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ORIGINAL ARTICLE

# Consecutive days of exercise decrease insulin response more than a single exercise session in healthy, inactive men

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## Abstract

**Purpose** It is reported that a single bout of exercise can lower insulin responses 12–24 h post-exercise; however, the insulin responses to alternate or consecutive bouts of exercise is unknown. Thus, the purpose of this study was to examine the effect of exercise pattern on post-exercise insulin and glucose responses following a glucose challenge.

**Methods** Ten male participants ($n = 10$, mean ± SD, Age 29.5 ± 7.7 years; BMI 25.7 ± 3.0 kg/m$^2$) completed three exercise trials of walking for 60 min at ~ 70% of $VO_{2max}$. The trials consisted of: three consecutive exercise days (3CON), three alternate exercise days (3ALT), a single bout of exercise (SB), and a no exercise control (*R*). Twelve to fourteen hours after the last bout of exercise or *R*, participants completed a 75 g oral glucose tolerance test (OGTT) and blood was collected at 30 min intervals for the measurement of glucose, insulin, and C-peptide.

**Result** Calculated incremental area under the curve ($_{i}$AUC) for glucose and C-peptide was not different between the four trials. Insulin $_{i}$AUC decreased 34.9% for 3CON compared to *R* ($p < 0.01$).

**Conclusion** Three consecutive days of walking at ~ 70% $VO_{2max}$ improved insulin response following an OGTT compared to no exercise. It is possible, that for healthy males, the effect of a single bout of exercise or exercise bouts separated by more than 24 h may not be enough stimulus to lower insulin responses to a glucose challenge.

**Keywords** Consecutive days · Exercise pattern · Glucose · Incremental area under the curve · Insulin · Single session

### Abbreviations

| | |
|---|---|
| AUC | Area under the curve |
| $_{i}$AUC | Incremental area under the curve |
| OGTT | Oral glucose tolerance test |
| 3ALT | Three alternate days |
| 3CON | Three consecutive days |

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## Introduction

Aerobic exercise is beneficial for improving insulin responses and glycemic control in both healthy people, and those with type 2 diabetes (T2D) (Bird and Hawley 2016; Umpierre 2011). Insulin resistance is also associated with the development of cardiovascular disease from a decrease in GLUT4 translocation, increased oxidative stress, and increased inflammatory responses (Ormazabal et al. 2018). Obesity and increased visceral fat can lead to an increase in inflammation, especially IL-6 and TNF-α. Increased TNF-α leads to a dysregulated phosphorylation of insulin receptor substrate-1, leading to a downhill cascade of reactions, ultimately leading to decreased GLUT4 translocation (Borst 2004). However, skeletal muscle glucose uptake has been shown to increase 25% in carbohydrate-deprived rats 3 h post-exercise. Subsequently, carbohydrate feeding appears to blunt the post-exercise increase in glucose uptake (Cartee et al. 1989). Following three swimming bouts, GLUT4 protein has been shown to increase twofold in rats up to 18 h post-exercise (Garcia-Roves et al. 2003). In humans, a single bout of exercise has also shown to elicit an improved insulin response 14 h post-cycle ergometry in nontrained males, similar to trained males following a control trial with no exercise (Young et al. 1989). It is well recognized that a lower insulin response to a glucose challenge is associated with reduced risk for developing insulin resistance and T2D (Hayashi et al. 2013). It has been shown that a single bout of moderate intensity (40–75% $VO_{2max}$) aerobic exercise lowers insulin responses to a glucose challenge 12–24 h post-exercise in normoglycemic men and women (Young et al. 1989; Ben-Ezra et al. 1995; Brestoff et al. 2009) and improves insulin response in people with T2D (Devlin et al. 1987; Burstein et al. 1990). While much of the literature supports improved insulin responses following a single bout of exercise, there are a few studies that did not find improvements in insulin sensitivity (Newsom et al. 2010, 2013; Hasson et al. 2010; Newsom et al. 2013) or decreases in insulin responses to carbohydrate ingestion (Weiss et al. 2008; Cononie et al. 1994; Short et al. 2012) in healthy men and women.

Studies using consecutive days of exercise have shown improvements in insulin response. For example, three consecutive days of walking at 70% $VO_{2max}$ for 50–60 min resulted in significant reductions in insulin response to a glucose challenge in healthy, young women (Jankowski et al. 2004), and young men (Rivas et al. 2016) compared to no exercise. In addition, Cononie et al. (1994) who found no effect of a single session of exercise on insulin responses, did find a significant decrease (− 20%) in insulin responses (indicating improved sensitivity to insulin) after seven consecutive days of exercise in older men and women. The American College of Sports Medicine (ACSM)-American Heart Association physical activity recommendations for healthy adults is a minimum of 150 min/week (30 min/days for 5 days) of moderate intensity aerobic exercise or 60 min/week (20 min/ days for 3 days) of vigorous intensity exercise for cardiovascular and metabolic health (ACSM 10th edition). It should be noted that the guidelines do not specify the pattern of exercise (i.e., are consecutive days better than alternate days). The ACSM-American Diabetes Association exercise recommendations for people with type 2 diabetes is 150 min per week of moderate to vigorous intensity exercise spread over 3 days with no more than two consecutive days between aerobic exercise sessions (Colberg et al. 2010), implying that the exercise benefit on glycemic control may be better if consecutive days are employed. The benefits of aerobic exercise on glycemic control when exercise is performed every other day compared to exercise performed on consecutive days, has yet to be determined. It seems worthwhile to identify exercise regimens that may be optimal to maintain or improve insulin responses even in healthy adults. Therefore, this study aims to determine the effect of exercise pattern (no exercise, [R], a single bout [SB], alternate days [3ALT], or consecutive days [3CON]) on glycemic control to a 75 g oral glucose challenge 12–14 h post-exercise. We hypothesized that 3CON would result in reduced insulin secretion, and subsequently improved insulin responses compared with SB or 3ALT.

## Materials and methods

Twelve healthy, inactive male participants (ages 19–44 years) were recruited from Texas Woman's University and the surrounding Denton, TX area. All participants completed an informed consent and PAR-Q prior to the study. Inclusion criteria included: no structured exercise or physical activity for the previous 3 months, and no known dyslipidemia, cardiovascular or metabolic disease. Participants arrived at the laboratory fasted ( > 10 h) on two separate mornings and completed a finger-tip capillary blood sample glucose check using a FreeStyle Freedom Lite glucometer (Abbot Diabetes Care, Alameda, CA). Participants were excluded if fasting glucose was ≥ 100 mg/dl for either screening. Height was measured using a stadiometer (Perspective Enterprises; Kalamazoo, MI), weight was measured to the nearest 0.1 kg using a digital scale (Tanita Corp.; Arlington Heights, IL), and body mass index (BMI, $kg/m^2$) was calculated. This study was approved by the Institutional Review Board of Texas Woman's University and all participants gave their signed, informed consent to participate. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

### Maximal aerobic capacity test

Participants performed a maximal oxygen uptake ($VO_{2max}$) test on a Quinton ST65 motor driven treadmill (Quinton Instruments Company, Bothell, WA) walking at a constant speed of 3.5 miles per hour. Treadmill elevation began at 0% with a 5% increase every 2 min until $VO_{2max}$ was achieved. Heart rate was continuously monitored by 12-lead electrocardiograph and was recorded during the last 10 s of each min. Thirty-second averages of expired gasses and expired ventilation were continuously collected by indirect calorimetry (TrueOne 2400; ParvoMedics, Sandy, UT). Two of the following three criteria were used to determine $VO_{2max}$: a plateau in $VO_2$ ( ≤ 150 ml/min) with an increase in workload, heart rate within 10 bpm of age-predicted HR max, or RER ≥ 1.10. Based on this criteria, all participants achieved $VO_{2max}$.

Approximately 7–10 days after initial testing, participants returned to the laboratory between 1600 and 1900 h to complete their first trial. The trials included: rest (*R*), a single exercise bout (SB), three consecutive days of exercise

(3CON), and three alternate days of exercise (3ALT). This study used a randomized, crossover design and each trial was separated by at least 7 days following completion of the previous trial.

### Exercise

Each exercise session consisted of 60 min of walking on a treadmill at 3.5 mph with a grade adjustment to achieve an individualized 70% $VO_{2max}$. $VO_2$ was measured during the first 10 min of exercise, 25–30 min, and the last 5 min of each exercise session. During exercise, heart rate was continuously monitored using a Polar heart rate monitor (Lake Success, NY).

Participants were instructed to follow their normal dietary patterns and maintain body weight for the duration of the study. To reduce the effect of macronutrient composition on oral glucose tolerance tests (OGTT) outcomes, participants consumed the same commercially available sandwich consisting of a minimum of 100 g of carbohydrate for the dinner meal prior to their OGTT. Three-day diet logs were also recorded for each participant to verify consistency. The participants were encouraged to maintain an intake of at least 150 g of carbohydrate/day. Dietary intake was analyzed using MyFitnessPal.com. Any change in the timing or meal composition excluded the trial and the trial was repeated a minimum of 1 week later.

### Oral glucose tolerance test (OGTT)

Twelve to fourteen hours following the completion of each exercise trial, participants returned to the laboratory after a 10–12 h fast and completed a 75 g OGTT. An intravenous catheter (21 g; Becton–Dickinson, Sandy UT) was placed in an antecubital vein and kept patent with a saline drip (1 drop/5 s, ~ 225 ml). Venous blood samples (5 ml) were collected into $K_2$-EDTA-treated vacutainers. A baseline sample was collected 5 min after placement of the catheter. Participants consumed the 75 g glucose beverage (Trutol 75; Fisher Diagnostics, Middletown, VA) within 2 min and thereafter, 5 ml blood samples were obtained at 30, 60, 90, 120, and 150 min. Blood samples were immediately centrifuged for 10 min (8 °C at 3000 rpm) and plasma was aliquoted and stored at − 80 °C pending analysis.

### Biochemical analysis

Plasma glucose was measured using enzyme electrode technology (YSI 2300/2900 Yellow Springs, OH). Insulin and C-peptide analyses were performed via radioimmunoassay (Webster, TX) using a Packard Riastar 5410 gamma counter ($n = 7$). Intra-assay coefficient of variation for insulin was 4.8% and 7% for C-peptide. Due to transitions in lab equipment, the remaining samples (n = 3) were measured using ELISAs (ALPCO, Salem, NH), with intra-assay coefficient of variation of 2.4% for insulin and 3.4% for C-peptide. Comparisons between insulin ELISA and RIA analysis have been determined to be comparable (Miller et al. 2009), and since each individual served as their own control, each participant's trial was analyzed by only one method.

### Calculations and statistical analysis

All descriptive data are reported as mean ± SD. Incremental area under the curve ($_i$AUC) for glucose, insulin, and C-peptide was calculated using the trapezoidal method. Insulin sensitivity was calculated using the Matsuda Index (Matsuda and DeFronzo 1999). Percent differences in $_i$AUC between trials were calculated as (Trial A–Trial B)/Trial A. A one-way repeated measures ANOVA was used to determine differences in $_i$AUC and dietary consumption between all trials. A two-way repeated-measures analysis of variance (trial × time; RM ANOVA) was used to determine significant differences in time points across all trials. If significant differences were found across trials, a Bonferroni post hoc comparison and corrections were employed. The criterion reference for significance was set at $p = 0.05$. All statistical calculations and analyses were performed using IBM SPSS Statistics (Version 25, IBM Corp., Armonk, NY).

## Results

A total of 12 participants provided informed consent and began the study. Two participants were eliminated from analysis. One was not included due to impaired glucose tolerance from his first OGTT (plasma glucose was above 200 mg/dl 2 h post-OGTT, and one due to lack of adherence to study protocols). Table 1 displays participant descriptive variables.

**Table 1** Participant characteristics

| | Males ($n = 10$) |
|---|---|
| Age (years) | 29.5 ± 7.7 |
| Height (m) | 1.79 ± 0.1 |
| Weight (kg) | 82.6 ± 13.2 |
| BMI (kg/m$^2$) | 25.68 ± 2.98 |
| $VO_{2max}$ (ml/kg/min) | 38.31 ± 4.68 |
| $VO_{2max}$ (L/min) | 3.13 ± 0.49 |
| CHO intake prior to OGTT (g) | 287.6 ± 102.1 |
| FAT intake prior to OGTT (g) | 90.7 ± 37.1 |
| PRO intake prior to OGTT (g) | 108.8 ± 30.8 |

Data are presented as mean ± SD. Nutrient data reflect mean macronutrient intake for the complete day prior to each OGTT

*CHO* carbohydrate, *PRO* protein

Table 2 provides all exercising data including heart rate, $VO_2$, and caloric expenditure. Table 3 displays data from fasting glucose, insulin, and C-peptide as mean ± SD across the four trials. There was no difference in fasting concentrations in glucose, insulin, or C-peptide across the four trials. There were also no differences in dietary intake within each participant's three exercise trials and control trial.

As shown in Fig. 1, calculated $_{i}$AUC for glucose was not different between the four trials. There were also no time-point differences for glucose or insulin across the four trials (Fig. 2) or significant changes in C-peptide $_{i}$AUC across each trial.

Insulin $_{i}$AUC was significantly decreased by 34.9% for 3CON (4100 ± 1717 µU/mL;) compared to *R* (6296 ± 2292 µU/mL; $p < 0.01$) as shown in Fig. 1. Insulin $_{i}$AUC was also decreased for SB (5034 ± 1716 µU/mL; − 20.0%, $p = 0.16$) and 3ALT (4407 ± 1155 µU/mL; − 30.0%, $p = 0.14$) compared to *R* however, these values did not reach statistical significance. Figure 2 displays time-point data for plasma insulin concentrations. All participants showed a decrease in plasma insulin $_{i}$AUC following 3CON (Fig. 3).

## Discussion

Acute aerobic exercise is an effective treatment that leads to improvements in glycemic control in healthy men and women (Young et al. 1989; Ben-Ezra et al. 1995). However, the differential effects of aerobic exercise pattern on insulin responses have yet to be fully determined. The aim of the current study was to examine changes in glucose and insulin responses following acute aerobic exercise performed every other day compared to consecutive days or a single bout. The primary finding from this study was that three consecutive days of walking for 60 min at 70% $VO_{2max}$ reduced post-exercise insulin $_{i}$AUC (34.9%) in response to a 75 g OGTT in 3CON compared to *R* in healthy, inactive males. Additionally, there were no significant changes in insulin and glucose $_{i}$AUC for SB or 3ALT compared to *R*.

The 34.9% decrease in insulin response after three consecutive days of exercise is consistent with previous studies that have examined the effect of short-term (≤ 7 days) aerobic exercise on insulin responses to an OGTT. Similarly, adults with impaired glucose tolerance who performed seven consecutive days of aerobic exercise at 68% of $VO_{2max}$ experienced significant reductions (32%) in insulin AUC (Rogers et al. 1998).Rivas et al. 2016 and Jankowski et al. 2004, using healthy untrained adults, both used three consecutive days of exercise at 70% $VO_{2max}$ for 50–60 min and found significant decreases (15–21%) in plasma insulin responses to a 75 g OGTT drink 12–16 h post-exercise. In addition, the results support our initial hypothesis that consecutive days of exercise, rather than a single bout or alternating days of exercise, would result in a greater reduction in insulin response compared to *R*.

Our C-peptide results are similar to previous reports that aerobic exercise in healthy individuals may not always elicit differences in C-peptide with concurrent differences in insulin concentrations. Forty-five minutes following either aerobic, resistance, or no exercise, serum C-peptide levels have shown no differences during a 75 g OGTT in men with significant decreases in insulin (Schell et al. 1999). In males with normal glucose tolerance, 1 h of cycle ergometry at

**Table 2** Exercise data

| | SB | 3ALT | 3CON |
|---|---|---|---|
| Average exercise HR (bpm) | 148.9 ± 12.4 | 146.8 ± 13.1 | 148.4 ± 13.7 |
| Average exercise HR (% $HR_{max}$) | 78.2 ± 6.3 | 77.0 ± 5.4 | 77.9 ± 5.6 |
| Average exercise $VO_2$ (L/min) | 2.20 ± 0.4 | 2.17 ± 0.4 | 2.29 ± 0.3 |
| Average exercise $VO_2$ (%$VO_{2max}$) | 72.3 ± 7.7 | 72.0 ± 7.7 | 73.0 ± 6.2 |
| Average caloric expenditure (kcal/h) | 660 ± 120 | 651 ± 120 | 687 ± 90 |

Data are presented as mean ± SD and are averages per bout of exercise for each day of each trial

*HR* heart rate, *kcal/h* kilocalorie/hour

**Table 3** Fasting glucose, insulin and insulin sensitivity for all four trials

| | Rest | SB | 3ALT | 3CON |
|---|---|---|---|---|
| Glucose (mmol/L) | 4.83 ± 0.31 | 4.68 ± 0.30 | 4.74 ± 0.29 | 4.78 ± 0.21 |
| Insulin (µIU/mL) | 8.03 ± 6.62 | 7.06 ± 4.01 | 7.38 ± 5.29 | 5.88 ± 2.95 |
| C-peptide (pmol/L) | 595.2 ± 219.6 | 578.7 ± 220.8 | 613.6 ± 239.8 | 644.4 ± 318.1 |
| Matsuda Index | 7.80 ± 4.96 | 7.81 ± 3.13 | 9.14 ± 4.75 | 9.45 ± 3.87 |

Data are presented as mean ± SD. Fasting values were collected at the zero time point, prior to glucose consumption

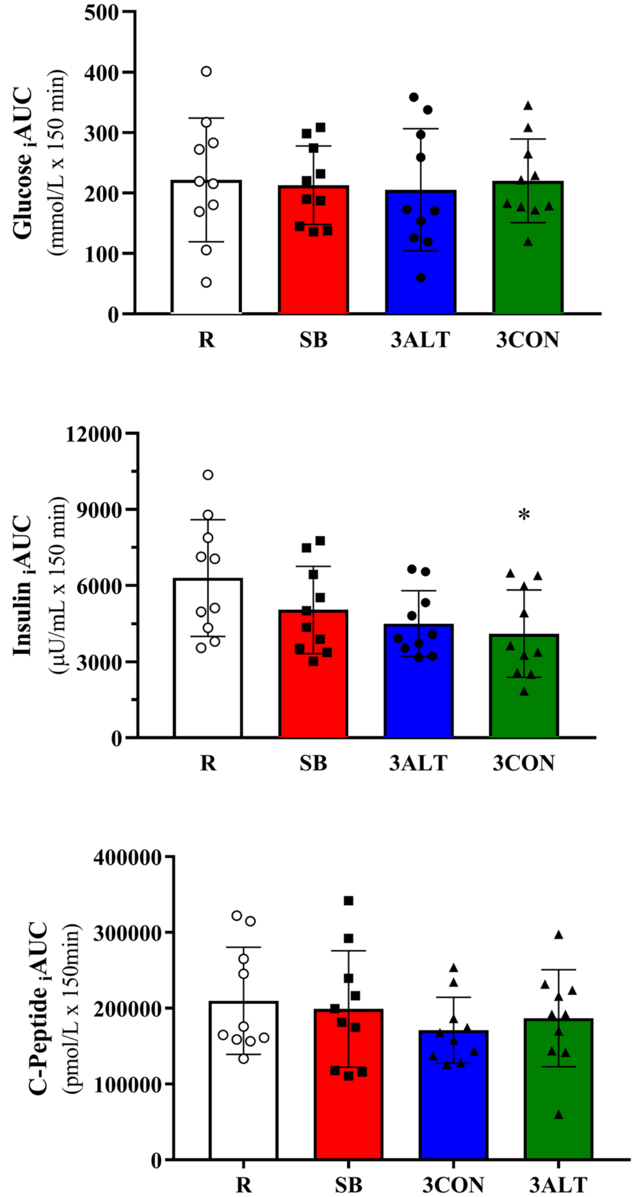


**Fig. 1** Glucose, insulin, and C-peptide Iauc. Insulin, glucose, and C-peptide $_{i}$AUC for all four trials with individual data shown during the 75 g OGTT

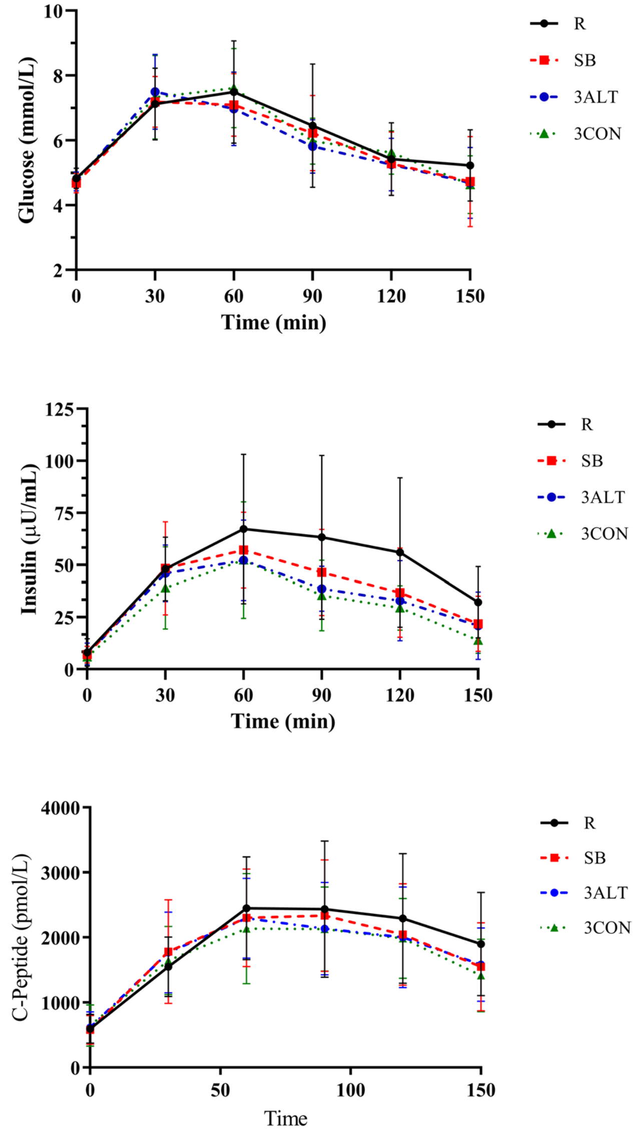


**Fig. 2** Mean timepoint concentrations for glucose, insulin, and C-peptide. Glucose and Insulin mean values across time for all four trials during the 75 g OGTT

50% $W_{max}$ did not elicit differences in C-peptide concentrations compared to no exercise (Knudsen et al. 2014). Since $_{i}$AUC for C-peptide was not different between *R* and 3CON, the reduction in insulin response in the 3CON trial could potentially be due to an increase in insulin clearance.

The results of the current study did not show significant improvements in insulin responses for SB or 3ALT 12–14 h post-exercise. These results are similar to other studies that used a single bout of aerobic exercise (Weiss et al. 2008; Short et al. 2012) and did not find changes insulin AUC. The fact that a SB did not stimulate a significant decrease in insulin responses is somewhat surprising since

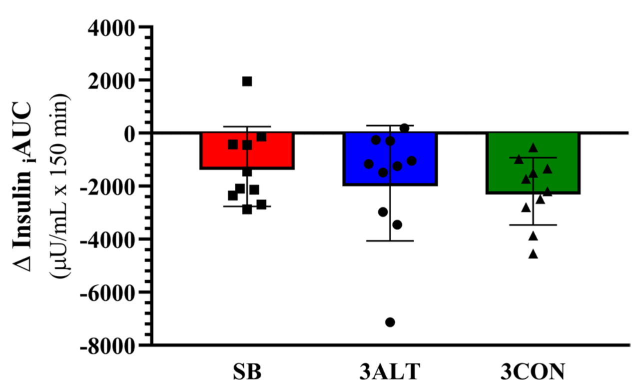


**Fig. 3** Change in insulin responses for all trials compared to the mean of R. Insulin $_{i}$AUC change compared to R for all treatment trials with individual data shown during the 75 g OGTT

the duration and intensity of exercise employed in the present study are the same in previously published data from our lab where significant decreases in insulin response were found (Ben-Ezra et al. 1995; Jankowski et al. 1999). As previously mentioned many studies have shown that a single bout of aerobic exercise at similar exercise intensities and durations improves post-prandial insulin responses (Young et al. 1989; Ben-Ezra et al. 1995; Rogers et al. 1998; Brestoff et al. 2009). Increases in skeletal muscle contraction accompanying treadmill walking are associated with increased AMPK activity. This deactivates TCB1D1, leading to increased translocation of GLUT4 to the cell membrane (Bird and Hawley 2016). Houmard et al. 2004 suggests that exercise duration of at least ~ 115 min per week is necessary to substantiate an improvement in insulin sensitivity (Houmard et al. 2004). Since we did not directly measure or see changes with the Matsuda Index for insulin sensitivity, we could associate our reduction in insulin response following 3CON to the duration of exercise across the week and to the consecutive days of exercise. Our results indicate all ten participants reduced insulin responses to 3CON.

There are a few factors that may explain why SB or 3ALT did not significantly reduce the insulin response. One explanation may be due to the individual variability in responses between trials. It is noteworthy to mention all participants (10/10) responded with a decrease in insulin concentrations following the 3CON compared to R with the smallest decrease in insulin of 14.1%; however, only 70% (7/10) of the participants responded with > 10% decrease in insulin to SB and 3ALT. Ben-Ezra et al. (1995) also found that 25% of their participants did not show a decrease in insulin response 12–14 h after a single bout of exercise. The variability could be due to skeletal muscle glucose transporter (GLUT 4) content, translocation, or expression resulting from acute exercise. There is a large individual variation in GLUT 4 expression 3–24 h following exercise (Coderre 1992). However, three consecutive days of aerobic exercise have been shown to increase GLUT 4 protein content, which may explain the improved insulin response in the 3CON (Green et al. 2008). Improvement in insulin responses post-exercise is associated with GLUT 4 translocation to the cell membrane. It is possible that consecutive days of exercise may have had a greater influence on this translocation, compared to single bout and alternate days of exercise. Moreover, glycogen depletion has been associated with decreased insulin response to glucose ingestion (Jensen et al. 1997; Richter and Hargreaves 2013; Zorzano et al. 1986). However, as shown in Table 1, carbohydrate ingestion the day prior to the OGTT should have negated any effect of glycogen depletion, as participants in the current study consumed ~ 287 g of carbohydrate the day prior to all OGTTs. It should be noted that carbohydrate replacement has been shown to attenuate post-exercise insulin sensitivity and impair glucose tolerance (Taylor et al. 2018). This could help explain the lack of significant reductions in insulin responses after SB and 3ALT but not after 3CON since the dietary carbohydrate were similar across all trials.

Last, Burstein et al. (1990) found that 1 h of exercise reduced post-exercise insulin responses in obese normoglycemic subjects with elevated fasting insulin levels (26 µU/mL) but exercise had no effect on the lean controls (7.4 µU/mL). The implication is that individuals who are already relatively insulin sensitive may not gain additional exercise-related benefits. Similar to Burstein et al. (1990) our participants had fasting insulin levels of 8.05 µU/mL in their resting trial. This point is further emphasized in the Jankowski et al. (1999) study. They found that Mexican–American women, who were pair matched on age and total body fat mass with non-Hispanic white women, had greater non-exercise insulin responses to an OGTT and significantly reduced their insulin responses 12–15 h post-exercise (70% $VO_{2max}$ for 50 min) where the non-Hispanic white women did not. This is somewhat supported by van Dijk et al. (2012) who found that glycemic control similarly improved in people with type 2 diabetes regardless whether exercise was performed on 1 day or two consecutive days. Our results, based on all ten participants reducing insulin responses to 3CON, support the notion that a more robust pattern of exercise may be needed to improve insulin responses in young, healthy men.

It should be stated there are limitations to this study. The small sample size and inherent variability in responses may have prevented finding significant effects in insulin response to the SB and 3ALT trials. However, the observed power for insulin $_{i}$AUC was 0.99 and the effect size of 0.73 provides us with confidence that our sample size was adequate to support our results (Cohen et al. 2015). The variability in insulin responses may in part be related to the range in BMI (25.68 ± 2.98) in our population. Future studies should consider selecting participants all of whom fit into a single BMI category. However, we found no relationship ($r = 0.006$) between BMI and insulin area at rest. Additionally, though our participants were instructed to maintain their dietary habits throughout the study and eat the same meal the night before each OGTT, dietary information was all self-reported. Physical activity was also self-reported to confirm that participants were indeed inactive but activity levels were not measured or analyzed. Also, because of the lengthy duration of the study and equipment replacement, $n = 7$ samples were stored for more than 3 years and we did not feel it was appropriate to re-analyze the samples after a freeze–re-freeze cycle using different techniques. Though it should be noted that all samples from an individual were analyzed using the same methodology. Last, since this study was conducted with healthy men, the results may not be generalizable to other populations. Future studies examining the effect of pattern of exercise using participants who are obese, pre-diabetic, or have type 2 diabetes may be needed to determine the most effective exercise prescription for individuals with impaired insulin responses.

It is well established that exercise is an important factor contributing to a healthy lifestyle, and regular exercise reduces risk factors for metabolic diseases such as type 2 diabetes. It appears based on our data that to reduce insulin responses to a glucose challenge for already apparently healthy men that consecutive days of exercise may be needed since three alternating days or a single bout of exercise did not decrease insulin responses to a glucose challenge.

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**Author contributions** TC was responsible for collecting and analyzing data, and writing the manuscript. CI and RG were responsible for collecting data, analyzing data, and manuscript revisions. SD collected data and reviewed and edited the manuscript. MB, MO, and JR collected and analyzed data. AD reviewed data and edited manuscript. VB designed the research study, collected the data, and edited the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors have no conflicts of interest to declare.

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