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Running Head: Insulin Resistance and Walking Training

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Effects of Patterns of Walking Training on Metabolic Health of Untrained Postmenopausal Women

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Abstract

Purpose: High-intensity aerobic interval training (AIT) has been reported to be more effective than continuous aerobic training (CoAT) to improve metabolic health. The aim of our study was to investigate whether moderate-intensity AIT is more effective than CoAT on metabolic health, when applied to a walking training program. **Design/Methods:** Thirty-two postmenopausal women (55.37 ± 3.46 years) were investigated for body composition, plasma glucose, insulin, lipids, adiponectin, HOMA-IR, HOMA-AD, aerobic fitness, dietary habits and spontaneous physical activity, and randomly assigned to one of two different walking training programs: CoAT or AIT. **Results:** CoAT and AIT elicited the same physiological benefits, including: reduction of plasma glucose, insulin, HOMA-IR and HOMA-AD, and increase of plasma HDL-C, adiponectin and aerobic fitness. **Conclusions:** An AIT scheme as part of an outdoor walking training program elicits the same physiological adaptations as a CoAT scheme, probably because walking does not promote exercise intensities that elicit greater effects.

Key words: HOMA-IR, HOMA-AD, adiponectin, continuous aerobic training, aerobic interval training

Introduction

Menopause is frequently associated with weight gain and a shift in body-fat accumulation from the hips and the thighs to the trunk. This is promoted by estrogen deficiency accompanied by a reduction in spontaneous physical activity, which is typical of ageing. This android obesity can contribute to a reduction in insulin sensitivity and development of dyslipidemia, insulin-resistance, and type 2 diabetes. These are important risk factors for cardiovascular disease (CVD), which represents the major cause of death among postmenopausal women (Zhang, Rexrode, van Dam, Li, and Hu, 2008). Increased body fat is also associated with modifications to adipocyte hormonal secretion, which can result in reduced production of adiponectin, one of the several adipokines that are produced by adipocytes (Arita et al., 1999). A reduction in adiponectin secretion is important because of its significant role in the onset of metabolic disorders, such as type 2 diabetes and metabolic syndrome; indeed, adiponectin has insulin sensitizing, anti-inflammatory, and anti-atherogenic properties (Villareal-Molina & Antuna-Puente, 2012). Due to the relationships between insulin and adiponectin, a new index has been suggested as a predictor of insulin resistance: the homeostatic model assessment (HOMA) corrected for adiponectin (HOMA-AD) (Matsuhisa et al., 2007), which has been shown to be a more sensitive predictor of insulin resistance in subjects with normal and impaired glucose tolerance, and in diabetic subjects with moderate hyperglycemia (Matsuhisa et al., 2007).

In the literature, there are reports on the important role of physical activity and healthy eating in the prevention and treatment of insulin resistance and android obesity, and in the reduction of plasma adiponectin levels. Indeed, a role for aerobic training in the reduction of insulin resistance is well established (Pedersen & Saltin, 2006), as also for increased plasma adiponectin levels (Bouassida et al., 2010). Aerobic training can be performed in different

modalities: in a continuous manner, or in an alternating manner, where bouts of physical exercise at higher intensities are alternated with bouts of physical exercise at lower intensities. New scientific evidence suggests that high-intensity aerobic interval training (AIT) has a greater efficacy for increases in aerobic fitness and insulin sensitivity, and for reduction of risk factors for metabolic syndrome, with respect to continuous aerobic training (CoAT) (Tjønnå et al., 2008). However, no scientific evidence has yet been reported to support the same effects for plasma adiponectin.

Although high-intensity AIT shows greater efficacy for the improvement of cardio-metabolic parameters, it can be unsafe, particularly in unhealthy and elderly people, for whom walking training must be recommended in any aerobic physical exercise program. Therefore, as walking outdoors on the flat does not allow the exercise to reach high-intensity effort, it appears unlikely to expect the same amplitude of benefits of high-intensity AIT in a walking program. However, interval organization of walking training might be a valid method to interrupt the monotony of a continuous walk and to increase the adherence to physical exercise of those people who consider continuous walking through a whole exercise period to be boring. The study of the effects of different walking training patterns on health is useful because it promotes the health benefits of movement according to the psychological (e.g., individual preferences) and physio-pathological characteristics of everyone. Movement to promote health is of great importance, especially during the climacteric period, which, as indicated, is linked with increased cardiometabolic risk that can lead to reduced quality of life from menopause to old age. As a consequence, to prevent and to treat the decline of both physical performance and health, it is important that exercise specialists have as many choices as possible to effectively adapt physical exercise to the relevant situations.

Thus, the aim of our study was to compare a moderate-intensity AIT walking program and a CoAT walking program in untrained post-menopausal women, with regard to insulin resistance and plasma levels of lipids and adiponectin, while controlling for the effects of anthropometry and behavioral variables and matching the training to the mean exercise intensity.

Methods

Participants

Fifty-five post-menopausal women (mean age, 57.09 ± 4.24 years) responded to a public advertisement placed in the offices of several general physicians in the Pescara area (Italy). A telephone interview verified whether the women met the inclusion criteria of: age <65 years; no estrogen replacement therapy; no history of pulmonary, myocardial or orthopedic diseases that would limit walking; and no participation in any controlled diet and regular exercise program. The women were considered post-menopausal if their menses had naturally ceased for at least 12 months, and if they had plasma estradiol <20 pg/ml. Thirty-two of these women (mean age, 55.37 ± 3.46 years) were considered eligible for the study.

The Ethics Committee of the ‘G. D’Annunzio’ University of Chieti–Pescara approved this study, and all of the participants gave their written informed consent.

Medical examinations and measurements were performed at the Centre of Sports Medicine of the ‘G. D’Annunzio’ University of Chieti–Pescara. The laboratory conditions were controlled for temperature (21-23 °C) and humidity (50%) (ACSM, 2005). The participants presented after overnight fasting, without having performed maximal muscle exertion the day before. The participants also abstained from alcohol consumption from 48 h before the tests and were standardized for fluid intake during the same period. Before starting the physical exercise training, they underwent medical history data collection, physical examination, blood sampling,

anthropometry, maximal stress tests, and dietary habits interviews. At the end of the basal measurements (T_0), the participants were randomly assigned to one of two different walking training programs, which were equalized for mean intensity and volume. After the tests, and before the beginning of the physical exercise program, the daily physical activity of the participants was recorded over seven consecutive days under free-living context. The same tests were repeated after 14 weeks of aerobic exercise training (T_1).

Blood sampling

After an 8-h overnight fast, venous blood samples were collected to measure total serum estradiol, glucose, insulin, adiponectin, total cholesterol, high-density (HDL-C) and low-density (LDL-C) lipoprotein cholesterol, and triglycerides. Glucose, HDL-C and triglycerides were assessed by enzymatic methods, LDL-C was calculated according to the Friedewald equation (Friedewald, Levy, and Fredrickson, 1972), and enzyme-linked immunosorbent assays were used to measure plasma estradiol, adiponectin and insulin (DRG Instruments GmbH, Marburg, Germany). The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as: $(\text{fasting insulin } [\mu\text{U/l}] \times \text{fasting glucose } [\text{mg/dl}]) / 405$ (Matthews et al., 1985). The HOMA-IR corrected for adiponectin (HOMA-AD) was calculated as: $(\text{fasting insulin } [\mu\text{U/l}] \times \text{fasting glucose } [\text{mg/dl}]) / \text{adiponectin } [\mu\text{g/ml}]$ (Matsuhisa et al., 2007).

Anthropometry and body composition

A first level anthropometrist of the International Society for the Advancement of Kinanthropometry carried out the body measurements of the participants while they were still under fasting conditions. Body weight and stretched stature were measured to the nearest 0.1 kg and 0.1 cm, respectively, with the subjects dressed in light clothing and without shoes (Marfell-Jones, Olds, Stewart, and Carter, 2006), using a stadiometer with a balance-beam scale (Seca

220, Seca, Hamburg, Germany). Their body mass indices were calculated according to the formula of body weight/ stature² [kg/m²]. Anthropometric tape (Seca 200, Seca) was used to measure their waist circumference that was measured as the smallest circumference between the rib cage and the iliac crest, at the end of normal expiration (Marfell-Jones et al., 2006). The body composition was assessed using an electrical bioimpedance technique, on a foot-to-foot, 50-kHz frequency, bioelectrical impedance scale (BC-420MA, Tanita, Tokyo, Japan). The impedance measurement error margin of the body composition analyzer was $\pm 2\%$, which corresponded to about $\pm 0.5\%$ change in the body fat measurement of a person with a regular figure. The test was performed 3 h after getting up and immediately after voiding, with the participants in an upright position, barefoot and without conducting garments (ACSM, 2005).

Aerobic fitness assessment and cardiovascular eligibility for walking training

A resting 12-lead electrocardiogram (P8000, Esaote, Genoa, Italy) was performed after 10 min of supine rest. As most of the participants had never used a treadmill before, a graded maximal exercise test on a cycle ergometer (SANA BIKE 150 F, Ergosana GmbH, Bitz, Germany) was used to assess the aerobic fitness (METs_{peak}) and eligibility of the participants for aerobic training. A medical doctor specialized in sports medicine supervised and conducted the stress tests. The participants were tested under continuous electrocardiogram monitoring (AT-10 plus, SCHILLER, Baar, Switzerland) coupled with blood pressure measurements at the end of each step of the graded maximal exercise test. The Astrand protocol was used to test the fitness of the participants, to the point at which there occurred absolute or relative indications for termination of a clinical graded exercise test, according to the American College of Cardiology/ American Heart Association guidelines (ACSM, 2005).

Dietary habits assessment

A dietician estimated the participant dietary habits and energy intake from 7-day dietary records, using the *WinFood 2.7* software (Medimatica, Colonnella, Italy). This provided qualitative (macro and micronutrient composition of daily meals of up to 70 components) and quantitative (kcal, g) information about the meals of the participants. The food portions were recorded as they were consumed, by self-measurement using a scale or household measures (cup, tablespoon), and they were recorded on an open-ended form that included a section that clarified how to draw up the dietary record. At the end of the recording period, the dietician reviewed the records with each participant to clarify the entries, to probe for forgotten foods, and to collect further details of the preparation of the foods. The mean value of each investigated dietary parameter was used for statistical analysis.

Daily physical activity measurements

Daily physical activity was measured under free-living conditions over 7 consecutive days using SenseWear Pro₂ armbands (BodyMedia, Pittsburgh, USA), which integrate the information gathered by two axis accelerometers and sensors (i.e., skin and near-body temperature, heat flux, galvanic skin response) with the sex, age, stature, weight, smoking status, and handedness of the user, to provide qualitative (intensity) and quantitative (number of daily steps, energy expenditure) information about the daily physical activity of the user. From the recorded data, we focused our attention on the mean intensity of daily physical activity ($METS_{m/die}$), daily steps ($STEPS_{m/die}$), time spent on physical activity with an intensity ≥ 3 METs and ≤ 6 METs (i.e., moderate-intensity physical activity; $MIPAT_{m/die}$) and with an intensity > 6 METs (i.e. vigorous-intensity physical activity; $VIPAT_{m/die}$). The energy expenditure from $MIPAT_{m/die}$ and from $VIPAT_{m/die}$ were also calculated and considered. In addition, the time spent

in physical activities with intensities ≤ 1.5 METs (SEDENTARY TIME_{m/die}) and with intensities between 1.5 METs and 3 METs (LIPAT_{m/die}) were also considered. The T₁ physical activity measurements were the only measurements taken 20 days prior to the end of the training. The weekdays included training days and non-training days. The mean of each parameter over the recorded days was used for the statistical analyses, for both T₀ and T₁. The T₁ physical activity measurements were also gathered for T₁ training days data and T₁ non-training days data, and they were also separately compared with the T₀ measurements. The participants wore their monitors throughout the recording days, except while bathing. The recordings did not include days when it was raining.

Physical exercise programs

The participants walked on 4 days per week, for 14 weeks, and were randomly assigned to one of two training subgroups: walking CoAT or walking AIT. The exercise intensity was assigned and monitored according to the rating of the perceived exertion (RPE) scale (Borg, 1998). The participants were familiarized with this scale before the beginning of the training and during the first week of the training. Each training session included a 10-min warm-up, a training period, and 5-min to 10-min cooling down. During the warm-up, the walking speed progressively increased until the given intensity was reached. During the cooling down, the walking speed progressively decreased until the RPE decreased from the target level intensity to a RPE <10. The subgroups had the same walking program from the first to the fourth week of training and the same warm-up and cooling down periods throughout the 14 weeks of training (Figure 1).

The walking CoAT participants ($n = 16$) were trained at moderate intensities (i.e., 11-13 RPE) in a continuous manner. The duration and intensity of their training was modulated according to the week of training (Figure 1).

Starting from the fifth week of training, the walking AIT participants ($n = 16$) trained with alternating periods of moderate intensity and low intensity, which were modulated according to the duration and the week of training (Figure 1).

We calculated the volume of each exercise session by multiplication of the duration of the session (min) by the RPE points (RPE). The sum of the volume of all of the completed exercise sessions provided the volume of the exercise program of each participant. All of the participants walked together according to their subgroup membership. An exercise trainer supervised all of the weekly sessions, and also checked on compliance with the training sessions. The exercise trainer monitored the exercise intensities of the participants through the talk test, which has been shown to be a relatively precise and consistent method for monitoring exercise intensity (Persinger, Foster, Gibson, Fater, and Porcari, 2004). In addition, to ascertain that the participants trained as precisely as possible at the given intensities, during every training session every participant wore a necklace with a print of the RPE scale as a pendant, to be able to correctly visualize the scale and to transform the perceived exertion into numbers. To eliminate the effects of seasonal changes on the amounts and patterns of physical activity (Buchowski et al., 2009), the study was conducted during the summer. The walking training was the only study intervention.

Statistical analysis

The data were tested for normality before statistical analysis (i.e., skewness and kurtosis tests for normality), using the STATA 10 software (StataCorp LP, College Station, USA), and are presented as means \pm standard deviations.

Because of the small sample size, non-parametric statistics were applied. The Wilcoxon test, performed on the whole sample, was used to detect the effect of aerobic walking training, not considering its organization (i.e., walking CoAT or walking AIT), on the investigated variables. When a significant change was detected, the Mann-Whitney test was used to compare the modification between subgroups (i.e., post-training value minus pre-training value, Δ). The same variables were also compared for their basal values with the Mann-Whitney test. To determine whether walking training promoted a reduction in spontaneous physical activity, we compared the mean weekly values of the variables of physical activity recorded at T_0 with those recorded at T_1 . Furthermore, the T_0 values were also separately compared with those recorded at T_1 during the training days and the non-training days. Statistical significance was set at $p \leq 0.05$.

Results

Table 1 shows the basal (T_0) characteristics of the participants and the effects of aerobic training on the investigated variables, not considering the effects of its organization. After 14 weeks of aerobic training, participants had a mean significant reduction of plasma insulin ($p = 0.01$) and glycemia ($p = 0.008$), and of HOMA-IR ($p = 0.005$), HOMA-AD ($p = 0.001$), and $LIPAT_{m/die}$ ($p < 0.001$), and a mean significant increase of adiponectin ($p = 0.04$), aerobic fitness ($p < 0.001$), daily energy intake ($p = 0.001$), $METS_{m/die}$ ($p = 0.003$), $STEPS_{m/die}$ ($p = 0.001$), $MIPAT_{m/die}$ ($p = 0.005$) and EE from $MIPAT_{m/die}$ ($p = 0.003$) (Table 1). To determine the effects of the organization of the experimented aerobic training that had the same volume and mean

intensity, we compared the Δ of the significantly modified variables (Table 1) (i.e., walking CoAT vs. walking AIT) (Table 2). Statistical analysis showed that the walking CoAT and walking AIT programs elicited different modifications of only $METS_{m/die}$ ($p = 0.05$), $STEPS_{m/die}$ ($p = 0.04$), $MIPAT_{m/die}$ ($p = 0.01$) and EE from $MIPAT_{m/die}$ ($p = 0.01$): the Δ values recorded for walking AIT members were significantly greater than those recorded for walking CoAT members (Table 2). When we compared the basal values of the previously described modifications, statistical analysis showed that the subgroups differed only for their basal spontaneous physical activity: before the beginning of the training period, walking CoAT members were more active than walking AIT members; indeed, they had significantly higher $STEPS_{m/die}$ ($p = 0.05$), $MIPAT_{m/die}$ ($p = 0.04$) and EE from $MIPAT_{m/die}$ ($p = 0.02$) (Table 3). As the literature indicates that the addition of a training program to daily physical activity of previous sedentary women can result in a reduction in their spontaneous physical activity, which counteracts some of the positive effects of the training (Izzicupo et al., 2013; Di Blasio et al., 2013; Di Blasio et al., 2012), we analysed the trend of spontaneous physical activity of our participants. Statistical analysis confirmed that the walking training did not result in any reductions in spontaneous physical activity; indeed, comparisons of the mean weekly values of the variables of physical activity recorded at T_0 with those recorded at T_1 during nontraining days showed no significant modifications (Table 3). On the contrary, when comparing the T_0 data with the data recorded at T_1 during training days, significant increases of $METS_{m/die}$, $STEPS_{m/die}$, $MIPAT_{m/die}$, and EE from $MIPAT_{m/die}$ were detected in both subgroups (Table 3).

The subgroups underwent the same volume of physical exercise (walking CoAT: 19537.19 ± 4258.26 min \times RPE; walking AIT: 20043.75 ± 3557.66 min \times RPE; $p = 0.83$). The choice to consider the volume of physical exercise instead of the number of training sessions

attended was mainly followed because the volume of physical exercise changed from the beginning to the end of the training programs. Indeed, the same adherence with a different distribution of non-attendance does not guarantee that participants had the same amount of physical exercise.

There were no significant modifications recorded across the anthropometric variables.

Discussion

The present study shows the positive effects of walking training on insulin resistance and plasma adiponectin, independent of the anthropometric and dietary habits parameters and of the patterns of walking training. Across these exercise subgroups, when exercising at the same volume and at moderate maximum intensity (RPE 14), walking AIT did not provide better positive metabolic adaptations with respect to a mean-intensity-matched walking CoAT program. Indeed, both of these subgroups showed reduced insulin resistance and increased aerobic fitness and plasma adiponectin levels, without significant differences in the magnitudes of these changes. Our results here are thus different from those of Tjønnå et al. (2008), who investigated the effects of high-intensity AIT with respect to moderate-intensity CoAT, and from those of Karstoft et al. (2012), who investigated the effects of walking AIT with respect to walking CoAT; in both of these other studies, AIT elicited better metabolic responses than CoAT. In terms of the Tjønnå et al. (2008) study, we appear to have obtained different results because the maximum achievable speed during walking training determines the physical effort that does not exceed the anaerobic threshold but lies between the aerobic and the anaerobic thresholds (Sentija & Markovic, 2009), even if we assume that these untrained postmenopausal women have a low anaerobic threshold (Gaskill et al., 2001; Herdy & Uhlendorf, 2011). This is of great relevance, because the key factor that confers greater efficacy to the high-intensity AIT in terms of the

variations of some of the physiological variables, including insulin resistance, appears to be the combination of high intensity (i.e., intensity greater than the anaerobic threshold) and duration of the bouts of exercise. The discrepancy between our results and those obtained by Karstoft et al. (2012) might be due to the different training intensities used, although we cannot really know whether the relative intensities used by Karstoft et al. (2012) (i.e., % of walking VO_2 peak) are lower, equal to, or greater than ours (i.e., RPE) because of the different methods. As reported by Karstoft et al. (2012), the walking speed of their walking CoAT subgroup was so close to normal walking speed as to probably not elicit any significant changes in aerobic fitness, and as a consequence, did not adequately stimulate the body metabolism. On the contrary, in the present study, the walking CoAT group always underwent brisk walking, which corresponds to a slight/moderate increase in the breathing rate, even during the lowest intensity of the training. So, it is likely that our walking CoAT group trained at higher intensities than that of Karstoft et al. (2012). However, taken all together, in terms of our results here and those recorded in these two cited studies (Tjønnå et al., 2008; Karstoft et al., 2012), this confirms that in order to optimize body stimulation through physical exercise, both the pattern of the training and the mean intensity of the training have to be considered. Indeed, these determine the choice, according to the pattern, of exercise intensity and duration, and the total duration of the exercise sessions.

With regard to adiponectin, our results add data to the literature that show that moderate-to-high-intensity physical exercise can have the greatest impact on adiponectin levels, without any well established dose-response relationship (Simpson, Fiatarone, and Singh, 2008). Indeed, the study of Tjønnå et al. (2008), which was conducted with moderate-to-high-intensity training, and our study, which was conducted on light-to-moderate-intensity training, suggest that to elicit

plasma adiponectin variations, the mean intensity and duration of the training are more important than the pattern of training.

Although the subgroups had the same positive modifications, neither of them showed any significant reductions in the anthropometric variables and the plasma lipids that could be explained by modifications to the spontaneous dietary habits that occurred; indeed, we detected an increase in the daily energy intake was seen, which probably prevented the achievement of a negative energy balance, a necessary step to induce body-fat reduction (Turner, Holman, Matthews, Hockaday, and Peto, 1979). The positive modifications to HOMA-IR, HOMA-AD and adiponectin reinforce the concept of the positive role of such physical exercise on insulin resistance and plasma levels of adiponectin, independent of body-fat variations.

There is a growing literature that indicates that increased or no modified spontaneous physical activity are not always the consequences of participation in physical exercise (Di Blasio et al., 2012; Manthou, Gill, Wright, and Malkova, 2010). Instead, there are several possible compensatory pathways (e.g., a reduction in spontaneous physical activity as a consequence of participation in a physical exercise program) that are under several controllers (Garland et al., 2011). As aerobic physical exercise has been shown not to be effective to induce improvements to some metabolic variables in the presence of some of these compensatory pathways (i.e., a reduction in spontaneous physical activity) (Di Blasio et al., 2013; Di Blasio et al., 2012), we also analyzed the data relating to the spontaneous physical activity of these participants, in a search for differences or supplementary explanations for the observed metabolic results. Our data show that in both subgroups, physical exercise participation did not elicit a reduction in spontaneous physical activity. Indeed, we did not detect any significant modification of physical activity parameters comparing T_0 with T_1 nontraining day data, while the T_0 with T_1 training day

data comparisons revealed a physical activity increase in both subgroups (Table 3). As a consequence, the observed metabolic results can only be attributed to the physical exercise programs.

The real limitation of our study is the small sample size that prompted the use of nonparametric statistics, to be more certain of the results obtained. Therefore, the consequent conclusions from our study are not generalizable and must be further verified with increased sample size. Following this real limitation, there are questionable limitations. Indeed, even if the use of subjective methods to monitor the intensity of training and the lack of intervention on behavioral variables that might influence the metabolic parameters can be considered limitations of the present study, both the literature and the study design reinforce these weak points. Indeed, the literature reports a strong correlation between the RPE scale and all of the objective parameters of exercise intensity, even if the use of the RPE scale requires the familiarization with both the scale and the method. The method was principally chosen because of its two important characteristics: it is low cost (i.e. no monitors are needed), and it allows automatic regulation of walking speed to maintain the given exercise intensity when aerobic fitness increases. In addition, the monitoring of both spontaneous physical activity and dietary habits allows possible confounding effects to be removed, even if the insertion of an intermediate control, between T0 and T1, is needed to be more certain about the real trend of the dietary and physical activity parameters from the beginning to the end of the study.

According to our data, walking outdoors in an interval pattern at moderate intensity and without running did not provide further gains in terms of positive metabolic effects with respect to those elicited by continuous walking training. Indeed, when matched for moderate intensity, the CoAT and AIT training elicited the same reductions in plasma glucose, insulin, HOMA-IR

and HOMA-AD, and the same increases in aerobic fitness and plasma adiponectin, independent of body fat reduction. Therefore, our results indicate that these different patterns of walking training achieved the same results, thus supplying a useful argument against sedentarism, in terms of the possibility to use a walking AIT scheme either alone or in rotation with a walking CoAT scheme in both adults and ageing people. At the same time, there is growing literature relating to the positive effects of high-intensity AIT on health. Thus, when appropriate, both in adults (Kessler, Sisson, and Short, 2012) and in seniors (Molmen, Wisloff, Aamot, Stoylen, and Ingul, 2012), a walking AIT scheme can be used as a light introduction to a full interval training scheme to psychologically and physiologically accustom the exercisers.

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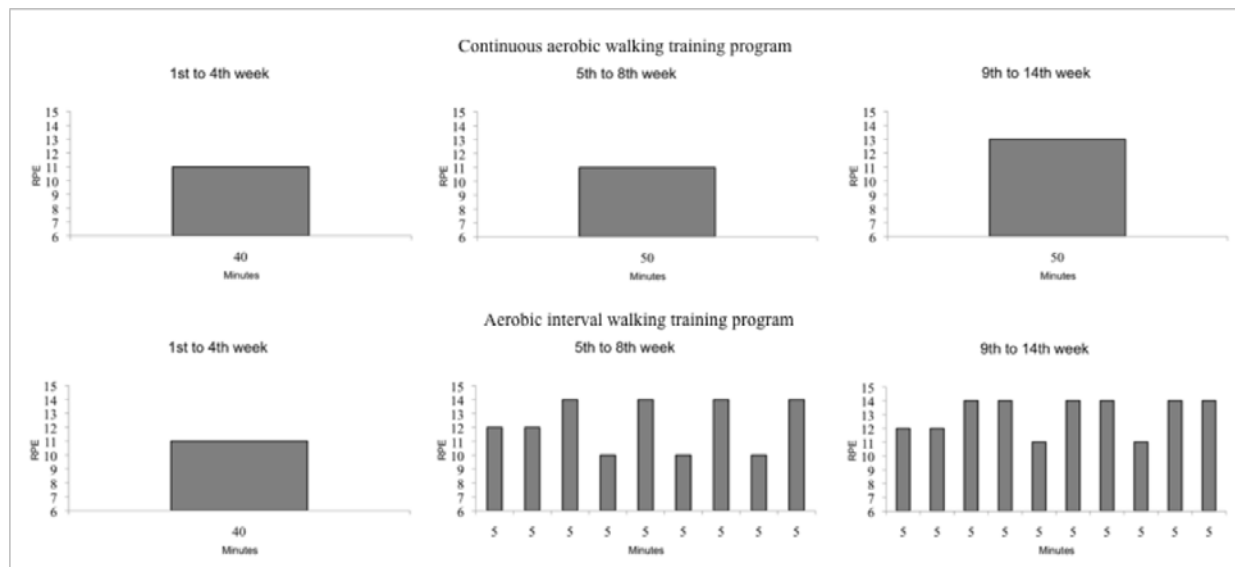


Figure 1. Training programs used in the study.

Table 1. Baseline characteristics (T_0) and effects of aerobic training (T_1) for all of the participants combined.

Characteristics	T_0 (N = 32)	T_1 (N = 32)	p
Age (yrs)	55.37 ±3.46		
Anthropometry and body composition			
Body mass index (kg/m ²)	26.67 ±3.75	26.45 ±3.53	0.21
Waist circumference (cm)	84.08 ±8.98	84.39 ±8.78	0.39
Fat mass (kg)	22.83 ±6.62	22.55 ±6.15	0.31
Dietary habits			
Protein (%)	16.96 ±3.08	16.57 ±2.23	0.61
Lipid (%)	38.15 ±7.55	34.61 ±5.95	0.12
Carbohydrate (%)	44.84 ±9.10	48.78 ±6.35	0.08
Energy intake (kcal)	1816.84 ±395.34	1970.11 ±365.27	0.001
Daily physical activity			
METS _{m/die} (METs)	1.41 ±0.21	1.53 ±0.26	0.003
STEPS _{m/die} (steps)	11314.98 ±3407.68	14259.91 ±4521.40	0.001
SEDENTARY TIME _{m/die} (min)	224.34 ±38.78	216.81 ±38.54	0.19
LIPAT _{m/die} (min)	852.10 ±92.98	718.51 ±137.04	<0.001
MIPAT _{m/die} (min)	84.44 ±65.48	128.10 ±100.62	0.005
EE from MIPAT _{m/die} (kcal)	361.28 ±287.12	561.23 ±411.98	0.003
Plasma values			
Insulin (μU/ml)	10.79 ±4.59	9.19 ±5.37	0.01
Glycemia (mg/dl)	82.28 ±6.06	79.09 ±6.90	0.008
Adiponectin (ng/ml)	49.62 ±28.12	64.65 ±38.09	0.04
HOMA-IR	2.22 ±1.06	1.83 ±1.17	0.005
HOMA-AD	25.96 ±21.13	16.02 ±14.80	0.001
Total cholesterol (mg/dl)	217.03 ±35.76	221.65 ±37.69	0.27
HDL-C (mg/dl)	61.41 ±14.92	64.12 ±13.33	0.06
LDL-C (mg/dl)	135.97 ±36.71	137.53 ±36.48	0.47
Triglycerides (mg/dl)	96.72 ±35.98	101.06 ±34.06	0.35

Characteristics	T₀ (N = 32)	T₁ (N = 32)	p
Aerobic fitness			
METS _{peak}	7.55 ±1.39	8.29 ±1.49	<0.001

Note: METS_{m/die}, mean intensity of daily physical activity; STEPS_{m/die}, daily steps; SEDENTARY TIME_{m/die}, time spent in physical activities with intensities ≤ 1.5 METs; LIPAT_{m/die}, time spent in physical activities with intensities between 1.5 and 3 METs; MIPAT_{m/die}, time spent in moderate-intensity physical activity; EE from MIPAT_{m/die}, energy expenditure from MIPAT_{m/die}; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-AD, homeostatic model assessment for insulin resistance corrected by adiponectin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2. Effects of subgroup membership on the modifications to the variables monitored

Characteristics	Walking CoAT (n = 16)	Walking AIT (n = 16)	p
Dietary habits			
Δ Energy intake (kcal)	199.78 ±233.52	106.75 ±272.17	0.46
Daily physical activity			
Δ MET _{s_m/die} (METs)	0.04 ±0.14	0.2 ±0.27	0.05
Δ STEPS _{m/die} (steps)	1186.16 ±3848.44	4777.81 ±4256.07	0.04
Δ LIPAT _{m/die} (min)	-143 ±88.67	-124.18 ±154.26	0.19
Δ MIPAT _{m/die} (min)	11.96 ±71.32	68.06 ±107.09	0.01
Δ EE from MIPAT _{m/die} (kcal)	69.53 ±282.67	330.37 ±493.26	0.01
Plasma values			
Δ Insulin (μU/ml)	-1.11 ±4.23	-2.08 ±3.56	0.72
Δ Glycemia (mg/dl)	-4.12 ±5.86	-2.25 ±6.07	0.72
Δ Adiponectin (ng/ml)	16.60 ±53.17	13.46 ±23.75	0.69
Δ HOMA-IR	-0.29 ±0.78	-0.47 ±0.8	0.75
Δ HOMA-AD	-12.31 ±21.09	-7.56 ±10.99	0.95
Aerobic fitness			
Δ MET _{s_{peak}}	1.52 ±1.65	1.94 ±0.47	0.95

Note: Δ, post-training value minus pre-training value; MET_{s_m/die}, mean intensity of daily physical activity; STEPS_{m/die}, daily steps; SEDENTARY TIME_{m/die}, time spent in physical activities with intensities ≤1.5 METs; LIPAT_{m/die}, time spent in physical activities with intensities between 1.5 and 3 METs; MIPAT_{m/die}, time spent in moderate-intensity physical activity; EE from MIPAT_{m/die}, energy expenditure from MIPAT_{m/die}; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-AD, homeostatic model assessment for insulin resistance corrected by adiponectin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 3. Basal differences and trends in the physical activity from T₀ to both T₁ non-training days and T₁ training days, according to subgroup membership.

Characteristics	T ₀	T ₁ non-training days	T ₁ training days
METS_{m/die}			
Walking CoAT	1.42 ±0.17 [^]	1.47 ±0.22	1.53 ±0.22 [^]
Walking AIT	1.40 ±0.24 ^{^^}	1.53 ±0.31	1.67 ±0.29 ^{^^}
STEPS_{m/die}			
Walking CoAT	11914.28 ±2656.71* [^]	12571.50 ±5544.39	14610.13 ±5832.47 [^]
Walking AIT	10715.69 ±4021.73* ^{^^}	13188.69 ±6579.54	17794.13 ±3411.50 ^{^^}
SEDENTARY TIME_{m/die}			
Walking CoAT	230.91 ±48.87	230.68 ±43.42	235.90 ±48.92
Walking AIT	219.75 ±27.79	204.90 ±30.74	214.50 ±40.34
LIPAT_{m/die}			
Walking CoAT	868.81 ±75.40 [^]	841.44 ±62.39	795.11 ±77.99 [^]
Walking AIT	825.68 ±97.77	827.00 ±147.01	776.56 ±162.14
MIPAT_{m/die}			
Walking CoAT	90.94 ±48.75* [^]	81.62 ±58.75	127.81 ±58.46 [^]
Walking AIT	77.94 ±79.97* ^{^^}	122.31 ±109.02	172.75 ±118.99 ^{^^}
EE from MIPAT_{m/die}			
Walking CoAT	409.00 ±238.20* [^]	364.78 ±278.75	559.21 ±264.96 [^]
Walking AIT	313.56 ±329.81* ^{^^}	500.37 ±467.39	787.12 ±524.84 ^{^^}

Note: METS_{m/die}, mean intensity of daily physical activity; STEPS_{m/die}, daily steps; SEDENTARY TIME_{m/die}, time spent in physical activities with intensities ≤ 1.5 METs; LIPAT_{m/die}, time spent in physical activities with intensities comprised between 1.5 and 3 METs; MIPAT_{m/die}, time spent in moderate-intensity physical activity; *significant differences between basal values, p ≤0.05; [^]significant differences between T₀ and T₁ values, p ≤0.05; ^{^^} significant differences between T₀ and T₁ values, p ≤0.01.