ORIGINAL ARTICLE

Cognitive effects of a 30-min aerobic exercise bout on adults with overweight/obesity and type 2 diabetes

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[Correction added on 21 July 2017, after first online publication: A change was made to the affiliation for P.A. Hall].

Summary

Background

Several studies document reliable brain health benefits of acute exercise bouts. However, no prior studies have explored such effects among those living with co-morbid overweight/obesity and type 2 diabetes (T2DM), both of which are conditions associated with cognitive performance decrements.

Purpose

To examine the impact of a 30-min bout of moderate-intensity aerobic exercise on executive function among adults with overweight/obesity and T2DM, employing a widely used experimental paradigm.

Methods

Thirty adults with overweight/obesity and T2DM were randomly assigned to moderate (30% maximal heart rate reserve) and minimal (r.p.m. 30–50; work load 5) intensity aerobic exercise. Pre-exercise to post-exercise changes in Stroop interference and Go/No-Go scores were compared across conditions.

Results

Primary analyses revealed no overall effect of exercise condition on changes in Stroop or Go/No-Go performance. Post-hoc moderation analyses indicated that Stroop interference scores were reduced, following moderate exercise among female participants and among those who were more physically active.

Conclusion

The current study revealed no reliable benefit of acute aerobic exercise for overweight and obese individuals living with T2DM overall. There may be limited benefits for women and more and active subgroups, but the precise nature of such benefits remains unclear.

Keywords: Aerobic exercise, executive function, self-regulatory fatigue, type 2 diabetes.

Type 2 diabetes mellitus (T2DM) is highly prevalent worldwide (8.3%) (1) with predicted rates reaching 10.1% by 2035 (2). The association of T2DM with a broad range of microvascular and macrovascular complications has made it a global health concern (3,4). In addition to these well-established consequences, recent evidence suggests an association between T2DM and impaired cognitive function (5–8). Among those cognitive functions most strongly affected is executive function (EF), a collection of intricately connected cognitive mechanisms that are responsible for control over thoughts, emotions and behaviours (9–19). A recent meta-analysis identified reliable decrements associated with T2DM that were relatively uniform across EF measurement approaches and conceptual subtypes of EF (8)

Impaired EF among the T2DM population is potentially problematic because of the role of EF integrity in the consistent implementation, assessment and adaptation

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behaviours necessary in diabetes self-care (20–24). For example, EF has been implicated in the ability to perform healthy dietary behaviours, consistently follow physical activity recommendations, adhere to medication regimens and maintain weight loss (21,25–36), all of which are required for effective management of T2DM (37). For this reason, effective disease management may rely partially on intact executive control capacities.

There exist several primary avenues for optimization of executive control abilities in adulthood, and aerobic exercise is one of the more promising options for the middle-aged and older adult populations. Recent meta-analyses have demonstrated enhancement of EF following both longer term aerobic training programmes (38) and immediately following a single bout of aerobic exercise (39,40) in non-diabetic older adult populations. Although the mechanism by which aerobic exercise is thought to benefit executive control is not conclusively known, it appears that improvement in the function of the prefrontal cortex is one potential route, with potential mediators including brain-derived neurotrophic factor, insulin-like growth factor or enhanced blood oxygenation (41,42)

This beneficial effect of aerobic exercise on EF may be moderated by a number of factors, including sex and physical fitness. Kramer and Erikson (42) noted that studies with larger proportion of female participants tended to show larger effect sizes of chronic exercise on improved cognition and hypothesized that this difference may be due to an interaction between oestrogen and brain-derived neurotrophic factor. Although acute aerobic exercise literature has failed to observe a difference between effects in male and female participants (40), to our knowledge, few studies have directly assessed these differences. Additionally, physical fitness has been shown to act as a moderator of the effect of exercise on cognition such that, during exercise, low-fit individuals displayed impaired cognition and hit fit displayed improved, while following exercise improvement was observed in both groups (40,43)

While studies have demonstrated evidence of EF enhancement following a single session of aerobic exercise in healthy young (39) and healthy older adults (44–46), the effect among those with T2DM has not been previously examined. Thus, the primary aim of the current study is to examine the effects of acute aerobic exercise on EF in a population of adults with overweight/obesity and T2DM. The potential moderating effects of sex, physical activity status and diabetes-related factor – disease duration, A1C and number of T2DM medications – were also assessed. It was hypothesized that in line with prior research in other populations, EF would be enhanced following moderate, but not minimal, intensity exercise and that such effects may be stronger for women and relatively older participants.

Methods

Participants

Thirty obese and overweight adults ($M_{BMI} = 33$ kg), age 40–69 ($M_{age} = 59.6$ years, SD = 5.7), with T2DM and not currently taking insulin were recruited from the community through a combination of (i) study posters, (ii) presentations to diabetes education classes and (c) study flyers distributed in endocrinologist offices. Informed consent was obtained from all participants included in the study; participant characteristics are presented in Table 1. Primary inclusion criteria were current diagnosis of T2DM, safety for exercise (according to the American Heart Association (47) and American College of Sports Medicine guidelines (48), using the Physical Activity Readiness Questionnaire and an additional medical questionnaire) and age 40-69. This age range was selected in order to target a middle-aged population and to allow for use of the Physical Activity Readiness Questionnaire screening tool, which is valid in individuals under 70 years of age. Additional exclusion criteria were mobility limitation precluding exercise or any of the following factors which could interfere with accuracy of EF testing: insulin use, anti-cholinergic drug use, severe vision impairment, colour blindness, substance abuse in the past 6 months or current diagnosis of a depressive disorder. The participants were asked not to consume caffeine for 3 h prior to participation and, if possible, to limit food consumption

Table 1 Sample characteristic

n	30
Age (years)*	59.6 (5.7)
Sex % female (n)	50% (15)
BMI ($n = 27$) (kg)	33.11 (7.3)
Ethnicity % (n)	
Caucasian	73.3% (22)
Aboriginal	6.7% (2)
Other	16.7% (5)
T2DM duration ($n = 29$) (years)	5.95 (4.9)
A1C (n = 16)	7.8 (1.8)
No. of T2DM medications % (n)	
None	16.7% (5)
One oral medication	33.3% (10)
Two oral medications	40% (12)
Three oral medications	10% (3)
Hours of vigorous activity in past week ($n = 28$)	1.75 (3.07)

*Values are mean (SD) unless otherwise specified.

© 2017 The Authors Obesity Science & Practice published by John Wiley & Sons Ltd, World Obesity and The Obesity Society. Obesity Science & Practice during the hour prior to participation. Of the 30 individuals who attended at least one session, 2 were unable to complete exercise protocol as a result of high blood pressure; thus, the final sample consisted of 28 participants.

Design

A within-subject design was employed to compare performance on EF tasks before and after two intensities of aerobic exercise. Each participant attended two laboratory sessions in counterbalanced order; both sessions were conducted at the same time of day and the same day of week. During each session, the participants completed an exercise bout and two computer tasks measuring response inhibition (Stroop and Go/No-Go, GNG), performed before and after the exercise session. The exercise session was moderate intensity (30% maximal heart rate reserve, MHRR) on one visit and minimal (minimal effort pedalling) on another. Informed consent was obtained at the start of the first session, and the participants completed about demographics questionnaires and health behaviours at the end of the second session. This study received ethics approval from the institutional research ethics review board, and the participants received \$50 gift cards in exchange for participation.

Executive function measures

Two EF tasks – GNG task and the Stroop task – were presented on a desktop computer by using E-PRIME software (Psychology Software Tools Inc). The participants responded via button press using a response box and were asked to respond as quickly and accurately as possible for each task. Both EF tasks were administered before and after the exercise session at each visit to measure change in EF resulting from the exercise bout. Order of tasks was randomized for each visit, but during a given visit, the order of tasks was the same before and after the exercise session in order to limit the effect of order of tasks on differences in pre-performance and post-performance.

Stroop task

In this version of the Stroop task (49), which has been modelled after the version in Miyake *et al.* (50), the participants were instructed to indicate (by button press) the colour of a stimulus, as quickly and accurately as possible. Stimuli were presented on the screen until the participant responded, followed by a response to stimulus interval of 1,000 ms minus the response time; all stimuli were presented in red, blue, green, yellow, orange or purple coloured font. Following 10 practice trials, the participants were presented with a mixed block of trials: 72 trials with a string of asterisks appearing in one of the six colours, 12 congruent colour word trials (e.g. the word red appearing in red coloured font) and 60 incongruent colour word trials (e.g. the word yellow appearing in purple coloured font). The Stroop task is one of the most widely used measures of inhibition (51,52). The dependent variable of interest was Stroop interference (calculated as reaction time (RT) on incongruent trials minus RT on asterisk trials (46)), where greater Stroop interference was indicative of weaker EF.

Go/No-Go task

The GNG task measures one's ability to inhibit a prepotent response (53). In this task, the participants were required to press a button, as quickly and accurately as possible, whenever a lower case letter was presented on the computer screen and withhold their response when an upper case letter was presented. The stimulus duration was set at 1,000 ms, with a 500-ms interstimulus interval. A total of 10 practice trials were followed by 4 blocks of 60 test trials. In half of the test blocks, upper case letters predominated (5:1), and in the other half of the test blocks, lower case letters predominated (5:1). The dependent variable of interest was RT on correct trials where longer RT indicated weaker EF.

Exercise protocol

Each participant completed two exercise bouts, one at each visit (moderate intensity for one and minimum for the other). Exercise sessions were conducted by using a recumbent cycle ergometer and were overseen by a Canadian Society for Exercise Physiology/American College of Sports Medicine certified personal trainer or exercise physiologist.

In the moderate-intensity exercise condition, the participants completed a 5-min warm-up, 20-min exercise at 30% MHRR and 5-min cool down for a total of 30 min of exercise. Target heart rate (THR) for the moderate-intensity condition (30% MHRR) was calculated by using the equation THR = resting heart rate (RHR) + 0.3 (MHR – RHR), where MHR = 220 – age (the equation MHR = 164 – 0.7 * age was substituted for individuals by using beta-blocker), and RHR was measured with participant seated on the cycle ergometer prior to exercise. The 5-min warm-up and cool-down were conducted at a workload of 5 r.p.m. of 50–70 (with cool down approaching 50 r.p.m.). THR was achieved and maintained throughout the 20-min exercise session

by adjusting the workload while maintaining r.p.m. between 50 and 70.

The minimal exercise condition – which can be thought of as equivalent to a 'movement only' condition, designed to control for body position and movement without any aerobic exercise component – required participants to cycle at a slow and steady rate at the lowest available setting: workload of 5 r.p.m. 30–50. Every effort was made to keep THR as close to RHR as possible. In order to maintain a consistent duration of exercise between the two sessions, the participants performed minimal exercise for a total of 30 min.

Exercise measures

Resting heart rate and blood pressure were taken on the cycle ergometer prior to the initiation of the exercise bout. Heart rate was monitored throughout the exercise session (and recorded every 5 min) to ensure that THR was achieved and maintained and to quickly identify any irregularities. Blood pressure, rated perceived exertion (RPE; using the Borg RPE scale 6–20) and workload were also be recorded at the end of each 5-min interval.

Moderators

Additional information about participant demographics and health behaviours was collected through survey response at the end of the second session. Recent physical activity status (assessed by using the question: 'How many hours of vigorous exercise have you completed over the past 7 d?'), T2DM duration (in years), most recently recorded A1C, number of diabetes medications and sex were included as potential moderators of the effect.

Statistics

Change scores were calculated as post-exercise score minus pre-exercise score, and separate one-way repeated measures analysis of variance (ANOVA) were used to assess whether greater increases in EF performance on Stroop and GNG tasks emerge following moderate as compared with minimal aerobic exercise. Potential moderating effects of order of sessions, sex, recent physical activity history, years with T2DM, recent self-reported A1C and number of diabetes medications were assessed by using interaction terms, and further ANOVA analyses were conducted to tease out the details of any significant relationships. One data point was omitted from Stroop analysis because of accuracy less than 0.5, as this suggests lack of understanding of the task instruction. Outcome frequency distributions for Stroop interference and GNG RT were skewed and therefore subject to a square root transformation to improve normality.

Results

Preliminary analyses

An initial comparison of the moderate and minimal conditions was conducted using repeated measures ANOVA to establish a difference between the moderate and minimal exercise conditions. A significant effect of condition was observed for both exercise intensity (calculated from average [MHR HR = RHR + _ RHR] × intensity) (F[1,22] = 194.88, p < 0.001) and RPE (F[1,27] = 53.589,p < 0.001), such that both were higher in moderate (intensity: M = 30.67, SD = 3.31 RPE: M = 11.95, SD = 1.72), as compared with minimal exercise (intensity: M = 10.80 SD = 6.72; RPE: M = 9.07 SD = 1.74).

One-way repeated measures ANCOVA of difference scores on moderate and minimal conditions demonstrated that there was no significant interaction between order of sessions (i.e. whether participants engaged in moderate exercise or minimal exercise in the first session) and condition indicating that the order of sessions did not significantly impact the effect of exercise condition on Stroop interference (*F*[1,25] = 0.524, p = 0.48) or GNG RT (p = 0.19).

Stroop effects

A one-way repeated measures ANOVA was conducted to examine the impact of condition (moderate vs. minimal) on change in Stroop interference score. Analysis revealed no significant main effect for condition (p = 0.34). See Table 2 for means.

Moderator analysis using repeated measures ANCOVA demonstrated a significant interaction between sex and condition (F[1,25] = 5.67, p = 0.025). To further understand the relationship between the effect of acute aerobic exercise on EF and sex, a stratified analysis was conducted in order to observe the effect of exercise on Stroop interference separately for male and female participants, using one-way ANOVA of Stroop interference change scores. For male participants, there was no significant effect of condition on change in Stroop interference (F[1,13] = 0.66, p = 0.43); however, for female participants, there was a significant main effect of condition on change in Stroop interference (F[1,12] = 21.52, p = 0.017; Figure 1) such that there was an increase in interference following minimal intensity exercise ($M_{min} = 1.3$, $SD_{min} = 1.99$) and a decrease

	Moderate		Min	imal
	Pre	Post	Pre	Post
Stroop interference (ms)*	192.40 (172.09)	154.78 (135.98)	150.84 (233.69)	160.65 (161.09)
GNG RT (ms)*	491.0 (61.77)	473.02 (56.44)	496.44 (62.6)	478.03 (51.76)

Table 2 Stroop interference and Go/No-Go reaction time (RT) by condition and time

*Values are mean (SD); higher values indicate weaker executive function.

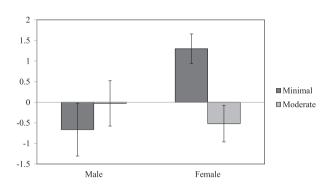


Figure 1 Stroop interference change by condition and sex. This figure depicts change in Stroop interference (Post – Pre) following minimal and moderate intensity exercise for male (n=14) and female (n=13) participants separately, such that a positive score indicates an increase in interference and a negative score indicates a decrease. Error bars represent standard error

following moderate ($M_{mod} = -0.518$, $SD_{mod} = 1.60$). Further, the increase in Stroop interference following the minimal intensity exercise ($M_{pre} = 0.219$, $SD_{pre} = 2.24$, $M_{post} = 1.52$, $SD_{post} = 1.44$) was significant (F[1,12] = 5.566, p = 0.036), but there was no significant change in Stroop interference following moderate exercise condition (p = 0.265). There was no significant interaction between order and condition for either male or female participants (F[1,12] = 0.481, p = 0.753 and F[1,11] = 0.549, p = 0.168 respectively), indicating that the order of sessions did not significantly impact the effect of exercise condition on Stroop interference.

Moderator analysis also demonstrated a significant interaction between hours of recent vigorous activity (as a continuous variable) and condition (F[1,25] = 4.738, p = 0.039). To further understand the relationship between the effect of acute aerobic exercise on EF and hours of vigorous physical activity in the past week, the participants were separated into two groups based on hours of physical activity reported. Those reporting more than 1 h of vigorous activity were considered active, while those reporting 1 h of vigorous activity or less were categorized as inactive. A stratified analysis was conducted in order to observe the effect of exercise on Stroop interference separately for those active and

inactive groups, using one-way ANOVA of change scores. For inactive participants, there was no significant effect of condition on change in Stroop interference (F[1,16] = 0.089, p = 0.770); however, for active participants, there was a significant effect of condition on change in Stroop interference (F[1.9] = 5.538. p = 0.043; Figure 1) such that there was an increase in Stroop interference following the minimal, but not moderate, condition ($M_{min} = 1.45$, $SD_{min} = 1.89$, $M_{\rm mod}$ = -0.3829, $SD_{\rm mod}$ = 1.82). This effect was characterized by a significant increase in Stroop interference following minimal intensity exercise $(F[1,9] = 5.87, p = 0.038, M_{pre} = 1.38, SD_{pre} = 3.30)$ and mitigation of this increase in the moderate condition $(p = 0.330, M_{post} = 2.84, SD_{post} = 2.16)$. There was no significant interaction between order and condition for inactive or active participants (F[1,15] = 2.040, p = 0.174 and F[1,8] = 0.674, p = 0.435 respectively).

There was no moderating effect of disease duration (F[25,1] = 0.590, p = 0.449), A1C (F[14,1] = 2.440, p = 0.141) or number of diabetes medications (F[25,1] = 0.520, p = 0.478).

GNG effects

A one-way repeated measures ANOVA was conducted to examine the impact of condition (moderate vs. minimal) on change in GNG RT score (calculated as post-score – pre-score). The analysis revealed no significant main effect of condition (*F*[1,27] < 0.001, p = 0.994). See Table 2 for means. Moderator analysis using repeated measures ANCOVA demonstrated that there was no significant interaction among condition and sex (*F*[1,26] = 0.597, p = 0.447), hours of vigorous physical activity in the past week (*F*[1,26] = 1.032, p = 0.319), T2DM duration (*F*[1,26] = 0.017, p = 0.898), A1C (*F*[1,14] = 0.590, p = 0.455) or number of T2DM medications (*F*[1,26] = 0.005, p = 0.942).

Discussion

The current study examined the effects of acute aerobic exercise (moderate and minimal) on EF task performance.

© 2017 The Authors Obesity Science & Practice published by John Wiley & Sons Ltd, World Obesity and The Obesity Society. Obesity Science & Practice Overall, there was no significant effect of moderate intensity acute exercise on EF. However, moderational analysis revealed that women (but not men) and active (but not inactive) individuals performed significantly better following moderate exercise as compared with minimal intensity exercise (the control condition). The effect of moderate exercise on cognition in women and among physically active individuals was characterized by a reduction of an increased Stroop interference effect in the minimal exercise condition.

The explanation for the mitigating effects of moderate exercise in relation to minimal-exercise-induced EF decrements in these groups is unclear. However, one possibility is that the minimal exercise condition - though less physically demanding - was somewhat mentally demanding due to its less engaging gualities and its requirement for attention to maintain a minimum pedalling speed without stopping altogether. Indeed, neuroimaging studies have demonstrated decreased activity in the anterior cingulate cortex (54) as well as the dorsolateral prefrontal cortex (DLPFC) (55) in association with fatigue induced by cognitively demanding activities. This is significant because of the DLPFC involvement in EF (and particularly Stroop performance), which has been demonstrated in both younger and older adults (41,46). Furthermore, increased activity in the PFC following exercise has been observed by using functional magnetic resonance imaging and functional near-infrared spectroscopy, and this increase in activity is associated with improved performance of Stroop (41) and a working memory task (56). Thus, decreased DLPFC activity due to selfregulatory fatigue may be mitigated by an increase in PFC activity following exercise.

Additionally, the T2DM population may be uniquely predisposed to self-regulatory fatigue following exercise, due to the possible mechanistic role of glucose in selfregulatory fatigue (57). Decreases in blood glucose following self-regulation, predictive ability of lower blood glucose for self-regulatory fatigue and restorative effects of glucose consumption have all contributed to the view that glucose depletion may serve as a mechanism for self-regulatory fatigue (57-61) and imply that a predisposition to decreased blood glucose may increase the likelihood of experiencing self-regulatory fatigue. Among individuals with T2DM - but not healthy lean adults - acute aerobic exercise has been shown to increase metabolic clearance of glucose (62) and decrease blood glucose (63). Together, these findings suggest that following acute aerobic exercise, individuals with T2DM may experience a greater decrease in glucose availability than is seen in the general population and, as a result, may be at greater risk of self-regulatory fatigue following exercise than healthy young or older adults.

A finding of self-regulatory fatigue following exercise was similarly proposed by Barella et al. (64), who suggested that self-regulatory fatigue or effort-reward imbalance contributed to null findings with respect to EF performance following 25 min of moderate intensity exercise and multiple repeated measures of the Stroop task in a group of older adults. Additionally, competing attention has been suggested as a possible explanation for the small decreased effect size in cognitive function during the initial phase of acute aerobic exercise (39). While other studies have failed to demonstrate a selfregulatory fatigue effect following acute aerobic exercise (41,46,56,65), ours is the first to examine this effect in a T2DM population, which has an arguably greater susceptibility to self-regulatory fatigue particularly following aerobic exercise.

With respect to the observed moderating effect of sex on the relationship, our findings illustrate that selfregulatory fatigue occurred among female, but not male, participants. Evidence suggests that sex differences exist with respect to the activation of neural pathways during self-control tasks (66). Similarly, Maluchenko et al. (67) observed that, while serotonin deficiency was associated with a lower threshold for self-regulatory fatigue in men, serotonin excess was associated with a lower threshold in women, thus suggesting that differences in mechanism of self-regulatory fatigue in men and women may exist. As such, it is possible that sex differences in degree of selfregulatory fatigue or interaction of self-regulatory fatigue with exercise condition may account for this finding. Future research should examine the possibility of sex differences in neural activity during self-regulatory fatigue, particularly as it pertains to the effects of aerobic exercise.

With respect to physical activity status, a similar moderating effect was found, whereby only physically active individuals displayed self-regulatory fatigue following minimal intensity aerobic exercise. In a meta-analysis of the effects of aerobic exercise on cognition, Chang et al. (40) found that physical fitness acted as a moderator of the effect such that, during exercise. low-fit individuals displayed impaired and high fit displayed improved cognition, while following exercise improvement was observed in both groups. While overall, the effects following exercise seemed to be consistent among hit-fit and low-fit individuals, differences seen during exercise provide some evidence of differences between these two groups. Given that these studies typically compared low fit and hit fit within a population of healthy individuals, the effects of physical fitness may be exacerbated in the current study as the overall level of fitness was low and the participants share a common disease status (T2DM). As such, we

hypothesize that, given the extreme lack of physical activity in our physically inactive group (less than or equal to 1 h of vigorous activity per week), it is possible that even a minimal intensity of aerobic exercise (at an intensity of approximately 11%) may have been enough to produce aerobic exercise-induced benefits. If this is the case, a lack of self-regulatory fatigue following minimal intensity exercise would be indicative of restorative effects resulting from minimal intensity exercise; however, future research is needed to establish whether acute minimal intensity exercise among a population of inactive individuals with T2DM induces cognitive benefits.

The discrepancy between GNG results and Stroop results in the current study suggests that, even among response inhibition tasks, there are differences in effect of exercise, a finding which is consistent with prior research (68). Specifically, while a number of studies have shown improvement in Stroop performance (40,41,46,69–71), results for GNG have been inconsistent (72,73). This finding suggests that Stroop, but not GNG performance, is significantly improved following acute aerobic exercise; however, future research is needed to confirm these findings.

The current study provides a preliminary look at the effects of acute aerobic exercise on EF in a population of adults with T2DM. The results of this study are strengthened by strict inclusion criteria to control for extraneous influences on EF and by the within-subject design, which limits the influence of between subject variability. However, while individuals currently taking insulin or with a tendency for hypoglycaemic events were excluded from this study, a limitation of the study is a failure to measure blood glucose following exercise to ensure that hypoglycaemia had not been induced. Furthermore, although self-regulatory fatigue induced by attentional focus during minimal exercise pedalling (in the minimal exercise condition) provides a plausible explanation of our findings, the study was not specifically designed to induce or measure selfregulatory fatigue as a mediating process. Future studies would be required in order to test this possible mechanism.

List of acronyms

T2DM type 2 diabetes mellitus EF executive function GNG Go/No-Go MHRR maximal heart rate reserve RHR resting heart rate MHR maximum heart rate

- THR target heart rate
- RPE rated perceived exertion

Conclusion

The findings revealed no overall benefit of moderate intensity aerobic exercise on EF among obese and overweight adults living with T2DM. Moderational analysis did reveal a possible selective benefit to those participants who were female and initially more active; such benefits of exercise took the form of mitigation of decreased EF following minimal intensity exercise, rather than a hypothesized EF-enhancement effect. Further examination of factors associated with effect size estimates for acute exercise on cognitive outcomes in T2DM is warranted. Future studies should also examine the potential benefits of extended exercise training programmes on cognitive outcomes in this group, as few such studies have been conducted at present.

Ethical approval

All procedures performed in studies involving human participants 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.

Conflict of Interest Statement

The authors declare no conflict of interest.

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