Title: EXERCISE INTENSITY, REDOX HOMEOSTASIS AND INFLAMMATION IN TYPE 2 DIABETES MELLITUS

Authors: Alistair R. Mallard, Siri Marte Hollekim-Strand, Jeff S. Coombes, Charlotte B. Ingul

PII: S1440-2440(17)30345-6
DOI: http://dx.doi.org/doi:10.1016/j.jsams.2017.03.014
Reference: JSAMS 1490

To appear in: *Journal of Science and Medicine in Sport*

Received date: 26-4-2016
Revised date: 17-2-2017
Accepted date: 2-3-2017

Please cite this article as: Mallard Alistair R, Hollekim-Strand Siri Marte, Coombes Jeff S, Ingul Charlotte B.EXERCISE INTENSITY, REDOX HOMEOSTASIS AND INFLAMMATION IN TYPE 2 DIABETES MELLITUS.*Journal of Science and Medicine in Sport* http://dx.doi.org/10.1016/j.jsams.2017.03.014

EXERCISE INTENSITY, REDOX HOMEOSTASIS AND INFLAMMATION IN TYPE 2 DIABETES MELLITUS.

Alistair R. Mallard[1], Siri Marte Hollekim-Strand[2], Jeff S. Coombes[1] and Charlotte B. Ingul[2]

Centre for Research on Exercise, Physical Activity and Health, School of Human Movement and Nutrition Sciences, The University of Queensland[1]; K.G. Jebsen Center for Exercise in Medicine - Cardiac Exercise Research Group, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology (NTNU), Trondheim, Norway[2]

Corresponding author:

Professor Jeff Coombes

Centre for Research on Exercise, Physical Activity and Health

School of Human Movement and Nutrition Sciences

University of Queensland

Brisbane, 4072

Australia

Tel: +61 (0)7 3365 6767

Fax: +61 (7) 3365 6877

Email: jcoombes@uq.edu.au

Abstract

Objectives: To compare 12 weeks of exercise training at two intensities on oxidative stress, antioxidants and inflammatory biomarkers in patients with type 2 diabetes (T2D).

Design: Randomized trial

Methods: Thirty-six participants with T2D were randomized to complete either 12 weeks of treadmill based high-intensity interval training (HIIT) or moderate-intensity continuous training (MICT), followed by 40 weeks of home-based training at the same intensities. Plasma inflammation, oxidative stress and antioxidant biomarkers (total F2-isoprostanes, protein carbonyls, total antioxidant capacity, glutathione peroxidase activity, interleukin-10, interleukin-6, interleukin-8 and TNF-α) were measured at baseline, 12-weeks and 1-year.

Results: There were no significant changes ($p>0.05$) in oxidative stress and inflammation biomarkers from baseline to 12-weeks in either intervention. A decrease in total antioxidant capacity in the MICT group from baseline to 1-year by 0.05 mmol/L ($p=0.05$) was observed. There was a significant difference ($p<0.05$) when groups were separated by sex with females in the MICT group having a 22.1% ($p<0.05$) decrease in protein carbonyls from baseline to 1-year.

Conclusions: HIIT and MICT had no acute effect on oxidative stress and inflammatory biomarkers in patients with T2D.

Keywords: High-intensity interval training, Moderate-intensity exercise, F_2-Isoprostanes, Interleukin-6, TNF-α, Protein carbonyls.

Introduction

Oxidative stress has been implicated in the pathogenesis of many diseases due to a significant increase in reactive species (RS).[1] An excess of RS damages cellular proteins, lipids, and DNA and disrupts regular cellular function.[1] Increased basal production of RS biomarkers is associated with T2D,[2] and subsequently T2D is associated with oxidative stress and inflammation.[3]

Chronic exercise training has been shown to decrease oxidative stress in many populations,[4] including individuals with T2D.[5] Exercise improves the cellular antioxidant defence systems ability to counteract increases in RS, which enhances metabolic status and improves insulin sensitivity.[5, 6] However, the intensity of exercise needed to produce the most favourable changes in redox homeostasis, the balance between RS and antioxidants, is still under debate.[7, 8, 9] High-intensity interval training (HIIT) is a time efficient way of increasing cardiorespiratory fitness with double the improvements in VO_2peak in a shorter exercise time.[10] It has been suggested that HIIT may provide greater improvements in redox homeostasis regulation than traditional moderate-intensity continuous training (MICT).[11] Comparing the effects of different exercise intensities on redox regulation is yet to be studied in patients with T2D.

Inflammation plays a major role in the pathogenesis of T2D and shares a cyclical relationship with oxidative stress.[12] Exercise training reduces pro-inflammatory biomarkers,[13] with several studies reporting that moderate intensity exercise decreases inflammation compared to low intensity exercise.[14, 15] However, the effects of higher intensity aerobic exercise on inflammatory biomarkers in patients with T2D has yet to be investigated.

Therefore, the aim of this study was to determine the effects of exercise intensity on redox homeostasis and inflammatory biomarkers in individuals with T2D. We hypothesised that HIIT will elicit a greater reduction in oxidative stress, antioxidant and inflammatory biomarkers than MICT.

Methods

A randomised, two-group, parallel design was used. Thirty-six participants with T2D were allocated to either a HIIT or a MICT group (Table 1). They completed 12 weeks of exercise training at the specified intensity with a combination of supervised and home based training. This was followed by a 40 week home-based training phase at the same intensity. Measurements were taken at baseline, 12-weeks and 1-year. The protocol was approved by the Regional Committee for Medical and Health Research Ethics of Central Norway, the Medical Research Ethics Committee of the University of Queensland and was registered with the Clinical Trials Registry (NCT01206725) in September 2010. Written informed consent was obtained from all participants and they were insured through the intervention period.

Inclusion criteria were; diagnosis of T2D less than ten years ago, non-insulin dependent, aged between 44 and 65 years, diastolic dysfunction (e'<8 cm/s) and achieving less than 210 minutes/week of exercise. Exclusion criteria included overt cardiovascular disease, coronary artery disease, moderate to severe valvular disease, atrial fibrillation, untreated hypertension, congenital heart disease, retinopathy, neuropathy, macroalbuminuria, ejection fraction < 40%, body mass index >35, ischemia at exercise echocardiograph or disease or disability making exercise training difficult. Participants were recruited through local newspaper advertising and from the outpatient population at St. Olav's University Hospital, Trondheim, Norway.

Upon arriving at the laboratory after a 12 hour fasting period, participants height and weight were measured, and a blood sample drawn from an antecubital vein. Informal questioning at the time of screening was performed to determine habitual physical activity time. Participants were asked to avoid strenuous exercise for 24 hours prior to testing.

The exercise testing protocol started with a self –selected 10 minute warm up (3-6 $km.h^{-1}$ at 0-5% incline). After the warm up a facemask was placed on the participants for expired air gas analysis using a metabolic system (Jaeger LE2000CE, Hochberg, Germany). Before measuring VO_2peak, participants were instructed to exercise to their maximum limit. The VO_2peak test was performed using

a ramp protocol where the speed was constant and the incline was increased 2% every two minutes until VO_2peak was reached. Test durations were between 8 – 12 minutes. Heart rate was constantly monitored using a Polar RS 400 (Polar Electro, Kempele, Finland) to obtain maximum heart rate (HRmax). A 12-lead ECG was used to monitor for any sign of silent ischemia during exercise. Post training exercise testing at 12 weeks and 12 months was performed at least 48 hours after the last training session at approximately the same time of day as at baseline. Exercise test supervisors provided verbal encouragement to the participant.

After initial assessment for inclusion into the study, participants were randomised into exercise interventions by the Unit of Applied Clinical Research at the Norwegian University of Science and Technology using a random number generator into either HIIT or MICT. Allocation sequence was blinded to investigators by using an online process that provided the participant's training group one at a time.

The HIIT sessions started with warming-up for 10 minutes at 70% of HRmax before performing 4x4 minute intervals at 90-95% of HRmax, interspersed with 3 minutes active recovery at 70% of HRmax and ending with a 5 minute cool-down period, giving a total of 40-min. HIIT training was performed 3 times per week. The exercise training sessions were supervised by an instructor and there was a doctor nearby in case of adverse reactions during training sessions. During the HIIT sessions, heart rate monitors (Polar RS 400; Polar Electro, Kempele, Finland) were used to ensure that the required exercise intensity was achieved and maintained. The training was performed at the test laboratory, at the Department of Circulation and Medical Imaging, St. Olav's University Hospital, Trondheim, Norway. A defibrillator and emergency medicine (including insulin, glucagon and free glucose) were available in the laboratory.

The MICT sessions were home based exercise sessions equivalent to the current exercise guidelines as set by the Norwegian Directorate of Health. In total, 210 minutes per week of unsupervised moderate intensity (~70% HRmax) exercise was accumulated in bouts that were a minimum of 10 minutes in duration. Participants were instructed verbally to exercise to the level of breathing and

sweating, but still be able to maintain a conversation of small sentences during exercise. Exercise adherence was self-reported and verbal encouragement to maintain the exercise regimen via telephone was performed twice during the intervention. Participants reported perceived effort and duration of exercise in minutes each day for the 12 week intervention.

Once participants had completed the initial 12 week training phase they were instructed to complete 40 weeks of unsupervised home based training. Participants were asked to continue with the same regiment of exercise they performed during the initial intervention. During this 40 week period participants received two phone calls or emails to monitor their exercise adherence.

Ethylenediaminetetraacetic acid vacutainers (Vacuette, Greiner Bio-One, Belgium) were used to collect all blood samples. Samples were immediately stored on ice before being centrifuged at 3000 rpm for 10 minutes at 4°C. Separated plasma was aliquoted and stored at -80°C until biochemical assays were performed. Samples were shipped on dry ice to the University of Queensland, Brisbane, Australia for inflammatory and oxidative biomarker analysis.

Plasma isoprostanes were analysed via gas chromatography mass spectrometry (Varian, Belrose, Australia) as we have previously described in Briskey et al.[16] Briefly, isoprostanes were extracted from plasma after saponification with methanolic NaOH. 8-iso-PGF2α-d4 (Cayman Chemicals, Ann Arbor, MI) was used as an internal standard and added to samples before incubation at 42 °C for 60 min. Samples were acidified to pH 3 with hydrochloric acid and hexane was added before 10 minutes of centrifugation. The supernatant was removed and the remaining solution extracted with ethyl acetate before being dried under nitrogen. Acetonitrile was used to reconstitute samples prior to drying in silanized glass inserts. Derivatization with pentafluorobenzylbromide and diisoproplyethylamine followed. Incubation for 30 minutes at room temperature preceded drying samples under nitrogen. Pyridine, bis(trimethylsilyl)trifluoroacetamide 99% and trimethlychlorosilane 1% were added and incubated at 45 °C for 20 minutes. Finally hexane was added and samples were mixed before analysis. Our laboratory coefficient of variation for this assay is 7%.[16]

Plasma protein carbonyls were analysed using an adaption of the Levine et al.[17] method previously described in Mullins et al.[18] Samples were incubated with 2,4 dinitrophenylhydrazine in 2.5 M HCl for 1 hour in the dark. Plasma black were incubated in 2.5M HCl only. Samples were precipitated with 20% trichloroacetic acid (TCA) on ice and centrifuged at 10000g for 10 minutes. Supernatants were discarded, and the pellets resuspended in 10% TCA and centrifuged as above. Supernatants were removed and the pellets resuspended in 1:1 ethanol:ethylacetate solution. After centrifugation as above, the pellets were washed twice more in ethanol:ethylacetate solution. Pellets were then resuspended in 6 M guanidinehydrocholride solution and 220 µL of samples and blanks were transferred to microplate wells and absorbance read at 370 nm with correction at 650 nm using a microplate reader (Fluostar Optima, BMG Labtech, Offenburg, Germany). PC concentration was normalised to plasma protein content measured using a Pierce BCA protein assay kit (Thermo Scientific, Victoria, Australia). Our laboratory coefficient of variation for this assay is 11.9%.[18]

Plasma glutathione peroxidase activity was measured using an adaptation of the method from Wheeler et al.[19] Glutathione peroxidase activity was measured as the rate of oxidation of nicotinamide adenine dinucleotide phosphate, reduced (NADPH) at 340 nm in a coupled reaction, cycling oxidised glutathione to reduced glutathione using glutathione reductase. The reduced glutathione is utilised by glutathione peroxidase when the reaction is started with the addition of t-butyl hydroperoxide. Our laboratory coefficient of variation for this assay is 2.4%.[18] These measures were performed on an auto analyser (Cobas Mira, Roche Diagnostica, Basel, Switzerland).

Total antioxidant capacity (TAC) was measured using a modified version of an assay preciously described,[20] and adapted for an auto analyser (Cobas Mira, Roche Diagnostica, Switzerland). Plasma was incubated with metmyoglobin and 2,2'-azino-biz(3-ethylbenzothiazoline-6-sulphonic acid). Post incubation hydrogen peroxide was added and the sample was again incubated. Absorbance was measured spetrophotometrically to determine TAC. Our laboratory coefficient of variation for this assay is 1.9%.[18]

Plasma cytokines (interleukins 6, 8, 10 and tumour necrosis factor-alpha) were measured using the Milliplex MAP Human High Sensitivity T Cell Magnetic Bead Panel Kit. The assay protocol was followed as set out by Millipore, and magnetic beads were analysed by a multiplex analyser (Athena Multi-lyte, Zeus Scientific, Raritan, NJ, USA). Our laboratory coefficient of variation for this assay is 12.3%.[21]

All data analyses were completed using SPSS software (version 22 for Windows, SPSS Inc., Chicago, IL). Normality testing of data using the Shapiro-Wilk test was performed. Outliers were identified using the Grubbs test. Data that was not normally distributed were analysed using Wilcoxon signed rank tests and Mann-Whitney U tests. No point measures or change values for redox homeostasis and inflammatory markers were normally distributed. Significance was assumed when $p<0.05$.

Results

Exercise session attendance was 93.7% for the HIIT intervention (supervised sessions) and 94% for MICT (self-reported) during the initial 12 week phase. There were no significant differences ($p>0.05$) between redox homeostasis and inflammatory biomarkers at baseline, 12-weeks or 1-year (Table 2). There were also no significant differences ($p>0.05$) in the change values from baseline to 12-weeks or from baseline to 1-year in any of the biomarkers (Figures A – H). The decrease in total antioxidant capacity from baseline to 1-year in the MICT group of 0.05 mmol/L was close to significant ($p=0.05$).

When groups were separated by sex, females in the MICT group were observed to have a decrease in protein carbonyls from baseline to 1-year ($p=0.04$). Males in the HIIT group had a decrease in protein carbonyls from 12-weeks to 1-year ($p=0.02$). Males in the MICT group had a decrease of total antioxidant capacity from baseline to 12-weeks ($p=0.01$).

Between groups, males in the MICT group had increased protein carbonyls compared to males in the HIIT group by 0.18 U/mg protein ($p=0.03$) at 1-year (Table 2). Females had increased protein

carbonyls in HIIT group by 0.26 U/mg protein (p=0.01) at 1-year than females in the MICT group (Table 2).

It has been proposed that individuals with elevated oxidative stress and/or inflammation may respond differently to exercise interventions therefore we examined groups separated into elevated oxidative stress (EOS) (F_2-isoprostanes > 250 pg/mL) and elevated inflammation (EI) (Interleukin-6 > 3.5 pg/mL). No differences in changes from baseline to 12-weeks or from baseline to 1-year were found between exercise interventions in either EI or EOS. EOS had changes of 29.50 pg/mL (-48.85 – 107.85) and 93.7 pg/mL (-5.62 – 133.80) in F_2-isoprostanes from baseline to 12-weeks in the HIIT and MICT groups, respectively. EI had changes of 1.91 pg/mL (0.75 – 2.89) and 2.57 pg/mL (2.15 – 2.96) in interleukin-6 from baseline to 12-weeks in the HIIT and MICT groups, respectively.

Discussion

This is the first study to compare the effects of HIIT with MICT on markers of oxidative stress and inflammation in patients with T2DM. The main findings were that there were no effects of either training intensity on circulating redox homeostasis or inflammatory biomarkers over the initial 12 week intervention period. We did find a trend (p=0.05) for HIIT maintaining total antioxidant capacity compared to MICT over the 1-year period, suggesting that a 1-year HIIT intervention is associated with maintenance of TAC in individuals with T2D. There were several differences when groups were separated by sex, namely that males in the MICT group had a decrease in TAC from baseline to 12-weeks and females in the HIIT group had increased protein carbonyls at 1-year compared with the MICT group.

Both males and females had alterations in protein carbonyl content. Of interest, these changes were seen in opposing intervention groups suggesting that the exercise interventions may be sex specific

when analysing for protein carbonylation. Previous research into protein oxidation has shown no sex differences after acute bouts of exercise.[22, 23]

It seems that neither HIIT nor MICT were able to improve oxidative stress or inflammatory status in individuals with T2D, which is in contrast to previous studies investigating antioxidant and anti-inflammatory properties of exercise interventions and exercise intensity.[4, 24, 25] In T2D populations, exercise training has been found to act as an antioxidant and anti-inflammatory stimulator/promotor.[26] A review article discusses the various benefits of exercise to inflammatory status in individuals with T2D, namely that regular exercise is associated with lower pro-inflammatory factors (C-reactive protein, IL-6, TNF-α) and higher anti-inflammatory factors (adiponectin, IL-10).[24] In our study we did not see any changes in IL-6, TNF-α or IL-10.

Even though the expected decrease in oxidative stress and inflammation biomarkers was not observed after the exercise interventions, there was also no observable increase in these biomarkers either. It seems that while the exercise interventions were unable to change oxidative stress and inflammation biomarkers in this population, they were able to maintain the biomarker levels whilst also providing a host of physiological improvements which have previously been published.[27]

Our study did not contain a non-exercise control group. If this group was included we may have seen increases in oxidative stress and inflammatory markers over the study period that would have been slowed by the HIIT and MICT. These findings may be somewhat limited, reflected by the small differences between groups, by the modest sample size and the narrow panel of oxidative stress and inflammation biomarkers, which should be improved in further studies. Whilst all appropriate measures were taken to reduce variability during the intervention there may have been outside factors during the 1-year follow up that could have influenced results. Recently there have been studies illuminating the limitations of spot measures of oxidative stress. Spot measures (single time point measure) provide only a snap shot of information which may not accurately represent redox homeostasis or inflammatory status.[18, 28] An assessment of the ability of the antioxidant and anti-inflammatory system to cope with a stress (e.g. exercise) may better evaluate the capacities of each system.[18]

Conclusion

In summary, both MICT and HIIT had no effect on oxidative stress and inflammation levels in this particular study group of individuals with T2D over a 12 week intervention period with a one year follow up. In addition the HIIT intervention was able to maintain TAC in the 12 month follow-up period whereas MICT was not. The inability for both MICT and HIIT to have a positive effect upon inflammation and redox homeostasis biomarkers during the 12 week supervised intervention period is in contrast to the current literature which suggests that exercise in individuals with T2D is beneficial to redox homeostasis and inflammatory health.

Practical implications

- Moderate intensity continuous training and high-intensity interval training have no effect on oxidative stress and inflammation in patients with type 2 diabetes
- High-intensity interval training is effective at maintaining total antioxidant capacity over a 12 month period, above that of moderate intensity continuous training
- Females who completed moderate intensity continuous training for 12 months had a greater decrease in protein oxidation, above that of high intensity interval training.

Declaration of interest

This study was funded by the Liaison Committee between the Central Norway Regional Health Authority (Stjørdal, Norway) and the Norwegian University of Science and Technology (Trondheim, Norway) and K. G. Jebsen Foundation for Medical Research, Center for Exercise in Medicine, at Norwegian University of Science and Technology (Trondheim, Norway). The funders had no role in the design and conduct of the study. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Acknowledgements

The authors acknowledge the work conducted by the Norwegian research team (Marit R. Bjørgaas, Grethe Albrektsen, Arnt E. Tjønna, Ulrik Wisløff) in collecting and handling all samples prior to analysis.

References

1. Valko, M., D. Leibfritz, J. Moncol, et al., Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*, 2007. **39**(1): p. 44-84.
2. Evans, J.L., I.D. Goldfine, B.A. Maddux, et al., Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocrine reviews*, 2002. **23**(5): p. 599-622.
3. Wright, E., J.L. Scism-Bacon, and L.C. Glass, Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. *Int J Clin Prac*, 2006. **60**(3): p. 308-314.
4. Bogdanis, G.C., P. Stavrinou, I.G. Fatouros, et al., Short-term high-intensity interval exercise training attenuates oxidative stress responses and improves antioxidant status in healthy humans. *Food Chem Toxicol*, 2013. **61**: p. 171-7.
5. Krause, M., J. Rodrigues-Krause, C. O'Hagan, et al., The effects of aerobic exercise training at two different intensities in obesity and type 2 diabetes: implications for oxidative stress, low-grade inflammation and nitric oxide production. *Eur J Appl Physiol*, 2014. **114**(2): p. 251-60.
6. Tjønna, A.E., S.J. Lee, Ø. Rognmo, et al., Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation*, 2008. **118**(4): p. 346-354.

7. Parker, L., T.A. McGuckin, and A.S. Leicht, Influence of exercise intensity on systemic oxidative stress and antioxidant capacity. *Clin Physiol Funct Imaging*, 2014. **34**(5): p. 377-83.
8. Bouzid, M.A., O. Hammouda, R. Matran, et al., Low intensity aerobic exercise and oxidative stress markers in older adults. *J Aging Phys Act*, 2014. **22**(4): p. 536-42.
9. Vezzoli, A., L. Pugliese, M. Marzorati, et al., Time-course changes of oxidative stress response to high-intensity discontinuous training versus moderate-intensity continuous training in masters runners. *PLoS One*, 2014. **9**(1): p. e87506.
10. Weston, K.S., U. Wisloff, and J.S. Coombes, High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br J Sports Med*, 2014. **48**(16): p. 1227-34.
11. Gabriel, B., A. Ratkevicius, P. Gray, et al., High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. *Clin Sci (Lond)*, 2012. **123**(5): p. 313-21.
12. Tucker, P.S., A.T. Scanlan, and V.J. Dalbo, Chronic kidney disease influences multiple systems: Describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. *Oxid Med Cell Longev*, 2015. **2015**: p. 806358.
13. Nimmo, M.A., M. Leggate, J.L. Viana, et al., The effect of physical activity on mediators of inflammation. *Diabetes Obes Metab*, 2013. **15 Suppl 3**: p. 51-60.
14. Abd El-Kader, S., A. Gari, and A. Salah El-Den, Impact of moderate versus mild aerobic exercise training on inflammatory cytokines in obese type 2 diabetic patients: a randomized clinical trial. *Afr Health Sci*, 2013. **13**(4): p. 857-63.
15. Balducci, S., S. Zanuso, A. Nicolucci, et al., Anti-inflammatory effect of exercise training in subjects with type 2 diabetes and the metabolic syndrome is dependent on exercise modalities and independent of weight loss. *Nutr Metab Cardiovasc Dis*, 2010. **20**(8): p. 608-17.
16. Briskey, D.R., G.R. Wilson, R.G. Fassett, et al., Optimized method for quantification of total F2-isoprostanes using gas chromatography–tandem mass spectrometry. *J Pharm Biomed Anal*, 2014. **90**(0): p. 161-166.

17. Levine, R.L., D. Garland, C.N. Oliver, et al., Determination of carbonyl content in oxidatively modified proteins, in *Methods in Enzymology*, A.N.G. Lester Packer. 1990, Academic Press. p. 464-478.

18. Mullins, A.L., S.P. van Rosendal, D.R. Briskey, et al., Variability in oxidative stress biomarkers following a maximal exercise test. *Biomarkers*, 2013. **18**(5): p. 446-54.

19. Wheeler, C.R., J.A. Salzman, N.M. Elsayed, et al., Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Anal Biochem*, 1990. **184**(2): p. 193-9.

20. Rice-Evans, C. and N.J. Miller, Total antioxidant status in plasma and body fluids. *Methods Enzymol*, 1994. **234**: p. 279-93.

21. Shing, C.M., J.M. Peake, C.L. Lim, et al., Effects of probiotics supplementation on gastrointestinal permeability, inflammation and exercise performance in the heat. *Eur J Appl Physiol*, 2014. **114**(1): p. 93-103.

22. Goldfarb, A.H., M.J. McKenzie, and R.J. Bloomer, Gender comparisons of exercise-induced oxidative stress: influence of antioxidant supplementation. *Appl Physiol Nutr Metab*, 2007. **32**(6): p. 1124-31.

23. Bloomer, R.J., P.G. Davis, L.A. Consitt, et al., Plasma protein carbonyl response to increasing exercise duration in aerobically trained men and women. *Int J Sports Med*, 2007. **28**(1): p. 21-5.

24. de Lemos, E.T., J. Oliveira, J.P. Pinheiro, et al., Regular physical exercise as a strategy to improve antioxidant and anti-inflammatory status: benefits in type 2 diabetes mellitus. *Oxid Med Cell Longev*, 2012. **2012**: p. 741545.

25. Tucker, P.S., K. Fisher-Wellman, and R.J. Bloomer, Can exercise minimize postprandial oxidative stress in patients with type 2 diabetes? *Curr Diabetes Rev*, 2008. **4**(4): p. 309-19.

26. Teixeira de Lemos, E., R. Pinto, J. Oliveira, et al., Differential effects of acute (extenuating) and chronic (training) exercise on inflammation and oxidative stress status in an animal model of type 2 diabetes mellitus. *Mediators Inflamm*, 2011. **2011**: p. 253061.

27. Hollekim-Strand, S.M., M.R. Bjørgaas, G. Albrektsen, et al., High-Intensity Interval Exercise Effectively Improves Cardiac Function in Patients With Type 2 Diabetes Mellitus and Diastolic Dysfunction: A Randomized Controlled Trial. *J Am Coll Cardiol*, 2014. **64**(16): p. 1758-1760.

28. Nikolaidis, M.G., A. Kyparos, K. Dipla, et al., Exercise as a model to study redox homeostasis in blood: the effect of protocol and sampling point. *Biomarkers*, 2012. **17**(1): p. 28-35.

Figure legend.

Figure 1. Changes in oxidative stress and inflammatory biomarkers from baseline to 12-weeks, and from baseline to 1-year in HIIT and MICT groups. Data are presented as median and interquartile range. HIIT group denoted as X, MICT group denoted as ■.

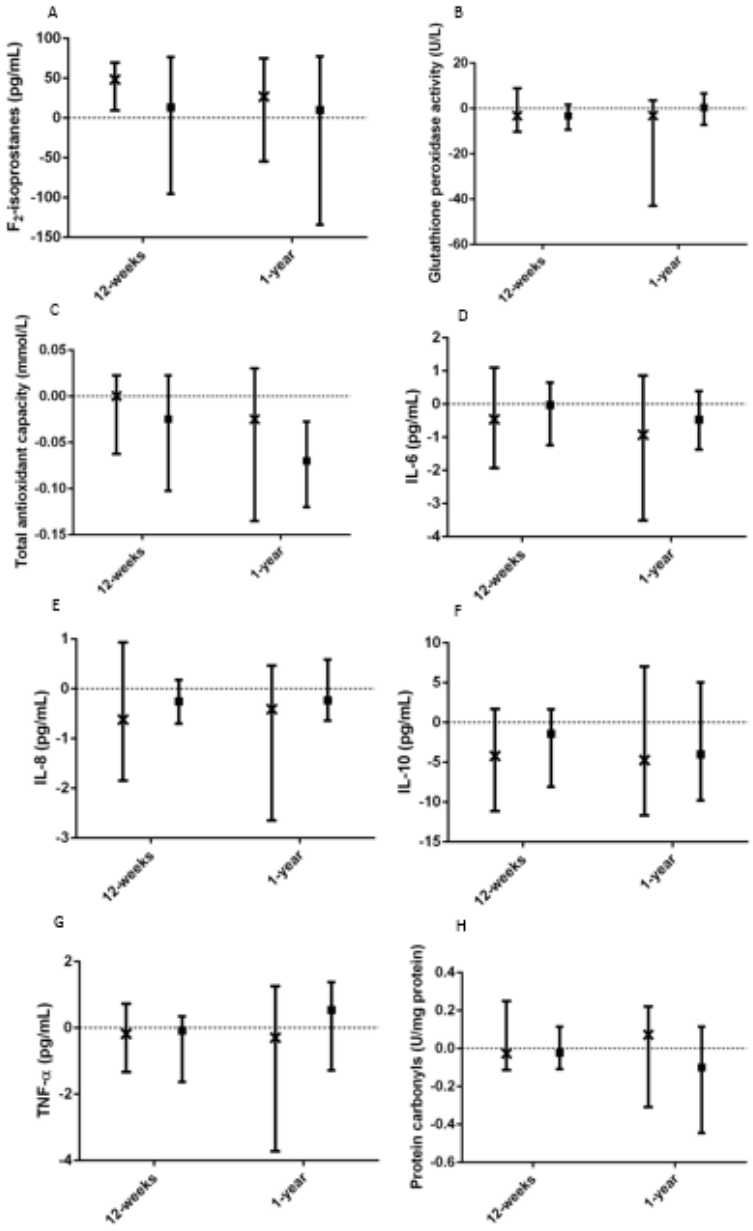

Table 1. Participant characteristics

	MICT (n=16)	HIIT (n=20)	P Value
Female, n (%)	6 (37.5)	8 (40.0)	0.88
Age (years)	54.9 ± 5.3	58.6 ± 5.0	0.04
T2D duration (years)	3.2 ± 2.6	4.3 ± 2.2	0.20
BMI (kg/m^2)	29.6 ± 3.6	30.2 ± 2.7	0.62
VO_2peak (ml/kg/min)	31.5 ± 6.0	33.2 ± 7.4	0.46
Smoking status, n (%)	0 (0)	1 (5)	0.38
Waist circumference (cm)	106.09 ± 8.79	108.60 ± 7.70	0.37
HbA1c (%)	6.65 ± 0.66	7.04 ± 1.19	0.26
Triglycerides (mmol/l)	1.88 ± 0.78	1.81 ± 0.79	0.77
HDL (mmol/l)	1.20 ± 0.29	1.30 ± 0.43	0.40
LDL (mmo/l)	3.21 ± 0.91	3.12 ± 0.89	0.77
Cholesterol (mmol/l)	5.25 ± 0.93	5.24 ± 1.03	0.96
HOMA-IR	2.89 ± 1.61	2.68 ± 0.67	0.61
Statin use	3 (18.75)	4 (20)	0.16
Antidiabetic medication	11 (68.75)	17 (85)	0.42
Antihypertension medication	7 (43.75)	6 (30)	0.39
Antiplatelet medication	7 (43.75)	4 (20)	0.99

Data are categorized in n (%) or mean ± SD. T2D, type 2 diabetes mellitus; BMI, body mass index; HDL, High-denisty lipoprotein cholesterol; LDL, Low-density lipoprotein cholesterol; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment – insulin resistance.

Table 2. *Oxidative stress, antioxidant, and inflammatory markers at baseline, 12-weeks, and 1-year*

		HIIT			MICT		
		Baseline	12-weeks	1-year	Baseline	12-weeks	1-year
F_2-isoprostanes (pg/mL)	All	170.0 (137.0-208.1)	199.3 (155.2-217.9)	201.4 (164.9-250.8)	223.6 (152.3-264.2)	202.5 (164.5-270.8)	227.3 (194.5-271.1)
	Males	175.1 (120.8-206.0)	183.0 (147.2-217.9)	194.6 (146.0-204.1)	223.6 (153.4-282.4)	193.9 (144.6-207.6)	194.6 (163.5-255.2)
	Females	169.5 (145.5-199.9)	201.1 (185.5-224.3)	251.8 (221.7-272.7)	218.4 (162.6-257.1)	275.8 (197.7-356.6)	240.6 (237.5-271.1)
Glutathione peroxidase activity (U/L)	All	96.9 (90.3-104.7)	96.3 (88.4-99.5)	94.8 (84.1-101.2)	96.5 (93.0-103.0)	94.6 (91.8-101.6)	100.0. (96.5-101.8)
	Males	99.9 (92.6-106.1)	96.3 (87.0-97.9)	95.4 (86.6-101.2)	97.3 (92.9-103.4)	96.7 (93.1-102.2)	100.1 (97.8-102.0)
	Females	93.8 (90.2-101.0)	96.3 (90.8-103.3)	94.1 (84.1-99.9)	96.5 (93.7-99.9)	92.9 (89.9-95.0)	99.0 (96.5-101.3)
Total antioxidant capacity (mmol/L)	All	1.71 (1.65-1.75)	1.72 (1.66-1.75)	1.71 (1.67-1.74)	1.76 (1.67-1.77)	1.69 (1.67-1.73)	1.71 (1.66-1.74)
	Males	1.74 (1.69-1.76)	1.74 (1.66-1.76)	1.71 (1.67-1.76)	1.76 (1.70-1.77)	1.69 (1.66-1.71)*	1.69 (1.63-1.70)
	Females	1.70 (1.62-1.72)	1.67 (1.65-1.72)	1.69 (1.67-1.71)	1.73 (1.63-1.84)	1.72 (1.67-1.76)	1.74 (1.72-1.75)
Protein carbonyls (U/mg protein)	All	0.54 (0.44-0.61)	0.54 (0.39-0.57)	0.46 (0.39-0.68)	0.48 (0.43-0.55)	0.44 (0.38-0.66)	0.54 (0.48-0.63)
	Males	0.61 (0.50-0.67)	0.54 (0.49-0.71)	0.40 (0.35-0.48)*†	0.48 (0.44-0.50)	0.43 (0.39-0.64)	0.58 (0.53-0.65)†
	Females	0.52 (0.44-0.54)	0.35 (0.29-0.55)	0.73 (0.69-0.80)†	0.54 (0.43-0.62)	0.46 (0.39-0.68)	0.47 (0.21-0.62)*†
Interleukin-10 (pg/mL)	All	12.2 (6.56-30.8)	13.0 (5.70-24.5)	18.3 (6.60-28.5)	12.4 (6.46-20.2)	7.77 (0.33-16.2)	14.0 (5.76-16.8)
	Males	23.2 (7.24-32.6)	14.2 (7.27-22.9)	19.3 (6.55-27.9)	16.1 (10.0-27.0)	10.4 (0.46-16.5)	14.5 (7.16-17.7)
	Females	10.5 (5.45-13.3)	11.5 (2.65-26.5)	17.8 (9.55-28.7)	7.67 (5.81-11.2)	7.46 (1.55-11.4)	10.8 (4.70-16.4)
Interleukin-6 (pg/mL)	All	2.42 (0.76-4.49)	1.79 (1.00-3.48)	2.23 (1.07-4.10)	1.94 (1.30-3.10)	1.79 (0.75-2.97)	1.98 (1.20-3.51)
	Males	2.75 (0.76-4.97)	2.12 (1.11-4.08)	2.1 (0.86-3.21)	2.21 (0.90-3.76)	1.05 (0.24-2.27)	2.58 (1.26-3.86)
	Females	2.42 (1.32-3.52)	1.4 (0.68-2.87)	3.92 (1.78-4.65)	1.89 (1.46-2.16)	2.40 (1.75-3.04)	1.46 (1.20-2.49)
Interleukin-8 (pg/mL)	All	2.25 (1.30-3.90)	1.91 (1.38-3.16)	2.29 (1.25-3.52)	1.95 (1.48-2.20)	1.63 (0.91-2.41)	2.04 (1.35-2.98)
	Males	3.32 (1.31-4.34)	2.05 (1.66-3.16)	2.05 (1.50-3.64)	1.90 (1.47-2.64)	1.35 (0.14-2.53)	2.20 (1.35-3.07)
	Females	2.04 (1.47-2.65)	1.67 (0.50-2.70)	2.53 (1.24-2.88)	1.95 (1.67-2.00)	1.97 (1.40-2.24)	2.04 (1.44-2.66)
TNF-α (pg/mL)	All	2.67 (1.57-4.27)	1.72 (0.79-4.39)	2.68 (1.34-4.64)	2.83 (1.54-4.19)	2.13 (0.24-3.39)	3.80 (1.50-4.50)
	Males	2.79 (1.76-5.88)	2.44 (1.30-4.87)	2.63 (1.27-3.77)	3.10 (1.83-4.54)	1.82 (0.22-3.66)	3.80 (2.58-4.20)
	Females	2.18 (1.45-3.72)	1.21 (0.52-4.22)	4.61 (1.42-4.84)	2.32 (1.49-3.21)	2.13 (1.13-2.41)	3.42 (1.50-4.50)

Data are presented as median and interquartile range. * denotes significantly different from 12-weeks within group ($p<0.05$), † denotes significantly different between sex ($p<0.05$).