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ORIGINAL ARTICLE

The improvement of cardiorespiratory fitness in healthy women after a 12-week Classical Pilates training

A melhoria da aptidão cardiorrespiratória em mulheres saudáveis depois de 12 semanas de treinamento no Pilates Clássico

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Abstract

Aims: to evaluate the effects of 12 weeks of Pilates training (Classical Method) on cardiorespiratory fitness and heart rate responses of healthy sedentary women.

Method: fifteen women (average age 29±4) performed 12 weeks of Pilates training for 60 minutes, three times per week (Pilates Group). Thirteen women (average age 29±5) as controls maintained their routine activities (Control Group). The exercises' introduction was by the degree of difficulty with changes in the plane of movements; smaller base of support; spinal rotations and extensions; use of abdominal muscles in prone position. Heart rate was monitored and recorded during all sessions using a heart frequency meter.

Results: ANOVA analysis results revealed significant difference in Pilates Group (p < 0.05) between pre and post measures of VO2peak (+13%), fat percentage (- 3.3%), free fat mass (+ 2.8 kg), and muscular endurance in the abdominal area (+61%), lower limbs (+75%) and upper limbs (+68%). The percent of Maximum Heart Rate achieved in the 6th (79.25%) and 12th (79.86%) weeks of training increased from the first week (73.4%). Physical fitness in Control Group remained unchanged.

Conclusion: twelve weeks of Pilates training positively affected overall physical fitness in previously sedentary healthy women. Furthermore, the increase in VO2peak and achieved Maximum Heart Rate was significant even with no specific aerobic training.

Keywords: cardiorespiratory fitness, body composition, muscle endurance, pilates.

Resumo

Objetivos: avaliar os efeitos de 12 semanas de treinamento de Pilates (Método Clássico) sobre o condicionamento cardiorrespiratório e respostas da frequência cardíaca de mulheres sedentárias saudáveis.

Métodos: quinze mulheres realizaram 12 semanas de treinamento de Pilates durante 60 minutos, três vezes por semana (Grupo Pilates), com média de idade 29±4 anos. Treze mulheres com idade compatível com os controles mantiveram suas atividades rotineiras (Grupo Controle), com média de idade de 29±5 anos. A

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introdução dos exercícios foi pelo grau de dificuldade com mudança nos planos de movimento; menor base de suporte; rotação e extensão da coluna; e ativação da musculatura abdominal em posição de decúbito ventral. A frequência cardíaca foi monitorada e gravada durante todas as sessões usando um frequencímetro.

Resultados: Os resultados da ANOVA revelaram diferença significativa no Grupo Pilates (p < 0,05) entre medidas pré e pós de VO2pico (+13%), percentual de gordura (- 3,3%), massa gorda livre (+ 2,8 kg), e resistência muscular da área abdominal (+61%), membros inferiores (+75%) e dos membros superiores (+68%). O percentual de Frequência Cardíaca Máxima alcançado na sexta (79,25%) e na décima primeira (79,86%) semanas de treinamento aumentou a partir da primeira semana (73,4%). A aptidão física do Grupo Controle permaneceu inalterada.

Conclusão: doze semanas de treinamento de Pilates afetaram positivamente o condicionamento físico geral em mulheres saudáveis anteriormente sedentárias. Além disso, o aumento do VO2pico e da Frequência Cardíaca Máxima foi significativo mesmo sem treinamento aeróbico específico.

Palavras-chave: aptidão cardiorrespiratória, composição corporal, resistência muscular, pilates.

ABBREVIATIONS: HRmax, Maximum Heart Rate.

Introduction

Joseph Pilates developed Classical Pilates training nearly a century ago, but its popularity and increasing number of practitioners has grown exponentially in recent years (1). The main goal of Pilates training is to improve physical function. It emphasis core (abdominal and back muscles) strength, posture, and coordination of breathing with movement, and it has been widely used in rehabilitation and fitness programs (2, 3). The exercises performed on a mat or apparatus follows a specific order progressing from basic and intermediate to advanced levels (4). Several studies have suggested that Pilates training improves balance (5, 6, 7), flexibility (8, 9, 10), dynamic muscular endurance (8, 9, 11), body composition (11, 12, 13) and peripheral (8, 14), inspiratory muscle strengths (15, 16, 17), and significant improvement in mean heart rate, respiratory exchange ratio, and oxygen equivalent in sedentary subjects (18).

According to The American College of Sports Medicine guidelines (19), it is necessary to exercise 30-60 minutes at moderate-intensity (five days per week) or 20-60 minutes at a vigorous intensity (three days per week) to develop and maintain cardiorespiratory fitness. Following such recommendations, a superior cardiorespiratory improvements was found after 16 weeks of aerobic (30 min), plus mat Pilates training (20 min), in heart failure patients (2). These improvements were compared to a conventional rehabilitation program (30 min of aerobic and 20 min of calisthenics exercises). Mat Pilates training also showed a significant effect as an adjunct therapy to reduce hypertensive women's blood pressure under an antihypertensive regimen (20). However, research on cardiorespiratory fitness and the truly Classical Pilates (mat and apparatus), as envisioned by Joseph Pilates, is still very scarce. It is unknown what level of cardiorespiratory adaptation can be achieved with this training Classical Pilates Method (without any modern adaptations), especially in young, healthy people, which makes the majority of practitioners.

Load progression in Classical Pilates is based on the level of difficulty to perform the exercises (mat) and on the spring resistance (apparatus) (2, 4). Empirically, this training method promotes an appropriate stimulus to develop the general physical function, including cardiorespiratory fitness. During the Pilates session, abdominal muscles activate continuously, mixing isometric and dynamic exercises with highly energetic demands (21).

Given the positive effects of mat Pilates training on physical function and cardiorespiratory adaptations in cardiac patients, we hypothesized that Classical Pilates training could be beneficial also for a healthy previously sedentary participant (2, 20). Therefore, this study's primary aim was to evaluate the effects of 12 weeks of Pilates training (Classical Method) on cardiorespiratory fitness and heart rate (HR) responses of healthy sedentary women. Furthermore, we also verified the effects of Pilates on body composition and dynamic muscular endurance.

Methods

Pilates is a suitable type of training to improve flexibility, balance, and muscular endurance in different populations. The current study was designed to evaluate the effects of 12 weeks of Pilates training (Classical Method) primarily on cardiorespiratory fitness.

This prospective nonrandomized parallel trial, with a convenience sampling, investigated healthy sedentary women divided into two groups: one performed by 12 weeks of Pilates training (Classical Method) and others remained as control. During training sessions, HR, and the energy intake was controlled in both groups.

All evaluations were performed twice before and after 12 weeks for the same evaluators. On the first day, a cardiorespiratory fitness test (peak oxygen consumption - VO2peak) was performed, and 48 hours later, we assessed body composition (weight, fat percentage, free fat mass) and dynamic muscular endurance (abdominals, upper limb, lower limb). The same evaluator appraised the parameters one week before and one week after the 12 weeks intervention.

Participants

The recruitment of participants came through print media and informal personal contacts. 48 women and one man were interviewed by phone. The International Physical Activity Questionnaire (IPAQ) extended version was applied to measure participants' physical activity level (22, 23). The inclusion criterion was <150 minutes of moderate or intense weekly physical activity (23, 24). Thenceforth, 30 participants were further screened for detailed inclusion criteria.

The inclusion criteria where they must not engage in any regular physical activity for at least six months, never having practiced Pilates session, without physical limitations, such as musculoskeletal or neurological illnesses that limited their practice. In addition, they should have be free of chronic diseases, such as diabetes, hypertension, and obesity.

The sample was assigned to the Pilates Training by schedule availability to the Pilates Group (PG) and Control Group (CG). Pilates Group was composed of 15 participants who underwent training three times a week for 12 weeks, in a private Pilates Studio in Porto Alegre – Brazil, with attendance control. Thirteen healthy controls maintained their routine activities. The study was approved by the Ethics Committee in the Clínicas Hospital of the Federal University of Rio Grande do Sul (record 08-202). Informed consent was obtained from all participants.

Procedures

Cardiorespiratory exercise test

The maximal test was performed on a treadmill (IMBRAMED, KT 4000, Porto Alegre, Brazil). The VO2peak, the respiratory exchange ratio, and maximum heart rate (HRmax) were analyzed breath-by-breath by a validated system (Metalyzer 3B, Cortex, Leipzig, Germany). Ramp protocol until volitional exhaustion was used, and one of the criteria should be achieved: respiratory exchange ratio >1.1 or nine points in Borg Rating of Perceived Exertion (1-10 scale) (25). First, it was performed 3 minutes of warming up (speed 2 km/h and 0% incline), then the test was initiated with 4 km / h up to 10 km / h, at 2% of initial inclination up to 10%, with exhaustion up to 12 minutes. Blood pressure was measured every 2 minutes by auscultation, and a standard 12-lead Electrocardiogram (ECG) continuously monitored HR. The highest average VO2 value during the test was recorded as the VO2peak (26). The evaluator 1 is trained and specialized in this type of test and was blind to each group's participants.

Body Composition and Energy Intake

Body density, fat percentage, fat mass, and free fat mass, according to the anthropometric equation, validated for Brazilian women were evaluated (27, 28). All skinfolds' measurements were performed on the body's right side as recommended by the scientific picometer (CES-CORF, Porto Alegre, Brazil). We used the mean of three randomized measures. The anatomical points were triceps, suprailiac, abdominal, and thigh, as described before (27). The fat percentage estimate was evaluated by the SIRI equation (28). The evaluator 2 is trained and specialized in this type of test and was blind to each group's participants. A three-day food diary (2 days of the week and one day of the weekend) was used to

measure the energy intake one week before the intervention, and one week after the intervention in the PG, in the CG, the diary was filed one week and after 12 weeks. Intake energy analysis was analyzed in accordance with the Brazilian Food Composition Table (29). The final analysis was performed with the mean delta of three days of consumption (the difference between pre and post registers).

Dynamic Muscular Endurance

The dynamic muscular endurance was assessed by measuring the maximum number of consecutive repetitions of upper limbs and shoulder exercise, abdominal and jumps, as described before (8, 30). The test was carried out with two evaluators: one was selected for counting repetitions and time control (evaluator 1), and the other also count the repetitions and observe the movement's quality (evaluator 2).

Pilates Training protocol

The exercise program followed the principles of the Classical Pilates Method (4,31). In the first three weeks, exercise series from the basic system was applied: The Mat (The Hundred was used to warm up), The Reformer, The Wall and The Weight (free weight exercises) (32, 33, 34). Participants performed ten repetitions of each exercise, and the training sessions lasted 55 minutes.

The basic system exercises remained unchanged during the first three weeks of training, for the participants to acquire greater control of breath and accomplished the appropriate transitions between exercises (3). During the 4th, 5th, and 6th week, the participants were introduced into the intermediary system, with three new exercises per session, for better assimilation (32, 33, 34). The order of introducing the exercise was by the degree of difficulty, such as introducing exercises for abdominal muscles, spins, stretch, and balance (4, 32, 33, 34). In the 6th week, all the intermediate system exercises were introduced, remaining unchanged up to 12 weeks (see supplemental material for more details – **Table 1**).

During all sessions, each participant's exercise intensity was monitored and recorded by HR mo-

nitors (Polar RS800 sd, Finland). The percentage of HRmax (%HRmax) measured during the Pilates sessions are expressed in this study as a percentage of the HRmax obtained in the Cardiorespiratory exercise test. The Pilates instructor specialized in the Classical Method for more than 10 years and was blinded to all evaluations. All sessions were single or double designed. The adherence and compliance to the program were controlled in PG, and an extra session every week was offered for those that could not attend the regular sessions.

CG was instructed to maintain their routine activities for the same 12 weeks period. The group was contacted by telephone, between 5th and 8th weeks, to monitor their routine and check their physical activity records. In the 12th week, they were invited to re-evaluate.

Statistical Analysis

All values were expressed as mean and standard deviation. Comparison between the Pilates Group and Control Group of baseline values of age, height, body mass, fat percentage, free fat mass, HR rest, HRmax, VO2 peak, muscle endurance (abdominal, upper limbs, and lower limbs), we used Independent T Student test. Comparisons of cardiorespiratory fitness, body composition, and dynamic muscular endurance variables were performed between PG and CG employing ANOVA two-way. Values were considered statistically significant at p <0.05. For the analysis of the %HRmax during sessions, the ANOVA one-way used multiple comparisons by Bonferroni test correction between 1st, 9th, 18th, and 36th sessions. All tests were performed in software IBM SPSS Statistics 21. A posteriori power calculation for the main output (VO2peak) was 94.3%.

Results

All participants completed the 12-weeks trail according to the study protocol. Fifteen participants from PG trained all of 36 training sessions. No adverse effects were reported during the session and only reported after the intervention of muscle pain and discomfort in each session.

Week 1-2-3 Exercises	NR	Week 4 Exercises	NR	Week 5 Exercises	NR	Week 6 -12 Exercises	NR
Basic Mat		Basic Mat		Basic Mat		Basic Mat	
The Hundred	10	Basic Reformer		Basic Reformer		Basic Reformer	
Roll up	5	The Wall		Basic The Wall		Basic The Wall	
Single-Leg Circle	5	Basic The Weight		Basic The Weight		Basic The Weight	
Roll Down	10	Intermediate Mat		Intermediate Mat week 4		Intermediate Mat week 4 and 5 Intermediate	
Rolling Like a Ball	10	Single Straight Leg Stretch	10	Intermediate Reformer week 4		Reformer week 4 and 5	
Single-Leg Stretch	10	Double Straight Leg Stretch	10	Intermediate Weight week 4		Intermediate Weight week 4 and 5	
Double Leg Stretch	10	Criss Cross	10	Intermediate Mat		Intermediate Mat	
Spine Stretch Forward	5	Saw Neck Pull	4	Neck Roll	3	Corkscrew I	5
Basic Reformer		Neck Pull	5	Single Leg Kick	10	Side Kicks – Leg Lifts	5
Footwork Series	10	Open Leg Rocker	6	Double Leg Kick	6	Teaser I	5
The Hundred	10	Intermediate Reformer		Side Kicks- front and back	10	Seal	6
Leg Circles	10	Short Box- Twist/Reach	3	Side Kicks- up and down	10	Intermediate Reformer	
Frog	10	Stomach Massage Twist	3	Side Kicks- small circle	5	Down Stretch	8
Stomach Massage Round	10	Intermediate The Weight		Intermediate Reformer		Up Stretch	5
Stomach Massage Hands Back	10	Sides	10	Short spine Back	5	Long Box- Teaser	3
Stomach Massage Reach							
Up	10	The Bug	10	Coordination	5	Semi Circle	6
Short Box- Round	8			Long Box – Pulling Straps	3	Side Split	5
Short Box- Flat	8			Long Box - The "T"	3	Front Splits	3
Short Box- Side to Side	3			Long Box- Backstroke	5		
Short Box- The Tree	3			Long Strech	8		
Elephant	10			Intermediate The Weight			
Knee Stretch Round	10			Zip-Up	10		
Knee Stretch Arches	10			Shaving	10		
Knee Stretch Knee Off	10						
Running	20						
Pelvic Lift	10						
The Wall							
Arm Circles	5						
The Roll Down	10						
Squats	5						
Basic The Weight							
90 Degrees	10						
Arms at Side	10						
Standing Curls	10						

TABLE 1 – Classical Pilates exercise execution protocol and repetition.

NR, number of repetitions. In the first column, we describe the exercises that are part of The Mat Basic, Reformer Basic, Wall, and Weights Basic (bold). In the subsequent columns, we have the description of the intermediate exercises added in the basic sequence.

More exercises were implemented in the four to six weeks, and tiredness was implemented after training from six weeks to 12 weeks. During follow-up 12 weeks after the first contact, two CG participants declined: one for pregnancy and the other could not attend the final evaluations. The physical and functional characteristics of all participants (PG and CG) are described in **Table 2**. Characteristics on Baseline did not differ between groups.

TABLE 2 – Baseline characteristics for Pilates Group and Control Group.

	Pilates Group	Control Group	P*
Age (years)	29±4	29±5	0.85
Height (cm)	162±4	165±6	0.43
Body mass (kg)	63±6	65±9	0.53
Fat percentage (%)	31.8±4.7	29.5±4	0.68
Free Fat Mass (kg)	42.7±2	45.8±5	0.07
HR rest (bpm)	81±9	81±7	0.17
Maximum HR (bpm)	101±7	103±6	0.50
VO2peak (mL·kg ^{-1,} min ⁻¹)	34.8±4.1	32.2±5.5	0.12
Abdominal muscle endurance (reps/min)	36±7	41±8	0.73
Upper limb (reps/min)	25±8	30±9	0.92
Lower limb (reps/min)	32±9	35±13	0.07

HR, heart rate; VO2peak, peak oxygen consumption. Data are shown as mean±standard deviation. Independent T student test.

Regarding the cardiorespiratory fitness, VO2peak increased significantly in PG (13%), whereas in CG did not change (**Table 3**). In the first training session, HRmax was 141 ± 18 bpm, approximately 73.47% of the achieved HRmax during the cardiorespiratory test. In 9th session, participants achieved 146 ± 18 bpm (75.58% of the HRmax). In the 18th session, the participants completed the intermediate series, and they achieved significantly higher HR (153 ± 17 bpm – 79.25% of HRmax than the other moments. In the last training session (36th), the average HR was also significantly higher than the first session, achieving 79.86% of the HRmax (**Figure 1**). The fat percentage decreased, and free fat mass increased in the PG participants (-10.4% and +6.5%, respectively), with no significant changes in CG (**Table 4**). The diaries energy intake analysis showed that the intervention group had an increase in total calories intake (average +140kcal; p=0.05), but a decrease in fat consumption (average -3.36g; p<0.01), and no differences in CG, and they did not receive any particular nutritional recommendation.

Considering intra-group comparisons, PG participants showed an increase in abdominal muscular endurance (+61%), lower limb (+75%), and upper limb (+68%) while the CG showed a significantly decreased (-10%, -6%, and -4% respectively).

TABLE 3 - Cardiorespiratory Variables.

	Pilates Group (before/after)	Δ	Control Group (before/ after)	Δ	p for Group*	p for Time*	p for Interaction*
HR Rest (bpm)	81±9/77±12	-4	81±7/80±6	-1	0.57	0.16	0.32
Resting systolic blood pressure	109±10/110±6	1	110±10/106±10	-4	0.78	0.26	0.049
Resting diastolic blood pressure	65±6/70±5	5	69±7/66±6	-3	0.86	0.35	0.002
VO2peak	34.82±4.1/39.3±4.9	4.5	32.2±5.5/31.55±6.4	-0.6	0.012	<0.001	<0.001
Respiratory exchange ratio	1.23±0.1/1.23±0.1	0	1.2±0.1/1.15±0.1	-0.04	0.07	0.54	0.48
Maximum HR	193±13/193±17	0	195±11/189±9	-6	0.91	0.30	0.33
Maximal systolic blood pressure	141±13/143±8	2	143±7/138±18	-5	0.71	0.72	0.32

HR, heart rate; VO2peak, peak oxygen consumption. Data are shown as mean±standard deviation. 'ANOVA two-way.

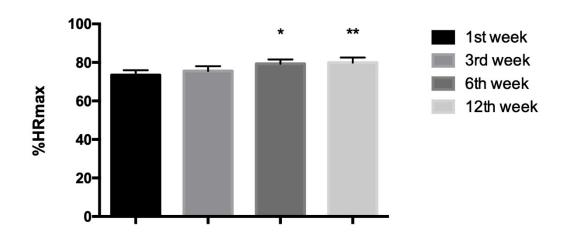


Figure 1 - Pecentage of Maximum Heart Rate (%HRmax) achieved at 1st, 3rd, 6th, 12th week of Pilates training expressed as mean±standard deviation with *p <0.005 and **p <0.049 to the 1st week. ANOVA one--way used multiple comparisons by Bonferroni test correction between 1st, 9th, 18th, and 36th sessions.

Discussion

The present study is critical to breaking new grounds in evaluating the effects of the Classical Pilates Method as truly cardiorespiratory fitness training. The primary purpose was to evaluate the effects of 12 weeks of Pilates training on VO2peak and HR responses. The secondary purpose was

to evaluate the training effect on body composition and dynamic muscular endurance. Our data confirm the hypothesis that Pilates training improves physical fitness in healthy women, including significant cardiorespiratory adaptations.

Intensity around 70% of HRmax can be considered sufficient to induce cardiorespiratory

and metabolic adaptations (19, 35). Our results showed that sessions of Pilates training could promote about 73-79% of HRmax. This exciting finding suggests that Pilates training can be used as an option to traditional aerobic exercises. Therefore, the intensity achieved during the training sessions can justify, at least in part, the increase of 13% to VO2peak in our PG. In agreement with our findings, have found² improvements of 19% in VO2peak after aerobic exercise combined with Mat Pilates in heart failure patients.

Body Composition	Pilates Group (before/ after)	Δ	Control Group (before/ after)	Δ	p for group*	p for time*	p for interaction*
Body mass (kg)	63±6/63.9±7	0.9	65.2±8.8/66±9.2	0.8	0.475	0.025	0.80
Fat percentage (%)	31.8±4.7/28.5±4.2	-3.3	29.5±4/30.3±4	0.8	0.871	<0.001	<0.001
Free fat mass (kg)	42.7±2.4/45.5±3.4	2.8	45.8±5/45.8±4.9	0	0.284	<0.001	<0.001
Dynamic muscle endurance							
abdominals	36±7/58±9	22	41±8/37±7	-4	0.005	<0.001	<0.001
Upper limb	25±8/42±6	17	30±9/29±8	-1	0.164	<0.001	<0.001
Lower limb	32±9/56±14	24	35±13/33±11	-2	0.020	<0.001	<0.001

Data are shown as mean±standard deviation. Independent T student test.

Pilates practice involved a link between controlled diaphragmatic breathing and movements. We believed that cardiorespiratory adaptation could also be mediated by increased strength and resistance of respiratory muscles. Pieces of evidence suggest that when these muscles are trained in heart failure patients, exercise tolerance increases, and this effect is associated with the attenuation of the inspiratory muscle metaboreflex (36, 37). Indeed, specific respiratory muscle training added to moderate continuous exercise training had shown a significantly higher effect on VO2peak (40%) when compared to aerobic training only (21%) (36).

The increase in VO2peak value in PG could also be explained by the fact that previously sedentary women with low physical function have a higher potential to increase their physical endurance performance than trained participants (38).

Although the participants of the PG have maintained their body mass unchanged, they showed variations of fat percentage (- 3.3%) and free fat mass (+ 2.8 kg). Similar body composition results were observed after 12 weeks of Mat Pilates training assessed through dual-energy x-ray absorptiometry (39). Also verified fat percentage decrease (-5%) without changes in total body mass after eight weeks of Mat Pilates (11). However, some previous studies showed no significant alteration in body composition, there is currently insufficient empirical quantitative evidence indicating a positive effect of Pilates exercises on body composition (8, 11, 12, 40). Poor standardization in the measurement protocols, insufficient or no control of the energy intake, and the lack of adequately certified Pilates instructors are pointed as the main factors to low-quality studies (40). Our study was performed, taking into account all these issues, and our results showed a positive effect of Classical Pilates Training on body composition.

The American College of Sports Medicine guidelines recommend that to improve dynamic muscle endurance is necessary eight to 15 repetitions of each muscle group, two to three times a week, with an average interval of 24 to 48 hours (41). Our training program agrees with

those principles, and our protocol was incremented regularly to attend the overload training principles (42). We found improvement in dynamic muscle endurance abdominals, dynamic muscle endurance, upper limbs (support), and dynamic muscle endurance, lower limbs (jump). The positive effect of Pilates training on muscle endurance was also observed in previous studies (8, 9, 43, 44). In our study, the PG has changed from 60 to > 90 in the percentile rank of abdominals and 70 to > 90 also in support. These results indicated an intense work of muscle endurance in the series of the Classical Pilates Method. The improvement in dynamic muscle endurance is considered an essential peripheral adaptation and could also explain the increase in VO2peak of PG (43).

The present study has some limitations. First, even if the participants had similar characteristics, it was impossible to truly randomize them because they were arranged according to their schedule availability. However, we tried to follow the CONSORT statement as much as possible (45). Second, the instructor that applied the Pilates Classical training was not blinded in this study; however, all evaluators and, most importantly, the VO2peak evaluator were blinded to the group interventions. Based on the Pilates principle, we believe that our results, in part, can be attributed to the instructors' participation/training program in this trial. Finally, the exclusiveness of the female sex makes our results gender-specific; nevertheless, it still represents most participants who subscribe to Pilates training.

Pilates training is recommended mainly to improve muscular strength, endurance, flexibility, and balance.

The present study showed that, beyond changes in these physical capacities, 12 weeks of Pilates Training monitored by HR 3 times a week positively impacts body composition and cardiorespiratory fitness.

Our findings support that Classical Pilates Training could be an excellent option to traditional fitness programs, especially for previous sedentary participants, since Pilates training could promote an intensity higher than 70% of the HRmax. This study's novelty was to present the effects of a Pilates HR monitored training on cardiorespiratory fitness.

The Classic Pilates Method training of 12 weeks positively affected overall physical function in previously sedentary healthy women. Furthermore, the increase in VO2peak was significant, even with no specific aerobic training.

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Conflicts of interest disclosure

The authors declare no competing interests relevant to the content of this study.

Authors' contributions

All the authors declare to have made substantial contributions to the conception, or design, or acquisition, or analysis, or interpretation of data; and drafting the work or revising it critically for important intellectual content; and to approve the version to be published.

Availability of data and responsibility for the results

All the authors declare to have had full access to the available data and they assume full responsibility for the integrity of these results.

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