The effect of exercise timing on glycaemic control in individuals with Type 2 Diabetes Mellitus

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Author’s Declaration

I declare that:

a) The thesis is my own account of my research, except where other sources are acknowledged.

b) The extent to which the work of others has been used is clearly stated in each chapter and certified by my supervisors.

c) The thesis contains as its main content, work that has not been previously submitted for a degree at any other university.

Shaun Teo

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Statement of Contribution of Others

This thesis has been developed in the format of Thesis by Publication. Chapters Two, Three, Four and Five within this thesis have been published or are currently in review or draft for submission to scientific journals. These chapters represent collaborative works; however, the PhD candidate for which this thesis represents has completed the majority of the study design, data collection, data analyses and interpretation, and drafting of the manuscript.

Percentage Contribution

Chapter Two

Title: Exercise timing in Type 2 Diabetes Mellitus: A systematic review

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Jeffrey Hebert	5%	0%	0%	5%	5%	5%
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Chapter Three

Title: The impact of diurnal exercise timing on the management of Type 2 Diabetes Mellitus: A systematic review

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Summer Cook	5%	20%	0%	5%	5%	5%
Jeffrey Hebert	5%	0%	0%	5%	5%	5%
Mitchell Forrest	0%	20%	50%	5%	5%	5%
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Chapter Four

Title: The impact of exercise timing on glycaemic control: A randomised controlled trial

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Timothy Fairchild	10%	0%	15%	10%	10%

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Chapter Five

Title: Effect of diurnal exercise timing on postprandial glucose responses: A randomised controlled trial

Name	Design	Data Collection	Data Analyses	Interpretation	Manuscript Development
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Kym Guelfi	10%	0%	0%	10%	10%
Kieran Marston	0%	20%	15%	5%	5%
Timothy Fairchild	10%	0%	15%	10%	10%

Confirmation of contribution

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Thesis Abstract

Background: Tight glycaemic control is a key target for the management of Type 2 diabetes mellitus (T2DM). Exercise is regarded as an important adjunct treatment and a principal lifestyle consideration for T2DM management. However, the association between the timing of exercise performance and both the timing of meal consumption and diurnal time has only been recently considered having an important role in maintaining glycaemic control. The overarching aim of this thesis therefore was to investigate the importance of exercise time on glycaemic control in individuals with T2DM and at risk of T2DM.

Specific Aims: Within this thesis, two systematic reviews were completed to understand the effect of exercise timing on both acute and chronic glycaemic control measures. The aim of Systematic Review One (Chapter 2) was to review the literature related to exercise timing, relative to meal consumption, and glycaemic control in individuals with T2DM. While the aim of Systematic Review Two (Chapter 3) was to assess the literature related to the diurnal timing of exercise performance and glycaemic control in T2DM individuals. In addition, a training study was conducted to examine the chronic effect of the diurnal timing of exercise performance (morning vs. evening) on glycaemic control measures. The study sought to determine the impact of diurnal exercise timing on i) glycaemic control and insulin sensitivity measures (Study Part One: Chapter 4) and; ii) postprandial glucose (PPG) and insulin (PPI) responses (Study Part Two: Chapter 5) in overweight non-T2DM/T2DM individuals enrolled into a 12-week supervised multi-modal exercise training program.

Methods: Systematic searches of online databases were performed to identify articles published in English from inception to October 2017 for both systematic reviews. Thereafter,

two authors independently extracted data and evaluated the quality of studies using the Cochrane Collaboration Data Collection Form and Cochrane Collaboration Risk of Bias Assessment Tool respectively. A qualitative synthesis was performed on the included studies with the results summarized in tables for both systematic reviews (Chapter 2 and 3). In addition, a training study was conducted to examine the chronic effect of the diurnal timing of exercise performance (morning vs. evening) on glycaemic control measures. In the training study (Chapter 4 and 5), individuals completed a 12-week supervised multi-modal exercise training program (3 days per week; each session: 30 minutes walking protocol and 4 resistance-based exercises for 3 sets of 12-18 repetitions).

Results: In Systematic Review One (Chapter 2), a total of 19 (346 participants) randomized controlled trials (RCTS) that employed either an acute crossover (n = 17) or longitudinal parallel-groups (n = 2) design were included in the qualitative synthesis. The main findings of this review were that postprandial exercise performed between 30 to 60 minutes after meal consumption appears to more consistently improve glycemia (glucose concentrations and glucose-AUC) and insulin-AUC when compared to an acute exercise bout performed prior to a meal. In Systematic Review Two (Chapter 3), 18 studies (321 participants: 17 crossover and 2 parallel-groups) were included for qualitative analysis. Comparisons of the studies indicate greater improvements in glucose concentrations when exercise was performed in the morning, however, similar improvements in glucose-AUC were seen for both morning and evening groups. In addition to the large heterogeneity in study design of the included studies, the observations from both systematic reviews were largely based on indirect comparisons between the studies given the limited number of studies that directly investigated the timing of exercise (meal consumption: 3 trials; diurnal timing: nil). Thus, it remains inconclusive to whether i) postprandial exercise is more effective in improving glycaemic control when compared to pre-

prandial exercise and ii) morning or evening exercise is more effective for glycaemic control management in T2DM individuals. However, there were no group differences for any variables (all $p \geq 0.4$). With regards to the impact of manipulating the diurnal timing of exercise performance on long term glycaemic control (Study Part One: Chapter 4), 12-weeks of multi-modal exercise training significantly reduced (main effect of time: all $p < 0.01$) glycosylated haemoglobin (amEX vs pmEX: -0.27 vs. -0.25%), fasting glucose (amEX vs pmEX: -0.9 vs. -1.18 mmol/L), fasting insulin (amEX vs pmEX: -23.8 vs. -22.35 pmol/L), HOMA2-IR (both groups: -0.5) and fructosamine (amEX vs. pmEX: -34.5 vs. -29.6 ± 51.2 μmol/L). However, these reductions were not different between the intervention groups for any variables (all $p \geq 0.4$). Thus, irrespective of the diurnal timing of exercise training, 12-weeks of multi-modal exercise training significant improved glycaemic control in both overweight non-T2DM and T2DM individuals. However, the diurnal (morning versus evening) timing of exercise training did not result in additional benefits to glycaemic outcomes. Given that tight glycaemic control necessitates the management of both fasting glucose and PPG concentrations, with accumulating evidence suggesting that PPG excursions better predict death from all causes and cardiovascular disease (CVD) compared to fasting glucose alone, Study Part Two (Chapter 5) showed similar results to that of Study Part One with regards to PPG and PPI responses. 12-weeks of multi-modal exercise training significantly reduced (main effect of time, $p < 0.01$) PPG and PPI concentrations during the mixed meal tolerance test (MMTT), with no group differences observed ($p = 0.69$). However, a significantly greater reduction in PPG-iAUC was observed for the pmEX group (-78.56 mmol/L) when compared to the amEX group (-33.22 mmol/L) at post-intervention ($p = 0.03$). Although a trend may exist to indicate that evening exercise performance may have a greater impact on PPG responses, results from Study Part Two indicate similar findings to that of Study Part One, in that the performance of a multi-

modal training program is an effective method to improve PPG and PPI responses. However, the diurnal timing of exercise performance may not provide any additional benefits.

Conclusions: The findings presented in this thesis provide evidence for effect of strategically manipulating the diurnal timing of exercise performance on glycaemic control for both overweight non-T2DM and T2DM individuals. Despite the diurnal manipulation of exercise timing not indicating any additional benefits for glycaemic control management, this thesis may have provided plausible explanations effect of diurnal timing of exercise on components of glucose variability observed by a T2DM individual on a daily basis. Specifically, morning exercise may potentially lower glucose excursions experienced during the extended dawn phenomenon while evening exercise may potentially allow for an overall reduction in glucose excursions throughout the day by lowering the early morning 'spike' in glucose resulting from the dawn phenomenon. This results provides a clinically meaningful explanation for strategically manipulating the timing of exercise performance for glycaemic control management in T2DM individuals, given that, failing to address both the dawn and extended dawn phenomenon has been proposed to potentially contribute to inadequate glycaemic control and increase the progression of diabetes related complications. More importantly, this thesis the performance of regular exercise based on current exercise guidelines is an effective management tool for i) improving glycaemic control and insulin sensitivity; ii) reducing body anthropometric measures and; iii) improving cardiovascular health. This indicates that the consistent performance of exercise is a vital component for both the prevention and management of T2DM.

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Teo SYM, Kanaley JA, Guelfi KJ, Cook S, Hebert JJ, Mitchell F and Fairchild TJ (2018). The impact of diurnal exercise timing on the management of Type 2 Diabetes Mellitus: A systematic review. *Submitted to Sports Medicine*

Teo SYM, Kanaley JA, Guelfi KJ, Marston KJ and Fairchild TJ (2018). The impact of exercise timing on glycaemic control: A randomised controlled trial. *Submitted to Medicine and science in sports and exercise*.

Teo SYM, Kanaley JA, Guelfi KJ and Fairchild TJ (2018). Effect of diurnal exercise timing on postprandial glucose responses: A randomised controlled trial. *Submitted to Medicine and science in sports and exercise*.

List of Abbreviations

ADA	American Diabetes Association
AER	Aerobic exercise
amEX	Morning exercise training group
AUC	Area under the curve
BF	Body fat
BMI	Body mass index
CI	Confidence interval
CMA	Comprehensive Meta-Analysis
CRF	Cardiorespiratory fitness
CVD	Cardiovascular fitness
DM	Diabetes mellitus
DXA	Dual energy X-ray absorptiometry
ES	Effect size
FFM	Fat free mass
FG	Fasting glucose
FI	Fasting insulin
FITT	Frequency, intensity, type and time
FRA	Fructosamine
GIP	Glucose dependent insulintropic peptide
GLP-1	Glucagon-like-peptide 1
HbA1c	Glycosylated haemoglobin
HOMA2-IR	Homeostasis model of insulin resistance
iAUC	Incremental area under the curve

IGT	Impaired glucose tolerance
LMM	Linear mixed models
MeSH	Medial Subject Headings
MET	Metabolic equivalents
MMTT	Mixed meal tolerance test
OW	Overweight
PA	Physical activity
pmEX	Evening exercise training group
PPG	Postprandial glucose
PPG	Postprandial glucose
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized controlled trial
RCF	Relative centrifugal force
RE	Resistance exercise
RM	Repetition maximum
SCN	Suprachiasmatic nucleus
SGLT-1	Sodium dependent glucose cotransporter 1
SMD	Standardized mean difference
T2DM	Type 2 diabetes mellitus
UKPDS	United Kingdom Prospective Diabetes Study
VAT	Visceral adipose tissue
VO_{2peak}	Peak oxygen consumption

Chapter 1 Introduction

1.1 Background

Diabetes mellitus (DM) has a global prevalence of approximately 9% among adults (1), accounts for approximately 12% of global health expenditure (1) and is the 6th leading cause of global mortality (2). Consequently, DM is a recognised 21st century epidemic (3). Nearly 90-95% of all DM cases are classified as type 2 diabetes mellitus (T2DM), a condition characterized by a relative insulin deficiency which is usually accompanied by insulin resistance (4), although the level of resistance is highly heterogeneous (5). Individuals with T2DM and who demonstrate poor glycaemic control are particularly susceptible to T2DM morbidities and mortality (3, 6, 7). Thus, improving glycaemic control, measured via a reduction in glycosylated haemoglobin (HbA_{1c}), to ≤7% (which may vary according to individual patient profiles) is the standard treatment goal for individuals with T2DM (7-9).

Increased levels of exercise have long been considered an important adjunct in treatment of T2DM and an effective method to improve long-term glycaemic control (10, 11). Yanai et al. (12) stated that structured exercise training was associated with a reduction in HbA_{1c} levels 0.67% when compared to control participants, with a further reduction of HbA_{1c} levels (-0.9%) associated with structured exercise durations exceeding 150 min/week. These results indicate that exercise i) is an effective method of glycaemic management for individuals with T2DM and; ii) the improvements observed in these exercise training programs are comparable with those observed using metformin monotherapy (-1.1%) (13). Lifestyle interventions (dietary and exercise therapy, coupled with weight-loss targets) have also been shown to be highly effective in the prevention of T2DM in individuals at-risk of T2DM. Indeed, the diabetes prevention program (14) demonstrated a 58% relative risk reduction for T2DM compared with a 31% reduction for those on Metformin (850 mg twice daily). Importantly, when the individual

contributions of diet and exercise were independently assessed in the Da Qing IGT and Diabetes Study (15), the exercise intervention demonstrated a greater relative risk reduction (46%) versus the diet-only intervention (31%). For this reason, exercise is a cornerstone in both the prevention and management of T2DM (16).

Exercise training of at least six weeks duration in individuals with T2DM consistently demonstrates relative risk reductions for development of T2DM and improvements in HbA_{1c} levels in individuals with T2DM (4, 17), however, there are large variances in the magnitude of improvements observed within and between studies (12, 17). Beyond inter-individual differences such as age, sex and duration since T2DM diagnosis of participants, these variations likely arise due to differences within programmatic components of the FITT (frequency, intensity, type and time) principle associated with exercise prescription (18). Umpierre et al. (19) found there was a potential for HbA_{1c} levels to be further reduced by approximately -0.39% for every extra session of exercise performance completed each week, indicating the dose-response effect of exercise performance frequency. Grace et al. (20) concluded in a review of the impact of different aerobic exercise training intensities in T2DM individuals on glycaemic responses that moderate and high intensity exercise resulted in differing levels of HbA_{1c} reductions. Concerning the type of exercise modalities used for T2DM interventions, aerobic and resistance exercises have traditionally been isolated within the training programs. However, Church et al. (21) reported the combination of both aerobic and resistance training resulted in significantly greater reductions in HbA_{1c} levels (-0.45%) as compared to aerobic (-0.25%) or resistance (-0.17%) training alone after 9 months. The time component of exercise prescription refers to the duration of exercise, with Yanai et al. (12) showing structured exercise durations of >150 mins/week resulted in significantly greater reductions in HbA_{1c} as compared to durations of ≤150 mins/week (-0.80% vs. -0.36%, respectively). Currently it is

acknowledged that multi-modal exercise (resistance and aerobic exercise training), of at least moderate intensity and >150 mins/week will significantly improve HbA_{1c} levels (4, 17, 20). Based on the varying differences in HbA_{1c} reductions associated with the different components of the FITT principle, strategically manipulating each of these variables allow for the optimal beneficial effects of exercise for the management of glycaemic control in T2DM individuals.

Despite the universal acknowledgement of exercise as an important component for the prevention and management of T2DM, the role of exercise timing in affecting glycaemic control has only been recently considered and is gaining considerable attention. The timing of exercise can be categorized into i) exercise timing in relation to meal consumption (i.e. preprandial and postprandial) and; ii) the diurnal timing of exercise (i.e. morning and evening). Recent evidence (22, 23) along with two reviews (24, 25) have shown that acute exercise performance in the postprandial period improves glycaemic control to a greater degree than exercise performed in the preprandial period. Moreover, comparison of acute exercise interventions performed at different times in the day suggest alterations in the glycemic response (22, 25). However, to the best of our knowledge, no studies have directly explored the impact of diurnal exercise timing on glycaemic control over the longer-term. As such, there is a need to examine the impact of exercise timing on glycaemic control and associated outcomes in individuals at risk of T2DM and individuals with T2DM.

1.2 Purpose of the Thesis

The role of diurnal feeding patterns have long been acknowledged, with the adage "Eat Breakfast Like a King, Lunch Like a Prince, and Dinner Like a Pauper" commonly used in popular culture. More recently, the role of time-restricted feeding has garnered increasing

interest (26-28). However, while clinicians and researchers struggle with how-best to engage individuals in physical activity programs, the role of diurnal exercise timing and potential benefits associated with "temporal optimization" of exercise has largely gone unexplored. Given diurnal variations in peripheral insulin sensitivity are well acknowledged (e.g. (28)), we sought to determine the importance of diurnal exercise timing on glycaemic control in individuals at risk of developing T2DM and glycemic management in individuals with T2DM.

Therefore, the purpose of this thesis was to i) systematically review the literature to determine the impact of exercise timing in relation to meal consumption (Chapter 2) and diurnal exercise timing (Chapter 3) on glycaemic control in individuals with T2DM; ii) determine the effect of diurnal exercise timing on the circadian rhythm and longer-term glycaemic-related outcomes (Chapter 4), as well as the postprandial glucose and insulin responses during an extended mixed-meal tolerance (Chapter 5), after 12-weeks of multi-modal exercise training. The results from the systematic reviews and the longitudinal study are expected to provide evidence in support of a role for diurnal exercise-timing in freely-living individuals at risk of developing T2DM or with T2DM.

Specifically, we hypothesised that i) based on current evidence, postprandial exercise (Chapter 2) and morning exercise (Chapter 3) will result in greater improvements in glycaemic control measures when compared to pre-prandial and evening exercise performance, respectively; ii) 12-weeks of multi-modal training performed in the morning will result in greater improvements in glycaemic control than evening exercise performance; and iii) 12-weeks of supervised evening exercise training will improve PPG and insulin responses to a greater degree than compared to morning exercise training. The development of each of these hypotheses are provided within each chapter.

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Chapter 2 Exercise timing in Type 2 Diabetes Mellitus: A systematic review

Teo SYM, Kanaley JA, Guelfi KJ, Cook S, Hebert JJ, Mitchell F and Fairchild TJ (2018). Exercise timing in Type 2 Diabetes Mellitus: A Systematic Review. *Medicine and science in sports and exercise*.

2.1 Abstract

Purpose: The timing of exercise relative to meal consumption has recently been identified as potentially moderating the effectiveness of exercise on glycemic responses in type 2 diabetes mellitus (T2DM). The aim of this study was to systematically review the literature related to exercise timing, relative to meal consumption, and glycemic control in individuals with T2DM. **Methods:** Systematic searches in PubMed, EMBASE, CINAHL, Cochrane Library and ClinicalTrials.gov Registry databases were performed to identify articles published in English from inception to October 2017. Two authors independently extracted data and evaluated the quality of studies using the Cochrane Collaboration Data Collection Form and Cochrane Collaboration Risk of Bias Assessment Tool respectively. A qualitative synthesis was performed on the included studies, and results summarized in tables. **Results:** 19 randomized controlled trials (RCTs) with a total of 346 participants were included. Improvements in glycemia (glucose concentrations and glucose-AUC) and insulin-AUC appeared more consistent when exercise was performed during the post-meal period as compared to the pre-meal period, however, this observation was largely based on indirect comparisons between studies. **Conclusions:** There is some evidence from RCTs that exercise performed 30 min after meal consumption may convey greater improvements in glycemic control for individuals with T2DM. However, there are only two studies which have directly assessed the role of exercise timing on glycemic management and adopted methodologies are heterogeneous. Future low risk trials in this field are warranted.

Key Words: Glycaemic control, exercise timing, postprandial, systematic review

Systematic review registration**:** PROSPERO CRD42017054666

2.2 Introduction

The maintenance of tight glycemic control is a key objective in the management of individuals with Type 2 Diabetes Mellitus (T2DM) (1). Tight glycemic control necessitates the management of fasting glucose (FG) as well as the postprandial glucose excursions (PPG) (2). Exercise will transiently improve the muscle's insulin sensitivity and insulin responsiveness over the subsequent hours and days (3, 4), resulting in reduced PPG excursions (5-7). This reduction in PPG excursions largely accounts for the consistent improvements in glycosylated hemoglobin (HbA_{1c}; (8)) observed with exercise training (-0.6% to -0.89% versus control; (9-12)). As a result of these clinically meaningful improvements in HbA_{1c}, exercise, and physical activity more broadly, are recognized as important adjuvant treatments in the management of T2DM (13). Together with dietary modifications (14), exercise is a principal lifestyle consideration within clinical management guidelines for T2DM (15).

The association between the timing of exercise and the timing of meals has long been acknowledged on blood lipid profiles (16), but only recently has this association been identified as playing a potentially important role in maintaining glycemic control (17-20). For instance, Heden et al. (20) showed exercise conducted in the postprandial period significantly reduced the glucose area under the curve (AUC) in individuals with T2DM relative to pre-prandial exercise. These improvements in glycaemia through postprandial exercise have been identified in some recent findings (18-20) and the current position statement by the American Diabetes Association (ADA) includes reference to differences in glucose control when exercise is performed in the fasted, pre-prandial or postprandial state (13). However, there is need for this evidence to be systematically reviewed. Therefore, the aim of this systematic review was to assess the effect of exercise timing in relation to meal consumption, on glycemia and glycemic control in individuals with T2DM.

2.3 Methods

Search Strategy

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta – Analyses (PRISMA) Statement (Appendix A) and registered with the International Prospective Register of Systematic Reviews (PROSPERO CRD42017054666) (21).

A systematic search was performed in Medline (PubMed), EMBASE, CINAHL, Cochrane Library and the ClinicalTrials.gov registry utilizing a combination of Medical Subject Headings (MeSH) and key words to identify potentially relevant studies. The search syntax was designed for PubMed (Appendix B) and adapted for use in the remaining databases. All databases were searched from inception to September 2017. In addition, reference lists of all retrieved papers were manually searched to identify any potentially relevant studies not identified by the database search.

We included randomized controlled trials examining the effects of exercise (acute exercise and exercise training) on glycemic control and insulin response in adults diagnosed with T2DM. For inclusion, the exercise protocols of potentially eligible studies had to comprise quantifiable (frequency, intensity, time and type) bouts of physical activity, which were planned, structured and repetitive in movement. All exercise durations ranging from acute single exercise bouts to extended exercise training programs were included. We excluded observational or uncontrolled studies and studies that did not report the timing of exercise performance with reference to meal consumption. In addition, studies that were not fully reported or written in English language were excluded from this systematic review.

Outcome measures

Glycemic control encompasses the three components of the glucose triad: (i) glycosylated hemoglobin, (ii) fasting glucose and, (iii) postprandial glucose (22). While glycosylated hemoglobin is the criterion measure for chronic glycemic control (12, 23, 24), reflecting the previous 8-12 weeks (25), it does not necessarily capture the magnitude of change in diurnal glucose (8, 26, 27). Suh et al. (28) reported the glucose AUC following a glucose challenge as a precise assessment of postprandial glucose (PPG) excursions. Therefore, to ensure a broad assessment of glycemic control, we included the following as primary review outcomes: (i) HbA_{1c}; (ii) FG; (iii) PPG (postprandial glucose concentrations reported after a glucose load); and, (iv) glucose AUC. Considering the importance of insulin and in particular, the association between hyperinsulinemia and increased cardiovascular disease (CVD) mortality rates (29-31), the secondary review outcomes were postprandial insulin concentrations and insulin AUC after a glucose load.

Data extraction and quality analysis

Potential studies were exported to Covidence Systematic Review Software (Veritas Health Innovation, Melbourne, Australia) for assessment. Following removal of duplicate references, two independent authors (ST and SC) completed the title and abstract screening process in a blinded manner against the eligibility criteria. Disagreements were resolved by a third author (TF). Thereafter, a full text assessment was completed in a blinded manner by two independent review authors (ST and MF). Similarly, all disagreements were resolved by a third author (TF). Reasons for excluding each study at the full-text stage were recorded. When insufficient information was available, we contacted corresponding authors to obtain additional information. Upon the completion of the full-text assessment, data extraction for the included studies was completed independently by two authors (ST and MF) utilizing the Cochrane

Collaboration Data Collection Form (32). The following information was extracted from each of the included studies: (i) experimental conditions; (ii) total number of participants; (iii) age; (iv) gender; (v) body mass index; (vi) T2DM duration; (vii) exercise protocols; (viii) exercise timing and; (ix) comparisons in outcome measures of glycemic control and insulin. Variations in data extraction were resolved by consensus (ST and MF). Seventeen authors were contacted for additional information.

The assessment of risk of bias for each study was completed by utilizing the Cochrane Collaboration's tool for assessing risk of bias (33). The tool assesses bias for each study from seven evidence-based domains: (i) random sequence generation; (ii) allocation concealment; (iii) blinding of participants and personnel; (iv) blinding of outcome assessment; (v) incomplete outcome data; (vi) selective reporting and; (vii) other sources of bias. Two independent authors (ST and MF) provided a judgement (low risk of bias/ high risk of bias/ unclear) for each of the aforementioned domains, and all disagreements were resolved by consensus.

Statistical analysis

A qualitative syntheses of trial results were summarized in tables stratified by study design and outcome type (HbA_{1c}, PPG, FG, glucose AUC; fasting and postprandial insulin concentration, insulin AUC). As a consequence of i) the heterogeneity in study methodology; ii) the high proportion of studies reporting on small sample sizes; iii) the low number of studies directly comparing exercise-timing (pre- versus post-meal) on outcome measures; we did not perform the planned random-effects meta-analysis. The direction of the observed association for each of the outcome measures was graphically presented by constructing forest plots (SigmaPlot, version 13.0; Systat Software, San Jose, CA) using standardized mean differences (SMDs, [Cohen's *d*]). The SMDs were calculated for each condition presented within respective studies

using comprehensive meta-analysis (version 3.3.070), and interpreted as small (d = 0.2), moderate (d = 0.5) or large (d = 0.8) (34).

2.4 Results

Search Results

Our search strategy identified 6,407 unique studies, of which, 19 randomized clinical trials (346 participants) were included following assessment of selection criteria (Figure 2-1) (20, 35-52). Included trials were published between 1997 and 2017, and employed either a crossover (n = 17) (20, 35-50) or parallel-groups design (n = 2) (51, 52).

Characteristics of Included Studies

At baseline, participants had a mean age of 55.4 years (95% CI [52.6, 58.1]), mean HbA_{1c} of 7.3 % [7.2, 7.4], mean body mass index (BMI) of 29.8 $kg.m^{-2}$ [28.0, 31.6] and a mean T2DM duration of 7.4 years [6.8, 8.0]. Within the 17 crossover trials, PPG concentration was used as a measure of glycemic control in 13 of the trials, while glucose AUC was used in 10 of the crossover trials (Table 1-1; Figure 2-2A, 2-2B). The percentage of female participants was 9.3%. Of the 19 included trials, 15 trials investigated the effects of aerobic-based exercises (cycle ergometer: n = 9; treadmill walk: n = 6) (35-42, 44-51) and 4 trials investigating resistance exercises (20, 43, 48, 52). With regards to the timing of exercise performance, three trials compared pre-meal exercise to a no-exercise control (38, 39, 44), 13 trials compared post-meal exercise to a no-exercise control (35, 37, 40-43, 45-51), 2 trials compared pre-meal exercise and post-meal exercise to a no-exercise control (20, 36), and one trial compared pre-meal exercise and post-meal exercise (52) for glycemia and glycemic control. A detailed summary of the results for both acute crossover and parallel-group trials are presented in Table 1-1 and 2-2, respectively.

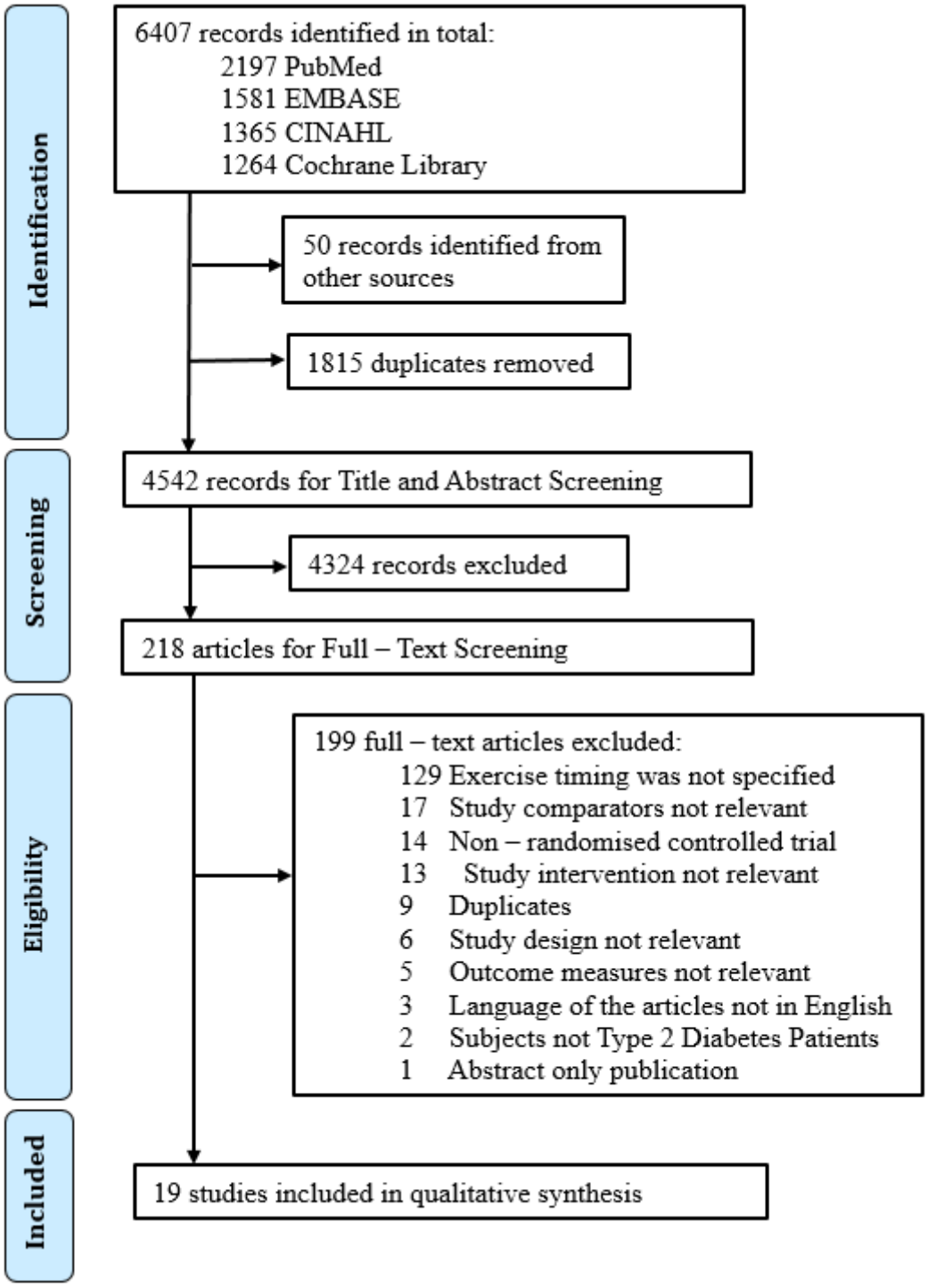


Figure 2-1. Study Selection Process

Table 2-1. Summary of included randomized controlled crossover trials

Author	Participants Characteristics	Standardized Meal	Exercise Characteristics	Study Outcomes (Exercise vs. Control)			
				Glycemic Control		Insulin Response	
				PPG	G_{AUC}	PPI	I_{AUC}
Colberg (2009)[36]	12 subjects (6 females) Age: 61 y BMI: 35 kg.m^{-2} T2DM duration: 11 y	430kcal dinner [t = 30] CHO: 50% Fat: 18% Protein: 32%	Control: no exercise Pre-meal exercise: 20mins of treadmill walking at a self-selected pace (3.5 METs) [t = 0] Post-meal exercise: 20mins of treadmill walking at a self-selected pace (3.5 METs) [t = 60]	←→ ←→	←→ ←→	NA NA	NA NA
Colberg (2014)[35]	12 subjects (9 females) Age: 59 y BMI: 35 kg.m^{-2} T2DM duration: 6 y	300kcal dinner [t = 30]	Control: no exercise Post-meal table tennis: 30mins of table tennis against an iPong robot (4 METs) [t = 60] Post-meal walking: 30mins of treadmill walking at a self-selected pace (4 METs) [t = 60]	↓ ↓	NA NA	NA NA	NA NA
Erickson (2017)[37]	8 subjects (3 females) Age: 60 y BMI: 34 kg.m^{-2} T2DM duration: NR	Breakfast meal [t = 0] CHO: 65% Fat: 25% Protein: 10%	Control: no exercise Post-meal interval walking: Three 10min bouts of interval treadmill walking at 50% VO_{2max} (3.5 METs) [t = 30]	↓	↓	NA	NA
Gill (2007)[38]	10 male subjects Age: 49 y BMI: 31 kg.m^{-2} T2DM duration: 4 y	1028kcal breakfast [t = 0] CHO: 43% Fat: 49% Protein: 1%	Control: no exercise Pre-meal exercise: 90mins of treadmill walking at 70% of HR_{max} (3.5 METs) [between t = -960 and -1080]	↓	←→	↓	↓
Heden (2014)[20]	13 subjects (8 females) Age: 49 y BMI: 37 kg.m^{-2} T2DM duration: 4 y	Dinner meal [t = 0] CHO: 50% Fat: 35% Protein: 15%	Control: no exercise Pre-meal exercise: 3 sets of 10 repetitions of 8 resistance exercises at 10RM (3.5 METs) [t = -75] Post-meal exercise: 3 sets of 10 repetitions of 8 resistance exercises at 10RM (3.5 METs)[t = 45]	←→ ←→	↓ ↓	←→ ←→	↓ ↓

PPG: postprandial glucose; $G_{AUC:}$ area under the glucose curve; PPI: postprandial insulin; I_{AUC}: area under the insulin curve; CHO: carbohydrates; RM: repetition maximum; NA: not assessed; ←→: no significant difference reported between conditions; ↓: significant difference between exercise and control conditions

Author	Participants Characteristics	Standardized Meal	Exercise Characteristics	Study Outcomes (Exercise vs. Control)			
				Glycemic Control		Insulin Response	
				PPG	G_{AUC}	PPI	I_{AUC}
Karstoft (2014)[39]	10 subjects (3 females) Age: 60 y BMI: 28 kg.m^{-2} T2DM duration: 6 y	450kcal MMTT [t = 105] CHO: 55% Fat: 30% Protein: 15%	Control: no exercise				
			Pre-meal interval walking: Twenty 3min bouts of slow (54% of VO_{2peak}; 3.5 METs) and fast (89% VO_{2peak}; 5 METs) intervals treadmill walking [t = 0]	←→	NA	←→	NA
			Pre-meal continuous walking: 60mins of treadmill walking at 70% of HR_{max} (3.5 METs) [t = 0]	←→	NA	←→	NA
Larsen (1997)[40]	9 male subjects Age: 60 y BMI: 29 kg.m^{-2} T2DM duration: NR	6.9kcal/kg breakfast [t = 0] CHO: 56% Fat: 30% Protein: 14% 14.1 kcal/kg lunch [t = 240] CHO: 53% Fat: 31% Protein: 16%	Control: no exercise				
			Post-meal exercise: 45mins of cycling on an ergometer at 50% of VO_{2peak} (3.5 METs) [t = 45]	NA	↓	NA	↓
Larsen (1999)[41]	8 male subjects Age: 60 y BMI: 29 kg.m^{-2} T2DM duration: NR	6.9kcal/kg breakfast [t = 0] CHO: 56% Fat: 30% Protein: 14% 14.1kcal/kg lunch [t = 240] CHO: 53% Fat: 31% Protein: 16%	Control: no exercise				
			Post-meal exercise: 4 intermittent cycling bouts on an ergometer at 50% (3.5 METs) and 100% (7 METs) of VO_{2peak} [t = 45]	←→	←→	←→	←→

PPG: postprandial glucose; $G_{AUC:}$ area under the glucose curve; PPI: postprandial insulin; I_{AUC}: area under the insulin curve; CHO: carbohydrates; NR: not reported; NA: not assessed; ←→: no significant difference reported between conditions; ↓: significant difference between exercise and control conditions

Author	Participants Characteristics	Standardized Meal	Exercise Characteristics	Study Outcomes (Exercise vs. Control)			
				Glycemic Control		Insulin Response	
				PPG	G_{AUC}	PPI	I_{AUC}
Manders (2010)[42]	9 male subjects Age: 57 y BMI: 29 kg.m^{-2} T2DM duration: 9 y	52.5kcal/kg breakfast [t = 0] CHO: 61% Fat: 26% Protein: 13% 28.9kcal/kg per meal for 3 subsequent meals [t = 330, 630 and 1410] CHO: 58% Fat: 30% Protein: 11%	Control: no exercise Post-meal low intensity exercise: 60mins of cycling on an ergometer at 35% of W_{max} (3.5 METs) [t = 60]	←→	↓	NA	NA
			Post-meal high intensity exercise: 30mins of cycling on an ergometer at 70% of W_{max} (6.8 METs) [t = 60]	←→	←→	NA	NA
Moreira (2012)[43]	9 male subjects Age: 47 y BMI: 29 kg.m^{-2} T2DM duration: 5 y	285kcal breakfast [t = -120] CHO: 63% Fat: 29% Protein: 8%	Control: no exercise Post-meal low intensity exercise: 3 sets of 30 repetitions of 6 resistance exercises at 23% of 1RM (3.5 METs) [t = 0]	NA	↓	NA	NA
			Post-meal moderate intensity exercise: 3 sets of 16 repetitions of 6 resistance exercises at 43% of 1RM (4 METs) [t = 0]	NA	↓	NA	NA
Oberlin (2014)[44]	9 subjects (5 females) Age: 60 y BMI: 36 kg.m^{-2} T2DM duration: NR	1600-2400kcal per meal for 6 meals [t = 0, 300, 600, 1440, 1740 and 2040] CHO: 51% Fat: 31% Protein: 18%	Control: no exercise Pre-meal exercise: Two 20min bouts of treadmill walking and 20mins of cycling on an ergometer at 60% of HRR (3.5 METs) [t = -90]	←→	←→	NA	NA

PPG: postprandial glucose concentration; $G_{AUC:}$ area under the glucose curve; PPI: postprandial insulin; I_{AUC}: area under the insulin curve; CHO: carbohydrates; NR: not reported; RM: repetition maximum; NA: not assessed; ←→: no significant difference reported between conditions; ↓: significant difference between exercise and control conditions

Author	Participants Characteristics	Standardized Meal	Exercise Characteristics	Study Outcomes (Exercise vs. Control)			
				Glycemic Control		Insulin Response	
				PPG	G_{AUC}	PPI	I_{AUC}
Rasmussen (1999)[45]	12 subjects (4 females) Age: 56 y BMI: 29 kg.m^{-2} T2DM duration: 8 y	436kcal breakfast [t = 0] CHO: 48% Fat: 38% Protein: 14%	Control: no exercise Post-meal exercise: 30mins of cycling on an ergometer at 40% of VO_{2peak} (3.5 METs) [t = 30]	NA	←→	NA	←→
Tobin (2008)[46]	8 male subjects Age: 59 y BMI: 29 kg.m^{-2} T2DM duration: NR	680kcal breakfast [t = 0] CHO: 8% Fat: 84% Protein: 8%	Control: no exercise Post-meal exercise: 60mins of cycling on an ergometer at 60% of VO_{2peak} (5 METs) [t = 90]	↓	NA	↓	↓
Van Dijk (2012)[48]	<u>INS</u> 15 male subjects Age: 60 y BMI: 30 kg.m^{-2} T2DM duration: 14 y <u>OGLM</u> 15 male subjects Age: 61 y BMI: 30 kg.m^{-2} T2DM duration: 7 y	Total of 2485kcal for 3 meals after exercise [t = 240, 570 and 1440] CHO: 57% Fat: 30% Protein: 13%	Control: no exercise Post-meal endurance exercise: 45mins of cycling on an ergometer at 50% of W_{max} (4 METs) [t = 150] Post-meal resistance exercise: 5 sets of 10 repetitions for 4 resistance exercises at 40-75% of 1RM (3.5 METs) [t = 150]	↓ ↓	NA NA	NA NA	NA NA

PPG: postprandial glucose; $G_{AUC:}$ area under the glucose curve; PPI: postprandial insulin; I_{AUC}: area under the insulin curve; CHO: carbohydrates; NR: not reported; INS: exogenous insulin treatment; OGLM: oral glucose-lowering medication; RM: repetition maximum; NA: not assessed; ←→: no significant difference reported between conditions; ↓: significant difference between exercise and control conditions

Author	Participants Characteristics	Standardized Meal	Exercise Characteristics	Study Outcomes (Exercise vs. Control)			
				Glycemic Control		Insulin Response	
				PPG	G_{AUC}	PPI	I_{AUC}
Van Dijk (2012)[49]	<u>NIDDM</u> 16 male subjects Age: 60 y BMI: 30 kg.m^{-2} T2DM duration: 5 y <u>IDDM</u> 14 male subjects Age: 60 y BMI: 31 kg.m^{-2} T2DM duration: 12 y	Total of 4922kcal for 6 meals [t = 240, 570, 1440, 1680, 2010 and 2880] CHO: 55% Fat: 31% Protein: 14%	Control: no exercise Post-meal single bout exercise: 60mins of cycling on an ergometer at 50% of W_{max} (4 METs) [t = 150] Post-meal multiple bouts exercise: Two 30min bouts of cycling on an ergometer at 50% of W_{max} (4 METs) [t = 0 and t = 1440]	NA NA	↓ ↓	NA NA	NA NA
Van Dijk (2013)[50]	20 male subjects Age: 64 y BMI: 30 kg.m^{-2} T2DM duration: 8 y	Total of 2342kcal for 3 meals [t = 0, 240 and 510] CHO: 50% Fat: 35% Protein: 15%	Control: no exercise Post-meal walking: Three 15min bouts of walking at approximately 3 METs [t = 45, 285 and 555] Post-meal cycling: 45mins of cycling on an ergometer at 50% of W_{max} (4 METs)[t = 45]	←→ ↓	↓ ↓	NA NA	↓ ↓
Van Dijk (2013)[47]	<u>NIDDM</u> 37 male subjects Age: 59 y BMI: 30 kg.m^{-2} T2DM duration: 7 y <u>IDDM</u> 23 male subjects Age: 60 y BMI: 30 kg.m^{-2} T2DM duration: 12 y	Total of 2413kcal for 3 meals [t = 240, 510 and 1440] CHO: 56% Fat: 30% Protein: 14%	Control: no exercise Post-meal exercise: 45 to 60mins of cycling on an ergometer at 35-50% of W_{max} (3.5 – 4 METs) [t = 90]	↓	NA	NA	NA

PPG: postprandial glucose; $G_{AUC:}$ area under the glucose curve; PPI: postprandial insulin; I_{AUC}: area under the insulin curve; CHO: carbohydrates; NIDDM: non-insulin dependent diabetes mellitus; IDDM: insulin dependent diabetes mellitus NA: not assessed; ←→: no significant difference reported between conditions; ↓: significant difference between exercise and control conditions

Table 2-2. Summary of included parallel-group studies

Author	Participants Characteristics	Exercise Characteristics	Study Outcomes (Exercise vs. Control)					
			Glycemic Control				Insulin Response	
			HbA1c	PG	FG	PPG	FI	IS
Vancea (2009)[51]	Control 20 subjects Age: 56 y BMI: 28 kg.m^{-2} T2DM duration: 6 y	Control: no exercise						
	3x/week exercise (G3) 14 subjects Age: 57 y BMI: 30 kg.m^{-2} T2DM duration: 5 y	G3: 30 min of treadmill walking performed 3 times per week for 20 weeks at 60-70% HR_{max} (3.5 METs). Each session was performed after a meal.	←→	NA	←→	←→	NA	NA
	5x/week exercise (G5) 9 subjects Age: 59 years, BMI: 30 kg.m^{-2} T2DM duration: 6 y	G5: 30 min of treadmill walking performed 5 times per week for 20 weeks at 60-70% HR_{max} (3.5 METs). Each session was performed after a meal.	←→	NA	↓	↓	NA	NA
Wycherley (2010)[52]	Post protein snack exercise (P0) 18 male and female subjects Age: 56 y BMI: 35 kg.m^{-2} T2DM duration: NR	P0: 2 sets of 8-12 repetitions of 8 resistance exercises performed 3 times per week after a protein snack at 70-85% 1RM (3.5 METs) for 16 weeks	↓	↓	NA	NA	↓	↓
	Pre protein snack exercise (P2) 16 male and female subjects Age: 56 y BMI: 36 kg.m^{-2} T2DM duration: NR	P2: 2 sets of 8-12 repetitions of 8 resistance exercises performed 3 times per week prior to a snack at 70-85% 1RM (3.5 METs) for 16 weeks	↓	↓	NA	NA	↓	↓

HbA1c: glycosylated hemoglobin; PG: plasma glucose concentration; FG: fasting glucose; PPG: postprandial glucose; FI: fasting insulin; IS: insulin sensitivity as measured by HOMA2-IR CHO; NR: not reported; NA: not assessed; RM: repetition maximum

Risk of Bias

The risk of bias assessment (Appendix C) revealed insufficient reporting across most categories being assessed. In particular, methods for generating the allocation sequence and the allocation concealment were unclear (or at high risk of bias in one study; (20, 37)). Although all studies were deemed to have a low risk of bias with respect to the blinding of participants and personnel, the blinding of outcome assessment was unclear due to insufficient information for all included studies with the exception of two, Heden et al. (20) and Karstoft et al. (39), which were judged to have a high risk of bias since the crossover assignments of the interventions were open-labelled. Treatment of incomplete data were judged as a low risk of bias in 15 studies, since sufficient information was provided for assessment of completeness of outcome data, including attrition and exclusions from the final analysis. Due to insufficient information, four trials received an unclear judgement for bias relating to incomplete data. Six studies (35, 43, 47, 51) were deemed to have a low risk of bias for selective outcome reporting and seven studies (37, 39, 47-50, 52) were deemed to have a low risk of bias for other sources; the remaining 12 studies were judged as having an unclear risk of bias for selective reporting and other sources due to insufficient information being presented. In addition, 13 of the 17 authors that were contacted for additional information responded.

Changes in Glucose Concentrations and AUC

Data from trials assessing the effect of exercise timing on PPG concentrations (Figure 2-2A) showed 5 (total of 8 trials) reported a significant benefit of exercise conducted in the post-meal period compared to a no-exercise control condition; while 2 trials (total of 5) demonstrated significant improvements when exercise was performed in the pre-meal period.

Data from trials assessing the effect of exercise timing on glucose AUC showed improvements in 2 of 4 trials when exercise was performed in the pre-meal period (relative to no-exercise control); and 4 of 9 trials when exercise was performed in the post-meal period (Figure 2-2B).

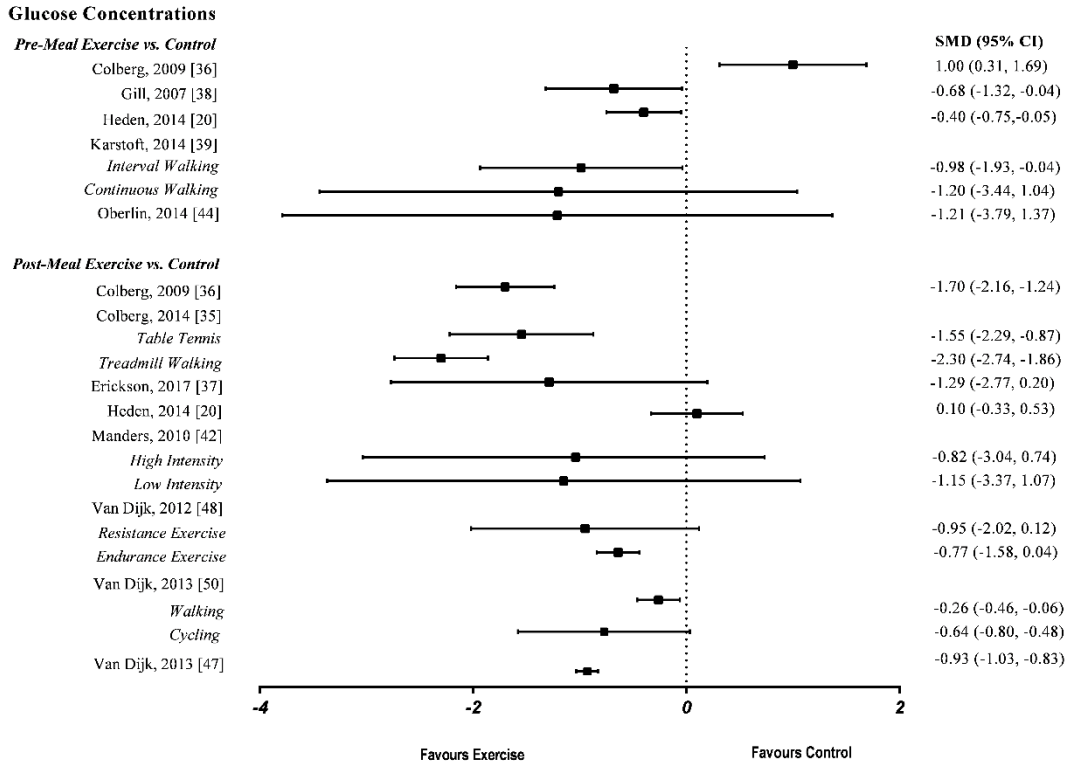
Of the two parallel-group trials, Vancea et al. (51) reported that 20 weeks of post-meal aerobic exercise training led to greater decreases in HbA_1c (-1.30% [-2.56, -0.04]), fasting glucose (-4.02 mmol/L [-5.64, -2.39]) and postprandial glucose (-3.09 mmol/L [-5.94, -0.23]) compared to a no-exercise control. Wycherley et al. (52) reported that 16 weeks of resistance exercise training performed prior to a protein snack resulted in no differences in HbA_1c or average glucose concentrations compared to similar resistance exercise training performed after a protein snack (-0.1% [-0.36, -0.16] and -0.1 mmol/L [-0.7, 0.5] respectively).

Changes in Insulin Concentrations and AUC

All 3 trials assessing the effect of exercise timing (Pre-meal versus control: 3 trials; Post-meal versus control: 1 trials) on postprandial insulin concentration showed significant reductions in insulin concentration when exercise was performed in the pre-meal period (Figure 2-3A). Insulin AUC (Figure 2-3B) was decreased in all (4 out of 4) trials assessing exercise in the post-meal period and the 2 trials comparing exercise in the pre-meal period to the no-exercise control.

Wycherley et al. (52) reported that 16 weeks of resistance training performed prior to a protein snack did not change fasting insulin concentration (-9.6 ρmol/L [-14.42, -4.78]) when compared to the resistance exercise training performed after a protein snack.

2A



2B

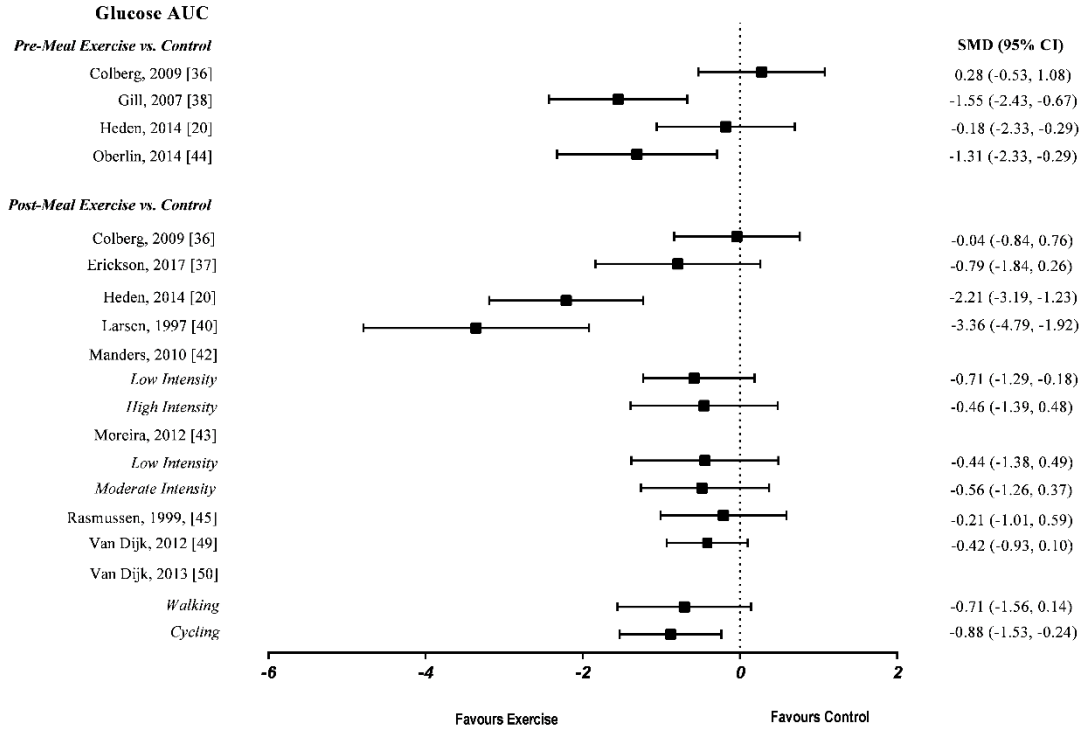
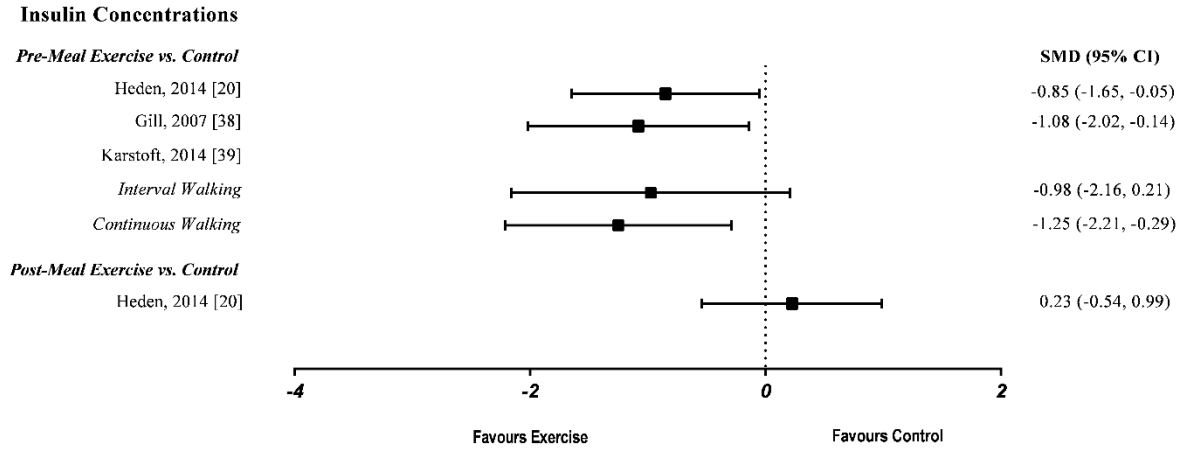


Figure 2-2. Pre-meal and post-meal exercise versus control on glucose concentrations (**2A**) and glucose AUC (**2B)**

3A



3B

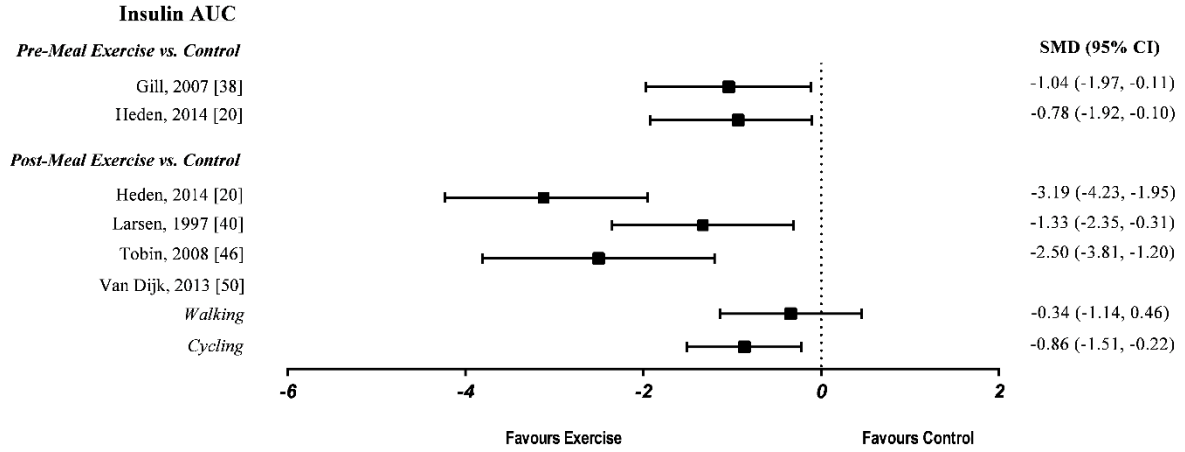


Figure 2-3. Pre-meal and post-meal exercise versus control on insulin concentrations (**3A**) and insulin AUC (**3B**)

2.5 Discussion

Exploratory evidence suggests a potentially important relationship between exercise-timing and meal consumption (18, 19). This systematic review of exercise-based interventions for T2DM included 19 randomized controlled trials, of which 17 were cross-sectional studies and 2 studies were parallel-group designs, to explore this question in greater detail. The findings of this review are i) acute postprandial exercise appeared to more consistently improve glycemia

(glucose concentrations and glucose-AUC) than when compared to an acute exercise bout performed prior to a meal; (ii) insulin concentrations are improved acutely following pre-meal exercise, while both preprandial and postprandial exercise improve the acute insulin AUC. Large heterogeneity in study design and methodology precluded a meta-analysis of included data; specifically, the timing and frequency of blood samples; exercise type and duration; composition and caloric content of the meal used during the glucose-challenge; and the timing of the exercise bout relative to the meal were too variable. No randomized controlled trials directly assessed the effect of manipulating exercise timing within a training program on long-term glycemic control; while only two studies (20, 52) directly assessed the effect of manipulating exercise timing on acute glucose and insulin concentrations.

Acute glucose and insulin responses to meals: Effect of exercise-timing

Large intra-study variability in glucose concentrations (Figure 2-2A and 2-2B) were identified in the studies of Karstoft, (39) Manders (42) and Oberlin (44). This variability may in part be explained by the time-span between meal consumption and exercise performance. Postprandial exercise performed within 30 min after meal consumption (35-37) lowered glucose concentrations levels by a greater amount (SMD: -1.76) as compared to postprandial exercise performed 30 to 60 min (20, 41, 50), 60 to 90 min (47) and 120 to 150 min (48) after the consumption of a meal (SMD: -0.27; -0.93 and; -0.95, respectively). Accordingly, exercise performed between 30 and 60 min (20, 36, 37, 40, 45, 50) after the consumption of a meal resulted in a greater change in glucose AUC (SMD: -1.25) when compared to postprandial exercise performed between 60 and 120 min (42) and 120 to 150 min (43, 49) after meal consumption (SMD -0.46 and; -0.43 respectively). These observations align with conclusions by Chacko (18) that a single bout of light to moderate intensity physical activty performed 30

min after a meal resulted in the simultaneous blunting of the glucose peak and risk of hypoglycaemia.

The effect of pre-meal exercise on blood glucose responses was not as consistent as that observed when exercise was performed in the postprandial period (Figure 2-2A and 2-2B). There was large variability in the glucose responses between-studies, which may in part be explained by the time-span between meal consumption and exercise performance. In particular, the two studies (38, 44) which resulted in significant improvements (SMD: -1.55 and -1.31, respectively) in glucose AUC, conducted the exercise the day prior (~16-18 h prior) to the breakfast meal, (38) or observed the improvement in the second meal (~4.5 h post-exercise) but not in the first meal (~30 min) post-exercise (44). There was a consistent and appropriate effect of acute exercise blunting the insulin response to meals (Figure 2-3A and 2-3B) which occurred irrespective of exercise timing.

Effect of exercise-timing within an exercise training program on glycaemic control: Clinical implications

Two studies meeting inclusion criteria into this systematic review assessed the longer-term effects (i.e., >12 weeks) of exercise on glycaemia. Structured aerobic exercise training (20 week intervention) conducted either 3 or 5 times per week after a meal, resulted in non-significant changes in HbA_1c when compared to a control group (spontaneous exercise encouraged; unsupervised and timing of exercise not discussed); however, fasting and postprandial glucose concentrations were significantly reduced (fasting: ~2.3 mmol/L; postprandial glucose: ~1.1 mmol/L) when training was performed 5 times per week (51). The effect of resistance exercise training performed either immediately after ingestion of a protein snack or 2 h prior to a protein snack on measures of glycaemia were assessed over the course

of a 16 week lifestyle intervention program (52). While both interventions resulted in significant reductions in HbA_{1c} (-1.1 ± 0.1%), fasting glucose (-1.9 ± 1.7 mmol/L) and insulin (-6.1 ± 6.7 mU/L), there were no between-group differences.

Exercise training has been shown to exert meaningful changes on glycaemia in individuals with T2DM (9, 12) and when training exceeds 150 min, HbA_{1c} has been shown to be reduced by 0.89% [-1.26%, -0.51%] (12). In combination with dietary changes, HbA_{1c} may indeed reduce by 1.1% with the addition of exercise training, which compares favourably to the ~1% improvement in HbA_{1c} with a single oral hypoglycaemic agent (53). However, based on available evidence (two studies), it appears manipulating exercise-timing within a training program does not appear to be critical for eliciting greater improvements in glycaemic control. Based on the limited evidence and large heterogeneity in the two studies addressing this question, further work in this field is warranted.

Exercise-timing relative to meal ingestion: Potential mechanisms explaining the interaction

An acute bout of exercise is associated with an increased rate of glucose uptake in individuals with normoglycaemia, impaired glucose tolerance (IGT) and T2DM (54, 55). However, acute exercise performed in the preprandial (and fasted) state is associated with a reduced or unaltered glucose tolerance in individuals with normoglycaemia, IGT and T2DM (56-59). This paradox is explained on account of increased endogenous glucose production (i.e. increased hepatic glucose release) and increased exogenous glucose appearance (54). Indeed, whole-body rate of glucose disappearance during a glucose tolerance test has been shown to increase 24% when exercise preceeds the test (30 min prior), however, glucose appearance from exogenous (primary) and endogenous (secondary) sources increased by ~25% (59). The

increased exogenous-glucose appearance after exercise is unlikely to result from altered gastrointestinal emptying rates (expected to decrease only during high-intensity exercise and effect does not appear to persist post-exercise (60)), but may arise as a response to increased intestinal permeability (61), although this is speculative and reflective of paracellular uptake rather than transport-specific uptake. The overwhelming majority of glucose is absorbed from the intestinal lumen via the sodium dependent glucose cotransporter (SGLT-1) which also plays the pivotal role in the release of incretins (glucose-dependent insulinotropic peptide, GIP; glucagon-like-peptide 1, GLP-1) (62). While dietary intake is known to alter SGLT-1 transporter density (63), whether exercise may modulate SGLT-1 activity (transport or incretin response) is unknown. An improved understanding of these mechanisms may have important clinical and practical implications, in particular with respect to the timing of meals relative to exercise or general physical activity.

Strength and Limiations of the Review

The strength of this systematic review is the inclusion of recent and relevant randomized controlled trials that investigated the temporal effect of exercise performance on critical measures of T2DM management. In addition, findings from this review have clinical applicability given that included participants were restricted to T2DM individuals. There is physiological evidence suggesting a potentially important role for the timing of exercise performance relative to meal ingestion on glycaemic and insulin variability; a better understanding of the optimal time for exercise performance relative to meal intake to allow more effective management of glycaemic control in T2DM individuals is clinically relevant and important. A limitation of this review is the exclusion of studies based on language (i.e. non-english studies).

The extant literature curently lacks homogenenity with respect to primary outcome measures (i.e. fasting glucose, HbA_{1c}, glucose AUC), analyses of outcome measures (i.e. incremental AUC, total AUC, time-period comprising AUC, number of samples performed during the AUC), types of exercise interventions (frequency, intensity, type and duration of exercise), test-meal composition and the reporting of outcome measures at different time-points. Specific to the exercise interventions, exercise characteristics of studies ranged from activities such as table tennis, walking (low-, moderate- and high-intensity), resistance exercise (low- and moderate-intensity) and combined aerobic-resistance exercise; wherein estimated metabolic equivalents (MET) ranged from 42 MET-min to 315 MET-min (mean ± SD; 167.9 ± 70.2 MET-min). Additionally, the findings of this systematic review are reliant on the quality of the included studies, whereby, majority of the studies were deemed unclear during risk of bias assessment (Appendix C). The large heterogeneity in prior studies precluded the performance of a meta-analysis. With respect to exercise training, it is noteworthy that acute changes in glycaemic control and insulin responses may not translate to chronic responses associated with longer-term interventions. Finally, only 9.3% of participants in included studies were female. It is recommended that future research investigates the longer-term (>12 weeks) effect of manipulating exercise-timing relative to meal ingestion (i.e., 30 min pre-meal versus 30 min post-meal), and adopt an exercise-intervention based on current best-evidence (i.e., supervised combination of aerobic and resistance-based exercise; at least 50 min per session, three times per week) with clinically relevant outcome measures (fasting glucose; oral glucose tolerance test; HbA_{1c}) in a gender-balanced cohort of individuals with T2DM.

2.6 Conclusions

Structured exercise exceeding the minimum recommendations (i.e., >150 min of exercise per week) can result in HbA_{1c} reductions of 0.89% (12) which is comparable to changes associated with diabetes medications adopted as monotherapy (HbA_{1c} reduction ~1.0%) (53). This systematic review explored the current evidence assessing the importance of exercise timing relative to meal ingestion from randomized controlled trials, and considered this evidence in light of known physiological mechanisms. The main findings were that postprandial exercise performed between 30 to 60 min after meal consumption appeared to result in more consistent reductions in glucose concentration and glucose AUC when compared to a control group. However, there is insufficient evidence to directly compare the benefits of exercise performed in the preprandial and postprandial periods on glucose concentration (acutely) or glucose control (longer-term; i.e., >12 weeks), or indeed, assess the relative importance of this variable (exercise-timing relative to meal ingestion) relative to other exercise programming variables. More broadly, insufficient studies have investigated the role of exercise-timing on glycemic control in female participants with T2DM. Clincally, postprandial exercise confers the advantage of reducing the risk of hypoglycemia when compared to preprandial exercise in individuals with T2DM. Whether strategic manipulation of exercise-timing results in clinically meaningful changes in glycemic control (i.e. HbA_{1c}) when adopted as part of a logitudinal exercise training protocol; and explain some of the individual variance observed in HbA_{1c} in response to exercise-training interventions, is currently not known and requires investigation.

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Chapter 3 The diurnal impact of exercise timing on the management of Type 2 Diabetes Mellitus: A systematic review

Teo SYM, Kanaley JA, Guelfi KJ, Cook S, Hebert JJ, Mitchell F and Fairchild TJ (2018). The impact of diurnal exercise timing on the management of Type 2 Diabetes Mellitus: A systematic review. *Submitted to Sports Medicine*.

3.1 Abstract

Background: The diurnal timing of exercise performance may potentially be an important variable in mediating glycemic control for the management of type 2 diabetes mellitus (T2DM). The objective of this systematic review was assess the literature related to the diurnal timing of exercise performance and glycemic control in individuals with T2DM.

Methods: Systematic searches of randomized controlled trials published in English were performed in PubMed, EMBASE, CINAHL, Cochrane Library and ClinicalTrials.gov Registry databases from inception to June 2018. Two authors independently utilized the Cochrane Collaboration Risk of Bias Assessment Tool and The Cochrane Collaboration Data Collection Form for the completion of study quality assessment and data extraction of articles, respectively. A qualitative synthesis was performed on the included studies, and results summarized in tables.

Results: Eighteen studies (321 participants) were included in the final data synthesis. No study directly assessed this question. Trial data between studies conducted in the evening or morning were compared and suggest both morning and evening exercise result in greater improvements in glucose outcomes, with the standardized mean difference (SMD) in glucose area under the curve (AUC) for morning exercise trials (8 included studies) ranging from -0.21 (95% CI: -1.01, 0.59) to -3.36 (95% CI: -4.79, -1.92) and the glucose AUC for evening exercise ranging from -0.12 (95% CI: -0.92, 0.68) to -1.20 (95% CI: -2.76, 0.36) relative to control (no exercise) conditions.

Conclusions: Current evidence regarding the importance of diurnal exercise-timing on glycaemic control is inconclusive. Despite the fundamental nature of the question, no studies

have addressed this in individuals with T2DM and future low risk trials in this field are warranted.

Registration: International Prospective Register of Systematic Reviews (PROSPERO CRD42017054666).

Key Points:

1. In this systematic review, irrespective of exercise timing, improvements in acute glycaemic outcomes consistently favoured exercise interventions as compared to control conditions. This may indicate that exercise performance is a paramount component in exercise prescription for increased benefits in glycaemia management for T2DM.
2. The diurnal timing of exercise performance may be an important component to further increase the benefits of exercise in T2DM management. However, the magnitude of its effect on glycaemic management remains elucidated and future low risk trials are warranted.

Key Words: Glycemic control, exercise, diurnal timing, systematic review

3.2 Introduction

Type 2 diabetes mellitus (T2DM) is one of the leading causes of mortality in most developed countries (1, 2), accounting for approximately 8.4% of global all-cause mortality in 2013 (3). Current estimates identify a continuing increase in global prevalence in T2DM (4), with the greatest rates of increased prevalence occurring in low- and middle-income countries (5).

Despite substantial phenotypic heterogeneity with T2DM (6), poor glycemic control with rates of morbidity and mortality increasing proportionally with poorer glycemic control (7, 8]) are characteristic of individuals with T2DM.

Maintenance of tight glycemic control is a critical objective in the management of T2DM. Increasing physical activity is an effective therapy for improving glycemic control (9-17) and therefore it is a recognized adjunct in the management of T2DM. Accordingly, the American Diabetes Association (ADA) guidelines recommend adults to engage in at least 150 min/week of physical activity, comprising both aerobic and resistance exercise training on a daily basis, or at least, not allowing more than two days to elapse between exercise sessions (18). The acute response of plasma glucose to exercise varies with alterations in exercise intensity, duration, frequency and type, and this has been well characterized in previous reviews (18-22). While the acute response of plasma glucose to exercise are well defined—albeit partly moderated by participant characteristics—the longer term glycemic effect of modifying the intensity, duration, frequency and type of exercise within training programs is less clear (18-22), and complicated by additional confounders such as diet and exercise adherence. The evidence supporting superiority of one training variable over another is limited, although increases in volume (particularly duration; (23)) appear particularly important in conveying the positive effects of exercise on glycemic management.

More recently, the association between poor sleep patterns and increased risk of T2DM (24) have prompted interest in exploring the role of the circadian timing system on glycemic control. The circadian timing system regulates metabolic, immunological, neurological and endocrine processes via a complex interaction between central (i.e., bilateral suprachiasmatic nuclei of the hypothalamus) and peripheral (i.e., liver, muscle, adipose, pancreatic cells) pathways (25,

26). The peripheral pathways—or molecular clocks within peripheral tissue—appear particularly susceptible to entrainment either directly or indirectly by feeding and exercise (25-28); wherein alterations in the timing of these can result in desynchronization between the various metabolic, neurologic and endocrine processes (26). This desynchronization can have negative health consequences, such as disrupting the rhythmic secretion of particular hormones (e.g. ghrelin (29)), although negative health consequences may be reversed once the circadian system is reset (30). The assessment of exercise timing on glycemic management in individuals with T2DM has emerged in the wake of this literature (31-34). The timing of exercise relative to nutrient ingestion (feeding) is an emerging area of interest, wherein preliminary support suggests beneficial glycemic responses for exercise performed in the postprandial period (32-35). With respect to diurnal timing of exercise, it is known that acute exercise performed at different times of the day alters metabolic processes (e.g. lipid metabolism (36)), endocrine concentration and responses (37) and autonomic function (38). The aim of this systematic review therefore, was to assess the available evidence from randomized controlled trials (RCTs) assessing effects of diurnal exercise timing on glycemic control in individuals with T2DM.

3.3 Methods

Protocol and Registration

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta – Analyses (PRISMA) Statement and registered with the International Prospective Register of Systematic Reviews (PROSPERO CRD42017054666) (39).

Data sources and searches

Potential studies were identified by systematically searching Medline (PubMed), EMBASE, CINAHL, Cochrane Library and the ClinicalTrials.gov Registry electronic databases. The search syntax was designed for PubMed (Appendix E) and applied to the remaining databases. All databases were searched from the inception to June 2018 for full reports written in the English language. In addition, reference lists of all retrieved papers were manually searched to identify any potentially relevant studies not identified by the database search.

Eligibility Criteria

The inclusion criteria were RCTs that examined the diurnal effects of exercise on measures of glycemic control and insulin response in adults diagnosed with T2DM. The exercise interventions of included studies had to be quantifiable based on the frequency, intensity, type and time of day that exercise was performed. To provide a comprehensive review of the available literature, exercise durations ranging from acute single exercise bouts to extended exercise training programs were included. Observational or uncontrolled studies and studies that did indicate the diurnal timing of exercise performance were excluded from this systematic review.

Outcome measures

Glycemic control encompasses the three components of the glucose triad: (i) glycosylated hemoglobin, (ii) fasting glucose (FG) and, (iii) postprandial glucose (40). While glycosylated hemoglobin (HbA_{1c}) is the criterion measure for chronic glycemic control (41-43), reflecting the previous 8-12 weeks (44), it may not necessarily capture the magnitude of change in diurnal glucose (45-47). While the magnitude change may best be presented by the change from fasting glucose concentration to the peak glucose concentration, the commonly adopted sampling

time-points do not capture peak glucose concentrations. Suh et al. (48) identified area under the curve (AUC) as an acceptable measure of postprandial glucose (PPG) excursions. Therefore, to account for varied perspectives of glycemic control, we included the following as primary review outcomes: (i) HbA_{1c}, (ii) FG, (iii) PPG (postprandial glucose concentrations reported after a glucose load) and, (iv) glucose AUC. Considering the association between hyperinsulinemia and increased cardiovascular disease (CVD) mortality rates (49-51), the secondary review outcomes were postprandial insulin concentrations and AUC after a glucose load.

Data extraction and quality assessment

Potential studies were exported to Covidence Systematic Review Software (Veritas Health Innovation, Melbourne, Australia) for assessment. Following removal of duplicate references, two independent authors (ST and SC) completed the title and abstract screening process in a blinded manner against the selection criteria. Disagreements were resolved by a third author (TF). Thereafter, a full text assessment was completed in a blinded manner by two review authors independently (ST and MF). Similarly, all disagreements were resolved by a third author (TF). Reasons for excluding each study at the full-text stage were recorded. When insufficient information was available, corresponding authors of included studies were contacted to obtain additional information.

Upon the completion of the full-text assessment, data extraction for the included studies was completed independently by two authors (ST and MF) utilizing the Cochrane Collaboration Data Collection Form (52). The following information was extracted from each of the included studies: (i) experimental conditions; (ii) total number of participants; (iii) age; (iv) gender; (v) body mass index; (vi) T2DM duration; (vii) exercise protocols; (viii) exercise timing and; (ix)

comparisons in outcome measures of glycemic control and insulin. Variations in data extraction were resolved by consensus (ST and MF). Seventeen authors were contacted for additional information.

The assessment of risk of bias for each study was completed by utilizing the Cochrane Collaboration's tool for assessing risk of bias (53). The tool assesses bias for each study from seven domains: (i) random sequence generation; (ii) allocation concealment; (iii) blinding of participants and personnel; (iv) blinding of outcome assessment; (v) incomplete outcome data; (vi) selective reporting and; (vii) other sources of bias. Two independent authors (ST and MF) provided a judgement (low risk of bias/ high risk of bias/ unclear) for each of the aforementioned domains, and all disagreements were resolved by consensus.

Data synthesis and analysis

A qualitative synthesis of trial results were summarized in tables stratified by study design and outcome type (HbA_{1c}, PPG, FG, glucose AUC) and insulin. As a consequence of i) the heterogeneity of studies; ii) the number of studies reporting on small sample sizes; iii) the insufficient number of studies completing direct comparisons of the effects of exercise-timing on outcome measures; we were unable to perform a quantitative synthesis with meta-analysis. Instead, the direction of the observed association for each of the outcome measures was graphically presented by constructing forest plots (SigmaPlot, version 13.0; Systat Software, San Jose, CA) to report standardized mean differences (SMD's [Cohen's d]) between exercise conditions. All analyses were performed with Comprehensive Meta-Analysis (CMA) software (version 3.3.070). SMDs were interpreted as small (d = 0.2), moderate (d = 0.5) or large (d = 0.8) (54).

3.4 Results

Study selection and characteristics

Our initial search identified 7333 trials, of which 18 RCTs (321 participants), published between 1997 and 2018, met our selection criteria and were included in the qualitative analysis (55-70) (Figure 3-1). Of these, either a crossover (n = 16) (56-68, 70) or parallel-groups design (n = 2) (55, 69) was employed.

At baseline, participants had a mean (95% CI) age 57.9 years (55.2, 60.5) years, HbA_{1c} of 8.2 % (8.1, 8.3), body mass index (BMI) of 30.6 (28.9, 32.2) $kg.m^{-2}$ and T2DM duration of 7.2 (5.2, 9.3) years. The percentage of male and female participants were 82.7% and 17.3% respectively. Of the 18 included trials, 16 trials investigated the effects of aerobic-based exercises (cycling: n = 7; walking: n = 6; cycle ergometer and treadmill walk: n =2) (55-57, 59-62, 64-70), and three resistance exercise protocols (resistance exercise only: n = 2; resistance exercise and cycling: n = 1) (58, 63, 68). With regards to the timing of exercise performance, 15 trials compared morning exercise to a no-exercise control (55, 59-70), and three trials compared evening exercise to a no-exercise control (56-58) for glycemia and glycemic control. A detailed summary of the results for both acute crossover and parallel-group trials are presented in Table 3-1 and 3-2 respectively.

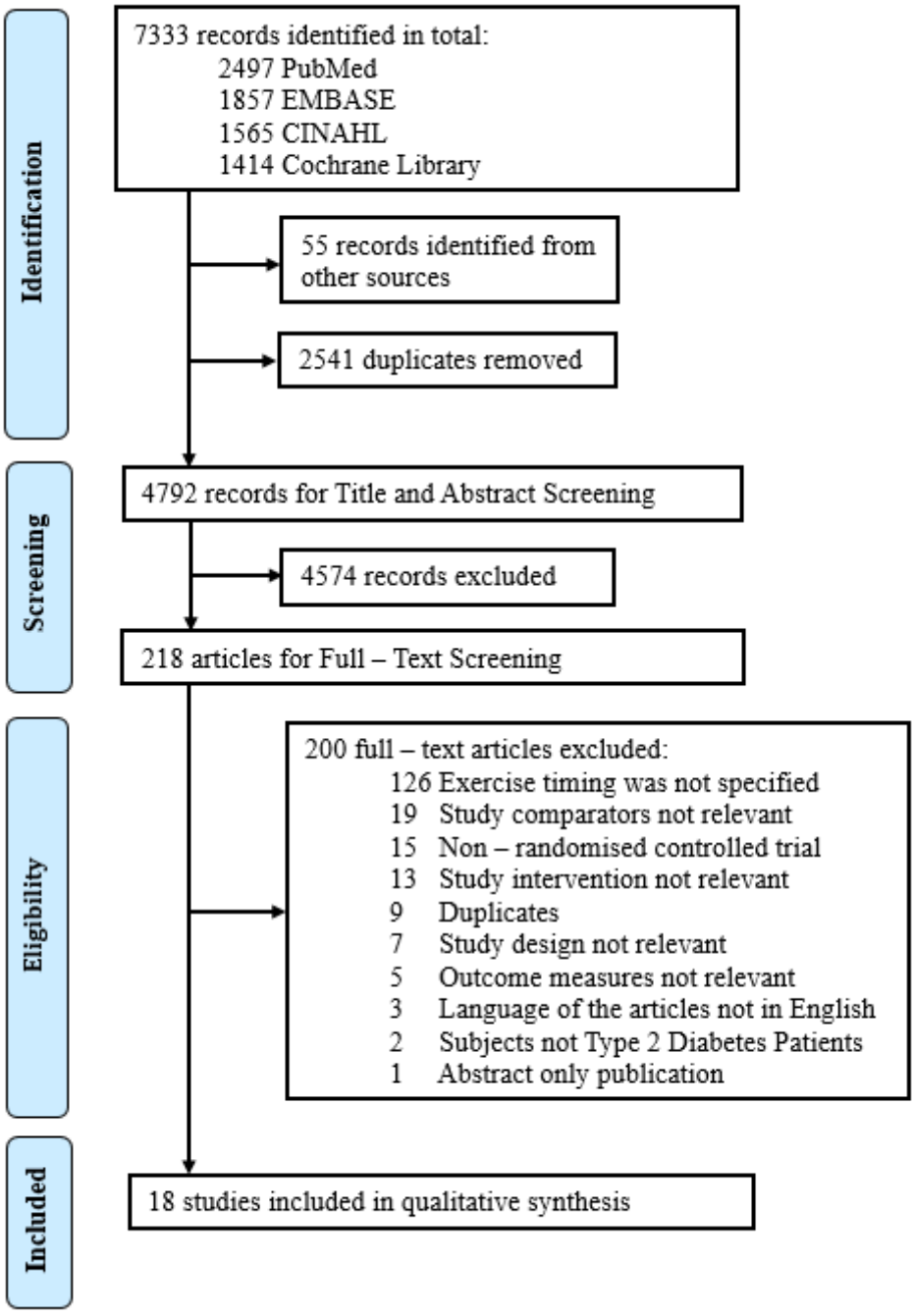


Figure 3-1. Study Selection Process

Table 3-1. Summary of included randomized controlled crossover trials

Author	Participants Characteristics	Exercise Timing; additional variables*	Exercise Characteristics				Study Outcomes (Exercise vs. Control)			
			Type	Duration	Intensity	METs	Glycemic Control		Insulin Response	
							GC	G_{AUC}	IC	I_{AUC}
Colberg (2009) [53]	12 subjects (6 females) Age: 61 y BMI: 35 kg.m^{-2} T2DM duration: 11 y	Evening [1800–1900h]; 2 conditions:	Control: NEx							
		Pre-Meal Exercise (PRE)	TM Walking	20 min	M-HR: 111bpm (40% of HRR)	3.5	←→	←→	NA	NA
		Post-Meal Exercise (POST)	TM Walking	20 min	M-HR: 113bpm (40% of HRR)	3.5	←→	←→	NA	NA
Colberg (2014) [52]	12 subjects (9 females) Age: 59 y BMI: 35 kg.m^{-2} T2DM duration: 6 y	Evening [1800–1900h]	Control: NEx							
			Table tennis: iPong robot	30 min	M-HR: 97 bpm (61% of HR_{max})	4	↓	NA	NA	NA
			TM Walking	30 min	M-HR: 122bpm (76% of HR_{max})	4	↓	NA	NA	NA
Erickson (2017) [66]	8 subjects (3 females) Age: 60 y BMI: 34 kg.m^{-2} T2DM duration: NR	Morning [NR]	Control: NEx							
			Interval walking	3x10 min	50% VO_{2max}	3.5	↓	↓	NA	NA
Heden (2014) [54]	13 subjects (8 females) Age: 49 y BMI: 37 kg.m^{-2} T2DM duration: 4 y	Evening [NR]; 2 conditions:	Control: NEx							
		Pre-Meal Exercise (PRE)	Resistance Training	3x10 reps 8 exercises	10 RM	3.5	←→	↓	←→	NA
		Post-Meal Exercise (POST)	Resistance Training	3x10 reps 8 exercises	10 RM	3.5	←→	↓	←→	NA

*Where multiple conditions are noted. METs: metabolic equivalents; GC: glucose concentration; $G_{AUC:}$ area under the glucose curve; IC: insulin concentration; I_{AUC}: area under the insulin curve; NEx: no exercise; TM: treadmill; M-HR: mean heart rate; NA: not assessed; NR; not reported; RM: repetition maximum

Author	Participants Characteristics	Exercise Timing; additional variables*	Exercise Characteristics				Study Outcomes (Exercise vs. Control)			
			Type	Duration	Intensity	METs	Glycemic Control		Insulin Response	
							GC	G_{AUC}	IC	I_{AUC}
Karstoft (2014) [55]	12 subjects (6 females) Age: 61 y BMI: 35 kg.m^{-2} T2DM duration: 11 y	Morning [0900h]	Control: NEx							
			Interval Walking	20x3 min	SB: 54% of VO_{2peak} FB: 89% of VO_{2peak}	3.5	←→	NA	←→	NA
			Continuous Walking	60 min	70% of HR_{max}	3.5	←→	NA	←→	NA
Larsen (1997) [56]	12 subjects (9 females) Age: 59 y BMI: 35 kg.m^{-2} T2DM duration: 6 y	Morning [0930h]	Control: NEx							
			Cycling Ergometer	45 min	50% of VO_{2peak}	3.5	NA	↓	NA	↓
Larsen (1999) [57]	8 subjects (3 females) Age: 60 y BMI: 34 kg.m^{-2} T2DM duration: NR	Morning [0930h]	Control: NEx							
			Cycling Ergometer	4 bouts	2x 50% of VO_{2peak} 2x 100% of VO_{2peak}	3.5	←→	NA	←→	NA
Manders (2010) [58]	13 subjects (8 females) Age: 49 y BMI: 37 kg.m^{-2} T2DM duration: 4 y	Morning [0900h]	Control: NEx							
			LI Cycling Ergometer	60 min	35% of W_{max}	3.5	↓	↓	NA	NA
			HI Cycling Ergometer	30 min	70% of W_{max}	6.8	←→	←→	NA	NA
Moreira (2012) [59]	9 male subjects Age: 47 y BMI: 29 kg.m^{-2} T2DM duration: 5 y	Morning [0800-0830h]	Control: NEx							
			LI Resistance Training	3x30 reps 6 exercises	23% of 1RM	3.5	NA	↓	NA	NA
			MI Resistance Training	3x16 reps 6 exercises	46% of 1RM	4	NA	↓	NA	NA

*Where multiple conditions are noted. METs: metabolic equivalents; GC: glucose concentration; $G_{AUC:}$ area under the glucose curve; IC: insulin concentration; I_{AUC}: area under the insulin curve; NEx: no exercise; SB: slow bout; FB: fast bout; LI: low intensity; MI: moderate intensity; HI: high intensity: NA: not assessed; NR; not reported; RM: repetition maximum

Author	Participants Characteristics	Exercise Timing; additional variables*	Exercise Characteristics				Study Outcomes (Exercise vs. Control)			
			Type	Duration	Intensity	METs	Glycemic Control		Insulin Response	
							GC	G_{AUC}	IC	I_{AUC}
Oberlin (2014) [60]	9 subjects (5 females) Age: 60 y BMI: 36 kg.m^{-2} T2DM duration: NR	Morning [0630h]	Control: NEx Combined: 1 bout x TM 1 bout x CE	40 min TM: 20 min CE: 20 min	TM: 60% of HRR CE: 60% of HRR	3.5	←→	←→	NA	NA
Rasmussen (1999) [61]	12 subjects (4 females) Age: 56 y BMI: 29 kg.m^{-2} T2DM duration: 8 y	Morning [1030-1100h]	Control: NEx Cycling Ergometer	30 min	40% of VO_{2peak}	3.5	NA	↓	NA	↓
Tobin (2008) [62]	8 male subjects Age: 59 y BMI: 29 kg.m^{-2} T2DM duration: NR	Morning [NR]	Control: NEx Cycling Ergometer	60 min	60% of VO_{2peak}	5	↓	NA	↓	↓
Van Dijk (2012) [64]	<u>INS</u> 15 male subjects Age: 60 y BMI: 30 kg.m^{-2} T2DM duration: 14 y <u>OGLM</u> 15 male subjects Age: 61 y BMI: 30 kg.m^{-2} T2DM duration: 7 y	Morning [1100–1145h]	Control: NEx Cycling Erogmeter Resistance Training	45 min 5x10 reps 4 exercises	35% of W_{max} 40-75% of 1RM	3.5 4	↓ ↓	NA NA	NA NA	NA NA

*Where multiple conditions are noted. METs: metabolic equivalents; GC: glucose concentration; $G_{AUC:}$ area under the glucose curve; IC: insulin concentration; I_{AUC}: area under the insulin curve; NEx: no exercise; SB: slow bout; FB: fast bout; LI: low intensity; MI: moderate intensity; HI: high intensity: NA: not assessed; NR; not reported; RM: repetition maximum

Author	Participants Characteristics	Exercise Timing; additional variables*	Exercise Characteristics				Study Outcomes (Exercise vs. Control)			
			Type	Duration	Intensity	METs	Glycemic Control		Insulin Response	
							GC	G_{AUC}	IC	I_{AUC}
Van Dijk (2012) [64]	<u>NIDDM</u> 16 male subjects Age: 60 y BMI: 30 kg.m^{-2} T2DM duration: 5 y <u>IDDM</u> 14 male subjects Age: 60 y BMI: 31 kg.m^{-2} T2DM duration: 12 y	Morning [1000h]	Control: NEx							
			Cycling Ergometer (Single)	60 min	50% of W_{max}	4	NA	↓	NA	NA
			Cycling Ergometer (Multiple)	2 x 30 min	50% of W_{max}	4	NA	↓	NA	NA
Van Dijk (2013) [63]	20 male subjects Age: 64 y BMI: 30 kg.m^{-2} T2DM duration: 8 y	Morning [0915–1000h]	Control: NEx							
			Walking	3 x 15 min	M-HR: 78bpm (50% of HR_{max})	3	←→	↓	NA	↓
			Cycling Ergometer	45 min	50% of W_{max}	4	↓	↓	NA	↓
Van Dijk (2013) [63]	<u>NIDDM</u> 37 male subjects Age: 59 y BMI: 30 kg.m^{-2} T2DM duration: 7 y <u>IDDM</u> 23 male subjects Age: 60 y BMI: 30 kg.m^{-2} T2DM duration: 12 y	Morning [1000–1100h]	Control: NEx							
			Cycling Ergometer	45 to 60 min	35-50% of VO_{2peak}	3.5 – 4	↓	NA	NA	NA

*Where multiple conditions are noted. METs: metabolic equivalents; GC: glucose concentration; $G_{AUC:}$ area under the glucose curve; IC: insulin concentration; I_{AUC}: area under the insulin curve; NEx: no exercise; M-HR: mean heart rate; NA: not assessed; NR; not reported

Table 3-2. Summary of included longitudinal parallel group studies

Author	Participants Characteristics	Exercise Characteristics	Study Outcomes (Exercise vs. Control)					
			Glycemic Control				Insulin Response	
			HbA1c	GC	FG	PPG	IC	IS
Belli (2011) [51]	Control 10 female subjects Age: 56 y BMI: 29.9 kg.m^{-2} T2DM duration: 3.7 y	Control: no exercise training						
	Training Group (TG) 9 female subjects Age: 53 y BMI: 32.2 kg.m^{-2} T2DM duration: 4.4 y	TG: 20 to 60 minutes of walking on an outdoor track 3 times per week for 12 weeks at VT velocity (5 METs). Each session was performed between 0800 and 1000h.	↓	NA	←→	NA	NA	NA
Vancea (2009) [65]	Control 20 subjects Age: 56 y BMI: 28 kg.m^{-2} T2DM duration: 6 y	Control: no exercise training						
	3x/week exercise (G3) 14 subjects Age: 57 y BMI: 30 kg.m^{-2} T2DM duration: 5 y	G3: 30 minutes of treadmill walking performed 3 times per week for 20 weeks at 60-70% HR_{max} (3.5 METs). Each session was performed after breakfast.	←→	NA	←→	←→	NA	NA
	5x/week exercise (G5) 9 subjects Age: 59 years, BMI: 30 kg.m^{-2} T2DM duration: 6 y	G5: 30 minutes of treadmill walking performed 5 times per week for 20 weeks at 60-70% HR_{max} (3.5 METs). Each session was performed after breakfast.	←→	NA	↓	↓	NA	NA

HbA1c: glycosylated hemoglobin; GC: glucose concentration; FG: fasting glucose; PPG: postprandial glucose; IC: insulin concentration; IS: insulin sensitivity; NA: not assessed; NR: not reported; VT: ventilatory threshold

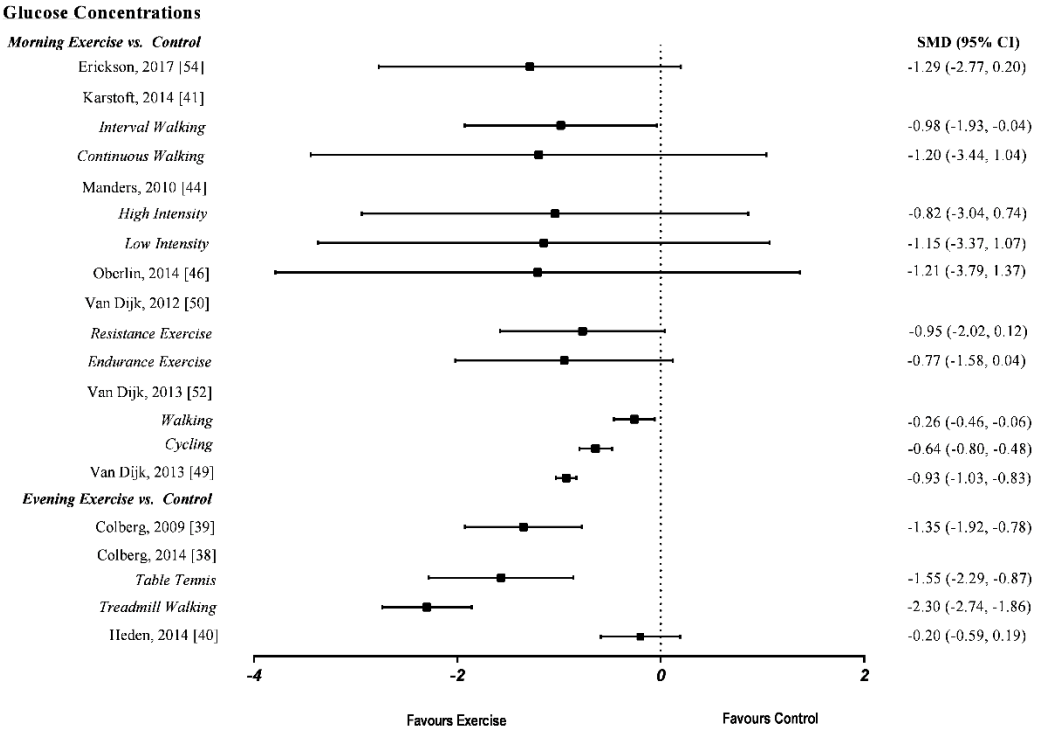
Risk of Bias Assessment

The 18 included trials were assessed for risk of bias and revealed insufficient evidence across most categories (Appendix F). In particular, methods for generating the allocation sequence and allocation concealment were unclear in 94% (17/18) and 89% (16/18) of the included studies. All studies presented adequate information with regards to the blinding of participants and personnel. However, the blinding of outcome assessors was unclear for 89% (16/18). The treatment of incomplete outcome data were deemed as a low risk of bias in 78% (14/18) of the trials due to sufficient information provided for the assessment of completeness of outcome data, which included information regarding attrition and exclusions from final analysis. Most of the studies were judged as having unclear risk of bias for both the assessment of selective reporting and other sources of bias due to insufficient information being presented, with only 38% (7/18) and 33% (6/18) of the included trials being judged as low risk of bias, respectively.

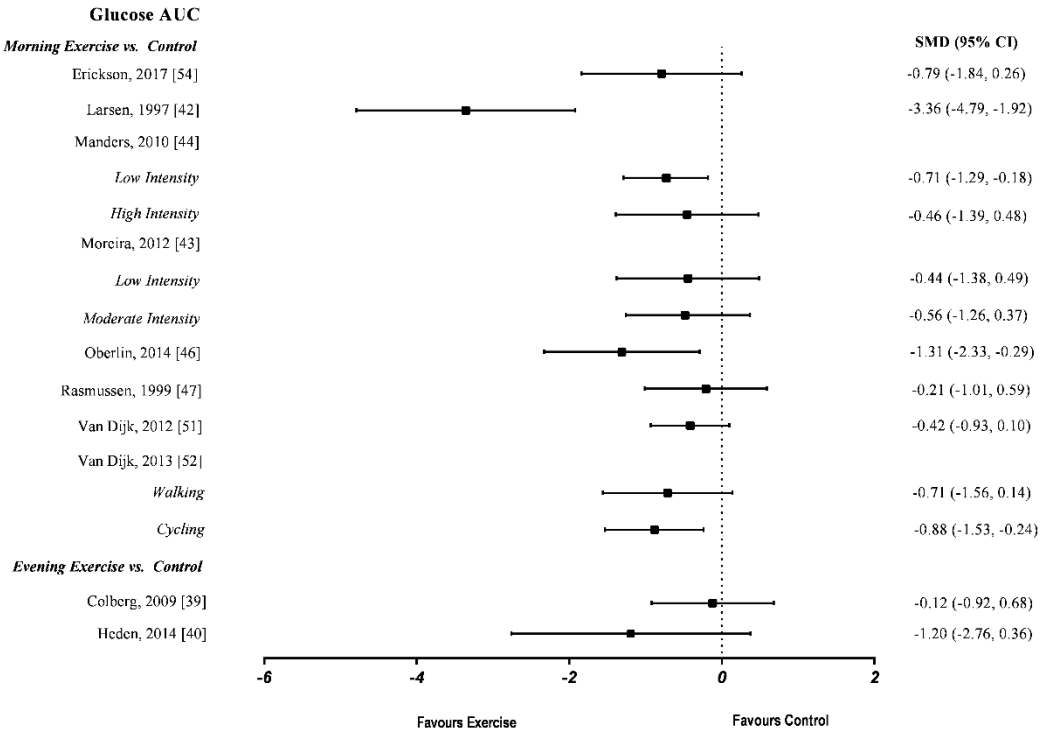
Changes in Postprandial Glucose Concentrations and AUC

Data from trials assessing the acute effect of exercise timing on glucose concentrations (Figure 3-2A) showed 88% (8/9) of trials reported a significant benefit of morning exercise when compared to a no-exercise control condition; while 33% (1/3) of trials demonstrated significant improvements when evening exercise was performed compared to a no-exercise control condition. Studies assessing the effect of exercise timing on glucose AUC showed significant improvements in 75% (6/8) of trials when exercise was performed in the morning (relative to no-exercise control); and 50% (1/2) of trials when exercise was performed in the evening (Figure 3-2B).

2A



2B



AUC: area under the curve; SMD: standardized mean difference; CI: confidence interval

Figure 3-2. Morning and evening exercise versus control on glucose concentrations (**2A**) and, glucose AUC (**2B**)

Of the two parallel-group trials, Belli et al. (55) reported that 12 weeks of morning aerobic exercise training led to a significant reduction in HbA1c (-1.3% [-1.50, -1.10]) but a non-significant reduction in FG (-1.77 mmol/L [-2.40, -1.00]) compared to a no-exercise control. Vancea et al. (69) reported that 20 weeks of morning aerobic exercise training led to greater decreases in HbA_1c (-1.30% [-2.56, -0.04]), FG (-4.02 mmol/L [-5.64, -2.39]) and PPG (-3.09 mmol/L [-5.94, -0.23]) compared to a no-exercise control.

Changes in Postprandial Insulin Concentrations and AUC

Of the three trials that assessed the effect of morning exercise on postprandial insulin concentrations, only one trial showed significant reductions in insulin concentrations when compared to a no-exercise control. No differences were reported in one trial that assessed the effect of evening exercise on insulin concentrations. Insulin AUC was significantly decreased in 75% (3/4) of the trials that assessed exercise in the morning when compared to a no-exercise control. No trials assessed the effect of evening exercise on insulin AUC.

3.5 Discussion

This systematic review of exercise-based interventions for T2DM included 18 randomized controlled trials, of which 16 were cross-over studies and 2 studies were parallel-group designs. Unfortunately, no RCTs assessed the direct effect of manipulating exercise timing within a training program on long-term glycemic control. Based on the available evidence, the findings of this review are i) irrespective of exercise-timing, acute glycemic outcomes consistently favoured exercise compared to control; ii) average glucose concentrations were improved in 4/11 trials conducted in the morning, 3/4 trials conducted in the evening; while, iii) glucose-AUC was improved in 4/11 trials conducted in the morning and 0/2 trials conducted in the

evening. Large heterogeneity in study design and methodology precluded a meta-analysis of included data. Specifically, the timing and frequency of blood samples, exercise type and duration, composition and timing of test-meals, and the timing of the exercise bout relative to meal consumption (i.e. before or after) were too variable for analyses. As a consequence, there is insufficient evidence to conclude the importance of diurnal timing of exercise on glycemic regulation or claim either morning exercise or evening exercise as conveying greater glycaemic benefits to individuals with T2DM.

Effect of exercise-timing on acute glycemic responses

Early morning exercise performance (0600-0900h) may potentially lower glucose by a greater amount than late morning exercise (0900-1200h) (glucose concentrations (59, 62, 64, 67, 68): SMD: -1.19 and -0.84, respectively; glucose AUC (60, 62-65, 67, 68): SMD: -1.39 and -0.50, respectively). Due to the heterogeniety in the composition and timing of meals, it was not possible to identify whether this effect was due to acute elevations in baseline levels, reduced peaks in glucose concentration, or more rapid return to baseline glucose concentrations.

Studies assessing the effect of evening exercise on glycemic responses included walking (56, 57), table tennis (56) and resistance training (58) exercise, with three of the four conditions demonstrating significant improvements in glucose concentrations (Figure 3-2A). However, the studies reporting glucose AUC (57, 58) did not demonstrate the same magnitude-effect, wherein each condition favoured exercise compared to control, but this was not significant in either condition (Figure 3-2B).

Effect of exercise-timing within an exercise training program on glycemic control

Exercise training has been shown to exert meaningful reductions in HbA_{1c} by 0.73% [-1.06% to -0.40%] (43, 71). Within these training programs, variables such as exercise type, frequency, intensity and duration have been exhaustively considered in regards to the management of glycemic control (71, 72). Accordingly, when training exceeds the recommended 150 min, HbA_{1c} has been shown to be reduced by 0.89% [-1.26% to -0.51%] (43). Only two studies assessing the longer-term effects of exercise on glycemic control, met the inclusion criteria of this systematic review. In one study, aerobic exercise training performed 3 mornings per week for 12 weeks resulted in a significant decrease in HbA_{1c} (~0.6%); however, non-significant changes were reported for fasting glucose (-0.5mmol/L) (55). Vancea et al. (69) investigated the effect of structured morning aerobic exercise training (20 week intervention) conducted either 3 or 5 times per week on blood glucose responses and reported non-significant changes in HbA_1c when compared to a control group (spontaneous exercise encouraged; unsupervised and timing of exercise not discussed). However, fasting and postprandial glucose concentrations were significantly reduced (fasting: ~2.3 mmol/L; postprandial glucose: ~1.1 mmol/L) when training was performed 5 times per week.

Effect of exercise-timing: Potential mechanisms and clinical implications

Despite the recognized importance of regular physical activity and exercise in managing individuals with T2DM, physical activity levels in individuals with T2DM remain below national norms (73). In particular, older adults (≥65 y.o.) with T2DM are 31-34% less likely to engage in physical activity than similarly aged individuals without DM (74). While increased frequency, duration and intensity of exercise are associated with improved glycemic management (23, 69-71, 75, 76), these findings have not translated well into clinical practice. If the diurnal timing of exercise is identified as an important training variable in future research,

the manipulation of this variable will likely present as an attractive alternative to patients and enhance translation into practice, although this is yet to be determined.

The regulation of glucose concentrations by the circadian system (25-28) along with the role of exercise in entraining circadian oscillations (28), provides an intriguing mechanism underlying potential influences of diurnal exercise timing in individuals with T2DM. Desynchronization of the circadian system in response to feeding, physical activity and sleep is linked with impaired insulin sensitivity (26) and may further explain, at least in part, the characteristic metabolic inflexibility in T2DM (77, 78). In contrast, time restricted feeding in mice was shown to reset the circadian clock which was associated with improvements in insulin sensitivity and lipid profiles (30). While exercise is known to entrain the circadian system, the potential impact of this in clinical patients, including T2DM require exploration. Further, it may indeed be plausible that individuals with T2DM but different chronotypes respond better to exercise performed at different times of the day. Unfortunately, there is limited available evidence from RCTs in the extant literature and further work is required to determine whether an optimal diurnal time for exercise exists and if so, how this may be moderated by an individual's chronotype.

Strength and Limiations of the Review

The strength of this systematic review is the specific inclusion of recent and relevant randomized controlled trials that investigated the temporal effect of exercise performance on critical measures of T2DM management. In addition, findings from this review have specific clinical applicability to the T2DM population given that included participants were restricted to T2DM individuals. Given that the difference in the timing of exercise performance may play a large role in glycemic and insulin variability, the diurnal timing of exercise performance may provide a better understanding of the optimal time for exercise performance to allow for

effective management of glycaemic control in T2DM individuals. A limitations of this review were the exclusion of studies based on langugage (i.e. non-english studies).

The extant literature curently lacks homogenenity with respect to primary outcome measures (i.e. fasting glucose, HbA_{1c}, glucose AUC), analyses of outcome measures (i.e. incremental AUC, total AUC, time-period comprising AUC, number of samples performed during the AUC), types of exercise interventions (frequency, intensity, type and duration of exercise), specific timing of exercise performance and the reporting of outcome measures at different time-points. Additionally, the findings of this systematic review are reliant on the quality of the included studies, whereby, the majority of the studies were deemed unclear during risk of bias assessment (Appendix F) and female participants were underrepresented in the included studies (i.e. males vs females: 83% vs. 17%). The limitations of these prior studies precluded the performance of a meta-analysis. With respect to exercise training, it is noteworthy that acute changes in glycemic control and insulin responses may not translate to chronic responses assocaited with longer-term interventions. It is recommended that future research investigates the longer-term (>12 weeks) effect of manipulating exercise-timing relative to diurnal timing (i.e., morning versus evening exercise interventions), and adopt an exercise-intervention based on current best-evidence (i.e., supervised combination of aerobic and resistance-based exercise; at least 50 min per session, three times per week) with clinically relevant outcome measures (fasting glucose; oral glucose tolerance test; HbA_{1c}) in individuals with T2DM.

3.6 Conclusions

Despite mechanistic rationale for an optimal diurnal timing of exercise from concurrent research in the exercise and circadian clock literature (25-28), the current evidence from RCTs is not sufficient to state whether morning or evening exercise is superior in improving glycemic

control. Indeed, there are no studies which have directly assessd this question, likely due to heterogenous methodology. Whether the strategic manipulation of exercise-timing results in clinically meaningful changes in glycemic control (i.e. HbA_{1c}) for individuals with T2DM is currently not known and requires investigation.

3.7 References

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Chapter 4 The impact of exercise timing on glycaemic control: A randomised controlled trial

Teo SYM, Kanaley JA, Guelfi KJ, Marston KJ and Fairchild TJ (2018). The impact of exercise timing on glycaemic control: A randomised controlled trial. *Submitted to Medicine and science in sports and exercise*.

4.1 Abstract

Despite acknowledgement of exercise as a cornerstone in the management of Type 2 diabetes mellitus (T2DM), the importance of exercise timing has only recently been considered. **Purpose:** This study sought to determine the effect of diurnal exercise timing on glycemic control in individuals enrolled in a 12-week supervised multi-modal exercise training program. A secondary aim was to determine the effect of diurnal exercise timing on the circadian rhythm of wrist sin temperature. **Methods:** Forty sedentary overweight men (n=17) and women (n =23) with and without (n=20) T2DM diagnosis (age: 51±13 years; BMI: 30.9±4.2 kg/m^2) were randomly allocated to either a morning (amEX) or evening (pmEX) exercise training group. The supervised 12-week (3 days/week) program, comprised 30 minutes moderate intensity walking and 4 resistance-based exercises (3 sets, 12-18 repetitions each). Glycemic outcomes (glycated haemoglobin, HbA_{1c}; fasting glucose, FG; fasting insulin, FI; homeostasis model assessment of insulin resistance, HOMA2-IR), body anthropometrics (weight, BMI; body fat), cardiorespiratory fitness (CRF) and wrist skin temperature were assessed at baseline and post-intervention. **Results:** No significant group (amEX versus pmEX) by time interactions were observed for any glycemic variables (all $p \geq 0.42$). However, exercise training improved (main effect of time: all $p < 0.01$) HbA_{1c}, FG, FI and HOMA2-IR. Exercise training significantly reduced body fat and increased CRF. There were no significant interaction effects or main effects of time observed in any of the circadian parameters assessed in the current study. **Conclusions:** Twelve weeks of multi-modal exercise training improves glycemic control in overweight non-T2DM and T2DM individuals. Under free-living conditions, no distinct glycemic benefits were associated with three exercise-training sessions performed either in the morning or evening and did not appear to alter circadian rhythm.

Key Words: diurnal timing, diabetes mellitus, glycemic control, insulin sensitivity

Trial Registration: ANZCTR ACTRN12616001172493

4.2 Introduction

Declining rates of physical activity (PA) have partially contributed to the increased prevalence in obesity over the past few decades (1, 2). This is concerning since obesity is acknowledged as the primary risk factor for the development of Type 2 diabetes mellitus (T2DM) (3) and a low level of PA is an independent risk factors for T2DM (4-6). Epidemiological evidence has shown vigorous physical activity performed at least once per week, can reduce the (up to) 8-year incidence of T2DM in both women (Age-adjusted Relative Risk, 0.67; Age and body-mass index adjusted Relative Risk, 0.84) (5) and men (Age-adjusted Relative Risk, 0.64; Age and body-mass index adjusted Relative Risk, 0.71) (6)) relative to no vigorous physical activity. Consequently, physical inactivity is regarded as an important modifiable risk factor for T2DM (7), with an increase in both general physical activity and scheduled exercise considered to be important adjuncts in both the prevention and the treatment of T2DM (8).

Exercise improves glycemic control via both local acute skeletal muscle responses and chronic adaptations (9), as well as systemic responses and adaptations in hepatic, neural, immune, endocrine and metabolic factors (10). Each of these responses are in turn moderated by the intensity, duration and type of exercise performed, and when embedded within a training program, these responses are moderated by the frequency of exercise (1, 11). The timing of exercise relative to meal ingestion has now emerged as potentially moderating the effect of exercise on glycemic control (12-17), with postprandial exercise appearing to be most beneficial to glycemic control. Whether diurnal exercise-timing (i.e., morning vs. evening) effects glycemia or glycemic control, has to the best of our knowledge, not been directly

compared. Therefore, the primary aim of this study was to determine the impact of diurnal exercise timing within the context of a 12-week supervised multi-modal exercise training program on glycemic control in sedentary, overweight individuals both with and without T2DM. Given the evidence that demonstrate a higher glucose tolerance occurring in the morning than in the evening in healthy individuals (12, 13), we hypothesised that morning exercise would result in greater improvements in glycemic control than exercise performed in the evening.

In a parallel line of research investigating the role of the circadian system in metabolic disturbance (i.e., obesity and T2DM) (18-22), exercise has been identified as a stimuli capable of affecting the circadian system (19-21). The circadian system in humans is complex, comprising a network of cellular clocks which possess the ability to autonomously maintain a circadian rhythm (20, 21). Despite their autonomous ability, the alignment of these clocks to both external cues (zeitgebers) such as light and dark cycles, as well as to each other (i.e., the clocks between tissues) is important (20, 23). Specifically, misalignment/disruption of these clocks may result in metabolic disturbance, while metabolic disturbance may lead to misalignment in clocks (18-20). Exercise (physical activity) is an important external cue able to shift the circadian rhythm (e.g., clock gene expression) in both the suprachiasmatic nucleus (SCN; central clock) of the hypothalamus (24) and skeletal muscle (19, 21) and if consistently maintained may entrain or align these clocks. A secondary aim of the study therefore, was to determine whether three days per week of exercise, performed either in the morning or evening is sufficient to shift the phase of the circadian rhythm of body temperature measured at the skin (25). We hypothesised that exercise would result in skin temperature entrainment, as evidenced by divergent responses in morning- and evening-exercise, respectively.

4.3 Methods

Using a parallel study design, participants were randomly allocated into 12-week multi-modal exercise training intervention either performed in the morning (amEX) or in the evening (pmEX). The study was approved by Murdoch University Human Research Ethics Committee, Western Australia, and written informed consent was obtained from all participants prior to commencement of the study. All investigations were conducted according to the principles expressed in the Declaration of Helsinki. The CONSORT checklist is available as supporting information (Appendix H).

Study Participants

The study recruited non-smoking, sedentary (< 150 min of exercise per week), overweight (BMI ≥ 27kg/m^2) men and women between the ages of 18 and 65 years. Participants were not eligible for this study if they were unable to exercise or had a condition known to be aggravated by exercise assessed using the Exercise and Sports Science Australia pre-exercise screening tool. In addition, participants were excluded if they: (i) were using insulin; (ii) have had surgery for weight loss; (iii) had prior history of heart, lung, kidney, endocrine or liver disease and; (vi) experienced recent weight loss ≥4kg in previous month. Participants with T2DM were allowed to continue their oral hypoglycemic medications at the usual dose, frequency and time while participating in the study.

Using a medium effect size (f = 0.25), a sample size of 34 participants was deemed sufficient to provide 80% power to detect (α-error probability value set at .05) within-between interactions (Two groups: amEX, pmEX) using measures taken at pre- and post-intervention time-points. To account for possible attrition, we increased the target sample size to forty participants (Appendix J). Participants were recruited via public advertisements and enrolled

between October 2016 and August 2017 and followed up until December 2017. The primary investigator of the study (ST) completed the recruitment of participants.

Experimental Procedures

At baseline, participants attended the Murdoch University Exercise Physiology laboratory after an overnight fast during which body anthropometrics (body composition, height, weight and waist circumference) were measured along with the assessment of peak oxygen consumption ($VO_{2\ peak}$). Additionally, venous blood samples were drawn for the assessment of glycosylated haemoglobin, glucose and insulin. Participants were then fitted with an accelerometer (Actigraph) and a wrist skin temperature device (Thermochron iBotton DS1922L, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) for 7 days prior to commencement of the intervention (exercise program). Thereafter, participants were assigned in a balanced treatment allocation ratio (i.e., 1:1) into either the amEX or pmEX training groups. The allocation to the training groups was completed in a blinded fashion using a computer-generated numbered list consisting of 1's and 2's that represented the amEX and pmEX groups, respectively. Each participant was assigned with a unique study ID for identification and allocation purposes. This ID was forwarded to an independent investigator (TF; blinded to the identity of the participants) who assigned each ID to a training group using randomly permutated blocks (each block n = 6-12; http://www.randomisation.com) with males and females counterbalanced across groups via generation of two separate lists (one for allocation of males; one for allocation of females). Mid-intervention $VO_{2\ peak}$ assessment was completed in Week 6 to determine improvements in participant's fitness levels and to adjust the training workload accordingly. In Week 12-13, participants completed their post-intervention assessments, which were identical to the baseline assessments, at least 24 h after the last training session, but no more than 96 h after the last training session.

Exercise Intervention

All participants completed three supervised (by a trained exercise physiologist) exercise training sessions per week, for a total of 12 weeks, at the Strength and Conditioning laboratory in Murdoch University. Participants in both the amEX and pmEX groups completed their training sessions between 0800-1000h and 1700-1900h, respectively. Participants were required to consume a snack/meal at least 1 hour prior to the start of each training session. Each training session consisted of both an aerobic (AER) and resistance (RE) exercise component, with an approximate session duration of 60 min. Each training session started off with the AER which consisted of 30 min of treadmill walking at 60-70% of $VO_{2\ peak}$. This intensity was prescribed in accordance to the American Heart Association scientific statement (26). Thereafter, participants performed four different RE involving the major muscle groups (i.e. Leg press, Bench press, Military press and Lat-pulldown). Three sets of each exercise were performed at 45%, 50% and 55% of individually tested one repetition maximums (1RM) for 18, 15 and 12 repetitions, with 60 s of rest between sets during Week 1 – 4, Week 5 – 8 and Week 9 – 12; respectively. These training intensities were shown to be effective in improving glycemic control with no adverse events being reported other than mild muscle soreness in obese and/or elderly diabetic patients (27). Prior to the commencement of the training intervention, the 1RM for each participant had to be determined. The exercises in the 1RM test were completed in the following order: Leg press, Bench press, Lat-pulldown and Military press. Two warm up sets (1^{st} set: 10 repetitions; 2^{nd} set: 5 repetitions) were completed with 2 minutes rest in between sets. Thereafter, participants attempted their 1RM for each exercise with 3 minutes recovery between each sets and 5 minutes recovery between exercise.

Outcome measures

The main outcome measures of the study were the change in glycemic control (HbA_{1c} and FG), insulin sensitivity (FI and HOMA2-IR) and fructosamine (as a short-term marker of glycemic control). The secondary outcome measures included changes in body anthropometrics, $VO_{2\ peak}$ and skin temperature (at wrist). Assessors of outcome measures were blinded to the treatment allocations.

Biochemical analyses. Glycosylated haemoglobin (HbA_{1c}) was measured by an independent pathology laboratory (Western Diagnostic Pathology, Perth, Western Australia), while plasma glucose (FG) and fructosamine (FRA; unadjusted for serum albumin) were measured using a COBAS analyser (COBAS Integra 400 plus, Roche Diagnostics Ltd, Switzerland). Plasma insulin (FI) was measured using enzyme linked immunoassay (Mercodia; Uppsala, Sweden). The computerized homeostatic model assessment (HOMA2-IR) was used as a surrogate measure of insulin resistance based on fasting glucose and insulin concentrations (28).

Body weight, body composition and cardiorespiratory fitness. Body weight was calculated using a calibrated electronic digital scale and body composition was measured using dual-energy X-ray absorptiometry (DXA) to assess for total body fat mass (BF) and fat free mass (FFM). Waist circumference was measured on a horizontal plane at the narrowest point between the lower costal border (10^{th} rib) and the uppermost lateral border of the iliac crest. Cardiorespiratory fitness (peak oxygen consumption; $VO_{2\ peak}$) was measured using a treadmill walking test protocol whilst simultaneously breathing through a mask connected to a metabolic cart (ParvoMedics TrueOne 2400) via plastic tubing. Rating of perceived exertion and heart rate were recorded throughout the test. The treadmill test protocol started at a speed of 3.5 km/hr at 0% incline, with the speed progressively increasing by 1 km/hr every 2 minutes. Once

a speed of 6.5km/hr was achieved, incline was increased by 2% every 2 minutes while the speed was maintained at 6.5 km/hr throughout these stages.

Peripheral skin temperature. Wrist skin temperature was continuously recorded (Thermochron iBotton DS1922L) for 7 days pre-intervention (prior to exercise training starting) and at the post-intervention time point (>24 h after the final training session). The use of iButtons for human skin temperature measurement has been previously reviewed (25) and shown to have an accuracy of -0.09°C with a precision of 0.05°C. For this study, the iButton resolution was set at 0.0625°C, with sampling every 15 min and the real-time clock synchronized with that of the computer.

Missing data and recording artefacts (recordings of skin temperature under 28°C) from the iButton were excluded from the analysis (less than 3% of the readings). The data from each participant collected across the 7 days of recording at the pre-intervention time period, and the post-intervention time period were then analysed for four rhythmic parameters (MESOR, rhythm adjusted mean; amplitude, half the range of daily excursions; acrophase, time of the daily peak; period, duration of each cycle) using the Cosinor software (available at: https://www.circadian.org/softwar.html). All data are presented relative to local time to minimise possible errors from the sleep-onset data recorded from the Actigraph.

Statistical Analyses

Statistical analyses were performed using SPSS (v.24, IBM, Chicago, IL, USA). Treatment effects were estimated using linear mixed models (LMM) to assess for any changes over time (pre- and post-intervention) in the primary and secondary outcome measures between the two intervention groups (amEX and pmEX). The primary hypothesis of interest was the group by

time interaction, which were modelled as fixed effects with a random intercept (to account for differences at baseline) and these were examined with pairwise comparisons of the estimated marginal means. Since this trial included only active comparators (i.e. amEX and pmEX), main effects for time were also of interest, and these were examined using pairwise comparisons of the estimated marginal means. Bivariate regressions were assessed using a Pearson correlational analysis (r_p). Statistical significance was set at $p < 0.05$. The magnitude of change for each outcome measure was reported using Hedge's *g*, and interpreted as small ($g = 0.2$), moderate ($g = 0.5$) or large ($g = 0.8$) (29). Data are presented as means ± standard deviation.

4.4 Results

Forty adults with (n = 20) or without T2DM (n = 20) diagnosis completed the study (Table 4-1; Appendix J). At baseline, there were no significant differences between the groups (Table 4-1; all $p \geq 0.25$), with the exception of age (amEX: 57 ± 5 years vs. pmEX: 51 ± 13 years; $p = 0.04$). No significant differences ($p = 0.48$) were reported for the adherence rate between amEX (32 ± 2 out of 36 sessions) and pmEX (31 ± 2 out of 36 sessions) during the 12-week training intervention.

Biochemical Analysis

There were no significant interaction effects between group (amEX, pmEX) and time (pre- and post-intervention) observed in any of the glycemic control measures (all $p \geq 0.42$; Table 4-2). However, significant improvements were observed in each of the primary outcome measures (HbA_{1c}, FG, FI, HOMA2-IR, fructosamine) in response to the exercise training (main effect of time, all $p < 0.01$), with effect sizes ranging from 0.23 to 0.9 in the amEX group and ranging

Table 4-1. Baseline characteristics

	amEX Group (n = 20)	pmEX Group (n = 20)	*p* value
Males/Females (n)	9/11	8/12	NA
T2DM (n)	10	10	NA
T2DM Duration (years)	13 ± 1	13 ± 2	0.62
Antidiabetic Medication (n)			
Metformin	6	6	
Thiazolidinediones	2	1	
Sulfonylureas	3	2	
Meglitinides	0	1	
Age (years)	57 ± 5	51 ± 13	0.04*
HbA1c (%)	6.9 ± 1.2	6.8 ± 1.7	0.89
HbA1c (mmol/mol)	51.1 ± 13.4	49.9 ± 17.5	0.82
Fasting Glucose (mmol/L)	7.7 ± 2.1	8.3 ± 3.7	0.51
Fasting Insulin (pmol/L)	88.3 ± 33.9	80.9 ± 29.7	0.47
HOMA2-IR	1.8 ± 0.7	1.7 ± 0.7	0.70
Fructosamine (µmol/L)	262.5 ± 45.2	259.8 ± 53.6	0.87
BMI (kg/m^2)	31.2 ± 3.8	30.9 ± 4.2	0.81
Total Body Fat Mass (kg)	27.3 ± 7.9	28.8 ± 7.4	0.55
Total Fat Free Mass (kg)	56.9 ± 12	55.2 ± 9.5	0.61
Total VAT Mass (kg)	9.1 ± 3.5	8.2 ± 2.2	0.32
VO_{2peak} (mL/min/kg)	22.5 ± 6.1	22.8 ± 4.5	0.86
Sleep Onset			
Pre-Intervention (h)	2317 ± 0.33	2301 ± 0.34	0.58
Post-Intervention (h)	2320 ± 0.20	2245 ± 0.03	0.37
Awakening			
Pre-Intervention (h)	0703 ± 0.03	0712 ± 0.90	0.52
Post-Intervention (h)	0657 ± 0.03	0634 ± 0.04	0.22

Data are mean ± SD.

*Significant difference between amEX and pmEX groups ($p < 0.05$).

amEX: morning exercise group; pmEX: evening exercise group; T2DM: type 2 diabetes mellitus; HbA1c: glycosylated haemoglobin; HOMA2-IR: homeostatic model of insulin resistance; BMI: body mass index; VAT: visceral adipose tissue; VO_{2peak}: peak oxygen consumption

Table 4-2. Changes in glycaemic control, insulin sensitivity and physiological measures from baseline to post-intervention for non-T2DM and T2DM individuals

		Overall (n = 40)					**T2DM** (n = 20)				
		amEX (n = 20)	**pmEX** (n =20)	***p* – value**			**amEX** (n = 10)	**pmEX** (n =10)	***p* – value**		
				Group x Time Effect	Time Effect	Group Effect			Group x Time Effect	Time Effect	Group Effect
HbA1c (%)	Week 0	6.85 ± 1.23	6.79 ± 1.66				7.91 ± 0.74	8.04 ± 1.44			
	Week 12	6.58 ± 1.1	6.54 ± 1.46				7.34 ± 0.81	7.64 ± 1.35			
	Change	-0.27 ± 0.24	-0.25 ± 0.23				-0.57 ± 0.13	-0.4 ± 0.12			
	ES	0.23	0.16	0.79	< 0.01†	0.90	0.57	0.32	0.86	< 0.01†	0.79
Fasting Glucose (mmol/L)	Week 0	7.68 ± 1.70	8.28 ± 3.72				9.02 ± 1.33	10.32 ± 4.33			
	Week 12	6.78 ± 1.45	7.10 ± 2.41				7.76 ± 1.33	8.52 ± 2.69			
	Change	-0.9 ± 0.68	-1.18 ± 1.40				-1.27 ± 0.75	-1.80 ± 1.77			
	ES	0.56	0.37	0.42	< 0.01†	0.55	0.91	0.53	0.39	< 0.01†	0.39
Fasting Insulin (pmol/L)	Week 0	88.34 ± 33.94	80.96 ± 29.74				81.75 ± 27.91	85.27 ± 24.58			
	Week 12	64.47 ± 23.45	58.62 ± 22.07				62.57 ± 21.50	64.41 ± 18.92			
	Change	-23.87 ± 27.78	-22.35 ± 22.72				-19.18 ± 17.76	-20.85 ± 17.18			
	ES	0.8	0.83	0.85	< 0.01†	0.40	0.74	1.06	0.83	< 0.01†	0.79
HOMA2-IR	Week 0	1.78 ± 0.66	1.70 ± 0.71				1.74 ± 0.57	1.94 ± 0.7			
	Week 12	1.26 ± 0.46	1.16 ± 0.45				1.28 ± 0.44	1.35 ± 0.4			
	Change	-0.52 ± 0.53	-0.54 ± 0.48				-0.46 ± 0.36	-0.59 ± 0.46			
	ES	0.9	0.9	0.92	< 0.01†	0.58	0.18	0.99	0.49	< 0.01†*	0.54
Fructosamine (µmol/L)	Week 0	262.5 ± 45.2	259.8 ± 53.6				286.5 ± 44.6	287.9 ± 57.7			
	Week 12	227.9 ± 77.8	230.2 ± 61.9				235.2 ± 108.5	267.9 ± 65.4			
	Change	-34.5 ± 78.9	-29.6 ±51.2				-51.3 ± 107.9	-20 ± 61.6			
	ES	0.53	0.5	0.70	< 0.01†*	0.99	0.61	0.32	0.10	0.09	0.50

Overall: combined results of non-T2DM and T2DM individuals; T2DM: only results of T2DM individuals

†Significant difference between baseline and post-intervention ($p < 0.05$). Data are mean ± SD.

T2DM: type 2 diabetes mellitus; amEX: morning exercise group; pmEX: evening exercise group; HbA1c: glycosylated haemoglobin; HOMA2-IR: homeostatic model of insulin resistance; ES: effect size (Hedge's *g*)

from 0.16 to 0.9 in the pmEX group. The HbA_{1c} level was significantly (All $p \leq 0.002$) correlated with FG ($r_p = 0.743$), Fructosamine ($r_p = 0.511$) and HOMA2-IR ($r_p = 0.346$).

Glycemic changes in the sub-cohort of individuals diagnosed with T2DM were assessed following the exercise training (Table 4-2). Similar to the overall cohort, there were no significant time by group interactions (all $p \geq 0.10$), but significant improvements in HbA_{1c}, FG, FI and HOMA2-IR after the 12-week intervention (main effect of time, all $p < 0.01$) were observed. Individual changes—identified by training group and T2DM status—in primary outcome measures (HbA_{1c}, FG, FI, HOMA-IR) are presented in Figure 4-1.

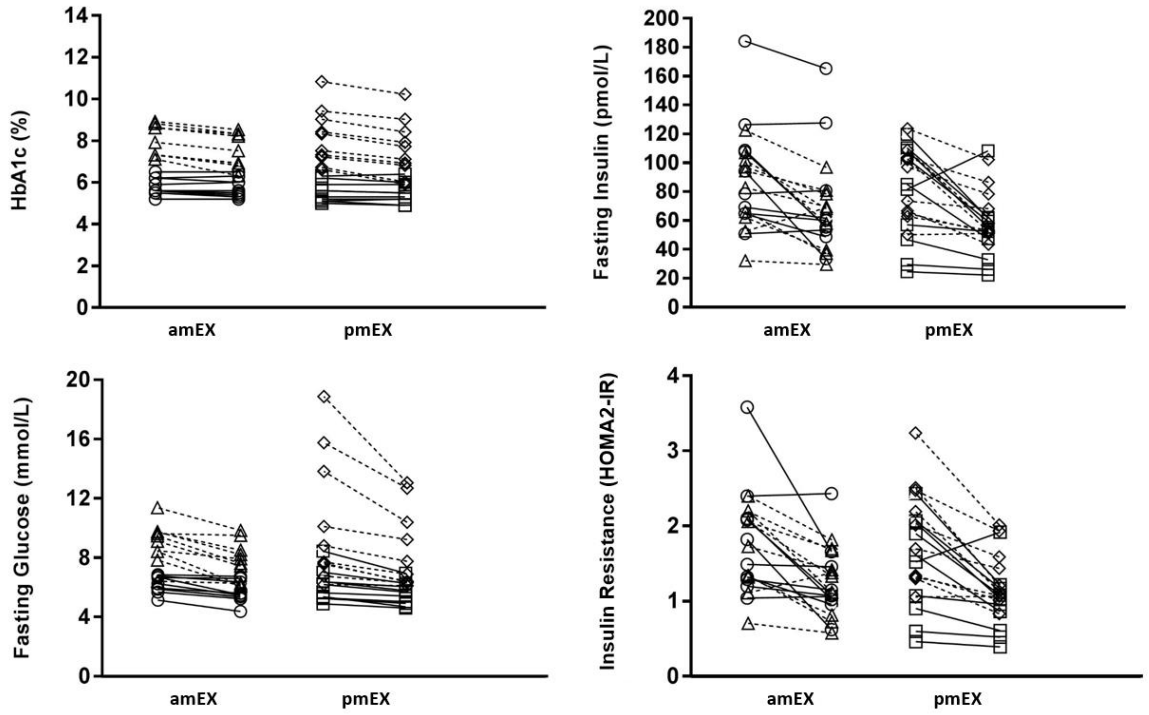

Figure 4-1. Individual changes in measures of glycaemic control and insulin sensitivity for both amEX and pmEX groups from baseline to post-intervention. △: T2DM individuals (amEX); ○: non-T2DM individuals (amEX); ◇: T2DM individuals (pmEX) and; □: non-T2DM individuals (pmEX).

Anthropometric measures and cardiorespiratory fitness

There were no significant time by group interaction effects observed in any measures of body anthropometrics (body weight, BMI, waist circumference, body fat and visceral adipose tissue mass; All $p \geq 0.27$) or $VO_{2\ peak}$ ($p \geq 0.36$) (Table 4-3). There were, however, significant pre- and post-intervention improvements (main effect of time, $p < 0.01$) in $VO_{2\ peak}$ and all anthropometric measures except FFM ($p = 0.22$; Table 4-3).

Secondary Analysis: Assessing glycemic changes in 'Responders' and 'non-responders'

To assess whether individuals demonstrating the greatest response to training were also those demonstrating the greatest improvements in glycemic control, data from all individuals were collapsed across groups (amEX T2DM and non-T2DM; pmEX T2DM and non-T2DM). Response to training was based on an increase in $VO_{2\ peak}$ above 3.5 ml/min/kg (Figure 4-2A) and a decrease in total body fat (kg; ≥ 2.1 kg body fat; Figure 4-2B). While *responders* tended to demonstrate more consistent improvements ($VO_{2\ peak}$ *responders*: $g = 0.18$-0.28, n = 23; total body fat *responders*: $g = 0.16$-0.46, n = 21) these did not reach statistical significance (All $p \geq 0.19$).

Peripheral skin temperature responses

There were no significant time (pre, post) by group (amEX, pmEX) interaction effects observed in any of the rhythmic parameters assessed in the overall cohort (All $p \geq 0.43$; Fig 4-3). When changes in peripheral skin temperature were graphed (Figure 4-3), there appear to be divergent responses to the exercise intervention between ~0630h to ~1430h, with the peripheral skin temperature in the pmEX group tending to decrease during this time period and the peripheral skin temperature increasing in the amEX group. Statistical analysis however, did not confirm this observation in the overall cohort. However, when the rhythmic parameters in only the

T2DM cohort were assessed, a significant interaction effect was observed in the Acrophase parameter (Figure 4-3).

Table 4-3. Changes in body anthropometrics and cardiovascular fitness from baseline to post-intervention in both amEX and pmEX groups

		amEX (n = 20)	**pmEX** (n =20)	***p* – value**		
				Group x Time Effect	Time effect	Group effect
Body weight (kg)	Week 0	84.3 ± 12.6	84.1 ± 14			
	Week 12	82 ± 12.1	81.9 ± 13.5			
	Change	-2.3 ± 1.4	-2.2 ± 1.8			
	ES	0.18	0.16	0.99	< 0.01†	0.97
BMI (kg/m^2)	Week 0	31.2 ± 3.8	30.9 ± 4.2			
	Week 12	30.4 ± 3.7	30.1 ± 4.1			
	Change	-0.8 ± 0.3	-0.8 ± 0.7			
	ES	0.21	0.19	0.92	< 0.01†	0.80
Waist circumference (cm)	Week 0	101 ± 9.9	103.8 ± 11.6			
	Week 12	97.6 ± 9.3	99.5 ± 11.9			
	Change	-3.4 ± 1.8	-4.3 ± 2.9			
	ES	0.35	0.36	0.27	< 0.01†	0.48
Total fat-free mass (kg)	Week 0	56.9 ± 12	55.2 ± 9.5			
	Week 12	57.4 ± 11.6	55.6 ± 9.7			
	Change	0.5 ± 2.7	0.4 ± 1.4			
	ES	0.04	0.04	0.97	0.22	0.60
Total body fat mass (kg)	Week 0	27.4 ± 8	28.8 ± 7.4			
	Week 12	25 ± 8	26.3 ± 7.7			
	Change	-2.4 ± 1.6	-2.5 ± 1.5			
	ES	0.29	0.32	0.72	< 0.01†	0.59
Total VAT mass (kg)	Week 0	0.9 ± 0.3	0.8 ± 0.3			
	Week 12	0.8 ± 0.3	0.7 ± 0.3			
	Change	-0.1 ± 0.1	-0.1 ± 0.01			
	ES	0.36	0.37	0.33	< 0.01†	0.36
VO_{2peak} (ml/kg/min)	Week 0	22.5 ± 6.1	22.8 ± 4.2			
	Week 12	26.5 ± 5.8	26.9 ± 4.1			
	Change	4 ± 1.7	4.1 ± 4.6			
	ES	0.66	0.88	0.89	< 0.01†	0.84

Data are mean ± SD

†Significant difference between baseline and post-intervention ($p < 0.05$)

amEX: morning exercise group; pmEX: evening exercise group; BMI: body mass index; VAT: visceral Adipose Tissue; VO_{2peak}: peak oxygen consumption; ES: effect size (Hedge's *g*)

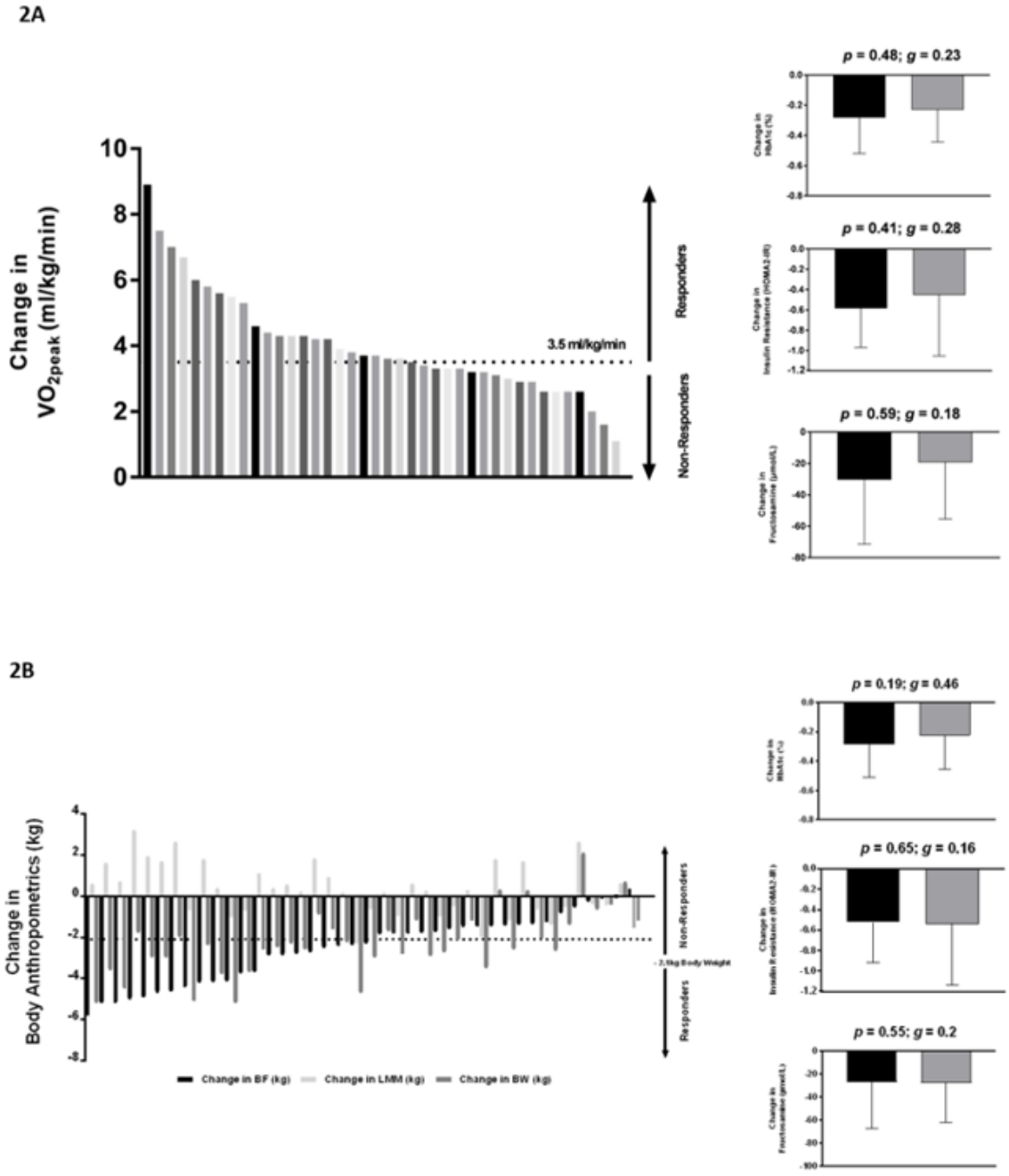

Figure 4-2. Individuals changes in VO_{2peak} (2A) and body composition (2B) from baseline to post-intervention and comparison of changes in HbA1c, insulin resistance and fructosamine between responders (black column) and non-responders (grey column).

	amEX		pmEX		*p*-value		
	Pre	Post	Pre	Post	Time	Group	Interaction
Overall							
MESOR (°C; SD)	33.72 (0.91)	33.92 (0.80)	33.54 (0.60)	33.51 (0.67)	*0.35*	*0.03*	*0.43*
Amplitude (°C; SD)	0.84 (0.33)	0.80 (0.32)	1.09 (0.48)	1.08 (0.41)	*0.88*	*0.14*	*0.84*
Acrophase (SD)	-127.05 (117.17)	-109.11 (92.82)	-69.16 (44.55)	-61.41 (33.35)	*0.37*	*0.14*	*0.74*
Time (h; SD)	8.42 (7.75)	7.24 (6.16)	4.60 (2.96)	4.07 (2.22)	*0.37*	*0.14*	*0.75*
T2D							
MESOR (°C; SD)	33.68 (0.89)	33.99 (0.57)	33.53 (0.61)	33.56 (0.78)	*0.15*	*0.76*	*0.65*
Amplitude (°C; SD)	0.74 (0.33)	0.70 (0.22)	0.97 (0.49)	0.87 (0.29)	*0.48*	*0.41*	*0.74*
Acrophase (SD)	-181.50 (125.38)	-132.60 (118.29)	-43.11 (15.13)	-42.29 (28.27)	*0.53*	*0.01*	*0.01*
Time (h; SD)	12.04 (8.31)	8.80 (7.85)	2.86 (1.00)	2.79 (1.87)	*0.53*	*0.01*	*0.01*

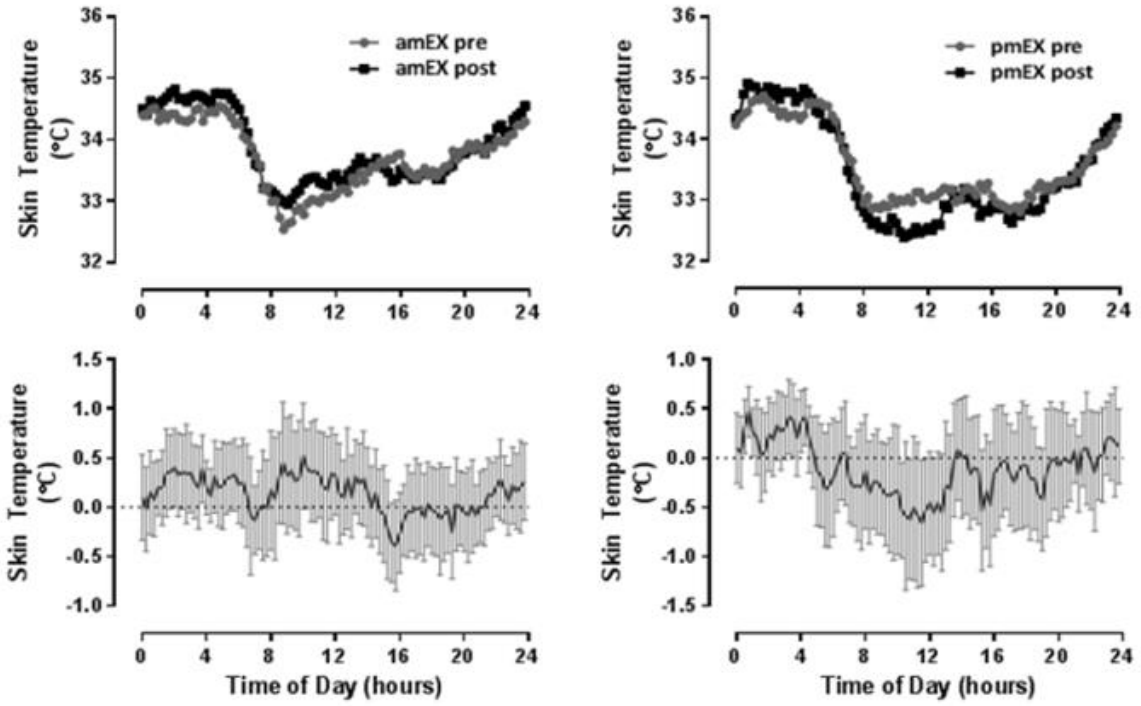

Figure 4-3. Changes in peripheral skin temperature from pre- to post-exercise training. Group mean data are presented in the top panels with difference (95% Confidence Intervals presented in the bottom two panels. Data are presented relative to the time of day (local time).

The rhythmic parameters at the pre-intervention time-point, were compared between non-T2D and T2DM. There were no significant differences between these groups (T2DM; non-T2DM) in either the MESOR (non-T2DM: 33.56 ± 0.79°C; T2DM: 33.65 ± 0.78°C; p = 0.71), Amplitude (non-T2DM: 0.93 ± 0.55°C; T2DM: 1.06 ± 0.40°C; p = 0.36) or the Acrophase (non-T2DM: -112.65 ± 112.72; T2DM: -82.6 ± 64.86; p = 0.32).

4.5 Discussion

The primary aim of this study was to determine the impact of diurnal timing of a multi-modal exercise program on glycemic control in previously sedentary, overweight individuals with and without T2DM. The main finding of this study was that 12-weeks of multi-modal exercise training in overweight T2DM and non-T2DM individuals resulted in significant improvements in measures of glycemic control (HbA_{1c}; Fasting insulin and glucose; HOMA2-IR), an effect which was independent of whether the exercise was performed in the morning (amEX) or evening (pmEX). Similarly, significant improvements in body anthropometrics (body weight, BMI, waist circumference, total body fat mass, VAT mass) and cardiorespiratory fitness ($VO_{2\ peak}$) were observed in response to the exercise training, but these responses were independent of exercise timing. In secondary analyses on *responders* and *non-responders* (according to changes in body-fat mass or $VO_{2\ peak}$), a non-significant trend (small-moderate effect size) was observed which favoured glycemic improvements in responders. A secondary aim of this study was to determine the effect of morning and evening exercise on the circadian rhythm of wrist skin temperature. The results of this study were that three days per week of exercise performed either in the evening or in the morning over the 12 week period did not cause significant changes in the circadian rhythm of wrist temperature.

Effect of exercise timing on glycemic control and insulin sensitivity

Glycemic control incorporates both FG and PPG excursions and may be quantified by HbA_{1c} levels, which together comprise the three components of the glucose triad (30). Accordingly, FG in the cohort recruited to this study explained 55% variance in the HbA_{1c} levels. In response to the 12 week exercise intervention, significant improvements in FG (-1.04 mmol/L) and HbA_{1c} (-0.26%) were observed, but there were no significant group by time interactions (Table 2). This finding suggests that under free-living conditions with no additional lifestyle

modifications (e.g. diet), the diurnal timing of exercise training may not be an important variable to consider, at least in the short-medium term (12 weeks). These findings in the overall cohort were reflected in the T2DM cohort, albeit that improvements in individuals with T2DM were considerably greater (FG: -1.54 mmol/L; HbA_{1c}: -0.45%). These improvements in HbA_{1c} are in agreement with previous findings from meta-analyses in individuals with T2DM (mean-weighted decrease in HbA_{1c} from 0.66% to 0.80%; (31, 32)) and a longer-term (9 month), randomized controlled exercise intervention (within-group HbA_{1c} change: -0.23%; (33)). Interestingly, the greatest within-group benefits in HbA_{1c} (~0.29% reduction) for individuals randomized to the combination exercise group in the study by Church *et. al.* (33), was observed at the 12-16 week training period. With respect to the clinical findings in the current study, it is noteworthy that 8 individuals with T2DM achieved the <7% HbA_{1c} recommendation (34) (pre-intervention: 2/20 individuals achieved recommendation; post-intervention: 10/18 individuals achieved recommendation) and this was independent of the timing of exercise.

Two additional markers of glycemia were included in the current study, the HOMA2-IR and fructosamine. Fructosamine is a short-term (1-3 weeks), non-specific marker of glycation, thereby complementing HbA_{1c} values in short-medium term intervention trials (35). Specifically, fructosamine appears to be a useful marker for assessment of chronic kidney disease, both in individuals with and without T2DM (35). Exercise training had a significant effect on fructosamine levels in the overall cohort, reducing the concentration by 32.05 µmol/L, which is a magnitude similar to that approaching the difference between individuals with or without a history of diabetes (44.2 µmol/L). The HOMA2-IR was also included in the current study and used as a marker of insulin sensitivity (26, 28). The HOMA2-IR is particularly useful in individuals at risk of T2D, and the finding in the current study reflect this with significant improvements observed in the overall cohort (-0.53 $mmol.L^{-1}$) and the largest magnitude

effects observed in this variable from pre- to post-intervention (g = 0.9). This change in the overall cohort largely reflects changes in insulin concentration.

Effect of exercise timing on body anthropometrics and cardiovascular fitness

There were no significant time by group interaction effects in any of the anthropometric measures or in the $VO_{2\ peak}$, indicating that under the current conditions, exercise timing was not shown to play a significant role in observed changes (Table 4-2). This is in contrast to results from Alizadeh et al (36), who showed significantly greater reductions in BMI and body weight after 6 weeks of exercise training (2 times per week) in the morning versus evening in overweight inactive females. The findings in Alizadeh may in part be explained by changes in caloric intake, with participants in the morning group reducing energy intake by a greater amount than participants in the evening group (Morning exercise: 362 kcal reduction from baseline; Evening exercise: 28 kcal reduction from baseline).

There were significant main effects for time in the VO_{2peak} response and all anthropometric measures except total fat-free mass (p = 0.22) in the current study. The observed improvements in body weight, waist circumference and body fat mass were comparable to the improvements reported by Church et al. (33) after 9 months of multi-modal exercise in T2DM individuals. Using a combined exercise training program in T2DM individuals, Yavari et al. (37) reported similar reductions in BMI (-0.8 kg/m^2) after 12-weeks. With regards to cardiorespiratory fitness, Park et al. (38) reported a significant improvement in $VO_{2\ peak}$ (4.6 ml/kg/min) after a 12 weeks combined exercise program in females with abdominal obesity, which were similar to the improvements in VO_{2peak} observed in our study (amEX: 4.0 ml/kg/min; pmEX: 4.1 ml/kg/min). These findings suggest combined exercise training is an effective tool for both the prevention and the management of T2DM, given that excess weight is an established risk factor

for T2D and the association between body fat accumulation and increased insulin resistance (39). It is noteworthy that the magnitude change in waist circumference and measures of fat mass (ES: 0.29 – 0.37) are greater than body weight (ES: 0.16 – 0.18) which can be attributed to the lack of change in total fat free mass (ES: 0.04). Additionally, improvements in $VO_{2\ peak}$ allows for the reduction in the risk of cardiovascular disease (40), given that T2DM individuals between the age of 55 and 60 years have a 10-year CVD risk of 7-11% (41).

The overall cohort were divided into *responders* and *non-responders* to the exercise program according to their improvements in $VO_{2\ peak}$ and total body fat mass. The 3.5 ml/kg/min cut point was based on the associated 10-25% reduction in mortality risk for every 1MET (3.5kg/ml/min) increase in exercise capacity (40). The cut-point (-2.1 kg) to identify *responders* and *non-responders* for total fat mass over 12 weeks was calculated using the following assumptions: ~600 kcal were expended per session, ratio of fat loss and muscle loss was 70:30. These assumptions result in an approximate 250g weight loss (175g fat loss) per week. Direct comparison of these groups, demonstrates favourable glycemic improvements in *responders* versus *non-responders*, but these were not significantly different.

Effect of exercise timing on circadian rhythm of skin temperature

Changes in the circadian rhythm of skin temperature were assessed in response to the exercise intervention, with the hypothesis being divergent responses in the circadian rhythm of wrist skin-temperature. While a circadian rhythm in skin temperature was evident, the rhythm parameters assessed (MESOR, Amplitude, Acrophase) did not demonstrate a significant interaction effect (All $p \geq 0.43$) or a main effect of time (All $p \geq 0.35$). Findings from this study therefore suggest that three supervised exercise sessions per week of moderate intensity adopted in isolation (i.e., no other lifestyle intervention), are insufficient to significantly change

the circadian rhythm parameters as assessed by the cosinor function of wrist skin temperature collected over 7 days. It may be plausible that higher intensity exercise or greater duration of exercise may result in a shift in the circadian rhythm—given that circulating metabolites may alter cellular clocks (and vice versa (19, 23)) and greater intensity is expected to yield greater shifts in metabolites—however, it is more likely that consistent exercise (i.e., increased frequency from 3 sessions per week) performed at a set diurnal time more likely leads to circadian entrainment, although this is an area which requires further work. Further analysis comparing individuals with T2DM and without T2DM did not reveal significant differences in the circadian parameters, excluding Acrophase, despite prior research suggesting reduced amplitudes and elevated MESOR in the circadian rhythm of body temperature (measured via thermometer) in T2DM individuals versus individuals with pre-diabetes (42). In individuals with metabolic syndrome however, reduction in the amplitude of the circadian rhythm of wrist skin temperature was revealed to be associated with triglycerides (43) which explained 33% of the variability in amplitude. Unfortunately the current study did not measure triglyceride concentrations. A plausible explanation for the significant group interaction ($p = 0.01$) observed for changes in Acrophase between the amEX and pmEX may be due to the changes in sleeping patterns associated with the T2DM individuals in each group, which is supported by the significant group interaction ($p = 0.01$) observed with the time component of circadian cycles presented.

Strengths and Limitations

The main strength of the study was implementation of a structured, supervised exercise training program comprising up to approximately 180 min per week of exercise in individuals with T2DM or at risk of developing T2DM. The multi-component (resistance training and aerobic training) exercise training program is associated with the greatest benefits glycemic benefits

(33), adheres with current guidelines (11) and allows modification of the FITT (frequency, intensity, type and time) principle to ensure individualisation (44). Research has shown that structured exercise durations of >150mins allowed for greater reductions in HbA_{1c} when compared to durations of ≤150mins (-0.80% vs. -0.36%, respectively) (45). An additional strength of the study was that every exercise session throughout the training program was supervised. There is evidence that exercise supervision results in improved glycemic control and insulin sensitivity while unsupervised exercise leads to a decline in exercise compliance and glycemic control (46).

While the study had a number of strengths, there were also limitations of the study. The main limitation of this study was the relatively small sample size and the absence of a no-exercise (non-active) control group. There is sufficient evidence demonstrating the benefits of exercise on glycemic control in individuals with T2DM (11) and therefore an active-control group is more appropriate, however, given the nature of the study aim (i.e. whether the timing of exercise is important), an appropriate comparator condition was challenging. Future research may seek to conduct a two-arm randomized controlled trial, where participants cross-over at the completion of the first stage (i.e., morning exercise for 12 weeks followed by evening exercise for 12 weeks). In addition, the size of the sample was not sufficient to allow sub-cohort analyses (i.e. four sub-groups: pmEX OW, pmEX T2DM, amEX OW, amEX T2DM) or explore individual differences more thoroughly using advanced multiple linear regression analyses. The absence of dietary control, and specifically controlling the diurnal timing of meal ingestion (beyond the postprandial-exercise requirement), may have masked the magnitude-change observed. In order to minimise potential confounders within the study (i.e., not introducing additional requirements) participants were asked to maintain their usual dietary

habits, however, change in circadian rhythm may require a concerted effort on both diet and exercise.

4.6 Conclusions

This study showed that 12-weeks of multi-modal exercise training can improve glycemic control, cardiorespiratory fitness and body-composition in overweight individuals with and without T2DM. This improvement occurs independent of exercise timing (morning or evening) and without additional dietary restriction. Current evidence supports an important role for exercise volume (47) and exercise intensity (11) in improving glycemic control for individuals with T2DM, and a burgeoning body of literature suggests a role for the timing of exercise relative to meal ingestion (13-17) on glycemic control, however, the evidence does not currently support an important role for manipulating the diurnal timing of exercise. It is important to note that the circadian rhythm of skin temperature was not affected under the conditions adopted in the current study. It may therefore be necessary for future research to increase the volume of morning or evening exercise to determine whether exercise alone is sufficient to entrain the circadian rhythm and how this may impact glycemic control. Additionally, simultaneous manipulation of the timing of meals (beyond the meal immediately preceding exercise) with the timing of exercise may help entrain the circadian system and ultimately glycemic control.

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Chapter 5 Effect of diurnal exercise timing on postprandial glucose responses: A randomised controlled trial.

Teo SYM, Kanaley JA, Guelfi KJ, Marston KJ and Fairchild TJ (2018). Effect of diurnal exercise timing on postprandial glucose responses: A randomised controlled trial. *Submitted to Medicine and science in sports and exercise*.

5.1 Abstract

Postprandial exercise has been shown to improve postprandial glucose (PPG) response to a greater degree than preprandial exercise, suggesting an important, yet under-acknowledged role for exercise timing on glycaemic control. Despite this accumulating evidence, the diurnal impact of exercise performance on glycaemic control remains unknown. **Purpose:** This study aimed to determine the diurnal effect of exercise timing on PPG response in individuals enrolled into a 12-week supervised multi-modal exercise training program. **Methods:** Forty sedentary overweight individuals (17 males, 23 female; age: 51 ± 13 years; BMI: 30.9 ± 4.2 kg/m2) with (n = 20) or without T2DM diagnosis were randomly allocated to either a morning (amEX) or evening (pmEX) exercise training group. All participants completed a 12-week supervised multi-modal exercise training program (3 days per week), which consisted of 30 minutes of aerobic exercise (walking protocol) and 4 resistance-based exercises (3 sets of 12-18 repetitions). The amEX and pmEX training sessions occurred between 0700-0900h and 1700-1900h, respectively. Changes in postprandial glucose (PPG) and insulin (PPI) responses, along with incremental area under the curve (iAUC), during a mixed meal tolerance test (MMTT) were the primary outcome measures of the study and were assessed at baseline and post-intervention at 12 weeks. All data is displayed as mean differences ± SD. **Results:** Exercise training reduced (main effect of time, $p < 0.01$) PPG and PPI responses during the MMTT, with no group differences observed (all $p \geq 0.65$). Reductions in PPI iAUC (main effect of time, $p < 0.01$) were observed at post-intervention, with no group differences reported ($p = 0.2$) **Conclusions:** Irrespective of the diurnal timing of exercise performance, 12-weeks of multi-modal exercise training significant improved PPG and PPI responses in both overweight non-T2DM and T2DM individuals.

Key Words: diabetes mellitus, postprandial, diurnal timing, multi-modal

Trial Registration: ANZCTR ACTRN12616001172493

5.2 Introduction

Skeletal muscle glucose uptake increases during and immediately after acute exercise via non-insulin dependent pathways, and this can persist for up to 48 h following exercise via insulin dependent pathways (1). When exercise is performed close to carbohydrate ingestion the increased skeletal muscle glucose uptake can cause a decrease in systemic glucose concentration relative to no exercise taking place (2-5), although this is not always the case (6-10) that the systemic glucose concentrations may remain stable or even be elevated by exercising close to carbohydrate intake are surprising. A number of detailed mechanistic studies have explored this issue and have shown that the increase in skeletal muscle glucose uptake (5, 10, 11) can be partly or fully compensated by an increase in endogenous glucose release (11) from the liver and an increased splanchnic release of exogenous glucose (10-13). The timing of exercise relative to meal ingestion has therefore become an area of interest (14-16) which has culminated in a number of reviews (17-20) suggesting improved postprandial glucose (PPG) responses when exercise is performed in the postprandial period (≤30 min post-ingestion). These acute benefits of postprandial exercise (2-5, 14, 16) have been shown to translate to longer-term improvements in glycemic control (20, 21) and PPG responses (22).

Effective management of glycemia in individuals with type 2 diabetes mellitus (T2DM) requires maintenance of circulating glucose within tight limits which is achieved via reducing both fasting glucose (FG) as well as the PPG excursions (23). The importance of managing PPG excursions throughout the day in individuals with T2DM or individuals at risk of developing T2DM, are increasingly acknowledged (24-31). Given exercise improves

glycaemic management via direct effects on both FG and PPG (32), a principal recommendation for management of individuals with T2DM is to increase exercise volume (duration and frequency) (23), intensity—with due consideration—and adopt multiple exercise types (33). Lifestyle interventions, comprising both dietary and physical activity education in individuals at risk of developing T2DM (overweight with impaired glucose tolerance), have demonstrated a 58% relative risk reduction in developing T2DM compared to 31% reduction through medication (31), while a 'physical-activity-only' intervention, resulted in a 46% relative risk reduction in development of T2DM in an at-risk cohort has been observed (34).

It is noteworthy the PPG response in prior research is classically assessed using a single meal (i.e., oral glucose tolerance test) and often using only a single time-point (e.g., 2 h post ingestion, (22)). However, the PPG response to one meal has been shown to be affected by prior food ingestion, such that the PPG response is blunted in a subsequent meal relative to the first meal (35-38). This response, coined the *second meal phenomenon*, is moderated by the composition of the meals (35), is evident in individuals with and without T2DM (35, 37, 39-41) and whilst likely moderated by diurnal changes in insulin sensitivity, occurs in the morning and evening (42, 43). Additionally, the *second meal phenomenon* is acutely moderated by exercise performed in the postprandial state between the first and second meal (37, 44). Specifically, the PPG response to the second meal has been shown to increase when exercise was performed relative to no exercise, indicating the second meal phenomenon may be acutely reduced with exercise (37, 44).

Given the associated benefits of exercise in individuals with T2DM (23) and individuals at-risk of developing T2DM (34), the aims of the current study were to determine the effects of a 12-week supervised exercise training program performed in the postprandial period on

glycemic control and the PPG response to two mixed-meal challenges. A second aim of this study was to determine the effect of conducting the exercise training at two different diurnal times (i.e., morning versus evening) on the habitual PPG response. We hypothesised that (i) the 12-week exercise training would improve glycemic control as measured by glycosylated haemoglobin (HbA_{1c}), as well as the fasting glucose and PPG (measured by the glucose area under the curve, glucose-AUC); (ii) that the improvements in HbA_{1c} would be more strongly associated with improvements in the total PPG response, than changes in fasting glucose; (iii) PPG and insulin responses would be improved to a greater degree in individuals performing the exercise training in the evening than compared to individuals performing the exercise training in the morning.

5.3 Methods

Experimental Design

This single blind, parallel-group clinical trial in overweight individuals with or without T2DM was conducted between October 2016 and December 2017 at Murdoch University (Western Australia). Findings from the study are currently under review (45) and details of the study design have been previously published (Trial Registration: ANZCTR ACTRN12616001172493).

Participants were randomly assigned to the morning (amEX) or evening (pmEX) training groups by an investigator (TF) using a unique study I.D. Specifically, a numbered list consisting of 1's and 2's was computer-generated using randomly permuted blocks (each block n = 2-6; http://www.randomisation.com) for males (with and without T2DM) and females (with and without T2DM). The final group allocation remained sealed in the envelope and revealed

only prior to the first training session by an independent individual (KM; blinded to the recruitment process). Recruitment for individuals with (n=20) and without T2DM (n=20) was closed when the respective numbers were achieved. Written informed consent was obtained from all participants prior to commencement of the study. All investigations were conducted according to the principles expressed in the Declaration of Helsinki, and the conduct of this study was approved by the Murdoch University Human Research Ethics Committee, Western Australia. The CONSORT checklist is available as supporting information (Appendix I)

Study Participants

Sedentary overweight males and females (defined as accrual of < 150min of exercise/week with a BMI ≥ 27kg/m^2) between the ages of 18-65 years both with and without a T2DM diagnosis were eligible for participation in the study. Individuals were not eligible for this study if they were unable to complete exercise or have a condition which is known to be aggravated by exercise assessed using the Exercise and Sports Science Australia pre-exercise screening tool. In addition, the exclusion criteria included the use of insulin, having had surgery for weight loss, previous history of heart, lung, kidney, endocrine or liver disease and recent weight loss ≥ 4kg in previous month.

Experimental Protocol

Participants were assessed at baseline (Week 0) and post-intervention (Week 12). The primary outcome measures of the study were the changes in postprandial glucose (PPG) and insulin (PPI) responses. Specifically, the maximum (Max, represented by the peak), the range (Max – Min, where Min represents the nadir), the AUC (4-h, 2-h) and the incremental AUC for glucose and insulin were the variables of interest in response to ingestion of a mixed meal tolerance test (MMTT). To assess the relative contributions of PPG to glycaemic control, associations

between glycosylated haemoglobin (HbA_{1c}), fasting glucose (FG) and the glucose-iAUC and glucose-AUC were analysed. Body anthropometrics and cardiovascular fitness were presented herein for descriptive purposes and secondary analyses comparing *responders* to *non-responders*. All participants continued with their normal daily dietary intake and oral hypoglycaemic medications (T2DM individuals) while participating in the study.

Exercise Intervention

Participants from both conditions completed three individually supervised (trained exercise physiologist) exercise training sessions per week, for a total of 12 weeks, at the Strength and Conditioning laboratory in Murdoch University. Participants in both the amEX and pmEX groups completed their training sessions between 0800-1000h and 1700-1900h, respectively. Participants were required to consume a snack/meal at least 1 hour prior to the start of the each training session.

Each training session consisted of both an aerobic (AER) and resistance (RE) exercise component, with an approximate session duration of 60 min. Each training session started off with the AER which was a walking protocol that was completed on a treadmill at 70% VO_{2max} for 30 min. This intensity was prescribed in accordance to the American Heart Association scientific statement (46). Thereafter, participants performed four different RE involving the major muscle groups on both the weight machines and free weights (i.e. Leg press, Bench press, Military press and Lat-pulldown). Three sets of each exercise were performed at 45%, 50% and 55% of 1RM for 18, 15 and 12 repetitions with 60s of rest in between sets during Week 1 – 4, Week 5 – 8 and Week 9 – 12; respectively. These training intensities has been shown to be effective in improving glycaemic control, with no adverse events being reported other than mild muscle soreness in obese and/or elderly diabetic patients (47).

Mixed Meal Tolerance Test Procedure

Participants were requested to refrain from any physical activity 24h prior to the MMTT. After an overnight fast, participants arrived at the Murdoch University Exercise Physiology laboratory between 7:00am and 7:30am. Upon arrival, a venous catheter for was inserted into the forearm vein for blood sampling. Thereafter, participants were given a 4-h meal challenge (Meal 1 and Meal 2). The standardized meals provided during the MMTT were liquid SUSTAGEN® Diabetic beverages (1057kJ; 52% carbohydrate, 22% fat and 24% protein) containing approximately 25g of carbohydrate. Blood samples were collected for Meal 1 (0, 15, 30, 45, 60, 90 and 120 min) and Meal 2 (130, 150, 165, 180, 195, 210, 225 240 min) for plasma glucose and insulin measurements (Appendix K)

Biochemical Analyses

The fasting blood samples collected prior to the ingestion of each 4-h meal challenge (pre-training, post-training) were used to assess HbA_{1c} via an independent, commercial pathology laboratory (Western Diagnostic Pathology, Perth, Western Australia). The assessment of glucose and insulin were conducted on plasma samples, which was separated by centrifugation at 1300 RCF (relative centrifugal force) for 10min immediately following blood collection and stored at -80^0C until analysis. Plasma glucose was measured using the COBAS analyser (COBAS Integra 400 plus, Roche Diagnostics Ltd, Switzerland). Plasma insulin (FI) was measured according to kit instructions using an enzyme-linked immunoassay (Mercodia; Uppsala, Sweden).

Body anthropometric and maximal oxygen consumption assessments

Body mass was calculated using a calibrated electronic digital scale and body composition was measured using dual-energy X-ray absorptiometry (DXA) to assess total body fat mass (BF) and fat free mass (FFM). Waist circumference was measured on a horizontal plane at the narrowest point between the lower costal border (10^{th} rib) and the uppermost lateral border of the iliac crest.

Cardiorespiratory fitness (VO_{2max}) was measured during a modified Bruce treadmill test protocol by breath-by-breath analysis of oxygen consumption and carbon dioxide production (ParvoMedics TrueOne 2400) as described previously (45). Attainment of VO_{2max} was accepted when two of three criteria were met: (i) a plateau in VO_2; (ii) a respiratory exchange ratio > 1.15 and/or; (iii) volitional exhaustion.

Sample Size

Prior to the commencement of the trial, we determined that a sample size of 34 participants would provide 80% power to detect a difference (Effect size, f = 0.25; repeated measures, within-between interaction) in PPG and glycaemic control where the α-error probability value was set at 0.05. To account for a potential attrition, the sample size of 40 was deemed to provide sufficient power to assess differences in these variables. The primary investigator of the study (ST), through public advertisements, recruited potential participants.

Data Analysis

Surrogate markers of muscle and liver insulin sensitivity were adopted using glucose and insulin concentrations (48). Muscle insulin sensitivity was calculated according to the slope (dG/dt) represented by the line of the least square fit from the peak to nadir glucose

concentration, divided by the mean plasma insulin concentration (herein calculated as: insulin-AUC/time). Hepatic insulin sensitivity was calculated according to the $glucose_{0\text{-}30}AUC$ multiplied by the $insulin_{0\text{-}30}AUC$.

Secondary analysis of *responders* and *non-responders* were based on all participants (n=40). Participants were stratified into respective *responder* and *non-responder* categories according to changes in $VO_{2\ peak}$ and body-fat. An increase in $VO_{2\ peak}$ above 3.5 ml/min/kg and a decrease in total body fat (kg; ≥ 2.1 kg body fat) was used as the *responder/non-responder* cut-point as previously described (45).

All statistical analyses were performed using SPSS (v.24, IBM, Chicago, IL, USA). Treatment effects were estimated using linear mixed models (LMM) to assess for any changes over time (pre- and post-intervention) in the primary and secondary outcome measures between the two intervention groups (amEX and pmEX). The hypothesis of interest was the group by time interaction (modelled as fixed effects; random intercept) which was examined with pairwise comparisons of the estimated marginal means. The magnitude of change for each outcome measure was reported using Hedge's g, and interpreted as small (g = 0.2), moderate (g = 0.5) or large (g = 0.8) (49). Pearson bivariate correlations and hierarchical linear regression models (two-step) adopting both forced and stepwise methods of entry were used to explore associations of interest. Statistical significance was set at $p < 0.05$. All data are presented as means ± standard deviation.

5.4 Results

As previously reported (45), sedentary overweight males (n = 17) and females (n = 23) (age: 51 ± 13 years; BMI: 30.9 ± 4.2 kg/m^2) with (n = 20) or without T2DM diagnosis completed the study with no drop-outs or adverse events reported (Appendix J). No significant between group differences were reported at baseline for T2DM duration (amEX: 13 ± 1 y; pmEX: 13 ± 2 y; p =.62), HbA_{1c} (amEX: 6.9 ± 1.2 y; pmEX: 6. 8± 1.7 y; p =.89), HOMA-IR (amEX: 1.8 ± 0.7 y; pmEX: 1.7 ± 0.7 y; p =.70), BMI (amEX: 31.2 ± 3.8 y; pmEX: 30.9 ± 4.2 y; p =.81), total body fat mass (amEX: 27.3 ± 7.9 y; pmEX: 28.8 ± 7.4 y; p=.55) or VO_2 peak (amEX: 22.5 ± 6.1 y; pmEX: 22.8 ± 4.5 y; p =.86). However, the amEX participants (57 ± 5 years) were older (p = .004) than pmEX (51 ± 13 years) participants at baseline. Adherence to the 12-week training intervention were similar for the amEX (32 ± 2 out of 36 sessions) and pmEX (31 ± 2 out of 36 sessions) training groups.

Changes in glycaemic control

When the entire cohort (n=40) were analysed, significant main effects for time (All p<.01) were observed in HbA_{1c} (pre to post change: amEX, -0.27 ± 0.24%; pmEX, -0.25 ± 0.23%), FG (pre to post change: amEX, -0.9 ± 0.68 mmol/L; pmEX, -1.18 ± 1.40 mmol/L) and FI (pre to post change: amEX, -23.87 ± 27.78 ρmol/L; pmEX, -22.35 ± 22.72 ρmol/L). There were however, no significant differences between groups (group x time interaction: All p ≥ 0.42).

When only individuals with T2DM (n = 20) were analysed, the response was similar to the entire cohort, with the exception that the magnitude of improvement was greater. Specifically, s significant main effects for time (All p<.01) were observed in HbA_{1c} (pre to post change: amEX, -0.57 ± 0.13%; pmEX, -0.4 ± 0.12%), FG (pre to post change: amEX, -1.27 ± 0.75 mmol/L; pmEX, -1.80 ± 1.77 mmol/L) and FI (pre to post change: amEX, -19.18 ± 17.76

ρmol/L; pmEX, -20.85 ± 17.18 ρmol/L). There were however, no significant differences between groups (group x time interaction: All $p \geq 0.39$).

Postprandial Glucose and insulin responses

Postprandial glucose responses are presented in Figure 5-1, with the raw data and outcomes of data analyses in Table 5-1. Overall, significant reductions in PPG concentrations (main effect of time, all $p < 0.01$) were observed in the postprandial period following the training intervention, with moderate effects in the maximum PPG response and total AUC for both amEX (maximum PPG: $g = 0.47$; 4-h AUC: $g = 0.46$) and pmEX (maximum PPG: $g = 0.39$; 4-h AUC: $g = 0.43$) identified. These effects were moderate-large when only the T2DM cohort were assessed (amEX: $g = 0.62$, $g = 0.51$; pmEX: $g = 0.75$, $g = 0.71$; maximum PPG, 4-h AUC, respectively). There were no time by group interactions reported in any primary outcome measure (all $p \geq .13$).

Postprandial insulin responses are presented in Figure 5-1 and the raw data and outcomes of data analyses in Table 5-2. Overall, significant reductions in PPI concentrations (main effect of time, all $p \leq 0.02$) were observed in the postprandial period following the training intervention, with magnitude of effect across all reported insulin variables ranging from $g = 0.35$ to $g = 0.76$ for both amEX and pmEX training groups. The primary variable of interest, AUC-insulin demonstrated moderate to large effects in both amEX (Overall cohort: $g = 0.76$; T2DM cohort: $g = 0.87$) and pmEX groups (Overall cohort: $g = 0.63$; T2DM cohort: $g = 0.60$). There were no time by group interactions reported in any primary outcome measure (all $p \geq .84$).

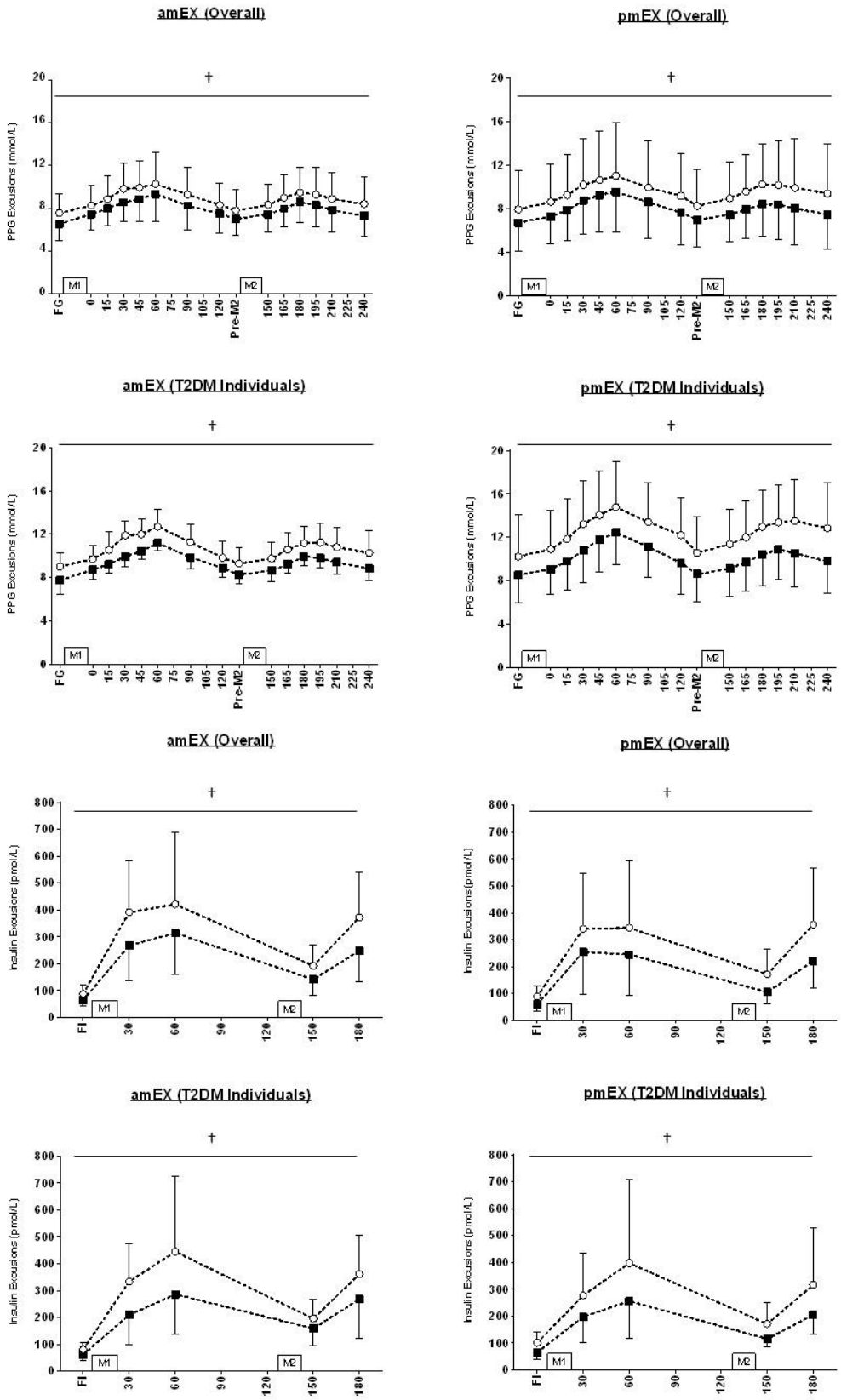

Figure 5-1. Changes in postprandial glucose and insulin during the 4-h mixed meal tolerance test for non-T2DM and T2DM individuals

Table 5-1. Changes in PPG responses from baseline to post-intervention for the overall cohort (non-T2DM and T2DM) and T2DM only.

		Overall (n = 40)					**T2DM** (n = 20)				
		amEX (n = 20)	**pmEX** (n =20)	***p* – value**			**amEX** (n = 10)	**pmEX** (n =10)	***p* – value**		
				Time Effect	Group Effect	Group Interaction			Time Effect	Group Effect	Group Interaction
Maximum PPG (mmol/L)	Week 0	10.74 ± 2.66	11.35 ± 4.63				13.07 ± 1.48	14.81 ± 3.41			
	Week 12	9.58 ± 2.21	9.69 ± 3.56				11.2 ± 0.86	12.44 ± 2.55			
	Change	-1.16 ± 1.36	-1.66 ± 1.44	< 0.01[†]	0.72	0.36	-1.87 ± 1.17	-2.37 ± 2.27	< 0.01[†]	0.22	0.56
Δ Max-Min PPG (mmol/L)	Week 0	3.18 ± 1.25	3.33 ± 1.85				4.09 ± 1.08	4.51 ± 1.87			
	Week 12	2.77 ± 0.96	2.91 ± 1.4				3.06 ± 0.56	3.87 ± 1.19			
	Change	-0.41 ± 1.12	-0.42 ± 1.28	0.04[†]	0.72	0.98	-1.03 ± 1.01	-0.64 ± 1.52	0.01[†]	0.25	0.53
4-h AUC (mmol/L/min)	Week 0	9.41 ± 2.35	10.12 ± 4.21				11.31 ± 1.46	13.3 ± 3.77			
	Week 12	8.39 ± 1.95	8.5 ± 3.17				9.9 ± 0.85	10.8 ± 2.89			
	Change	-1.02 ± 0.98	-1.62 ± 1.52	< 0.01[†]	0.68	0.15	-1.41 ± 1.05	-2.5 ± 1.68	< 0.01[†]	0.22	0.11
2-h AUC *Meal 1* (mmol/L/min)	Week 0	10.04 ± 2.51	10.68 ± 4.53				12.12 ± 1.42	14.1 ± 4.08			
	Week 12	8.98 ± 2.13	9.15 ± 3.38				10.61 ± 0.91	11.65 ± 2.99			
	Change	-1.06 ± 1.1	-1.53 ± 1.63	< 0.01[†]	0.7	0.32	-1.51 ± 0.90	-2.45 ± 1.87	< 0.01[†]	0.23	0.22
2-h AUC *Meal 2* (mmol/L/min)	Week 0	9.49 ± 2.43	10.33 ± 4.26				11.38 ± 1.73	13.55 ± 3.83			
	Week 12	8.43 ± 1.95	8.48 ± 3.21				9.95 ± 1.06	10.79 ± 1.51			
	Change	-1.06 ± 1.02	-1.85 ± 1.57	< 0.01[†]	0.65	0.07	-1.43 ± 1.19	-2.76 ± 1.73	< 0.01[†]	0.22	0.07
4-h iAUC (mmol/L)	Week 0	390 ± 237	456 ± 367				504 ± 258	657 ± 312			
	Week 12	378 ± 198	308 ± 192				441 ± 179	475 ± 191			
	Change	-11.86 ± 211	-88.4 ± 268	0.21	0.68	0.34	-62.53 ± 281	-181 ± 324	0.11	0.31	0.42
2-h iAUC *Meal 1* (mmol/L)	Week 0	226 ± 121	254 ± 173				289 ± 127	367 ± 173			
	Week 12	226 ± 113	228 ± 117				265 ± 96	295 ± 113			
	Change	No change	-26.18 ± 145	0.56	0.7	0.54	-23.64 ± 154	-72.28 ± 178	0.24	0.27	0.54
2-h iAUC *Meal 2* (mmol/L)	Week 0	176 ± 130	217 ± 150				232 ± 149	312 ± 158			
	Week 12	162 ± 96	152 ± 87				189 ± 94	196 ± 91			
	Change	-13.68 ± 117	-66.98 ± 140	0.06	0.66	0.21	-45.52 ± 156	-116 ± 169	0.05	0.36	0.35

Overall: combined results of non-T2DM and T2DM individuals; T2DM: only results of T2DM individuals. Data are mean ± SD. †Significant difference between baseline and post-intervention ($p < 0.05$). T2D: type 2 diabetes mellitus; amEX: morning exercise group; pmEX: evening exercise group; HbA1c: glycosylated haemoglobin; PPG: postprandial glucose; AUC: area under the curve (values above absolute zero); iAUC: incremental area under the curve (values above baseline value).

Table 5-2. Changes in the PPI responses to meal ingestion from baseline to post-intervention for the overall cohort (non-T2DM and T2DM) and T2DM only.

		Overall (n = 40)					**T2DM** (n = 20)				
		amEX (n = 20)	**pmEX** (n =20)	***p* – value**			**amEX** (n = 10)	**pmEX** (n =10)	***p* – value**		
				Time Effect	Group Effect	Group Interaction			Time Effect	Group Effect	Group Interaction
Average PPI (pmol/L)	Week 0	344 ± 89	303 ± 76				333 ± 89	290 ± 81			
	Week 12	243 ± 63	206 ± 59				230 ± 50	193 ± 50			
	Change	-101 ± 111	-97 ± 120	< 0.01[†]	0.32	0.90	-103 ± 62	-97 ± 169	< 0.01[†]	0.46	0.92
Maximum PPI (pmol/L)	Week 0	501 ± 240	432 ± 269				501 ± 249	409 ± 305			
	Week 12	371 ± 146	309 ± 165				342 ± 152	268 ± 132			
	Change	-130 ± 157	-123 ± 206	< 0.01[†]	0.3	0.92	-159 ± 117	-141 ± 296	< 0.01[†]	0.37	0.86
Δ Max-Min PPI (pmol/L)	Week 0	315 ± 211	268 ± 204				320 ± 242	239 ± 248			
	Week 12	250 ± 129	204 ± 147				217 ± 130	152 ± 127			
	Change	-65 ± 150	-64 ± 181	0.02[†]	0.37	0.98	-103 ± 139	-87 ± 254	0.06	0.37	0.87
Maximum PPI Increment (pmol/L)	Week 0	395 ± 225	344 ± 254				420 ± 244	308 ± 295			
	Week 12	307 ± 143	250 ± 153				279 ± 155	202 ± 124			
	Change	-88 ± 154	-94 ± 197	< 0.01[†]	0.36	0.93	-141 ± 107	-106 ± 273	0.02[†]	0.31	0.73
4-h PPI AUC (pmol/L/min)	Week 0	230.23 ± 104.8	199.75 ± 109.38				227.82 ± 92.45	201.21 ± 124.18			
	Week 12	165.66 ± 54.65	133.23 ± 97.14				157.81 ± 57.2	125.65 ± 115.38			
	Change	-64.57 ± 75.65	-66.52 ± 28.91	< 0.01[†]	0.3	0.92	-70.01 ± 45.22	-75.56 ± 29.83	< 0.01[†]	0.22	0.76
4-h PPI iAUC (pmol/L/min)	Week 0	164 ± 95.34	136.45 ± 63.61				166.5 ± 85.9	133.48 ± 50.74			
	Week 12	117.25 ± 53.55	91.99 ± 56.11				110.88 ± 55.58	84.36 ± 46.12			
	Change	-46.75 ± 79.28	-44.46 ± 18.86	< 0.01[†]	0.2	0.90	-55.62 ± 37.36	-49.12 ± 19.13	< 0.01[†]	0.36	0.65

Overall: combined results of non-T2DM and T2DM individuals; T2DM: only results of T2DM individuals. Data are mean ± SD. [†]Significant difference between baseline and post-intervention ($p < 0.05$). T2DM: type 2 diabetes mellitus; amEX: morning exercise group; pmEX: evening exercise group; PPI: postprandial insulin; iAUC: incremental area under the curve.

No significant interaction (time x group; where group = amEX or pmEX) was found in any of the surrogate markers of insulin sensitivity (muscle or hepatic insulin sensitivity). However, consistent with the raw glucose and insulin results there was a significant improvement in hepatic insulin sensitivity associated with the exercise training intervention for the overall cohort (amEX: g = 1.05; pmEX: g = 0.82), individuals with T2DM (amEX: g = 1.35; pmEX: g = 1.16) and individuals without T2DM (amEX: g = 0.80; pmEX: g = 0.57) (All $p \leq .01$), however, no improvements were observed in muscle insulin sensitivity in any groups (All $p \geq .14$), albeit the pmEX training was associated with greater effects than the amEX training (Overall cohort; amEX: g = 0.11; pmEX: g = 0.27).

There was an observed second meal phenomenon with the iAUC response to meal 2 (196.6 ± 143.2 mmol/L.120min) being smaller ($p < .01$) than the response to the first meal (240.3 ± 150.4 mmol/L.120min) in the overall cohort. This phenomenon persisted post-training (Meal 1: 227.5 ± 115.6 mmol/L.120min; Meal 2: 156.3 ± 92.3 mmol/L.120min; $p \leq .01$) and occurred in both T2DM (Meal 1: 226.8 ± 113.4 mmol/L.120min; Meal 2: 162.5 ± 96.3 mmol/L.120min; $p \leq .01$) and non-T2DM (Meal 1: 228.3 ± 117.8 mmol/L.120min; Meal 2: 156.3 ± 87.5 mmol/L.120min; $p \leq .01$) cohort.

Associations between HbA_1c, FG and PPG

Significant associations (All n = 80; pre- and post-training) between HbA_1c and FG (r = .741), HbA_1c and 4-h glucose AUC (r = .842) and HbA_1c and iAUC (r = .551) were observed. Regression modelling revealed the best predictor of HbA_{1c} was the 4-h glucose AUC value (Adjusted r^2 = .705). Addition of FG with either the 4-h glucose AUC (Adjusted r^2 = .702) or 4-h glucose iAUC (Adjusted r^2 = .690) did not significantly improve either fit (F change associated with addition of FG to the regression model between HbA_{1c} and 4-h AUC: p = .723).

The 2-h glucose AUCs from meal 1 (r = .853) and meal 2 (r = .823) demonstrated similar correlations with HbA_{1c} as compared with the 4-h glucose AUC.

Improvements in HbA_{1c} (All n = 40; pre-training subtract post-training) were associated with improvements in the 4h glucose AUC (r = .470) and FG (r = .469). When changes in HbA_{1c} were compared with changes in the 2-h glucose AUC for meal 1 (r = .480) and 2 (r = .436), the responses were similar.

Glycaemic outcomes in responders and non-responders

While *responders* tended to demonstrate more consistent improvements ($VO_{2\ peak}$ *responders*: g = 0.33-1.16, n = 23; total body fat *responders*: g = 0.46-0.67, n = 21; across stated glycaemic measures) versus *non-responders* ($VO_{2\ peak}$ *non-responders*: g = 0.48-0.65, n = 17; total body fat *non-responders*: g = 0.34-0.68, n = 19) these did not reach statistical significance (All $p \geq 0.21$) (Figure 5-2A and 5-3A). However, with T2DM individuals, there were no significant differences across stated glycaemic measures (All $p \geq 0.27$) between *responders* ($VO_{2\ peak}$ *responders*: g = 0.49-0.76, n = 12; total body fat *responders*: g = 0.62-0.71, n = 14; across stated glycaemic measures) and *non-responders* ($VO_{2\ peak}$ *non-responders*: g = 0.65-0.92, n = 8; total body fat *non-responders*: g = 0.7-0.74, n = 6) (Figure 5-2B and 5-3B).

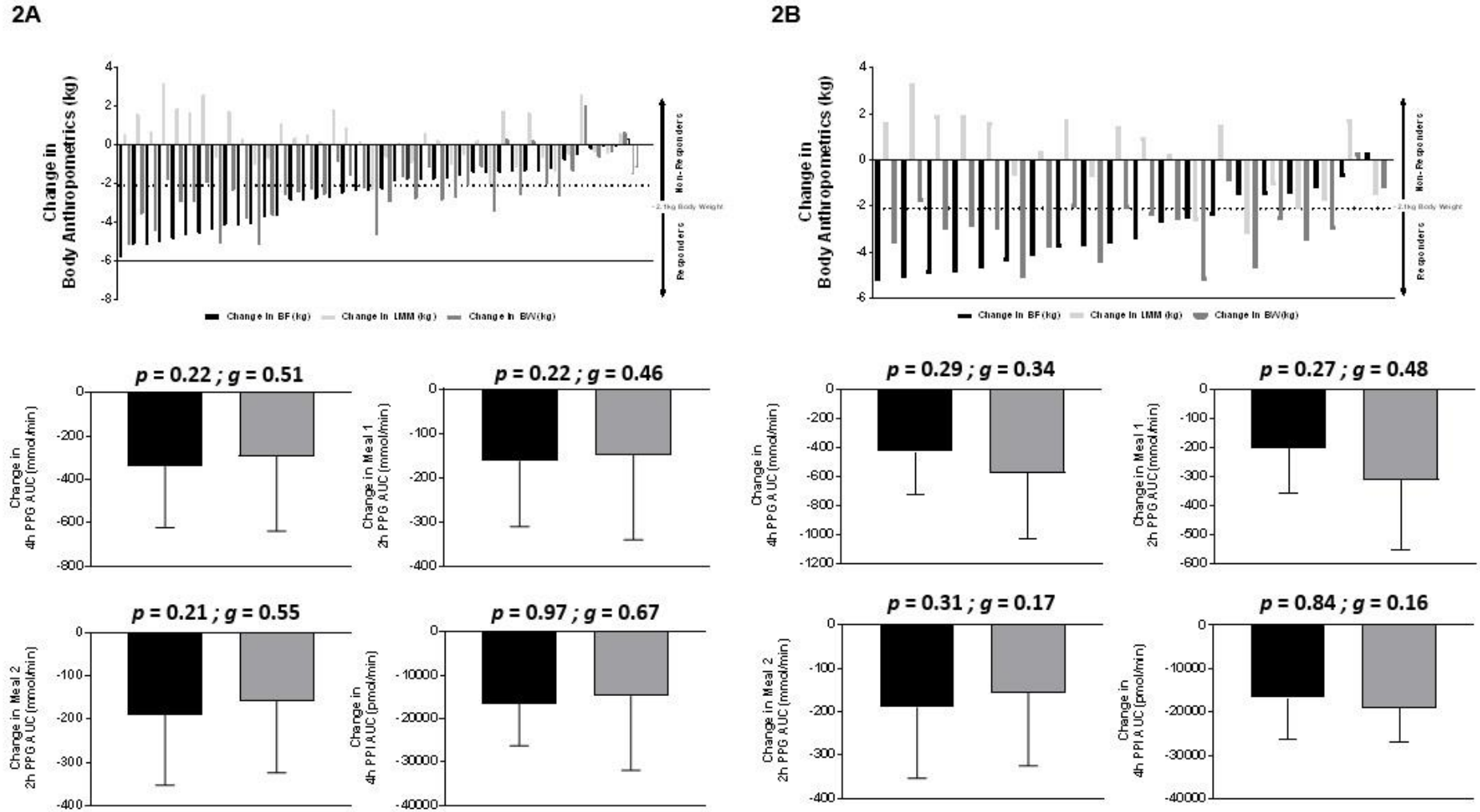

Figure 5-2. Individuals changes in body composition for both the overall (n = 40; 2A) and T2DM (n = 20; 2B) cohort from baseline to post-intervention and comparison of changes in 4-h PPG AUC (postprandial glucose area under the curve), 2-h PPG AUC for Meal 1 and Meal 2 and 4-h PPI (postprandial insulin) AUC responses between responders (black column) and non-responders (grey column).

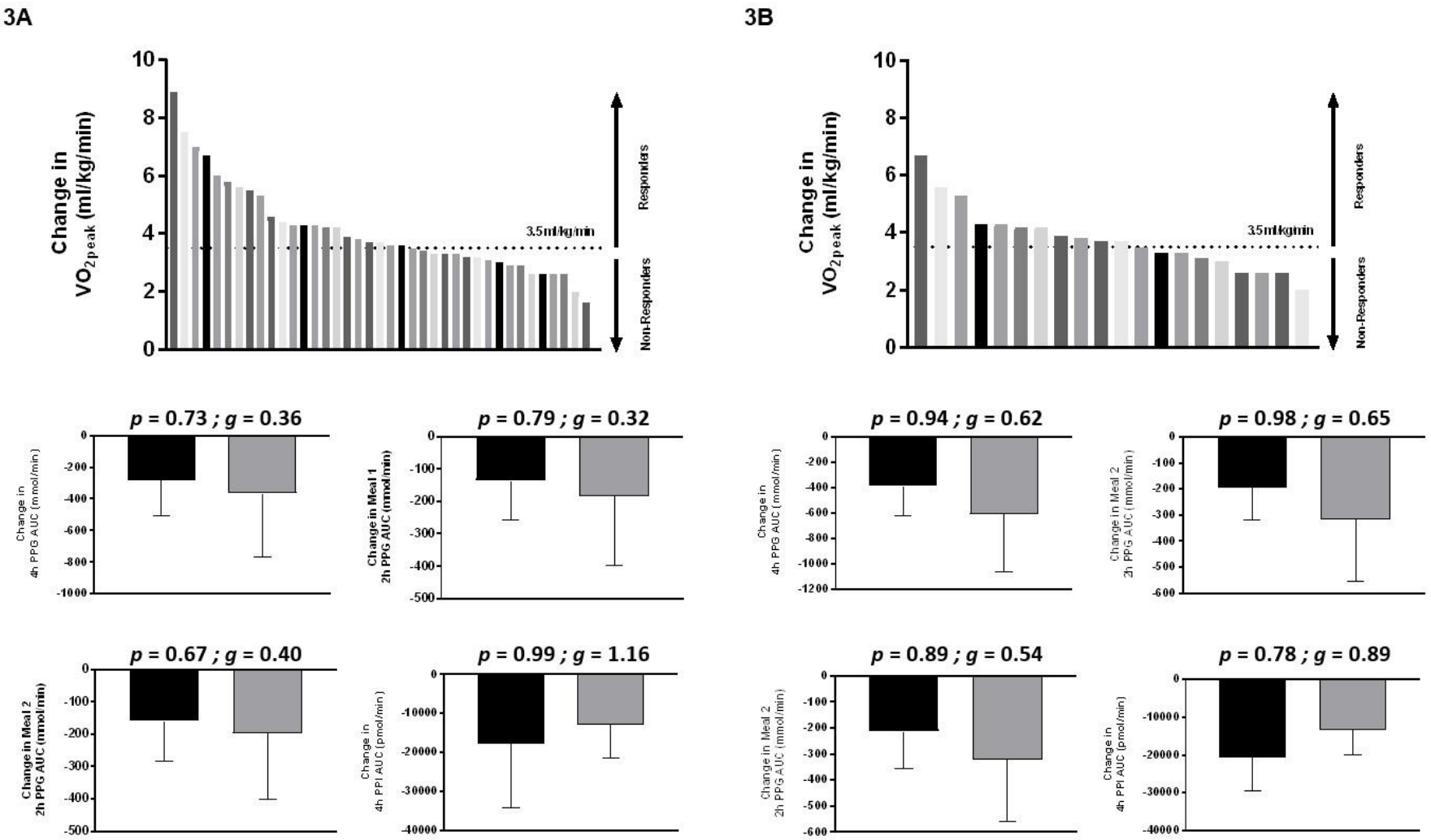

Figure 5-3. Individuals changes in VO_{2peak} for both the overall (n = 40; 3A) and T2DM (n = 20; 3B) cohort from baseline to post-intervention and comparison of changes in 4-h PPG AUC (postprandial glucose area under the curve), 2-h PPG AUC for Meal 1 and Meal 2 and 4-h PPI (postprandial insulin) AUC responses between responders (black column) and non-responders (grey column).

5.5 Discussion

The main findings of this study were i) 12-weeks of multi-modal exercise training resulted in significant improvements in glycaemic control (HbA_{1c}) along with significant improvements in PPG (glucose-AUC) and PPI (insulin-AUC) responses; ii) HbA_{1c} and glucose-AUC were more strongly correlated than HbA_{1c} and FG overall, however, the improvements in HbA_{1c} were associated to a similar extent with improvements in FG and glucose-AUC; iii) under the current free-living conditions, there appeared to be no benefit in performing the exercise in the morning or evening on any glycaemic outcomes measured; iv) the *second meal phenomenon* was observed in both the overall and T2DM cohorts, and was preserved post-training with no apparent effect of exercise timing (morning versus evening) on this response. Consistent with previous research and in accordance with our hypotheses, the adopted 12-week exercise training program was sufficient to significantly improve HbA_{1c} levels and the postprandial glucose response. In contrast to our hypotheses, improvements in HbA_{1c} were similarly correlated with improvements in glucose-AUC and FG, while the glycaemic benefits occurred independent of the timing of exercise.

The postprandial benefits resulting from exercise training were a 2.0 mmol/L and a 0.7 mmol/L decrease in glucose concentrations over the 4-h study period in individuals with T2DM and without T2DM, respectively. Unsurprisingly, the improvements in insulin (4-h AUC g = 0.60–0.87) were greater than those observed with glucose (4-h AUC g = 0.43–0.71), wherein insulin concentrations were reduced by 100.1 ρmol/L and 98.2 ρmol/L over the 4-h study period in individuals with and without T2DM, respectively. The similar insulin responses to the exercise training in individuals with T2DM and without T2DM suggests both the presence of some level of insulin resistance in individuals without T2DM in this cohort, and highlights the continued

responsiveness in those with T2DM in the current cohort. These observations are supported by changes in the surrogate measures of insulin sensitivity adopted herein. Specifically, the hepatic insulin sensitivity was significantly improved in response to training in both the non-T2DM and T2DM cohorts, and this occurred independent of the timing of exercise. The lack of change in insulin sensitivity, according to the adopted measure, was surprising given we anticipated a greater change in muscle insulin sensitivity than hepatic insulin sensitivity. However, it accords with the observed improvements in fasting glucose. A plausible explanation may relate to the intensity of exercise not being sufficient to stimulate the necessary metabolic or molecular changes required to up-regulate insulin sensitivity, despite the exercise intensity meeting current guidelines (23, 33). The significant postprandial benefits associated with the exercise training program occurred independent of the timing of exercise. The lack of effect associated with the exercise-timing is contrary to our hypothesis, however, as indicated in our previous study (45), this hypothesis was underpinned by proposed changes in the circadian rhythm, which was not observed (as measured by peripheral temperature) in the current study.

Ketema *et al.* (50) systematically reviewed the correlations between FG and PPG with HbA_{1c} and concluded that PPG (r = 0.68) had a closer association with HbA_{1c} than FG (r = 0.61). Within these associations, the relative contributions of PPG and FG vary according to HbA_{1c} levels (glycaemic control), with greater contributions from PPG when HbA_{1c} levels < 7.3% (i.e., good control; ~70% contribution from PPG) and greater contributions from FG when HbA_{1c} levels >10.2% (i.e., poor control; ~70% contribution from FG) (41, 51). These support the associations observed in our cohort (HbA_{1c} levels ~6.8%), wherein the 4-h glucose AUC was more strongly correlated with HbA_{1c} (r = 0.84) than FG (r = 0.74) although both were significant, explaining 71% and 55% of the variance in HbA_{1c}, respectively. These associations

are stronger than those reported in the meta-analysis of Ketema *et al*. (50), which included studies in individuals with T2DM (n = 11) and studies recruiting both T2DM and type 1 diabetes (n = 3). The strong association between HbA_{1c} and the AUC-glucose in the current study is likely to be associated with the adopted test-meal (mixed-meal), given the association between HbA_{1c} and the 4-h glucose AUC was similar to the 2-h glucose AUC (r = 0.85). In contrast to previous observations of an increased contribution of PPG response to [excess] hyperglycaemia in subsequent meals (41), we found similar associations between the PPG response in meal 1 and meal 2. However, the analysis, meal composition and time-interval (each meal separated by 4 hours) were quite different between studies and likely explain this difference. The multiple linear regression models suggested there was no benefit of combining FG with either the glucose-iAUC or glucose-AUC in the current study, although given the strength of the existing associations this may not be completely surprising. In contrast to our stated hypothesis, the improvement in HbA_{1c} demonstrated similar associations between improvements in FG (r = 0.46) and the glucose-AUC (r = 0.47).

In accordance with expectations of the *second meal phenomenon*, and despite the relatively short interval between eating occasions, the glucose response to the second meal (glucose-AUC) was significantly blunted. The circadian pattern in glucose tolerance has long been acknowledged (52), with a general worsening of tolerance (i.e., exaggerated glucose AUC) in the late afternoon and evening, culminating in a characteristic rise in circulating glucose during the early morning period (i.e. dawn phenomenon) in individuals with T2DM (53-55). Superimposed on this circadian rhythm, is the *second meal phenomenon,* which is presumed to be associated with changes in the gastric emptying rate (i.e., slowed following the first meal), or an altered plasma metabolic or endocrine milieu following the previous meal (37). While acute exercise has been shown to affect the second meal response (37, 44), the findings of the

current study are that this response appears to remain unaffected (relative to the first PPG meal response) following a 12-week exercise training program.

To assess whether individuals demonstrating the greatest response to training were also those demonstrating the greatest improvements in glycaemic measures (4-h glucose AUC, Meal-1 2h glucose AUC, Meal-2 2h glucose AUC, 4-h insulin AUC), data from all individuals were collapsed into two categories (*responders and non-responders*). These categories were based on changes in $VO_{2\ peak}$ and body-fat, wherein an increase in $VO_{2\ peak}$ above 3.5 ml/min/kg and a decrease in total body fat (kg; ≥ 2.1 kg body fat) was the *responder/non-responder* cut-point (45). While *responders* tended to demonstrate more consistent improvements ($VO_{2\ peak}$ *responders*: g = 0.33-1.16, n = 23; total body fat *responders*: g = 0.46-0.67, n = 21; across stated glycaemic measures) versus non-responders ($VO_{2\ peak}$ *non-responders*: g = 0.48-0.65, n = 23; total body fat *non-responders*: g = 0.34-0.68, n = 21) these did not reach statistical significance (All $p \geq 0.29$).

The main strength of the study were the implementation of a multimodal, supervised exercise training protocol and the adoption of a 4 h study period comprising two separate, mixed-meals. The different components of the exercise training program allow for optimal beneficial effects of exercise for the management of glycaemic control based on the strategic manipulation of the FITT (frequency, intensity, type and time) principle (56). The total duration of exercise performance per week in this study was approximately 180 min, which is expected to achieve greater reductions in HbA_{1c} levels when compared to durations of ≤150mins (-0.80% vs. -0.36%, respectively) (57). The multi-modal exercise protocol has been shown to result in greater improvements in HbA_{1c} as compared to aerobic and resistance exercise performed

individually (58), while supervision has been identified as being important to improve adherence (59).

The main limitations of this study were the relative small sample size and the absence of a sedentary control group. These factors were attributed to the difficulty in recruiting T2DM individuals that were able and willing to participate in the intervention for a period of 12 weeks. An increase in sample size would have allowed additional sub-cohort analyses (i.e. four sub-groups: pmEX OW, pmEX T2DM, amEX OW, amEX T2DM) or to explore individual differences more thoroughly using advanced multiple linear regression analyses, particularly given the main outcome measure of glucose control. As previously identified (45), the absence of a more rigid dietary control, and specifically controlling the diurnal timing of meal ingestion (beyond the postprandial-exercise requirement), may have masked the magnitude-change observed. It is however important to note, the impetus behind inclusion of a morning and evening exercise training protocol was to identify whether the timing of exercise was important for glucose control. While the study did not specifically control dietary intake, which can *post Hoc* be identified as a limitation, we adopted a pragmatic study design, specifically whether the diurnal-timing of exercise of three supervised exercise training sessions per week over 12 weeks were important. Based on the findings herein, we can conclude that the exercise training resulted in significant improvements in glycaemic control and PPG responses, but that without additional dietary controls, the diurnal timing of exercise did not moderate this relationship.

5.6 Conclusions

The 12-week multi-modal exercise training program adopted in the present study was effective in improving PPG and PPI responses to ingestion of a mixed meal in both T2DM and non-T2DM individuals. These improvements occurred independent of exercise timing (morning or

evening), suggesting that the diurnal timing of exercise performance may not be a significant factor in improving glycemic control. While the *second meal phenomenon* was observed, the PPG improvements were similar between the first and second meal. Given the importance of exercise intensity (60) in increasing insulin sensitivity, and the likely importance of frequency or routine in altering circadian patterns (61), it may be prudent for future research to increase the intensity and frequency (i.e., 5 sessions per week) of exercise sessions to comprehensively exclude a role for diurnal timing of exercise in altering glycemic control. The strong association between improvements in HbA1c and PPG-AUC evident in the present study supports the importance of addressing both FG and PPG, in order to better improve glycemic control for both T2DM individuals and individuals who are at risk of developing T2DM (62). Three supervised exercise sessions per week performed either in the morning or in the evening were equally as effective in improving FG, PPG and overall glycemic control in just 12 weeks in individuals with T2DM and at risk of developing T2DM.

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Chapter 6 General Discussion

6.1 Conclusions

Obesity is considered the single most imperative risk factor for the development of type 2 diabetes mellitus (T2DM) (1). The classical progression from obesity to T2DM is characterised by overlapping manifestations of insulin resistance, hyperinsulinemia, hyperglycaemia, dyslipidaemias and inflammation (2). With the global prevalence of obesity and DM estimated at 650 and 415 million respectively (3), along with the economic burden associated with both conditions, they are considered a major health problem of pandemic proportions. Management of T2DM requires the maintenance of tight glycaemic control as measured by glycosylated haemoglobin (HbA_{1c}) levels of <7% (4). In addition, a United Kingdom Prospective Diabetes Study (5) showed that for every 1% reduction in HbA_{1c} level resulted in significant reductions in the risk of diabetes related complications for T2DM individuals. Management of individuals identified as overweight or obese includes achieving a body mass index (BMI) of < 25kg/m^2 which has been shown to reduce the risk of T2DM by approximately 50-75% (6).

Exercise training is an important adjunct in the prevention of T2DM (7) and a principal recommendation within glycaemic control management guidelines of T2DM (8). Despite the universal acknowledgement of exercise being a cornerstone of T2DM prevention and management, the importance of exercise timing and its influence on glycaemic control has only been recently considered. This is surprising, given i) the extensive research in the sports nutrition literature concerning the optimal timing of energy intake and macronutrient content for performance (before, during and after) (e.g., (9)); ii) " the best time of day to exercise" is often discussed and debated in popular media (e.g., https://www.webmd.com/fitness-exercise/features/whats-the-best-time-to-exercise#1).

From a clinical perspective, the association between the timing of exercise and meal consumption has only been recently identified to potentially having an important role in maintaining glycaemic control (10-13). The current position statement by the American Diabetes Association (ADA) includes reference to differences in glycaemic control when exercise is performed in the fasted, pre-prandial or postprandial state (14). Furthermore, recent findings have indicated that postprandial exercise appears to result in greater improvements in glycaemia as compared to pre-prandial exercise (10-12). More importantly, there is limited research that have explored the potential association between exercise and diurnal time on glycaemic control, given the acknowledgement of a circadian rhythm that exist with diurnal glucose fluctuations (15).

Despite existing evidence suggesting an important role for exercise timing relative to meal ingestion, these have not been systematically reviewed for individuals with T2DM. In addition, and to the best of our knowledge, the importance of diurnal exercise timing playing a critical role in glycaemic control has not been previously investigated. Therefore, the overreaching aim of this thesis was to identify the impact of exercise timing, in relation to both meal ingestion and diurnal time, on glycaemic control and associated outcomes in individuals with T2DM and individuals at risk of T2DM from both an acute and chronic perspective.

To this end, we first sought to systematically review the literature examining the effect of exercise timing relative to meal ingestion on glycaemic control in individuals with T2DM (Chapter 2) and the effects of diurnal exercise timing on glycaemic control in individuals with T2DM (Chapter 3). Despite having insufficient evidence to directly compare the benefits of exercise performance in the pre-prandial and postprandial periods on acute glycaemia and long-term glucose control, comparing outcomes between studies in Chapter 2 showed that the

performance of postprandial exercise 30-60 minutes after meal consumption appeared to result in more consistent improvements on acute glycaemia as compared to pre-prandial exercise. Indeed, pre-prandial exercise was sometimes associated with elevations in the glucose area under the curve, if the meal was ingested close to exercise performance (16). This is likely due to the combination of increased free fatty acids in circulation when exercise is performed in the fasted (or pre-prandial) state, which is known to decrease insulin sensitivity (17); increased glucose output from the liver and concerted increase in hepatic gluconeogenesis (18) and increased rate of exogenous glucose uptake from the splanchnic region (18).

With respect to the importance of diurnal exercise timing on glycaemic control in individuals with T2DM, there was insufficient evidence to support a role for either morning or evening exercise. In spite of the mechanistic rational for an optimal exercise-time from research conducted in exercise and circadian clock literature (19-22), there were no studies which had previously investigated this question. As such, clinical relevance of the diurnal timing of exercise performance remained to be explored.

Based on the aforementioned systematic reviews, it is clear that the importance of exercise timing in the management of glycaemic control has not been unequivocally established. Given the indirect evidence on exercise timing relative to meal ingestion was strong, yet the evidence behind the diurnal timing of exercise on glycaemic control was absent, we decided to assess the effects of a medium-term (12-week) multi-modal exercise-training program, which was performed either in the morning or evening on longer-term glycaemic control (Chapter 4) and changes in postprandial glucose (PPG) responses (Chapter 5). In accordance with current best-evidence, we implemented the training during the postprandial period. The findings from Chapter 4 and Chapter 5 indicate that, irrespective of the diurnal timing of exercise

performance, 12-weeks of multi-modal exercise training is an effective strategy for improving long term glycaemic control. The potential temporal optimization of exercise prescription via the manipulation of diurnal exercise timing appears to provide little to no additional benefits. However, it is important to note that the chronic circadian rhythm—as measured via peripheral skin temperature—was unaffected by the training program. This may indicate that more intensive exercise training (most likely greater volume through increased frequency) may be required to induce a shift in the diurnal rhythmicity and once achieved, it may be plausible that one diurnal training pattern is deemed superior relative to the other.

Given the important role that postprandial hyperglycaemia plays in the aetiology of DM and diabetes associated complications (23), findings from Chapter 5 indicated that 12-weeks of multi-modal exercise training significantly improved PPG responses. However, the diurnal timing of exercise performance did not result in additional benefits to changes in PPG responses, which may be due to the aforementioned factor that no significant shift in circadian rhythm was observed during the 12-week intervention. In addition, the strong association between improvements in HbA1c and PPG responses present in Chapter 5 supports the importance of controlling PPG levels being a vital component for the achievement of recommended HbA1c targets in the management of T2DM (24). More importantly, given that the changes in HbA1c were strongly correlated to both changes in FG and PPG, this suggests that both FG and PPG should be addressed concurrently, rather than individually, for improved glycaemic control for both T2DM individuals and individuals at risk of developing T2DM (25).

In conclusion, the findings presented in this thesis indicate that 12-weeks of multi modal exercise training is an effective tool in improving measures of glycaemic control in both T2DM and non-T2DM individuals. When specifically considering the impact of exercise time on

glycaemia, evidence from existing literature and the findings of this thesis demonstrate that the timing of exercise in relation to meal ingestion (i.e. postprandial exercise) may be an effective complementary tool in prescribing exercise for glycaemic control management. Conversely, the diurnal timing of exercise performance may not have a profound effect on glycaemic control. Considering, however, chronic circadian patterns were not significantly shifted during the intervention, numerous factors such as: i) the timing and quantity of meals consumed; ii) individual chronotypes and; iii) the influence of sleep, may potentially have masked some of the differences that may exist with the effect of diurnal exercise timing. Thus, with respect to exercise prescription for glycemic control management, the intensity and frequency of exercise seem to be the key components for improvements in glycaemic control, while the timing of exercise performance may potentially be an effective supplementary tool to further enhance the benefits of exercise for the management of glycaemic control in T2DM individuals and individuals at risk of T2DM.

6.2 Clinical Significance

There were a number of significant clinical outcomes arising from this study. While it is acknowledged that exercise provides benefits to individuals with T2DM, there are a number of key findings which are worthy of highlighting. This is particularly true, given the intervention included three supervised exercise sessions only, with no additional dietary information provided or restrictions placed on participants. This was decided *a priori* and based on the difficulty in monitoring dietary adherence and actual dietary changes. In addition, while greater exercise volume in this freely-living population may likely have increased some of the outcomes within the study, approximately 56% of the population in Australia do not obtain 150 minutes of PA (inclusive of any movement such as for transport) (26) and therefore the three

sessions per week were identified as realistic, but not excessive. Nevertheless, the primary findings of clinical significance were:

1. The performance of a multi-modal exercise training program similar in prescription to that outlined in the American Diabetes Association guidelines (27) is an effective management tool for both the management and prevention of T2DM in overweight T2DM/non-T2DM individuals. At baseline, 45% (n = 18) of T2DM individuals had HbA1c levels of >7%, but at post-intervention, it was reduced to 25% (n = 10) of T2DM individuals, representing a 56% reduction in T2DM individuals having HbA1c levels that are considered as poor management (Figure 6-1). In addition, 20% (n = 4) of T2DM individuals had HbA1c levels of < 6.5% (HbA1c ≥ 6.5%; baseline vs. post-intervention (n): 20 vs. 16) (Figure 6-2A). For the non-T2DM individuals (Figure 6-2B), 63% (n = 5) of IFG/IGT individuals at baseline, had HbA1c levels of < 6% at post-intervention (HbA1c between 6.0 and 6.4%; baseline vs. post-intervention (n): 8 vs. 3).

2. Ning et al. (28) reported that a 2h-PPG concentration level that is higher than fasting glucose concentration is associated with a 10 to 20% increased risk for heart disease or stroke. Our findings showed that after 12-weeks of exercise training, 27% (n=5) of the individuals who had 2h-PPG levels lower than FG level at post-intervention (2h-PPG > FG; baseline vs. post-intervention (n): 27 vs. 22) (Figure 6-3).

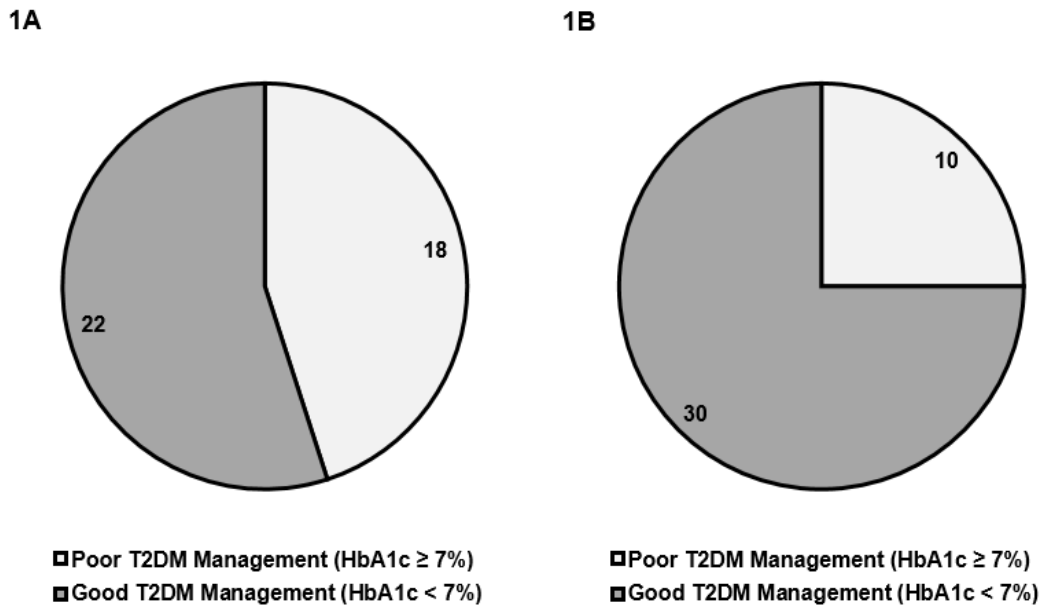

Figure 1. T2DM management based on HbA1c levels. 1A: Number of individuals with poor/good T2DM management at baseline; 1B: Number of individuals with poor/good T2DM management at post-intervention.

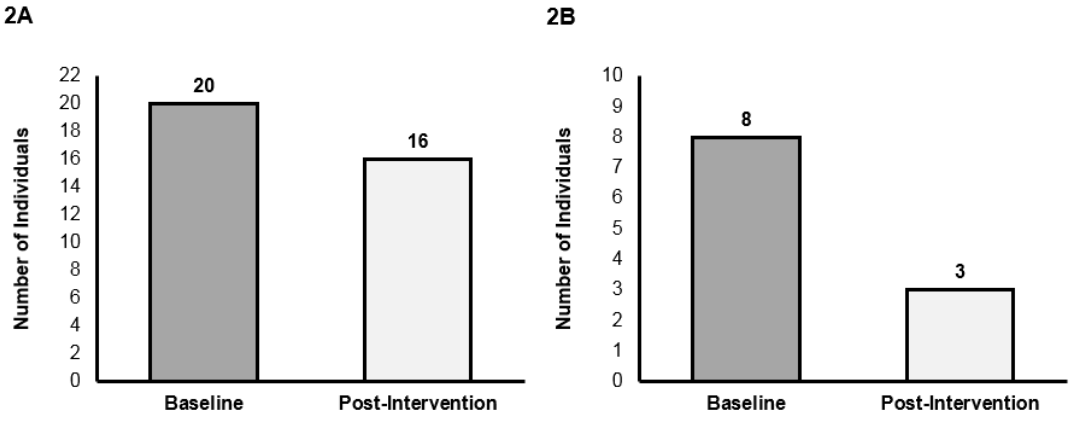

Figure 2. Number of individuals with HbA1c levels categorise as T2DM (HbA1c ≥ 6.5%; 2A) or IFG/IGT (HbA1c between 6.0 and 6.4%; 2B) at baseline (dark grey bars) and post-intervention (grey bars).

3. Kokkinos et al. (29) stated that for every 1MET (3.5ml/kg/min) increase in cardiovascular fitness, mortality risk is reduced by approximately 10 to 25%. As such, 12-weeks of multi-modal exercise training can be used as a complementary

management tool for the reduction in overall mortality risk based on improvements in cardiovascular health, with 55% (22 out of 40 participants) reporting an improvement of ≥ 3.5ml/kg/min in maximal oxygen consumption (VO_{2max}).

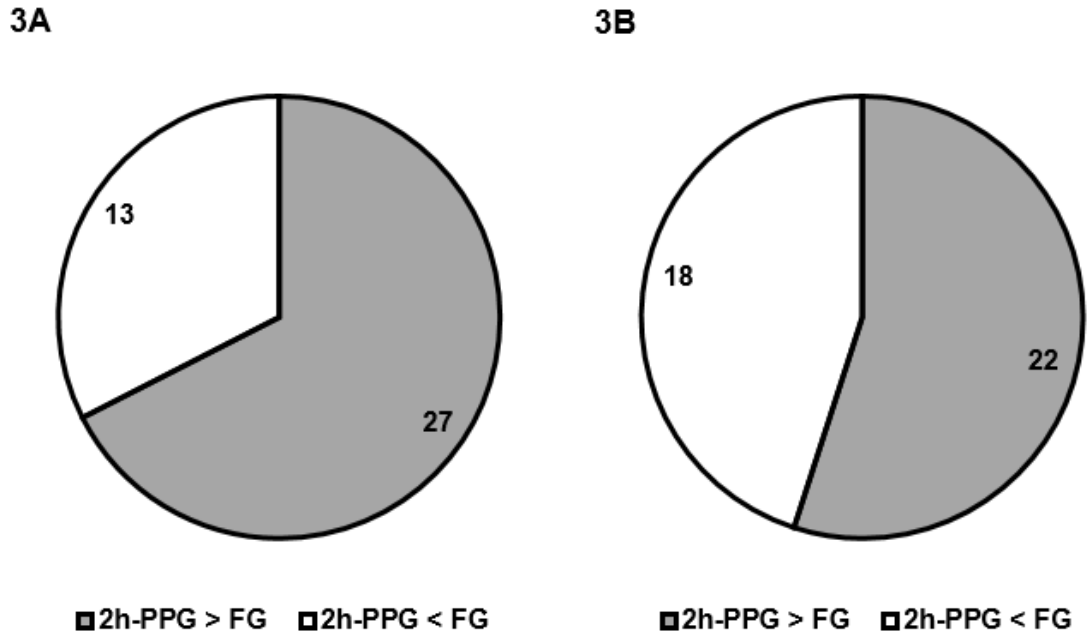

Figure 3. 2h-postprandial glucose vs. fasting glucose concentration. 3A: baseline results; 2B: post-intervention results.

6.3 Future Research Directions

Despite the growing body of evidence indicating the possible temporal optimization of exercise prescription for improved glycaemic control outcomes through the strategic manipulation of exercise timing, the results from this thesis has shown that the diurnal timing of exercise performance may not have any additional benefit to glycaemic control management in T2DM and non-T2DM individuals.

With regards to exercise time relative to meal ingestion, current evidence (11, 30) along with the findings from Chapter 2 suggests that postprandial exercise (when compared to a control condition) significantly improves glycaemic control (11, 30, 31). However, findings from the limited number of studies (12, 32) that have directly compared preprandial and postprandial exercise on glycaemic control showed mixed results indicating that the impact of exercise time (in relation to meal ingestion) on glycaemia remains inconclusive. In regards to diurnal exercise time, findings of Chapter 4 and 5, along with the limited number of studies that have investigated the diurnal time of exercise (Chapter 3) indicate that the extent to which diurnal exercise time impacts upon glycaemic control management remains inconclusive.

Based upon the findings highlighted in the aforementioned paragraph, and given that a pragmatic approach was utilised in the study design of Chapter 4 and 5 to investigate the effect of exercise timing on glycemic control, a more viable option, which future research should consider, would be to include additional intervention groups (i.e. amEX-preprandial vs. amEX-postprandial vs. pmEX-preprandial vs. pmEX-postprandial) to specifically identify which facet of exercise time that has the greatest impact on glycaemia. To do so, the proposed study design would require a greater number of participants (approximately n = 25 per group), which was deemed impractical for us due to restraints that include: i) limited resources, ii) time line and; iii) limited participant enrolment. In addition, individuals recruited to the proposed study would have to be primarily T2DM to identify any changes in glycaemia. Again, this was a limitation for the current study due to the underwhelming interest from T2DM individuals in participating in the intervention.

In regards to the exercise prescription for glycemic control management, numerous studies have indicated that the intensity of exercise training is considered one of the main determinants of subsequent improvements in glycemic control (33-36). In addition, it has been stated that the frequency at which exercise is performed represents another impact factor that may modulate the impact of exercise on glycemic control (37). These findings are supported by the results presented in Chapter 4 and 5, whereby our findings indicate that the intensity and frequency of the exercise intervention may have been insufficient, consequently, masking any potential differences that may exist with the diurnal timing of exercise performance on chronic glycaemia. Our exercise intervention of 3 supervised multi-modal exercise session per week (each session: approximately 60mins) was chosen, firstly, due to it meeting the current exercise guidelines (38) for T2DM management and, secondly, it was deemed as a pragmatic program given that the participants of the study were sedentary individuals (weekly physical activity of <150mins). However, in the context of exercise frequency, Umpierre et al. (39) showed that each additional exercise session per week corresponded to an additional 0.39% improvement in HbA1c levels. Additionally, a review by Grace et al. (40) stated that higher intensity exercise may result in superior fitness benefits and greater reductions in HbA1c levels as compared to moderate intensity exercise. Taking the aforementioned factors into account and the relatively small changes we observed for glycaemic control measures in Chapter 4 and 5, future research should consider increasing the intensity and frequency of exercise performance to truly observed the impact that diurnal exercise time has on glycemic control.

Other factors such as diet has also been shown to positively affect glycaemic control in both the prevention and management of T2DM (41). Consequently, in Chapter 4 and 5 participants were instructed to continue with their normal dietary habits throughout the 12-week intervention, resulting in a large variabilities in meal composition, quantity and times within

participants that could potentially have affected glycaemic control improvements observed in the study. As such, future research should consider maintaining participants' meal caloric intake and composition within a set range while restricting meal times based on their intervention groups (i.e. morning or evening) to isolate the effect that diurnal exercise timing has on glycaemia. In addition, the use of a mixed meal tolerance test (MMTT) in Chapter 5 as compared to a traditional oral glucose tolerance test (OGTT) was primarily chosen to as it was considered more 'ecological' and represented a typical daily dietary behaviour of an individual. However, the glucose concentration in the MMTT (24.5g) was approximately half the amount compared to the glucose solution in a 2-h OGTT (50g). This difference in glucose concentration may have resulted in blunted PPG responses, as such, future research should consider choosing a MMTT of greater glucose concentration to allow for a clearer representation of changes in PPG responses.

Within circadian clock literature, sleep quantity and quality has been shown to play a critical role in circadian rhythm through the phase advancement/delay of diurnal melatonin onset (42). As such, variability in participants' sleep patterns may potentially have an effect on changes in diurnal glucose rhythm. Findings from Chapter 4 and 5 did not indicate a significant shift in chronic circadian rhythm (as measured through peripheral skin temperature), consequently, this may explain the insignificant differences observed in glycaemic control when comparing between morning and evening exercise. Future research should consider controlling habitual sleeping behaviour to isolate the impact exercise has on chronic circadian rhythm. Lastly, it may be feasible that an individual's chronotype influences the efficacy of morning or evening exercise, whereby, the impact of chronotypes on circadian rhythm remains remains currently unknown given that the participants' chronotypes were not identified in Chapter 4 and 5, and

that future research should consider identifying individual chronotypes (e.g. Munich Chronotype Questionnaire) to control for circadian rhythm changes.

In all, despite the growing interest in this field of research, many questions remain. Whether the strategic manipulation of the diurnal timing of exercise performance may result in meaningful changes in glycaemic control, remains to be explored. Further research adopting complimentary techniques that could potentially isolate the differences that may exist between morning and evening exercise performance are required, in order to better understand the impact of exercise timing on glycaemic control for the prevention and management of T2DM

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Appendices

PRISMA 2009 Checklist

Appendix A

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 10
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 11
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 12
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 12
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Page 13
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 14
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 13
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix B
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 14
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 15
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 14
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 15
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 14-16

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Page 15
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 15
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 15
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 16-17
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 16-17
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 22
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 18-23
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 24-25
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 22
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 22-25
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 25-29
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 29-30
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 31
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

Search Strategy

The search strategy below was conducted on Medline [PubMed]. All keywords used in the search are free text terms unless stated otherwise; MeSH = Medical subject heading; * = for any character

Search for Type 2 Diabetes Mellitus (T2DM)

MeSH Terms	Type 2 diabetes, Non-insulin-dependent diabetes, Gestational diabetes, Diabetes insipidus
Keywords	Type 2 diabetes, Type 2 diabetes mellitus, Type II diabetes, Type II diabetes mellitus, Impaired glucose tolerance, Insulin resistance, MODY, NIDDM, T2DM, Non-insulin-dependent diabetes mellitus, Gestational diabetes, Diabetes insipidus
Combined Search (a)	((((type 2 diabetes[MeSH Terms]) OR non-insulin-dependent diabetes[MeSH Terms]) OR ((((((((((type 2 diabetes) OR type 2 diabetes mellitus) OR type II diabetes) OR type II diabetes mellitus) OR impaired glucose tolerance) OR insulin resistance) OR MODY) OR NIDDM) OR T2DM) OR non-insulin-dependent diabetes mellitus))) NOT ((((gestational diabetes) OR gestational diabetes[MeSH Terms]) OR diabetes insipidus) OR diabetes insipidus[MeSH Terms])
Results	217, 784

Search for Exercise

MeSH Terms	Exercise, Physical training, Physical fitness, Sports
Keywords	Exercis*, Physical activity, physical fitness, walking, weight lifting, strength training, resistance training, circuit weight training, sports, physical* active*, physical training, weight training, aerobic training, circuit training, interval training, combine* exercise training
Combined Search (b)	(((((exercise[MeSH Terms]) OR physical training[MeSH Terms]) OR physical fitness[MeSH Terms]) OR sports[MeSH Terms])) OR ((((((((((((((exercise) OR physical activity) OR physical fitness) OR walking) OR weight lifting) OR strength training) OR resistance training) OR circuit weight training) OR sports) OR weight training) OR endurance training) OR aerobic training) OR circuit training) OR interval training) OR combine exercise training)
Results	692, 043

Search for Glucose Control

MeSH Terms	Glucose control

Keywords	Glucose control, Blood glucose control, Glycaemic control, Glycemic control, Glycaemic variability, Glycemic variability, Glucose tolerance, Glucose metabolism, Postprandial glucose, Postprandial blood glucose, Fasting glucose, Fasting blood glucose, Glycosylated haemoglobin, Glycosylated, hemoglobin, Glycated haemoglobin, Glycated haemoglobin
Combined Search (c)	(glucose control[MeSH Terms]) OR ((((((((((((((((((glucose control) OR blood glucose control) OR glycaemic control) OR glycemic control) OR glycaemic variability) OR glycemic variability) OR glucose tolerance) OR glucose metabolism) OR postprandial glucose) OR postprandial blood glucose) OR PPG) OR fasting glucose) OR fasting blood glucose) OR glycosylated haemoglobin) OR glycosylated hemoglobin) OR glycated haemoglobin) OR glycated hemoglobin) OR HbA1c) OR A1c)
Results	422, 531

Filter for Randomized Controlled Trials (RCTs) & Humans

Publication Type	Randomized – controlled trials, Controlled – clinical trials, Clinical trials
MeSH Terms	Randomized – controlled-trials, Double – method, Single – blind method, Random allocation, Clinical trials, Placebo, Research design, Human
Keywords	Clinical trial, Placebo*, Random* Latin Square
Combined Search (d)	((((((((((randomized-controlled-trials[MeSH Terms]) OR double-blind method[MeSH Terms]) OR single-blind method[MeSH Terms]) OR random allocation[MeSH Terms]) OR clinical trials[MeSH Terms]) OR placebos[MeSH Terms]) OR research design[MeSH Terms])) OR ((((clinical trial) OR placebo) OR random) OR latin square))) AND humans[MeSH Terms]
Results	1, 313, 551

Complete Search Filtered for RCTs and Humans

Combined Search (e)	(a) AND (b) AND (c) AND (d)
Results	2, 197

Assessment for risk of bias in included randomized controlled trials

Authors	Sequence Generation	Allocation Concealment	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Sources of Bias
Colberg (2009)[36]	?	?	+	?	+	?	?
Colberg (2014)[35]	?	?	+	?	?	?	?
Erickson (2017)[37]	?	-	+	?	+	?	+
Gill (2007)[38]	?	?	+	?	+	?	?
Heden (2014)[20]	?	-	+	-	+	+	?
Karstoft (2014)[39]	?	?	+	-	+	+	+
Larsen (1997)[40]	?	?	+	?	+	?	?
Larsen (1999)[41]	?	?	+	?	+	?	?
Manders (2010)[42]	?	?	+	?	+	?	?
Moreira (2012)[43]	?	?	+	?	?	?	?
Oberlin (2014)[44]	?	?	+	?	+	?	?
Rasmussen (1999)[45]	?	?	+	?	+	?	?
Tobin (2008)[46]	?	?	+	?	+	?	?
Vancea (2009)[51]	?	?	+	?	?	?	?
Van Dijk (2012)[48]	?	?	+	?	+	+	+
Van Dijk (2012)[49]	?	?	+	?	+	+	+
Van Dijk (2013)[50]	?	?	+	?	?	+	+
Van Dijk (2013)[47]	?	?	+	?	+	+	+
Wycherley (2010)[52]	?	?	+	?	+	?	+

(+): Low risk of bias; (-): High risk of bias; (?): Unclear

PRISMA 2009 Checklist

Appendix D

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 39
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 40
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 41-43
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 41-43
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Page 43-44
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 44
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 43-44
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix E
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 45-46
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 45-46
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 44
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 46
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 46

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Page 46
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 46
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 46
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 47-48
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 47-48
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 54
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 49-53
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 55
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 54
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 56
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 56-59
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 59-60
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 60-61
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

Appendix E

Search Strategy

The search strategy below was conducted on Medline [PubMed]. All keywords used in the search are free text terms unless stated otherwise; MeSH = Medical subject heading; * = for any character

Search for Type 2 Diabetes Mellitus (T2DM)

MeSH Terms	Type 2 diabetes, Non-insulin-dependent diabetes, Gestational diabetes, Diabetes insipidus
Keywords	Type 2 diabetes, Type 2 diabetes mellitus, Type II diabetes, Type II diabetes mellitus, Impaired glucose tolerance, Insulin resistance, MODY, NIDDM, T2DM, Non-insulin-dependent diabetes mellitus, Gestational diabetes, Diabetes insipidus
Combined Search (a)	((((type 2 diabetes[MeSH Terms]) OR non-insulin-dependent diabetes[MeSH Terms]) OR ((((((((((type 2 diabetes) OR type 2 diabetes mellitus) OR type II diabetes) OR type II diabetes mellitus) OR impaired glucose tolerance) OR insulin resistance) OR MODY) OR NIDDM) OR T2DM) OR non-insulin-dependent diabetes mellitus))) NOT ((((gestational diabetes) OR gestational diabetes[MeSH Terms]) OR diabetes insipidus) OR diabetes insipidus[MeSH Terms])
Results	217, 784

Search for Exercise

MeSH Terms	Exercise, Physical training, Physical fitness, Sports
Keywords	Exercis*, Physical activity, physical fitness, walking, weight lifting, strength training, resistance training, circuit weight training, sports, physical* active*, physical training, weight training, aerobic training, circuit training, interval training, combine* exercise training
Combined Search (b)	(((((exercise[MeSH Terms]) OR physical training[MeSH Terms]) OR physical fitness[MeSH Terms]) OR sports[MeSH Terms])) OR ((((((((((((((exercise) OR physical activity) OR physical fitness) OR walking) OR weight lifting) OR strength training) OR resistance training) OR circuit weight training) OR sports) OR weight training) OR endurance training) OR aerobic training) OR circuit training) OR interval training) OR combine exercise training)
Results	692, 043

Search for Glucose Control

MeSH Terms	Glucose control

Keywords	Glucose control, Blood glucose control, Glycaemic control, Glycemic control, Glycaemic variability, Glycemic variability, Glucose tolerance, Glucose metabolism, Postprandial glucose, Postprandial blood glucose, Fasting glucose, Fasting blood glucose, Glycosylated haemoglobin, Glycosylated, hemoglobin, Glycated haemoglobin, Glycated hemoglobin
Combined Search (c)	(glucose control[MeSH Terms]) OR ((((((((((((((((((glucose control) OR blood glucose control) OR glycaemic control) OR glycemic control) OR glycaemic variability) OR glycemic variability) OR glucose tolerance) OR glucose metabolism) OR postprandial glucose) OR postprandial blood glucose) OR PPG) OR fasting glucose) OR fasting blood glucose) OR glycosylated haemoglobin) OR glycosylated hemoglobin) OR glycated haemoglobin) OR glycated hemoglobin) OR HbA1c) OR A1c)
Results	422, 531

Filter for Randomized Controlled Trials (RCTs) & Humans

Publication Type	Randomized – controlled trials, Controlled – clinical trials, Clinical trials
MeSH Terms	Randomized – controlled-trials, Double – method, Single – blind method, Random allocation, Clinical trials, Placebo, Research design, Human
Keywords	Clinical trial, Placebo*, Random* Latin Square
Combined Search (d)	((((((((((randomized-controlled-trials[MeSH Terms]) OR double-blind method[MeSH Terms]) OR single-blind method[MeSH Terms]) OR random allocation[MeSH Terms]) OR clinical trials[MeSH Terms]) OR placebos[MeSH Terms]) OR research design[MeSH Terms])) OR ((((clinical trial) OR placebo) OR random) OR latin square))) AND humans[MeSH Terms]
Results	1, 313, 551

Complete Search Filtered for RCTs and Humans

Combined Search (e)	(a) AND (b) AND (c) AND (d)
Results	2, 497

Supplementary Table 2. Assessment for risk of bias in included randomized controlled trials

Authors	Sequence Generation	Allocation Concealment	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Sources of Bias
Belli (2011)[37]	+	?	+	?	+	+	?
Colberg (2009)[38]	?	?	+	?	+	?	?
Colberg (2014)[39]	?	?	+	?	?	?	?
Erickson (2017)[54]	?	-	+	?	+	?	+
Heden (2014)[40]	?	-	+	-	+	+	?
Karstoft (2014)[41]	?	?	+	-	+	+	+
Larsen (1997)[42]	?	?	+	?	+	?	?
Larsen (1999)[43]	?	?	+	?	+	?	?
Manders (2010)[44]	?	?	+	?	+	?	?
Moreira (2012)[45]	?	?	+	?	?	?	?
Oberlin (2014)[46]	?	?	+	?	+	?	?
Rasmussen (1999)[47]	?	?	+	?	+	?	?
Tobin (2008)[48]	?	?	+	?	+	?	?
Vancea (2009)[53]	?	?	+	?	?	?	?
Van Dijk (2012)[50]	?	?	+	?	+	+	+
Van Dijk (2012)[51]	?	?	+	?	+	+	+
Van Dijk (2013)[52]	?	?	+	?	?	+	+
Van Dijk (2013)[49]	?	?	+	?	+	+	+

(+): Low risk of bias; (-): High risk of bias; (?): Unclear

Murdoch UNIVERSITY

Monday, 12 October 2015

Division of Research & Development
Research Ethics and Integrity

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Dr Timothy Fairchild
School of Psychology and Exercise Science
Murdoch University

Dear Timothy,

Project No. 2015/170
Project Title The effect of exercise timing on glycemic control in individuals with Type 2 diabetes mellitus

Thank you for addressing the conditions placed on the above application to the Murdoch University Human Research Ethics Committee. On behalf of the Committee, I am pleased to advise the application now has:

OUTRIGHT APPROVAL

Please note, Co-Investigators Prof Jill Kanaley and A/Prof Kim Guelfi will be added to the permit once a signature or an email indicating participation in the study has been received.

Approval is granted on the understanding that research will be conducted according the standards of the ***National Statement on Ethical Conduct in Human Research* (2007), the *Australian Code for the Responsible Conduct of Research* (2007)** and **Murdoch University policies** at all times. You must also abide by the **Human Research Ethics Committee's standard conditions of approval (see attached).** All reporting forms are available on the Research Ethics and Integrity web-site.

I wish you every success for your research.

Please quote your ethics project number in all correspondence.

Kind Regards,

Dr. Erich von Dietze
Manager
Research Ethics and Integrity

cc: Prof Jill Kanaley, A/Prof Kym Guelfi and Shaun Teo

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Page 71
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Page 72
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	Page 73-74
	2b	Specific objectives or hypotheses	Page 73-74
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Page 75
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Page 75
Participants	4a	Eligibility criteria for participants	Page 75
	4b	Settings and locations where the data were collected	Page 76
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Page 77
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Page 78-79
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	Page 75
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Page 76
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Page 76
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Page 76-77
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Page 76-77
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Page 76-77
	11b	If relevant, description of the similarity of interventions	NA

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	Page 79
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	NA
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Appendix J
	13b	For each group, losses and exclusions after randomisation, together with reasons	Appendix J
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Page 76
	14b	Why the trial ended or was stopped	Page 76
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 4-1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Appendix J
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Page 82-87
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Page 82-87
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Page 82-87
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Page 92-93
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Page 93
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	Page 88-92
Other information			
Registration	23	Registration number and name of trial registry	Page 72
Protocol	24	Where the full trial protocol can be accessed, if available	Page 72
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	NA

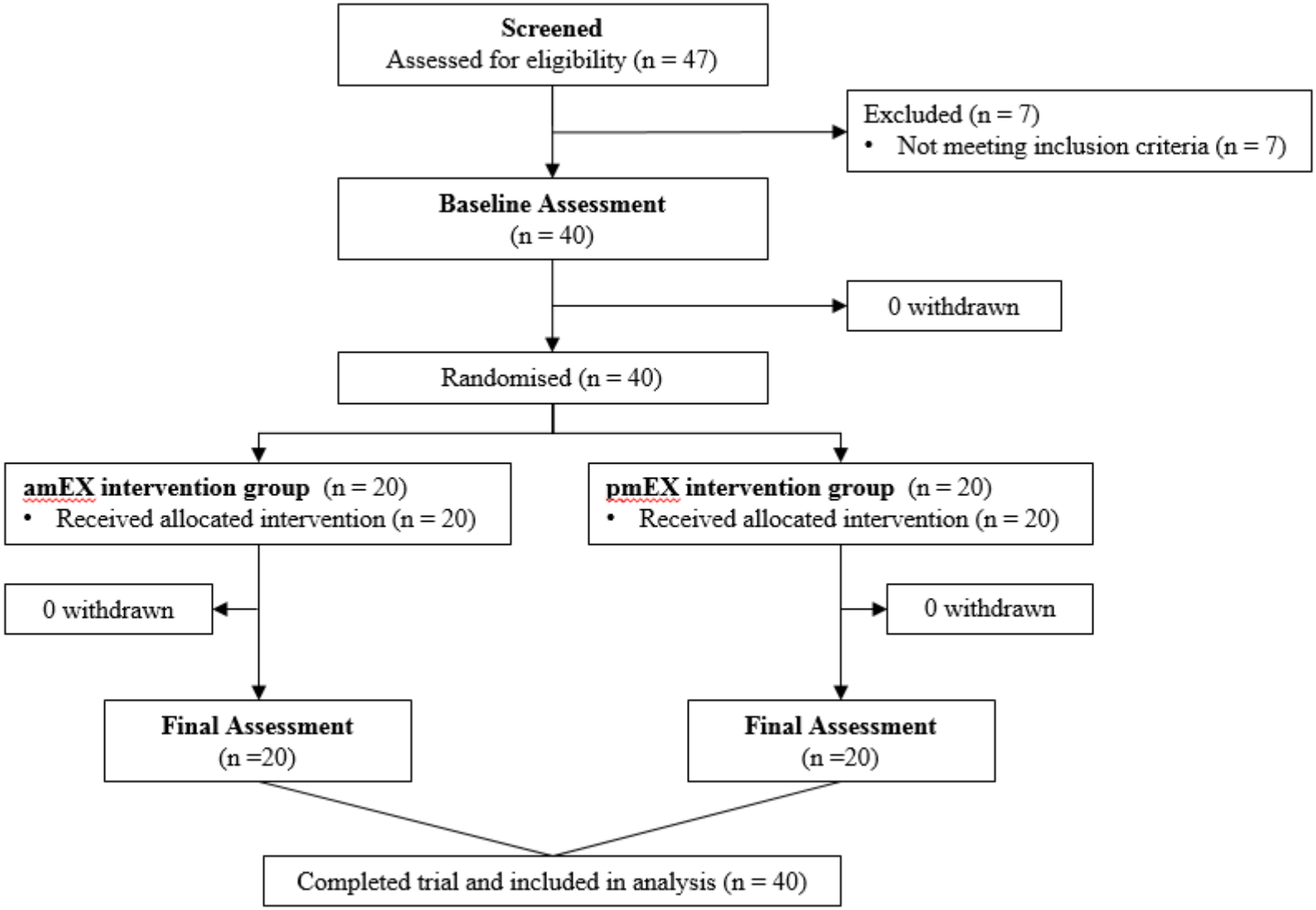
*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

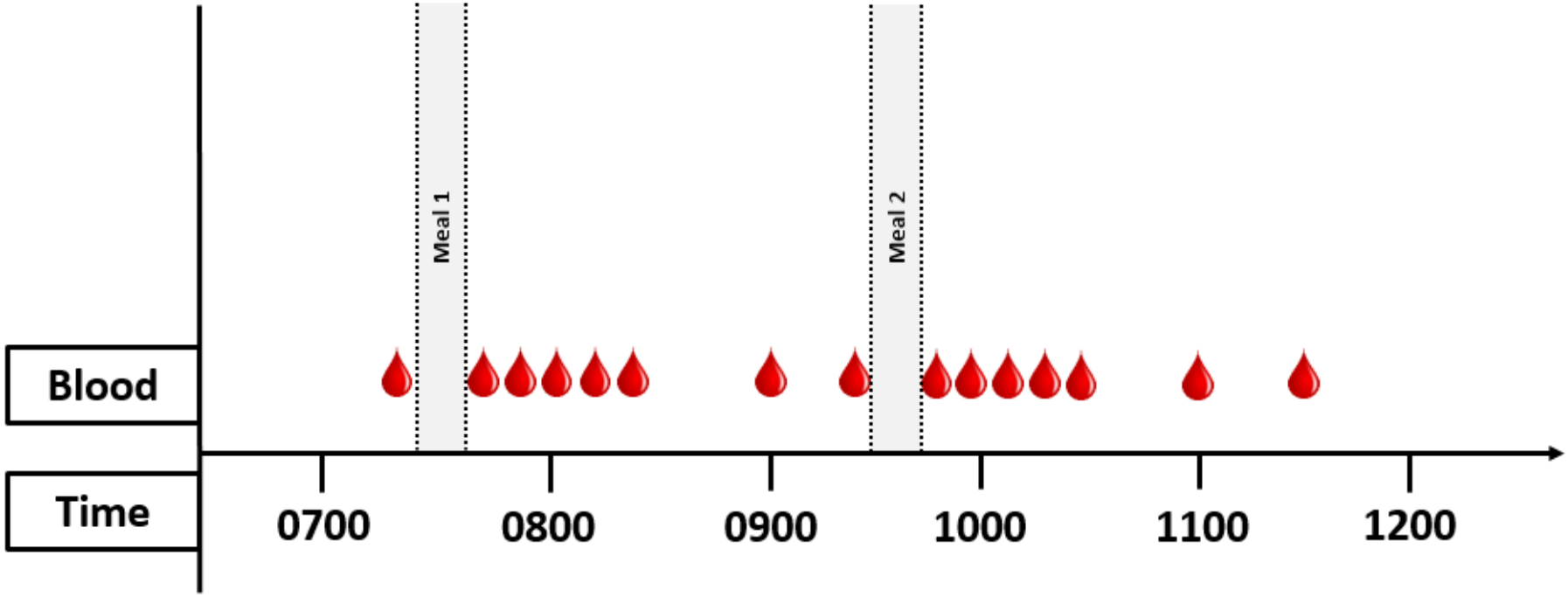
CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	Page 101
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Page 102
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	Page 103-105
	2b	Specific objectives or hypotheses	Page 103-105
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Page 105
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	Page 106
Participants	4a	Eligibility criteria for participants	Page 106
	4b	Settings and locations where the data were collected	Page 106-107
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Page 106-107
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Page 108-109
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	Page 109
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Page 105-106
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Page 105-106
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Page 105-106
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Page 105-106
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Page 105-106
	11b	If relevant, description of the similarity of interventions	NA

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	Page 109
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	NA
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Appendix J
	13b	For each group, losses and exclusions after randomisation, together with reasons	Appendix J
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Page 105
	14b	Why the trial ended or was stopped	Page 105
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Figure 114-116
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 114-116
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Page 113-120
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Page 113-120
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Page 113-120
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	Page 124-125
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Page 121-125
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	Page 121-125
Other information			
Registration	23	Registration number and name of trial registry	Page 103
Protocol	24	Where the full trial protocol can be accessed, if available	Page 103
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	NA

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.


Screened
Assessed for eligibility (n = 47)
Excluded (n = 7)
• Not meeting inclusion criteria (n = 7)
Baseline Assessment
(n = 40)
0 withdrawn
Randomised (n = 40)
amEX intervention group (n = 20)
• Received allocated intervention (n = 20)
pmEX intervention group (n = 20)
• Received allocated intervention (n = 20)
0 withdrawn
0 withdrawn
Final Assessment
(n =20)
Final Assessment
(n =20)
Completed trial and included in analysis (n = 40)


Meal 1
Meal 2
Blood
Time
0700
0800
0900
1000
1100
1200