Title: High-intensity interval walking in combination with acute green tea extract supplementation reduces postprandial blood glucose concentrations in physically inactive participants.

Authors: Joseph M. Bulmer [1], Thomas R. McBain [2], Daniel J. Peart [1]*

1. Department of Sport, Exercise and Rehabilitation, Northumbria University, Newcastle-upon-Tyne, UK
2. Academy of Sport and Physical Activity, Sheffield Hallam University, Sheffield, UK

*Correspondence:

Dr Daniel J. Peart, Department of Sport, Exercise and Rehabilitation, Northumbria University, Newcastle-upon-Tyne, UK, Email: Daniel.peart@northumbria.ac.uk, Tel: +44 (0)191 227 3712

Abstract

Background: Exercise and green tea supplementation have been shown to have the potential to improve postprandial blood glucose concentrations, but past interventions have not often investigated attainable and time effective exercise protocols.

Aim: The purpose of this study was to investigate the effects of interval walking exercise and acute green tea extract supplementation on the glycaemic response to an oral glucose tolerance test (OGTT).

Methods: Twelve physically inactive participants (9 male, 3 female, age: 22 ± 1 y; body mass: 81.2 ± 16.3 kg; stature: 175.7 ± 9.6 cm; body mass index (BMI; in kg/m^2): 26.2 ± 4.3) underwent a 2-hour OGTT immediately following i) no intervention (REST), ii) placebo and exercise (EX-PLAC), ii) green tea extract supplementation and exercise (EX-GTE), in a random order. The walking exercise consisted of 6 x 1-min of brisk walking (7.92 ± 0.56 km/h) separated by 1-min of slower walking (4.8 km/h). Differences between groups were identified using magnitude based inferences.

Results: The EX-GTE intervention resulted in a ~9% most likely beneficial effect on blood glucose area under the curve response to the OGTT (702.18 ± 76.90 $mmol/L^{-1}.120\ min^{-1}$) compared to REST (775.30 ± 86.76 $mmol/L^{-1}.120\ min^{-1}$), and a very likely beneficial effect compared to the EX-PLAC (772.04 ± 81.53 $mmol/L^{-1}.120\ min^{-1}$).

Conclusion: These data suggest that an EX-GTE intervention can reduce postprandial glucose concentrations in physically inactive individuals.

Key words: Interval training, nutrition, blood glucose, supplement, green tea, walking

Introduction

Glycaemic control is vital in the management and prevention of insulin resistant related diseases such as metabolic syndrome and type 2 diabetes mellitus (T2DM) (American Diabetes Association, 2015). Control of postprandial hyperglycaemia is essential for achieving long-term glycaemic control, defined using recommended HbA_{1c} goals. Peak glucose concentrations typically occur ~60-90 min postprandially and, in individuals with insulin resistance, are sustained for several hours (American Diabetes Association, 2015). Glycaemic excursions, such as those following meals, correlate with HbA_{1c} levels and have a detrimental effect, inducing oxidative stress and inflammation (Brownlee, 2005). Furthermore, HbA_{1c} levels are directly associated with increased cardiovascular disease (CVD) risk and all cause-mortality (Brownlee, 2005); with CVD accounting for more than 65% of all diabetic deaths (Lloyd-Jones et al., 2009). T2DM prevalence continues to increase among the adult population and presents a major public health challenge (Zghebi et al., 2017).

Obesity and a sedentary lifestyle are modifiable risk factors for the development of T2DM. Lifestyle interventions (exercise and diet modification) are therefore obvious cost-effective methods to prevent the development of T2DM and obesity. Both resistance and endurance-based exercise increase whole-body glucose uptake (Koopman et al., 2005; Larsen et al., 1997). However, a major barrier to exercise participation and adherence is reported ‘lack of time’, regardless of sex, age, socioeconomic status, and fitness level (Trost et al., 2002). Low-volume high-intensity interval training (HIT) has been shown to be a time-efficient stimulus to improve blood glucose in healthy and insulin resistant individuals, via a number

of different modalities (Adams, 2013). Little et al. (2011) conclude that HIT training increases muscle mitochondrial capacity and GLUT-4 protein content, rapidly improving glucose control (10 x 60-s cycling bouts). Additionally, regular HIT training (two weeks cycling intervention) may reduce obesity risk, by increasing energy expenditure and fat oxidation, enhancing weight loss, aiding in the prevention of T2DM (Whyte et al., 2010). Lower intensity interval training, such as interval-walking has also been found to be a feasible training method in T2DM participants. Karsoft et al. (2013) report high adherence rates (89 ± 4%) and significant improvements in $\dot{V}O_{2max}$ (16.1 ± 3.7%) and glycaemic control. Moreover, Francois et al. (2014) found that even brief bouts of incline walking (6 x 1 min bouts at ~90% HR_{max}) prior to meals significantly improved glycaemic control in individuals with insulin resistance.

Pragmatic lifestyle interventions combining physical activity and diet modifications are effective at promoting weight loss, and improve glycaemic control, potentially reducing the risk of developing T2DM and cardiovascular disease (Hordern et al., 2012). However, there is a need for more research to establish optimal strategies that are both cost-effective and attainable. Interestingly, after investigating diabetic patients' perceptions of illness and treatments, Broadbent et al. (2011) report that 86% of patients adhered to medication, whereas, just 22% report to adhere to nutritional advice. Suggesting that nutritional supplementation may be an effective alternative to diet manipulation. Recent research has found that green tea catechin (GTC) supplementation in humans may improve risk factors related to metabolic syndrome, including increased insulin sensitivity and reduced cholesterol and adiposity (Bogdanski et al., 2012; Suliburska et al., 2012). An accessible concentrated form of the catechins that are linked to lower disease risk (Kao et al., 2006) can be found in green tea extract (GTE). Specifically, the most biologically active molecule in GTE,

epigallocatechin gallate (EGCG), is of a high concentration, accounting for ~50-80% of the total catechin content (Khan and Mukhtar, 2007). Importantly, a recent meta-analysis concluded that GTC ingestion lowers fasting blood glucose (-1.48 mg/dL; 95% CI: -2.57, -0.40 mg/dL) in human adults (n = 1584) (Zheng et al., 2013), and Venables et al. (2008) have reported that just 24-hrs of green tea extract (GTE) supplementation improves glycaemic control (-15 ± 4% serum insulin AUC) after an oral glucose load in healthy men (n =11) at rest.

There is limited research on the use of GTE in combination with exercise. A single study has reported that GTE supplementation attenuates the glucose and insulin responses to an oral glucose load 1 hr after a graded exercise test but not at rest (Martin et al., 2016). The exercise employed by Martin et al. (2016) was also appropriate to control workload between conditions and analyse substrate oxidation. However the translation of results from such an exercise may be limited, as individuals are unlikely to complete a graded exercise test within their regular physical activity for practical and comfort reasons. Further work is needed to build upon this proof of principle research of Martin et al. (2016), and examine if the results from laboratory tests hold true for more attainable and time efficient physical activity such as low-volume interval-walking. The aim of this study was to examine the effect of interval-walking exercise, and any additive effects of GTE, on glycaemic control.

Materials and methods

Participants

Twelve participants (9 male, 3 female, age: 22 ± 1 y; body mass: 81.2 ± 16.3 kg; stature: 175.7 ± 9.6 cm; body mass index (BMI; in kg/m^2): 26.2 ± 4.3) were recruited for the study. All participants were considered to be physically inactive after completing a Global Physical Activity Questionnaire (GPAQ); defined by not meeting national guidelines to achieve a healthy lifestyle – *150 minutes of moderate-intensity exercise per week or 75 minutes of vigorous-intensity exercise per week*. All participants gave written informed consent to participate in the study, and the study and its protocol received full ethical approval from the Faculty of Health and Life Sciences Research Ethics Committee at Northumbria University. The study contained no drop out of participants.

Preliminary testing

Basic anthropometric measures were taken, as well as safety measures, including, fasting blood glucose (4.41 ± 0.17 mmol/L) and systolic blood pressure (SBP; 124.7 ± 14.3 mmHg) (Omron M6 AC Blood Pressure Monitor, Omron, United Kingdom). Fasting blood glucose was collected following an overnight fast (> 8 hr) using finger capillary blood sampling, followed by blood analysis (Biosen 5030 lactate analyser, Cardiff UK). No participants presented a blood glucose over 7 mmol/L and/or a systolic blood pressure over 160 mmHg. Following recording of preliminary measures, participants completed a graded exercise test on an incline treadmill (Woodway, Waukeska WI). Participants started at 5 km/h and 4% incline, and gradually increased treadmill speed (1 $km/h/min^{-1}$) and treadmill incline (1

%/min^{-1}) in order to achieve a target RPE of 16 (Borg's Perceived Rate of Exertion). Participants wore a Polar Electro heart rate monitor (Polar, Finland) throughout preliminary and intervention exercise testing periods to quantitatively monitor work rate alongside RPE. Average HR was measured as 170 ± 6 bpm after participants achieved an RPE score of 16.

Study design

A within-groups, double blind, crossover design was used to compare the effects of green tea extract to a placebo, and to a resting condition. A familiarisation visit took place prior to participant completion of three randomly ordered experimental trials. The experimental trials included (1) resting conditions (REST), (2) acute exercise with GTE (EX-GTE), and (3) acute exercise with a placebo (EX-PLAC). All trials were conducted in the morning following an overnight fast (10-12 hrs). At least 3 days separated each trial day (5.7 ± 1.7 days), acting as a washout period.

Supplementation

Participants were provided with capsules prior to each exercise trial of either decaffeinated GTE powder (EGCg Green Tea Extract, Now Foods, Bloomingdale IL) or a plain-flour placebo to colour match the capsules, and then the opposing capsules the following exercise test day.

Due to the pharmacokinetic evidence that the bioavailability of ingested catechins is greater in a fasted state (Chow et al., 2005), and considering a half-life of ~4 hr (Lee et al., 2002),

participants were asked to ingest each capsule with 500 ml of water ~1 hr before the provided dextrose solution, and also ~1 hr before breakfast, lunch and dinner the day prior to each trial day. Therefore, participants ingested a total of 4 GTE capsules, and 4 PLA capsule each. Each 400 mg GTE capsule (98% total polyphenols, 80% catechins, 50% EGCG) contained 320 mg of catechins per capsule.

Study controls

Participants were asked to maintain a habitual diet, and to not consume alcohol or excessive amounts of caffeine the day before each trial. A 24-hr food diary was completed by each participants on these days to monitor intake. Participants were also asked not to perform any exercise the day prior to each trial.

Experimental protocol

The resting trial consisted of a 5-minute sitting rest period followed by a 2-hr oral glucose tolerance test (OGTT). OGTT protocol involved a baseline capillary blood sample (minute 0) followed by the ingestion of a 250 ml 75g oral glucose beverage (Dextrose powder, MyProtein Ltd., Cheshire UK) in a fasted state (10-12 hr overnight fast), then capillary blood sampling for 2 hrs following ingestion (at minutes 15, 30, 45, 60, 90, 120).

After preliminary testing, each participant's treadmill speed and incline was noted, after achieving an RPE score of 16 (speed: 7.92 ± 0.56 km/h; incline 6.88 ± 1.17 %). The trial

exercise protocol consisted of 6 x 1-min long bouts at a speed that elicited an RPE of 16, interspersed with 'slow' walking (4.8 km/h (3 mph)) for 1-min (total exercise time = 12 mins). This exercise protocol was modified from the work of Francois et al. (2014), who found 'exercise snacking' to be a time-efficient and effective approach to improve glycaemic control. RPE was used as a simple and inexpensive alternative to HR_{max} as it is easier to measure in a real-world setting. This study aimed for participants to achieve an RPE score of 16 (hard - very hard) to mimic the research of Francois et al. (2014) which targeted a measure of 90% HR_{max}. A typical RPE response in the Francois et al. (2014) study resulted in the mean RPE of 16 in high-intensity bouts 4-6, this is in accordance with the work of Francois and Little (2015) which suggests its take ~3-4 intervals to accurately determine intensity. Following the exercise bout, a baseline blood glucose sample was taken prior to the administration of the oral glucose load and 2-hr OGTT.

Statistical analysis

A sample size calculation was conducted using a custom made spreadsheet (Will Hopkins; www.sportsci.org), based on glucose AUC reproducibility data from previous work (Gordon et al., 2011), who found increases greater than 63.5 $mmol/L^{-1}.120\ min^{-1}$ and decreases greater than 80.9 $mmol/L^{-1}.120\ min^{-1}$ to exceed daily variation. A between subject standard deviation of 100 $mmol/L^{-1}.120\ min^{-1}$ was taken from Venables et al. (2008), and a within subject standard deviation of 98 $mmol/L^{-1}.120\ min^{-1}$ was calculated by taking 13% (upper 95% CI of normal daily variation; Gordon et al., 2011) of the average glucose AUC reported by Venables et al. (2008). These values resulted in a sample size of ten being required to achieve 90% power.

Glucose area under the curve (AUC) was calculated using the incremental method. All data were log-transformed prior to analysis. The descriptive summary for all variables comprised of the geometric mean and dispersion shown as standard deviation (SD) (Hopkins et al., 2009). An analysis of variance (ANOVA) model was used on peak and AUC glucose data. Following this, a magnitude-based inferences approach (Hopkins et al., 2009), was used to analyse the mean effect of the intervention (EX-GTE), versus placebo (EX-PLAC) and rest (REST). Inferences were based on the disposition of the 90% confidence limits (CL) for the mean difference to the minimal clinically important difference (MCID). Log-transformed data were back transformed to provide percent differences between conditions. The probability (percent chances) that differences in glucose AUC between EX-GTE, EX-PLAC and REST were beneficial (>MCID), harmful (>MCID with opposite sign), or trivial (within ± MCID) was calculated (Hopkins et al., 2009). Robust clinical data for the MCID on all variables is scarce, therefore, MCID was determined using a standardised mean difference of 0.2 times between subjects' standard deviations (Cohen, 1988). Subsequently, the percent chances were defined via probabilistic terms assigned using the following scale; <0.5%, most unlikely or almost certainly not; 0.5 to 5%, very unlikely; 5 to 25%, unlikely or probably not; 25 to 75%, possibly; 75 to 95%, likely or probably; 95 to 99.5%, very likely; >99.5%, most likely or almost certainly (Batterham and Hopkins, 2006). Inferences were categorised as clinical, with the default probabilities for declaring an effect clinically beneficial being <0.5% (most unlikely) for harm and >25% (possibly) for benefit (Hopkins et al., 2009). Additionally, in the case of an effect being possibly beneficial (>25%) an unacceptable risk of harm (>0.5%) and with an odds ratio for benefit: harm of <66, would be classified as unclear.

Results

The heart rate (170 ± 13 vs. 166 ± 13 beats.min^{-1}) and RPE (13 ±2 vs. 14 ± 2) were comparable between exercise trials. Comparison between conditions for glucose AUC and peak glucose can be seen in Table 1. When compared to the REST condition (775.30 ± 86.76 mmol/L^{-1}.120 min^{-1}), there was a most likely beneficial effect of EX-GTE (702.18 ± 76.90 mmol/L^{-1}.120 min^{-1}) on glucose AUC and a very likely beneficial effect compared to EX-PLAC (772.04 ± 81.53 mmol/L^{-1}.120 min^{-1}). The effect was unclear between EX-PLAC and REST. The average response to the OGTT at all time points is presented in Fig 1. There was a very likely beneficial effect of EX-GTE (7.51 ± 0.91 mmom/L) when compared to REST on peak glucose (8.30 ± 0.92 mmol/L). The effect was unclear on all other outcomes.

[Insert Figure 1.]

Discussion

This study aimed to investigate the effect of high-intensity walking exercise on glycaemic control, and any additive effect of an acute GTE supplementation strategy. The main finding was that the walking exercise alone did not influence the glycaemic response during a 2-h OGTT, but the combined walking exercise with GTE had a 'most likely', and 'very likely' beneficial effect on glucose AUC and peak glucose respectively.

Previous research has suggested that high-intensity interval walking may be effective at reducing mean postprandial blood glucose concentrations (Francois et al., 2014; Jakobsen et al., 2016). Francois et al. (2014) reported that 6 x 1 min bouts of inclined interval walking (90% HR_{max}) interspersed with periods of slow walking significantly reduced mean 3 hr postprandial glucose before breakfast (-1.4 ± 1.5 mmol/L, p = 0.02) when compared to traditional continuous exercise (30 min moderate-intensity; 60% HR_{max}), a 17% reduction in 3 hr post-breakfast AUC (interval walking: 1,090 ± 178 mmol/l vs. continuous exercise: 1,307 ± 337, p = 0.04) . The present study aimed to emulate the exercise protocol of Francois et al. (2014) whilst using RPE to measure effort, as opposed to HR_{max}, to give a reliable (Ciolac et al., 2015) but simple and inexpensive method that could be replicated more easily in the real world, to simplify the translation of our findings to practice. However, this study did not find a worthwhile effect between postprandial glucose concentrations of exercise alone with placebo and the resting condition. More specifically, we aimed for participants to achieve an RPE score of 16 throughout exercise testing, in an attempt to replicate the 90% HR max targeted by Francois et al. (2014). However, average trial RPE failed to give the desired effect (RPE = 16) with an average RPE score of 13.4 ± 1.7 (HR: 170 ± 13.1 bpm) and 13.7 ± 1.6 (HR: 165.8 ± 13 bpm) during the EX-PLAC and EX-GTE trials, respectively. Moreover, the average HR during the high-intensity intervals was 170 ± 6, ~85% of HR_{max}, lower than the desired 90% of HR_{max} (~178 bpm, $p < 0.01$). This suggests that the study duration, and/or intensity may not have been high enough to induce the desired physiological changes. Similarly, Jakobsen et al. (2016) suggest that altering the intervention to 3 min long bouts of high-intensity walking may improve glycaemic control, specifically, by reducing postprandial glucose concentrations. The study found no difference between mean glucose after 1 min walking cycles compared to control, whereas 3 min bouts attenuated glucose response following a 4 hr liquid mixed meal tolerance test. The inclusion of a step test in

place of a ramp test would be recommended in future, with sufficient breaks between steps to reduce the effect of cumulative fatigue during the graded exercise test and increase the walking speed at an RPE of 16. Whilst the current study found no benefit to high-intensity interval walking alone for glycaemic control, these results are contrary to the limited previous research.

The addition of GTE to the walking intervention did reduce postprandial glucose concentrations, and the ~9% reduction in glucose AUC in the EX-GTE trial can be interpreted as being 'most likely beneficial' compared to REST and 'very likely beneficial' compared to exercise alone (Table 1). The effect of this intervention was greater than the typical 6% daily variation of OGTT results identified by Gordon et al. (2011). This would suggest that the study intervention may improve insulin sensitivity of the skeletal muscle, agreeing with the work of Martin et al. (2016) who suggest that GTE may alter skeletal muscle glucose uptake in humans. Possibly due to the increased translocation of glucose transporters which is apparent in rodent studies, specifically, green tea has shown to have a similar effect to exercise, in that, prolonged consumption increases GLUT-4 translocation in normal and insulin resistant skeletal muscle, in addition to increased adipocyte insulin-receptor binding (Wu et al., 2004).

A limitation of the present study is the absence of a GTE group without the exercise intervention to give further context to the combined effect of GTE and exercise, however, previous research has indicated that GTE alone may not sufficiently reduce postprandial glucose concentrations. As mentioned, Martin et al. (2016) state that GTE attenuated glucose response to an oral glucose load following acute exercise, however, the study found no effect

under resting conditions (Glucose AUC: GTE = 394 ± 70, PLA = 409 ± 78 mmol/L^{-1}·60 min^{-1}, $p = 0.51$). Venables et al. (2008) also found that GTE significantly lowered insulin AUC (-15 ± 4%, $p < 0.01$) and increased insulin sensitivity (insulin sensitivity index (ISI): 13 ± 4%, $p < 0.05$), albeit with no difference in glucose concentrations ($p > 0.05$). Furthermore, a combined intervention should be recommended where possible due to the further reaching benefits of physical activity. Importantly, this study presents evidence that a combined walking and GTE intervention can improve glycaemic control. This offers insight in to a potentially more real world applicable and achievable exercise in physically inactive people than has been researched in the past, as previous studies have for example used higher intensity cycling protocols (Little et al., 2011; Whyte et al., 2010). It should also be considered when interpreting our results that although the participants were physically inactive, their glycaemic control was good under all testing conditions, and fasted blood glucose was 4.41 ± 0.17 mmol/L. The results may be different in populations with poorer glycaemic control, and further research is warranted in this area.

In conclusion low-volume interval-walking exercise combined with GTE supplementation was found to reduce postprandial glucose concentrations in physically inactive individuals. A combined walking and green tea routine may be an achievable and translatable intervention for physically inactive people.

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Authors' contributions

XXX and XXX conceived the study. XXX recruited participants and collected the data. XXX performed the statistical analysis. XXX, XXX and XXX contributed to drafts of the manuscript, and all authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of conflicting interests

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Figure caption

Fig 1. Average post-prandial blood glucose (mmol/L) response at each time point of the OGTT for the REST, EX-PLAC and EX-GTE conditions. Error bars for EX-PLAC have been removed for clarity.

454 **Table 1 Clinical inferences of differences in glycaemic control between treatment groups**

Variable	Comparison	Difference between groups (% mean; 90%CL)	Likelihood (%) of intervention being beneficial / trivial / harmful	Clinical inference
Glucose AUC ($mmol.min^{-1}$)	EX-GTE to REST	-9.4 ±4.9	99.8 / 0.1 / 0.1	Most likely beneficial
	EX-GTE to EX-PLAC	-9.1 ±7.1	98.0 / 0.0 / 2.0	Very likely beneficial
	EX-PLAC to REST	-0.37 ±50	50.5 / 0.0 / 2.0	Unclear
Peak glucose (mmol/l)	EX-GTE to REST	-9.8 ±7.5	98.2 / 0.0 / 1.7	Very likely beneficial
	EX-GTE to EX-PLAC	-7.23 ±12	84.1 / 0.2 / 15.2	Unclear
	EX-PLAC to REST	-2.79 ±50	53.7 / 0.1 / 46.2	Unclear

AUC = area under the curve; REST = resting condition; EX-PLAC = exercise intervention with placebo; EX-GTE exercise intervention with green tea extract supplementation

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