ESM Methods

Participants and study design Ninety-nine individuals diagnosed with MetS according to the International Diabetes Federation criteria, were recruited (January 2013 to August 2015). This is a sub-study of the ‘Exercise in prevention of Metabolic Syndrome (EX-MET) multi-center trial. The present sub-study reports data collected exclusively at the Brisbane site where participants were also requested to undertake OGTT. The fasting intact proinsulin concentration and other pancreatic beta cell function indices, and parameters derived from OGTT are the endpoints specific to the present study. All other outcome measures reported in the current study are background information/endpoints that are either part of the EX-MET trial (MetS risk factors, CRF, and HOMA-IR), or have been published previously (body composition) [1]. Fig. 1 presents a consort diagram of participant flow throughout this sub-study. Sample size was calculated using an anticipated mean difference in intact proinsulin concentration reduction of 0.62 (power=0.80, alpha=0.05 for 2-tailed test) between the HIIT and MICT groups. This was based on 1) a previous meta-analysis showing a similar mean difference in reduction of IR between HIIT and MICT in MetS or type 2 diabetes [2] and 2) that the continuous demand for insulin in the face of IR, negatively affects the quality of insulin processed by the pancreatic beta cells [3].

Participants were recruited through several avenues: i) referrals from medical practitioners at the Princess Alexandra Hospital; ii) advertising through posters, newspapers, television news and flyers placed around the University and local health care centers; and iii) a website was created to serve as a recruitment link for social platforms and the University’s online magazine. Exclusion criteria were: unstable angina, recent myocardial infarction (last four weeks), severe valvular heart disease, uncompensated heart failure, pulmonary disease, uncontrolled hypertension, kidney failure, and cardiomyopathy. Participants provided written and oral consent to participate in the study before inclusion and were randomized into the
following exercise groups (stratified by age and sex): i) MICT (n=30); ii) 4HIIT (n=29); iii) 1HIIT (n=28). Lead investigators of this multi-center trial from the Norwegian University of Science and Technology performed the randomization procedure via randomisation software employing random permuted blocks. Details of participants eligible were entered into an online system to obtain group allocation.

Participants underwent several tests at the university’s laboratory (Human Movement and Nutrition Sciences Building, St Lucia Campus, The University of Queensland, QLD, Australia) before and after the 16-week interventions to measure the following: (1) MetS risk factors and body composition; (2) fasting intact proinsulin concentration indices; (3) CRF; (4) insulin sensitivity; and (5) other beta cell function indices. All tests were performed at approximately the same time of the day (morning, ±2 hours), with participants instructed to refrain from strenuous activities for at least 48 hours, and caffeine and alcohol for at least 24 hours before each examination. Post-intervention measures were obtained at 2-3 days after the last exercise session. Medication dosages were monitored during the study. Participants were instructed to take medications at times prescribed by their medical doctor (am/pm) to ensure they were taken at approximately the same time during pre- and post-intervention testing. In participants taking metformin medication (n=19), 47% consumed this drug in the morning of both testing time-points. The Medical Research Ethics Committee, University of Queensland, Brisbane, Australia approved this study.

**MetS risk factors** The following assessments were conducted at a 12-hour fasted state to determine the participants’ eligibility for the study: (1) fasting lipid profile and glucose level; (2) brachial systolic blood pressure (SBP) and diastolic (DBP) blood pressure; and (3) anthropometric measures (weight, height, waist circumference [WC], and hip circumference [HC]). In-depth details of these assessments have been reported previously [4]. After the 16-
week training interventions, a tester blinded to the training group allocation performed the subsequent series of tests.

**Proinsulin, insulin sensitivity, and other beta cell function measures** Serum and plasma samples were obtained from blood collected from the participants’ antecubital vein following a 12 h overnight fast. Serum samples were derived from whole blood collected into tubes without anticoagulants. These tubes were left at room temperature for 30 minutes to allow blood to clot before centrifugation. Plasma samples were obtained by collecting whole blood into tubes with anticoagulants and were placed immediately into ice following the blood draw. Both tubes were centrifuged at 2500rpm for 10 minutes at 4°C for serum/plasma separation. Aliquots were then stored at -80°C. Plasma samples were used for later analysis of fasting glucose concentration (Rx Daytona Plus, Randox Laboratories, Crumlin, County Antrim, UK), whilst serum samples were used for the analysis of fasting intact proinsulin concentration (human intact proinsulin ELISA, EZHIPI-17K, Merck Millipore, Darmstadt, Germany), C-peptide, and insulin concentrations (electrochemiluminescence immunoassay [ECLIA], Cobas e411 immunoassay analyzer, Roche Diagnostics, Indianapolis, IN, USA). HbA1c was also measured from a whole blood sample (Rx Daytona Plus, Randox Laboratories Ltd, UK). All parameters were measured in duplicate and if the co-efficient of variation (%) between repeated measures were more than the accepted range for each parameter, analyses were repeated from the same sample. The coefficient of variations (%) for each parameter at each time-point were as follows: i) fasting glucose (Pre=1.29%; post=0.6%); ii) fasting insulin (pre=2.4%; post=2.2%); iii) C-peptide (pre=2.0%; post=1.5%); iv) intact proinsulin concentration (pre=5.7%; post=4.9%); and v) HbA1c (pre=2.0%; post=1.8%). A value of 0.1pM was assigned for statistical analysis if the intact proinsulin concentration was below the detection limit of the assay (0.5-100pM). Ratios of fasting intact
proinsulin concentration to insulin or C-peptide concentration (proinsulin:insulin and proinsulin:C-peptide) were calculated.

HOMA-IR and HOMA of beta cell function (HOMA-B) were used to determine IR and insulin secretion at a steady state, calculated via the HOMA2 calculator version 2.2 [5]. The basal disposition index (basal DI=HOMA-B/HOMA-IR) was then calculated to determine beta cell function at a steady state condition, given that the amount of insulin secreted is highly dependent upon the prevailing IR magnitude.

A 2 h OGTT was also performed in a subgroup of participants (n=32) to determine IR and beta cell function in a dynamic condition. Following collection of a fasted sample, participants consumed 75 grams of sugar dissolved within a factory produced 300mL drink (Fronine Carbotest, Thermo Fisher Scientific Australia Pty Ltd). Small blood samples (~4mL) were then collected at four time points every 30 minutes up to 2-hours (30, 60, 90, and 120 minutes). Whole blood derived from each time point was treated as described above to derive plasma samples that were later analyzed for insulin and glucose concentrations. The trapezoidal method was used to determine insulin and glucose AUC [6]. The Matsuda index (M_index) was used to assess insulin sensitivity at a dynamic state [7]. The ratio of insulin AUC to glucose AUC during the first 30 (I_{0-30}/G_{0-30}) and last 60 minutes (I_{60-120}/G_{60-120}) was determined to represent first- and second-phase glucose-stimulated insulin secretion (GSIS), respectively [8]. First- and second-phase oral DI was then calculated as the product of first/second-phase GSIS and insulin sensitivity (I_{0-30}/G_{0-30} or I_{60-120}/G_{60-120} \times M_{index}).

Cardiorespiratory fitness CRF depicted as the peak oxygen uptake (\dot{V}O_{2peak}) was measured via indirect calorimetry using the Metamax II system (Cortex, Leipzig, Germany) or Parvo Medics TrueOne 2400 system (Parvomedics Inc., Sandy, UT, USA) during a graded maximal exercise test. \dot{V}O_{2peak} was calculated as the highest 15-sec time averaged \dot{V}O_2 A treadmill or
cycle ergometer was used during the test, according to the participant’s orthopedic limitations or preferred training method during the supervised exercise sessions. A liquid nutritional supplement (Sustagen, 250mL, Dutch Chocolate, Nestle, Gympie QLD, Australia) was consumed two hours before the test to standardize nutrition. An 8-minute warm-up consisting of 2 stages (stage 1 warm-up: 4km/h at 0% incline or 50-60rpm at 0W; stage 2 warm-up: 4km/h at 4% incline or 50-60rpm at 25W) preceded all tests to familiarize participants to the test protocol. The maximal exercise test was then initiated at a workload slightly harder than the warm-up stages (treadmill speed individualized at 4-6km/h or cycling at 60-70rpm; load: ~6% incline or 50W). Thereafter, the speed (individualized: within 6-9km/h) and load (2% incline or 25W) increased each minute until exhaustion. To help the participants reach maximal effort, standardized verbal cues were provided throughout the duration of the exercise test. A tester blinded to the training group allocation provided these motivational cues at post-training intervention.

**Body composition** Body composition (total body fat percentage [BF%], trunk fat percentage [TF%], and fat-free mass [FFM, in kg]) was assessed at baseline and after the 16 week training programme via dual-energy x-ray absorptiometry (Hologic Discovery W Apex software version 3.3; Hologic, Bedford, MA, USA). The percentage of fat mass from total mass was deemed as the total BF%. TF% was calculated as the percentage of fat mass from total trunk mass. A 3 day food diary was also administered before and after the training program to determine total energy intake. Data was analysed using diet analysis software (FoodWorks 8 Professional, Xyris Software, Australia).

**Training protocol** The HIIT groups trained three times per week, whilst the MICT group trained five times per week. An Accredited Exercise Physiologist supervised two out of the three prescribed weekly sessions at the University of Queensland exercise laboratory. The residual sessions were completed in an unsupervised environment (i.e. home). Participants
performed the supervised sessions either on a treadmill or cycle ergometer, depending on
their orthopedic limitations or preference. Unsupervised sessions included outdoor/indoor
exercises involving large muscle groups (walking, running, swimming, and rowing). Each
MICT was 30 minutes in duration at a continuous pace and target intensity of 60-70%
HRpeak or rate of perceived exertion (RPE) of 11-13 on the Borg scale (Fig. 2a). The 4HIIT
group trained for 38 minutes per session (Fig. 2b) whilst each session of the 1HIIT protocol
was only about half of this duration (17 minutes) (Fig. 2c). HIIT sessions were preceded by a
10-minute warm-up and terminated with a 3-minute cool-down at 60-70%HRpeak (Fig. 2b
and 2c). The 1HIIT protocol consisted of only one 4-minute interval at 85-95%HRpeak/15-
17RPE. The 4HIIT included 4 bouts of 4-minute intervals at 85-95%HRpeak/15-17RPE,
separated by 3 minutes of recovery at 50-70%HRpeak. The target HR for each 4-minute
interval was required to be reached within 2 minutes. Both HR and RPE were monitored
using a HR monitor (Polar electro, Kempele, Finland) and the Borg 6-20 scale, respectively
[9]. Training logs were provided to all participants to monitor adherence and target exercise
intensity (HR and RPE) during both supervised and unsupervised exercise sessions.
Participants were trained to fill out the training logs during the supervised sessions to ensure
accurate reporting of unsupervised training sessions. The metabolic equivalent (MET) of the
average workload throughout the intervention was calculated [10], and used to calculate
energy expended per minute based on the following assumptions: 1MET=3.5ml kg\(^{-1}\) min\(^{-1}\)
and 1 l of O\(_2\) = 20.92 kJ (5 kcal). Total kcal was calculated as the product of kcal/session and
the number of sessions completed during the 16-week training program. Incidental moderate
to vigorous physical activity (MVPA) time (mins/day) was also measured before and after the
exercise program using the activPAL device (Version 3, Pal Technologies Ltd, Glasgow,
UK) as described previously [11, 12].
Statistical analysis  The SPSS version 22 software package (IBM, New York, NY, USA) was used to analyse all data. To determine the suitability of parametric tests, the assumption of normality was tested via Shapiro-Wilk test. Log transformation of the data was applied when the assumption of normality was violated. A non-parametric test equivalent was then used when the assumption of normality was still violated after log transformation of the data. One-way ANOVA or Kruskal-Wallis, and $\chi^2$ tests were used to compare baseline values, as well as training adherence between intervention groups (MICT, 4HIIT, and 1HIIT). Within-group differences in continuous variables were analysed via a paired $t$ test or Wilcoxon test. Significant within-group changes in intact proinsulin concentration indices over time were further assessed via mixed linear model regression analysis to determine whether the changes in these variables were associated with factors contributing to a glucolipotoxic environment (glucose, lipid profile, insulin resistance, body fat indices, and CRF).

Group × time interaction effects were examined via ANCOVA, with the difference/$\Delta$-value assigned as the dependent variable, whilst the baseline value was entered as the covariate. A Bonferroni post hoc test was then employed to identify significant differences between the intervention groups, where appropriate. $\eta^2$ group × time interaction effect sizes were calculated as the between-group sum of squares divided by the total sum of squares and interpreted as follows: ‘small’ effect (0.01); ‘small-to-medium’ effect (0.01-0.10); ‘medium-to-large’ (0.10-0.25) [13]. The McNemar’s test was used to determine whether there was a significant difference between the number of participants diagnosed with MetS according to the IDF criteria from pre to post intervention. Significance level was set at $p<0.05$.

Continuous and categorical variables are reported as mean ± standard deviation (SD) and frequencies, respectively.
References

[1] Ramos JS, Dalleck LC, Ramos MV, et al. (2016) Twelve minutes/week of high-intensity exercise significantly decreases aortic reservoir pressure in individuals with metabolic syndrome. Accepted in Journal of Hypertension, 10 June 2016


