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Eight sessions of endurance training decrease fasting glucose and improve glucose tolerance in middle-aged overweight males

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Running title: Endurance training rapidly improves metabolic health

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Abstract

Exercise improves metabolic regulation and reduces the risk for developing type 2 diabetes and other metabolic diseases. The recommendations for exercise are rather general and the health benefits of controlled training studies are important to make better recommendations. In the present study, we report that eight endurance training sessions over three weeks reduced fasting glucose, and improved glucose tolerance and plasma lipids in sedentary middle-aged males (44-64 years) with overweight or obesity (BMI 27-38). The decrease in fasting glucose was substantial (from 5.3±0.3 to 4.8±0.2 mM; $p<0.001$). The training sessions consisted of 60-minute indoor-cycling at ~83 % of peak heart rate divided in four blocks of 15 minutes cycling, with 2 minutes rest between blocks. Maximal oxygen uptake did not increase (38.8±1.8 *versus* 39.0±1.6 $ml \cdot kg^{-1} \cdot min^{-1}$). In conclusion, three weekly sessions of moderate/high intensity endurance training can be recommended for untrained males with overweight or obesity to improve glucose homeostasis.

Keywords: Exercise, insulin, diabetes, cholesterol, LDL, HDL

Introduction

Obesity is a worldwide problem and today more than half the middle-aged population is overweight or obese in many countries (Ng *et al.*, 2014). Overweight and obesity disposes for elevated fasting glucose and reduced glucose tolerance, and increases the risk of developing type 2 diabetes mellitus (T2DM) (Abdullah, Peeters, de Court, & Stoelwinder, 2010; Ross et al., 2011). Treatment of T2DM is costly and effective intervention strategies are necessary (Leung, Pollack, Colditz, & Chang, 2015; Leung, Carlsson, Colditz, & Chang, 2017). Importantly, exercise reduces fasting glucose and improves insulin sensitivity and glucose tolerance (Colberg, 2007; Sandvei et al., 2012; Smith, Crippa, Woodcock, & Brage, 2016; Langleite et al., 2016; Karstoft et al., 2013). However, optimising the exercise protocols for people with overweight and obesity seem necessary to reach more people and improve metabolic health.

Inactivity and overweight/obesity predispose for increased plasma triacylglycerol and LDL, and low HDL, which are major risk factors for development of cardiovascular diseases (CVD). It has been known for years that exercise decreases the risk for cardiovascular disease (Paffenbarger, Jr. et al., 1993; Blair et al., 1989), and several studies have reported increased HDL and reduced LDL after endurance training (Kraus et al., 2002; Slentz et al., 2007). The exercise recommendations to improve metabolic health are physical activity (endurance) of moderate intensity for a minimum of 30 minutes five days a week, or vigorous intensity for a minimum of 20 minutes three days a week (Haskell *et al.*, 2007). Recently, recommendations have also included some strength training. Indeed, the combination of high intensity endurance exercise and strength training can effectively improve insulin sensitivity in both lean and overweight people (Langleite *et al.*, 2016).

The interest in the effect of high intensity interval training (HIIT) on glucose homeostasis has been high lately. The classical model for HIIT is 30-second all-out exercise with 2-5 minute rest between repetitions of 4 to 10 sets (MacDougall *et al.*, 1998). Studies have shown that HIIT reduces fasting glucose (Sandvei *et al.*, 2012), increases glucose tolerance (Sandvei et al., 2012; Babraj et al., 2009) and improves insulin sensitivity (Richards *et al.*, 2010). Glycogen breakdown in skeletal muscle is substantial during 30-second all out cycling (Birk & Wojtaszewski, 2006), and glycogen content in skeletal muscles is a key regulator of insulin sensitivity (Jensen, Aslesen, Ivy, & Brørs, 1997; Jensen et al., 2006; Kolnes et al., 2015). In both lean, obese and T2DM patients, 45 minutes of endurance exercise reduces glycogen content and activates glycogen synthase (Jensen *et al.*, 2012),

which contributes to improvement in insulin sensitivity and metabolic regulation (Jensen, Rustad, Kolnes, & Lai, 2011).

Carbohydrate is the main energy substrate during exercise at intensities ~75% of maximal oxygen uptake (Hermansen, Hultman, & Saltin, 1967; Romijn et al., 1993; Rustad et al., 2016; Sollie et al., 2018), and prolonged training at such intensities improves glucose tolerance. Moreover, exercise at such intensity effectively improves skeletal muscle oxidative capacity and expression of many proteins involved in glucose uptake. Most previous training studies have used training periods of 6 to 12 weeks to investigate the effect of exercise on glucose tolerance and insulin sensitivity (Sandvei et al., 2012; Bruce et al., 2006; Malin et al., 2013a; Malin et al., 2013b). Importantly, high intensity training has been reported to improve insulin sensitivity and glucose tolerance after only two weeks of training (Babraj et al., 2009; Richards et al., 2010). Improved glucose tolerance has also been reported after 7 to 10 consecutive days of endurance training (Holloszy, Schultz, Kusnierkiewicz, Hagberg, & Ehsani, 1986; Rogers et al., 1988; Angelopoulos, Schultz, Denton, & Jamurtas, 2002). The improved glucose tolerance after short training period is attractive from health promotion as well as motivational perspectives, and need further investigation.

The purpose of the present study was to evaluate the effect of short-term prolonged moderate/high intensity endurance training on glucose tolerance and blood lipid profile in untrained obese and overweight middle-aged men. We hypothesized that glucose tolerance and lipid profile would improve after eight training sessions of moderate/high intensity endurance training.

Methods

Subjects.

Key inclusion criteria were as follows: untrained middle-aged Caucasian males (40-65 years), body mass index (BMI) > 25, non-smoking, with no history of cardiovascular disease, diabetes or taking any medication. In addition, participants should not have participated in systematic training during the last two years (less than one session per week). Preliminary screening by questionnaire verified that participants did not have any known cardiovascular or metabolic disease. An adherence of ≥ 75 % to training was requested (6 out of 8 training sessions). Participants were informed about the study before they gave their written consent to participate. This study was approved by the Regional Ethics Committee (REK) in Norway (2012/970/REK, Sør-øst A) and was performed according to the Declaration of Helsinki. All research materials were stored as described in the approval from REK. The study was

conducted at Norwegian School of Sport Sciences. Ten participants were recruited to the study and fulfilled the exercise adherence criteria.

Study design. The study was designed as a short-term training intervention. Subjects performed familiarization and physiological tests prior to the intervention. Before and after the training period of three weeks oral glucose tolerance and physiological tests were conducted. Participants served as their own controls. All participants were asked to refrain from other strenuous physical activity throughout the intervention period and maintain their normal diet.

Preliminary testing

Relationship between workload, oxygen uptake (VO_2) and heart rate (HR)

Prior to the intervention, the relationships between workload, oxygen uptake (VO_2) and heart rate (HR) were established. A Lode bike (Lode, Groningen, Netherlands) was used for laboratory testing. The incremental test started with 5 minutes warm up at a load corresponding to 7-10 (easy) on the Reported Perceived Exertion (RPE) of Borg scale (Borg, 1970). After the warm up, participants cycled 5 minutes at 4-5 loads, depending of fitness level. The work load started at 105-135 W and was increased by 15 W every 5 minutes. Between 2 and 4 minutes on each load, oxygen uptake was measured through a two-way mouthpiece (Hans Rudolph Instr., Kansas, USA) connected to a Jaeger (Champion, Jaeger Instr., Germany) through a mixing chamber (Oxygon Pro: Jaeger, Hoechberg, Germany), and oxygen and carbon dioxide were measured and reported as 30-second averages. Expiration volume was measured with a turbine (TripleV volume transducer) calibrated manually with a 3 L pump before the tests. Oxygen and carbon dioxide were calibrated against room air and a standard gas. After 4 minutes cycling at all loads, lactate was measured in capillary blood (YSI 1500 SPORT; Yellow Springs Instruments Life Sciences, Yellow Springs, OH).

Test of VO_{2peak} and HR_{peak}

After completing the incremental test, participants rested or performed easy cycling for 5 minutes before the VO_{2peak} test. The test started at a load corresponding to 7-10 on the Borg scale. The load was increased with 15 W every 30 seconds. Subjects could choose to continue on the same load or increase by 7.5 W when they started to fatigue until voluntary exhaustion, where the RPE on Borg scale was reported to be 19. Encouragement was given verbally throughout the test and VO_{2peak} was defined as the highest oxygen uptake achieved during 1

minute. HR was measured during the test (Polar S610i, Polar Electro Oy, Kempele, Finland), and the highest heart rate registered during the test was defined as HR_{peak}. The tests were performed twice and the highest values were used to describe VO_{2peak} and HR_{peak}.

After the intervention the test was repeated for measurement of VO_{2peak} and the relationship between oxygen uptake and HR was determined.

Body composition

Body composition was determined with Inbody 720 (Biospace Co, Ltd., Seoul, Korea), after an overnight fast prior to blood sampling. Measurements were done prior to and after the training interventions.

Blood sampling

Participants were asked to refrain from strenuous physical activity and training three days before the OGTT prior to the intervention. On the evening before the first OGTT, participants were instructed to eat a carbohydrate rich evening meal and register their diet. They were instructed to eat the same diet the evening prior to the OGTT after the training intervention. Participants arrived at the laboratory at 06:30 a.m. after overnight fast (9-11 hours) and a veneflon was inserted in v. antecubital for blood sampling. The catheter was kept patent by flushing with 0.9 % saline solution after each blood sample collection.

Fasting samples

Fasting blood samples for glucose, insulin, total cholesterol, LDL, HDL and triglycerides were collected in serum separator tubes (serum gel 8.5 ml, BD Vacutainer, UK) and coagulated for 30-45 minutes at room temperature before centrifugation (Eppendorf 5072R, Hamburg, Germany) at 2500 rpm for 10 minutes and refrigeration at 4°C. Fasting samples for $HbA1_c$ were collected in EDTA tubes (3 ml, BD Vacutainer, UK) and immediately put on ice before stored at 4°C. Samples were analysed at Fürst Laboratory, Oslo, Norway (Advia Centaur XPT, Siemens Medical Solutions Diagnostics, Tokyo, Japan).

Oral glucose tolerance test.

Oral glucose tolerance tests (OGTT) were conducted prior to and after the training intervention. Samples were collected at 20, 40, 60, 90 and 120 minutes after ingestion of 75g of glucose, dissolved in 300 ml of water. The solution was ingested within a two-minute

timeframe. Samples were collected in 8.5 ml tube (serum gel 8.5 ml, BD Vacutainer, UK), coagulated at room temperature for 30 minutes before centrifuging at 2500 rpm for 10 minutes (Heraeus Megafuge 16 R centrifuge, Thermo Scientific, UK). Samples were stored at 4°C, before analyses at Fürst Laboratory, Oslo, Norway (Advia Centaur XPT, Siemens Medical Solutions Diagnostics, Tokyo, Japan). Catheters were not inserted in two participants; blood glucose was measured in capillary blood (HemoCue Glucose 201^{+}, Ängholm, Sweden) sampled from a fingertip. These samples were collected in cuvettes and immediately analysed for glucose concentration (Hemocue glucose 201^{+}, Ängelholm, Sweden).

Training protocol

During the three-week training period, the subjects participated in eight training sessions, where at least one day of rest was given between each training sessions. All training sessions were group-based and were performed on indoor-bicycles (Body Bike Classic, Body Bike International A/S, Frederikshavn, Denmark), supervised by a qualified instructor. Training intensity was determined from the relationship between heart rate and VO_2 as well as the information about VO_{2peak}, and HR_{peak}. Training sessions consisted of 5-minute warm up at a HR no higher than 80% of HR_{peak}, followed by 60 minutes of cycling at 82-87% of HR_{peak}. Each session was divided into 4 blocks of 15 minutes, with 2-minute breaks between blocks. During the breaks participants cycled without load or rested. After each training session the participants cycled slowly 5 minutes without load as cool down. A heart rate monitor (Polar S610i, Polar Electro Oy, Kempele, Finland) was used to control intensity and duration. Data from the heart rate monitors was stored for later analysis.

Statistics and calculations.

Statistical analysis was performed using “Predictive Analytics Software” (PASW Statistics 18). All data were tested for normality with a Shapiro-Wilk test before analysis. A paired t-test was used to compare training effect within group when the data were normal distributed. If data was not normal distributed, a Wilcoxon signed rank-test was used to compare training effect within group. Statistical significance was accepted at $p < 0.05$ level. Data are presented as mean ± SEM. Anthropometric data are given as median and range.

Insulin resistance was calculated by ‘Homeostasis model assessment of insulin resistance’ (HOMA-IR) by using the following formula: fasting glucose x fasting insulin / 22.5 (Wallace, Levy, & Matthews, 2004). Area under the curve (AUC) was calculated

geometrically by the trapezoidal method. Energy expenditure was estimated assuming that one litre of oxygen corresponds to 5 kcal.

RESULTS

Training

All included participants fulfilled the exercise adherence criteria and completed the training intervention. Eight of the participants completed all eight training sessions. One participant completed seven training sessions and one participant completed six training sessions.

Heart rate during training sessions

Average HR during the intervals in the training sessions was 149.5 ± 4.3 beats/min, and did differ significantly during the eight sessions (Figure 1A). The average HR corresponded to ~83% (range: 82-86) of HR_{peak}. During the 2-minute breaks between the four 15-minute cycling intervals, HR decreased to 139.4 ± 4.2 beats/min corresponding to 77% of HR_{peak} (range: 72-81). The average energy expenditure during training session was 1088 ± 29 kcal. Accumulated energy expenditure for the eight training sessions, was 8358 ± 257 kcal. The subjects reported a mean RPE of 16.2 ± 0.3 on average on the Borg scale (Figure 1B). Borg scale: 16 corresponds to hard.

Body weight and body composition

There was a significant reduction in body weight (99.3 ± 3.2 to 98.5 ± 2.9 kg; $p<0.05$) and BMI (30.4 (27.1-38.3) to 30.1 (27.0-37.7) kg/m^2; $p<0.05$). Body composition estimated as muscle mass and body fat did not change during the training period (Table 1).

VO_{2peak} and lactate profile

VO_{2peak} did not increase during this short training intervention. Participants rated perceived exertion to 19 on the Borg scale during the test, before and after the training period. All participants cycled at loads of 135, 150 and 165 W during the VO_{2peak} test, before and after the training period. HR did not decrease significantly at any of the aforementioned loads after the training intervention (Figure 2A). Blood lactate concentration was significantly lower at all loads after the training intervention compared with concentrations prior to the intervention (Figure 2B).

Oral glucose tolerance test (OGTT)

Fasting glucose decreased during the training intervention (from 5.4 ± 0.3 to 4.8 ± 0.2 mM; p=0.001; Table 2). During the OGTT, glucose concentration (as AUC) was significantly lower (893.2 ± 74.3 to 773.5 ± 66.5 mM·120 min^{-1}; p= 0.001) after the training intervention (Figure 3A). The training intervention reduced the early phase of glucose AUC (AUC_{0-20}) (132.8 ± 7.8 to 119.3 ± 4.3 mM·20 min^{-1}) (p= 0.012) and the late phase of glucose AUC (AUC_{60-120}: 421.6 ± 39.8 to 361.1 ± 37.8 mM·60 min^{-1}; p= 0.01). The 2-hour glucose concentration during the OGTT was lower after the intervention, compared to baseline (5.7 ± 0.3 to 5.1 ± 0.4 mM; p<0.05; Figure 3A).

Fasting insulin did not change after the training intervention (p=0.247). Insulin responses during the OGTTs are shown in Figure 3B. Total insulin AUC (AUC_{0-120}) was significantly reduced (41388 ± 7334 to 30382 ± 4989 pM·120 min^{-1}; p=0.044) after the training intervention. The 2-h insulin concentration was lower after the intervention (321 ±80 versus to 155 ± 33 pM; p<0.05), and late phase of insulin AUC (AUC_{60-120}) tended to be lower comparing prior to and after training intervention (p=0.063). Inspecting the graph indicates that plasma insulin increased faster after the training intervention, but the early insulin response was not significantly improved. However, insulin concentration reached its highest level at an earlier stage after the training intervention than before (Figure 3B).

HOMA-IR and glycosylated hemoglobin

HOMA-IR did not change significantly during the training intervention, and HbA_{1c} was unchanged (Table 2).

Cholesterol and triglycerides

Total cholesterol (5.96 ± 0.20 to 5.48 ± 0.28 mM; p=0.029) and LDL-cholesterol (3.92 ± 0.16 to 3.46 ± 0.20 mM; p=0.015) decreased after the training intervention, whereas HDL-cholesterol increased (1.24 ± 0.07 to 1.32 ± 0.08 mM; p=0.037; Table 2). Fasting triglyceride levels were unaffected after the training intervention.

Discussion

The main finding of this study was that three weeks with eight sessions of moderate/high intensity endurance training reduced fasting glucose and improved glucose tolerance in previously untrained middle-aged males with overweight or obesity. In addition, the short training period increased HDL cholesterol and reduced LDL and total cholesterol.

The effect of eight high intensity endurance training sessions on glucose homeostasis was convincing, since the intervention reduced fasting glucose as well as AUC for both glucose and insulin. In the present study, fasting glucose decreased by 0.5 mM, which may have large health benefits. Fasting glucose increases gradually by age, and cross-sectional data indicate an increase of 0.15 mM per decade (Ko, Wai, & Tang, 2006). In the prospective Framingham study, non-fasting glucose increased by 0.3 mM per decade (Yashin *et al.*, 2009) and maintaining low plasma glucose is associated with longevity (Yashin et al., 2009; Yi et al., 2017). Several studies have previously reported reduced fasting glucose (Holloszy et al., 1986; Angelopoulos et al., 2002; Sandvei et al., 2012; Malin et al., 2013a). The hepatic glucose production is the main contributor to elevated fasting glucose (Abdul-Ghani, Tripathy, & DeFronzo, 2006) and fasting insulin elevates over time to counteract hyperglycaemia. Fasting plasma insulin did not decrease significantly in the present study, agreeing with many previous studies (Holloszy et al., 1986; Sandvei et al., 2012) whereas other find reduced plasma insulin after training interventions (Bruce *et al.*, 2006). In the present study, we speculate that the lower fasting glucose results from training-induced improvement of hepatic insulin sensitivity.

The rapid improvement in glucose tolerance observed after eight high intensity endurance exercise sessions is noteworthy and emphasises the importance of training to improve glucose metabolism. Short-term HIIT training has previously shown to improve glucose tolerance and insulin sensitivity in untrained individuals after only six sessions during two weeks (Babraj et al., 2009; Richards et al., 2010). Improved glucose tolerance has previously been reported after endurance training for seven (Rogers et al., 1988) or ten consecutive days (Angelopoulos et al., 2002; Denton, Schultz, Jamurtas, & Angelopoulos, 2004). It is worth to note that training was conducted every second day (three session per week) in the present study, and the training program will for many be easier from practical and motivational perspectives.

Indeed, not all studies have reported improved glucose tolerance after endurance training. Kang et al. observed unchanged plasma glucose response area during the OGTT in obese men after seven days of endurance training at 50% VO_{2max} for 70 minutes and after training at 70 % of VO_{2max} for 50 minutes (Kang et al., 1996). However, the insulin response was reduced after seven days of endurance training at 50 % VO_{2max} (Kang et al., 1996), which agree with our finding. Several studies have suggested that the beneficial effect of training on glucose metabolism is impaired in people with impaired metabolic regulation. Malin and Kirwan found that fasting hyperglycemia blunted the reversal of impaired glucose tolerance

after exercise training (Malin & Kirwan, 2012). However, two participants in the present study had fasting glucose above 6 mM before the intervention, but their plasma glucose decreased from 7.6 to 6.2 and from 6.1 to 4.9 mM, respectively, which implies a reduced risk of developing T2DM. In addition, ten consecutive days of treadmill walking for one hour per day at 70-75 % of peak aerobic capacity also improved glucose tolerance in people with metabolic syndrome, but not in participants without metabolic syndrome (Baynard, Carhart, Jr., Ploutz-Snyder, Weinstock, & Kanaley, 2008). The intensity of the training also influences the effect of exercise on metabolic regulation, and Karstoft et al. have reported that high intensity interval walking was superior to continuous low intensity for improving glucose tolerance (Karstoft et al., 2013; Karstoft et al., 2014).

Increased glucose tolerance with normal or reduced insulin response most likely results from increased insulin sensitivity in skeletal muscle (Abdul-Ghani et al., 2006; Malin et al., 2013a). Regular exercise training, in addition to loss of body mass, increases pancreatic β-cell function in adults (Malin et al., 2013b) and in agreement with the finding in the present study, several studies have reported that both glucose and insulin responses decrease after endurance training (Holloszy et al., 1986; Rogers et al., 1988; Bruce et al., 2006; Malin et al., 2013b). In the present study, the energy utilization was about 1,000 kcal per training session, and high exercise dose is required to improve β-cell function (Malin *et al.*, 2013b). Adaptations of skeletal muscles occur after short period of high intensity endurance training (Burgomaster, Hughes, Heigenhauser, Bradwell, & Gibala, 2005), and we found reduced glucose tolerance without reduction in insulin response after high intensity sprint interval training (Sandvei et al., 2012). The insulin response to the OGTT was also reduced after the training intervention in the present study, which supports the β-cell function improved after this short training intervention.

In this study, OGTT was performed 15-17 hours after the final training session as in many other studies (Holloszy et al., 1986; Rogers et al., 1988; Angelopoulos et al., 2002). Indeed, improved glucose tolerance has been found 36 hours after training interventions (Sandvei *et al.*, 2012), but a single acute bout of exercise has been reported to improve glucose tolerance in some (King et al., 1995; Bonen, Ball-Burnett, & Russel, 1998; Oberlin et al., 2014) but not other studies (Knudsen, Karstoft, Pedersen, van, & Solomon, 2014; Rose, Howlett, King, & Hargreaves, 2001). The energy utilization during the training sessions in the present study was 1,000 kcal at 85 % of HR_{peak}, and oxidation of ~150 g of the carbohydrate is a fair estimate. It is therefore likely that the improvement in glucose tolerance resulted from both acute and chronic effects of exercise. However, whether these beneficial effects of the

training intervention on glucose tolerance are partly explained by the last training session, does not eliminate the importance of the present study. Three sessions with exercise training per week will still increase glucose tolerance at least three days per week.

The eight sessions of endurance training with moderate/high intensity improved the lipid profile. High levels of total cholesterol and LDL-cholesterol, and low levels of HDL cholesterol have been associated with increased risk of cardiovascular diseases (Huxley et al., 2011; Singh, Sharma, Kumar, & Deedwania, 2010; Ahmed et al., 2016; Badimon & Vilahur, 2012) and exercise improves lipid profile (Tambalis, Panagiotakos, Kavouras, & Sidossis, 2009). In the present study, HDL increased whereas both total cholesterol and LDL-cholesterol were reduced following training intervention, indicating that the included participants reduced their risk of developing cardiovascular diseases following the intervention. Many other studies have reported improved lipid profile after training (Kraus et al., 2002; Slentz et al., 2007). In a randomised control trial, Kraus et al. reported that high amount (23 kcal $\cdot$ kg^{-1} per week) of high intensity training caused the greatest improvement (Kraus *et al.*, 2002). In the present study, participants exercised ~30 kcal $\cdot$ kg^{-1} per week and the intensity was at least as high as in the previous study, and the improvement was substantial after only eight training sessions. Indeed, a single bout of exercise has a potential to transient increase in HDL-cholesterol, but duration and intensity of the training seems to be critical for the changes that can be observed (Pronk, 1993). In the present study, plasma cholesterol was measured ~16 hours after the last training session, whereas elevated HDL has been reported 14 days after a six months intervention with high amount high intensity endurance exercise (Slentz *et al.*, 2007). Although triglyceride levels did not decrease significantly, the post intervention triglyceride was below primary prevention levels.

There are some limitations in the present study that must be taken into consideration. The present study was a pre-post comparison, where subjects served as their own control. Furthermore, only ten subjects took part in and completed the study. Although there were no dropouts, a small sample size often involves uncertainty, and a greater sample size had been a strengthened. It is a strength that heart rate was controlled at all training sessions and the intensity related VO_{2peak}. Energy expenditure during exercise was calculated using heart rate to calculate oxygen consumptions, and the calculations are associated with assumptions.

In conclusion, our findings show that eight sessions of endurance training with moderate/high intensity are sufficient to improve glucose tolerance in previously untrained men with overweight or obesity. Further, this study shows that the endurance training increased HDL cholesterol and reduce LDL and total cholesterol. For middle-aged men, who

possess or are in the process of developing abnormal glucose tolerance and unfavourable lipid profile, endurance training of moderate/high intensity can be recommended to improve metabolic health.

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Declaration of interest

The authors report no conflict of interest

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Legends

Figure 1. Heart rate and perceived exertion during the training sessions. (A) Heart rates are means during the training sessions; the 2-minute resting periods are excluded. N=8-10 on different days. (B) Perceived exertion was scored on Borg scale after the session. N=9-10 on different days. Data are presented as mean ± SEM.

Figure 2. Heart rate and blood lactate at standard workloads before and after 8 weeks of training. Participants cycled for 5 minutes at 135, 150 and 165 Watt. Heart rate and blood lactate was measured at the end of each 5 minute period. Data are presented as mean ± SEM. n = 10.

Figure 3. Plasma glucose and insulin during an OGTT, before and after 8 sessions with moderate/high intensity endurance training. Blood samples were taken before (0), 20, 40, 60, 90 and 120 minutes after consuming 75 gram of glucose. (A) Plasma glucose concentration during the OGTT, before and after the training intervention. n=10 for all time points. For two of the subjects, capillary blood was sampled and measured with Hemocue; data for these two participants are corrected plasma values. (B) Plasma insulin during the OGTT before and after the training intervention. n=8 for all time points. Data are mean ± SEM.

Tables

Table 1. Selected characteristics of participants before and after 3 weeks of training.

Variable	Before	After
Age (years)	54.1 (44-64)	-
Height (cm)	181.1±1.7	
Body mass (kg)	99.3 ± 3.2	98.5 ± 2.9*
BMI (kg•m^{-2})	30.4 (27.1-38.3)	30.1 (27.0-37.7)*
Muscle mass (%)	40.9 ± 1.2	41.1 ± 1.1
Body fat (%)	27.9 ± 2.1	27.5 ± 2.0
VO_{2peak} (ml•kg^{-1}•min^{-1})	38.8 ± 1.8	39.0 ± 1.6

Data are presented as mean ± SEM and median (range). n=10. BMI, body mass index. VO_{2peak}, highest level of oxygen consumption during test of VO_{2maks}. *= $p<0.05$ compared with before the intervention.

Table 2. Selected plasma metabolites before and after 3 weeks of training.

Variable	Before	After	Before vs After P value
Fasting glucose (mM)	5.3 ± 0.3	4.8 ± 0.2*	0.001
Glucose AUC_{0-120}	893.2 ± 74.3	773.5 ± 66.5*	0.001
Glucose AUC_{0-20}	132.8 ± 7.8	119.3 ± 4.3*	0.012
Glucose AUC_{60-120}	421.6 ± 39.8	361.1 ± 37.8*	0.000
Fasting insulin (pM)	61.2 ± 11.6	50.1 ± 7.8	0.247
Insulin AUC_{0-120}	41388 ± 7334	30382 ± 4989*	0.044
Insulin AUC_{0-20}	2660 ± 619.8	3365 ± 744.2	0.376
Insulin AUC_{60-120}	25226 ± 5321.3	14844 ± 1719	0.063
Triglycerides (mM)	1.76 ± 0.27	1.47 ± 0.38	0.131
Total cholesterol (mM)	5.96 ± 0.20	5.48 ± 0.28*	0.029
HDL-cholesterol (mM)	1.24 ± 0.07	1.32 ± 0.08*	0.037
LDL-cholesterol (mM)	3.92 ± 0.16	3.46 ± 0.20*	0.015
HbA_{1c} (%)	5.3 ± 0.2	5.3 ± 0.2	0.591
HOMA-IR	2.0 ± 0.3	1.5 ± 0.2	0.127

Data are presented as mean ± SEM. AUC, area under the curve. HbA_{1c}, glycosylated hemoglobin. HDL-cholesterol, High Density Lipoprotein-cholesterol. LDL-cholesterol, Low Density Lipoprotein-cholesterol. HOMA-IR, the homeostasis model assessment-insulin resistance. n=10 for fasting glucose, fasting insulin, glucose AUC, triglycerides, cholesterol, HbA_{1c} and HOMA-IR. n=8 for insulin AUC. *= $p<0.05$ compared with before the intervention.

Figure 1

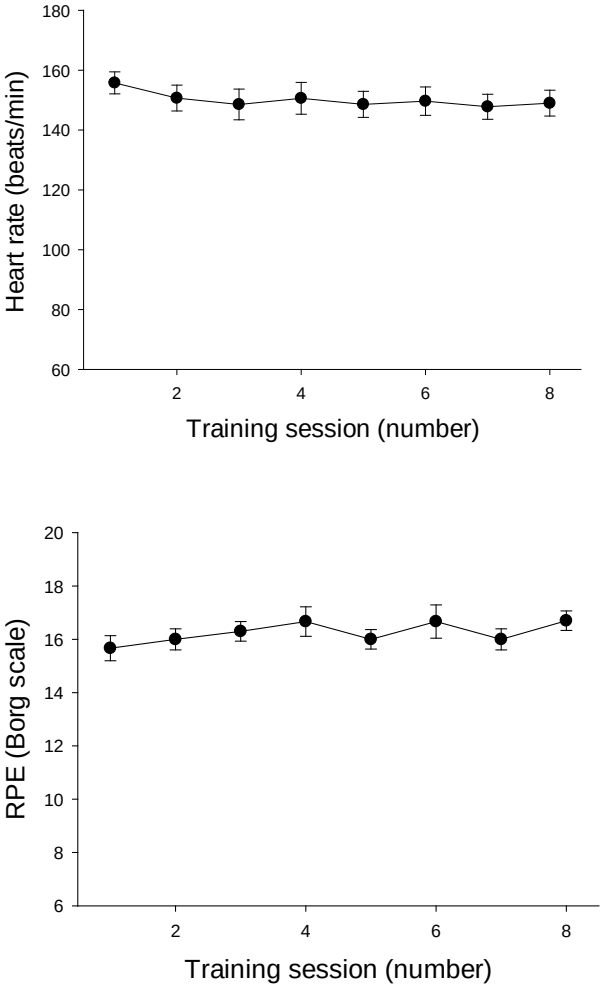

180
160
140
120
100
80
60
Heart rate (beats/min)
2
4
6
8
Training session (number)
20
18
16
14
12
10
8
6
RPE (Borg scale)
2
4
6
8
Training session (number)

Figure 2

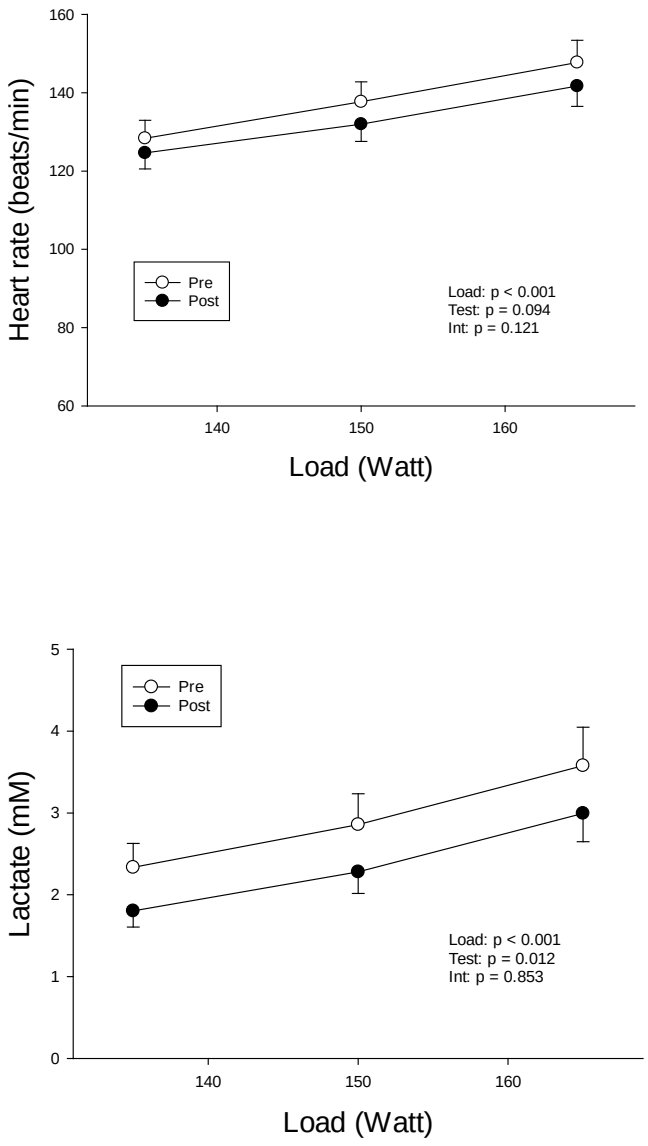
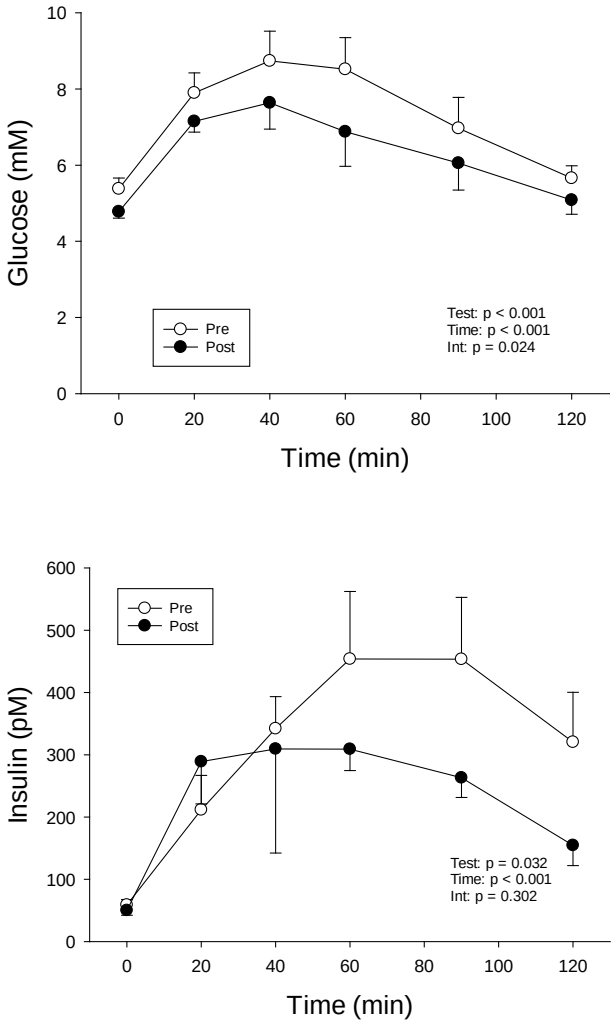

Heart rate (beats/min)
160
140
120
100
80
60
Pre
Post
Load: p < 0.001
Test: p = 0.094
Int: p = 0.121
140
150
160
Load (Watt)
Lactate (mM)
5
4
3
2
1
0
Pre
Post
Load: p < 0.001
Test: p = 0.012
Int: p = 0.853
140
150
160
Load (Watt)

Figure 3


Glucose (mM)
Time (min)
Pre
Post
Test: p < 0.001
Time: p < 0.001
Int: p = 0.024
Insulin (pM)
Time (min)
Pre
Post
Test: p = 0.032
Time: p < 0.001
Int: p = 0.302