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ABSTRACT

Purpose: Exercise-nutrient timing is of interest for people with type 2 diabetes (T2D) as a potential method to optimize glycemic control. However, the optimal nutritional environment for exercise is not well understood over the long term. The Fasted Exercise for Type 2 Diabetes (FED) Trial compared 16 weeks of fasted versus postprandial morning exercise on glycated hemoglobin (HbA1c) and liver proton density fat fraction (PDFF). **Methods**. Twenty adults with T2D were recruited and randomized to complete exercise after an overnight fast versus after their morning meal. Participants walked three mornings per week, progressing to 180 minutes per week over 16 weeks. **Results:** Groups were balanced with 5 males and 5 females each. Sixteen participants completed the trial (8 in each group, 50% female). Age, HbA1c, and PDFF were 59.8±9.0 years, 7.2±0.7%, and 9.3±4.1%, respectively. On average, both groups completed 98% of their walking sessions but there was no change in HbA1c (-0.2%, p=0.588). However, one participant from each group had changes in their glucose lowering medication during the trial and, when excluded, the fasted training group had greater improvements in HbA1c compared to the postprandial group (-0.3% versus 0.0%, p=0.033). There was no difference in changes in liver PDFF between groups (-1.6% versus 0.3%, p=0.221) but visceral fat and intramuscular fat decreased to a greater extent following fasted exercise. **Conclusions:** Although our study had a small sample size, it suggests that exercise after an overnight fast can have high adherence and represents an option for people with T2D to improve longer-term indicators of glycemia and ectopic fat depots.

Key Words: FASTING, LIVER FAT, MAGNETIC RESONANCE IMAGING, PHYSICAL ACTIVITY, TYPE 2 DIABETES

INTRODUCTION

Regular exercise and dietary improvements are widely recommended for individuals with type 2 diabetes (T2D) to improve insulin sensitivity and glycemic control (1-3). However, the optimal nutritional environment in which exercise should be delivered is not well understood, particularly over the long term. The most recent American College of Sports Medicine position statement for exercise and T2D was the first to include a section on 'exercise timing', and recommended exercise after a meal for patients with T2D (4). These recommendations are partly based on acute studies where exercise was performed in the afternoon or evening (5-7) making it difficult to discern the impact of time of day versus the impact of fasting versus postprandial exercise. Acute reductions in glycemia are important, particularly during the postprandial period, yet the longer-term training adaptations that may occur with regular fasted exercise are not well understood in this population.

Exercise after an overnight fast takes place at a time when liver glycogen stores are reduced (8, 9). Alternatively, when exercise is performed in the postprandial period, exogenous carbohydrate oxidation is increased, supplying about 25% of the energy (10, 11). This greater reliance on exogenous glucose may be beneficial in reducing postprandial blood glucose concentrations, yet leads to a corresponding decrease in endogenous glucose or fat oxidation (11) which over time, could reduce some metabolic adaptations to exercise, as discussed in recent reviews (12-14).

In healthy males, 6 weeks of fasted exercise (300 minutes per week), but not morning postprandial exercise, led to improvements in oral glucose tolerance (15). More recently, a

greater improvement in insulin sensitivity following 6 weeks of fasted compared to morning postprandial exercise (150 minutes per week) was also demonstrated in males with overweight or obesity (16). However, to our knowledge, only 2 studies have compared the effect of fasted versus postprandial morning exercise on glycated hemoglobin (HbA1c) in T2D. Brinkmann et al randomized 30 adults with T2D into 8 weeks of combined aerobic and resistance exercise, either after an overnight fast or after breakfast (17). Results indicated an overall reduction in HbA1c (-0.3%) but no difference in HbA1c between the two groups (17). In a second study in 25 males with T2D, 12 weeks of aerobic exercise caused greater improvements in HbA1c in the morning postprandial exercise group (-0.3%) compared to the fasted group (-0.08%) (18). Notably, neither of the two studies in people with T2D met the recommended 150 minutes per week of aerobic activity (1, 3).

Furthermore, none of the above studies examined changes in ectopic fat (e.g., in muscle or liver), which may be more directly targeted through fasted exercise. These fat depots represent a smaller proportion of total body fat compared to subcutaneous fat and are not directly measured by most conventional body composition techniques, including DXA. Measurements of liver fat, visceral fat, intermuscular fat and intramuscular fat can be performed non-invasively through magnetic resonance imaging (MRI) and provide insight on how fasted exercise may differ from postprandial exercise. Although traditionally assessed by biopsies, fatty liver disease can be defined as >5% fat in the liver as assessed by MRI (19) and has been found to affect over half of those living with T2D; more than double the rate of the general population (20). Fat stored within and around skeletal muscle fibers or muscle groups is not necessarily pathological,

but excess of some forms can contribute to insulin resistance in T2D (see (21) for a more thorough review).

The objective of this study is to compare pre-breakfast (i.e., fasting) to post-breakfast exercise on HbA1c and liver fat in adults with T2D. It was hypothesized that fasted exercise training would lead to greater improvements in HbA1c and liver fat compared to morning postprandial exercise training.

METHODS

Trial design

This trial was a 16-week, 2 arm, randomized control trial comparing fasted versus morning postprandial exercise in adults with T2D. The co-primary outcomes are HbA1c and liver fat. Secondary outcomes include MRI derived measures of pancreatic and skeletal muscle fat, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT). The trial was registered with clinicaltrials.gov (NCT03908281) and was approved by the University of Alberta Health Research Ethics Board (Pro00088195).

Following completion of the pre-screening and baseline measures, and immediately prior to the study intervention, participants were randomly assigned to one of two groups: 1) exercise performed after an overnight fast (FAST) or 2) exercise performed in the postprandial period after breakfast (FED). Each individual participant allocation was completed by an individual not directly involved with the study who used a computer-generated list that only they had access to. Randomization was linked to participant ID numbers and stratified according to sex. Allocation

was further concealed using variable block size (e.g., 2, 4). While participants were told if they would exercise pre or post breakfast, individuals collecting the blood samples, acquiring the MRI data, and calculating ventilatory threshold were blinded to group allocation (e.g., single blind for the primary outcomes). In addition, participants were not randomized until baseline assessments were completed.

Participants

Participants were recruited in the Edmonton area in Alberta, Canada. Recruitment strategies included radio advertisement, posters in community settings, and recruitment through existing databases of past research participants. To be eligible, participants were required to be between the ages of 30-75 years, diagnosed with T2D for a least 6 months with an HbA1c <9.0% (74.9 mmol/mol). Eligible participants were achieving <150 minutes of moderate to vigorous intensity aerobic exercise/week (self reported), had a BMI ≥25.0 kg/m^2, and central obesity as determined by waist circumference according to the Diabetes Canada cut-offs (22). Exclusion criteria included previous myocardial infarction, stroke, or coronary artery disease; resting blood pressure (BP) ≥160/100 mmHg; resting heart rate (HR) ≥100bpm; using exogenous insulin; inability to walk continuously for 50 minutes.

Initial Assessment

Participants underwent preliminarily pre-screening questions over the phone. They were then invited to the Physical Activity and Diabetes Laboratory at the University of Alberta to undergo further screening and baseline assessments. At this first visit, participants completed written informed consent and were invited to ask questions about the study. Participants

completed questionnaires regarding medical history, sleep behaviour (Pittsburgh Sleep Quality Index), physical activity (Godin Leisure-time Exercise Questionnaire), and depression (Patient Health Questionnaire). Resting HR and BP, height, weight, waist and hip circumference were measured to confirm eligibility. Participants were also given a requisition form to complete fasted bloodwork at a local DynaLife laboratory. The panel included HbA1c (used to confirm eligibility), fasting glucose, fasting insulin, aspartate transaminase (AST), alanine transaminase (ALT), total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Homeostatic Model Assessment of Insulin Resistance (HOMA IR) was calculated according to the formula fasting insulin(microU/L) x fasting glucose (mmol/L)/22.5 (23).

Fitness Test

Participants completed a graded aerobic exercise test on a treadmill to assess cardiorespiratory fitness at baseline and following the 16-week exercise intervention. The Parvo Medics TrueOne® 2400 Metabolic Measurement System (Sandy, Utah, USA) was used for this test and calibrated for air volume and gas concentrations prior to each test. Participants started the test at a comfortable, but brisk self-selected walking pace, determined with the assistance from the exercise physiologist, and a grade of 0%. Each minute, the incline was increased by 1.0%, while the speed remained the same throughout the test. The test was terminated after a respiratory exchange ratio (RER) of 1.0 was consistently exceeded, or if the participant experienced any pain or discomfort or signs of exercise intolerance. Heart rate (Polar HR monitors, Polar Electro, Kempele, Finland) and ratings of perceived exertion (RPE) using a 6–20 point scale were monitored during exercise and recorded every minute. Blood pressure was taken

and recorded every 2-3 minutes to ensure a normal response to exercise. Pre and post exercise results were compared to assess changes in cardiorespiratory fitness and substrate utilization.

MRI protocol

MRI scans were completed at baseline and following the 16-week exercise intervention. Scans were completed on a 3-T system (PRISMA; Siemens Healthineers, Erlangen, Germany) at the Peter Allen MR Centre at the University of Alberta Hospital. The scans were completed in the morning after a minimum 8-hour fast both at baseline and following the exercise intervention. All MRI images were acquired using a multi-echo chemical shift encoded pulse sequence to enable the calculation of water and fat separated images (24, 25) for liver, pancreas, and skeletal muscle (26).

Liver: Proton density fat fraction (PDFF) (percent) in the liver and pancreas were calculated as $F/(F+W)\cdot 100$, where F and W are the fat and water signal intensities from fat and water separated images (Figure 1). Three axial slices with a 6 mm thickness and 2.5 mm by 2.5 mm in-plane resolution were acquired with [1.09, 2.45, 3.81, 5.17, 6.53, 7.89] ms echo times, 9.2 ms repetition time, 13° flip angle and rate 2 parallel imaging (GRAPPA). The liver was manually traced on all three slices using custom software (MATLAB), with automated removal of blood vessels.

Pancreas: Six slices with a 6 mm thickness and 2.5 mm by 2.5 mm in-plane resolution were acquired with [1.09, 2.45, 3.81, 5.17, 6.53, 7.89] ms echo times, 9.2 ms repetition time, 13° flip

angle, rate 2 parallel imaging (GRAPPA). Multiple regions of interest within the pancreas were manually selected using custom software (MATLAB).

Skeletal Muscle: Fat volumes in skeletal muscle (thigh) were measured in intermuscular (between muscles) and intramuscular spaces (within the muscle), and corresponding quality measures were calculated with normalization to muscle volume (quality = fat volume /muscle volume ·100%). Five axial slices were acquired (centered 17 cm superior to the distal head of the femur) with a 3.5 mm thickness (12.5 mm gap), 2 mm by 2 mm in-plane resolution, [2.51 3.51 4.51 4.78 5.78 6.78] ms echo times, 9.0 ms repetition time, 30° flip angle, rate 2 parallel imaging (GRAPPA). A custom machine learning segmentation approach was used to identify subcutaneous fat, intermuscular fat, muscle and bone regions. Calculated parameters included volumes of subcutaneous fat, muscle, intermuscular fat and intramuscular fat (fat content in the muscle region) (Figure 1.).

Abdomen: A similar approach to skeletal muscle was used for assessment of abdominal body composition measured at the third lumbar vertebra. Abdominal fat and water separated images were used to calculate the volume of VAT, SAT, muscle volume and intramuscular fat volume (Figure 1). Eight slices with a 6 mm thickness were prescribed centered on the third lumbar vertebra with 2 mm by 2 mm in-plane resolution, [1.09, 2.45, 3.81, 5.17, 6.53, 7.89] ms echo times, 9.2 ms repetition time, 25° flip angle, rate 2 parallel imaging (GRAPPA). A custom machine learning segmentation approach was used to identify regions of VAT, SAT, muscle, and intermuscular fat from three central slices (Figure 1).

Exercise intervention

The exercise program was designed as a 16-week exercise program with supervised walking sessions 3 mornings per week, spread across the week (e.g., Monday, Wednesday, Friday). The duration of the exercise sessions progressed from 30 minutes/session during the first 2 weeks to 40 minutes/session at week 3. Sessions increased to 50 minutes/session at week 5, and increased to 60 minutes/session at week 13. Exercise intensity was initially prescribed at a workload corresponding to 70-80% of ventilatory threshold from the baseline exercise test. Ventilatory threshold was determined using the V-slope method (27). In some cases ventilatory threshold was difficult to determine using the V-slope method and in these cases, the ventilatory equivalent method was also used to help identify threshold, which has been shown to have good agreement with the V-slope method across different fitness levels (28). In March 2020, due to local health restrictions during the COVID-19 pandemic and the risk associated with in-person interactions, the protocol changed to a home-based walking program. The home-based protocol was similar to the supervised program in that it progressed in duration over the 16-week period, but the intensity of the walking sessions could not be as tightly controlled in the home-based program. Participants who walked outdoors were instructed to walk at a brisk walking pace to increase their breathing rate. For participants completing the full home-based walking program, a supervised session in the laboratory was completed every 2 weeks. This supervised session did not apply to those walking between March 2020 – August 2020 during the most severe COVID-19 restrictions (n=8).

The FAST group was instructed to walk in the morning after an overnight fast and prior to their breakfast meal, while the FED group was asked to begin their walk within 1 hour after

consuming breakfast. When unsupervised, participant adherence and the time of each exercise session was tracked though the use of pedometers (Yamax DigiWalker 200) and text messaging of images of pedometer step count immediately before and after each walking session. The study coordinator spoke or texted with participants regularly to remind them of any changes to their walking session (e.g., increase in walking duration).

Wrist worn accelerometers (ActiGraph wGT3X+) were worn for 7 days prior to the start of the intervention (i.e., 'free-living' conditions), during the first 7 days of the intervention, and during the last 2 weeks of the intervention (i.e., weeks 15 and 16). Wrist worn accelerometers were chosen for reduced burden on participants and for improved compliance(29). In addition to tracking total activity, the accelerometers were used to verify exercise times. Food logs were also used during the same weeks as the accelerometers to assess the breakfast mealtime in relation to the exercise sessions, as well as to assess the macronutrient and fiber content of the breakfast meals. Food was analysed using the Canadian Nutrient File by 2 separate individuals.

Statistical analysis

Analysis was performed through Statistical Package for Social Sciences (SPSS) software version 24.0. Outcomes of interest were compared between the FAST and FED exercise groups using a 2x2 ANOVA to compare the main effect of time (pre vs post), the main effect of group (FAST vs FED) and the interaction. Data from the accelerometers and food logs were analysed using a 2x3 ANOVA to compare the main effect of time (pre intervention, week 1 of exercise, and weeks 15 & 16 of exercise), the main effect of group (FAST vs FED) and the interaction. Values are shown as mean±standard deviation except for insulin and HOMA-IR which are

shown as median [interquartile range]. Insulin and HOMA-IR were log transformed prior to analyses to better approach normality.

RESULTS

Recruitment & Participants

Between September 2019 and July 2022, 123 individuals were screened for inclusion (Figure 2). The main reasons for exclusion were participants being above the age range (n=14, using insulin (n=16), or a history of a cardiovascular event (n=12). Twenty interested and eligible participants were randomized, and 16 participants completed the study (8 FAST, 8 FED, 50% female). Two participants dropped out due to concerns with the COVID-19 pandemic in March 2020, 1 dropped out due to family concerns, and 1 dropped out for reasons unknown.

Participant characteristics are presented in Table 1. Participants were diagnosed with T2D for 10.1±6.9 years and included 10 males and 10 females. Two female participants were pre-menopausal, 3 female participants had hysterectomies (time since hysterectomy 27±9.5 years), and 5 participants were post-menopausal (time since last menstrual cycle 10.2±5.8 years). Eighty-five percent of participants were prescribed metformin, and the average number of prescribed hypoglycemic agents was 2 (range: 1-3). During the trial, two participants stopped taking one of their hypoglycemic agents; one in the FAST group and one in the FED group. Therefore, due to medication instability during the trial, per-protocol analysis was also performed to assess changes HbA1c.

Blood Panel

There was no difference in HbA1c pre vs post exercise training (main effect of time, p=0.588) and no difference between the FAST and FED groups (-0.1% vs +0.1%, time by group interaction p=0.286, main effect of group p=0.312). However, when 2 participants were removed from analysis due to changes in their glucose lowering medications during the trial (FAST=1, FED=1) the FAST group had a reduction in HbA1c compared to the FED group (-0.3%, 0.0%, time by group interaction p=0.033, Figure 3). Individual changes in HbA1c are shown in Figure 3a. There were no differences in fasting plasma glucose, serum insulin concentrations, or HOMA-IR pre vs post exercise training. HDL improved pre vs post exercise training (main effect of time p=0.029), but there was no difference between groups (time by group interaction p=0.142, main effect of group p=0.739). Similarly, there were no changes in liver enzymes alanine transaminase and aspartate aminotransferase pre vs post intervention. There were baseline differences for many of the plasma lipid concentrations, which were elevated in FED compared to FAST and persisted to the end of the intervention (i.e., main effect of group). However, compared to FAST, total cholesterol improved in the FED group only (+0.1 vs -0.2, time by group interaction p=0.018, main effect of group p=0.002, main effect of time p=0.805). Details of metabolic profiles are presented in Table 2.

Magnetic Resonance Imaging

Details of MRI data are presented in Table 2 and Figure 3. For MRI analysis, 7 participants were included in the FAST group as 1 participant did not complete the follow up scan due to COVID-19, while 8 were included in the FED group. There were no differences in liver or pancreas PDFF pre vs post exercise intervention, and changes were not statistically

different between groups (liver: -1.6% vs +0.3%, time by group interaction p=0.221), (pancreas: -1.6% vs -0.4%, time by group interaction p=0.521). Visceral adipose tissue decreased to a greater extent in the FAST group (-58.3ml vs +34.2ml, time by group interaction p=0.035).

Muscle volume decreased to a greater extent in the FAST group compared to the FED group (-35.3ml vs +1.6ml, time by group interaction p=0.033, main effect of time p=0.047), and intramuscular fat was reduced to a greater extent in the FAST group (-0.1ml vs +0.6ml, time by group interaction p=0.035). Overall, when both groups were considered together, there was a positive correlation between changes in muscle mass and changes in intramuscular fat (r=0.84, n=15, p<0.001).

Exercise Adherence & Changes in fitness.

One participant completed the full supervised exercise protocol prior to the COVID-19 pandemic and 8 participants started with the supervised program before transitioning to the home-based program in March 2020. Four participants transitioned between weeks 7 and 8 of the intervention, 2 participants in week 9, and 2 in the first week of the intervention (2 dropout). Ten participants completed the full home-based program (2 dropout). A total of forty-eight walking sessions were prescribed to each participant over the 16-week intervention. The average number of walking sessions in both the FAST and FED groups was 47, corresponding to 98% adherence.

During the graded exercise test, there was no difference in VO_2 measured when RER reached 1.0 (i.e., near the termination of the exercise test), see Table 3 for details. However, time required to reach an RER of 1 increased pre vs post exercise training in both groups (FAST =

+1.9 minutes, FED= +1.0 minutes, main effect of time p=0.001, main effect of group p=0.222, time by group interaction p=0.231). Data from wrist worn accelerometers indicated a main effect of group (p=0.047), for 24-hour physical activity levels (i.e., light physical activity and moderate to vigorous physical activity) between the free-living week, week 1, and weeks 15 & 16 of the intervention. There was no effect of time (p=0.631), and no time by group interaction (p=0.761). There was no effect of time (p=0.295) group (p=0.998) or time by group interaction (p=0.312) for MVPA across the three time points.

Dietary Intake

Data from food logs for the breakfast meal indicate no differences in energy intake between the free-living week, week 1, and weeks 15 & 16 of the intervention (main effect of time p=0.358), and no time by group interaction (p=0.877).. There were no differences in energy intake, carbohydrate, fat, or protein content across the three time points (main effect of time for energy intake p=0.358, carbohydrate p=0.441, fat p=0.587, protein p=0.491), no main effect of group (energy intake p=0.115, carbohydrate p=0.802, fat p=0.086, protein p=0.347) and no time by group interaction (energy intake p=0.887, carbohydrate p=0.329, fat p=0.951, protein p=0.602). Overall, the macronutrient distribution of the breakfast meal was 50.5±11.1% carbohydrate, 32.5±7.8% fat, and 19.1±7.3% protein. There were no differences in fibre intake across the three timepoints (main effect of time p=0.085), no effect of group (p=0.972), and no time by group interaction (p=0.672). See Supplemental Table 1 (Supplemental Digital Content, http://links.lww.com/MSS/D84) for complete details.

DISCUSSION

This is the first study in people with T2D to compare fasted vs postprandial morning exercise with a sufficient duration of exercise to meet the physical activity guidelines and to include novel measures of ectopic fat stores as measured by MRI. Sixteen weeks of fasted aerobic exercise resulted in greater reductions in HbA1c compared to morning postprandial exercise. The difference was not statistically significant in the overall analyses but became significant after excluding a participant from each group due to changes in their glucose lowering medications during the trial. No differences between groups were observed in liver, pancreas, or total muscle fat, while intramuscular fat and VAT were decreased to a greater extent in the fasted exercise group.

To date, there are only 2 studies directly comparing fasted vs postprandial morning exercise training on HbA1c in T2D and neither of these studies found greater reductions in HbA1c with fasted exercise (17, 18). Brinkman et al attributed this to a potential elevation in blood insulin concentrations in their T2D population compared to previous studies in healthy populations, with an increase in circulating insulin thought to downregulate catabolic pathways elicited through fasted exercise. The 30 minutes of aerobic exercise per session in the study by Brinkman et al., and the 45 minutes per session in the study by Verboven et al., may not have been sufficient to elicit fasted adaptations. Previous fasted studies in people without T2D have largely focused on longer periods of endurance-based exercise (16, 30). Our study included up to 180 minutes/week for the last 4 weeks of the intervention which may have been more effective in depleting endogenous lipid stores than the shorter duration exercise sessions, therefore leading to greater improvements in insulin sensitivity, and subsequently HbA1c. Since substrate utilization

during exercise depends on the intensity and duration of exercise (31), this could be an important aspect to consider when designing fasted exercise protocols for this population. Although the present study did not assess acute substrate utilization during fasted versus fed exercise, previous work from our lab in people with type 2 diabetes has demonstrated significantly lower RER when 60 minutes of continuous exercise was performed in the fasted state vs after breakfast (RER = 0.82 vs 0.85 at the midpoint, and RER = 0.81 vs 0.85 at the end of exercise)(32). We also compared 50 minutes of fasted exercise to afternoon and evening exercise in people with type 2 diabetes and found RER to be significantly lower in the fasted condition (0.78) compared to the afternoon (0.83) and evening (0.84) exercise (33). This greater reliance on lipid oxidation during fasted exercise may explain some of the longer-term adaptations observed in our study. Our study also included an even split of males and females, whereas the previous study by Verboven et al., included only males. Females have been shown to have a greater reliance on fat oxidation during exercise compared to males (34), potentially augmenting the effects of fasted exercise. Our sample was too small to properly examine sex differences, but males and females seemed to respond similarly for the primary outcomes.

The present study was also 16 weeks in duration whereas the previous studies were 8 and 12 weeks (17, 18). It is possible that adaptations of fasted exercise may take longer to fully realize compared to postprandial exercise, where there are more immediate and acute reductions in postprandial glycemia. While these immediate reductions in postprandial glucose are beneficial in the short term, if the observed changes in intra-organ fat continue to improve with recurrent fasted exercise training, reductions in postprandial glucose may be further accentuated

over time due to improved peripheral insulin sensitivity. Although, this is speculative and deserves further investigation.

Reductions in ectopic fat could be one mechanism by which fasted exercise may be more beneficial for improving insulin sensitivity in T2D. Our results suggest a greater reduction in intramuscular fat following fasted exercise training. In a study by Edinburgh et al fasted vs fed exercise was acutely compared in males with overweight or obesity. Reductions in intramuscular fat, as measured by muscle biopsy, was observed in their fasted exercise group, but not the fed exercise group (16). Similarly, in a study by De Bock et al (35), reductions in intramyocellular lipid stores were demonstrated acutely in healthy individuals following fasted exercise, but not after fed exercise. These are both acute examples, and this study is the first training study to observe reductions in intramuscular fat in T2D as measured by MRI.

In the present trial, and contrary to our hypothesis, fasted exercise did not decrease liver fat compared to postprandial morning exercise. Although not significant, the fasted exercise group tended to have a decrease in both liver and pancreatic fat compared to the postprandial morning group. Previous meta-analysis (36-38) have reported significant overall reductions in liver fat following exercise compared to control, regardless of weight loss, however, little is known about the longer-term effect of exercise timing on liver fat in T2D. With a larger sample size, it could be possible that these data reach statistical significance. Interestingly, there appears to be some inter-individual differences in response, with the change in liver fat being more pronounced in some individuals, despite minimal changes in body weight. For example, 3/7 (43%) in the FAST group and 3/8 (38%) in the FED group did not show improvements in liver

fat. Work by Magalhães et al examined inter-individual differences to changes in fat mass following one year of exercise training (either moderate intensity exercise, high intensity exercise, or control) in type 2 diabetes. Although this study was assessing total fat mass as measured by DEXA, they found that 50% of participants in either the moderate or high intensity exercise groups appeared to be 'non-responders' to changes in fat mass(39). A more thorough discussion on sources of interindividual variability in responses to exercise in people with T2DM can be found here(40). Further investigation of the impact of fasted exercise on liver and pancreas fat is needed to better understand the potential impact in depleting these ectopic fat stores.

Surprisingly the fasted group had a decrease in total muscle volume. This was not expected and is difficult to explain in the absence of weight loss. Although, it should be highlighted that the decrease in total muscle volume was strongly correlated with the decrease in intramuscular fat (r=0.84). However, the total volume of intramuscular fat loss was much less than the reduction in muscle volume, and therefore cannot explain the muscle volume loss on its own. Several studies have also found that fasted training can lead to greater post exercise increases in muscle glycogen (30, 35), so it is not clear why muscle volume decreased with fasted exercise. With this decrease in muscle volume, careful consideration for dietary and protein intake following fasted exercise and/or the addition of strength training may be necessary to attenuate the loss of muscle mass, but further investigation is needed. Interestingly VAT decreased only in the fasted group, while there was a greater decrease in SAT in the FED group. A reduction in either of these adipose tissue depots would be considered beneficial, although a reduction in VAT could lead to more favourable changes in insulin sensitivity over time.

The observed changes in body composition could also be impacted by nutritional intake during the study. Since diet was not controlled, it could also be possible that the nutritional intake differed between groups. Although, data from participant breakfast food logs indicated no difference in macronutrient or total energy intake between groups. A limitation of our study was the absence of measurement of dietary intake overall several days, making it difficult to understand the impact of exercise timing on overall energy intake and diet quality. Data on meals outside of breakfast (i.e., lunch and supper) were not collected and this could have identified differences between groups. However, it could be speculated that energy intake was relatively consistent throughout the trial as weight did not change in either group.

Other limitations of the current study include a small sample size. Power calculations determined a priori indicated that in order to detect a clinically significant difference in HbA1c (e.g., -0.3%) between fasted versus fed exercise with a SD of 0.3, a sample size of 34 (17 per group) would have been required to provide 80% power. Given our budgetary constraints, a sample size of 20 was selected to obtain preliminary data for a more definitive trial. There were also 2 participants who had medication changes during the trial, further decreasing sample size for per protocol analyses of our primary outcome, HbA1c. Another limitation was the unsupervised nature of the exercise sessions due to the protocol change during the COVID-19 pandemic which could have led to a lower intensity of exercise than initially prescribed. Despite these limitations, this study had many strengths including the novel magnetic resonance imaging outcomes and high adherence to the exercise sessions.

CONCLUSIONS

In conclusion, this is the first study to suggest that regular fasted exercise training could be more beneficial than postprandial morning exercise for improving HbA1c in adults with T2D. The high adherence rate and the absence of negative impacts on breakfast quality or physical activity throughout the rest of the day support the feasibility of exercise before breakfast. To our knowledge, it is also the first study to compare fasted vs postprandial exercise training on changes in ectopic lipid stores in the liver, pancreas, and skeletal muscle. Greater reduction in some ectopic fat depots following fasted training (e.g., VAT and intramuscular fat), was encouraging, but should be confirmed in larger trials. The potential for reductions in muscle volume with fasted training should also be further explored.

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FIGURE LEGENDS

Figure 1. Magnetic Resonance Imaging of Fat and Muscle Volumes. Illustrative water and fat separated images in skeletal muscle (thigh), liver, pancreas and abdomen (third lumbar vertebra). PDFF – proton density fat fraction (%).

Figure 2. FED Trial Consort Flow Chart

Figure 3. Changes in glycated hemoglobin (**A**) and liver proton density fat fraction (**B**) pre vs post exercise training between the fasted and postprandial exercise groups. Individual changes are shown as dots and group average are shown as bars. The open circles indicate the 2 participants who had glucose lowering medication changes during the trial and were not included in the 2×2 ANOVA.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplementary material.docx

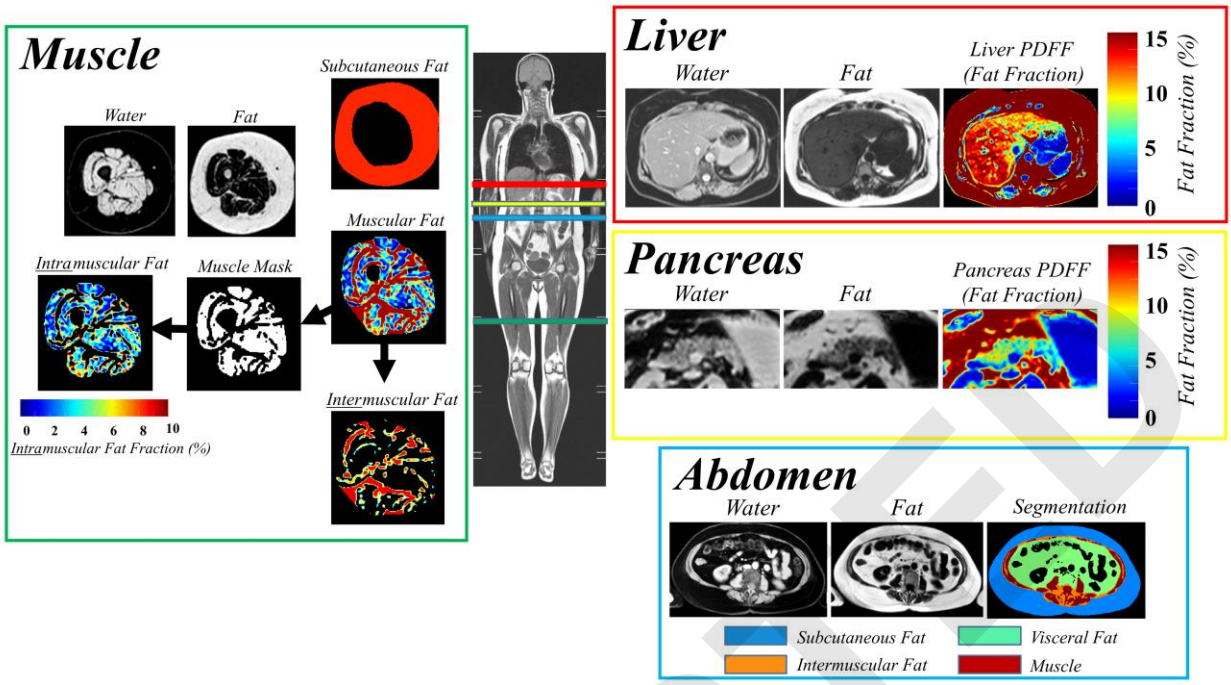

Figure 1

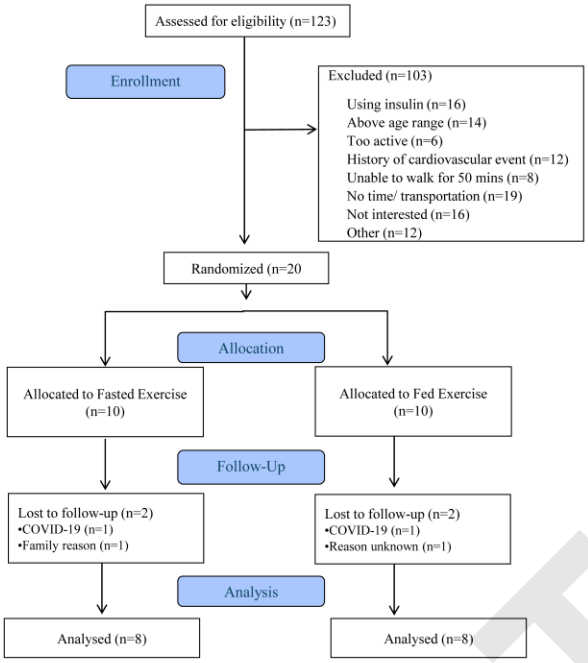

Figure 2

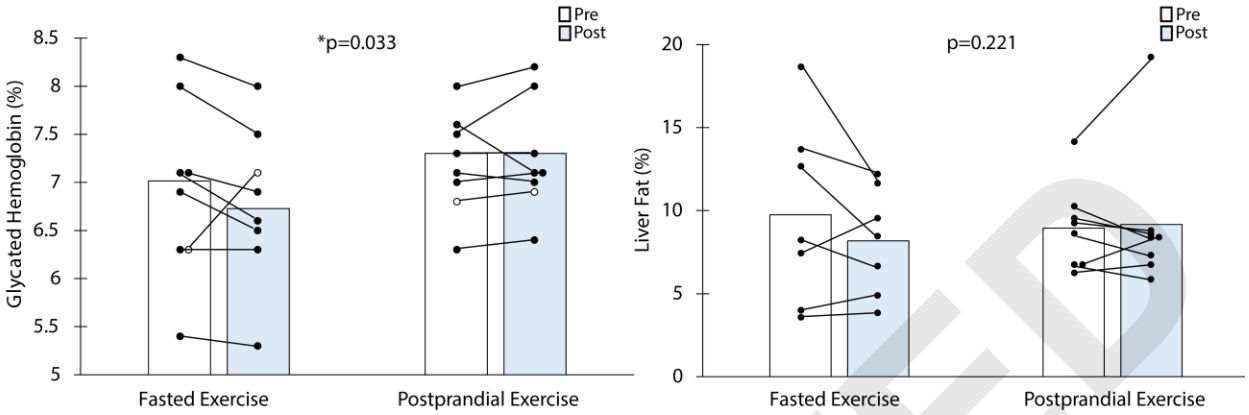

Figure 3

Table 1. Participant Baseline Characteristics

	All (n= 20)	**FAST** (n=10)	**FED** (n=10)
Sex, n (M/F)	20 (10/10)	10 (5/5)	10 (5/5)
Age (yrs)	59.8 ±9.0	60.9 ±9.0	58.6 ±9.2
Duration of Diabetes (yrs)	10.1 ±6.9	12.2 ±7.2	7.7 ±6.1
HbA1c (%)	7.2 ±0.7	7.0 ±0.9	7.4 ±0.6
Resting SBP (mmHg)	126 ±13	124 ±15	129 ±10
Resting DBP (mmHg)	77 ±9	74 ±10	79 ±9
Resting HR (bpm)	76 ±11	73 ±10	79 ±11
Height (cm)	169.1 ±8.9	168.9 ±8.6	169.4 ±9.6
Weight (kg)	93.8 ±16.3	91.5 ±17.3	96.0 ±15.9
BMI (kg/m^2)	32.5 ±4.6	31.8 ±4.0	33.2 ±5.3
Waist circumference (cm)	111.6 ±11.8	110.9 ±13.4	112.2 ±10.8
Hip circumference (cm)	114.8 ±11.3	114.0 ±9.8	115.5 ±12.9
Sleep (PSQI)	4.3 ±3.0	3.7 ±2.6	4.9 ±3.4
Depression (PHQ-8)	2.7 ±2.7	1.6 ±0.8	3.8 ±3.4
PA (GLTEQ)	23 ±12.8	26.8 ±15.2	19.2 ±9.1
Glucose medications (%)			
Metformin	85%	90%	80%
Sulphonylurea	35%	30%	40%
DPP-4 inhibitor	25%	30%	20%
SGLT2 inhibitor	30%	30%	30%
GLP-1 agonist	30%	30%	30%
TZD	5%	10%	0%
BP medications (%)			
Beta blocker	5%	0%	10%
ARB	30%	30%	30%
ACE inhibitor	30%	30%	30%
Diuretic	10%	20%	0%
Calcium channel blocker	10%	10%	10%
Lipid medications (%)			
Statin	55%	70%	40%
Fibrate	5%	0%	10%

n=sample size, M=male, F=female, yrs=years, HbA1c =glycated hemoglobin, %=percent, SBP=systolic blood pressure, DBP=diastolic blood pressure, mmHg=millimeters of mercury, HR= heart rate, bpm=beats per minute, cm=centimeters, kg=kilograms, BMI=body mass index, PSQI = Pittsburgh Sleep Quality Index, PHQ-8 = Patient Health Questionnaire, PA=physical activity, GLTEQ = Godin Leisure-Time and Exercise Questionnaire (physical activity), DPP-4 inhibitor=dipeptidyl peptidase 4 inhibitor, SGLT2 inhibitor=sodium glucose tranporter-2 inhibitor, GLP-1 agonist =glucagon-like peptide 1 receptor agonist, ARB=angiotensin receptor blocker, ACE=angiotensin converting enzyme inhibitor.

Table 2. Changes in cardiometabolic markers and magnetic resonance imaging outcomes, pre vs post exercise training

	FASTED (n=8)			FED (n=8)			p	p	p
	Baseline	**End of training**	**Change from baseline**	**Baseline**	**End of training**	**Change from baseline**	**Group**	**Time**	**Group x Time**
CARDIOMETABOLIC MARKERS									
A1C (%)	6.9±0.9	6.8±0.8	-0.1 ±0.4	7.2±0.5	7.3±0.6	0.1 ±0.3	0.312	0.588	0.286
Fasting glucose (mmol/L)	7.0±1.4	7.0±0.9	0.0 ±1.2	8.3±2.1	9.0±2.2	0.7 ±1.1	0.068	0.195	0.195
Fasting insulin (mmol/L)	58.5 [40.3-63.8]	66.5 [45.0-78.0]	8.4 [-2.8-15]	57.0 [45.5-97.25]	68 [37.0-70.8]	-15 [-38.8-17]	0.750	0.669	0.238
HOMA IR	2.4 [1.7-2.7]	2.6 [2.2-3.3]	0.2 [-0.2- 1.0]	3.2 [2.0-5.8]	4.0 [1.6-5.0]	-0.7 [-1.5-1.1]	0.809	0.974	0.460
HDL (mmol/L)	1.1±0.3	1.2±0.3	0.1 ±0.1	1.2±0.3	1.2±0.2	0.0 ±0.1	0.739	*0.029	0.142
LDL (mmol/L)	1.7±0.5	1.7±0.5	0.0 ±0.2	2.6±0.5	2.7±0.5	0.1 ±0.4	*0.002	0.671	0.789
Non-HDL (mmol/L)	2.2±0.7	2.2±0.6	0.0 ±0.7	3.7±0.7	3.5±0.7	-0.2 ±0.1	*0.001	0.188	0.075
TC (mmol/L)	3.3±0.8	3.4±0.8	0.1±0.2	4.9±0.7	4.7±0.7	-0.2 ±0.2	*0.002	0.805	*0.018
TG (mmol/L)	1.0±0.4	1.1±0.4	0.1 ±0.2	2.3±0.8	1.8±0.7	-0.5 ±0.9	*0.002	0.221	0.176
ALT (IU/L)	29.0±13.8	27.1±7.2	-1.9 ±9.5	31.8±6.2	28.9±9.0	-2.9 ±4.7	0.616	0.218	0.790
AST (IU/L)	21.0±4.4	23.4±4.4	2.4 ±3.1	22.9±4.0	23.9±5.6	1.0 ±4.3	0.607	0.105	0.480
Body weight (kg)	87.4±17.3	86.0±16.3	-1.4 ±3.4	94.3±17.1	94.9±15.1	0.6 ±4.1	0.365	0.713	0.339
SBP (mmHg)	120±15	121±11	-1 ±13	128±11	128±11	0 ±8	0.173	0.848	0.955
DBP (mmHg)	74±8	73±7	-1 ±6	78±8	79±7	1 ±3	0.201	0.961	0.467
Resting HR (bpm)	74±10	72±10	-2 ±7	75±8	75±7	0 ±5	0.617	0.299	0.571
MAGNETIC RESONANCE IMAGING OUTCOMES									
	FASTED (n=7)			**FED (n=8)**					
Liver PDFF (%)	9.8 ±5.5	8.2±3.2	-1.6 ±3.1	8.9 ±2.6	9.2 ±4.2	0.3 ±2.3	0.962	0.353	0.221
Pancreas PDFF (%)	12.9 ±8.3	11.3 ±6.1	-1.6 ±3.3	12.9 ±7.4	12.5 ±6.6	-0.4 ±3.4	0.860	0.279	0.521
Muscle Volume (ml)	455.3 ±70.1	420.0 ±64.6	-35.3 ±30.5	401.2 ±54.5	402.8 ±44.3	1.6 ±29.2	0.246	0.047*	0.033*
Muscle Fat (ml)	83.6 ±22.8	81.8 ±23.4	-1.8 ±2.3	89.5 ±25.3	89.4 ±25.5	-0.1 ±3.7	0.602	0.265	0.306
Intermuscular fat (ml)	64.4 ±19.4	63.6 ±20.1	-0.8 ±3.2	70.2 ±20.6	69.5 ±20.3	-0.7 ±3.1	0.584	0.377	0.942
Intramuscular fat (ml)	19.2 ±3.7	18.2 ±3.5	-1.0 ±1.2	19.3 ±5.3	19.9 ±5.5	0.6 ±1.4	0.714	0.572	0.035*
SAT (ml)	650.8 ±319.5	647.0 ±290.0	-3.8 ±88.7	653.9 ±242.7	614.7 ±176.3	-39.2 ±115.7	0.913	0.439*	0.522*
VAT (ml)	723.2 ±189.4	664.9 ±204.8	-58.3 ±63.0	693.4 ±314.0	727.6 ±283.1	34.2 ±85.5	0.902	0.550	0.035*

Abdominal intramuscular fat (ml)	100.5 ±17.3	96.4 ±15.1	-4.1 ±5.7	101.1 ±31.38	107.3 ±34.6	6.2 ±12.8	0.677	0.701	0.072

Values shown as mean±standard deviation with the exception of insulin and HOMA IR which are shown as median [interquartile range]. A1C =glycated hemoglobin, %=percent, mmol/L=millimole per litre, ln=log transformed, HOMA IR=homeostatic model assessment of insulin resistance, HDL=high density lipoprotein cholesterol, LDL=low density lipoprotein cholesterol, TC=total cholesterol, TG=triglycerides, ALT=alanine transaminase, AST=aspartate aminotransferase, kg=kilogram, mmHg=millimeters of mercury, bpm=beats per minute, PDFF=proton density fat fraction, SAT=subcutaneous abdominal adipose tissue, VAT=visceral adipose tissue, ml=millilitres, %=percent

Table 3. Metabolic fitness data

	FASTED (n=6)			FED (n=8)			p	p	p
	Baseline	**End of training**	**Change from baseline**	**Baseline**	**End of training**	**Change from baseline**	**Group**	**Time**	**Group x Time**
VO_2 at RER 1.0	21.9±4.7	23.3±4.4	1.4±3.2	21.4±5.0	22.3±4.5	0.91±3.5	0.769	0.233	0.803
HR at RER 1.0	138±13	139±14	1.5±6.1	144±14	146±13	2±4.9	0.361	0.256	0.867
Mean test duration	10.4±1.2	12.3±1.7	1.9±1.3	9.1±2.5	10.0±2.7	0.8±2.7	0.133	0.001*	0.121
RER @70% VT	0.86±0.05	0.83±0.04	-0.02±0.06	0.86±0.04	0.82±0.05	-0.03±0.05	0.832	0.052	0.642
HR @70% VT	121±11	113±13	-7±8	125±12	123±14	-1±14	0.337	0.037*	0.119
VO_2 @70% VT	16.5±3.3	17.0±7.8	0.5±1.6	16.5±3.5	16.6±3.6	0.1±3.6	0.860	0.419	0.648

VO_2=volume of oxygen, HR=heart rate, RER=respiratory exchange ratio, VT=ventilatory threshold. The treadmill test was completed when participants reached an RER of 1.0 or asked to terminate the test.

Supplementary Material

	FASTED (n=7)			FED (n=7)			p	p	p
	Free living	Start of Training	End of Training	Free living	Start of Training	End of Training	group	Time	Time x group
LPA & MVPA (min)	288±90	273±47	275±71	216±24	209±42	228±55	0.047*	0.631	0.761
MVPA (min)	64±22	66±22	58±19	52±17	66±28	70±46	0.998	0.295	0.312
Breakfast meal energy intake (kcal)	446±102	429±124	496±167	362±157	350±88	357±114	0.115	0.358	0.877
Carbohydrate (g)	46.8±8.4	47.4±7.3	47.7±12.2	50.5±11.69	51.8±15.6	44.4±21.0	0.802	0.441	0.329
Protein (g)	17.4±3.0	18.2±4.4	16.8±5.6	20.8±10.4	21.7±10.2	22.1±11.0	0.347	0.491	0.602
Fat (g)	37.2±8.1	35.7±8.0	37.4±8.3	30.7±7.8	28.2±9.6	30.0±10.6	0.086	0.587	0.951
Fibre (g)	9.8±7.4	7.3±5.6	10.7±12.5	8.8±6.1	7.7±6.4	10.9±15.5	0.972	0.085	0.672

LPA=Light physical activity, MVPA=Moderate to vigorous physical activity, Min=minutes