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Original Article

The volume and order of starting exercise modulates the glucose-lowering effect of a single session of combined exercise in middle-aged and older adults with type 2 diabetes

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Abstract:
Problem Statement: Although combined exercise (CE) is frequently prescribed for glycemic control of Type 2 Diabetes Mellitus (T2DM) subjects, beginning CE session with aerobic or resistance exercises can lead to distinct responses in capillary glucose, especially when training prescriptions differ in intensity and volume. **Approach:** This study aimed to evaluate the effect of exercise intensity, volume, and order within combined exercise sessions on glycemic response. **Purpose:** Forty T2DM subjects participated in this study. The subjects were divided into three different protocols: standard (S-P, n = 13), high intensity (HI-P, n = 16), and high volume (HV-P, n = 11) in two counter-balanced orders each [i.e., resistance exercise followed by aerobic exercise (R-A) or vice versa (A-R)]. The capillary glucose was evaluated before and after each CE session. **Results:** All protocols of combined exercise reduced glucose concentrations ($p < .001$). HV-P induced a glycemic reduction that was 2-fold higher than that observed in HI-P (34% vs. 18%; $p < .05$). Both exercise orders within combined exercise session reduced glucose concentrations (S-P: 25%, 28%; HI-P: 17%, 19%, and HV-P: 31%, 36% for A-R and R-A, respectively); however, R-A induced slightly larger (Cohen's d was 13-33% higher in R-A compared to A-R), but clinically relevant, reductions in all combined exercise protocols. Pre-exercise glucose concentrations were correlated to exercise-induced glucose reduction only in the A-R order (r = 65 to .79, $p < .05$). **Conclusion:** All combined exercise protocols were effective in decreasing glucose concentrations. Nonetheless, a higher magnitude of glucose-lowering effect occurred when a higher exercise volume was performed and when performing resistance instead of aerobic exercise in the first place.

Keywords: Concurrent exercise; Blood glucose; training workload; Exercise volume; Exercise intensity; Diabetes mellitus

Introduction

Type 2 diabetes (T2DM) is the most prevalent type of Diabetes Mellitus, accounting for nearly 95% of the cases, and is characterized by abnormally high glucose levels caused by impaired insulin action accompanied or not by impaired insulin secretion (American Diabetes Association, 2020). Physical exercise has been shown to promote health, adequate the clinical management of disease (Colberg et al., 2016), and prevent mortality in T2DM subjects (Silva et al., 2019). A simultaneous combination of aerobic and resistance, known as combined exercise (CE), has been the most indicated type of training for T2DM management since it combines stimuli in complementary metabolic pathways (Consitt et al., 2019). CE improves glycemic control and several other health outcomes (Bacchi et al., 2012; Balducci et al., 2010, 2012; Egger et al., 2013; Silveira-Rodrigues et al., 2018, 2021). Abnormally high glucose levels (*i.e.*, hyperglycemia) are a hallmark of T2DM by their wide frequency throughout the day, regardless of drug therapy (Van Dijk et al., 2012). For that, an appropriate glycemic control obtained by reducing elevated of glycated hemoglobin levels is a leading therapeutic target in T2DM (American Diabetes Association, 2020).

Assuming that hyperglycemia is directly related to vascular complications, a pivotal contributor to mortality in these patients (Ceriello, 2005), interventions that promote glycemic reductions are clinically relevant. One exercise session provokes immediate and prolonged glycemic reductions (Figueira et al., 2013), reducing the mean glycemia and the prevalence of hyperglycemic episodes over the next 24h (Van Dijk et al., 2012). Nonetheless, the recurrence of these acute effects is crucial to lowering postprandial glycemia (Van Dijk et al., 2012) and improving glycemic control (Monnier et al., 2003), which may contribute to chronic ameliorations in this specific therapeutic target.

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The training process is commonly marked by oscillation in training workload parameters. Among these parameters, are the exercise intensity and exercise volume, substantial determinants of the training-induced metabolic adaptations (Garber et al., 2011). Exercise intensity is defined as the energy used per time unit, and exercise volume is the total amount of energy used in a training session (Garber et al., 2011). T2DM subjects usually have low exercise capacity (Patterson et al., 2018).

Therefore, the training parameters must be considered in relative terms, not in absolute terms. Thus, the intensity and volume should be adequately chosen, considering their low conditioning levels. Progressing the intensity and volume of aerobic exercise seems crucial to maintaining the glucose-lowering effect of a single session of aerobic exercise in T2DM subjects (Delevatti et al., 2016). When the training load management is not sufficiently during a training period, the glucose-lowering effect of aerobic exercise drops (Delevatti et al., 2019). Concerning the exercise intensity, it has been shown that low (Hu et al., 2018; Terada et al., 2013), moderate (Bacchi, Negri, Trombetta, et al., 2012; Figueira et al., 2013; Terada et al., 2013) and high-intensity aerobic exercise reduces the capillary glucose (CG) in T2DM subjects (Gillen et al., 2012). Indeed, low (Moreira et al., 2012), moderate (Moreira et al., 2012; Ogando et al., 2022), and high intensities of resistance exercises (Ogando et al., 2022) also were effective in lowering the CG of T2DM subjects. The exercise volume also influences the glycemic reduction in both aerobic (Terada et al., 2013) and resistance exercise (Ogando et al., 2022). However, the effects of CE exercise intensity, volume, and the order, if aerobic or resistance exercise at first place in the training bout, on glycemic response of T2DM remains unclear.

Beyond the exercise volume and intensity, the order of exercises performed in the CE-session, *i.e.*, the type of exercise performed in the first place, provokes distinct acute physiological outcomes, including different hormonal responses, such as cortisol and testosterone, that may influence CG concentrations (Eduardo Lusa Cadore, Izquierdo, dos Santos, et al., 2012; Jones et al., 2017). Fewer investigations focused on glycemic responses under different orders in the CE-sessions, reported no effects in trained subjects (Inoue et al., 2016) and a reduction of CG in adults with T1DM (Yardley et al., 2012). Nevertheless, the glycemic responses of T2DM subjects that have a higher prevalence of hyperglycemic events (Van Dijk et al., 2012) remain unknown.

Thus, this study aimed to compare the effect of the intensity, volume, and order of exercises performed within a CE session on glucose response assessed by a portable glucometer in T2DM subjects. We hypothesized that the CE session with higher exercise intensity would mitigate the glucose-lowering effect of the exercise session, whereas the highest exercise volume would exacerbate this effect. Furthermore, we also assumed that performing resistance exercise in the first place within a CE session would elicit a more considerable glycemic reduction.

Material and Methods

The study was conducted through an exploratory analysis of two previously published studies. One of those was a clinical trial registered in the Brazilian Registry of Clinical Trials (ReBEC), no. RBR-86hfz5. The Research Ethics Committee of the Federal University of Minas Gerais approved the protocol of both studies (registration nos. 15352613.9.0000.5149 and 66804817.8.0000.5149). All study protocols followed Helsinki's Declaration for human studies. After explaining the research procedures, risks, and benefits, subjects signed a consent form to participate in the study.

Sample size calculation and participants

A pilot investigation conducted in our laboratory with T2DM subjects of similar clinical conditions and exercise workloads adopted in the present study showed a significant reduction of CG (Cohen's *d*= 1.33), and this reduction correlated with pre-exercise CG values (Pearson's correlation coefficient= 0.74). Therefore, following the *a priori* sample size estimation recommendations (Beck, 2013), this data was entered in Gpower™ (v3.1.9.4, (v.11.0, Kiel, GER) considering: $1-\beta = 80\%$ and $\alpha = 5\%$. The minimum sample size obtained was 9 subjects for each experimental condition.

Forty both sexes subjects participated in this study (Table II). To be included, the participants should have at least two years of a physician confirmed T2DM diagnosis. In addition, they did not have skeletal muscle injury in the last three months before the commencement of the study. Subjects in standard (S-P) and high intensity (HI-P) protocols were not enrolled in a physical exercise program. In high volume protocol (HV-P), they had a minimum of 6 months of physical training history.

Study design

Three counter-balanced, crossover pilot studies performed at different times were designed to evaluate the acute influence of the order of CE-session (*i.e.*, A-R: aerobic followed by resistance exercise and R-A: resistance exercise followed by aerobic exercise), its exercise intensity, and volume (*i.e.*, S-P, HV-P, and HI-P) on glycemic responses of T2DM subjects. Each pilot study contained one distinct CE-protocol (Table I), and CG was assessed before, during (only in HP-V), and immediately after the CE-protocol exercise session. At least two but no more than five days were granted to perform each session in CE-protocol.

2.3 Combined exercise protocols

Table 1. Summary of combined exercise sessions

	S-P (*n=13*)	HI-P (*n=16*)	HV-P (*n=11*)
Resistance			
Intensity	50% of $1\text{-RM}_{estimated}$	Maximal repetitions	50% of $1\text{-RM}_{estimated}$
Volume	2x15 reps	2x13-15 reps	3x15 reps
Exercise no.	8 exercises	6 exercises	8 exercises
Cadence	self-controlled	self-controlled	self-controlled
Pause	30 seconds	60 seconds	30 seconds
Aerobic			
Equipment	Treadmill	Treadmill	Treadmill
Duration	20 min	20 min	30 min
Intensity	self-controlled	ms6MWT	self-controlled
RPE	9 at 13	11 at 15	9 at 13

S-P: Standard protocol, HI-P: High intensity protocol, HV-P: High volume protocol, $1\text{-RM}_{estimated}$: One repetition maximum estimated, RPE: rate of perceived exertion, ms6MWT: mean walk speed in 6-min walk test.

The CE-protocols (Table I) were planned according to the exercise prescriptions for T2DM subjects (Colberg et al., 2016). The following resistance exercises were included: bench press machine, leg press, lateral pulldown, calf raise machine, abdominal curl, and lateral raises (S-P and HV-P), or overhead press (HI-P). For S-P and HV-P, hip flexion and leg extension were added. In both S-P and HV-P, the estimated 1-RM (repetition maximum) was used for prescription of resistance exercise intensity, calculated as follow: 1-RM = kg * [1+(0,025 * r)], where: kg = kilograms of external resistance; r = repetition number (O'Connor et al., 1989). For the HI-P, exercise intensity was considered the heaviest possible weight that subjects could sustain for the designated repetitions. In the HV-P group, subjects performed both resistance and aerobic exercises at a higher volume than the two groups (*i.e.,* 50% more exercise volume of both resistance and aerobic exercise). The subjects should maintain aerobic exercise intensity from 'very light' to 'somewhat hard' using the rate of perceived exertion (RPE, 9-13) (Borg, 1998), except in HI-P, where the exercise intensity was settled as the 6MWT average speed (Rikli & Jones, 1999) and was classified as 'light' to 'hard' (RPE, 11-15) (Borg, 1998).

2.4 Capillary glucose assessment

CG was measured using Accu-Chek Active™ (Roche, Basel, Switzerland). To improve the accuracy of glycemic measurements, the subjects had their hands washed with water and liquid soap, and dried with absorbent paper before each measurement (Sagkal Midilli et al., 2019). Also, all measurements were performed by the same researcher using the same glucometer (Schifman et al., 2014). Experimental conditions only occurred whether the glycemic values were between 80 and 250mg/dL according to recommendations of ACSM and ADA for T2DM subjects to exercise (Colberg et al., 2016). In a range from 57-319 mg/dl, the variations coefficient of CG measurements by Accu-Chek™ meter varies approximately between 6.9 and 8.8% (Schifman et al., 2014).

Food records

Since nutritional intake can interfere in the exercise-induced glycemic reductions (Terada et al., 2013), only in the last performed condition, HV-P, all subjects answer a record for food intake for 24 h before the two CE sessions. At this time, food intake was analyzed for total energy content (Kcal) and macronutrient intake (g) to ensure that dietary intake was similar before each different trial. The Brazilian food composition table (TACO) was used to estimate the macronutrient and total energy content of the meals with the software DietBox™ v.4.0 (Dietbox Informatica, Porto Alegre, Brazil).

Statistical analysis

Shapiro-Wilk's and *Levene*'s tests confirmed the normality and homoscedasticity of data. Data were expressed in mean ± standard deviation or relative frequency. Quantitative baseline characteristics were compared by *one-way* ANOVA and qualitative baseline characteristics by *Chi-square* tests. CG into different exercise order (time: pre- vs. post-exercise *or* post 1st exercise *vs*. post 2nd exercise and order: A-R *vs*. R-A) and the isolated effects of exercise modalities (only in HV-P, time: pre- vs. post-exercise and order: A-R *vs*. R-A) were compared by t*wo-way* ANOVA with repeated measures. The percentual of glycemic reductions (order: A-R *vs*. R-A and protocol: S-P *vs*. HI-P *vs*. HV-P) were compared using t*wo-way* ANOVA. In both conditions, *Tukey's* post-hoc identified pairwise differences, when a significant *F value* was obtained. Pearson's coefficient measures the relationship between pre and post CE-session glycemic values. The food ingestion parameters and glycemic reduction were compared using *Student's t-test.* The significance level was 5%. The clinical relevance of CE-induced glycemic reduction was calculated by the *Cohen's d* effect size: $\frac{x^1-x^2}{\sigma}$ where x^1 is capillary glucose immediately after CE-session, x^2 is capillary glucose before exercise, and σ represents the pooled standard deviation. The interpretation of magnitude of treatment was as follows: small: *d*= .2 to .49, medium: *d*= .5 to .79 and large effects: *d* >.8 (Cohen, 1988). Sample size calculation was performed in Gpower™ (v3.1.9.4, (v.11.0, Kiel, GER). Statistical analyses were performed with Sigma Plot 11.0™.

Results

Forty Brazilian T2DM subjects were recruited to perform the three distinct exercise protocols (Table II). Among the three protocols, there was no statistical difference between the baseline characteristics of the participants. All subjects completed the exercise protocol and were included in the statistical analysis. Two hypoglycemic episodes, considered as CG below 70 mg/dl according to previous criteria (American Diabetes Association, 2020) were reported in this study, representing only 2.5% of all experimental sessions. These events occurred for the same subject after the end of HI-P in both A-R and R-A exercise orders. There were no other adverse effects throughout the study.

Table 2. Participant's characteristics

	S-P (*n*=13)	HI-P (*n*=16)	HV-P (*n*=11)	*p-value*
Age (years)	64.2 ± 9.0	64.6 ± 7.5	65.1 ± 7.2	.58
Diagnosis time (years)	12.7 ± 8.5	12.3 ± 8.8	10.3 ± 7.8	.86
Body mass (kg)	74.8 ± 8.9	75.8 ± 12.2	76.1 ± 14.1	.67
BMI ($kg \cdot m^{-2}$)	28.7 ± 3.6	28.9 ± 3.7	30.3 ± 5.6	.31
Sex (women, %)	77%	63%	82%	.49
Hipertension (%)	69%	69%	73%	.97
Biguanides (%)	92%	88%	66%	.16
Sulfonylureas (%)	15%	31%	9%	.18
Thiazolidinediones (%)	8%	19%	9%	.65
Insulin users (%)	46%	31%	55%	.85
6MWT (m)	485 ± 77	523 ± 87	513 ± 46	.52

S-PROT: Standard protocol, HI-PROT: High intensity protocol, HV-PROT: High volume protocol, BMI: body mass index, and 6MWT: 6 minutes-walk test.

The pre-exercise CG were not different in A-R and R-A order in S-P (F= 1.40; p >.05), HI-P (F= 0.77; p >.05), or HV-P (F= 1.25; p> .05) (Figure I), suggesting that the different CE-sessions can be compared regarding their glucose-lowering effects. A significant (p<.05 for all) glucose-lowering effect of CE occurred in S-P (25% in A-R and 28% in R-A), HI-P (17% in A-R and 19% in R-A), and HV-P (31 in A-R and 36% in R-A). Interestingly, the clinical magnitude analysis showed that R-A induced a slightly larger effect size in all protocols (Cohen's d was 13 to 33% higher in R-A rather than A-R). Concerning CE-intensity and volume, larger effect size for glucose-lowering was observed in HV-P than in S-P and HI-P (Figure I) since HV-P and S-P presented large effects sizes (d > .80), while HI-P had a moderate effect size (d = .50 to .79).

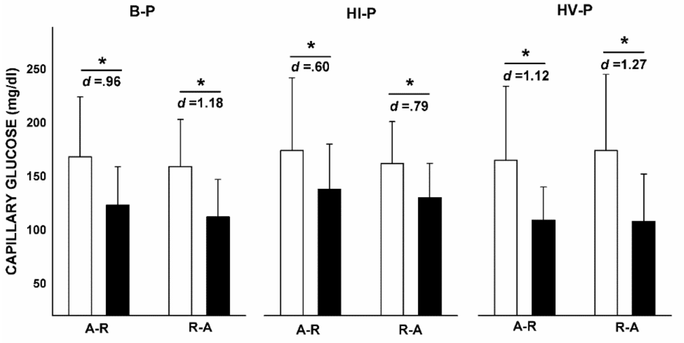

Figure 1. Capillary glucose values in different exercise protocols. White and black bars are pre- and post-exercise capillary glucose concentrations, respectively. A-R, aerobic exercise performed at the first place within a CE session; R-A, resistance exercise at the first place within a CE session. *indicates statistical time effect in *two-way RM ANOVA* (p<.05). S-P: Standard protocol, HI-P: High intensity protocol, HV-P: High volume protocol. d= *Cohen's d* effect size.

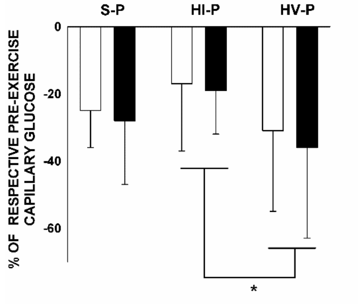

Figure 2. Percentage of variation on glucose concentrations concerning pre-exercise condition in different exercise orders of the three experimental protocols. White bars represent A-R exercise order and Black bars represents R-A exercise order. S-P: Standard protocol, HI-P: High intensity protocol, HV-P: High volume protocol. *indicates a statistical effect of protocol in two-way ANOVA (p<.05)

Because pre-exercise CG concentrations can determine exercise-induced lowering effects on glucose concentrations (Terada et al., 2013), we analyzed the relative effect (*i.e.*, % change) of pre-exercise glucose concentration in each CE-protocol. The percentage of variation on glucose concentrations induced by HV-P was an almost 2-fold increase compared to HI-P (34 ± 25% *vs.* 18 ± 15%; *F*= 4.6, *p*= .01) (Figure II). There was no difference between S-P and HV-P (*p*= .41) or HI-P (*p*= .19). The percentage of glucose concentration variation were not significantly different between exercise orders, probably because of a substantial intragroup variability (Figure II). Thus, CE-session with high exercise volume induced a higher glucose-lowering effect.

The separate analysis between exercise modalities (aerobic or resistance type) in HV-P revealed that pre-exercise CG in both aerobic (*r*= .93, *p*<.001) and resistance (*r*= .87, *p*<.001) was strongly correlated to the observed glucose-lowering effect induced by each exercise modality. However, when combining aerobic and resistance exercise in the same CE-session, this association solely remained when performing aerobic exercise in the first place (*i.e.*, A-R order) (Table III).

Table 3. *Pearson's* correlation coefficient between pre-exercise capillary glucose concentrations and CE-induced glucose-lowering effect.

	A-R	*p*-value	R-A	*p*-value
S-P	.77	<.01	.54	.06
HI-P	.65	<.01	.20	.46
HV-P	.79	<.01	.48	.15

A-R: aerobic exercise performed in the first place and R-A: resistance exercise performed at the first place within a CE-session. S-P: Standard protocol, HI-P: High intensity, HV-P: High volume protocol.

To further investigate the contribution of aerobic and resistance exercise to the glucose-lowering effect of CE during HV-P, CG was also measured during the CE-session, *i.e.*, after the first exercise type (aerobic or resistance) and before the second one. Both reduced CG when compared to pre-exercise (*F* = 18.9, *p*> .001). However, when performed in the first place, the aerobic exercise-induced a glycemic reduction 1.7-fold higher than when resistance exercise was performed in the first place (*t*= 2.88, *p* =.02) (Table IV). Also, when performing resistance exercise firstly, the glucose-lowering effect of aerobic exercise continues to occur (*t*= 8.70, *p* <.001), but when performing aerobic firstly the resistance exercise, there was no additional reduction in CG (*t*= 0.95, *p* =0.36). Therefore, aerobic exercises seem to be the main contributor to the glucose-lowering effect of CE-session, and this effect remained even when performed after resistance ones. Nonetheless, we observed a slightly higher magnitude of the exercise-induced glucose-lowering effect in R-A than in A-R order in all protocols, as shown in Figure I.

Table 4. Capillary glucose reductions related to previous measures in both exercise orders of HV-P.

	A-R (mg/dl)	%	R-A (mg/dl)
Pre-exercise	170 ± 70	-	182 ± 69
1st exercise *vs.* Pre-exercise	119 ± 43*	-28[#]	147 ± 56
2nd exercise *vs.* 1st exercise	109 ± 33*	-5	108 ± 46*
Post- *vs.* Pre-exercise	-	-31[#]	-

A-R: aerobic exercise performed at the first place and R-A: resistance exercise performed at the first place within a CE-session. % indicate the average percentual of glycemic reduction related to previous measurement, *indicate differences in *two-way RM ANOVA* (*p*<.05), [#]indicate differences in *Student's t-test* respective to pre-exercise capillary glucose (*p*<.05).

Since nutritional intake can interfere with exercise-induced glycemic responses (Terada et al., 2013), subjects of the HV-P group, the most responsive group concerning glycemic reductions after CE-protocol, answered a food record. There was no difference regarding total energy intake (A-R: 1544kcal *vs.* R-A: 1500kcal, *p*= 0.71), the amounts of carbohydrates (A-R: 206g *vs.* R-A: 180g, *p*= 0.26), protein (A-R: 70g *vs.* R-A: 77g, *p*= 0.41), and total fat ingested (A-R: 47g *vs.* R-A: 50 g, *p*= 0.68) between A-R and R-A.

Discussion

Our results confirm our hypothesis that the glucose-lowering effect of CE-session in T2DM subjects depends on exercise volume, intensity, and exercise type order (whether aerobic or resistance exercise performed in the first place). HV-P may substantially potentiate the exercise-induced glucose-lowering effect, whereas HI-P mitigates it. Also, performing resistance exercise in the first place had a greater magnitude of lowering glucose concentrations than when CE began with aerobic exercises.

Although CE has been the most prescribed exercise training, to the best of our knowledge, this is the first study exploring the influence of its intensity, volume, and exercise order on the capillary glucose responses of T2DM subjects. The exercise volume is commonly determined by the duration, and the number of sets and reps for aerobic and resistance exercises, respectively. Indeed, a longer time (higher exercise volume) requires more energy to the active muscles, which may exacerbate the glycemic reduction observed in response to exercise compared to more robust muscle contractions during a shorter time. In addition, augments in the exercise volume increase the number and time of muscle contractions, requiring more energy, hence, more glucose as a substrate to maintain these contractions. Our findings agree with previous data from our group showing that the increase in exercise volume through an increase in the number of exercise sets exacerbates exercise-induced glucose-lowering effect in T2DM subjects submitted to either moderate or high-intensity resistance exercise (Ogando et al., 2022). Likewise, Turner and colleagues increased the exercise volume by adding one set in each strength exercise of the resistance exercise protocol. This exercise volume manipulation further reduced CG after a moderate-intensity resistance exercise session in T1DM subjects (Turner et al., 2015). Moreover, the increase in exercise volume by adding 15 min per month in an exercise training program prescribed to T2DM subjects exacerbated the glucose-lowering effect of an aerobic exercise session (Terada et al., 2013). Therefore, exercise volume in a CE-session seems crucial for the magnitude of the CG reduction in T2DM subjects.

In contrast to alterations in exercise volume, our results demonstrated that the higher exercise intensity seems to mitigate the glycemic reduction observed after the CE-session. The aerobic exercise intensities for CE-session were settled according to average speed or % of maximal heart rate and for resistance exercises by the weight lifted in previous tests. It has been shown that under equivalent workloads (*i.e.*, isocaloric conditions), similar glycemic reductions were observed after high and low-intensity exercise in non-insulin-treated T2DM subjects (Kang et al., 1999). Higher intensity resistance exercise in 8 weeks of CE training induces a more pronounced strength and glycemic control enhancement in T2DM subjects (Egger et al., 2013). On the other hand, the findings concerning CE-intensity and glycemic reduction under non-matched workloads in T2DM subjects are inconclusive. We have recently shown that in T2DM subjects, higher workloads produced by increases in resistance exercise intensity did not potentialize the CG reduction compared to a moderate-intensity resistance exercise, which produced a lower workload (Ogando et al., 2022). It is known that high-intensity exercises elicit higher sympathetic activity compared to moderate-intensity. This higher sympathetic activity raises adrenaline and noradrenaline levels that, in turn, increase glucose production by the liver, which may somehow override the capillary glucose reduction in response to exercise in T2DM subjects (Marliss & Vranic, 2002). Otherwise, under the same duration, the higher intensity of aerobic exercise in a cycle ergometer resulted in a higher workload that can enhance the glycemic reduction in T2DM subjects (Asano et al., 2017). Thus, the present study takes a step forward, evidencing that the glucose-lowering effect of a CE-session was mitigated at high intensity. From a professional perspective, it is tempting to speculate that when prescribing CE programs to obtain a more pronounced reduction of glycemia in the T2DM population, one should choose resistance exercise with moderate-intensity and high volume of exercise associated with moderate-intensity aerobic exercise.

Both exercise orders (A-R and R-A) substantially reduced the CG of T2DM subjects. Noteworthy, a slightly higher magnitude of CG reduction with clinical relevance was observed when resistance exercise was performed firstly in a CE-session, regardless of CE-protocol. A mechanical and metabolic hypothesis may support our results. Residual fatigue may reduce the force that a muscle can generate (García-Pallarés & Izquierdo, 2011) when there is no allowed reduction in the volume of resistance exercise, as was the case after performing the aerobic exercise. Then, for anyone to lift the same weight, more muscle fibers should be recruited, and these could increase glycolytic phenotype leading to elevated lactate production and raising glycemia. Also, it may be hypothesized that when performed in the first place in the CE-session, the muscle contractions during resistance exercise can lead to an early increase in ATP consumption, causing a decrease in the energetic state of the musculoskeletal cells. This low energetic state would activate AMPK (AMP-activated protein kinase), increasing the glucose uptake during the following aerobic exercise to fulfill the energy demand to accomplish the prescribed exercise in this modality (Pesta et al., 2017). Indeed, in normoglycemic trained adults, when performing the combined exercise, resistance exercise in the first place induced higher elevations in the concentrations of lactate and cortisol compared to the reverse order (Jones et al., 2017). Despite this, increases in lactate and cortisol are often accompanied by blood glucose increase, although it was not found when subjected T1DM subjects performed both exercise orders within a CE-session (Yardley et al., 2012). However, as we have already pointed out, until now, the glycemic responses to a CE-session under different orders, have not been investigated in T2DM subjects, a population known to pursue substantial metabolic and clinical differences compared to T1DM subjects (Colberg et al., 2016). Our results demonstrated that although the exercise order in CE session did not show a main statistical effect in glycemic reduction induced by the CE-session, a substantial clinical reduction of CG was observed when resistance exercise was performed in the first place, regardless of CE protocol.

Our results indicate that aerobic exercise was the major contributor to glycemic reduction within the CE-session in T2DM subjects. Previous data reported similar findings, Bacchi and colleagues (2012) subjected T2DM subjects to aerobic and resistance exercise on separate days and observed that aerobic but not resistance exercise induced a glucose-lowering effect in the T2DM subjects. Akin, Yardley, and colleagues (2012) (Yardley et al., 2012), studying physically active T1DM subjects, revealed that only aerobic exercise reduced plasma glucose regardless of exercise order within a CE-session. Compared to resistance exercise, aerobic exercise is continuously performed, requiring the involvement of a higher number of muscle groups. That allows greater energetic consumption and glucose uptake by the active muscles, overcoming the hepatic glucose output that may potentialize the observed glycemic reduction. Furthermore, our findings also revealed that the glucose-lowering effect of aerobic exercise seems to remain when performed after resistance exercise in CE-session (contrary to observed in A-R order), likely contributing to the slight but relevant higher magnitude of CG reduction observed in R-A in all protocols.

Pre-exercise capillary glucose concentrations was directly related to glycemic reduction induced by CE-session solely at the A-R order. Within a physiological range, the glucose uptake by active musculoskeletal cells enhances proportionally to the increase in glycemia (Rose & Richter, 2005). In T2DM subjects the glycemic reduction after exercise has been related to pre-exercise capillary glucose in moderate and high-intensity aerobic exercise (Terada et al., 2013) and resistance exercise (Ogando et al., 2022) in T2DM. Unprecedentedly, our study showed that even when combining aerobic and resistance exercise the glycemic reduction induced by CE-session is related to the pre-exercise capillary glucose, regardless of CE-protocols. However, this relationship appears to be modulated by exercise type order into CE-session, since it only occurred when aerobic exercise was performed in the first place. Thus, T2DM subjects with higher pre-exercise glycemic values, as long as within recommended limits for the practice of exercises (Colberg et al., 2016), may obtain more remarkable benefits from CE-session to lower their glycemia. However, the exact mechanisms underpinning these distinct responses regarding the exercise type order and its effects on the glucose-lowering outcomes are still unknown.

Concerning practical applications, manipulating exercise order can be conducted when increasing a given parameter of physical quality is a priority. It seems to be very important for the diabetic subjects since improvements in maximal aerobic capacity or muscle strength after CE-training can predict, in distinct ways, reductions in clinical parameters including glycated hemoglobin and waist circumference (Balducci et al., 2012b). This point is relevant to the elderly population that shares with diabetics, among others, some metabolic, cardiovascular, and muscular impairments. Two studies in older men comparing the effect of 12 weeks of CE performed at a moderate intensity for aerobic exercise, and maximal repetitions for resistance exercise suggested that performing aerobic exercise firstly induced higher improvement rates of maximal endurance performance (Cadore et al., 2018). Conversely, performing resistance exercise firstly increased muscle quality (which was not observed in reverse order) without reducing gains in endurance performance of older adults (Cadore et al., 2012). Thus, it is possible that performing the R-A in CE-session is beneficial not only to obtain maximal improvements in muscle quality but also for inducing aerobic performance improvements. However, more studies are needed to elucidate the metabolic pathways and functional responses from training parameters manipulation (*i.e.,* exercise intensity, volume, and order) within CE-session in T2DM subjects.

Some strengths should be emphasized, such as comparing glycemic responses to distinct CE sessions and comparing the magnitude of these responses, which have a substantial ecological relevance regarding the exercise prescription for T2DM subjects that often present poorly controlled glycemia. Nonetheless, this study has some limitations. The control of physical (*e.g.,* time under tension) and physiological variables (*e.g.,* heart rate responses) into different exercise orders could refine the results. In addition, the CE-sessions were not conducted at the same time nor on the same subjects, disregarding the influence of individual training status. However, it has been shown that the repetition of training sessions does not increase the glucose-lowering effect of an aerobic exercise session in subjects with T2DM (Delevatti et al., 2019). In the current study, the pre-exercise glycemic values were not different between groups. Also, the exercise protocols were prescribed under relative intensities, allowing the comparison between different CE sessions regarding their glucose-lowering effects.

Conclusion

Distinct CE-protocols (i.e., varying intensity and exercise volume) induced acutely significant glucose-lowering effects in T2DM subjects, and higher volumes of exercise exacerbated it. In addition, both exercise orders (R-A, A-R) within a CE-session effectively reduced capillary glucose. However, performing resistance exercises in the first place can contribute to the magnitude of this reduction. From a professional perspective, the current study findings may contribute to prescribing CE programs aiming for a potentialized reduction of glycemia in the T2DM population.

Conflicts of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Author's contribution Statement (CRediT taxonomy)

All authors read and approved the final version of the manuscript. Conceptualization: JGSR, ASA, WP, Data Curation: JGSR, PHMO, Formal Analysis: JGSR, PHMO, DAG, Funding Acquisition: IMSA, DDS, Investigation: JGSR, ASA, WP, Methodology: JGSR, ASA, WP, Project Administration: JGSR, IMSA, ASA, WP, Resources: IMSA, DDS, Software: JGSR, Supervision: JGSR, WP, IMSA, Validation: DAG, RSD, DDS, Visualization: DAG, RSD, DDS, Writing – Original Draft Preparation: JGSR, PHMO, DAG, RSD, DDS, Writing – Review & Editing: JGSR, PHMO, DAG, RSD, IMSA, DDS

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