Effects of 12-week overground walking training at ventilatory threshold velocity in type 2 diabetic women

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A B S T R A C T  
This study analyzed the effects of overground walking training at ventilatory threshold (VT) velocity on glycaemic control, body composition, physical fitness and lipid profile in DM2 women. Nineteen sedentary patients were randomly assigned to a control group (CG; n = 10, 55.9 ± 2.2 years) or a trained group (TG; n = 9, 53.4 ± 2.3 years). Both groups were subjected to anthropometric measures, a 12-h fasting blood sampling and a graded treadmill exercise test at baseline and after a 12-week period, during which TG followed a training program involving overground walking at VT velocity for 20-60 min/session three times/week. Significant group × time interactions (P < 0.05) in glycaemic hemoglobin (HbA1c), body mass, body mass index (BMI), peak oxygen uptake (VO2peak) and exercise duration were observed as effects of training exercise, whereas intervention did not induced significant changes (P > 0.05) in fasting blood glucose, submaximal fitness parameters and lipid profile. Our results suggest that overground walking training at VT velocity improves long term glycaemic control, body composition and exercise capacity, attesting for the relevance of this parameter as an effective strategy for the exercise intensity prescription in DM2 population.  
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1. Introduction

Glycaemic control is a fundamental component in the management of type 2 diabetes mellitus (DM2) and its complications [1,2], with convincing evidence showing physical training to be an effective strategy for this purpose [3–5] since it increases both glucose transport and skeletal muscle insulin sensitivity [6].

In this framework, walking has been considered a safe, accessible and convenient exercise type for individuals with DM2 without peripheral neuropathy. Given the familiar pattern of movement, walking exercise is easily added into the daily physical activity routine of a patient with DM2 [7,8]. Furthermore, this type of exercise involves large skeletal muscle mass, which plays a major role in peripheral glucose uptake [9], thus triggering more effective improvement in glycaemic homeostasis [10].

In addition to the type of exercise, other key components (i.e., volume, frequency and intensity) must be considered in training programs that intend to maximize health benefits while minimizing risks in DM2 subjects, with special attention being devoted to the exercise intensity [11–15]. In a meta-analysis by Boulé et al. [16], reductions in glycated hemoglobin
(HbA1c) after training in these patients were better predicted by exercise intensity than volume. Yet, although current position statements from different world leading organizations [17–19] provide exercise intensity recommendations for such population, these suggestions should be viewed with caution given the wide range of moderate and vigorous intensities based on percentages of maximal oxygen consumption (VO_{2max}) (40–59 and 60–84%) and/or maximal heart rate (HR_{max}) (50–69 and 70–89%). It is well known that exercising at a given percentage of VO_{2max} or HR_{max} can elicit different physiological responses for different individuals and that submaximal parameters such as lactate or gas exchange thresholds are better markers of the relative stress induced by exercise [20].

Regardless of the controversy about its physiological background [21], ventilatory threshold (VT), or the exercise intensity above which ventilation begins to increase disproportionately relative to oxygen uptake, has been considered a direct, simple and useful parameter for optimal exercise intensity prescription in DM2 patients [22–24]. While acute exercise at VT reduces plasma glucose [25], training at this intensity improves both the aerobic capacity and the cost-effectiveness of treatment [26]. Moreover, Belli et al. [24] showed the feasibility of VT determination during graded treadmill test in women with DM2 without complications. In spite of this, in most studies adopting walking exercise for DM2 patients, intensity was either subjective [7,27,28] or based on %HR_{max} [8,11,29–31].

Although VT accessed in a graded exercise test can be expressed in both performance (i.e., power or velocity) and physiological (i.e., %VO_{2max} and %HR_{max}) terms [24], heart rate (HR) at VT has been the preferred parameter for exercise prescription in clinical settings [20,26]. However, despite the increasing availability of portable monitors, continuous HR assessment during training sessions is not without limitations [32] and can be quite complex due to technical and learning reasons. Alternatively, intensity control by means of velocity in cyclic activities such as running and walking is very practical in field settings, where subjects are commonly aware about covering known distances in particular times. Yet, the effectiveness of training overground at VT velocities derived from treadmill tests in patients with DM2 remains to be investigated. Thus, the purposes of this study were to assess the effects of supervised overground walking training at ventilatory threshold velocity on HbA1c, blood glucose, body composition, physical fitness and lipid profile in type 2 diabetic women.

2. Materials and methods

2.1. Subjects

After approval by the São Carlos Federal University Ethics Committee for Human Research (no 034/04), 24 sedentary middle-aged women with DM2 diagnosis [33] were recruited from local health facilities and signed an informed consent volunteering to take part in this investigation. All of them were housewives, non-smokers and not engaged in regular exercise practice, with current physical activity <1 h/week. Patients were included in the study if their diabetes was treated by diet (n = 5) or oral agents (sulphonylurea, n = 6; metformin, n = 3; and sulphonylurea + metformin, n = 10), but not if they were treated with insulin given the tendency of an advanced disease state in this case. Absence of long term complications (retinopathy, microalbuminuria, nephropathy, peripheral and autonomic neuropathy) as well as blood pressure greater than 160/95 mmHg were confirmed by clinical history, clinical examination and laboratory tests. After a blinded randomization by means of computer-generated random numbers subjects were allocated in a control (CG) or a trained group (TG), each one with 12 volunteers. Five of the 24 women initially enrolled in the study were not able to conclude the experimental protocol (2 from CG and 3 from TG) due to family responsibilities (n = 3), thrombosis (n = 1) and spinal disc herniation (n = 1), being these last events not attributed to the study procedures. Therefore, our final sample was composed by 10 and 9 subjects in the CG and TG, respectively. No significant differences were found between groups for age (55.9 ± 2.2 vs 53.4 ± 2.3 years, P = 0.48), height (155.0 ± 1.6 vs 152.0 ± 2.4 cm, P = 0.30) and disease time (3.7 ± 0.8 vs 4.4 ± 1.2 years, P = 0.71).

2.2. Experimental procedures

Using a longitudinal approach, all patients were submitted to anthropometric measurements, a 12-h fasting blood sampling, dietary evaluation and a graded exercise test on a motorized treadmill at baseline and after a 12-week period. Exercise test (TG only) and dietary evaluation (TG and CG) were also performed at the 6th week. Subjects from the trained group were evaluated 48–72 h after the last training session in order to avoid residual influence of acute exercise.

2.3. Anthropometry

Height was accessed using a calibrated stadiometer (Filizola®, Brazil), while body mass and body composition including percent body fat, fat and fat free mass, were determined using a bioelectric impedance system with the electrodes in contact with soles and heels of both feet (Tanita Body Composition Analyzer TBF-310) [34]. Measurements were performed in a quiet environment after a 12-h overnight fast, being the subjects in the standing position without shoes and using light clothes. Body mass index (BMI) was calculated as body mass divided by squared height (kg/m²). Waist circumferences were taken at narrowest circumference between the lowest rib and the iliac crest by a single trained evaluator using an inextensible metallic tape (Sanny®, Brazil) placed directly on the skin, perpendicularly to the long axis of the body and horizontally to the floor at the end of normal expiration. For this purpose, subjects stood with feet together, looking straight ahead with the arms hanging by the side of the body [35]. Average values from two measurements were considered.

2.4. Blood analysis

Following anthropometrical measures, 4 mL blood samples were taken from subject’s antecubital vein into tubes (Vacuette, Greiner Bio-One) with coagulation enhancer for posterior
analysis of blood glucose and lipid profile. Blood glucose, total cholesterol, high density lipoprotein cholesterol (HDLC) and triglycerides were determined by enzymatic-colorimetric procedures (Labolor®) using a spectrophotometer (VERSA-Max® microplate reader). Low density lipoprotein cholesterol (LDLC) was determined using Friedwald’s equation for triglycerides < 400 mg/dL. Additional 2 ml blood samples were taken in tubes containing EDTA for HbA1c determination using high-performance liquid chromatography (BioRad Dia-STAT Analyzer®).

2.5. Dietary evaluation

For dietary evaluation, a 1-day food record was adopted in a face to face interview conducted by an experienced dietitian. Thereafter, records were analyzed using the “Nutrition Support Program” software from the Escola Paulista de Medicina–EPM (DIS-EPM, version 2.5a, UNIFESP, Brazil). It was recommended a dietary education according to the American Diabetes Association (ADA) position statement [36] to all subjects to minimize dietary variability among groups.

2.6. Graded exercise tests

After a familiarization session, graded exercise tests were conducted using a previously described protocol [24] on a treadmill (Movement – LX-160, Brazil) without inclination. Initial velocity was set at 1 km/h and increased by 1 km/h every 2 min until voluntary exhaustion. During exercise, 20 s average ventilation (VE), oxygen uptake (VO₂) and carbon dioxide production (VCO₂) were monitored with subjects using a nose clip and breathing into a mouthpiece (VO2000 Gas Analyzer, MedAnalyzer, MedGraphics, USA). The system was automatically calibrated before each test and subjects were allowed to lightly rest their hands on the treadmill bar during exercise to maintain their balance. The highest VO₂ attained during the test was taken as the peak oxygen uptake (VO₂peak). VT was assessed by two independent investigators through VE/VO₂ increase without a concomitant rise in VE/VCO₂ [37]. After 20 min sitting rest preceding the exercise test, participants had assessed their blood pressure by the auscultatory method using an aneroid sphygmomanometer (Tycos, USA®) and a stethoscope (Becton Dickinson, USA®). Furthermore, blood pressure and heart rate (Polar S810, Polar Electro Oy, Finland®) were measured at the end of each stage for safety reasons during the test.

2.7. Training protocol

Women from TG were subjected to a 12-week walking training program, which was performed in a flat 350 m outdoor track at the individual VT velocity, three times a week (Monday, Wednesday and Friday). All training sessions were supervised and performed between 8:00 and 10:00 a.m. Stretching exercises involving main muscle groups of upper and lower limbs were performed before and after walking and individual velocities were controlled by the first author during all training sessions. For this purpose, sonorous signals were provided in order to drive subjects to pass by marks placed 50 m apart in the track in pre-determined times. After a familiarization session prior to the first training bout, all subjects were able to keep the prescribed velocity using this procedure within few minutes, which has been previously adopted in field rehabilitation [38]. In the first week sessions lasted 20 min, being duration increased by 10 min/week until 60 min at the 5th week. This duration was kept until the end of training period. A graded exercise test was carried out at the end of the 6th week in order to adjust training speeds. Furthermore, in the last training session of each two weeks, pre and post exercise fingertip blood samples were taken in order to verify the acute effects of the bout and assure that blood glucose (Optimum – Medisense Product, Abbott Lab®) was kept in the safe range suggested by ADA [39]. Volunteers from the CG continued their normal lives without any systematic exercise during the experimental protocol.

2.8. Statistical analysis

Statistical analysis was carried out using a statistical software package (Statistic 7.0, Statsoft, Tulsa, USA). Mean and standard error of mean (SEM) were calculated for all studied variables. Normal distribution and homogeneity of the data were verified by the Shapiro-Wilk and Levene’s tests, respectively, and before using parametric analysis blood glucose, triglycerides, maximal and submaximal velocities were log transformed. Group comparisons at baseline were carried out using unpaired t tests. Two-way 2 × 2 (group × time) mixed analysis of variances (ANOVAs) were used to investigate main effects and group × time interactions. Dietary parameters were analyzed by means of 2 × 3 (group × time) mixed ANOVAs, being Greenhouse-Geisser correction applied if violation of sphericity was pointed by Mauchly’s test. Unequal n Tukey post hoc test was performed when appropriate. Statistical significance was set at P < 0.05 in all cases.

3. Results

No significant differences (P > 0.05) were found between trained and control groups for any studied variable at baseline (Tables 1 and 2, Fig. 1), and high attendance to the supervised training program was observed for the TG, with volunteers performing 92 ± 2% of 36 offered sessions. Capillary blood glucose significantly decreased (P < 0.01) after all selected bouts in this group, being averaged pre and post exercise values respectively 162 ± 8 vs 118 ± 7 mg/dL. No significant changes (P > 0.05) were found in fasting blood glucose before and after experimental period (TG: 119 ± 9 and 110 ± 8 mg/dL; CG: 132 ± 16 and 142 ± 20 mg/dL). On the other hand, while CG showed similar HbA1c levels after training, a pronounced 11.6% decrease was found in TG, leading to a significant (P < 0.05) group × time interaction (Fig. 1).

Anthropometric measures, lipid profile and resting blood pressure at baseline and after the 12-week period for both groups are presented in Table 1. Greater reductions in body mass and BMI were found in TG after the experimental period and waist circumference increased in CG, being group × time
interactions statistically significant for all these parameters \((P < 0.01)\). While fat free mass remained rather stable in both groups, fat mass and percentage body fat decreased to a great extent in TG, but only significant time effects \((P < 0.01)\) were found for these changes. Regarding lipid profile, a significant group \(\times\) time interaction was found for HDLC \((P < 0.05)\), with a greater increase in control vs trained group. In contrast, both groups showed lower total cholesterol and LDLc after 12-weeks, with a significant time effect \((P < 0.01)\) and no significant changes were found for triglycerides as well as resting blood pressure \((P > 0.05)\).

Table 2 shows fitness parameters accessed during the graded exercise tests at baseline and after intervention. Greater increases in peak treadmill velocity and exercise duration were observed in the TG, but a significant interaction was found only for the latter \((P < 0.05)\). A significant group \(\times\) time interaction \((P < 0.05)\) was also observed for \(\text{VO}_{2}\text{peak}\), with a 6% increase in the TG but an 8% reduction in the CG. In addition, while no significant changes were found for \(\text{HR}_{\text{peak}}\), slightly higher respiratory exchange ratio \(\text{(RER)}\) values in both groups after 12 weeks led to a significant time effect \((P < 0.05)\). Regarding submaximal parameters, velocity at VT increased to a great extent in TG \((~17\% vs 7\%)\), but only significant time effect \((P < 0.01)\) were found for this change. On the other hand, no significant main effects or interactions were observed for VT expressed in physiologic terms \((P > 0.05)\).

Energy intake at baseline, at the 6th week and after the experimental period in TG and CG were respectively 7569 \(\pm\) 753 and 7971 \(\pm\) 791 kJ/day, 6514 \(\pm\) 506 and 5912 \(\pm\) 594 kJ/day, and 6828 \(\pm\) 456 and 5912 \(\pm\) 414 kJ/day. A significant time effect \((P < 0.05)\) was found for this variable, with post-hoc analysis showing lower energy intake at 6th and 12-weeks \((P < 0.05)\) compared to baseline values. On the other hand, no significant group effect or group \(\times\) time interaction
(P > 0.05) were observed. No significant differences or changes were found for macronutrient composition of the diet (data not shown).

4. Discussion

The main findings of the present study were that 12-weeks of supervised walking training at VT velocity led to significant improvements in long term glycaemic control, body composition and exercise capacity in middle-aged DM2 women. On the other hand, such intervention had no significant effects on fasting blood glucose or lipid profile in these patients.

In line with existing evidence [24], when expressed relative to VO2peak and HRpeak, higher inter-individual variability of VT was observed in our volunteers (~60 to 85%), but despite this intensity has been claimed as a marker for optimal exercise prescription in DM2 subjects [22–24] to our knowledge this is the first study addressing the chronic effects of a full supervised walking program at VT velocity in these patients.

Interestingly, the reduction in HbA1c observed in our TG (11.6%) was greater than those found in the only two previous studies showing improvements in long term glycaemic control after walking training in patients with DM2 [7,31]. Walker et al. [7] showed 12-weeks of self-paced walking for 60 min, five times a week, to lower HbA1c by 7.6% in postmenopausal DM2 women, while Shenoy et al. [31] found a 9.7% decrease in this parameter after an 8-week exercise program at ~75 to 83% HRmax for 35 to 40 min, five times a week, in Asian Indians of both genders with DM2.

Contrary to these studies however, reduced HbA1c in the present investigation was not accompanied by lower fasting blood glucose. Given that hemoglobin glycation depends on mean blood glucose concentration over the ~120-day lifespan of red blood cells [40], this apparently contradictory finding could result from the acute effects of each exercise bout in lowering blood glucose. In line with this, Manders et al. [41] found a single bout of exercise to reduce average blood glucose concentration and the prevalence of hyperglycemia during the 24-h post exercise period. Mean capillary glycaemia reduction observed in our subjects in selected training bouts was 27%, being these values in the adequate range (70–300 mg/DL) for exercise practice suggested by ADA [39], attesting for the safety our walking program.

Corroborating previous studies, our results showed walking training to reduce body mass and BMI in DM2 patients [7,27–29,31]. In addition, we found a tendency to greater reduction in fat mass without changes in fat-free mass as a result of exercise, that also prevented waist circumference increase, which in turn is strongly related to visceral adipose tissue, insulin resistance and cardiovascular complications in DM2 [42–44]. Together, these findings indicate a healthier anthropometric profile and consequent protection against obesity related co-morbidities in our subjects after training.

Enhancement on lipid profile including reductions on total cholesterol and LDLC has been observed in some but not all walking training studies [7,8,26,27]. Abnormal lipid profile was observed in our subjects at baseline, however, unexpectedly changes towards target values were found after experimental period in both groups. These results may be partially explained by diet characteristics that were not accounted for in our study protocol, such as reductions in saturated fat, trans fat and cholesterol intake or also increased viscous fiber and n-3 fatty acids ingestion [45]. In addition, despite the lack of significant group or group × time interaction, total energy intake decreased to a great extent in control vs trained group through the experimental period (~25 vs 10%). This can also elucidate the greater HDLC increase in control vs trained group after intervention.

The significant group × time interactions for VO2peak and exercise duration corroborate data from previous studies [7,30] and can be considered evidences that our walking training protocol was able to improve exercise capacity/tolerance in DM2 subjects. These are relevant adaptations since low cardiopulmonary fitness is a common feature related to female gender, family history of coronary heart disease [46] and an independent predictor of mortality [47] in people with DM2. Contrary to the hypothesis that training would elicit a greater increase in VT compared to VO2peak, we found the former to be unchanged after the intervention when expressed in physiological terms. In support of this, Larose et al. [48] observed that workload at VT increased while %VO2peak at VT remained unchanged after 6-months of walking and cycling at 60–75% HRmax for 25–45 min, three times a week in patients with DM2.

Yet regular walking has been considered an interesting adjunct therapy for DM2 subjects, controversial findings have been reported regarding the chronic effects of this type of exercise on heart related parameters in such population [7,8,11,26–31]. These divergences may result from the diversity of adopted training periods (few weeks up to a year), exercise frequencies (2–5 days/week), session durations (30–60 min) and intensities (subjective self paced, brisk walking, %HRmax), as well as sample characteristics (age, gender, race).

Since our subjects exercised at VT velocities derived from treadmill tests one could question whether training was performed at VT intensity expressed in physiological terms (i.e., %HRpeak or %VO2peak) due to environmental and specificity concerns. In an attempt to minimize the climatic differences between laboratory and field conditions, testing and training sessions were performed at approximately the same time of the day. In addition, training program was deliberately conducted during the spring, with rather stable climatic conditions in our subtropical region. Albeit these cautions do not warrant identical environments, differences were probably too small to result in rather discrepant physiological strains. On the other hand, from the results of Parvataneni et al. [49] indicating lower metabolic requirement in overground compared to treadmill walking at the same velocity in older adults, it is plausible that our volunteers’ VT velocity could be greater if determined on the track. A limitation of the current investigation however was that we could not quantify the extent to which overground walking differed from treadmill walking in physiological terms.

Extending findings from a previous study showing the feasibility of VT determination during graded treadmill test in DM2 women [24] we showed overground walking training at VT velocity to promote a remarkable decrease in HbA1c as well as improvements in body composition and exercise capacity in these patients. Importantly, since volunteers presented
little comorbidity and short disease duration, reaching such low HbA1c values without hypoglycaemia episodes may provide important reductions in the risk of microvascular complications [45]. While these results point out the effectiveness of a field walking program at the laboratory based VT velocity in DM2 subjects, indices such as the maximal level of lipid oxidation, which is lower than VT, have been also suggested for optimizing exercise intensity in patients with metabolic diseases [50]. Nevertheless, the issue of which individualized target is more efficient or even whether individualization is superior than standard procedures for patients with DM2 is a relatively new research area and further work on this topic is warranted.

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Conflict of interest

The authors declare that they have no conflict of interest.

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