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## Accepted Manuscript

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The effects of interval- vs. continuous exercise on excess post-exercise oxygen consumption and substrate oxidation rates in subjects with type 2 diabetes

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**ABBREVIATIONS:** EPOC, Excess post-exercise oxygen consumption; CON, control; CW, continuous walking; IW, interval walking; MMTT, mixed meal tolerance test; T2D, type 2 diabetes mellitus; DXA, dual x-ray absorptiometry; RMR, resting metabolic rate; HR, heart rate; FFA, free fatty acid; RER, Respiratory exchange ratio; Ra, rate of appearance; Rd, rate of disappearance;

**ABSTRACT**

*Background:* For unknown reasons, interval training often reduces body weight more than energy-expenditure matched continuous training. We compared the acute effects of time-duration and oxygen-consumption matched interval- vs. continuous exercise on excess post-exercise oxygen consumption (EPOC), substrate oxidation rates and lipid metabolism in the hours following exercise in subjects with type 2 diabetes (T2D).

*Methods:* Following an overnight fast, ten T2D subjects (M/F: 7/3; age =  $60.3 \pm 2.3$  years; body mass index (BMI) =  $28.3 \pm 1.1$  kg/m<sup>2</sup>) completed three 60-min interventions in a counterbalanced, randomized order: 1) Control (CON), 2) continuous walking (CW), 3) interval-walking (IW – repeated cycles of 3 min of fast and 3 min of slow walking). Indirect calorimetry was applied during each intervention and repeatedly for 30 min per hour during the following 5 hours. A liquid mixed meal tolerance test (MMTT, 450 kcal) was consumed by the subjects 45 min after completion of the intervention with blood samples taken regularly.

*Results:* Exercise interventions were successfully matched for total oxygen consumption (CW =  $1641 \pm 133$  ml/min; IW =  $1634 \pm 126$  ml/min,  $P > 0.05$ ). EPOC was higher after IW ( $8.4 \pm 1.3$  l) compared to CW ( $3.7 \pm 1.4$  l,  $P < 0.05$ ). Lipid oxidation rates were increased during the MMTT in IW ( $1.03 \pm 0.12$  mg/kg/min) and CW ( $0.87 \pm 0.04$  mg/kg/min) compared with CON ( $0.73 \pm 0.04$  mg/kg/min,  $P < 0.01$  and  $P < 0.05$ , respectively), with no difference between IW and CW. Moreover, free fatty acids and glycerol concentrations, and glycerol kinetics were increased comparably during and after IW and CW compared to CON.

*Conclusions:* Interval exercise results in greater EPOC than oxygen-consumption matched continuous exercise during a post-exercise MMTT in subjects with T2D, whereas effects on substrate oxidation and lipid metabolism are comparable.

**KEYWORDS**

Walking, interval walking, EPOC, lipid metabolism, lipolysis, free fatty acid, glycerol

## 1. INTRODUCTION

Most subjects with type 2 diabetes mellitus (T2D) are overweight or obese, and this is considered to be a key element in the pathogenesis of T2D (1). Moreover, weight loss leads to improved insulin sensitivity and glycemic control in T2D subjects (2). As such, interventions that effectively promote weight loss are warranted.

Exercise training may improve insulin sensitivity and glycemic control both directly and indirectly, the latter partly via the induction of a weight loss, and the ability for a training intervention to induce and sustain a weight loss may be greater if training is performed with higher intensity (3). In that respect, we have shown that 4 months of interval-walking (IW) training results in a substantial weight loss in opposition to continuous walking (CW) training matched for training-contingent energy-expenditure (4). The reason for this discrepancy is unclear, but since no differences between or changes within intervention groups on energy intake was found (4), the explanation might be sought in differences in non-training-contingent energy expenditure.

Excess Post-exercise Oxygen Consumption (EPOC) is defined as the elevated oxygen consumption the hours following an exercise bout and consists of an acute and a prolonged component (5). A large EPOC means greater total energy-expenditure, which, if energy intake does not change, leads to a greater weight loss. The magnitude of EPOC is linearly related to exercise volume, but exponentially related to exercise intensity (6). Whereas EPOC after interval-training has been highlighted as a potentially underestimated factor for the weight governing effect seen (7), it is, to our knowledge, so far unknown whether IW produces the same EPOC as CW when exercise is matched with regards to energy expenditure and duration.

Subjects, who have a low lipid oxidation capacity, are potentially at risk of gaining weight over time (8-10). Since exercise results in increased lipolysis and lipid oxidation in healthy subjects the hours following an exercise bout (11-14), the weight governing effect of exercise may, at least partly, be dependent on the capacity of the exercise bout to increase lipolysis and lipid oxidation. There is some indication that exercise with higher intensity promotes post-exercise lipolysis and lipid oxidation to a greater extent than exercise with lower intensity (15;16), although evidence for this is conflicting (12). Subjects with T2D have dysregulated lipid metabolism in general (17), as well as during and after exercise (18), and although exercise-related increases in lipolysis and lipid oxidation are apparent in diabetic subjects (19), neither the influence of exercise intensity or the effects of interval-type exercise on lipolysis and lipid oxidation in T2D patients is known (20).

Furthermore, little is known about the effects of exercise on lipid metabolism in the postprandial state, which is considered to be the period mainly responsible for the increased atherosclerosis seen in T2D subjects (21).

Although IW mediates a substantial amount of metabolic effects directly and independent of changes in body composition (22), our recent evidence (that IW training results in greater weight loss than CW training) warrants further investigations. Thus, the objective of this study was to compare the effects of an acute bout of IW with an acute bout of oxygen-consumption and time-duration matched CW vs. no exercise on EPOC, substrate oxidation and lipolysis rates during a meal ingested in the hours following exercise in T2D subjects. We hypothesized that exercise would increase both EPOC and post-exercise lipid oxidation rates and that IW would result in greater EPOC and post-exercise lipid oxidation rates compared to CW.

## **2. METHODS**

### **2.1 Subjects**

Subjects with T2D (23) were recruited and underwent a medical screening. Exclusion criteria were the use of exogenous insulin,  $\beta$ -blocking agents, smoking, pregnancy, evidence of liver, renal, cardiopulmonary disease and diseases contraindicating physical activity (24). Written informed consent was obtained from all subjects. The study was approved by the ethical committee of the Capital Region of Denmark and registered at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) (NCT01987258). Other parts of the study have previously been published (25).

At the screening day, a Dual X-ray Absorptiometry (DXA; Lunar Prodigy Advance; GE Healthcare, Madison, WI, USA) scan and a graded walking  $\text{VO}_2$ peak-test (4;26) with a portable indirect calorimetric system (Cosmed K4b<sup>2</sup>, Rome, Italy) was performed. Moreover, familiarisation to the exercise interventions was performed on a treadmill (Technogym Runrace, Gambettola, Italy) using a stationary indirect calorimetric system (Cosmed Quark, Rome, Italy).

All subjects completed all trials and baseline characteristics were as follows: N = 10; Males = 7; Age =  $60.3 \pm 2.3$  years; Body mass =  $85.9 \pm 3.6$  kg; Body mass index [BMI] =  $28.3 \pm 1.1$  kg/m<sup>2</sup>; Lean body mass =  $58.8 \pm 3.2$  kg; Body fat percentage =  $32.6 \pm 2.2$  %;  $\text{VO}_2$ peak =  $2.1 \pm 0.2$  l O<sub>2</sub>/min.

### **2.2 Trials**

Three trials were performed in a randomised, counterbalanced order with 1-2 weeks in between. Trials were identical except for the following interventions: 1) One hour of IW, 2) One hour of oxygen-consumption matched CW, and 3) One hour of rest (no walking; CON). Subjects were instructed to abstain from antidiabetic medication and avoid vigorous physical activity, caffeine consumption and alcohol from 48 hours before the intervention day to after each trial. Compliance to this was ensured by activity monitoring (Actiheart®) and food records for 24 hours prior to initiation of the intervention day. Subjects were provided copies of their food record during the first trial and were instructed to ingest the same food for 24 hours prior to the intervention day in the following 2 trials.

### **2.3 Intervention day**

Subjects met fasting ( $\geq 8$  hours) in the laboratory at 8:00 am. Body weight was measured and bilateral antecubital venous lines for tracer infusion and blood sampling were placed. A primed ( $1.5 \mu\text{mol/kg}$ ), continuous ( $0.1 \mu\text{mol/kg/min}$ ) infusion of  $[1,1,2,3,3\text{-}^2\text{H}_5]$ glycerol tracer was initiated and subjects rested in a supine position for a 1 hour tracer load period. Forty-five min into this tracer load period, a 15 min resting metabolic rate (RMR) measurement was performed using indirect calorimetry (Cosmed Quark) with a ventilated hood. The 1-hour intervention was then begun with both IW and CW performed at the treadmill with 1% incline (except for one subject who, due to unpleasantly high walking speeds, walked with 7% incline during both IW and CW). Indirect calorimetry (Cosmed Quark) was applied throughout the session using breath-by-breath measurements. The goal was to match IW and CW with regards to mean oxygen consumption. As such, IW consisted of alternating 3-min slow and fast intervals aiming for oxygen uptake rates at 54 and 89% of the individual peak  $\text{VO}_2$ -rate, respectively, and the oxygen uptake rate of continuous-walking was aimed at 73% of the individual peak  $\text{VO}_2$ -rate as previously reported beneficial (4). To ensure correct oxygen uptake rates, walking speed was increased if oxygen uptake rates were too low and decreased if oxygen uptake rates were too high. Heart rate (HR) was monitored during the entire intervention (Cosmed, Wireless HR monitor).

After the intervention, subjects were placed on a chair keeping the indirect calorimetric system on for additionally 30 min, in order to assess the acute EPOC component. During the CON intervention, subjects sat on the chair for the entire duration but were also continuously monitored with indirect calorimetry. Forty-five minutes after cessation of the intervention, subjects were moved to a bed and a 4 hour liquid mixed meal tolerance test (MMTT; 300 ml, 450 kcal; energy

distribution: 15% protein, 55% carbohydrate [of which 25% sugar and 75% starch], 30% fat) was ingested over maximally 5 min. Indirect calorimetric measurements were performed for 30 min per hour for the following 4 hours, in order to assess the prolonged EPOC component. All investigations were performed in a temperature-controlled room, with a fixed temperature of 17°C.

#### **2.4 Blood and urine sampling and analyses**

Blood was sampled at the intervention day at baseline, during and after the intervention, and regularly during the MMTT. Lactate was measured in heparinized blood at a bedside analyser (ABL 7 series, Radiometer, Herlev, Denmark). Blood for tracer analyses was collected in sodium fluoride plasma tubes and blood for free fatty acid (FFA) analyses was collected in serum tubes. Plasma tubes were immediately placed on ice whereas serum tubes were left at room temperature for 30 min. Blood samples were centrifuged (2000g, 15 min, 4°C), and stored at -80°C until analyses. Glycerol concentration and tracer analyses were performed by a liquid chromatography–mass-spectrometry hexabenzoyl derivative method tracer (27). FFA was measured using a commercially available kit (Wako Diagnostics, VA, USA).

Urine was collected in two fractions: from the beginning of the intervention to the beginning of the MMTT and during the MMTT. Urine was analysed for urea by absorption photometry (Cobas 8000, Module c702, Roche Diagnostics, IN, USA).

#### **2.5 Calculations**

Mean oxygen consumption and HR were measured for the entire duration of the interventions. EPOC was calculated in each of the 30 min periods of indirect calorimetry measurements after CW and IW, by subtracting the corresponding CON oxygen consumption rate from the IW/CW oxygen consumption rate. We defined the acute component as the 30 min immediately after the intervention) and the prolonged component as the 1-5½ hours after the intervention. To calculate the total EPOC from the indirect calorimetry measurements, the prolonged EPOC component was multiplied with 300 min and added to the acute EPOC component multiplied with 30 min. The respiratory exchange ratio (RER; carbon dioxide production/oxygen consumption) was used to estimate carbohydrate and lipid oxidation using the equations from Frayn (28). The rate of protein oxidation was estimated from urinary nitrogen excretion. Glycerol rate of appearance (Ra) and disappearance (Rd) was calculated using non-steady-state assumptions, as previously described (29;30).



## 2.6 Statistics

Variables relevant only to the IW and CW trials were compared using Student's paired t-tests.

Variables relevant to all trials were compared using one-way repeated-measure analysis of variance (RM-ANOVA), and where significant interactions arose, Bonferroni post hoc tests were applied to identify significant differences between trials. All statistical analyses were performed by Prism v6 (Graphpad, San Diego, CA). Results are reported as mean  $\pm$  SEM. Statistical significance was accepted with  $P < 0.05$ .

## 3. RESULTS

### 3.1 Trials

No differences in physical activity (mean for all trials:  $278 \pm 31$  kcal/day) or dietary intake (mean for all trials:  $1647 \pm 105$  kcal/day; energy distribution:  $22 \pm 1\%$  protein,  $49 \pm 2\%$  carbohydrate,  $29 \pm 1\%$  fat) were seen between trials the day before the intervention day, and body mass did not differ between any of the intervention days (data not shown).

### 3.2 Interventions (Table 1)

As previously described (25), mean oxygen consumption during the intervention was not different between IW and CW ( $76.5 \pm 2.6\%$  of  $\text{VO}_2\text{peak}$  vs.  $76.6 \pm 2.5\%$  of  $\text{VO}_2\text{peak}$ ,  $P=0.98$ ), whereas oxygen consumption was lower and higher during slow ( $60.7 \pm 2.5\%$  of  $\text{VO}_2\text{peak}$ ,  $P < 0.001$ ) and fast ( $92.8 \pm 2.9\%$  of  $\text{VO}_2\text{peak}$ ,  $P < 0.001$ ) IW compared to CW. Oxygen consumption during IW and CW was at all times higher than during the CON intervention ( $14.4 \pm 1.0\%$  of  $\text{VO}_2\text{peak}$ ,  $P < 0.001$  for all). The energy expenditure during the interventions was  $473 \pm 38$  kcal,  $471 \pm 36$  kcal and  $86 \pm 4$  kcal, respectively, for CW, IW and CON.

Mean HR during the intervention was not different between IW and CW ( $73.5 \pm 2.0\%$  of  $\text{HRmax}$  vs.  $73.3 \pm 1.9\%$  of  $\text{HRmax}$ ,  $P > 0.92$ ), with HR being lower ( $66.9 \pm 2.4\%$  of  $\text{HRmax}$ ,  $P < 0.001$ ) and higher ( $81.5 \pm 2.0\%$  of  $\text{HRmax}$ ,  $P < 0.001$ ) during slow and fast IW compared to CW. HR during IW and CW was at all times higher than during the CON intervention ( $39.6 \pm 2.1\%$  of  $\text{HRmax}$ ,  $P < 0.001$  for all).

Mean walking speed was lower during IW compared to CW ( $4.68 \pm 0.26$  km/h vs.  $5.04 \pm 0.27$  km/h,  $P < 0.001$ ), with slow IW walking speed being lower ( $3.40 \pm 0.24$  km/h,  $P < 0.001$ ) and fast IW walking being higher ( $5.97 \pm 0.29$  km/h,  $P < 0.001$ ) than CW walking speed.

Mean RER values were higher during IW ( $0.86 \pm 0.01$ ) and CW ( $0.86 \pm 0.01$ ) compared to CON ( $0.82 \pm 0.01$ ,  $P < 0.01$  for both), with no differences between IW and CW ( $P = 0.29$ ).

### 3.3 Lactate

Lactate concentrations during the intervention were higher in IW ( $2.1 \pm 0.2$  mmol/l, no difference between slow and fast IW) compared to CW ( $1.4 \pm 0.1$  mmol/l,  $P < 0.001$ ), and both IW and CW resulted in higher lactate concentrations than CON ( $0.9 \pm 0.1$  mmol/l,  $P < 0.01$  for both). When initiating the MMTT, lactate concentrations did not differ between any of the interventions ( $0.9 \pm 0.1$ ;  $1.0 \pm 0.1$ ;  $1.1 \pm 0.1$  mmol/l for CON; CW; IW, respectively,  $P > 0.99$ ;  $P = 0.59$ ;  $P = 0.90$  for CON vs. CW; CON vs. IW; CW vs. IW), nor were there any differences at the end of the MMTT ( $0.7 \pm 0.1$  mmol/l for all).

### 3.4 RMR and EPOC (Table 2 and Figure 1)

No baseline differences in RMR were found between any of the interventions ( $P > 0.05$  for all).

Overall, the oxygen consumption rate during the entire EPOC period was higher after IW ( $308 \pm 11$  ml/min) than after CW ( $290 \pm 9$  ml/min,  $P < 0.05$ ), and both IW and CW resulted in higher mean oxygen consumption rate than CON ( $272 \pm 10$  ml/min,  $P < 0.001$  and  $P < 0.05$ , respectively).

The oxygen consumption rate during the acute EPOC was higher after IW ( $430 \pm 20$  ml/min) than after CW ( $385 \pm 18$  ml/min,  $P < 0.05$ ), and both IW and CW resulted in higher oxygen consumption rates during the acute EPOC than CON ( $300 \pm 15$  ml/min,  $P < 0.001$  for both).

The oxygen consumption rate during the prolonged EPOC was higher after IW ( $280 \pm 10$  ml/min) than after CON ( $265 \pm 9$  ml/min,  $P < 0.05$ ), and tended to be higher than after CW ( $269 \pm 8$  ml/min,  $P = 0.09$ ), whereas no difference was seen between CW and CON ( $P > 0.99$ ).

Total EPOC was higher after IW ( $36 \pm 5$  ml/min) than after CW ( $18 \pm 5$  ml/min,  $P < 0.05$ ). When separated, IW resulted in larger acute EPOC than CW ( $129 \pm 18$  vs.  $85 \pm 17$  ml/min,  $P < 0.01$ ) and tended to result in larger prolonged EPOC than CW ( $15 \pm 4$  vs.  $4 \pm 4$  ml/min,  $P = 0.09$ ).

The total EPOC magnitude for the 5 hours measured was  $8.4 \pm 1.3$  l for IW and  $3.7 \pm 1.4$  l for CW ( $P < 0.05$ , IW vs. CW), corresponding to 8.6 and 3.8% of the oxygen consumed during the IW and

CW interventions, respectively. When assuming a mean oxygen equivalent of 4.8 kcal/l O<sub>2</sub> (31), the total EPOC corresponded to 40.3 ± 6.2 kcal for IW and 17.8 ± 6.7 kcal for CW.

### 3.5 RER and Substrate Oxidation Rates (Table 2 and Figure 2+3)

No baseline differences in RER values were found between any of the interventions ( $P > 0.05$  for all). Also, RER values did not differ between interventions during the acute EPOC measurement. During the prolonged EPOC measurement (during the MMTT), RER was lower in IW ( $0.79 \pm 0.01$ ) compared to CON ( $0.82 \pm 0.01$ ,  $P < 0.01$ ) and tended to be lower in CW ( $0.80 \pm 0.01$ ) compared to CON ( $P = 0.09$ ). No differences were seen between IW and CW ( $P = 0.42$ ).

During the intervention, absolute carbohydrate and lipid oxidation rates were higher in IW ( $9.93 \pm 1.03$  and  $3.06 \pm 0.20$  mg/kg/min) and CW ( $9.24 \pm 0.92$  and  $3.26 \pm 0.28$  mg/kg/min) compared to CON ( $1.54 \pm 0.18$  and  $0.72 \pm 0.11$  mg/kg/min,  $P < 0.001$  for all), whereas no differences were seen between IW and CW ( $P > 0.99$  for both). Relative carbohydrate oxidation rates were also higher during IW ( $55.5 \pm 2.5\%$  of total) and CW ( $52.9 \pm 2.6\%$ ) compared to CON ( $38.1 \pm 4.6\%$ ,  $P < 0.05$  for both), with no difference between IW and CW, whereas relative lipid oxidation rates did not differ between the interventions ( $39.2 \pm 5.8\%$  vs.  $42.7 \pm 2.8\%$  vs.  $39.9 \pm 2.5\%$ ,  $P > 0.99$  for all comparisons). Absolute protein oxidation rates did not differ between any of the interventions ( $P > 0.05$  for all), whereas relative protein oxidation rates were higher in CON ( $22.7 \pm 2.0\%$ ) compared to IW ( $4.6 \pm 0.6\%$ ) and CW ( $4.5 \pm 0.7\%$ ,  $P < 0.001$  for both).

During the MMTT, absolute carbohydrate oxidation rates were higher in CON ( $1.24 \pm 0.10$  mg/kg/min) compared to IW ( $0.86 \pm 0.12$  mg/kg/min,  $P < 0.05$ ), whereas CW ( $1.03 \pm 0.12$  mg/kg/min) did not differ from CON ( $P = 0.31$ ) or IW ( $P = 0.53$ ). Relative carbohydrate oxidation was also higher in CON ( $34.3 \pm 2.7\%$ ) compared to IW ( $22.9 \pm 2.9\%$ ,  $P < 0.01$ ), with no difference between CW ( $28.0 \pm 2.6\%$ ) and the two other interventions ( $P = 0.19$  and  $P = 0.37$  compared to CON and IW, respectively). Absolute lipid oxidation rates during the MMTT were higher in IW ( $1.03 \pm 0.12$  mg/kg/min) and CW ( $0.87 \pm 0.04$  mg/kg/min) compared to CON ( $0.73 \pm 0.04$  mg/kg/min,  $P < 0.01$  and  $P < 0.05$ , respectively), with no differences between IW and CW ( $P = 0.26$ ), and this pattern was also seen for relative lipid oxidation rates ( $58.0 \pm 2.4\%$  vs.  $54.4 \pm 2.6\%$  vs.  $45.5 \pm 2.6\%$  for IW, CW and CON, respectively). Protein oxidation rates (absolute and relative) did not differ between any of the interventions.

### 3.6 Lipid Metabolism (Table 3 and Figure 4)

No baseline differences in FFA or glycerol concentrations were found between any of the interventions ( $P>0.05$  for all).

During and after the intervention, FFA and glycerol concentrations were higher or tended to be higher during IW and CW compared to CON ( $P<0.01$  to  $P=0.09$ ), with no differences between IW and CW. During the MMTT, FFA levels were lower in IW compared to CON ( $P<0.05$ ), with no differences between CW and the other interventions. Glycerol concentrations during the MMTT tended to be lower in IW compared to CON ( $P=0.08$ ), again with no differences between CW and CON ( $P=0.99$ ) or CW and IW ( $P=0.11$ ).

Glycerol Ra and Rd were higher during the IW and CW interventions compared to CON ( $P<0.05$  for all) and remained or tended to remain higher after IW compared to CON ( $P<0.05$  to  $P<0.10$ ) before the MMTT, with no differences between CW and the other interventions ( $P>0.23$  for all comparisons). No significant differences in glycerol Ra and Rd were seen between any of the interventions during the MMTT ( $P>0.99$  for all comparisons).

#### 4. DISCUSSION

Several findings are to be highlighted from this study: First, an acute session of aerobic interval training results in greater EPOC than an acute session of continuous training matched with regards to time-duration and oxygen consumption. Second, an acute exercise session increases lipid oxidation rates during a mixed meal tolerance test in the recovery period, and this is preceded by increased lipid turnover during and after the exercise session. Third, interval- and continuous exercise does not result in major differences in substrate oxidation rates or lipid metabolism during and after the exercise performed. This is of relevance in the T2D subjects studied, since these variables have all been linked to the weight governing effect of exercise and since weight loss is recommended for overweight/obese T2D subjects (23).

The total EPOC was clearly higher in IW compared to CW, with both the acute and the prolonged component contributing to the difference seen. With increased lactate levels immediately after the exercise bout but not when initiating the MMTT, some of the acute but not the prolonged EPOC may potentially be ascribed to oxygen debt, although the causality between EPOC and lactate has been questioned (32). Whereas most of the EPOC after exercise is produced the first 5 hours after

the exercise session (33;34), the duration of the EPOC period has been reported to be up to 24 hours long (6;35). For logistical reasons, we did not have the opportunity to prolong the measurement period beyond 5½ hours. When looking at the last oxygen consumption measurement performed, there was a significant difference in oxygen consumption rate between IW ( $271 \pm 10$  ml/min) and CON ( $252 \pm 9$  ml/min,  $P < 0.01$ ), whereas CW ( $259 \pm 9$  ml/min) did not differ between the other interventions. Thus, oxygen consumption after IW was potentially increased beyond the measurement period and the EPOC period was probably of longer duration than the five hours measured. As such, the magnitude of the EPOC calculated is a minimum estimate. Although the magnitude of the EPOC after IW was probably not larger than 10-15% of the oxygen consumed during the intervention (36), the differential EPOC after IW and CW can likely explain parts of the larger weight loss seen after an IW training intervention compared to a CW training intervention (4). Still, bearing the measured difference in energy expenditure due EPOC between IW and CW (~23 kcal) in mind, other mechanisms are probably also involved in the differential weight loss seen after IW/CW training interventions. The EPOC results found are in line with previous research. As such, Laforgia *et al.* have shown that supramaximal interval running results in higher EPOC compared to submaximal running in athletes (37), and Larsen *et al.* have found that aerobic interval training results in higher EPOC compared to continuous training in males with metabolic syndrome (38). Our results are interesting since they extend previous findings to subjects with type 2 diabetes and since the matching of the exercise interventions with regards to mean intensity and duration is unique. Thus, the interval exercise pattern *per se* is responsible for the differential EPOC effects seen.

The exercise interventions resulted in comparable effects on substrate oxidation rates with increases in both carbohydrate and lipid oxidation during both IW and CW compared to CON. During the subsequent MMTT, carbohydrate oxidation decreased and lipid oxidation increased in the exercise interventions compared to CON, again with no major differences between IW and CW. As previously published (25), both IW and CW resulted in increased glucose Rd during the MMTT, and thus the reduced carbohydrate oxidation during the MMTT after the exercise interventions can probably be explained by increased non-oxidative glucose metabolism. Although we did not take muscle biopsies to support this explanation, we speculate that the exercise interventions were followed by increased glycogen synthase activity with accompanying increased glycogen storage during the MMTT (39;40).

The comparable effects on substrate oxidation rates in IW and CW were in both interventions preceded by increased lipid turnover during and after the exercise. As such, the increased FFA and glycerol concentrations and glycerol kinetics during and after IW and CW, may have primed the body for subsequently increased lipid oxidation during the MMTT. Contributing to this, since glucose levels were numerically lower during the MMTT after the exercise interventions compared to after the CON intervention (25), lipid oxidation during the MMTT's following exercise may have been increased due to the competition between glucose and fatty acids as substrates which has been described by Randle *et al.* (41).

As previously stated, T2D have dysregulated lipid metabolism with decreased lipid oxidation rates and increased FFA levels (17), and this dysregulation is also found during and after exercise (18). Our study indicates, like previous studies (19;42), that lipid oxidation does increase during and after exercise in T2D subjects, but we did not observe any differences between the exercise interventions. This has also been found in healthy subjects (43;44), whereas post-exercise lipid oxidation has been shown to increase more after exercise with higher intensity (43) or longer duration (45).

An unexpected observation from the study was the lack of increased RER values during the MMTT in the CON intervention. The MMTT resulted in increased glucose and insulin levels in all subjects (25), and we would have expected RER levels to increase as indicative of increased utilization of glucose (46). T2D subjects are however known to be metabolic inflexible (47), and the dynamic nature of the MMTT with varying insulin and glucose levels may have contributed to a lack of increases in RER. Moreover, the carbohydrate composition in the MMTT (25% sugar and 75% starch), may have influenced the substrate utilization. Still, the complete lack of increase in RER in the fed compared to the fasting state is unexpected and interesting.

A limitation in the study includes the small sample size. When looking at the substrate oxidation rates and lipid metabolism data, there might be vague indications of differences in FFA and glycerol concentrations and lipid oxidation rates during the MMTT between the IW and CW interventions, but the study was underpowered to elucidate such potential differences. Also, if a true difference between IW and CW existed in these variables, the difference is probably small and therefore of limited clinical importance.

Another consideration was that the prolonged EPOC component was measured during the MMTT. While the reason for this design was to let the study conditions be as close to normal and realistic conditions as possible, the oxygen consumption during the MMTT was probably increased due the thermal effect of food. However, since EPOC was calculated by subtracting the oxygen

consumption during the CON trial from the IW/CW trial and since we assume that the thermal effect of food is equal between trials, we do not believe that the EPOC measurements are influenced by the MMTT.

In conclusion, this study has shown that whereas both interval and continuous exercise increased lipid oxidation and lipolysis during a standardised MMTT consumed after the exercise, no major differences were seen between the exercise bouts. Conversely, an acute session of aerobic interval-training matched with regards to time-duration and oxygen consumption to an acute session of continuous training, resulted in greater EPOC in T2D subjects. This observation is important as it may help us understand why interval exercise has a greater propensity to induce weight loss than continuous exercise.

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**DISCLOSURES**

The authors have nothing to disclose.

**AUTHOR CONTRIBUTIONS:**

KK and TS designed and initiated the study. KK implemented the study, performed analyses and wrote the manuscript. All authors edited and approved the final manuscript before submission. KK had access to all data and takes responsibility for the integrity of the analysis.



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## TABLES

*Table 1: Intervention variables*

	<u>CON</u>	<u>CW</u>	<u>IW total</u>	<u>IW slow</u>	<u>IW fast</u>
Oxygen consumption rate (ml/min)	297 ± 13	1641 ± 133#	1634 ± 126*	1284 ± 84†	1985 ± 160†
Heart rate (bpm)	62 ± 3	115 ± 4#	115 ± 3*	104 ± 3†	128 ± 3†
Walking speed (km/h)	NA	5.04 ± 0.27	4.68 ± 0.27†	3.40 ± 0.24†	5.97 ± 0.29†
RER (Fraction)	0.82 ± 0.01	0.86 ± 0.01#	0.86 ± 0.01*	0.86 ± 0.01†	0.87 ± 0.01

Data are Mean ± SEM.

RMR = Resting Metabolic Rate; EPOC = Excess Post-exercise Oxygen Consumption; RER = Respiratory Exchange Ratio; CON = Control; CW = Continuous Walking; IW = Interval Walking;

Statistical differences were assessed by one-way repeated-measures analysis of variance (ANOVA) or Student's paired t tests (see text for details). Statistical differences are indicated by \* (CON vs. IW), # (CON vs. CW) and † (CW vs. IW).

Table 2: RMR, EPOC and RER variables

	<u>CON</u>	<u>CW</u>	<u>IW</u>
Oxygen consumption rate (ml/min)			
Baseline (RMR)	249 ± 10	253 ± 9	258 ± 12
Acute EPOC	300 ± 15	385 ± 18#	430 ± 20*†
Prolonged EPOC	265 ± 9	269 ± 8	280 ± 10*(†)
RER (Fraction)			
Baseline (RMR)	0.83 ± 0.01	0.81 ± 0.01	0.83 ± 0.01
Acute EPOC	0.82 ± 0.02	0.82 ± 0.01	0.82 ± 0.01
Prolonged EPOC	0.82 ± 0.01	0.80 ± 0.01(#)	0.79 ± 0.01*

Data are Mean ± SEM.

RMR = Resting Metabolic Rate; EPOC = Excess Post-exercise Oxygen Consumption; RER = Respiratory Exchange Ratio; CON = Control; CW = Continuous Walking; IW = Interval Walking. Variables were compared using one-way repeated-measures ANOVA with Bonferroni-corrected post hoc tests. Statistical differences ( $P < 0.05$ ) are indicated by \* (CON vs. IW), # (CON vs. CW) and † (CW vs. IW). (#) and (†) indicate  $P < 0.10$ .

Table 3: Lipid metabolism variables

	<u>CON</u>	<u>CW</u>	<u>IW</u>
FFA ( $\mu\text{mol/l}$ )			
Basal (pre intervention)	705 $\pm$ 75	698 $\pm$ 67	645 $\pm$ 51
Post intervention	597 $\pm$ 35	804 $\pm$ 74#	718 $\pm$ 77(*)
Resting	601 $\pm$ 51	795 $\pm$ 63#	834 $\pm$ 99*
MMTT	310 $\pm$ 29	345 $\pm$ 32	373 $\pm$ 36*
Glycerol ( $\mu\text{mol/l}$ )			
Basal (pre intervention)	117 $\pm$ 23	104 $\pm$ 19	114 $\pm$ 23
Post intervention	94 $\pm$ 95	335 $\pm$ 34#	366 $\pm$ 49*
Resting	103 $\pm$ 13	173 $\pm$ 19#	197 $\pm$ 21*
MMTT	59 $\pm$ 9	59 $\pm$ 9	69 $\pm$ 10(*)
Glycerol Ra ( $\mu\text{mol/kg/min}$ )			
During intervention	5.31 $\pm$ 0.88	10.30 $\pm$ 0.98#	10.09 $\pm$ 0.93*
Resting	3.88 $\pm$ 0.46	4.68 $\pm$ 0.40	5.16 $\pm$ 0.39(*)
MMTT	2.63 $\pm$ 0.31	2.37 $\pm$ 0.24	2.64 $\pm$ 0.37
Glycerol Rd ( $\mu\text{mol/kg/min}$ )			
During intervention	5.30 $\pm$ 0.88	10.20 $\pm$ 0.98#	9.96 $\pm$ 0.92*
Resting	3.85 $\pm$ 0.46	4.85 $\pm$ 0.41	5.36 $\pm$ 0.39*
MMTT	2.65 $\pm$ 0.31	2.39 $\pm$ 0.24	2.65 $\pm$ 0.37

Data are Mean  $\pm$  SEM.

FFA = Free Fatty Acids; MMTT = Mixed Meal Tolerance Test; Glycerol Ra = rate of glycerol appearance; Glycerol Rd = rate of glycerol disappearance; CON = Control; CW = Continuous Walking; IW = Interval Walking. Variables were compared using one-way repeated-measures ANOVA with Bonferroni-corrected post hoc tests. Statistical differences ( $P < 0.05$ ) are indicated by \* (CON vs. IW), # (CON vs. CW) and † (CW vs. IW). (\*) indicate  $P < 0.10$ .

**FIGURE LEGENDS**

Figure 1. Oxygen consumption and EPOC of the exercise interventions.

Panel A shows the oxygen consumption rates following the interventions, Panel B shows the total EPOC, Panel C shows the acute EPOC component (30 min immediately after the intervention) and Panel D shows the prolonged EPOC component (hour 1-5½ after the intervention). In Panel A, the shaded area indicates the intervention (t = 0–60 min), whereas the solid vertical line indicates start of the MMTT (t = 105 min).

Data is presented as mean  $\pm$  SEM. Differences were analysed by Student's paired t-test with significant differences indicated by asterix. One symbol indicates  $P < 0.05$  and two symbols indicate  $P < 0.01$ .

IW = interval-walking; CW = continuous walking; CON = control.

Figure 2. Absolute macronutrient oxidation rates.

Macronutrient oxidation rates were calculated according to the algorithms from Frayn (28) during the intervention (Panel A-C) and during the MMTT (Panel 4-6).

Data is presented as mean  $\pm$  SEM. Differences were analysed by one-way repeated-measures ANOVA with Bonferroni-corrected p-values and with significant differences indicated by asterix.

One symbol indicates  $P < 0.05$  and three symbols indicate  $P < 0.001$ .

IW = interval-walking; CW = continuous walking; CON = control.

Figure 3. Relative macronutrient oxidation rates.

Macronutrient oxidation was calculated according to the algorithms from Frayn (28) during the intervention (Panel A) and during the MMTT (Panel B). Data is presented as fractionated contribution of the total energy expenditure (mean). Please see main text for SEM levels and statistical tests.

IW = interval-walking; CW = continuous walking; CON = control.

Figure 4. Lipid concentrations and lipid turnover.



Profiles (mean  $\pm$  SEM) of free fatty acid concentrations (FFA - Panel A), glycerol concentrations (Panel B), Glycerol rate of appearance (Ra - Panel C) and Glycerol rate of disappearance (Rd - Panel D) are shown. The shaded area indicates the intervention (t = 0–60 min), whereas the solid vertical line indicates start of the MMTT (t = 105 min). For statistical analyses, see text and Table 3. IW = interval-walking; CW = continuous walking; CON = control.

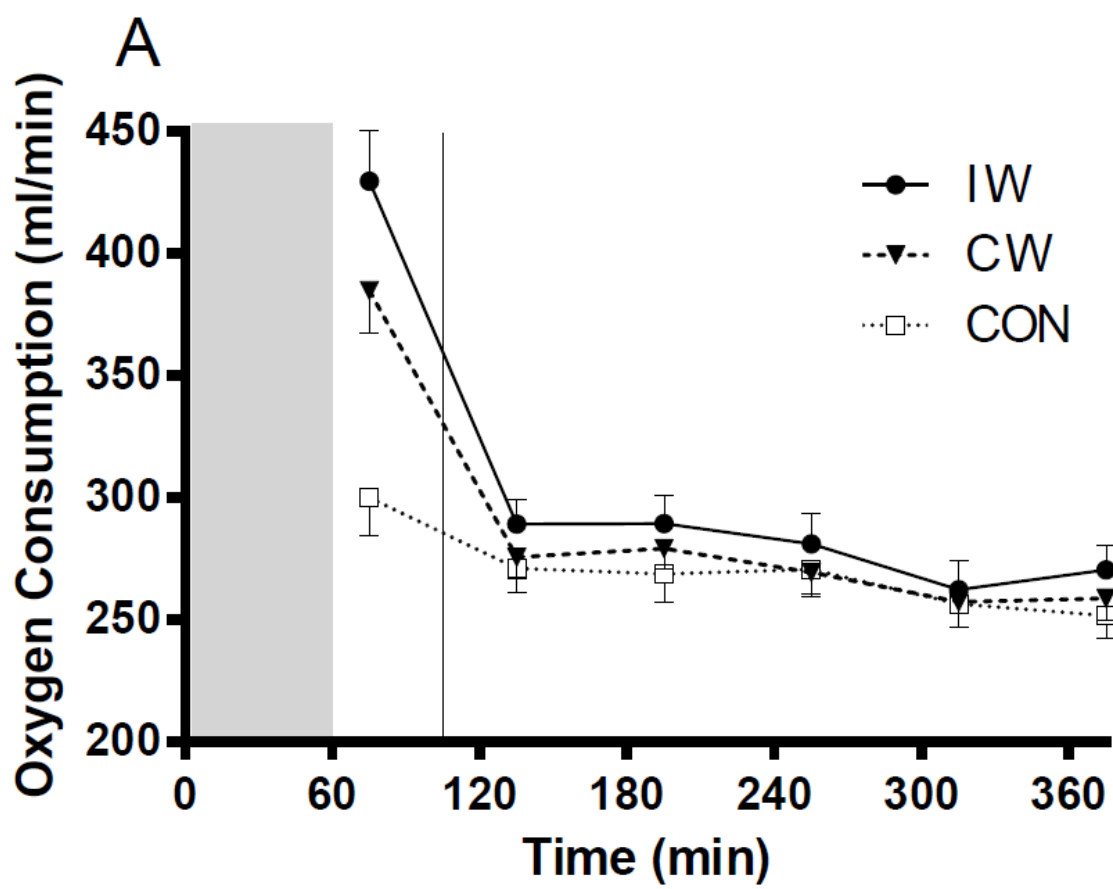


Figure 1a

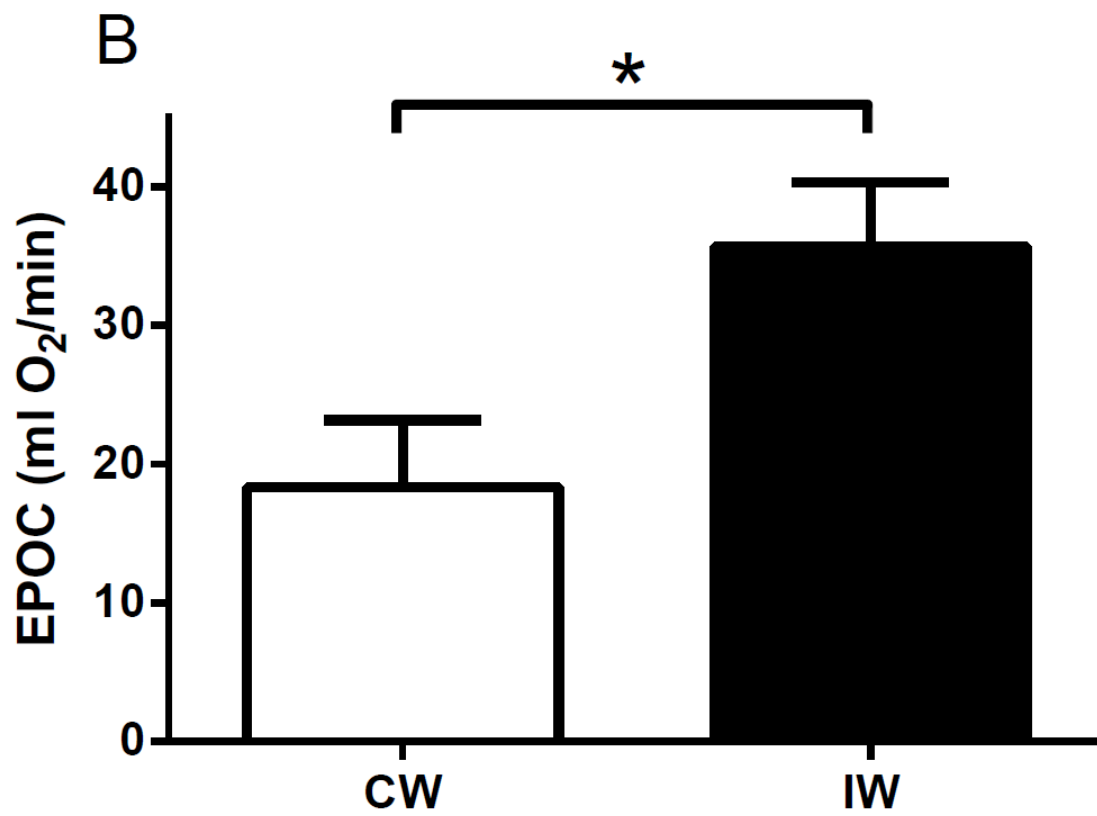


Figure 1b

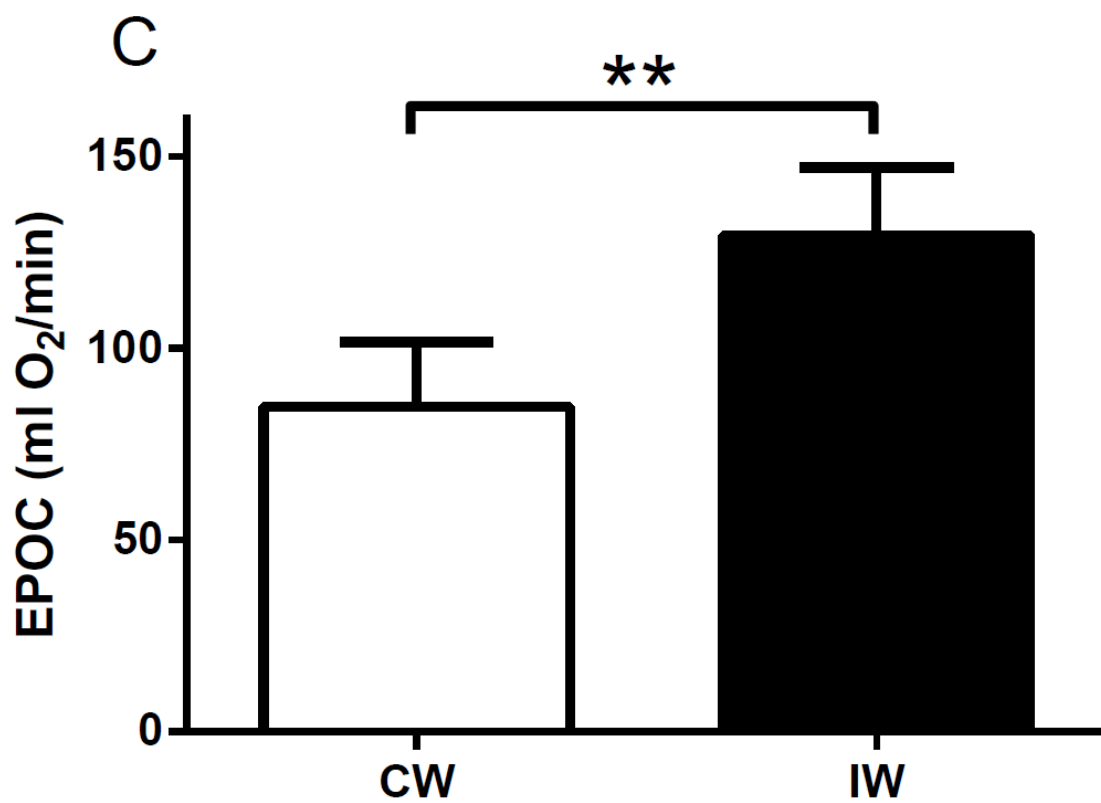


Figure 1c

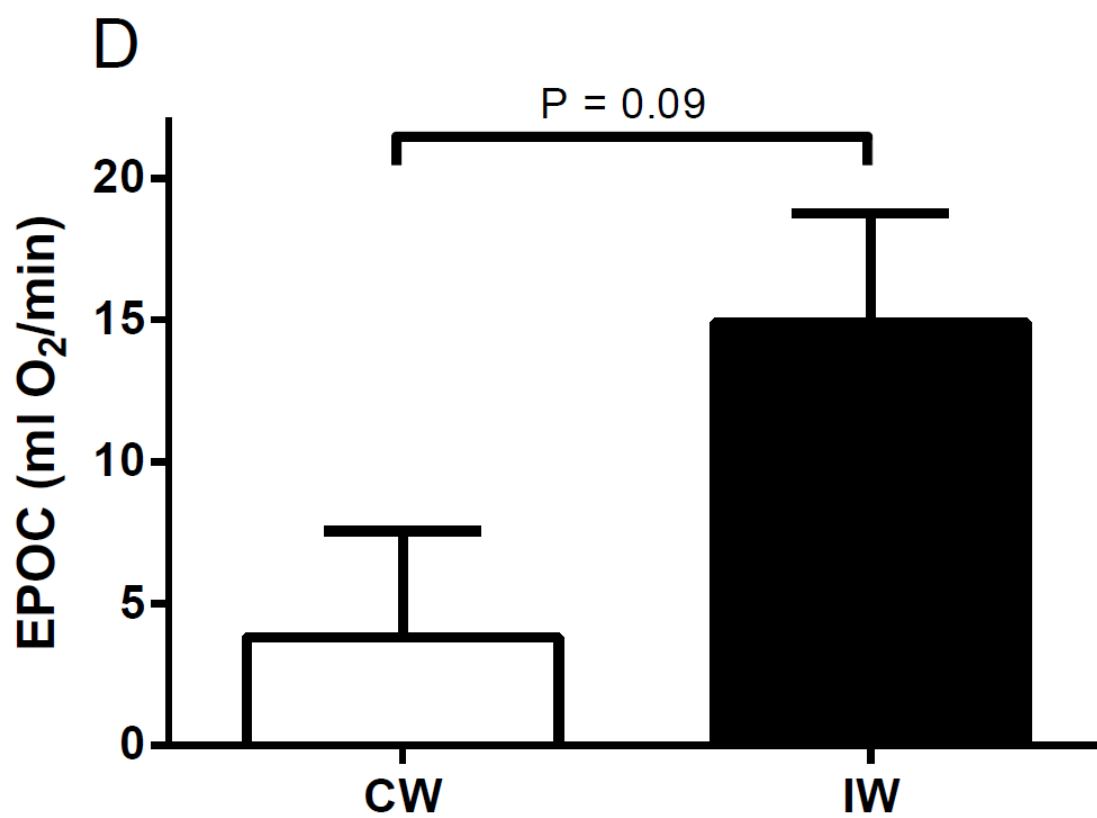


Figure 1d

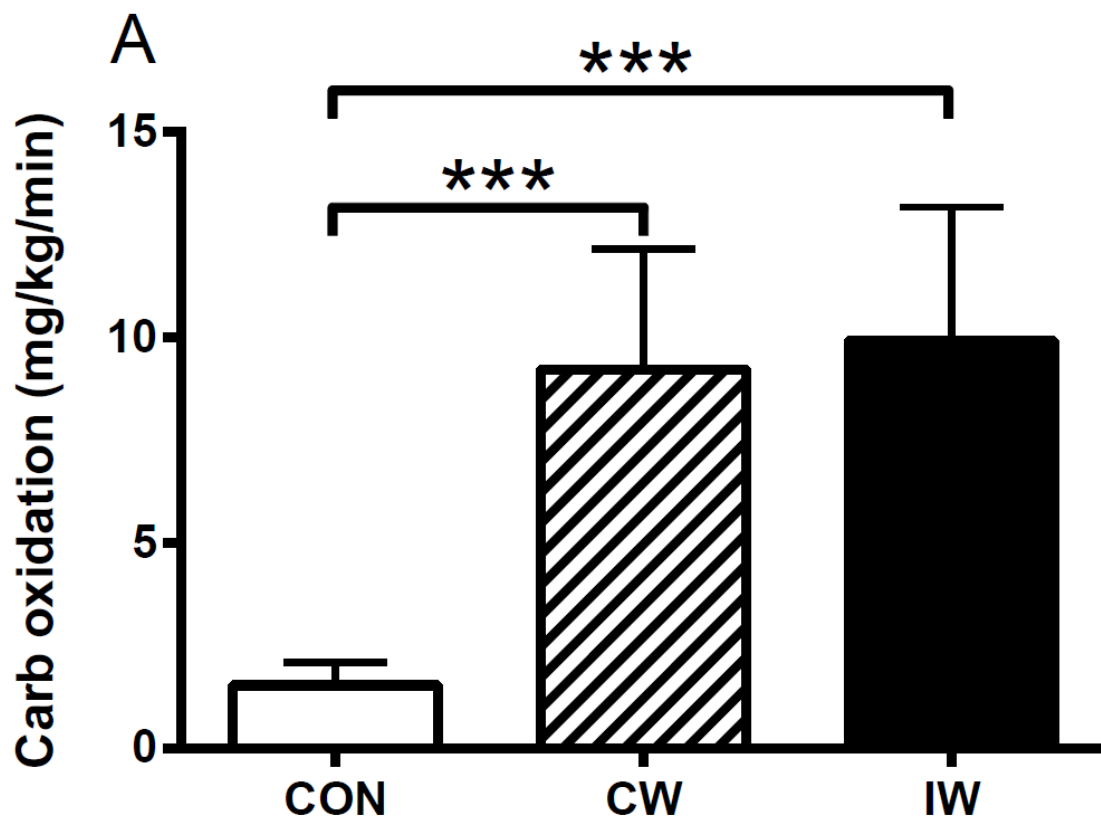


Figure 2a

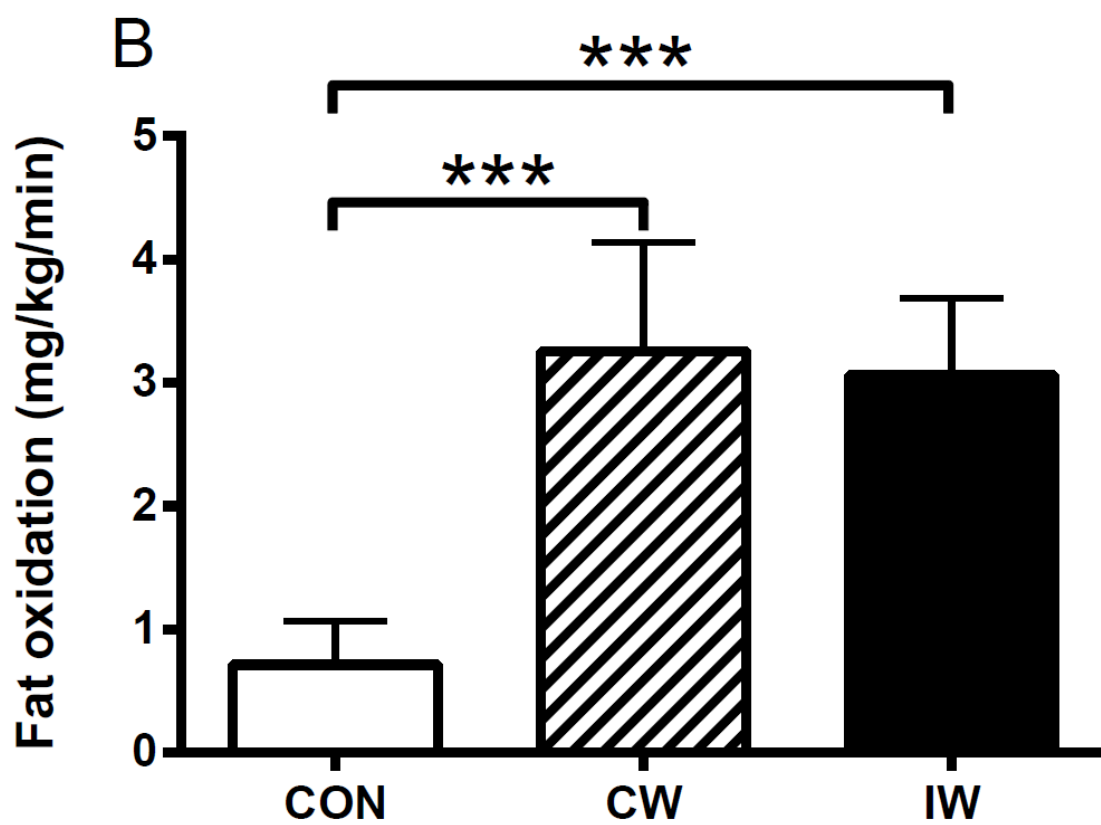


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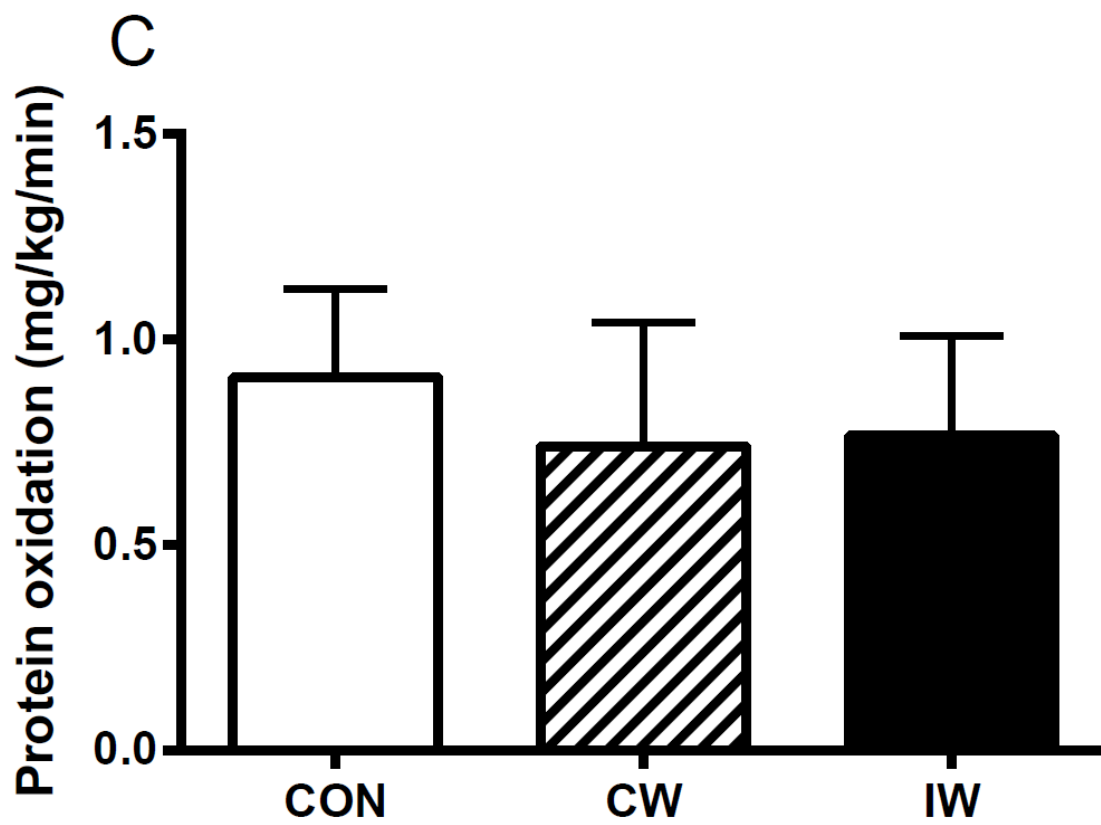


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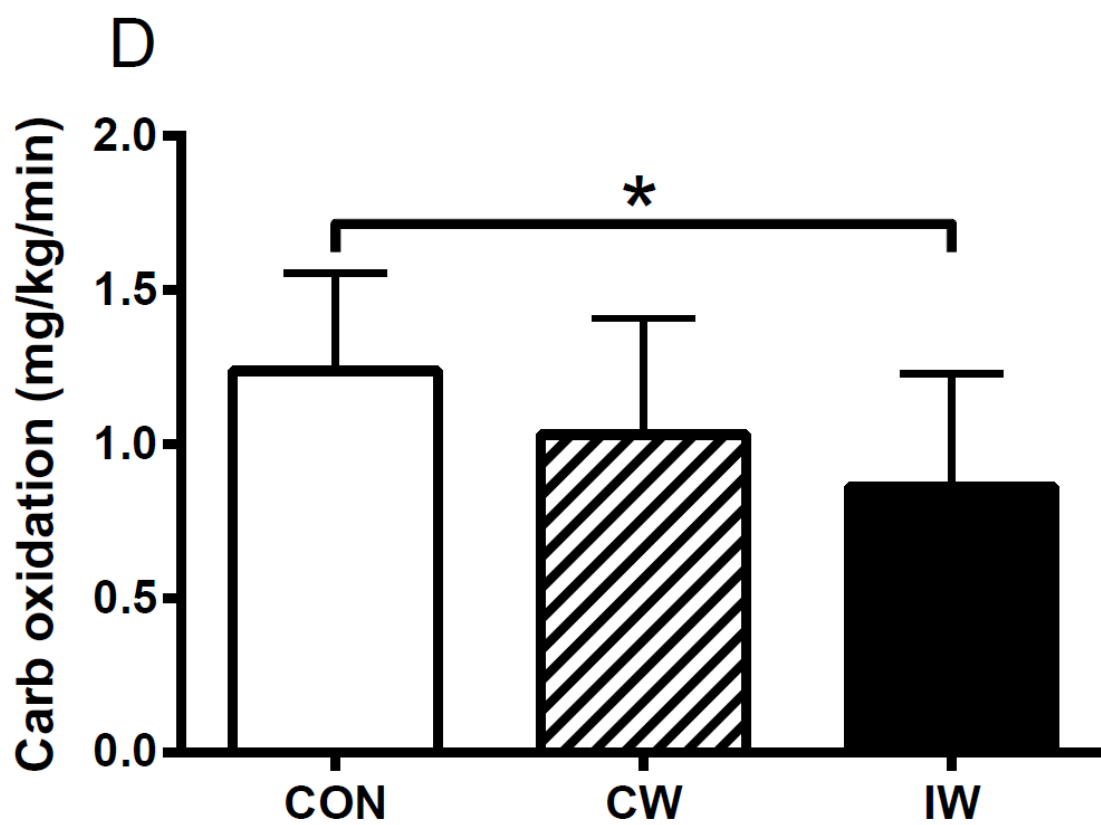


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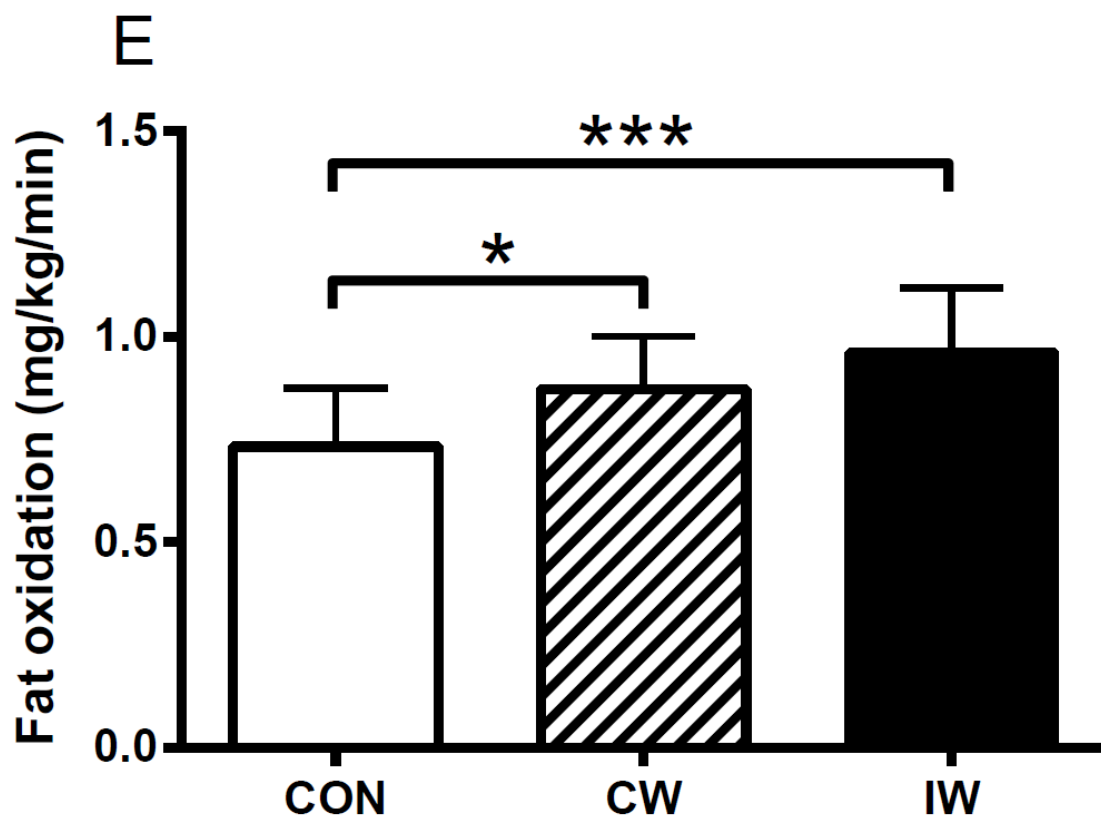


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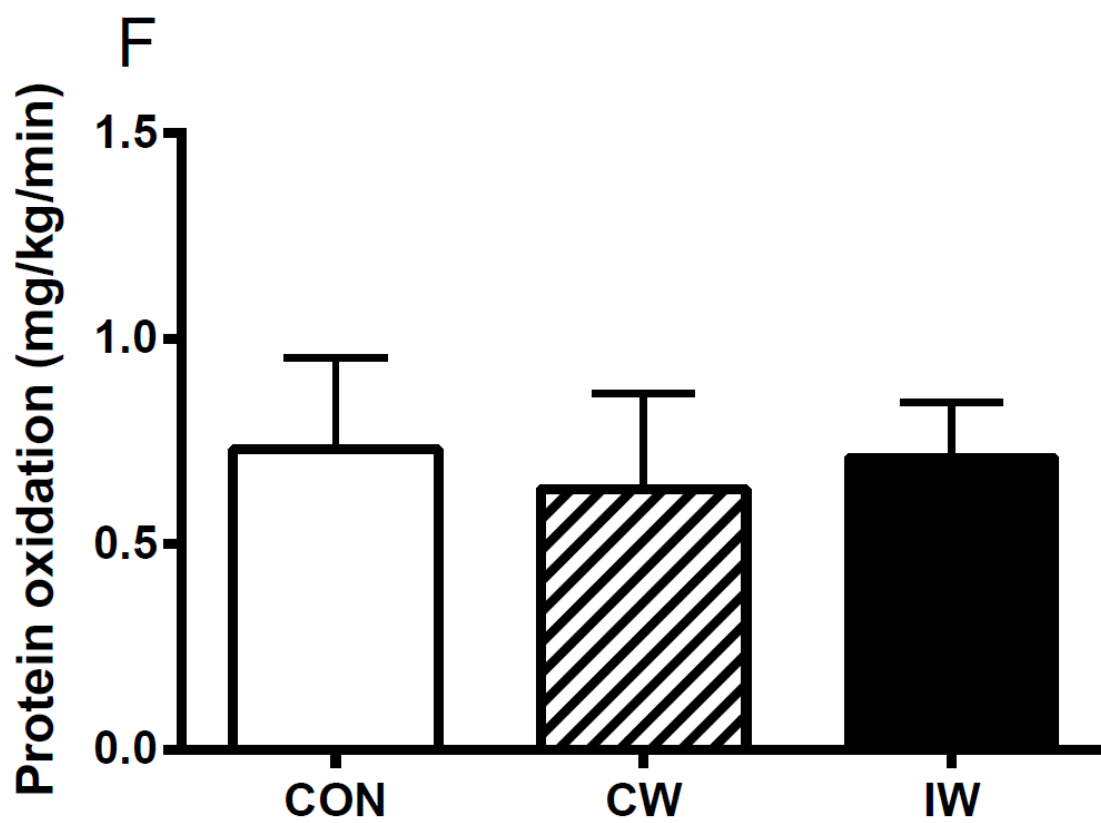


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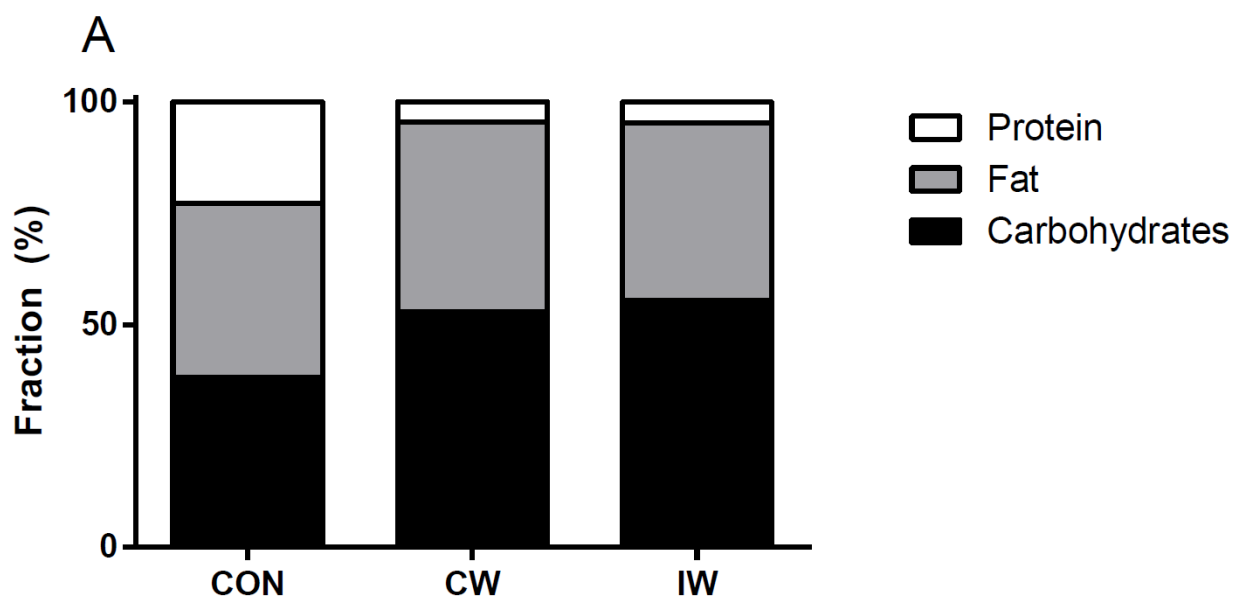


Figure 3a

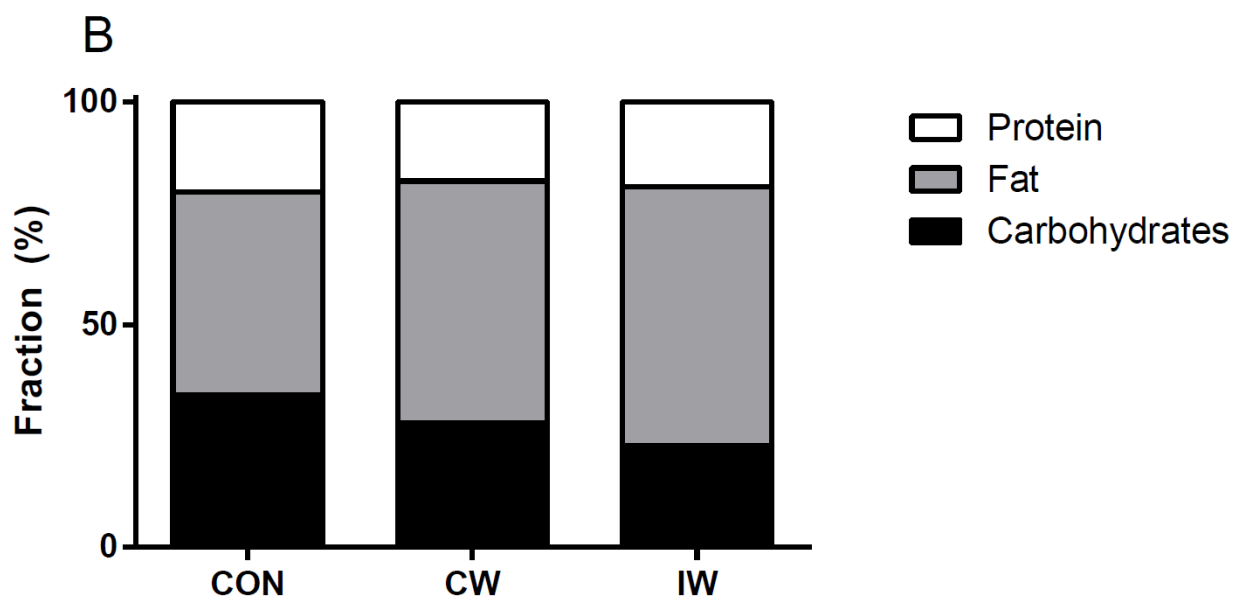


Figure 3b

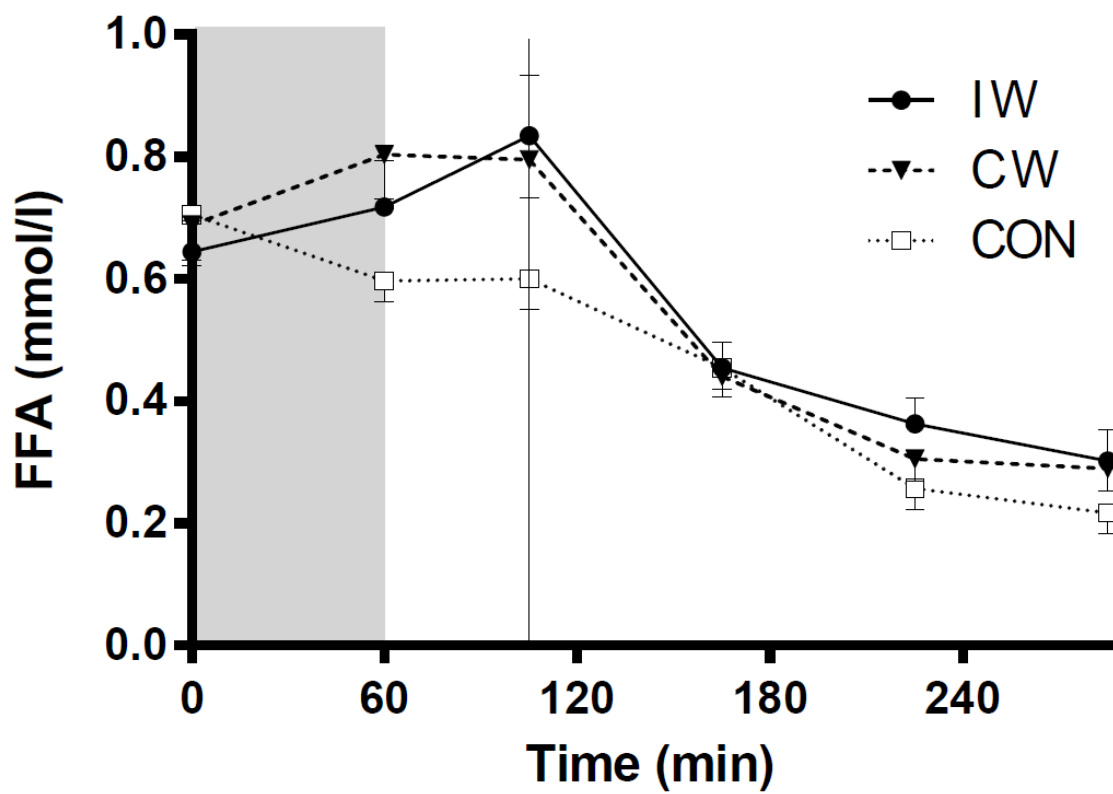


Figure 4a

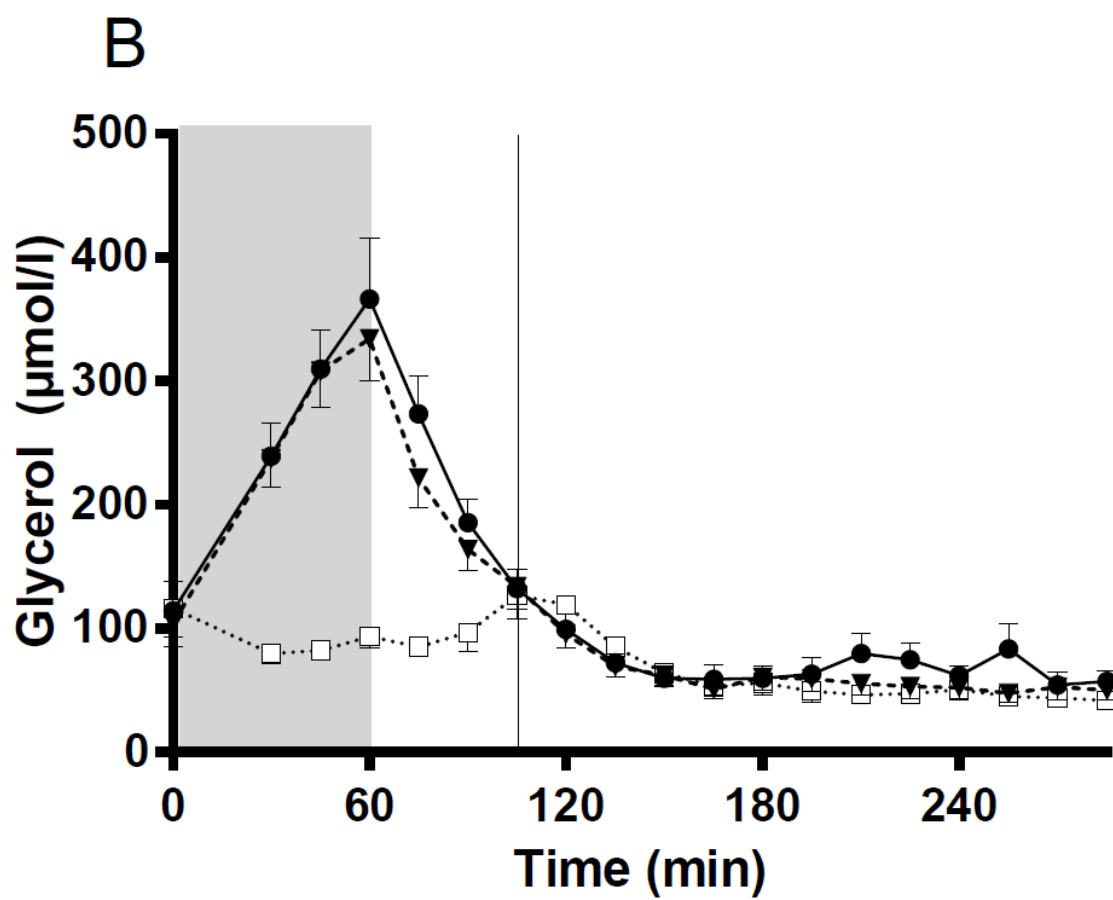


Figure 4b

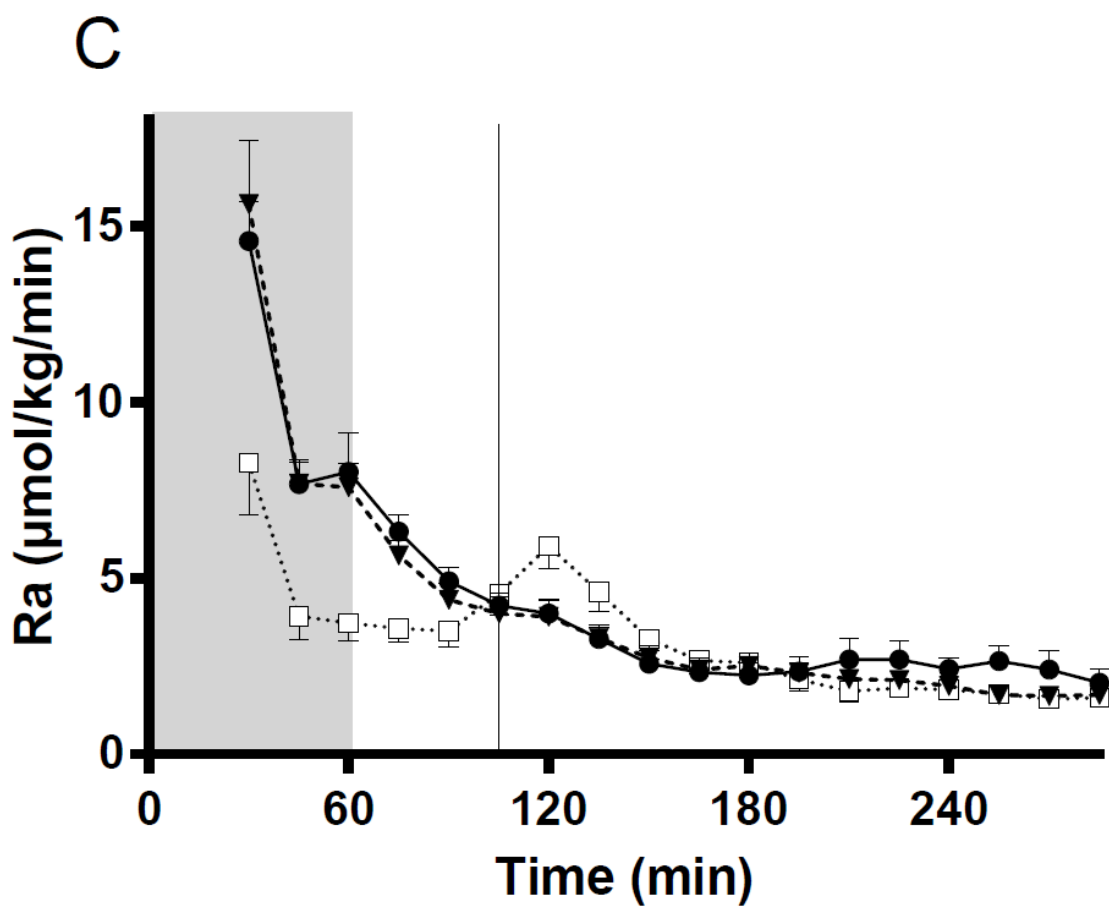


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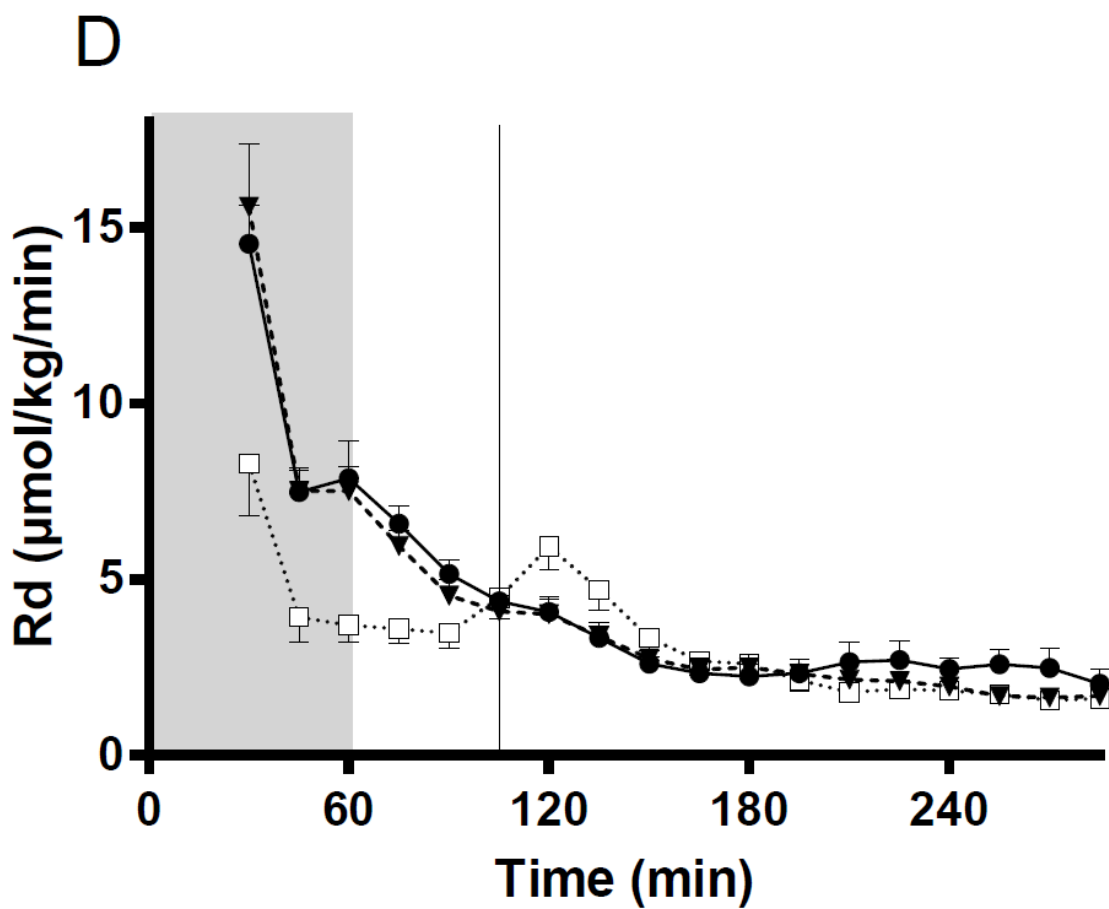


Figure 4d

**HIGHLIGHTS**

- Interval exercise results in greater EPOC than continuous exercise in T2D patients
- Exercise increases lipid oxidation rates during a mixed meal in the recovery period
- Interval- and continuous exercise results in comparable effects on lipid metabolism