

Research article

Two Weeks of Interval Training Enhances Fat Oxidation during Exercise in Obese Adults with Prediabetes

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Abstract

Prediabetes is associated with impaired oxidative capacity and altered substrate utilization during exercise. The effects of continuous (CONT) versus interval (INT) exercise training on fat oxidation during an acute exercise bout at the same absolute and relative intensities are unknown in this population. Obese females/males ($n = 17$, $n = 5$) with prediabetes (BMI 32.2 ± 1.2 kg·m⁻²; age 62.8 ± 1.6 y; fasting glucose 103.4 ± 1.6 mg·dL⁻¹; 2-hour glucose 153.7 ± 7.1 mg·dL⁻¹; VO_{2peak} 19.9 ± 1.0 mL·kg⁻¹·min⁻¹) were screened with a 75g OGTT. Subjects completed a peak oxygen consumption test and a submaximal exercise substrate utilization test consisting of 5min stages at absolute (30W) and relative (70%HR_{peak}) intensities before and after randomization to 12 sessions (60min each) of CONT (70% HR_{peak}) or INT (alternating 3min 90% HR_{peak}, 3min 50% HR_{peak}) over a two-week period. Body mass decreased and VO_{2peak} increased more after INT than CONT (INT: -0.6 ± 0.2 kg, CONT: -0.1 ± 0.2 kg; $p = 0.04$; INT: 1.9 ± 0.6 mL/kg/min, CONT: 0.1 ± 0.6 mL·kg⁻¹·min⁻¹; $p = 0.04$). Training increased fat oxidation by 0.7 ± 0.2 mL·kg⁻¹·min⁻¹ during the absolute intensity test ($p < 0.001$), independent of intensity. During the relative intensity test, fat oxidation increased more after INT than CONT (INT: 1.3 ± 0.4 mL·kg⁻¹·min⁻¹, CONT: 0.3 ± 0.3 mL·kg⁻¹·min⁻¹; $p = 0.03$), with no difference in exercise energy expenditure between groups. Enhanced fat oxidation during the relative test was correlated with increased VO_{2peak} ($r = 0.53$ $p = 0.01$). High intensity INT training enhances fat oxidation during the same relative intensity exercise in people with prediabetes.

Key words: Exercise training, oxidative metabolism, substrate utilization, exercise intensity, hyperglycemia.

Introduction

Prediabetes is characterized by elevated blood glucose levels that do not meet criteria for type 2 diabetes (T2D) (American Diabetes Association, 2015). It is estimated that over 318 million adults in the world have prediabetes, and that figure is projected to rise to 481 million by 2040 (Ogurtsova et al., 2017). This is concerning because prediabetes increases risk for T2D, cardiovascular disease and all-cause mortality (Huang et al., 2016). Although prediabetes is likely the result of multiple factors, impaired mitochondrial function is a leading candidate that contributes to the etiology of insulin resistance, a hallmark of the disease (Fabbri et al., 2016). Interventions designed to increase oxidative capacity in adults with prediabetes therefore may

alleviate insulin resistance and improve glucose tolerance.

Aerobic exercise training ranging from 12–16 weeks increases fat oxidation during rest and/or exercise in obese adults with prediabetes or T2D (Goodpaster et al., 2003; Solomon et al., 2008; Meex et al., 2010; Malin and Braun, 2013; Arad et al., 2015). However, some of these studies also report clinically meaningful weight and fat loss (Goodpaster, Katsariaras and Kelley, 2003; Solomon et al., 2008), making it difficult to isolate the independent effects of exercise on fat oxidation. Most (Talanian et al., 2006; Venables and Jeukendrup, 2008; Hood et al., 2011; Lanzi et al., 2015), but not all (Skleryk et al., 2013) 2–4 week moderate to high intensity exercise training studies report increased fat oxidation at rest or during exercise at the same absolute workload in normal weight or overweight/obese subjects, independent of weight loss. Additionally, high intensity interval training may augment oxidative capacity when compared to continuous training in healthy individuals (Daussin et al., 2008), suggesting that fat oxidation changes may be intensity-dependent. In contrast, others report that short-term continuous, but not interval exercise training increases fat oxidation during exercise in obese individuals (Venables and Jeukendrup, 2008). This later observation is clinically relevant as exercise fat oxidation was directly related to insulin sensitivity. Interestingly, our group recently reported that interval and continuous exercise training resulted in comparable reductions in insulin resistance (Gilbertson et al., 2018). While this improvement was partly attributable to post-prandial carbohydrate oxidation, it is worth acknowledging that bio-active lipids such as ceramides and diacylglycerols interfere with insulin signaling (Fabbri et al., 2016). Thus, it remains possible that the increased oxidation of lipids during exercise could contribute to reductions in insulin resistance. However, whether exercise training intensity impacts fuel selection during exercise at the same absolute and/or relative workload is unclear in obese adults with prediabetes, and how this change in exercise fuel selection relates to insulin sensitivity independent of clinically meaningful weight loss has yet to be systematically determined. Therefore, we tested the hypothesis that high intensity interval training (INT) would increase fat oxidation during submaximal exercise more than moderate continuous intensity training (CONT) at the same absolute and relative intensities in obese adults with prediabetes. We also hypothesized that enhanced fat oxidation would correlate with improved clinical health and aerobic fitness.

Methods

Subjects

Twenty-two (female $n = 17$; male $n = 5$) obese ($BMI = 32.2 \pm 1.2 \text{ kg} \cdot \text{m}^{-2}$) adults (age = $62.8 \pm 1.6 \text{ y}$, range 43 – 74 y) were enrolled in this block-randomized two-week exercise intervention. Prediabetes was screened for using a standard 2-hour 75 g oral glucose tolerance test (OGTT) and/or HbA_{1c} after a 10-12 hour overnight fast according to American Diabetes Association criteria (American Diabetes Association, 2015). Subjects were characterized as having impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or IFG+IGT. There were a similar number of individuals with IFG, IGT, and IFG+IGT in CONT (4, 3, and 4, respectively) and INT (2, 3, and 6, respectively) by design. Subjects were sedentary (i.e. $< 60 \text{ min/wk}$ of structured exercise), non-smoking, and free of cardiovascular disease, cancer (within the last 5 years), respiratory complications, or known metabolic disease (e.g. T2D, non-alcoholic fatty liver disease, etc.). Subjects were excluded if they took medication affecting substrate metabolism, blood flow, or insulin sensitivity (e.g. metformin, ACE inhibitors, angiotensin II receptor blockers). Females were all postmenopausal. All subjects underwent a resting electrocardiogram, clinical biochemistries, and a physical exam by a study physician to ensure subject safety during the intervention. All subjects consumed a mixed diet and were instructed to not change their diet or habitual physical activity throughout the study. Subjects gave verbal and written informed consent before participating and the study was approved by our Institutional Review Board.

Metabolic control

Subjects were instructed to refrain from strenuous non-exercise physical activity 48 hours prior to the OGTT. Subjects were also instructed to refrain from caffeine, alcohol, medications and dietary supplements 24 hours prior to the OGTT. On the day prior to OGTTs, subjects were instructed to record their diets and consume approximately 250 g of carbohydrate (CHO) to minimize the influence of muscle glycogen on insulin sensitivity. Food intake was assessed using 24-hour food logs before and after training, and data were processed using ESHA (Version 11.1, Salem, OR). The last exercise training bout was performed approximately 24 hours before the OGTT.

Peak aerobic fitness

Subjects performed an incremental peak oxygen consumption ($VO_{2\text{peak}}$) test on a cycle ergometer (Lode Corival, Groningen, Netherlands). Subjects began cycling at 0W, and power output was increased by 25W every 2 min until volitional exhaustion. $VO_{2\text{peak}}$ was confirmed by a respiratory exchange ratio (RER) > 1.1 and volitional fatigue. Respiratory gases (i.e. VO_2 and VCO_2) were collected throughout the test using indirect calorimetry (Vmax Encore, CareFusion, Yorba Linda, CA). HR via electrocardiogram and rating of perceived exertion (RPE) were recorded during the last 30 sec of each stage. $VO_{2\text{peak}}$ was determined as the highest VO_2 value achieved during the incremental test.

Anthropometrics and body composition

Body mass was measured on an electronic scale (Health-O-Meter, Neosho, MO). Waist circumference was measured in duplicate 2 cm above the umbilicus using a standard tape measure. Values within 0.5 cm were averaged. If a value was not within 0.5 cm, a third measure was recorded. Body fat and fat-free mass (FFM) were measured using bioelectrical impedance (InBody 770 Body Composition Analyzer, InBody, Cerritos, CA).

Oral glucose tolerance test

After an approximate 10 hour overnight fast, subjects reported to the Clinical Research Unit in the morning and an intravenous catheter was placed in an antecubital vein as previously described by our group. Blood samples were drawn before and during a 75 g OGTT. Circulating glucose, FFA, and insulin were determined at fasting and every 30 minutes up to 120 minutes and then at 180 minutes to assess glucose tolerance and insulin resistance. Glucose tolerance was defined as the glucose total area under the curve (tAUC) at 180 minutes using the trapezoidal model. Whole-body and adipose insulin resistance were calculated as previously described (Gilbertson *et al.*, 2018), and we estimated liver insulin resistance by HOMA-IR (fasting insulin multiplied glucose divided by 405).

Resting and submaximal exercise substrate metabolism

On the first and last days of exercise training (*see below*), subjects reported to the Applied Metabolism & Physiology Laboratory between 07:00–09:30 after a 10–12 hour fast. Subjects rested on a cycle ergometer for 5 min, then cycled for 5 min at 30W (absolute), and finally for 5 min at 70% HR_{peak} (relative) (based on pre-training HR_{peak}). Respiratory gases were collected using indirect calorimetry during each 5 min stage (resting, absolute, and relative intensities). During the last 30 sec of each stage, HR via telemetry (Polar Electro, Kempele, Finland) and RPE were recorded. VO_2 and VCO_2 were averaged over the last 2 min of each stage for determination of fuel selection. Fat and CHO oxidation rates (Peronnet and Massicotte, 1991) and energy expenditure (Kuo *et al.*, 2005) were calculated from these respiratory gases as:

$$\text{Fat oxidation } \left(\frac{\text{g}}{\text{min}} \right) = [(1.695) \left(VO_2 \left(\frac{\text{L}}{\text{min}} \right) \right) - (1.701) (VCO_2 \left(\frac{\text{L}}{\text{min}} \right))]$$

$$\text{CHO oxidation } \left(\frac{\text{g}}{\text{min}} \right) = [(4.585) \left(VO_2 \left(\frac{\text{L}}{\text{min}} \right) \right) - (3.226) (VCO_2 \left(\frac{\text{L}}{\text{min}} \right))]$$

$$\text{Energy expenditure } \left(\frac{\text{kcal}}{\text{min}} \right) = \left\{ \left(\frac{\%CHO}{100} \right) \left(VO_2 \left(\frac{\text{L}}{\text{min}} \right) \right) \right\} \left\{ 5.05 \left(\frac{\text{kcal}}{\text{L } O_2} \right) \right\} + \left\{ \left(1 - \frac{\%CHO}{100} \right) \left(VO_2 \left(\frac{\text{L}}{\text{min}} \right) \right) \right\} \left\{ 4.7 \left(\frac{\text{kcal}}{\text{L } O_2} \right) \right\}$$

Exercise training

In order to isolate the effect of training intensity on our outcomes, we designed our exercise sessions to have the same the average HR to equate energy expenditure. Subjects were randomized to 12 days of fully supervised isocaloric CONT or INT exercise training on a cycle ergometer over a 13-day period. INT consisted of alternating intervals of 3 min at 90% HR_{peak} and 3 min at 50% HR_{peak} for 60 min. CONT consisted of 70% HR_{peak} for the entire 60 min. Days

one and two consisted of 30 min and 45 min training sessions, respectively, to allow acclimation and promote adherence to training. HR via telemetry and RPE were recorded throughout each training session to ensure the appropriate intensities were performed.

Biochemical analysis

Plasma glucose samples were analyzed immediately using the YSI 2300 StatPlus Glucose Analyzer system (Yellow Springs, OH). All other samples were centrifuged for 10 minutes at 4°C and 3000 rpm, aliquoted, and stored at -80°C until later analysis. All measurements pre- and post-training were analyzed on the same plate to minimize inter-assay variability. Plasma insulin and FFA was placed in vacutainers containing EDTA and the protease inhibitor aprotinin. Insulin was analyzed using an enzyme-link immunosorbent assay kit (Millipore, Billerica, MA) and circulating FFAs were analyzed using an enzymatic colorimetric assay (Wako Diagnostics, Richmond, VA).

Statistical analysis

Data were analyzed using SPSS (Version 24, IBM, Armonk, NY). Unpaired two-tailed *t*-tests were used to analyze baseline differences between group characteristics. A 2-way analysis of variance with repeated measures (group x test) was used to analyze main outcomes. Pearson correlation was used to determine associations. Data are mean \pm SEM, and significance was determined as $P < 0.05$.

Results

Subject characteristics

There were no significant differences at baseline between CONT and INT in key demographics (Table 1). Both groups experienced a non-clinically significant loss of body mass (INT: 89.2 \pm 4.6 kg vs 88.6 \pm 4.6 kg; CONT: 89.1 \pm 5.1 kg vs 89.0 \pm 5.1 kg) and statistically, the decrease after INT was more than after CONT exercise (group x test interaction; $p = 0.04$) but there was no significant change in waist circumference. Among both groups, body fat was unchanged and fat-free mass decreased ($p < 0.01$; Table 1). VO_{2peak} increased after INT significantly more than after CONT (group x test interaction; $P=0.04$). Although there was no change in fasting glucose, 2-hour glucose decreased after training (20.9 \pm 6.5 mg·dL⁻¹)

among all subjects (main effect of test; $p = 0.005$) (Table 1). There were no differences in CHO intake in the days prior to the OGTTs before the intervention (INT: 287.6 \pm 19.6 g; CONT: 273.3 \pm 20.1 g; $p = 0.62$) or after (INT: 280.3 \pm 20.5; CONT: 304.3 \pm 17.1; $p = 0.38$) and no group x test interaction ($p = 0.29$).

Submaximal exercise characteristics

HR, RPE, oxygen consumption, RER, and energy expenditure were not statistically different during resting, absolute, or relative tests before or after training, with the exception of an increase in oxygen consumption at rest after training (main effect of test; $p = 0.04$) and a decrease in RER after training during absolute and relative intensity tests (main effect of test; $p < 0.001$, $p < 0.05$, respectively). During the absolute intensity test, the percentage of HR_{peak} attained by subjects was 62.8 \pm 2.1% (range: 42.5 – 79.8%) pre-intervention and 62.5 \pm 2.0% (range: 46.4 – 79.0%) post-intervention and was not different between groups at either timepoint ($p = 0.82$, $p = 0.65$, respectively). During the relative intensity test, the percentage of HR_{peak} attained by subjects was 72.0 \pm 1.0% (range: 64.1 – 87.1%) pre-intervention and 72.3 \pm 1.0% (range: 66.9 – 82.8%) post-intervention and was not statistically different between groups at either timepoint ($p = 0.55$, $p = 0.39$, respectively). RPE was not different between groups at rest, absolute, or relative conditions ($p = 0.56$, $p = 0.59$, $p = 0.35$, respectively) (Table 2).

Resting substrate oxidation

Resting fat oxidation prior to training was lower in CONT than INT (0.7 \pm 0.1 vs. 1.1 \pm 0.1 mg/kg/min; $p = 0.002$). However, resting fat oxidation increased after CONT and INT exercise comparably (CONT: 0.9 \pm 0.1 mg/kg/min; INT: 1.2 \pm 0.1 mg·kg⁻¹·min⁻¹; $p = 0.02$) and was not significantly different after training ($p = 0.09$).

Submaximal exercise substrate oxidation

CONT and INT groups increased fat oxidation during the absolute (main effect of test; $p < 0.001$) and relative (main effect of test; $p = 0.003$) intensity tests. INT increased fat oxidation more than CONT during the relative intensity test (group x test interaction; $p = 0.03$; Figure 1). Carbohydrate oxidation decreased reciprocally during the absolute intensity test (main effect of test; $p = 0.01$) and did not change statistically during the relative test (Figure 1).

Table 1. Subject characteristics.

	CONT		INT	
	PRE	POST	PRE	POST
N (male/female)	11 (3/8)	-	11 (2/9)	-
Age (years)	65.6 \pm 1.9	-	60.1 \pm 2.3	-
Height (cm)	164.5 \pm 2.2	-	166.1 \pm 3.0	-
Waist Circumference (cm)	104.2 \pm 3.7	104.2 \pm 3.7	106.2 \pm 4.4	105.4 \pm 4.3
Body Mass (kg)	89.1 \pm 5.1	89.0 \pm 5.1*	89.2 \pm 4.6	88.6 \pm 4.6†
BMI (kg·m ⁻²)	31.8 \pm 1.8	31.7 \pm 1.8*	32.7 \pm 1.6	32.4 \pm 1.6*
Fat-free Mass (kg)	53.3 \pm 3.2	52.6 \pm 3.2*	51.2 \pm 3.0	50.4 \pm 3.0*
Fat Mass (kg)	29.0 \pm 2.2	28.8 \pm 2.3*	26.7 \pm 2.4	28.0 \pm 2.3*
FPG (mg·dL ⁻¹)	105.9 \pm 2.5	103.1 \pm 3.1	100.9 \pm 1.7	103.3 \pm 2.4
2-hr Glucose (mg·dL ⁻¹)	152.3 \pm 10.3	131.8 \pm 10.0*	155.0 \pm 10.4	133.8 \pm 12.1*
VO_{2peak} (mL·kg ⁻¹ ·min ⁻¹)	19.4 \pm 1.6	19.4 \pm 1.8*	20.3 \pm 1.2	22.3 \pm 1.3*†

BMI, body mass index; FPG, fasting plasma glucose. 18 mg·dL⁻¹ = 1 mM of glucose. Data are mean \pm SEM. *Significant main effect of test, $p < 0.01$; †Significant group x test interaction, $p < 0.05$.

Table 2. Submaximal exercise characteristics.

		CONT		INT	
		PRE	POST	PRE	POST
HR (bpm)	Rest	76.8 ± 2.0	74.6 ± 2.3	74.5 ± 3.0	74.1 ± 3.0
	30W	93.2 ± 2.8	95.3 ± 3.7	95.9 ± 4.1	93.2 ± 3.1
	70% HR _{peak}	107.9 ± 3.7	109.1 ± 3.7	110.6 ± 2.8	109.5 ± 3.7
RPE (AU)	Rest	6.1 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.0 ± 0.0
	30W	9.6 ± 0.7	9.5 ± 0.5	9.2 ± 0.4	9.3 ± 0.6
	70% HR _{peak}	13.1 ± 1.2	11.5 ± 0.5	11.9 ± 0.4	11.3 ± 0.5
VO ₂ (mL·kg ⁻¹ ·min ⁻¹)	Rest	2.4 ± 0.2	2.6 ± 0.2 [‡]	3.0 ± 0.1	3.2 ± 0.1 [‡]
	30W	7.4 ± 0.5	8.2 ± 0.7	9.8 ± 0.4	9.7 ± 0.4
	70% HR _{peak}	11.4 ± 1.5	11.8 ± 1.6	12.7 ± 0.8	13.7 ± 1.3
RER (AU)	Rest	0.85 ± 0.02	0.81 ± 0.02	0.79 ± 0.01	0.79 ± 0.02
	30W	0.87 ± 0.03	0.84 ± 0.02	0.85 ± 0.02	0.79 ± 0.02
	70% HR _{peak}	0.93 ± 0.02	0.93 ± 0.03	0.92 ± 0.02	0.87 ± 0.02
EE (kcal·min ⁻¹)	Rest	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
	30W	3.6 ± 0.3	4.0 ± 0.3	4.2 ± 0.2	4.1 ± 0.3
	70% HR _{peak}	5.7 ± 0.7	5.8 ± 0.8	5.6 ± 0.4	5.8 ± 0.5

HR, heart rate; RPE, rating of perceived exertion; EE, energy expenditure. 1 kcal = 4.184 kJ. Data are mean ± SEM. ‡ Significant main effect of test, $p < 0.05$.

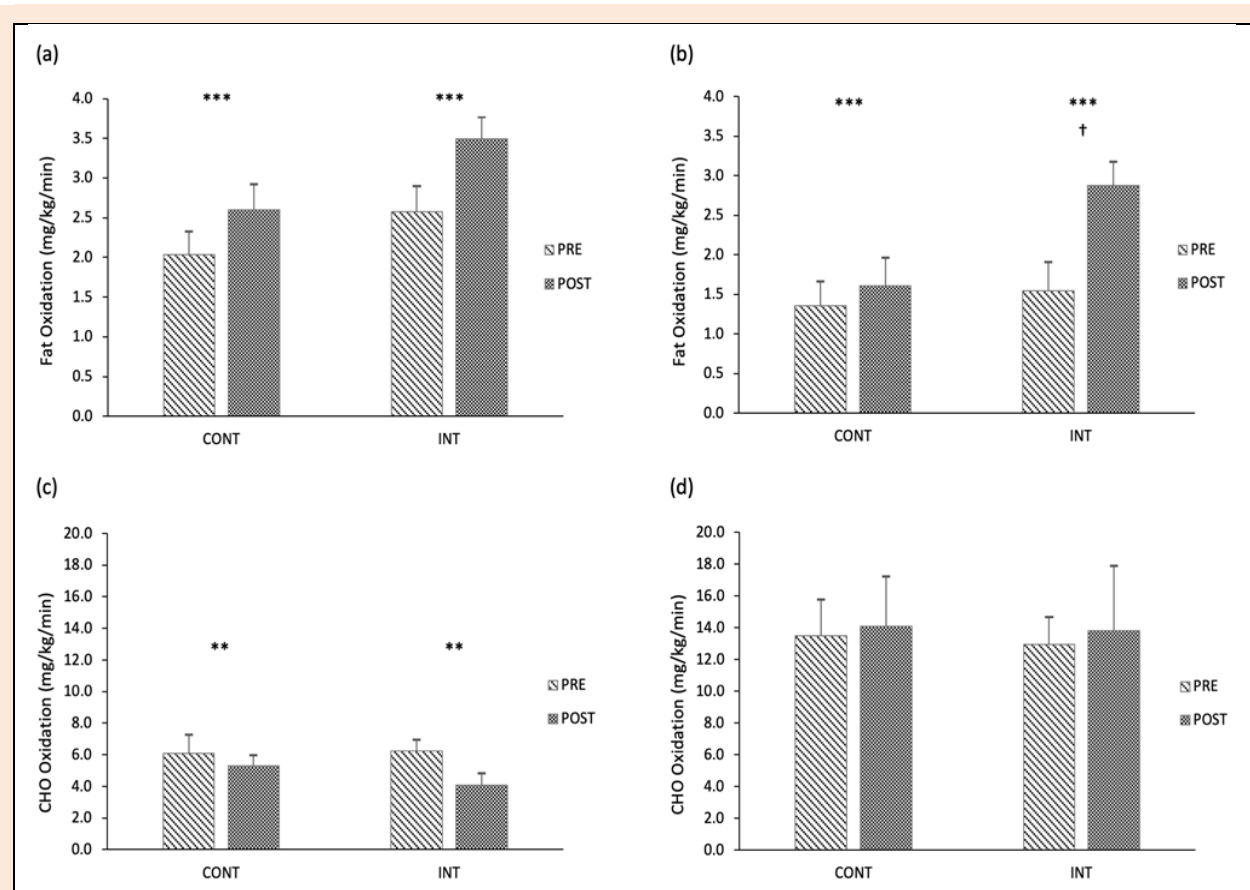


Figure 1. Substrate utilization during submaximal exercise. Substrate utilization during exercise. Fat oxidation during 30W (a) and 70%HR_{peak} (b), and carbohydrate oxidation during 30W (c) and 70%HR_{peak} (d). Data are mean ± SEM. **Significant main effect of test, $P < 0.01$; ***Significant main effect of test, $p < 0.001$; †Significant group x test interaction, $p < 0.05$.

Correlations

There were no significant correlations between resting fat oxidation and exercise fat oxidation during pre-intervention (absolute; $r = 0.11$, $p = 0.61$ and relative; $r = 0.03$, $p = 0.89$) or following training (absolute; $r = 0.17$, $p = 0.44$ and relative; $r = 0.28$, $p = 0.21$). There were also no significant correlations between the change in dietary CHO intake and fat oxidation during rest ($r = 0.30$, $p = 0.18$) or exercise (absolute $r = -0.25$, $p = 0.27$; relative $r = -0.16$, $p = 0.46$).

There were no significant correlations between fat oxidation during the relative test for any outcomes. Increased fat oxidation during the relative test correlated with elevated VO_{2peak} (Figure 2; $r = 0.53$, $p = 0.01$), but did not relate significantly with weight loss ($r = -0.22$, $p = 0.32$). Enhanced fat oxidation during the relative intensity test also did not relate to improved glucose tolerance or reductions in insulin resistance after treatment (Figure 3).

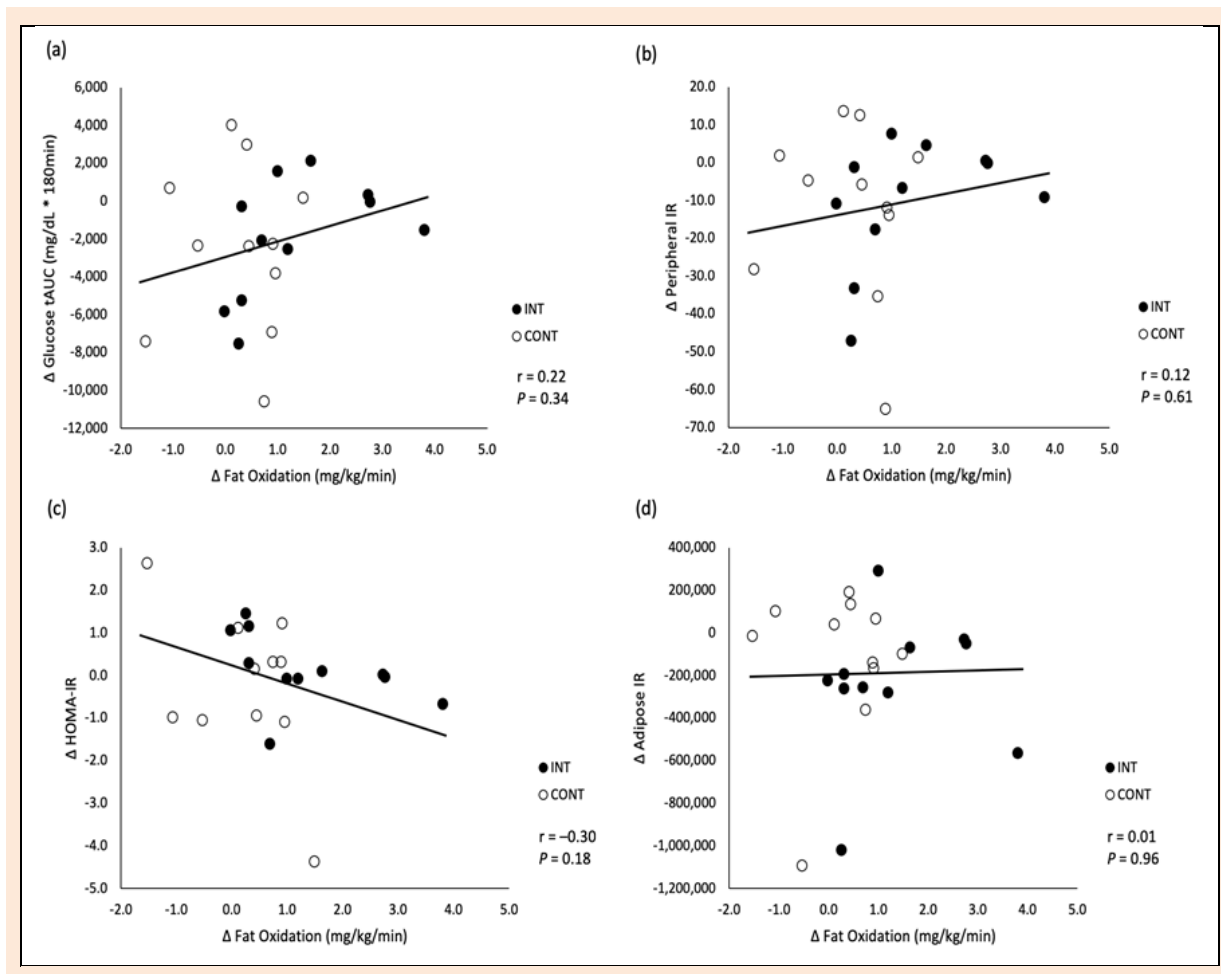


Figure 3. Correlations between changes in fat oxidation during 70%HR_{peak} and glucose regulation. Glucose tolerance, 180min OGTT glucose AUC (a); peripheral insulin resistance, 180min OGTT glucose AUC x insulin AUC (b); HOMA-IR (c); adipose IR, 180min OGTT insulin AUC x FFA AUC (d).

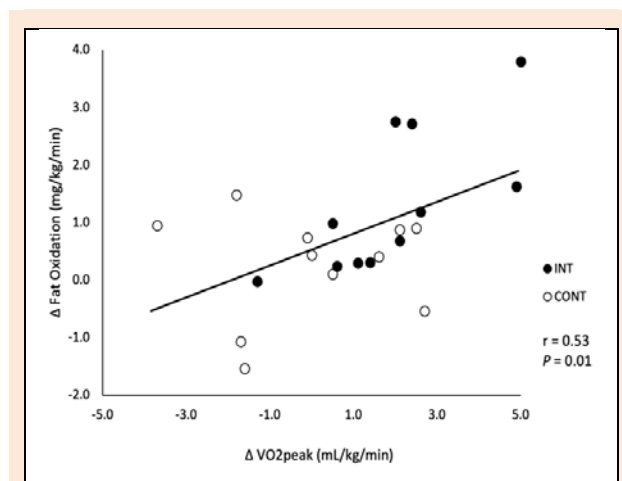


Figure 2. Correlation between changes in fat oxidation during 70%HR_{peak} and VO₂peak.

Discussion

The major finding of this study is that two weeks of INT exercise training results in enhanced fat oxidation at the same relative intensity after training compared with work-

matched CONT in obese adults with prediabetes. Additionally, both CONT and INT exercise training increased fat oxidation during exercise at the same absolute intensity. Various studies ranging from 2–16 weeks of sprint interval (e.g. 30 – 60 sec, “all out”), high intensity interval (e.g. 60 sec, 90%HR_{peak}), moderate intensity interval (e.g. 5 min, 65% VO₂peak), and/or continuous moderate intensity (30 – 60 min, 60 – 95% HR_{peak}) have reported increased fat oxidation during exercise at the same absolute workload in overweight and obese adults after exercise training (Van Aggel-Leijssen et al., 2002; Crampes et al., 2003; Pruchnic et al., 2004; Alkahtani et al., 2013; Astorino et al., 2013; Arad et al., 2015; Lanzi et al., 2015). However, few studies have examined the effects of exercise training on fuel selection at the same relative intensity, when greater reliance on CHO might be expected due to an increase in absolute workload. Prior work has shown that increasing the ability to use fat for energy during exercise may be clinically relevant, as it was associated with greater insulin sensitivity in obese, but healthy, males (Venables and Jeukendrup, 2008). Herein, we report that training-induced increases in fat oxidation did not correlate with reductions in insulin resistance or improved glucose tolerance. Our results suggest that exercise fat oxidation may not be a primary mecha-

nism for improved glucose regulation. The reason for conflicting results between Venables and Jeukendrup's work and the current study is not entirely clear but may be due to differences in study design. Their study recruited only obese but otherwise healthy males who trained for 4 weeks while ours included mostly females that exercised for 2 weeks. Furthermore, our study population was specifically characterized as having prediabetes which may alter substrate utilization (Braun et al., 2004; Malin et al., 2013). Nonetheless, the present findings are consistent with prior work showing increased whole-body fat oxidation during the same absolute exercise workload after 10 weeks of exercise training in people with prediabetes does not correlate with clamp-derived measures of insulin sensitivity (Malin and Braun, 2013). Taken together, the present data in combination with our prior report (Gilbertson et al., 2018), suggest that enhancing metabolic flexibility during feeding may be more important for glucose regulation than exercise-stimulated substrate utilization despite training inducing exercise fat oxidation adaptation in just two weeks.

Since exercise training had no effect on RPE, oxygen consumption, or energy expenditure during the submaximal exercise test, it is unlikely that differences in workload between interventions during testing explain the rise in fat oxidation. Moreover, although individuals in the INT treatment oxidized more fat than CONT at rest prior to the intervention, resting fat oxidation did not improve to a greater extent after CONT exercise than INT, nor was resting fat oxidation correlated to exercise fat oxidation during absolute or relative tests prior to or following treatment. Together, these findings suggest that the baseline group differences in resting fat oxidation did not impact training-induced exercise fat oxidation adaptation. There are, however, several potential mechanisms by which INT training could have accentuated fat metabolism compared to CONT exercise. It is possible that INT increased fat oxidation directly by improving oxidative capacity, or indirectly by increasing fat availability. While we did not measure circulating free fatty acids during exercise in this study, it seems unlikely that circulating fat availability would have a major role; the substrate metabolism tests were performed for 5 minutes at each exercise intensity and within this timeframe, lipid mobilization from adipose tissue was likely limited (Bülow et al., 2006). It remains possible that training reduced hepatic glucose production. This would limit plasma glucose availability and prompt a compensatory rise in skeletal muscle fat oxidation (Friedlander et al., 1997). Nonetheless, our data show that during the same relative intensity (i.e. higher absolute workload after training), CHO oxidation rate remained constant (Figure 1d), so it is unlikely that plasma glucose was a limiting factor. However, high intensity interval and sprint interval training increase markers of mitochondrial biogenesis, function, and enzyme activity after only 1–6 sessions in healthy and hyperglycemic adults (Burgomaster et al., 2008; Little et al., 2010, 2011), thereby promoting a rise in oxidative capacity and intramuscular triglyceride use. While we did not obtain direct mitochondrial or intramuscular measures, our results show improved aerobic capacity. In fact, VO_2peak improved 9.5% in INT, while remaining essentially unchanged in CONT (0.3%). Furthermore,

enhanced fat oxidation during exercise at the same relative intensity was correlated with increased VO_2peak (Figure 2), suggesting that increased oxidative capacity contributed to enhanced fat metabolism.

Since others have shown that weight loss contributes to increases in fat oxidation (Corpeleijn et al., 2008; Tsujimoto et al., 2012), we scaled fat oxidation to body mass to isolate the effects of exercise. Our results demonstrate that increased fat oxidation following CONT and INT was independent of clinically meaningful weight change (~ 0.1 and ~ 0.6 kg for CONT and INT, respectively). While we did not observe a significant correlation between enhanced fat oxidation during exercise and reduced body weight, recent work suggests energy expenditure, not intensity, is key for body fat reduction (Cowan et al., 2018). In line with this, exercise intensity has no effect on 24-hour fat metabolism (Saris and Schrauwen, 2004) and oxygen consumption (Hazell et al., 2012). Thus, it would seem exercise fat oxidation is not critical for explaining weight loss after the intervention. Additional work is warranted to determine the mechanism by which exercise intensity promotes fat utilization and whether this enhanced fat oxidation is clinically relevant in the long-term glycemic control in adults with prediabetes.

Exercise at the same absolute workload would be expected to induce less metabolic stress after exercise training as a result of aerobic fitness adaptation and reduced sympathetic nervous system activity. Consistent with this hypothesis, CONT and INT increased fat oxidation and decreased reliance on carbohydrate to a similar degree during the absolute intensity condition. However, HR and RPE were unchanged after training during the absolute intensity test (Table 2). This suggests that our results may be independent of sympathetic nervous system adaptation and downstream effects on fat mobilization, hepatic glucose production, and glucose metabolism (Friedlander et al., 1998; Friedlander et al., 1998). We chose a workload of 30W for the absolute intensity test because this workload constituted a light to moderate intensity for our participants that would not elicit a heart rate over $70\% \text{HR}_{\text{peak}}$.

This study has some limitations and strengths to consider. The majority of subjects tested were post-menopausal females, limiting the generalizability of our findings. We were not able to test for differences between males and females with adequate statistical power. Therefore, additional work is required to understand how sex affects metabolic adaptations to improve precision exercise therapy. Although substrate utilization testing was performed in a fasted state, we did not standardize meal intake in days prior to these tests. Differences in carbohydrate intake could influence glycogen concentrations and impact fuel selection during exercise. However, based on health history questionnaires, all subjects consumed a mixed diet and food intake analysis indicates that subjects consumed over 250g of carbohydrate in CONT and INT pre- and post-training. Moreover, there was no relation between changes in carbohydrate intake pre and post-intervention with fat oxidation. Therefore, low glycogen concentrations following exercise training are unlikely to explain the rise in fat oxidation we observed. Subjects were tested during each submaximal test stage (absolute 30W, relative $70\% \text{HR}_{\text{peak}}$)

for only 5 min. While this was sufficient to reach steady state oxygen consumption, fat oxidation may change with increasing duration of constant load exercise (Alkahtani et al., 2013). The slight reduction in FFM should be interpreted with caution, as we used multifrequency bioelectrical impedance to assess body composition and this is susceptible to changes in hydration status. Nonetheless, a strength of our study design is that participants in both groups were similar in age, sex, BMI, fitness, and glycemic status, suggesting that alterations in fat oxidation following CONT and INT training were not confounded by these factors.

Conclusion

In conclusion, two weeks of INT exercise training augmented fat oxidation at the same relative intensity compared to CONT in obese adults with prediabetes. Furthermore, CONT and INT increase fat oxidation during exercise at the same absolute intensity. The shift in reliance on fat metabolism was related to increased aerobic fitness, but not insulin sensitivity. These findings suggest that training-induced increases in oxidative capacity may contribute to cardio-metabolic risk reduction, and support use of INT as an effective therapy to prevent and/or delay the onset of T2D and cardiovascular disease.

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Key points

- Interval exercise training augments fat oxidation in adults with prediabetes
- Elevated exercise fat oxidation correlates with improved aerobic fitness
- Fat oxidation changes were not correlated with glucose regulation
- Enhanced fat oxidation and fitness occur before clinically significant weight loss

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