of physical training on telomere length may not be representative[16].

To our knowledge, few studies investigate changes on telomere length in PCOS, and the results are controversial leading to ambiguous conclusions[13], probably due to the heterogeneity of this syndrome. The first study was carried out by Li et al (2014) and the authors observed leukocytes telomere shortening in PCOS women[26]. Then, we reported no alterations in leukocyte's telomere length from PCOS, however, a negative correlation of inflammatory biomarkers with telomere length was observed[11]. In agreement, Wei et al. (2017) did not find changes in telomere length in leukocytes of PCOS women[27]. Contradictorily, an increasing in leukocytes' telomeres from women with PCOS was reported by Wang et al. (2017). The authors also reported a reduction of the TERRA (Telomeric repeat-containing RNA) gene expression that was negatively correlated with testosterone, whereas, telomere length had a positive correlation with testosterone[12].

We previously reported that physical resistance training (PRT) improved hyperandrogenism, reproductive function, and body composition in women with PCOS, with no changes on BMI or metabolic parameters of PCOS[6]. The PRT was performed three times per week during a four-week period in PCOS and non-PCOS women. We also observed that PRT reduced telomere length and increased homocysteine level in all participants, irrespective of them having PCOS or not. Considering aging and senescence, the PRT should be practiced with caution. In addition, a positive correlation between androstenedione and telomere length was observed in PCOS[5], suggesting that the hyperandrogenism may be an important protective factor for telomere erosion, irrespective of metabolic disturbance that contribute to telomere shortening in PCOS[10]. Short term PRT seems to change telomere content, however the same period of continuous or intermittent aerobic training did not affect telomere biology.

Loprinzi and Sng (2016) evaluated the effect of nine types of unsupervised exercise on telomere length and suggested that running was the most effective exercise with positive impacts on telomere biology[28]. In young and elderly populations, the endurance exercise practice increases maximum aerobic capacity (VO₂max) that consequently increases telomere length[14, 29]. Unfortunately, we did not measure the VO₂max in our groups. Tucker (2017)[30] evaluated the metabolic equivalent (MET), the ratio of the work metabolic rate, to the resting metabolic rate, using frequency, intensity and duration, observed that higher level of physical activity meant longer telomeres were reported in men and women. Differently, shortening of telomeres in endurance athletes after exposure to acute exercise was observed, due to oxidative damage in DNA[31].

Physical exercise can improve the cardiometabolic profile in women with PCOS without weight loss. High intensity interval training reduced insulin resistance and body composition in women with PCOS, whereas resistance training improved the body composition, and these improvements were independent of weight loss[32]. We also investigated the effects of aerobic exercise on metabolic, hormonal and anthropometric parameters which also may interfere in telomere biology. Both CAT and IAT showed benefits to women with PCOS, decreasing total testosterone level and anthropometric indexes in PCOS women, even though only CAT reduced lipids parameters. Mario et al. (2017)[33] reported that habitual physical activity improves anthropometric parameters and hyperandrogenism in women with PCOS. According to non-

supervised data, intense physical activity reduces metabolic syndrome in PCOS, irrespective of age, BMI, or total energy expenditure[19].

As a molecular marker of aging, progressive telomere shortening with each cell division is a common biological process, leading to chromosomal instability and cell senescence or apoptosis. Age-related diseases and some metabolic alterations might contribute to an accelerated shortening of telomeres [34] while others conditions, as increased estrogens levels [10] and physical activity prevent against progressive telomeric loss [35]. As reported in this study, Mason et al. (2013) did not observe changes on telomere biology after aerobic physical exercise, and maybe the time of exposition was relatively shorter to observe changes in telomere length [36]. Since telomere changes are progressive and a relatively slow process (~52 base pair/year) [37], 16 weeks of intervention may be not sufficient to observe the effect of the aerobic intervention on telomere biology. Sanberoth et al. (2015) suggested that at least 10 years of regular physical activity will be necessary for improvements on telomeres[38], that may explain our results, since four months of CAT or IAT might be insufficient to observe differences on telomere biology, and an increase in exposure time might provide additional information. Despite no differences in telomeres, in the non-obese population, physical exercise may prevent telomere shortening [14, 29].

In the adjusted model of our data, none of the confoundable variables seemed to interfere in telomere length in PCOS. However, a negative correlation was observed between telomere length and age, as expected, and the obesity index BMI and WC and the inflammatory biomarkers CRP and homocystein. A cross-sectional meta-analysis of 87 observational studies reported that telomere length is negatively correlated with BMI, since higher BMI is related with reduced telomeres, and this association appeared to be stronger in the young individuals[39]. This negative correlation may be due to the increased inflammation and oxidative stress, a common characteristic of PCOS that is positively correlated with BMI[40]. Nevertheless, the inflammatory biomarkers did not change after aerobic exercises, but a negative correlation with telomere length was observed in PCOS as previously reported [11]. Increased WC is related to reduced telomeres in obese women and physical activity is an important strategy for obesity treatment, by reducing anthropometric indices and improving body composition [35, 41]. Since both exercises reduced WC and hyperandrogenism in our BMI randomized study, the differences in telomere length might not be observed in a short time of training. Associated with the well-controlled study, as a repetitive molecular marker, telomeres have a great variability

Despite the important findings of this manuscript, one of the limitations of our clinical trial is that we used a platform chemiluminescence for the measurement of steroid sex hormones, which may not be as sensitive as mass spectrometry for detecting androgen levels in women. Still, we did not control the diet of the participants or evaluated the levels of habitual physical activity related to work and leisure. It's important to consider that intensity of the protocols was defined in terms of HRmax (%). Although the literature shows which there is a linear relationship between VO₂max and HRmax at submaximal exercise intensities, the VO₂max (%) or metabolic equivalent (METS) are considerate the gold standard to determine exercise intensity (19). It's may have underestimated the intensity. However, in this controlled

clinical trial study the effect of aerobic physical exercises evaluated were supervised by a physical education professional, which strengthens our results.

Conclusion

Short-term aerobic physical intervention, continuous and intermittent training, did not promote changes in telomere length and inflammatory parameters, while it reduces testosterone levels and improved anthropometric indices in PCOS women. Nevertheless, telomeres were negatively affected by obesity indices, as BMI and WC, and inflammatory biomarkers CRP and homocysteine. Also, after the CAT, the WC and HC were reduced, as well as total cholesterol, LDL, and total testosterone, whereas the IAT only showed a reduction in WC and WHR, and a decrease in total testosterone level and FAI. The non-exercise practice in the CG increased WC and body fat. Finally, both aerobic physical training protocols should be considered as an effective strategy in the treatment of metabolic disorders and hyperandrogenism in PCOS women, but the implications on telomere biology should be investigated over a longer period of time than the one in this study, with the use of these aerobic training protocols.

Abbreviations

Polycystic Ovary Syndrome (PCOS), Continuous Aerobic Training (CAT), Intermittent Aerobic Training (IAT), Control Group (CG), Body Mass Index (BMI), Waist Circumference (WC), C-Reactive Protein (CRP), Insulin Resistance (IR), Progressive Resistance Training (PRT), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Thyroid-Stimulating Hormone (TSH), Sex Steroid Hormone-Binding Globulin (SHBG), Fasting Insulin, And 17-Hydroxyprogesterone (17-OHP), Fasting High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Free Androgen Index (FAI), Homeostatic Model Assessment Of Insulin Resistance (HOMA-IR), Hip Circumference (HC), Waist-To-Hip Ratio (WHR), Body Fat (BF), American College Of Sport Medicine (ACSM), General Linear Mixed Model (GLMM), Dual Energy X-Ray Absorptiometry (DXA), Metabolic Equivalent (MET)

Declarations

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AUTHORS' CONTRIBUTIONS

VBR, conceived, designed the study and wrote the manuscript; DCCP, performed telomere length measurements; GSK, conceived the study and data analyzes; VBR and IPL, supervised and guided the performance of physical activities in the participants; ASM, responsible for PCOS clinical diagnosis; BAS, participate in telomere length measurements; HCDS, revised and supervised the aerobic protocols; RAF, contribute to participant's laboratorial measurements; RTC, supervised telomere data analysis; CLMF and RMR, conceived and designed the study, analyzed the data and critically improved the manuscript. All authors critically revised the manuscript and approved the final version and submission of this manuscript.

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Ethical Approval and Consent to Participate

This study was approved by the Institutional Review Board of the University Hospital (UH), Ribeirão Preto Medical School - University of São Paulo (FMRP-USP) (Protocol number nº 9640/2014), and all ongoing and related trials for this intervention were registered in the Brazilian Clinical Trials Registry (ReBec; RBR-78qtwy) and the International Controlled Randomized Controlled Trial Registry (ISRCTN10416750). Each participant has signed an informed consent prior to the initiation of physical activity.

Consent for Publication

The consent declarations have been completed.

Competing interests

The authors declare no conflict of interest in this study.

References

1. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. *Endocr Rev.* 2016;10(5):467–520. 37(.
2. Ding T, Hardiman PJ, Petersen I, Wang FF, Qu F, Baio G. The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: a systematic review and meta-analysis. *Oncotarget.* 2017 Nov;8(56):96351–8.
3. Yilmaz B, Vellanki P, Ata B, Yildiz BO. Metabolic syndrome, hypertension, and hyperlipidemia in mothers, fathers, sisters, and brothers of women with polycystic ovary syndrome: a systematic review and meta-analysis. *Fertil Steril.* 2018;02(2):356–64.e32. 109(.
4. Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. *Hum Reprod.* 2003 Sep;18(9):1928–32.
5. Miranda-Furtado CL, Ramos FK, Kogure GS, Santana-Lemos BA, Ferriani RA, Calado RT, et al. A Nonrandomized Trial of Progressive Resistance Training Intervention in Women With Polycystic Ovary Syndrome and Its Implications in Telomere Content. *Reprod Sci.* 2016 May;23(5):644–54.
6. Kogure GS, Miranda-Furtado CL, Silva RC, Melo AS, Ferriani RA, De Sá MF, et al. Resistance Exercise Impacts Lean Muscle Mass in Women with Polycystic Ovary Syndrome. *Med Sci Sports Exerc.* 2016 Apr;48(4):589–98.
7. Sagvekar P, Kumar P, Mangoli V, Desai S, Mukherjee S. DNA methylome profiling of granulosa cells reveals altered methylation in genes regulating vital ovarian functions in polycystic ovary syndrome. *Clin Epigenetics.* 2019;04(1):61. 11(.
8. Kalmbach KH, Antunes DM, Kohlrausch F, Keefe DL. Telomeres and Female Reproductive Aging. *Semin Reprod Med.* 2015 Nov;33(6):389–95.
9. Prasad KN, Wu M, Bondy SC. Telomere shortening during aging: Attenuation by antioxidants and anti-inflammatory agents. *Mech Ageing Dev.* 2017;06:164:61–6.
10. Calado RT, Yewdell WT, Wilkerson KL, Regal JA, Kajigaya S, Stratakis CA, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood.* 2009 Sep 10;114(11):2236–43.
11. Pedroso DC, Miranda-Furtado CL, Kogure GS, Meola J, Okuka M, Silva C, et al. Inflammatory biomarkers and telomere length in women with polycystic ovary syndrome. *Fertil Steril.* 2015 Feb;103(2):542-7 e2.
12. Wang C, Shen F, Zhu Y, Fang Y, Lu S. Telomeric repeat-containing RNA (TERRA) related to polycystic ovary syndrome (PCOS). *Clin Endocrinol (Oxf).* 2017 Apr;86(4):552–9.

13. Vasilopoulos E, Fragkiadaki P, Kalliora C, Fragou D, Docea AO, Vakonaki E, et al. The association of female and male infertility with telomere length (Review). *Int J Mol Med*. 2019 Aug;44(2):375–89.
14. LaRocca TJ, Seals DR, Pierce GL. Leukocyte telomere length is preserved with aging in endurance exercise-trained adults and related to maximal aerobic capacity. *Mech Ageing Dev*. 2010 Feb;131(2):165–7.
15. Puterman E, Weiss J, Lin J, Schilf S, Slusher AL, Johansen KL, et al. Aerobic exercise lengthens telomeres and reduces stress in family caregivers: A randomized controlled trial - Curt Richter Award Paper 2018. *Psychoneuroendocrinology*. 2018 12;98:245–52.
16. Denham J, O'Brien BJ, Charchar FJ. Telomere Length Maintenance and Cardio-Metabolic Disease Prevention Through Exercise Training. *Sports Med*. 2016 Sep;46(9):1213–37.
17. Dankel SJ, Loenneke JP, Loprinzi PD. The impact of overweight/obesity duration and physical activity on telomere length: An application of the WATCH paradigm. *Obes Res Clin Pract*. 2017 2017 Mar - Apr;11(2):247-52.
18. Hutchison SK, Stepto NK, Harrison CL, Moran LJ, Strauss BJ, Teede HJ. Effects of exercise on insulin resistance and body composition in overweight and obese women with and without polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2011 Jan;96(1):E48–56.
19. Greenwood EA, Noel MW, Kao CN, Shinkai K, Pasch LA, Cedars MI, et al. Vigorous exercise is associated with superior metabolic profiles in polycystic ovary syndrome independent of total exercise expenditure. *Fertil Steril*. 2016 Feb;105(2):486–93.
20. Ribeiro VB, Kogure GS, Lopes IP, Silva RC, Pedroso DCC, de Melo AS, et al. Effects of continuous and intermittent aerobic physical training on hormonal and metabolic profile, and body composition in women with polycystic ovary syndrome: A randomized controlled trial. *Clin Endocrinol (Oxf)*. 2020 Apr.
21. Group REA-SPCW. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004 Jan;81(1):19–25.
22. International Society for the Advancement of Kinanthropometry. *International Standards for Anthropometric Assessment* - Australia: The University of South Australia; 2001.
23. American College of Sports Medicine A. *Guidelines for Exercise Testing and Prescription*. 9^a ed. Philadelphia: Guanabara Koogan; 2014.
24. Calado RT, Brudno J, Mehta P, Kovacs JJ, Wu C, Zago MA, et al. Constitutional telomerase mutations are genetic risk factors for cirrhosis. *Hepatology*. 2011 May;53(5):1600–7.
25. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res*. 2009 Feb;37(3):e21.
26. Li Q, Du J, Feng R, Xu Y, Wang H, Sang Q, et al. A possible new mechanism in the pathophysiology of polycystic ovary syndrome (PCOS): the discovery that leukocyte telomere length is strongly associated with PCOS. *The Journal of clinical endocrinology metabolism*. 2014 Feb;99(2):E234-40.

27. Wei D, Xie J, Yin B, Hao H, Song X, Liu Q, et al. Significantly lengthened telomere in granulosa cells from women with polycystic ovarian syndrome (PCOS). *Journal of assisted reproduction and genetics*. 2017 Jul;34(7):861–6.
28. Loprinzi PD, Sng E. Mode-specific physical activity and leukocyte telomere length among U.S. adults: Implications of running on cellular aging. *Prev Med*. 2016 Apr;85:17–9.
29. Williams DM, Buxton JL, Kantomaa MT, Tammelin TH, Blakemore AIF, Järvelin MR. Associations of Leukocyte Telomere Length With Aerobic and Muscular Fitness in Young Adults. *Am J Epidemiol*. 2017;04(7):529–37. 185(.
30. Tucker LA. Physical activity and telomere length in U.S. men and women: An NHANES investigation. *Prev Med*. 2017 Jul;100:145–51.
31. Borghini A, Giardini G, Tonacci A, Mastorci F, Mercuri A, Mrakic-Sposta S, et al. Chronic and acute effects of endurance training on telomere length. *Mutagenesis*. 2015 Sep;30(5):711–6.
32. Almenning I, Rieber-Mohn A, Lundgren KM, Shetelig Løvvik T, Garnæs KK, Moholdt T. Effects of High Intensity Interval Training and Strength Training on Metabolic, Cardiovascular and Hormonal Outcomes in Women with Polycystic Ovary Syndrome: A Pilot Study. *PLoS One*. 2015;10(9):e0138793.
33. Mario FM, Graff SK, Spritzer PM. Habitual physical activity is associated with improved anthropometric and androgenic profile in PCOS: a cross-sectional study. *J Endocrinol Invest*. 2017 Apr;40(4):377–84.
34. Armanios M. Telomeres and age-related disease: how telomere biology informs clinical paradigms. *J Clin Invest*. 2013 Mar;123(3):996–1002.
35. Brandao CFC, Nonino CB, de Carvalho FG, Nicoletti CF, Noronha NY, San Martin R, et al. The effects of short-term combined exercise training on telomere length in obese women: a prospective, interventional study. *Sports Med Open*. 2020 Jan;16(1):5. 6(.
36. Mason C, Risques RA, Xiao L, Duggan CR, Imayama I, Campbell KL, et al. Independent and combined effects of dietary weight loss and exercise on leukocyte telomere length in postmenopausal women. *Obesity (Silver Spring)*. 2013 Dec;21(12):E549-54.
37. Calado RT, Cooper JN, Padilla-Nash HM, Sloand EM, Wu CO, Scheinberg P, et al. Short telomeres result in chromosomal instability in hematopoietic cells and precede malignant evolution in human aplastic anemia. *Leukemia*. 2012 Apr;26(4):700–7.
38. Saßenroth D, Meyer A, Salewsky B, Kroh M, Norman K, Steinhagen-Thiessen E, et al. Sports and Exercise at Different Ages and Leukocyte Telomere Length in Later Life—Data from the Berlin Aging Study II (BASE-II). *PLoS One*. 2015;10(12):e0142131.
39. Gielen M, Hageman GJ, Antoniou EE, Nordfjall K, Mangino M, Balasubramanyam M, et al. Body mass index is negatively associated with telomere length: a collaborative cross-sectional meta-analysis of 87 observational studies. *Am J Clin Nutr*. 2018;09(3):453–75. 108(.
40. Sabuncu T, Vural H, Harma M, Harma M. Oxidative stress in polycystic ovary syndrome and its contribution to the risk of cardiovascular disease. *Clinical biochemistry*. 2001 Jul;34(5):407–13.

Figures

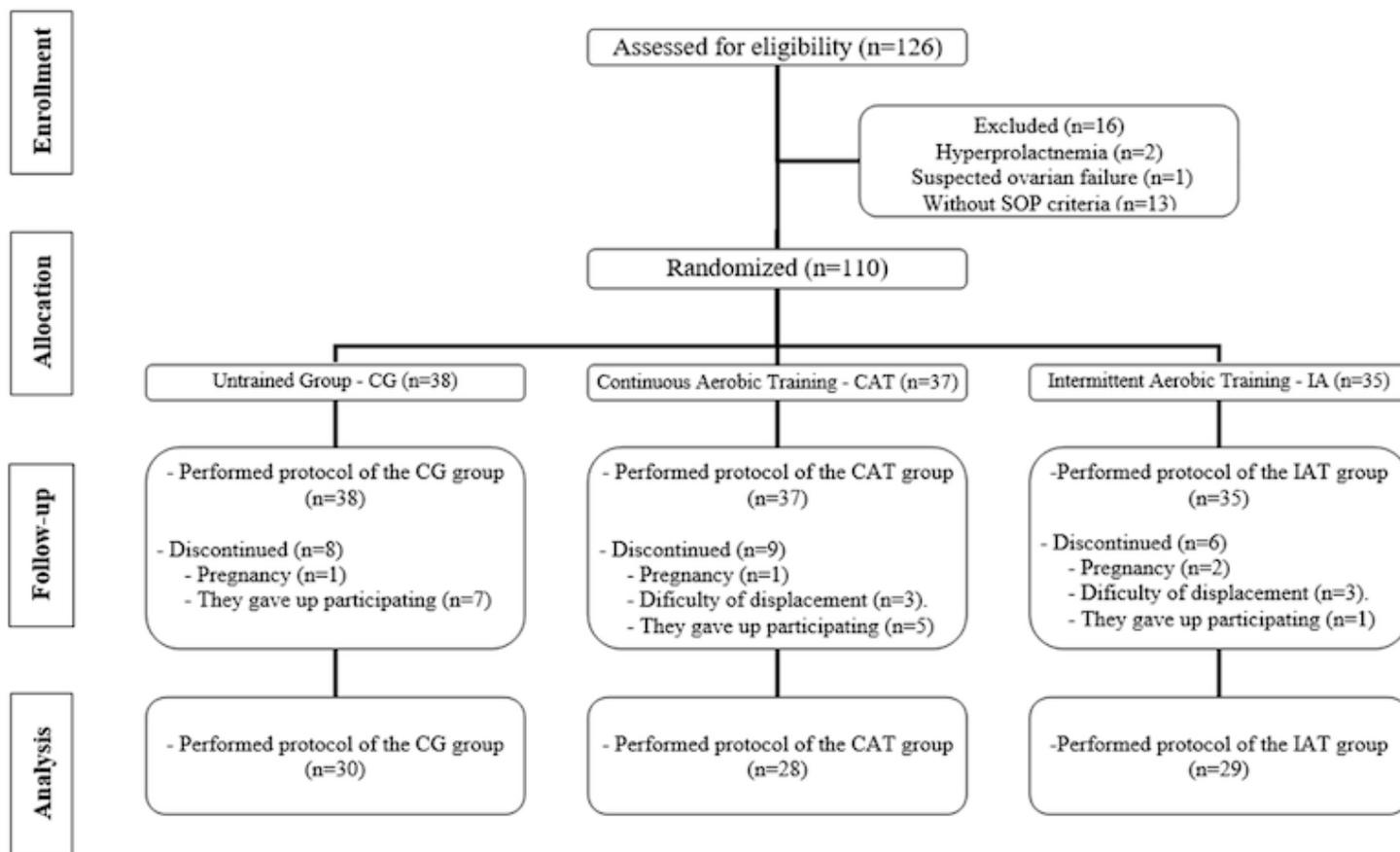


Figure 1

Flowchart of the study.

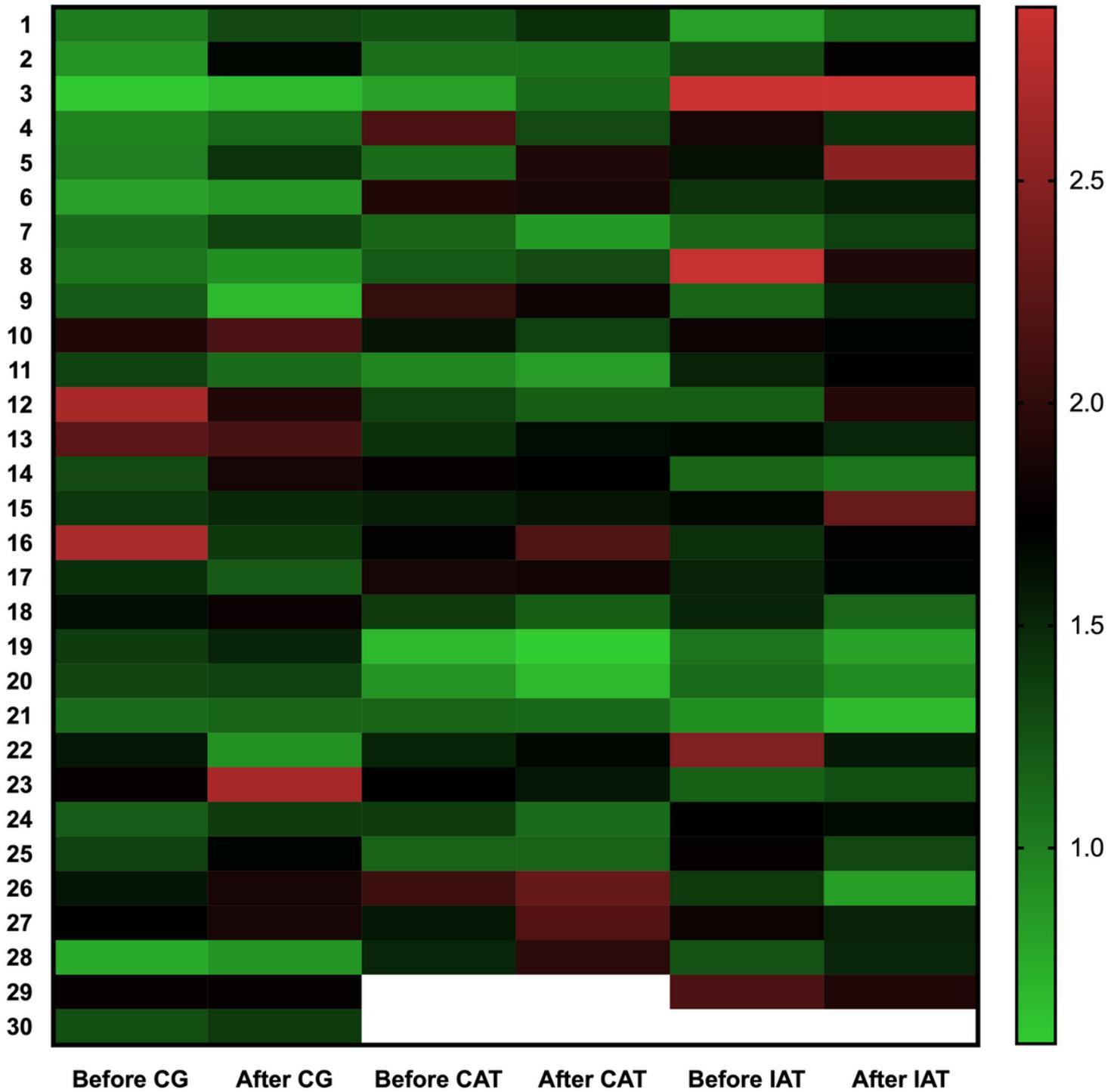


Figure 2

Heatmap of telomere length (T/S ratio) variation before and after 16 weeks of continuous and intermittent aerobic physical training or observation period.

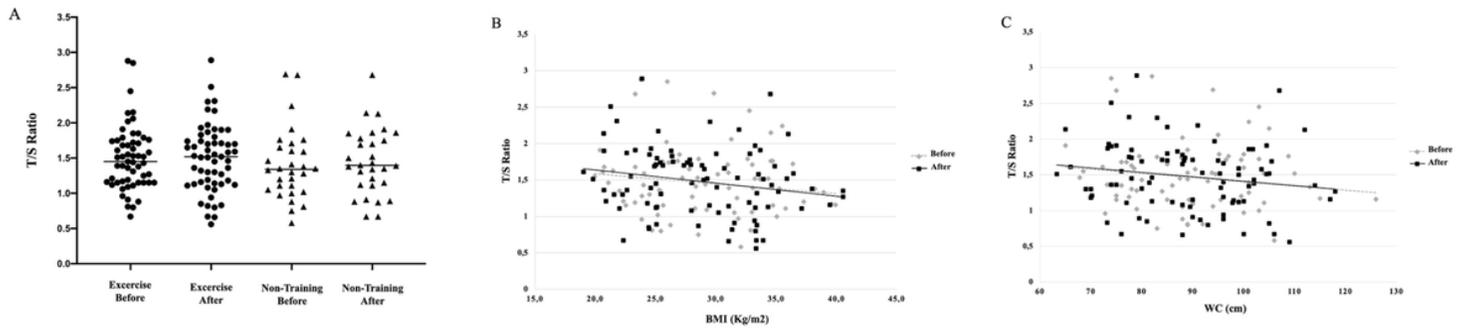


Figure 3

Telomere length (T/S ratio) before and after 16 weeks of aerobic physical training or observation period ($p > 0.05$) (A). Spearman correlation between telomere length vs BMI ($p=0.0192$) (B) and WC (C) ($p=0.0334$). CG, Control group; CAT, continuous aerobic training; IAT, intermittent

Supplementary Files

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