COMMENTARY

It has been well established that physical exercise is an effective therapy to reverse metabolic disorders associated with overweight/obesity. However, there is a lack of information about which exercise intensity might be more suitable. Dr Thaane's PhD study examined the "Effects of high or moderate intensity training in overweight/obese adults with insulin resistance. Study participants completed 10 supervised exercise sessions of high intensity interval training (HIIT) or continuous moderate intensity training (CMIT) at the Human Performance Laboratory (HPL) in the University of KwaZulu-Natal (UKZN). Her study findings showed a greater reduction in insulin resistance among the participants who were assigned to HIIT training compared to those assigned to CMIT training (31.95% vs. 9.40%). She also observed the improvements occurred without changes in aerobic fitness and/or body fat percentage, factors which have been found to independently improve insulin resistance. Furthermore, Cohen's (*d*) effect sizes (ES) indicated that the improvements in insulin resistance produced by HIIT training were large, while those of CMIT training were unclear. Dr Thaane, therefore, concluded that HIIT training is associated with superior improvements in insulin resistance when compared with CMIT training and recommended the use of HIIT training as an alternative therapeutic approach when the primary goal is to improve insulin resistance in overweight/obese adults.

EFFECTS OF HIGH OR MODERATE INTENSITY TRAINING ON INFLAMMATION AND ENDOTHELIAL FUNCTION IN INSULIN RESISTANCE

BY

TSHIDI THAANE (212515377)

SUPERVISOR: PROFESSOR ANDREW J. McKUNE
CO-SUPERVISOR: PROFESSOR AYESHA A. MOTALA

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UNIVERSITY OF KWAZULU-NATAL

2019

DECLARATION

I, Tshidi Thaane, declare that

(i) The research reported in this thesis, except where otherwise indicated is my original research.

(ii) This thesis has not been submitted for any degree or examination at any other university.

(iii) This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) Their words have been re-written, but the general information attributed to them has been referenced;

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Signed:  14 August 2019

DEDICATION

I dedicate this thesis to my late father Thaane J. Thaane and my mother Mathabiso B. Thaane
For your love and patience throughout my studies and forever
Ke a leboha Bakoena

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To the Almighty God who granted me the opportunity, strength and wisdom to accomplish this piece of work in my lifetime, I give all the Glory.

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ACRONYMS

ADA	American Diabetes Association
ACSM	American College of Sport Medicine
CMIT	Continuous Moderate Intensity Training
CRP	C-Reactive Protein
ELISA	Enzyme-Linked Immunosorbent Assay
GLUT	Glucose Transporter
HIIT	High Intensity Interval Training
HOMA	Homeostatic Model Assessment
IDF	International Diabetes Federation
IR	Insulin Resistance
IS	Insulin Sensitive
RH	Relative Humidity
UKZN	University of Kwazulu-Natal
$\dot{V}O_2max$	Maximal Oxygen Consumption
WHO	World Health Organization

ABSTRACT

Exercise training improves insulin resistance (IR) via numerous mechanisms including improved endothelial function and reduced systemic inflammation. However, it is unclear whether high intensity interval training (HIIT) offers superior benefit when compared with continuous moderate intensity training (CMIT) in a clinical population. This thesis reports findings from a clinical trial designed to determine the effects of short-term high or moderate intensity training on inflammation and endothelial function in overweight/obese adults with IR. Furthermore, the thesis provides a report on factors associated with IR in this population. Clinical examinations (body composition), blood tests [serum insulin and serum C-reactive protein (CRP), plasma glucose] and physiological tests (microvascular function and aerobic fitness tests) were undertaken on consenting individuals who volunteered to participate in the study. Study participants were stratified into IR and insulin sensitive (IS) groups based on their homeostatic model assessment (HOMA-IR) scores. The IR and IS groups were each further randomized into control (CNT), HIIT or CMIT sub-groups. The HIIT and CMIT sub-groups underwent baseline measures and exercise training for 10 consecutive days, followed by a repeat of baseline measures. The CNT sub-group was tested at baseline and post follow-up without taking part in the exercise intervention. When compared with CMIT, HIIT produced a greater improvement in markers of IR [(HOMA-IR); 32% vs. 9% by CMIT] and endothelial function [(PORHmax and MV); 37.59% and 27.45%, respectively, vs. 8.24% and -25.71%, respectively, by CMIT]. These improvements occurred without changes in body fat or aerobic fitness. Cohen's (*d*) effect sizes indicated that the improvements produced by HIIT were large, while those of CMIT were unclear, suggesting that HIIT may be the ideal mode of training when the primary goal is to improve IR and endothelial function in overweight/obese adults. Cross-sectional analysis of baseline data indicated that the acute-phase inflammatory protein, CRP, was strongly associated with IR in black Africans. However, this association was only significant in males. Suggesting that CRP has potential clinical application as a maker of systemic inflammation in this population. The gender of the patient, however, must be taken into consideration.

KEYWORDS: Africa, exercise training, peripheral vascular disease, physical activity, reactive hyperaemia

ABSTRACT (IsiZulu)

Ukuzivocavoca kuyasiza ekusebenzeni kwe-insulin ngezindlela eziningi okubalwakuzo, ukusebenza kangcono kwama-*endothelial cell* (ama-seli athololakala ngaphakathi kwemithambo yegazi) bese kwehlisa ukuvuvuka kwemithambo. Kodwa-ke akucacile noma ukuzivocavova ngokusheshisa phecelezi (HIIT) ikona okusizayo ukwedlula ukuzivocavoca okujwayelekile lapho umuntu ethatha isikhathi ukuqeda (CMIT). Lolucwaningo lwethula imiphula etholakale kwi-c*linical trial* ebiqhathanisa i-HIIT kanye ne-CMIT ekuvuvukeni kwemithambo yegazi kubantu abakhuluphele abane-*insulin* engasebenzi kahle. Kanti-ke lolucwaningo luphinde lwaxilonga izimbangela ezahlukahlukane ezihambelana nokungasebenzi kahle kwe-*insulin*. Amavolontiya avuma ukuba yinxenye yalolucwaningo ahlolelwa lokhu: ukukalwa komzimba okuhlukahlukene, izinga lwe-*insulin* egazini, kanye namamnye ama-thesti axilonga ukuqina komzimba. Amavolontiya ahlukaniswa ngokusebenza kwe-ins*ulin*, i.e. abene-i*nsulin* esebenza kahle nabane *insulin* engasebenzi kahle. Lamaqoqo amavolontiya aphinda ahlukaniswa ngolundelayo abangazivocavoci, (pecelezi i-control), HIIT kanye ne- CMIT. Amaqoqo e-HIIT kanyene CMIT bakalwa ngaphambi kokuthi bazivocavoce base bazivocavoca ngononina izinsuku iziyishumi baphinde bakalwa futhi. Iqoqo le-control alizange lizivocavocele lona kepha lakalwa njenge HIIT kanyene CMIT. I-HIIT ikhombise okukhulu ukuthuthukuswa komaka i-HOMA-IR, 32% uma iqhathaniswa no-9% we CMIT], ukusebenza kwama seli emithambo pecelezi i- [(PORHmax kanye ne MV); 37.59% kanye no 27.45%, ngokulandelana, vs. 8.24% kanye no -25.71%, ye CMIT]. Lemiphumela yenzeke ngaphandle kokushintsha kwamafutha omzimba kanye nokuqina kwawo. I-Cohens ikhombise ububanzi, nobukhulu bemiphumela eyenziwe yi-HIIT kanti imiphumela yea CMIT ibingacacile, lokhu kucacisa ngokusobal ukuthi i-HIIT iyonadlela yokuzivocavoca engasiza ukusebenza kwe-*insulin* kubantu abanemizimba emikhulu/emikhulu ngokweqile. Imiphumela ye-*baseline* (ngaphambi kokuzivocavoca) akhomise ukuthu iphrotheni eliwumaka wokuvuvuka kwemithambo i-CRP liyahambisana nokungasebenzi kahle kwe-*insulin* kubantu besilisa abamnyama. Lemiphumela ikubeka kucace ukuthi i-CRP ingaba umaka wokuvuvuka kwemithambo kulababntu, kepha kuqikelelwe ubulili bamavolontiya.

Amagama abalulekile: i-Afrika, ukuzivocavoca, ukuvuvuka kwemithambo

CHAPTER I: INTRODUCTION

1.1. Background

Overweight and obesity are characterized by body mass index [(BMI) ≥ 25 kg/m^2 and ≥ 30 kg/m^2, respectively] [1]. The World Health Organisation (WHO) reported that 39% of the world's adult population is overweight and 13% is obese [1]. South Africa has the highest prevalence of obesity in sub-Saharan Africa (69.3% in females and 39% in males) [2]. Excess body weight, specifically an increase in adiposity (fat tissue) can be a result of genetic and/or environmental factors including poor dietary habits and lack of physical activity [3]. Enlarged adipose tissue release pro-inflammatory cytokines including interlukin-6 (IL-6) which has been found to inhibit insulin action [4]. Obesity-induced inflammation, therefore, is associated with insulin resistance which is characterized by a decrease in sensitivity or response of muscle, adipose and liver tissue to cellular actions of insulin. Under normal physiological conditions, insulin maintains glucose homeostasis and vascular tone by promoting gluconeogenesis and glycogenesis, and producing vasoactive agents, including the vasodilator nitric oxide (NO) and vasoconstrictor endothelin-1 (ET-1). A defect in the metabolic and/or haemodynamic insulin-signalling pathway therefore, leads to cardio-metabolic disorders including endothelial dysfunction [5]. Endothelial dysfunction results impaired release of vascular factors such as endothelin-1 (ET-1) and nitric oxide (NO) [6, 7]. Impaired production of these vasoactive substances, particularly NO, results in low shear stress (reduced vascular blood flow) which is a marker of early stages of atherosclerosis [8]. Furthermore, compromised NO bioavailability results in systemic inflammation which has been reported to exacerbate endothelial dysfunction [9].

Management strategies for insulin resistance include regular screening of high-risk individuals. The American Diabetes Association (ADA) recommends that asymptomatic individuals who are physically inactive, overweight and/or relative of person with diabetes should be screened every 3 years [6]. Screening for insulin resistance presents an opportuning for early detection and treatment which improves treatment outcomes and may delay progression to overt diabetes [7]. The hyperinsulinemic euglycemic clamp is the gold standard for testing insulin sensitivity [8]. The clamp however, is invasive, time-consuming, and costly and is therefore considered impractical for clinical application [9]. Indirect measures of insulin sensitivity such as homeostatic model assessment of insulin resistance (HOMA-IR), acute phase inflammatory proteins (C-reactive protein), body composition [BMI and waist-to-height ratio (WHtR)] which are easier to administer and relatively inexpensive, have therefore found wide application in primary health-care setting and epidemiological studies [10-12].

Guidelines for diabetes care recommend the use of pharmacologic agents such as metformin and/intensive lifestyle modification (ILM) programmes (cessation of tobacco smoking, diet and exercise) to manage obesity-induced metabolic disorders including insulin resistance [13]. Pharmacological agents however, are limited due to patient non-compliance partly attributed to side effects such as gastrointestinal intolerance, nightmares and abnormal dreams [14, 15]. Exercise is a critical component of ILM and is recommended as the first line of treatment for insulin resistance by major diabetes organisations including the ADA and International Diabetes Federation (IDF) [16, 17]. Previous studies have shown that increasing exercise levels can achieve the same therapeutic effect as pharmacological agents without associated side-effects [18, 19]. Asymptomatic adults with insulin resistance are encouraged to achieve at least 150 minutes of moderate to vigorous exercise training 5 days per week, with more hours increasing benefit [20]. Although there is substantial evidence on the health benefits of regular physical activity for the prevention and management of various diseases of lifestyle including insulin resistance, levels of physical activity continue to decline across ages and gender [21]. Studies show that lack of time is the number one barrier cited by people who are physically inactive [22]. Thus, time-efficient exercise prescription is imperative to offer an alternative for time-pressed individuals who are at risk for insulin resistance.

High intensity interval training (HIIT) which requires approximately 10% of the time commitment of continuous moderate intensity training (CMIT) has been reported to produce similar and sometimes superior benefit compared with CMIT despite major differences in training volume and time commitment [23, 24]. This form of training (HIIT) is characterized by a work rate near "the maximal" effort (i.e. between 90 and 100% of maximal oxygen consumption ($\dot{V}O_2$max)[29]. The protocols for HIIT can vary based on the target population, however, the protocols generally involve work rate near maximal for 20 seconds up to 4 minutes separated by active or complete rest with a total exercise time of approximately 10-15 minutes [30]. Metabolic effects of HIIT on athletes are well documented and HIIT is now increasingly being investigated in clinical populations [26, 27]. Available research on

exercise in persons with impaired metabolic function is often based on long-term programmes. Chronic exercise training is associated with improvements in body composition and aerobic fitness, factors which have been found to independently improve insulin sensitivity [28]. This phenomenon presents a challenge to study the effects of exercise in isolation. Short-term (7-10 days) studies have gained the attention of researchers because they provide an opportunity to study the effects of exercise without significant changes in body composition and aerobic fitness. Reports from available short-term studies are mixed, some studies have reported improvement in insulin sensitivity while some have reported modest or no change [29, 30]. Furthermore, there is paucity of randomized controlled trials which have clearly defined insulin resistance status of participants and compared exercise intervention with a no exercise control group.

1.2. Research questions

Study research questions were as follows:

1. Do mucosal markers of inflammation correlate with circulating (plasma) markers?
2. Is there association between inflammatory markers and laser Doppler markers of endothelial function?
3. To what extent do HIIT or CMIT decrease inflammation and improve endothelial function in IR?
4. Do changes in inflammatory markers parallel to the changes in laser Doppler markers of endothelial function?

1.3. Study aim, objectives and hypothesis

1.3.1. Aim

The aim of this study was to evaluate the effects of high intensity interval training and continuous moderate intensity training on inflammation and endothelial function in overweight/obese adults with insulin resistance.

1.3.2. Objectives

Primary objective

The primary objective was to determine the effects of short-term (10 consecutive days) high intensity interval training and continuous moderate intensity training on inflammation and endothelial function in overweight/obese adults with insulin resistance.

Secondary objectives

The secondary objectives were to;

1. Determine if there was an association between mucosal (saliva) and systemic markers of systemic inflammation.
2. Determine if there was an association between markers of systemic inflammation and laser Doppler markers of endothelial function.
3. Establish whether changes in inflammatory markers parallel the changes in laser Doppler markers of endothelial function.

1.3.3. Hypothesis

The following hypotheses were identified for the study;

H_{01}: HIIT or CMIT decrease inflammation and improve endothelial function in insulin resistance.

H_{02}: Mucosal marker of inflammation correlate with those found in plasma, in insulin resistant patients.

H_{03}: Elevated levels of mucosal and systemic inflammatory markers are associated with impaired endothelial function in insulin resistant patients.

H_{04}: The reduction of mucosal and systemic inflammatory markers correlates with improved laser Doppler markers of endothelial function.

1.4. Research design

This study was a randomized controlled experimental study with two phases, a cross sectional (phase 1) and interventional (phase 2) phase as shown is Fig 1. Phase 1 involved collecting baseline data from consenting individuals who met the inclusion criteria. In phase 2, an exercise intervention was implemented and was followed by a repeat of the tests conducted at baseline to allow for comparison of the data.

1.5. Study population

Students and staff members from the University of KwaZulu-Natal (UKZN), Durban, South Africa, were invited via posters and social media platforms. Participants were considered for inclusion into the study if they were 1) 18-35 years old, 2) sedentary or physically inactive which was defined as failure to accumulate at least 30 minutes of physical activity 5 days per week for the past 3 months [31] and 3) overweight or obese which were classified as BMI $\geq$ 25 kg/m^2 or $\geq$ 30 kg/m^2, respectively [1]. Participants were excluded if they had a history of smoking (current or chronic smokers), taking treatment for diabetes, lipid disorders or human immunodeficiency virus or have an injury/disability that restricted their ability to participate in cycling.

1.5.1. Sample size

The target sample size for the study (based on power statistics) was one hundred and fifty (n = 150). This was calculated from the study population using an online sample size calculator (Raosoft®) with the margin of error and confidence interval set 5 and 95%, respectively. However, ninety-five (n = 95) participants were enrolled into the study due to financial constraints. The enrolled participants (n = 95) were entered into phase 1 of the study. Of these, sixty (n = 60) were randomly selected to take part in phase 2.

1.5.2. Ethical consideration

The study protocol was approved by the University of KwaZulu-Natal Biomedical Research Ethic Committee-BREC; reference number BFC098/16 (Appendix I).

1.6. Methods

The study consisted of 2 phases, phase 1 (cross-sectional) and phase 2 (interventional), as shown in the study outline flow chart in (Fig 1), below.

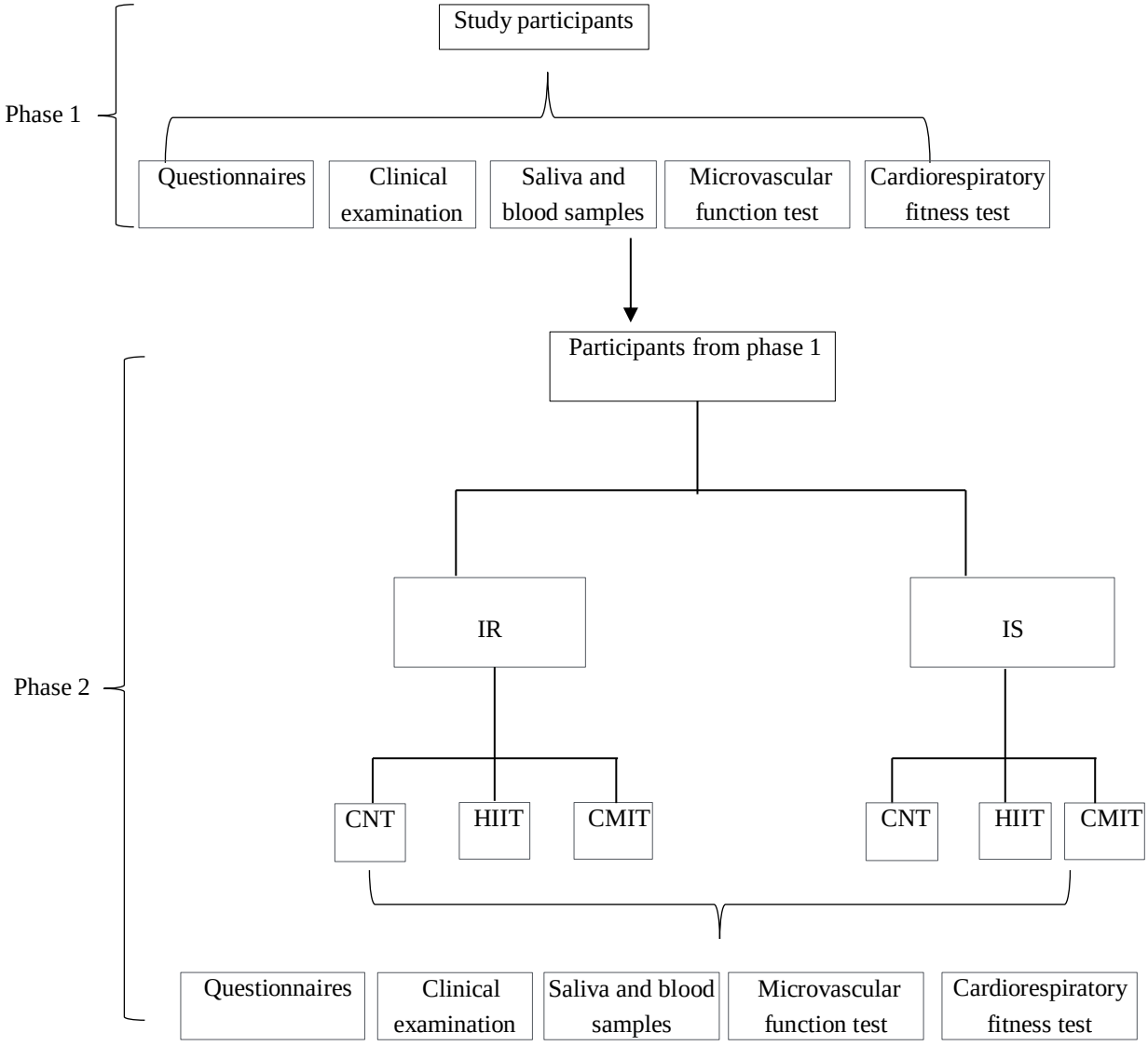

Fig 1: Study outline. Insulin resistant (IR), insulin sensitive (IS), control (CNT), high intensity interval training (HIIT), continuous moderate intensity training (CMIT).

1.6.1. Phase 1 (Cross-sectional study)

In phase 1, the study participants' readiness for exercise, risk for insulin resistance, body composition, insulin resistance status, microvascular function and cardiorespiratory fitness were assessed using a) questionnaires, b) clinical examination c) biochemical tests and d) physiological tests. Data collected in phase 1 served as baseline for phase 2 of the study.

a) Questionnaires

1. Physical activity screening questionnaire (PASQ)

Physical activity screening questionnaire (PASQ) (themes; activity at work/school, travel to and from places, recreational activities and sedentary behaviour) (Appendix VII) adapted from the World Health Organization (WHO) global physical activity questionnaire (GPAQ) was used to screen the participant's physical activity.

2. Insulin resistance screening questionnaire (IRSQ)

Insulin resistance screening questionnaire (IRSQ) (themes; body mass index, waist circumference and physical activity) (Appendix VIII) adapted from the International Diabetes Federation (IDF) Finnish type 2 diabetes risk assessment questionnaire was used to screen for insulin resistance.

3. Pre-exercise intervention questionnaire (PEIQ)

Pre-exercise intervention questionnaire (PEIQ) (themes; history of cardiovascular events, symptoms, other health issues and cardiovascular risk factors i.e. age, blood pressure and cholesterol) (Appendix IX) adapted the American Council for Sport Medicine (ACSM) pre-participating questionnaire was used for pre-exercise intervention assessment.

b) Clinical examination

A bio-electrical impedance analysis machine (Omron®) was used to measure body mass index (BMI) which served as a measure of total body obesity. Waist (cm) and hip (cm) circumference (measured by tape) and waist-hip ratio were used as measures of central obesity. Blood pressure was measured by a digital blood pressure device (Omron®) and light-weight Polar heart rate monitor (Polar S810™ HRM) with a chest strap was used to monitor heart rate (HR).

c) Laboratory tests

After a 12-hour overnight fast, saliva and blood samples were collected. Saliva samples were collected via passive drool into cryovials (Salimetrics®) over 4 minutes for the calculation of saliva flow rate. Blood samples were drawn from the antecubital region of the arm by a phlebotomist, collected into ethylenediamine tetraacetic (EDTA) tubes and centrifuged to obtain plasma for biochemical analysis which were conducted at the Lancet Pathology Laboratory (Durban, South Africa). Serum insulin was measured by insulin radioimmunoassay and plasma glucose by the glucose hexokinase method on Roche automated analyser. Serum C-reactive protein (CRP) was measured by enzyme-linked immunosorbent assay (ELISA).

Table 1: Summary of laboratory tests

Variable	Laboratory test
Serum insulin	Insulin radioimmunoassay
Plasma glucose	Glucose hexokinase method
Salivary CRP	ELISA
Serum CRP	ELISA
Blood total cholesterol	Accutrend Plus Meter
Blood triglycerides	Accutrend Plus Meter

CRP; C-reactive protein, ELISA; Enzyme-linked Immunosorbent Assay

Definition of insulin resistance

Fasting plasma glucose (FPG) and insulin (FPI) were used to calculate (HOMA Calculator© The University of Oxford 2013) derived indices for IR and pancreatic (β) cell secretory function; HOMA-IR and HOMA-β, respectively. Study participants with a HOMA-IR score ≥ 2 were considered to be IR and participants with a HOMA-IR score ≥ 1,1 – < 2 were considered to be IS [32].

d) Physiological measurements

Microvascular function test

A non-invasive laser Doppler flowmetry system (moorVMS-Instruments®) was used to measure cutaneous blood flow. A blood pressure cuff and laser were placed on the distal portion of upper arm and right palmer forearm, respectively. The cuff was inflated to a pressure to 50 mmHg above resting systolic blood pressure. Occlusion was maintained for 3 minutes. Post occlusive reactive hyperaemia (PORH) was monitored upon the release of the cuff until full recovery to basal level. Computerised software; moorVMS-PC V4.0 (moorVMS-Instruments®) was used to measure resting flux (RF), peak flow (PORHpeak) and time-to-peak (Tp).

Cardiorespiratory fitness test

The functional limit of the subject's cardiorespiratory system and exercise tolerance was assessed by an individualised maximal test, since maximal oxygen consumption ($\dot{V}O_2$max) is directly linked to power output, the maximum work load achieved during the $\dot{V}$max test was used to set exercise intensities required to elicit 90-100% of $\dot{V}O_2$max for the HIIT or 60-70% of $\dot{V}O_2$max for MIT. On the day of the max test, participants were given 15 minutes to acclimatize to laboratory conditions (20-22°C) following which body composition, resting blood pressure and heart rate were measured. Participants peak oxygen consumption ($\dot{V}O_2$peak) was assessed via a 2-minutes incremental cycle test performed on a specialised ergometer (Lode Excalibur Sport, Groningen, The Netherlands) with oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$), minute ventilation (VE), breathing frequency, and tidal volume analysed by an online breath by breath gas collection system (Cortex MetaMax 3b gas analyser). Briefly, participants cycled at a workload of 50 Watts for 5 minutes and the workload was increased by 25 watts every 2 minutes until volitional fatigue. The final score was the highest level (Watts) attained while completing the full 2-minutes increment. Participants were asked to maintain 60 revs per minute (RPM) throughout the max test. RPM below 60 for longer than 15 seconds will result in termination of the test. Blood pressure, heart rate and rate of perceived exertion were examined at each 2-minute increment. Participants were closely monitored during recovery until their blood pressure and heart rate returned to baseline. The following criteria was used to determine true maximal effort; 1) plateau in oxygen consumption ($\dot{V}O_2$) defined as an increase of less than 1.5 ml/kg/min despite progressive increases in exercise intensity, 2) a final respiratory exchange ratio (RER) of 1.1 or above and 3) a final heart rate (HR) above 95% of the age-related maximum.

1.6.2. Phase 2 (Intervention study)

Participants assigned to exercise intervention performed supervised HIIT or CMIT for 10 consecutive days. For HIIT the 60 seconds of cycling over 75 seconds of rest that was proposed initially was amended to 60 seconds of cycling over 30 seconds of rest exercise protocol following further review of literature which showed that during HIIT, a 2:1 work to rest ratio yields appropriate training stimulus and is perceived as less difficult by overweigh/obese participants [33]. The protocol amendment approval letter is shown is appendix III. Over the 10-day intervention period, the HIIT group trained at the " near maximal" effort (90 and 100% $\dot{V}O_2$max) while the CMIT group trained at moderate intensity (70 and 80% $\dot{V}O_2$max) [29]. The total duration of an exercise session was 21 minutes, similar to protocols studies by others [32, 39]. HIIT and CMIT exercise sessions are summarised in the Table 2 and Table 3, respectively.

Table 2: Summary of high intensity interval training (HIIT) sessions

HIIT sessions (Days)									
1	2	3	4	5	6	7	8	9	10
Warm-up for 3 minutes									
60 sec of cycling at 90% $\dot{V}O_2$peak with 30 sec of rest 8 sets				60 sec of cycling at 95% $\dot{V}O_2$peak with 30 sec of rest 10 sets				60 sec of cycling at 100% $\dot{V}O_2$peak with 30 sec of rest 12 sets	
Cool-down for 3 minutes									

Table 3: Summary of continuous moderate intensity training (CMIT) sessions

CMIT sessions (Days)									
1	2	3	4	5	6	7	8	9	10
Warm-up for 3 minutes									
12 min of cycling at 70% $\dot{V}O_2$peak with no rest 1 set				15 min of cycling at 75% $\dot{V}O_2$peak with no rest 1 set				18 min of cycling at 80% $\dot{V}O_2$peak with no rest 1 set	
Cool-down for 3 minutes									

Post-intervention measures

Post-training assessments were conducted at least 24 hours after the last exercise bout, within a 48-hour period. Post-training blood draws were conducted at least 24 hours before $\dot{V}O_2$peak test.

1.7. Statistical analysis

Data was analyzed using SPSS version 24 (IBM, USA). Data was tested for normal distribution using Shapiro-Wilk test. For data that were normally distributed, means and standard deviations were calculated for demographic characteristics and Student's t-test were used to determine differences in demographic characteristics between the insulin resistant and insulin sensitive (IS) groups. For data that were not normally distributed, non-parametric test (Mann-Whitney) was used and median and interquartile range (IQR) was reported. Multiple-linear regression (MLR) was used to explore the association between several characteristics (age, gender, body composition, blood pressure, endothelial function, cardiorespiratory fitness) and insulin resistance status. A repeated measure multiple analysis of variance was used to determine the effects of the different exercise modes (HIIT or CMIT) on markers of inflammation, endothelial function and insulin resistance as well as between group differences. Cohen's (*d*) effect sizes and 95% confidence intervals (CI) were calculated for all outcome measures. Magnitudes of the standardized effects were interpreted using thresholds of $d \leq 0.2$ (small), $d \geq 0.5$ (moderate) and $d \geq 0.8$ (large) [34]. Effect size was only accepted if the confidence interval did not cross both the positive and negative 0.2 ES thresholds otherwise were considered to be unclear [34].

1.8. Layout of the thesis

This thesis comprises of a preliminary section, background, research design and methods sections (Chapter I), chapters II-VI which are formatted in accordance with the journals to which they have been submitted and Chapter VII which is a synthesis chapter summarizing the findings of the study and is presenting study strengths and limitations, contributions to literature as well as recommendations for future studies.

Chapter II is manuscript 1 and serves as the literature review in the form of a narrative review.

Chapter III is manuscript 2 which is a systematic review of randomized controlled trials examining the effects of short-term exercise training on insulin resistance.

Chapter IV is manuscript 3 which presents findings from the intervention phase (phase 2) of the study and reports of effects of high intensity interval training vs. continuous moderate intensity training on insulin resistance in overweight/obese adults.

Chapter V is manuscript 4 which presents findings from the intervention phase (phase 2) of the study and reports of effects of HIIT vs. CMIT on endothelial function in overweight/obese adults with insulin resistance.

Chapter VI is manuscript 5 which presents data from the cross-sectional phase (phase 1) of the study and reports on the associations between inflammation and insulin resistance in overweight/obese individuals.

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CHAPTER II: MANUSCRIPT 1 - NARRATIVE REVIEW

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REVIEW ARTICLE

Lifestyle modification in the management of insulin resistance states in overweight/obesity: the role of exercise training

Tshidi Thaane[a]†*, Ayesha A Motala[b]‡ and Andrew J Mckune[a,c,d]‡

[a]*Discipline of Biokinetics, Exercise and Leisure Sciences, University of KwaZulu-Natal, Durban, South Africa;*
[b]*Department of Diabetes and Endocrinology, University of KwaZulu-Natal, Durban, South Africa;*
[c]*University of Canberra Research Institute for Sport and Exercise Science, University of Canberra, Canberra, Australia;*
[d]*Collaborative Research in Bioactives and Biomarkers (CRIBB) Group, University of Canberra, Canberra, Australia*
Corresponding author, email: t.thaane@gmail.com

Physical inactivity is a major contributor to overweight/obesity and associated disorders including cardiovascular diseases (CVDs), diabetes, and insulin resistance (IR). Intensive lifestyle modification (ILM) is recommended as first-line treatment for obese individuals at risk for IR. Exercise is considered to be a critical component of ILM. This narrative review discusses the role of exercise in the management of IR in overweight/obesity.

PubMed and Google Scholar were searched for articles published between January 1990 and January 2019 that examined mechanisms behind the effects of exercise on IR states associated with overweight/obesity. Studies examining and/comparing effects of exercise mode, volume and/intensity on IR were also retrieved. Medical Subject Headings (MeSH) used were 'metabolic diseases' OR 'chronic diseases' AND 'exercise' and their related terms. Text words used in conjunction with the MeSH terms were 'aerobic training/exercise' OR 'resistance training/exercise' OR 'high intensity interval training/exercise', OR 'low volume training/exercise'. Reference lists of retrieved articles were also searched for appropriate studies.

Aerobic exercise training (AET) and resistance exercise training (RET) appear to produce comparable effects on obesity-induced IR states. RET, however, appears to be associated with greater improvements in glucose disposal in skeletal muscle, which is usually the primary site for IR. This is partly attributed to greater increases in key proteins involved in the insulin signalling pathway including protein content of glucose transporter 4 (GLUT-4) following RET. A study on individuals with impaired glucose tolerance (IGT) showed that RET improved glucose disposal by 23%, primarily due to a 27% increase in non-oxidative glucose metabolism, suggesting that RET may delay the manifestation of diabetes in patients with IGT. Furthermore, studies reviewed here show that components of exercise including the mode, volume and intensity of exercise training are an integral element in exercise prescription and must be recommended in accordance with the desired outcome.

Keywords: exercise, insulin resistance, lifestyle modification, overweight, obesity

Introduction

Lack of exercise and sedentary behaviour (prolonged sitting) are major risk factors for insulin resistance.[1–3] Typically coupled with excessive intake of energy-dense foods, low-energy expenditure (physical inactivity) is associated with overweight/obesity.[4] In the development of obesity, macrophages infiltrate adipose tissue and alter its endocrine and metabolic functions to produce abnormal levels of adipokines and cytokines such as leptin and interleukin 6 (IL-6).[5, 6] This results in systemic inflammation, which is strongly associated with diminished response of liver, muscle and adipose tissue to cellular actions of insulin.[7] Liver, muscle and adipose tissue play a critical role in glucose homeostasis, thus a defect in insulin action in these tissues results in impaired glucose uptake and storage.[8] Consequences of impaired glucose homeostasis include dyslipidaemia, type 2 diabetes (diabetes) and cardiovascular diseases (CVDs).[9]

Lifestyle modification including cessation of tobacco smoking, changes in diet and exercise are recommended as non-pharmacological therapeutic approaches for the management of obesity-associated diseases including cancer, diabetes and insulin resistance.[10] Below we discuss therapeutic effects of exercise on impaired insulin signalling, impaired glucose metabolism, systemic inflammation and endothelial dysfunction; and clinical states of insulin resistance in overweight/obesity.

Impaired insulin signalling

Impaired insulin signalling as shown in Figure 1 occurs at the level of insulin receptor substrate 1 (IRS-1), which leads to impaired glucose transport. Under normal physiological conditions, the insulin signalling pathway is initiated when insulin binds to its receptors located on the cell surface of insulin-sensitive tissue including skeletal muscle and adipose tissue. Activation of the insulin receptor leads to phosphorylation of IRS-1 which in turn activates the enzyme phosphatidylinositol 3-kinase (PI3 K).[11] Activation of PI3 K leads to the activation of protein kinase B/Akt, which is responsible for the phosphorylation of the substrate AS160 and activation of Rab proteins required for the translocation of vesicles containing glucose transporter 4 (GLUT4) to the cell membrane.[12] Once at the cell

†Denotes graduate student author
‡Denotes professional author
‡Denotes professional author

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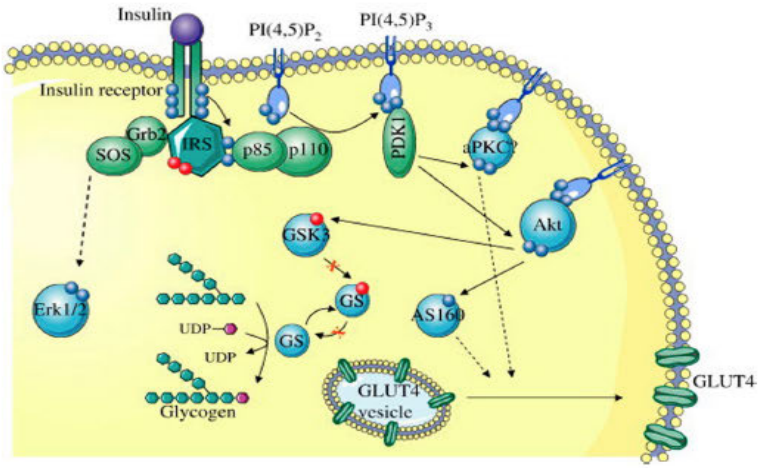

Figure 1. Schematic representation of the insulin signalling pathway showing defects in the signalling cascade in insulin resistance (Adapted from Fröjdö *et al.*).[13] IRS: insulin receptor substrate; Grb2: growth factor receptor-bound protein 2; SOS: Son of Sevenless; Erk/12: extracellular signal-regulated kinases 1/2; PIP_3: phosphatidylinositol 3 kinase; PDK1: phosphoinositide-dependent kinase-1; PKC: protein kinase C; Akt: serine-threonine protein kinase; GSK3: glycogen synthase kinase 3; UDP: uridine diphosphate; GLUT4-glucose transported 4.

surface, GLUT4 shuffles glucose into the cell where it is stored or metabolised.

Thus when insulin signalling is impaired, hyperglycaemia ensures. Chronic hyperglycaemia has been linked to diabetes-related micro- and macrovascular complications including neuropathy, retinopathy and nephropathy.[14,15]

Impaired insulin signalling is caused by a variety of factors. In the skeletal muscle, it is attributed to the accumulation of fat in muscle fibres.[16] Increased skeletal muscle fat content, particularly inside the muscle fibre (intramyocellar fat-IMCL) is strongly correlated with impaired glucose transport even though IMCL contributes a smaller portion (~1%) of the total fat content compared with fat accumulated outside the muscle cell (extramyocellar fat-EMCL).[17,18] The mechanism behind accumulation of IMCL in the muscle is unclear. Some studies, however, have suggested that mitochondrial dysfunction contributes to increased IMCL fat content.[19–21]

Skeletal muscle insulin resistance has been identified as a primary defect in insulin resistance and diabetes; the skeletal muscle therefore presents a critical target site for therapies aimed at controlling glycaemia.[22,23] Therapeutic effects of exercise in the skeletal muscle are via acute and chronic adaptations. Acutely, exercise results in the translocation of GLUT 4, which results in improved glucose uptake by the muscle.[24] This exercise-induced GLUT 4 expression occurs via insulin-independent pathways including the calcium (Ca^{2+}), 5'AMP-activated-kinase (AMPK) and nitric oxide synthase (NOS) kinases. Chronically, exercise results in increased mitochondrial content and muscle fibre transformation (from more glycolytic type IIb/IId/x to more oxidative type IIa fibres), which are associated with increased oxidative capacity.[25,26] A high muscle oxidative capacity is associated with optimal glucose metabolism and has been found to be predictive of metabolic health including low fat mass and optimal insulin sensitivity.[27,28]

Impaired glucose metabolism

Impaired glucose metabolism (pre-diabetes) is a transition state between normal glucose homeostasis and diabetes. This metabolic state is characterised by impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), which are attributed to skeletal muscle and hepatic insulin resistance, respectively.[29–31] Effects of exercise on human skeletal muscle are well elucidated and mechanisms have been highlighted in the preceding paragraph. Hepatocellular mechanisms, however, are unclear. Studies on animal models show that exercise reduces liver fat content, stimulates hepatic mitochondrial adaptations and increases 5' adenosine monophosphate-activated protein kinase (AMPK) activity.[32] Increased AMPK activity inhibits transcription factors including hepatocyte nuclear factor 4 (HNF4) and CREB regulated transcription coactivator 2 (CRTC2) which maintain glucose homeostasis by inhibiting gluconeogenesis.[33,34] Thus, exercise-induced AMPK activity may offer therapeutic benefit to patients with IFG who exhibit excessive rise in post-prandial glucose due to impaired hepatic glucose production.[35]

Chronic systemic inflammation

The inflammatory process is a normal part of the body's natural defence to infection or trauma and results in the expression of inflammatory mediators such as IL-6, TNF-α and CRP. Although this process is pivotal in immunological response, chronic inflammation (persistent elevated levels of inflammatory markers), as seen in total and abdominal obesity, is a major risk factor for chronic diseases including insulin resistance.[36] Pharmacological interventions such as the use of statins have been shown to decrease obesity-induced inflammation.[37,38] Behavioural interventions including exercise (acute and chronic), however, are increasingly being shown to also have clinically significant benefits.[39,40] It is important to note that, although exercise is associated with increased muscle-derived inflammatory cytokines acutely, these cytokines are of physiological benefit.[41] IL-6 secreted after an acute bout of exercise has been found to initiate the secretion of the anti-inflammatory

cytokine (IL-10) from monocytes and lymphocytes.[41] Chronic (regular, long-term) exercise has been shown to have an inverse, independent dose–response relation with inflammation.[42] In a 12-month study, Marfella *et al.* reported that exercise decreased IL-6, CRP and TNF by 62%, 44% and 31%, respectively.[43] The mechanism by which exercise training mitigates inflammation has been suggested to be through the reduction of adipose tissue, which is the main secretory organ responsible for the production of inflammatory cytokines.[7,43] Furthermore, exercise has been found to increase the expression of anti-inflammatory agents such IL-1 receptor antagonist (IL-1ra), IL-10 and adiponectin, which promote various physiological benefits including glucose metabolism and suppression of inflammatory markers such as TNF-α, which may reduce risk for insulin resistance.[44–46]

Endothelial dysfunction

Obesity, particularly abdominal obesity, is a primary risk factor for impaired endothelial function, which results in abnormal regulation of vasoactive substances including nitric oxide (NO).[47] Nitric oxide is considered to be the most important endothelium-derived substance due to its antiatherogenic effects including the inhibition of inflammation and vascular smooth cell proliferation and migration. Endothelial dysfunction (ED), therefore, is strongly associated with chronically elevated levels of the pro-inflammatory markers including CRP.[48] Thus, ED is considered an initial step in the pathogenesis of insulin resistance and associated cardiovascular complications.[49] Research indicates that exercise training results in significant reduction in CRP and may produce cardioprotective effects. A recent meta-analysis of randomised controlled trials found that exercise was associated with a significant decrease in CRP (−0.66 mg/l (95% CI, −1.09 to −0.23 mg/l; −14% from baseline), suggesting that exercise could be a therapeutic alternative for the management of abnormal inflammation levels.[50]

The effects of exercise on insulin resistance that are outlined in the preceding paragraphs are generally dependent on various components including the mode, volume and intensity of exercise training.[51] These components are integrated with patient health characteristics identified from the exercise pre-participation screening process, which involves assessing the patient's level of physical activity and identifying any presence of signs or symptoms of cardiovascular and metabolic disease.[51]

Exercise mode

The mode of exercise refers to the type of exercise. Aerobic exercise training (AET), which includes exercise types such as walking, jogging, bicycling and rowing, is the most commonly studied mode of exercise in patients with obesity and related metabolic disorders.[52] Long-duration AET has traditionally been prescribed to obese patients to aid in weight loss due to high energy demands associated with prolonged (> 60 minutes per day) exercise.[53] Recent studies, however, show that the effects of AET are amplified when it is combined with resistance exercise training (RET), which has previously not been recommended for obese patients.[54] In a 14-year study, Shiroma *et al.* reported that RET decreased the incidence of diabetes and cardiovascular disease by 30% and 17%, respectively.[54] Furthermore, the authors observed that people who performed RET reduced their risk of diabetes and CVDs by 30% and 17%, respectively.[54] This could be due to the fact that, unlike AET, RET maintains fat-free mass (FFM) and resting energy expenditure (REE), factors that are pivotal in weight loss and weight maintenance.[55] Resistance exercises included shoulder press, squats, lunges and deadlifts, which are performed with progressive resistance machines or bands and/or free weights.[56,57] These exercises were performed at ~70–85% one-repetition maximum (1RM), which has been reported to preserve or improve FFM and REE while decreasing body fat.[58]

Exercise volume and intensity

Exercise volume and intensity refer to the amount and effort required for the exercise. Exercise intensity (moderate, vigorous or high) is characterised by objective and subjective measures such as percentage of heart rate reserve (HHR) and rate of perceived exertion (Borg's 6–20 [RPE]), respectively.[59,60] Exercise training at 40–60% of HRR or 12–13 RPE is considered moderate intensity training (MIT). Vigorous-intensity exercise is performed at 60–85% of HRR or RPE $\leq$ 14 while high-intensity training (HIT) is performed at near maximal to maximal effort, 90–100% HHR and 19–20 on the RPE scale.[51]

The intensity of exercise determines the volume (amount) of exercise. The lower the intensity or load the higher the volume of exercise required to achieve therapeutic effect and vice versa.[61] High load (low volume) RET is traditionally considered to produce superior physiological adaptations including skeletal muscle hypertrophy when compared with low load (high volume) RET. A recent study by Morton *et al.* however, reported comparable increases in lean muscle mass in trained and untrained individuals following high and low load (high and low volume, respectively).[62] The authors speculated that the similar increases may have been due to the fact that the participants who performed low load (high volume) RET achieved a greater exercise volume since they were able to exercise until volitional failure, which allowed for maximal activation of their motor units and ultimately led to the similar increases in skeletal muscle adaptation seen in the high load (low volume) group, suggesting that the gains in muscle mass are not dependent on the load when the exercise is performed to volitional fatigue.[62]

There is general consensus that exercise training produces therapeutic effects on health outcomes including improvements in insulin sensitivity and cardiorespiratory fitness. Nonetheless, levels of physical inactivity continue to rise.[63] Time commitment (30–60 minutes) required for high-volume moderate-intensity training (HVMIT) has been cited as the most common barrier by people who are physically inactive.[64] Low-volume high-intensity training (LVHIT), which requires approximately 10% of the time required for HVMIT, is therefore increasingly being studied as a time-efficient alternative.[65]

High-intensity interval training (HIIT) and sprint interval training (SIT) are the most commonly studied forms of LVHIT. Literature evidence shows that HIIT and SIT, which are characterised by brief periods of high-intensity exercise separated by active or complete rest, produce similar and sometimes superior benefits in patients with cardiometabolic disorders despite major differences in time commitment.[66,67] In a 12-week study, Gillen *et al.* reported that three sets of 20-second SIT three times per week produced comparable improvements in cardiorespiratory fitness, insulin sensitivity and mitochondrial content as 45 minutes of MIT despite the fivefold lower exercise volume.[68] Studies have suggested that training at high intensity is associated with greater cardiac output and stroke volume as well as activation of peripheral factors that enhance the extraction of oxygen from the blood to tissues, factors associated with enhanced cardiorespiratory fitness.[69]

Conclusion

In conclusion, studies discussed in this narrative review show that exercise improves insulin resistance by improving insulin signalling and glucose metabolism. These improvements, however, are generally dependent on various components including the mode, volume and intensity of the exercise. The beneficial effects of exercise, which have been studied thoroughly, provide support for current physical activity guidelines that recommend 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic physical activity per week, which are endorsed by diabetes organisations including the American Diabetes Association (ADA) and International Diabetes Federation (IDF). However, considering that 'lack of time' is the main contributor to the physical inactivity pandemic, public health guidelines ought to reflect time-efficient exercise training strategies for the prevention and management of insulin resistance and other metabolic disorders. Emerging research is increasingly showing that HIIT is a time-efficient and effective mode of exercise training that has potential to be an alternative strategy to encourage individuals to adopt and maintain a physically active lifestyle. However, comprehensive clinical trials are needed to advance knowledge on HIIT.

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ORCID

Tshidi Thaane http://orcid.org/0000-0002-2420-2905
Andrew J Mckune http://orcid.org/0000-0002-5479-1544

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CHAPTER III: MANUSCRIPT 2 - SYSTEMATIC REVIEW

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Review Article Open Access

Effects of Short-Term Exercise in Overweight/Obese Adults with Insulin Resistance or Type 2 Diabetes: A Systematic Review of Randomized Controlled Trials

Tshidi Thaane[1*], Ayesha A Motala[2] and Andrew J McKune[1,3,4]

[1]*Discipline of Biokinetics, Exercise and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, South Africa*

[2]*Department of Diabetes and Endocrinology, School of Clinical Medicine, University of KwaZulu-Natal, South Africa*

[3]*Discipline of Sport and Exercise Science, University of Canberra Research Institute for Sport and Exercise Science, Faculty of Health, University of Canberra, Australia*

[4]*Collaborative Research in Bioactives and Biomarkers (CRIBB) Group, University of Canberra, Australia*

***Corresponding author:** Tshidi Thaane, Discipline of Biokinetics, Exercise and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, 34 Somme Road, Musgrave, Durban, KwaZulu-Natal 400, South Africa, Tel: +27788871384; E-mail: t.thaane@gmail.com

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Abstract

Background: Chronic exercise training is associated with improvements in body composition and/aerobic fitness. This presents a challenge to study effects of exercise in isolation. Metabolic effects of short-term exercise training (≤ 12 weeks) are unclear, with studies reporting improvements or no change in insulin sensitivity and/or glucose control. This review systemically examined randomized controlled trials (RCTs) to establish whether short-term exercise training improves insulin resistance and type 2 diabetes (diabetes).

Methods: Following the PRISMA guidelines, a systematic review was conducted on nine electronic databases (BMC Endocrine Disorders, Clinical Key, Cochrane Library, EBSChost, PubMed, Scopus, Sabinet_SA Publications, The Lancet and Web of Science) to identify randomized controlled human trials (2005-2018) examining effects of short-term exercise training (≤ 12 weeks) in overweight/obese adults with insulin resistance or diabetes. Search terms included: insulin resistance, type 2 diabetes, short-term, exercise or energy expenditure and randomized controlled trial. Studies were only included if they provided sufficient data on: insulin sensitivity, glycemic control, body composition and aerobic fitness.

Results: From 374 articles, three met the inclusion criteria. Of these, two prescribed moderate intensity training (MIT); rate of perceived exertion (RPE) 12-13 and 60% lactic threshold (LT) for four and twelve weeks, respectively while one prescribed vigorous exercise training at 70% VO2max for seven days. Duration of exercise sessions was 40-60 minutes. Twelve weeks of MIT was associated with improved glycemic control vs. no change in the 7-d and four weeks studies. Seven days of vigorous training was associated with greater improvement in insulin sensitivity, 44.4% vs. no change in the four- and twelve-weeks studies.

Conclusion: Short-term exercise appears to improve insulin sensitivity and glucose control independent of body fat loss or gains in aerobic fitness. Vigorous exercise training was associated with superior improvements in insulin sensitivity. More RCTs are needed to confirm these findings.

Keywords: Metabolic stress; Peripheral vascular disease; Physical activity

Introduction

Insulin maintains glucose homeostasis by promoting gluconeogenesis and glycogenesis [1]. A defect in the metabolic insulin-signalling pathway therefore, leads to disorders including peripheral vascular disease (PVD) and type 2 diabetes mellitus (diabetes) [2]. The pathogenesis of insulin resistance is unclear, however, the disorder has been reported to be a precursor for diabetes which has reached epidemic proportions and shows no sign of abatement [3]. Diabetes is characterized by hyperglycemia that results from the inadequate islet beta (β) cell and adipose-tissue responses to chronic fuel excess which results in metabolic stress that causes macro- and microvascular complications including nephrology, retinopathy and neuropathy [4].

Emerging evidence indicates that susceptibility to diabetes may be acquired early in life due to foetal and neonatal programming that occurs via epigenetics phenomena, suggesting that interventions to improve maternal and early child health may be crucial for diabetes prevention. In adults, intensive lifestyle modification (ILM); diet and exercise, is recommended as prevention and management strategy for insulin resistance and diabetes [5]. ILM has been reported to offer long-term clinical benefits such as improvement of insulin sensitivity, glucose control, body composition and cardiorespiratory fitness [6-8]. Diabetes patients are encouraged to accumulate at least 150 minutes of physical activity per week, with more hours increasing benefit [9]. Previous reviews and meta-analyses have reported that exercise training improves glycaemic control in patients with insulin resistance or diabetes [10]. In these reviews, however, both short-term and chronic exercise training interventions are included [11]. In chronic studies, exercise-induced improvements in insulin resistance or

diabetes are generally accompanied by improvements in body composition and aerobic fitness. This presents a challenge to study the effects of exercise in isolation. Thus, short-term exercise interventions are increasingly being investigated to better understand independent effects of exercise on insulin resistance and diabetes. Findings from available short-term studies vary; some studies have reported that exercise improves in insulin sensitivity and glucose control while others have reported modest or no change [12,13].

The current review therefore, systemically examined randomized controlled trials with insulin resistance or diabetes patients to determine whether short-term exercise training of 12 weeks or less improves insulin resistance and diabetes in overweight/obese adults.

Methods

Search strategy

This review was conducted in accordance with the Preferred Reporting for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [9]. Details of the protocol for this systematic review were registered on PROSPERO and can be accessed at *http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018086777* Electronic searches were conducted in nine databases; BMC Endocrine Disorders, Clinical Key, Cochrane Library, EBSChost, PubMed, Scopus, Sabinet_SA Publications, The Lancet and Web of Science for prospective studies published from 2005 to 2018. The database searching was conducted using the keywords; insulin resistance, type 2 diabetes, exercise, aerobic training, overweight, obesity, adults, randomized controlled trial, energy expenditure, impaired glucose tolerance. The search was restricted to peer reviewed original research with full-text published in English. Full-text of articles relevant to the current review was available. The reference list of key articles was manually searched for potentially eligible studies to ensure that all trials relevant to this review had been identified.

Search terminology

Medical subject heading used in the current review included: insulin resistance OR type 2 diabetes OR obesity AND exercise OR energy expenditure OR aerobic fitness AND glucose control OR lipid profile.

Inclusion and exclusion criteria

Studies were considered for inclusion if they met the following criteria: 1) Overweight/obese study participants with clearly defined insulin resistance or diabetes status and without co-morbidity; 2) exercise training of any mode, frequency, duration and intensity but follow-up period of twelve weeks or less; 3) studies must have reported on pre and post measures of at least one marker of insulin sensitivity or glucose control, body composition or aerobic fitness; 4) studies must have included details of the outcome measure including instrument, and method as well as methods of data collection and analysis; 5) studies must have randomly assigned participants to exercise and no exercise control groups. Studies with combined exercise and dietary interventions were only considered for inclusion if dietary intervention was uniform across all groups. Review articles and/meta-analysis, author manuscripts and letters to editors were excluded.

Outcome measures

The primary outcomes of the current review included markers of insulin resistance and glucose control (HOMA-IR, QUICKI, HbA1c, FPG), anthropometry (body mass index, body fat) and aerobic fitness (VO_2max, VO_2peak). Data in the studies included in the review were expressed as mean and standard deviation/standard error of the mean and percentage change. The selected outcomes were chosen because they are interrelated. Insulin resistance contributes to impaired glucose control which can cause hyperlipidemia and systemic inflammation. Improvements in body fat and aerobic fitness have been found to improve insulin sensitivity [10-12].

Data extraction

A standardized pre-piloted data extraction form [Joanne Briggs Institute (JBI) Data Extraction Form for Experimental/Observational Studies] was used to extract data from the included studies [13]. Extracted information included study method, study setting and population, sample size, details of the intervention (exercise mode, frequency and duration as well as duration of the intervention period) and measured outcomes.

Study quality assessment

The JBI Critical Appraisal Checklist for Randomized Controlled Trials was used to assess the methodological quality and to determine the extent to which a study addressed the possibility of bias in its design, conduct and analysis [13]. The tool consists of 13 items rated as 'no, 0; unclear, 0; and yes, 1' and includes criteria such as: clear description of the aims, interventions, outcome measurements and participants, representativeness of participant groups, appropriateness of statistical analyses. The checklist included the following questions: 1) was true randomization used; 2) were participants and/ assessors blinded to assignment; 3) were participants analysed in the groups to which they were randomized. The outcome of the critical appraisal was used to inform synthesis and interpretation of the results.

Data analysis

A narrative synthesis of the results was conducted due to the heterogeneity in the measurements of insulin sensitivity and follow-up period of studies included within this review. An increase in the output from research on short-term exercise in insulin resistant or type 2 diabetes will allow for meta-analysis to be conducted.

Results

Identification and selection of studies

Electronic searches yielded 374 articles while manual search yielded two. When duplicates and studies that did not meet the inclusion criteria were removed, three studies remained for quantitative analysis. The most common reasons for exclusion were; topic not relevant to the current review, study not a randomized controlled trial, insulin resistance or diabetes status not clear and intervention longer than twelve weeks (Figure 1).

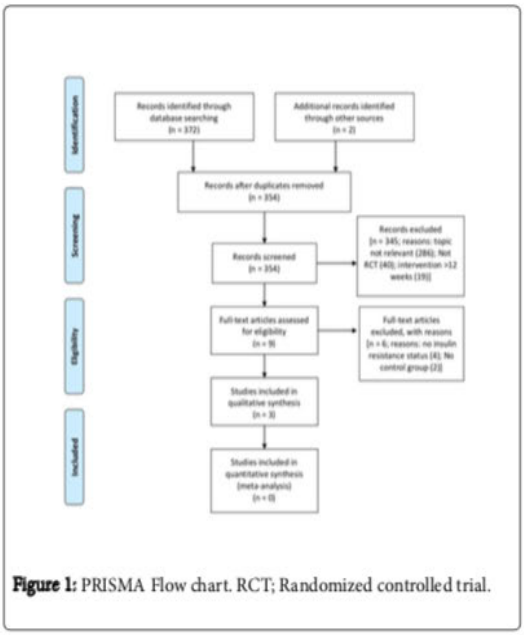

Figure 1: PRISMA Flow chart. RCT; Randomized controlled trial.

Study characteristics

Trial settings and participants: Table 1 shows the characteristics of studies included in the review. Exercise programme settings included University and University hospital [14,15] and clinical research centres [16]. The total number of participants in the included trials ranged from 18 to 132. The mean age ranged from 48 and 65 years.

Intervention: Studies included in this review prescribed individualized aerobic and/or resistance exercise. Of the three studies, one study prescribed aerobic training combined with a balanced (BAL) diet which consisted of 50% carbohydrates, 30% fat and 20% protein [16]. Two studies prescribed moderate intensity exercise while the remaining study prescribed vigorous intensity exercise.

The mode of aerobic exercise used included walking, jogging and running. Progressive resistance training used light weights with exercises designed to target major muscle groups such as legs, shoulders and back.

The duration of exercise sessions in all three trials was 40-60 minutes. Combined aerobic and exercise training groups performed the exercises at half the time required for aerobic and resistance training alone. Of the three trials, two studies prescribed 3 non-consecutive exercise sessions per week for a duration of four and twelve weeks [14,15]. The remaining trial prescribed seven consecutive 50 minutes exercise sessions [16].

Source	Sample size (M/F)	Age (years)	Study quality	Intervention			Outcome measure	Findings
				Group	Intensity	Frequency and duration		
[15]	12 (5/7)	52.09	Moderate	AET	LTHR	3d/week-12 weeks	HOMA-IR, HbA1c, FPG, PPG, TG, hs-CRP, BMI, VO2peak	AET significantly improved fasting glucose, TG and inflammation by 15.6%, 11.2% and 10.8%, RET lowered these parameters by 17.0%, 52.9% and 15.0%, while AET+RET improved them by 9.0%, 19.8% and 10.6% respectively. These improvements occurred without changes in BMI and WC in adults with type 2 diabetes
	12 (5/7)	54.1		RET				
	12 (4/8)	57.9		AET + RET				
	12 (4/8)	53.42		CNT				
[14]	68 (36/32)	57	Moderate	AET + RET	RPE 12-13	3d/week-4 weeks	HOMA-IR, HOMAβ-cell, BG, QUICKI, TG, BMI, VO2max	Combined AET and RET produced a statistically significant 0.2% decrease in TG with a small (1.4%) increase in VO2max but no change in BMI in moderately healthy patients with type 2 diabetes
	64 (34/30)	65		CNT				
[16]	9 (6/3)	48.4	Moderate	AET + BAL diet	70% VO2max	7d	WBIS, PIS, HIS, FPG, BF, VO2max	AET + BAL diet significantly improved WBIS and PIS by 37.5% and 44.4%, respectively without changes in body fat or aerobic fitness in obese adults with mild type 2 diabetes
	9 (7/2)	50.9		BAL diet				

AET: Aerobic exercise training; RET: Resistance exercise training; LTHR: Lactate threshold heart rate; BAL: Balanced; CNT: Control; RPE: Rate of perceived exertion; HOMA-IR: Homeostasis model assessment of insulin resistance; QUICKI: Quantitative insulin check index; WBIS: Whole body insulin sensitivity; PIS: Peripheral insulin sensitivity; HIS: Hepatic insulin sensitivity; BG: Blood glucose; FPG: Fasting plasma glucose; PPG: Post prandial glucose; HbA1c: Hemoglobin A1c; TG: Triacylglycerols; hs-CRP: High sensitivity C-reactive protein; BW: Body weight; BMI: Body mass index; WC: Waist circumference; BF: Body fat; SBP: Systolic blood pressure, DBP: Diastolic blood pressure; VO2max: maximal aerobic capacity; VO2peak: Peak aerobic capacity.

Table 1: Characteristics of studies included in the review (n:3).

Study quality

Overall the studies included in this review scored positively on the JBI Critical Appraisal Checklist for Randomized Controlled Trials [13]. All the included studies treated groups identically other than the intervention of interest. Follow-up was complete, participants were analyzed in the to which they were randomized, and outcomes were measured in a reliable way.

Study outcomes

Insulin resistance: Three studies reported on markers of insulin resistance [14-16]. Of these, one study reported positive findings. Winnick et al reported that during a high-dose insulin post-intervention clamp, exercise plus balanced diet for seven consecutive days significantly improved whole body and peripheral insulin sensitivity by 37.5% ($p<0.05$) and 44.4% ($p<0.0001$), respectively. These variables were unchanged in the balanced diet only group [16]. The remaining two studies reported no change in homeostasis model assessment for insulin resistance (HOMA-IR) and quantitative insulin check index (QUICKI) following four and twelve weeks of exercise training [14,15].

Glucose control: All three studies reported no difference in fasting glucose levels across groups following 7-d, four and twelve weeks of exercise training [14-16]. However, Jorge et al reported that within group comparisons showed that aerobic, resistance and combined aerobic plus resistance exercise training for twelve weeks significantly ($p<0.05$) lowered fasting glucose by 15.6%, 17.0% and 9.0%, respectively [15].

Blood lipids: Two studies assessed lipid profile. Of these, one study reported positive findings; Hordern et al. [19] reported that when compared with usual care, four weeks of exercise training produced a small but significant decrease in TG; 0.2% ($p<0.003$). By contrast, Jorge et al [20] reported no change in TG across groups following twelve weeks of exercise training, although within group comparisons showed that aerobic, resistance and combined aerobic plus resistance exercise training for twelve weeks significantly ($p<0.05$) decreased TG by 11.2%, 52.9% and 19.8%, respectively.

Inflammation: One trial examined inflammatory markers. While there was no statistical difference in high sensitivity C-reactive protein (hs-CRP) across groups, within group comparisons indicated that twelve weeks of aerobic, resistance and combined aerobic and resistance exercise training for twelve weeks significantly ($p<0.05$) decreased hs-CRP by 10.8%, 15.0% and 10.6%, respectively [15].

Anthropometry: All three studies included in the current review described anthropometric data and reported that when compared with a no exercise control group, exercise training had small or no significant effect on body composition including body weight (BW), body mass index (BMI) body fat (BF).

Aerobic fitness: Three studies reported on aerobic fitness. Hordern et al reported small but significant increase in maximal aerobic capacity (VO_2max) of 1.4% ($p<0.011$) following four weeks of exercise training [14] while the remaining two reported no change [15,16]. Jorge et al however, reported that although there was no significant difference across the groups, within groups comparison showed that aerobic exercise training for twelve weeks significantly increased peak oxygen consumption (VO_2peak) by 14.0% ($p<0.05$), while aerobic and/resistance training did not [15].

Discussion

The current review demonstrates that short-term exercise improves insulin sensitivity and glucose control in overweight/obese adults without changes in body composition and/aerobic fitness. Thus, short-term exercise may be applicable as the first line of treatment for insulin resistance and type 2 diabetes. Short-term exercise interventions offer opportunity for close supervision which has been associated with better adherence to exercise programs. Furthermore, the improvements observed with short-term exercise training may encourage individuals to adopt a physically active lifestyle [17].

The current review shows that exercise training for seven consecutive days increased whole body insulin sensitivity, reported in the study by Winnick et al [16] while longer duration studies of four and twelve weeks reported no change [14,15]. The differences could be attributed to higher intensity exercise prescription in the study by Winnick et al [16]. Previous studies have shown that when compared with moderate intensity training, higher intensity training produced superior benefits in insulin sensitivity [18,19]. Recent reports have shown that during high intensity training there is recruitment of a larger proportion of muscle fibres and greater number of type 2 glycolytic muscle fibres, adaptations which contribute to glycogen depletion-induced insulin sensitivity [20,21]. Also, the study by Winnick et al used direct measures of insulin sensitivity while the other studies used surrogate markers (HOMA indices) [14,15]. Indices of insulin resistance have been found to correlate well with direct measures such as the hyperinsulinemic clamp but are not necessarily equivalent [22].

This review also shows that short-term exercise improves triglycerides even at moderate intensity. This is in agreement with a previous review which reported that exercise improves dyslipidaemia in physically inactive individuals [23]. The mechanism by which exercise improves dyslipidaemia remains unclear. Studies however, have suggested that this could be due to exercise-induced activation of lipoprotein lipase (LPL) which is responsible for the hydrolysis of triglycerides [24]. Lipoprotein lipase can be active for up to 24 hours following one hour of exercise [25]. Thus, short-term exercise may be effective for instantaneous improvements in triglycerides which would avertedly improve the prognosis cardiovascular disorders associated with insulin resistance [26].

Studies in this review have shown that short-term exercise does not result in measurable changes in body composition and aerobic fitness. However, aerobic training was found to produce favourable effects on cardiorespiratory fitness compared with resistance training [14,15]. This was in contrast with previous review and meta-analysis which reported that resistance training improved cardiorespiratory fitness in a similar manner as aerobic training [27].

This review contributes to information for future research in understanding effects short-term exercise protocols on metabolic disorders associated with overweight/obesity in physically inactive adults. Our findings are to be interpreted with caution however, because the current review describes a small sample size and studies published in English only.

In the current review, only three studies met the inclusion criteria, suggesting that despite evidence of benefit of short-term exercise, randomised controlled trials are lacking. Trials included in this review were mostly on older adults, and the exercise prescriptions were moderate intensity. Since diabetes is increasingly affecting younger people and exercise training at high intensity is increasingly reported

to produce superior improvements on metabolic parameters; with half the time required for moderate intensity training, future randomized controlled trials to investigate short-term effects of high intensity exercise in younger insulin resistance/diabetes patients are warranted.

In conclusion, the current review provides useful information on the possible clinical applications of short-term exercise to improve insulin sensitivity and control glycaemia. Findings of the current review also show that the therapeutic effects of exercise can occur without changes in body composition and aerobic fitness. Therefore, in addition to encouraging changes in body composition of overweight/obese patients through exercise, improvements in metabolic health which precede changes in body composition should be emphasized.

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CHAPTER IV: MANUSCRIPT 3 - INSULIN RESISTANCE MANUSCRIPT

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Original Research

Effects of Short-Term High versus Continuous Moderate Intensity Training on Insulin Resistance in Overweight and Obese Adults: A Randomized Controlled Trial

TSHIDI THAANE †[1], AYESHA A. MOTALA ‡[2] and ANDREW J. MCKUNE ‡[1,3,4]

[1]Discipline of Biokinetics, Exercise and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, Durban, SOUTH AFRICA; [2]Department of Diabetes and Endocrinology, School of Clinical Medicine, University of KwaZulu-Natal, Durban, SOUTH AFRICA; [3]Discipline of Sport and Exercise Science, University of Canberra Research Institute for Sport and Exercise Science, Faculty of Health, University of Canberra, Canberra, AUSTRALIA; [4]Collaborative Research in Bioactives and Biomarkers (CRIBB) Group, University of Canberra, Canberra, AUSTRALIA

†Denotes graduate student author, ‡Denotes professional author

ABSTRACT

International Journal of Exercise Science 1(2): 1-14, 2019. In long-term exercise training studies (> 6 weeks), improvements in insulin resistance (IR) are amplified by decreased body fat and/or increased cardio-respiratory fitness. This presents a challenge in studying the independed effects of exercise training. Our study purpose was to determine the effects of short-term continuous moderate intensity training (CMIT) and high intensity interval training (HIIT) on IR in overweight/obese adults.

Participants were stratified into insulin sensitive (IS) and IR groups, and randomized into non-exercise control (CNT), CMIT and HIIT sub-groups that underwent baseline and post testing. Exercise sessions were 18-24 minutes for 10 consecutive days. The CMIT sub-group continuously cycled at 60-70% of peak oxygen consumption ($V\dot{O}_2$peak) while the HIIT sub-group performed 60s of cycling at 90-100% $V\dot{O}_2$peak interspersed with 30 seconds of rest.

Ninety-five participants (mean age and BMI 23.9 $\pm$ 3.9 years and 32.1 $\pm$ 5.0 kg/m^2) were enrolled into the study. Of these, 63% were IS and 37% had IR. CMIT or HIIT did not result in statistically significant improvements in IR. However, the reduction (32.4%) in IR observed with HIIT may be of clinical relevance. Cohen's (*d*) effect size (ES) for HIIT on IR was large (ES: *d* = -0.9; 95% CI: -1.7, -0.1) while that of CMIT was unclear (ES: *d* = -0.2; 95% CI: -1.0, 0.6). In the current study, CMIT or HIIT did not result in statistically significant improvements in insulin resistance. Future large-scale studies to clarify and confirm our findings are warranted.
KEY WORDS: Obesity, Chronic disease, Metabolic disorder, Physical inactivity

INTRODUCTION

Insulin resistance commonly arises from a chronic energy surplus associated with overweight and obesity [1]. Individuals who are overweight/obese are at increased risk of cardiometabolic disorders associated with insulin resistance including dyslipidaemia, type 2 diabetes and cardiovascular diseases (CVDs) [2]. Current strategies for the managements of insulin resistance and associated disorders include lifestyle modification (diet and exercise) and pharmacological agents such as insulin, metformin and statins [3-5]. Pharmacological agents however, are often associated with undesirable side effects such as diarrhea, abnormal dreams and myopathy [6].

Exercise and dietary interventions such as high-fat-low-carbohydrate (HFLC) diet, high protein diet and the Mediterranean diet have been reported to result in superior therapeutic effects compared with pharmacological agents [7, 8]. Challenges with dietary interventions, however, include high cost and weak sustainability [9-11]. Conversely, exercise offers an efficient and cost-effective alternative for the management of chronic diseases including insulin resistance [12]. A large body of evidence indicates that exercise training results in improved insulin sensitivity, and consequently, the concentration of insulin required to bring about 50% of its maximal effect on glucose transport is lower [13, 14]. As a result, a maximal insulin stimulus produces a larger increase in glucose transport. Exercise, therefore, improves impaired glucose homeostasis associated with insulin resistance by regulating insulin action.

Metabolic effects of exercise training are primarily dependent on the intensity and duration of the intervention [15, 16]. Chronic training studies have shown that high intensity interval training (HIIT) characterized by brief bouts of all-out effort interspersed with recovery, leads to similar improvements in metabolic outcomes when compared with traditional continuous moderate intensity training (CMIT) currently prescribed for individuals with metabolic disorders [17, 18]. Asymptomatic individuals with insulin resistance or type 2 diabetes are encouraged to achieve a minimum 30 minutes of moderate intensity (less than 60% of heart rate reserve) exercise per day, on at least 5 days per week [19]. Conversely, HIIT requires only 10-20% of the 150 minutes required for CMIT, with the same or possibly better therapeutic effects [20, 21]. However, with long-term training, effects of exercise on insulin action are influenced by decreases in body fat and/or improvements in cardiorespiratory fitness, factors which have been found to independently improve insulin resistance [22, 23]. As a result, short-term interventions (7-10 days) are increasingly being studied to better understand independent effects of exercise on insulin resistance. Despite being less understood, short-term HIIT has been reported to improve metabolic health in insulin resistant and diabetic patients [24, 25]. In these studies, however, the cohorts are often of a single gender, with no distinction made between individuals with and without insulin resistance, and no comparison to a non-exercise control and CMIT group [26]. The aim of the current randomized controlled trial, therefore, was to assess short-term effect of CMIT and HIIT on markers of beta

cell function, insulin sensitivity and insulin resistance in physically inactive overweight/obese adults.

METHODS

Participants
Study participants were male and female adults aged between 18 and 35 years who were physically inactive (less than 30 minutes of physical activity five days a week), overweight/obese (BMI ≥ 25 kg/m2), non-smokers and did not use medications that are known to affect body composition and metabolism.

Staff and Students of the University of KwaZulu-Natal were invited to volunteer to take part in the study through posters and social media outlets. Consenting individuals provided written consent for participation and publication of the results. The study was conducted between August 2016 and October 2017 at the Human Performance Laboratory (HPL) of the University of KwaZulu-Natal, Durban, South Africa. A target sample size of 150 was calculated using an online sample size calculator (Raosoft®) with the confidence level and confidence interval set at 95% and 5% respectively. Recruitment of study participants was stopped at 95 (63% of target sample size) due to financial constraints. The study protocol of this was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (reference: BFC098/16).

Protocol
This was a 10-day, multiple-arm randomized controlled trial designed to determine the effects of short-term CMIT and HIIT on insulin sensitivity (HOMA-IS), beta cell function (HOMA-β) and insulin resistance (HOMA-IR) in physically inactive, overweight/obese adults. Enrolled participants were screened for insulin resistance and categorized as insulin sensitive (IS; HOMA-IR > 1.1 - < 2) or insulin resistant (IR; HOMA-IR > 2) [34]. Using a pseudo-random number generator software (Research Randomizer version 4.0), participants in the IS and IR groups were assigned to a control group (CNT), continuous moderate intensity training (CMIT) or high intensity interval training (HIIT) sub-group. Participants in the CNT sub-group underwent baseline and follow-up evaluation without taking part in the intervention. The CMIT and HIIT sub-groups underwent baseline assessments, ten consecutive days of either CMIT or HIIT, and had follow-up evaluation after the exercise intervention period. Primary outcome measures included body mass index [BMI (kg/m^2)], percentage body fat (%BF), HOMA-IS, HOMA- β, HOMA-IR and peak oxygen consumption ($V\dot{O}_2peak$).

Baseline measurements included exercise pre-participation questionnaire, clinical examination, laboratory and physiological tests. A repeat of baseline measures was conducted at follow-up which was undertaken at least 24 hours after the last exercise bout, within a 48-hour period. Post-training blood draws were conducted at least 24 hours before $V\dot{O}_2peak$ test.

The exercise pre-participation health screening questionnaire focused on the participant's current level of physical activity as well as screening for signs or symptoms of cardio-metabolic disease; physical activity screening questionnaire (PASQ) and pre-exercise intervention questionnaire (PEIQ). The PASQ (themes; activity at work/school, travel to and from places, recreational activities and sedentary behaviour) was adapted from the World Health Organization (WHO) global physical activity questionnaire (GPAQ). The PEIQ (themes; history of cardiovascular events, symptoms, other health issues and cardiovascular risk factors i.e. age, blood pressure and cholesterol) was adapted the American Council for Sport Medicine (ACSM) pre-participating questionnaire.

Height (cm) was measured on a stadiometer without shoes. Waist circumference [WC (cm)] was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest [27]. Hip circumference [HC (cm)] was measured around the widest part of the buttocks [27]. Height, WC and HC were all measured to the nearest centimeter. Waist and hip circumference were used to calculate waist-hip ratio (WHR). An electronic body composition monitor (Omron® BF511 Body Composition Scale) was used to measure body weight [BW (kg)], BMI, %BF, muscle mass percentage [MM (%)] and visceral fat (VF).

Laboratory analyses were conducted at the Lancet Pathology Laboratory (Durban, South Africa). Following a 12-hour overnight fast by participants, venous blood samples were drawn from the ante cubital region of the arm, collected into plain tubes and NaF tubes for the analysis of insulin and glucose, respectively. The samples were stored at -80 °C until further biochemical analysis. Fasting serum insulin was measured by radioimmunoassay [28]. Fasting plasma glucose was measured using a glucose hexokinase method on a Roche automated analyzer [29, 30]. The fasting insulin and glucose were used to calculate HOMA-%β, HOMA%S and HOMA-IR (HOMA2 Calculator© The University of Oxford 2013) [31].

Physiological parameters, $\dot{V}O_2$peak and power output (PO) were assessed by a 2-minute continuous incremental cycling test performed on an electronically braked ergometer (Lode Excalibur Sport, Groningen, The Netherlands) [32, 33]. After participants pedalled at 50 watts for 3 minutes (warm-up), the workload was increased by 25 watts every 2 minutes until volitional fatigue. Blood pressure (mmHg) (Omron® MIT Elite Plus Blood Pressure Monitor) and heart rate (bpm) (Polar S810TM HRM) were measured at rest (before the exercise test), every 2 minutes during the $\dot{V}O_2$peak test and post exercise testing until the values returned toward resting measures. Oxygen consumption ($\dot{V}O_2$) was analyzed by an online breath by breath gas collection system (Cortex MetaMax 3b gas analyser). The highest $\dot{V}O_2$ achieved during the last stage was recorded as the $\dot{V}O_2$peak [34]. Studies have reported that acute exercise can influence insulin up to 72 hours post-exercise [35]. Thus, to minimize the effects of the $\dot{V}O_2$peak test on insulin, cardiorespiratory fitness testing was conducted no less than 4 days before blood samples were collected [35]. Study participants were asked to refrain from exercise before blood samples were collected for insulin assay.

The HIIT exercise protocol was designed based on findings from previous studies which indicated that during HIIT, a 2:1 work to rest ratio yields appropriate training stimulus and is perceived as less difficult by participants [36]. The CMIT group was matched for time. Exercise sessions were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) for 10 consecutive days, with at least 24 hours in-between sessions. Study participants were afforded 3 minutes of warm-up and 3 minutes of cool-down on the ergometer at 50W and 60rpm. During active exercise, participants were required to maintain a workload that corresponded with a wattage that solicited 60-70% $\dot{V}O_2$peak for the CMIT sub-group and 90-100% of $\dot{V}O_2$peak for the HIIT. The workloads were determined from pre-intervention $\dot{V}O_2$peak assessments.

The total time for exercise sessions started at 18 minutes and progressed to 24 minutes over the intervention period. In the current study, we were exploring one of the numerous work-to-rest ratios of HIIT. In studies on trained athletes, HIIT sessions are generally 15-30 minutes. However, little is known about the optimal tolerable combination of intensity and volume necessary for adaptations in clinical populations. The gradual progression in time and intensity was in accordance with *American College of Sports Medicine Guidelines for Exercise testing and Prescription* [37]. Over the 10 days of exercise intervention, the HIIT group spent 33.3% less time in active exercise than the CMIT group, 96 minutes versus 144 minutes of CMIT. Exercise sessions were performed under standard laboratory conditions. The primary investigator documented attendance and ensured that sessions were performed as prescribed. Post-training assessments (repeat of baseline measures) were conducted at least 24 hours after the last exercise bout, within a 48-hour period. A summary of procedures conducted at baseline, during the intervention and post intervention is shown in Table 1, below.

Table 1. Procedures conducted at baseline, during the intervention and post intervention

Baseline	Physical activity questionnaire	Conducted only at baseline		
	Clinical examination	Conducted between 07:00-09:00 AM		
	Venipuncture			
	Aerobic fitness test	Conducted 24 hrs after venipuncture		
Intervention	Days	1-4	5-8	9-10
	Warm-up at 50W and 60rpm for 3 minutes			
	CMIT sessions	12 min cycling with no rest 1 set	15 min cycling with no rest 1 set	18 min cycling with no rest 1 set
	HIIT sessions	60 sec cycling/30 sec rest 8 sets	60 sec cycling/30 sec rest 10 sets	60 sec cycling/30 sec of rest 12 sets
	Cool-down at 50W and 60rpm for 3 minutes			
Post meas	Clinical examination	Conducted between 07:00-09:00 AM, 24 hrs after the last exercise bout		
	Venipuncture			

	Aerobic fitness test	Conducted 24 hrs after venipuncture within 48 hrs of the last exercise bout

Note: CMIT; continuous moderate intensity training, HIIT; high intensity interval training, RPM; revolutions per minute, Hrs; hours

Statistical Analysis

Data was analyzed using SPSS version 24 (IBM, USA). Values are expressed as means and standard deviations (SD). Descriptive statistics were conducted to examine the general characteristics of study participants such as age, BMI, HOMA-IR and VO_2peak. The independent t-test was applied to compare the means at baseline between the sub-groups (CNT, CMIT and HIIT) to ensure no significant differences at the baseline. The comparison was also made between insulin sensitive and insulin resistant participants in the sub-groups. A repeated measure analysis of variable (ANOVA) was used to examine the interaction effect (sub-group × time) for all variables. Where appropriate, pairwise multiple comparison were performed using the Bonferroni post-hoc test. The significance level was set at $p < 0.05$. Percentage changes from baseline to follow-up were calculated for all outcome variables. To determine the magnitude of the changes from baseline to follow-up, Cohen's (d) effect sizes 95% confidence intervals (CI) were calculated. Magnitudes of the standardized effects were interpreted using thresholds of $d \leq 0.2$ (small), $d \geq 0.5$ (moderate) and $d \geq 0.8$ (large) [38]. An effect size was only accepted if the confidence interval did not cross both the positive and negative 0.2 ES thresholds otherwise were considered to be unclear [38].

RESULTS

A total of 95 participants were enrolled into the study. Of these, 38 (40%) were male and 57 (60%) were female. The mean age and BMI were 23 ± 3.9 years and 32.1 ± 5.0 kg/m^2, respectively. Figure 1 outlines the consort diagram. Of the total study group (n = 95), 60 participants (63%) were categorized as insulin sensitive (IS) and 35 (37%) as insulin resistant (IR). When the IS and IR groups were randomised into sub-groups, the CNT and HIIT sub-groups each included 31 participants: IS (n = 20) and IR (n = 11); the CMIT sub-group had 33 participants: IS (n = 20) and IR (n = 13).

Sixty participants were included in the pre-versus post-intervention analysis; of these, 31 were from the insulin sensitive group [CNT (n = 9); HIIT (n = 11); CMIT (n = 11)] and 29 from the insulin resistant group [CNT (n = 5); HIIT (n = 11); CMIT (n = 13)].

Thirty-five participants were lost to follow-up, of whom 29 were from the insulin sensitive group [CNT (n = 10); HIIT (n = 8); CMIT (n = 11)], and 6 were from the insulin resistant CNT group (n = 6). Reasons for discontinuing the study were, relocation (to study at other institutions or for employment) and busy schedule. There was no statistically significant difference in baseline characteristics between participants who completed the study and those who did not.

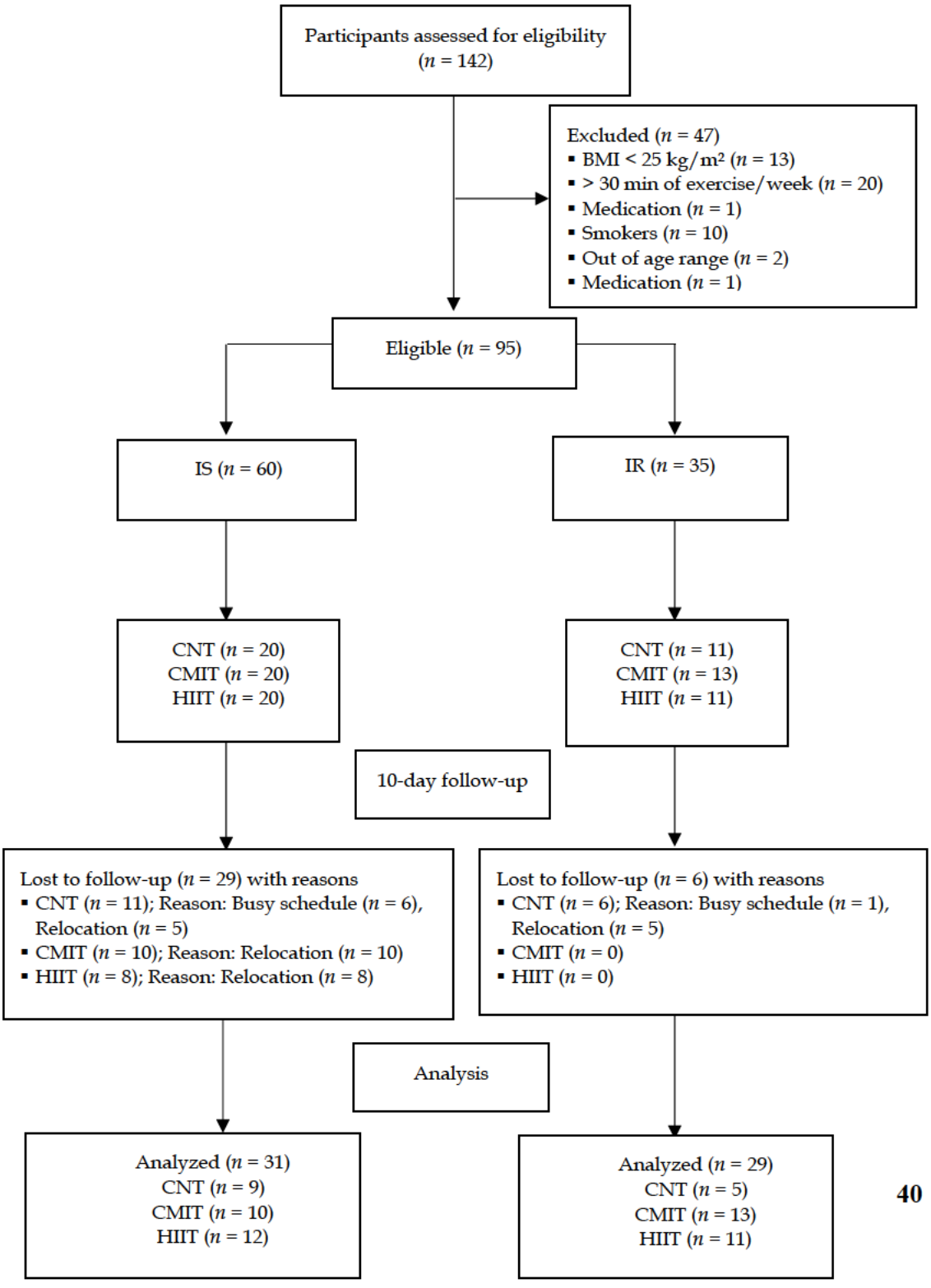
Participants assessed for eligibility (n = 142)
Excluded (n = 47)
▪ BMI < 25 kg/m² (n = 13)
▪ > 30 min of exercise/week (n = 20)
▪ Medication (n = 1)
▪ Smokers (n = 10)
▪ Out of age range (n = 2)
▪ Medication (n = 1)
Eligible (n = 95)
IS (n = 60)
IR (n = 35)
CNT (n = 20)
CMIT (n = 20)
HIIT (n = 20)
CNT (n = 11)
CMIT (n = 13)
HIIT (n = 11)
10-day follow-up
Lost to follow-up (n = 29) with reasons
▪ CNT (n = 11); Reason: Busy schedule (n = 6), Relocation (n = 5)
▪ CMIT (n = 10); Reason: Relocation (n = 10)
▪ HIIT (n = 8); Reason: Relocation (n = 8)
Lost to follow-up (n = 6) with reasons
▪ CNT (n = 6); Reason: Busy schedule (n = 1), Relocation (n = 5)
▪ CMIT (n = 0)
▪ HIIT (n = 0)
Analysis
Analyzed (n = 31)
CNT (n = 9)
CMIT (n = 10)
HIIT (n = 12)
Analyzed (n = 29)
CNT (n = 5)
CMIT (n = 13)
HIIT (n = 11)

Figure 1. Consort diagram. IS-insulin sensitive; IR-insulin resistant; CNT-control; CMIT-continuous moderate intensity training; HIIT-high intensity interval training; BMI-body mass index.

Table 2 shows baseline characteristics of the sub-sample that was followed-up (n = 60). When compared with IS participants, those with IR had significantly higher HOMA-IR in all sub-groups; CNT (p = 0.00), CMIT (p = 0.01) and HIIT (p = 0.02). IR participants had significantly lower aerobic fitness (p = 0.01) compared with IS participants in the CNT sub-group.

Table 2. Baseline characteristics of the sub-sample that was followed-up (n:60) by training intensity.

Characteristics	CNT (n = 14)		CMIT (n = 23)		HIIT (n = 23)	
	IS mean (SD)	IR mean (SD)	IS mean (SD)	IR mean (SD)	IS mean (SD)	IR mean (SD)
n (M; F)	9 (4; 5)	5 (2; 3)	12 (6; 6)	11 (5; 6)	10 (4; 6)	13 (7; 6)
BMI (kg/m^2)	31.6 (4.0)	36.4 (4.4)	30.1 (6.7)	35.3 (4.6)	30.3 (4.2)	32.7 (3.4)
BF (%)	40.2 (7.6)	48.7 (8.0)	39.3 (11.1)	45.1 (8.5)	37.9 (6.9)	43.7 (8.7)
FBG (mmol/L)	4.6 (0.3)	4.8 (0.4)	4.8 (0.5)	4.9 (0.3)	4.7 (0.2)	5.0 (0.4)
HOMA-%ß	118.6 (29.2)	161.7 (27.6)	90.4 (20.0)	151.5 (30.2)	104.0 (12.2)	151.8 (35.4)
HOMA-%S	105.6 (41.8)	54.0 (12.7)	138.3 (50.6)	57.6 (12.4)	106.2 (14.2)	57.2 (18.4)
HOMA-IR	1.4 (0.4)	2.6 (0.4)*	1.2 (0.3)	3.1 (0.7)*	1.5 (0.3)	3.4 (1.3)*
$\dot{V}O_2$peak (ml/min/kg)	26.4 (7.2)	18.8 (1.8)*	26.2 (5.6)	23.0 (4.0)	24.1 (4.5)	22.1 (2.9)

Note: Data are expressed as mean (SD). CNT: control; CMIT: continuous moderate intensity training; HIIT: high intensity interval training; IS: insulin sensitive; IR: insulin resistant; BMI: body mass index; BF: body fat; FBG: fasting blood glucose; HOMA-%ß: homeostasis model of assessment for beta-cell function; HOMA: %S: Homeostasis Model of Assessment for insulin sensitivity; HOMA-IR: homeostatic model of assessment for insulin resistance; $\dot{V}O_2$peak: peak oxygen consumption. *p < 0.05: IS vs IR. Fasting glucose reference range: 3.3-6.0 mmol/L.

Table 3 shows percentage changes in the insulin resistance (IR) group at follow-up (n = 24). High intensity interval training increased HOMA-%S by 33.3% vs. 9.33% in the continuous moderate intensity training (CMIT) sub-group. Insulin resistance (HOMA-IR) was reduced by 32.4% in the HIIT sub-group vs 6.45% in the CMIT sub-group.

Cohen's effect sizes (ES) showed that the effects of CMIT on HOMA-%S and HOMA-IR were unclear in the insulin resistant group while HIIT produced a large (ES: d = 0.9; 95% CI: 0.04.7, 1.8) increase in HOMA-%S and large (ES: d = -0.9; 95% CI: -1.7, -0.1) decrease in HOMA-IR.

Table 3. Changes in characteristics of the insulin resistant (IR) group from baseline to follow-up

Characteristics	Sub-group	Baseline	Follow-up	% change
BMI (kg/m^2)	CNT	36.4 (4.4)	36.2 (4.4)	-0.55
	CMIT	35.3 (4.6)	35.8 (4.5)	1.42
	HIIT	32.7 (3.4)	32.6 (3.4)	-1.21
BF (%)	CNT	48.7 (8.0)	49.2 (7.7)	1.03
	CMIT	45.1 (8.5)	45.7 (8.5)	1.33
	HIIT	43.7 (8.7)	44.0 (8.6)	0.69
FBG (mmol/L)	CNT	4.8 (0.4)	4.9 (0.4)	2.08
	CMIT	4.9 (0.3)	4.9 (0.3)	0.00
	HIIT	5.0 (0.4)	4.8 (0.3)	-4.00
HOMA-%ß	CNT	161.7 (27.6)	166.5 (56.4)	2.97
	CMIT	151.5 (30.2)	143.9 (28.7)	-5.02
	HIIT	151.8 (35.4)	130.5 (24.7)	-14.0
HOMA-%S	CNT	54.0 (12.7)	51.8 (14.9)	-4.07
	CMIT	57.6 (12.4)	63.1 (18.5)	9.93
	HIIT	57.2 (18.4)	76.4 (23.1)	33.3
HOMA-IR	CNT	2.6 (0.4)	3.2 (1.2)	23.1
	CMIT	3.1 (0.7)	2.9 (0.9)	-6.45
	HIIT	3.4 (1.3)	2.3 (1.2)	-32.4
VO_2peak (ml/min/kg)	CNT	18.8 (1.8)	19.3 (2.5)	2.66
	CMIT	23.0 (4.0)	23.2 (3.9)	0.87
	HIIT	22.1 (2.9)	21.5 (4.3)	-2.71

Note: Data are expressed as mean (SD). CNT: control; CMIT: continuous moderate intensity training; HIIT: high intensity interval training; IS: insulin sensitive; IR: insulin resistant; BMI: body mass index; BF: body fat; FBG: fasting blood glucose; HOMA-%ß: homeostasis model of assessment for beta-cell function; HOMA: %S: Homeostasis Model of Assessment for insulin sensitivity; HOMA-IR: homeostatic model of assessment for insulin resistance; VO_2peak: peak oxygen consumption.

DISCUSSION

This study on physically inactive overweight and obese adults has shown that CMIT or HIIT for 10 consecutive days does not result in statistically significant

improvements in beta cell function, insulin sensitivity and insulin resistance. Cohen's (*d*) effects sizes showed that in insulin resistant individuals, HIIT leads to a large (33.3%) increase and a large (32.4%) decrease in insulin sensitivity and insulin resistance, respectively. Thus, although not statistically significant, the effects of HIIT on insulin sensitivity and insulin resistance may be of clinical relevance.

The absence of statistically significant changes (based on *p*-value statistics) in cardiometabolic parameters may be due to small sample size and may not directly indicate that an effect does not exist [39, 40]. Cohen's effect sizes indicated that HIIT produced a large (d = 0.9) increase in HOMA%S and a large (d = -0.9) decrease in HOMA-IR while those of CMIT were unclear. Previous studies have indeed reported that when compared with CMIT, HIIT results in similar improvements in insulin sensitivity and insulin resistance even with the significantly reduced time spent exercising for the HIIT groups [41-43]. In those studies, however, there was concomitant improvement in cardiovascular fitness and decreases in body fat following HIIT. Improved cardiovascular fitness and decreased body fat have been reported to independently improve insulin sensitivity. Consequently, this presents a challenge in drawing conclusions on whether the improvements were from the exercise *per se*. In the present study, the positive effects of HIIT on insulin sensitivity and insulin resistance occurred in the absence of changes in body composition and cardiorespiratory fitness, suggesting that the observed improvements may be related to HIIT training. Possible mechanism for HIIT-induced metabolic adaptations include enhanced insulin signaling in skeletal muscle via increased insulin-stimulated phosphorylation of AS160 which regulates GLUT4 translocation [44]. Our findings however, are to be interpreted cautiously as other studies have found no clear advantage in the improvement of insulin sensitivity in short-term studies [45].

The strengths of this study include the randomized controlled design, categorization of participants into insulin sensitive and insulin resistant groups and use of Cohens (*d*) effect sizes to quantify the effects of the exercise intervention. Our study findings, however, need to be interpreted with caution. Limitations included high attrition rate, small sample and uncontrolled diet. Relocation to areas outside the study site and frequent visits to the laboratory may have contributed to the high attrition rate. The small sample size (63% of the estimated sample) which decreased the statistical power of the study. Participants were asked to maintain their regular diet, some individuals however, may have been encouraged to make healthier dietary choices upon enrollment into the study, this may have influenced the results.

Future large-scale studies with controlled diet and a broader representation of the South African population are necessary to enable a much wider interpretation and application of the findings. Furthermore, the current study explored one of the numerous possible combinations of work to rest ratio during HIIT. The ideal HIIT exercise prescription for individuals with insulin resistance/diabetes remains to be elucidated. Studies to establish the ideal combination of work to rest ratio, frequency and duration during HIIT training are needed.

In conclusion, the present study revealed that CMIT or HIIT for 10 consecutive days does not result in statistically significant improvements in beta cell function, insulin sensitivity and insulin resistance. However, future large-scale studies to clarify and confirm the effects of CMIT and HIIT in physically inactive overweight/obese adults.

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CHAPTER V: MANUSCRIPT 4 - ENDOTHELIAL FUNCTION MANUSCRIPT

Submitted to Clinical Science

Short-term High Intensity Interval Training Improves Microvascular Endothelial Dysfunction in Overweight/Obese Adults with Insulin Resistance: A Randomized Controlled Trial

Tshidi Thaane[1@], Ayesha A Motala[2] and Andrew J McKune[1,3,4]

[1]Discipline of Biokinetics, Exercise and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, South Africa; E-Mail: t.thaane@gmail.com

[2]Department of Diabetes and Endocrinology, School of Clinical Medicine, University of KwaZulu-Natal, South Africa; E-Mail: motala@ukzn.ac.za

[3]Discipline of Sport and Exercise Science, University of Canberra Research Institute for Sport and Exercise Science, Faculty of Health, University of Canberra, Australia; E-Mail: andrew.mckune@canberra.edu.au

[4]Collaborative Research in Bioactives and Biomarkers (CRIBB) Group, University of Canberra, Australia

[@]Author to whom correspondence should be addressed: Ms. Tshidi Thaane

E-Mail: t.thaane@gmail.com (TT)

Trial registration

Pan African Clinical Trial Registry: PACTR201802003021680

Abstract

Background

Exercise enhances cardiovascular health via numerous mechanisms including improved systemic inflammation and microvascular endothelial function. It is unclear whether high intensity interval training (HIIT) is superior to continuous moderate intensity training (CMIT) in a clinical population.

Objective

Determine the effects of HIIT or CMIT on inflammation and microvascular endothelial function in overweight/obese adults with insulin resistance (IR).

Methods

Participants were stratified into insulin sensitive (IS) and IR groups and randomized into control (CNT), HIIT or CMIT sub-groups. Exercise sessions were 18-24 minutes. The HIIT sub-group cycled for 60s at 90-100% peak oxygen consumption ($\dot{V}O_2$peak) interspersed with 30s of rest. CMIT sub-group cycled continuously at 60-70% $\dot{V}O_2$peak. Microvascular post-occlusive reactive hyperaemia (PORHmax and PORHpeak) and mean velocity (MV) were assessed by laser Doppler flowmetry (LDF) and serum C-reactive protein (CRP) by enzyme-linked immunosorbent assay (ELISA), pre and post-10days of exercise.

Results

Ninety-five participants were enrolled, 60 (63%) (IS/IR; 31/29) completed the study. In IR participants, HIIT improved PORHmax, PORHpeak and MV by 21.63%, 17.93% and 28.07%, respectively, while CMIT increased PORHmax and PORHpeak by 8.77% and 10.21%, respectively, and decreased MV by 20.53%. Cohens (*d*) effect size of HIIT on PORHmax, PORHpeak and MV was large (*d*=1.2; 95%CI: 0.01,2.4, *d*=1.1; 95%CI: 0.04,2.3 and *d*=1.2;

95%CI: 0.07,2.3, respectively), while that of CMIT was unclear (*d*=0.8; 95%CI: -0.1,1.9, *d*=0.8; 95%CI: -0.1,1.9 and *d*=0.7; 95%CI: -0.3,1.7, respectively).

Conclusion

High intensity training is associated with sizeable improvements in endothelial dysfunction in adults with insulin resistance. Future large-scale studies to confirm our findings are warranted.

Keywords: Africa, atherosclerosis, peripheral vascular disease, physical activity, skin blood flow

Abbreviations list

CMIT	Continuous moderate intensity training
CNT	Control
CRP	C-reactive protein
HIIT	High intensity interval training
LDF	Laser Doppler flowmetry
MV	Mean velocity
PORH	Post occlusive reactive hyperaemia
IR	Insulin resistance
IS	Insulin sensitive

INTRODUCTION

Insulin resistance is increasingly affecting young adults due to chronic physical inactivity [1-3]. Insufficient energy expenditure causes overweight and obesity which have been found to be principal contributors in the pathogenesis of insulin resistance [4]. Consequences of insulin resistance include dyslipidemia, type 2 diabetes and cardiovascular diseases (CVDs) [5]. Current strategies for the managements of insulin resistance and associated disorders includes lifestyle modification (diet and exercise) and pharmacological agent such as insulin, metformin and statins [6-8]. Pharmacological agents however, may be associated with poor patient compliance due to undesirable side effects [9].

Previous studies have shown that exercise and dietary interventions such as high-fat-low-carbohydrate (HFLC) diet, high protein diet and the Mediterranean diet can result in superior therapeutic effects compared with pharmacological agents [10, 11]. Dietary interventions, however, are limited due to concerns about their sustainability, efficacy and safety [12-14]. Conversely, exercise offers an efficient and cost-effective alternative for the management of diseases of lifestyle including insulin resistance [15]. Exercise induced adaptations result in improved insulin sensitivity, and consequently, the concentration of insulin required to bring about 50% of its maximal effect on glucose transport is lower [16, 17]. A study by Nassis et al., reported that 12-weeks of exercise training improves insulin sensitivity by reducing insulin-like growth factor-1 (IGF-1) [18]. Consitt et al., reported that exercise training for 12-weeks may inhibit insulin-induced phosphorylation of AS160 on specific sites in skeletal muscle contributes to the insulin resistance [19]. In another study, exercise training was found to improve insulin responsiveness by increasing glucose transporter 4 (GLUT4) in skeletal muscle [20].. As a result, a maximal insulin stimulus produces a larger increase in glucose transport. Exercise, therefore, improves impaired glucose homeostasis associated with insulin resistance by regulating insulin action.

Metabolic effects of exercise training are primarily dependent on the intensity and duration of the intervention [21, 22]. Chronic training studies have shown that high intensity interval training (HIIT) characterized by brief bouts of all-out effort interspersed with recovery, leads to similar improvements in metabolic outcomes when compared with traditional continuous moderate intensity training (CMIT) currently prescribed for individuals with metabolic disorders [23, 24]. Asymptomatic individuals with insulin resistance or type 2 diabetes are encouraged to achieve a minimum 30 minutes of moderate intensity (less than 60% of heart rate reserve) exercise per day, on at least 5 days per week [25]. Conversely, HIIT requires only 10-20% of the 150 minutes required for CMIT, with the same or possibly better therapeutic effects [26, 27]. However, with long-term training, effects of exercise on insulin action are influenced by decreases in body fat and/or improvements in cardiorespiratory fitness, factors which have been found to independently improve insulin resistance [28, 29]. As a result, short-term interventions (7-10 days) are increasingly being studied to better understand independent effects of exercise on insulin resistance. Despite being less understood, short-term HIIT has been reported to improve metabolic health in insulin resistant and diabetic patients [30, 31]. In these studies, however, the cohorts are often of a single-gender, with no distinction made between individuals with and without insulin resistance, and no comparison to a no exercise control and CMIT group [32].

The aim of the current randomized controlled trial, therefore, was to assess short-term effect of HIIT or CMIT on markers of beta cell function and insulin resistance in physically inactive

Materials and methods

Study design

The study was a 10-day randomized controlled trial designed to determine the effects of short-term HIIT and CMIT on systemic inflammation and microvascular endothelial function in overweight/obese adults with IR.

Procedure

Enrolled participants were screened for IR and stratified into insulin sensitive (IS) or IR groups. Participants in the IS and IR groups were assigned to a control (CNT), HIIT or CMIT sub-group using a pseudo-random number generator software (Research Randomizer version 4.0). Participants in the CNT sub-group underwent baseline and follow-up evaluation without taking part in the intervention. Participants in the HIIT and CMIT sub-groups underwent baseline assessments, 10 consecutive days of either HIIT or CMIT, and had follow-up assessments after the intervention period.

Participants and study setting

The inclusion criteria were overweight/obese (BMI $\geq$ 25 kg/m^2) adults between 18 and 35 years who were physically inactive (less than 30 minutes of physical activity five days a week), non-smokers and did not use medications that are known to affect body composition and metabolism. Students and staff of the University of KwaZulu-Natal were invited to volunteer to take part in the study through posters and social media. Consenting individuals provided written consent for participation and publication of the results. The study was conducted between August 2016 and October 2017 at the Human Performance Laboratory (HPL) of the University of KwaZulu-Natal, Durban, South Africa. A target sample size of 150 was calculated using an online sample size calculator with the confidence level set at 95%. The protocol of this was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (reference: BFC098/16).

Clinical examination

Height was measured on a stadiometer without shoes. Waist circumference (WC) and hip circumference (HC) were measured at the level of the umbilicus and iliac crest, respectively. Height, WC and HC were all measured to the nearest centimeter. The WC and HC were subsequently used to calculate waist-hip ratio (WHR). A bio-electrical impedance analysis machine (Omron® BF511 Body Composition Scale) was used to measure body weight (BW), body mass index (BMI), percentage body fat (%BF), muscle mass percentage (%MM) and visceral fat (VF). Blood pressure (mmHg) was measured using a digital blood pressure device (Omron® MIT Elite Plus Blood Pressure Monitor). A light-weight heart rate monitor (Polar S810™ HRM) was used to monitor heart rate (BPM).

Blood tests

Following a 12-hour overnight fast, venous blood samples were drawn from the ante cubital region of the arm, collected into plain tubes and NaF tubes for the analysis of insulin and glucose, respectively. The samples were stored at -80 °C until further biochemical analysis. Laboratory tests were conducted at the Lancet Pathology Laboratory (Durban, South Africa). Fasting serum insulin and serum C-reactive protein (CRP) were measured by radioimmunoassay and ELISA, respectively. Plasma glucose was measured using a glucose hexokinase method on a Roche automated analyzer. Serum insulin and plasma glucose were used to calculate HOMA-IR (HOMA2 Calculator© The University of Oxford 2013) [14]. Insulin resistance was defined as HOMA-IR ≥ 2. Participants with HOMA-IR ≥ 1.1 - < 2 were considered to be insulin sensitive [15].

Endothelial function test

Laser Doppler flowmetry (LDF) system (moorVMS-Instruments®, United Kingdom) with computerized software; moorVMS-PC V4.0 (moorVMS-Instruments®, United Kingdom) was used to assess microvascular endothelial function at baseline and post follow-up using a protocol adapted from Morales et al., 2005 [16]. Briefly, participants were asked to assume a supine position with the right arm measured at a 45°C using a goniometer in a supinated position. The left arm was placed on the chest. Participants were allowed 15 minutes to acclimatize to laboratory conditions regulated at 22 ± 1°C and 50 ± 5 relative humidity. A blood pressure cuff [(CUFF-ARM RD) moorVMS-Instruments®, United Kingdom] and a fibre skin laser probe [(VP1T/7) (moorVMS-Instruments®, United Kingdom] were placed on the distal portion of upper arm and right palmar forearm (4 cm distal to the antecubital crease), respectively. An additional 15 minutes was allowed before the measurement of baseline cutaneous flux recorded for 1 minute. The cuff was inflated to a pressure of 50 mmHg above resting systolic blood pressure; the occlusion was maintained for 3 minutes. The cuff was rapidly released and reactive hyperemic response was measured using the laser doppler probe. Variables measured included resting flux (RF), amplitude of peak flux (PORHpeak), maximum increase in post-occlusive hyperaemia (PORHmax), time-to-peak (Tp) flux and mean velocity of the hyperemic response (PORHmax/Tp). Peak flux and RF (PORHpeak-RF) were used to calculate PORHmax. PORHmax and TP (PORHmax/Tp) were used to compute mean velocity (MV).

Pre-exercise training protocol

Study participants underwent a continuous incremental cycling test on an electronically braked ergometer (Lode Excalibur Sport, Groningen, The Netherlands) for peak oxygen consumption ($\dot{V}O_2peak$) and power output (PO). After participants cycled at 50 watts for 3 minutes, the

workload was increased by 25 watts every 2 minutes until volitional fatigue. Oxygen consumption ($\dot{V}O_2$) was analyzed by an online breath by breath gas collection system (Cortex MetaMax 3b gas analyser). The highest $\dot{V}O_2$ achieved during the last stage was recorded as the $\dot{V}O_2$peak. Ventilatory threshold 1 (VT1) and ventilatory threshold 2 (VT2) were determined by the software. The $\dot{V}O_2$peak test was conducted no less than 4 days before the blood samples were collected to minimised its effects of insulin levels.

Intervention protocol

Study participants performed a total of 18-24 minutes (including warm up and cool down for 3 minutes) cycling on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) for 10 consecutive days, with at least 24 hours in-between sessions. Participants were required to maintain a workload that corresponded with a wattage that solicited 90-100% of $\dot{V}O_2$peak for the HIIT or 60-70% for the CMIT group. On days 1-4, the HIIT group performed 8 bouts of 60 seconds all-out cycling at 100% of $\dot{V}O_2$peak separated by 30 seconds of active or complete rest while the CMIT group cycled continuously at 70% $\dot{V}O_2$peak for 12 minutes. On days 5-8, the HIIT group performed 10 bouts of 60 seconds all-out cycling at 95% of $\dot{V}O_2$peak separated by 30 seconds of active or complete rest while the CMIT group cycled continuously at 65% $\dot{V}O_2$peak for 15 minutes. On days 9-10, the HIIT group performed 12 bouts of 60 seconds all-out cycling at 90% of $\dot{V}O_2$peak separated by 30 seconds of active or complete rest while the CMIT group cycled continuously at 60% $\dot{V}O_2$peak for 18 minutes. The gradual progression in time and intensity was in accordance with *American College of Sports Medicine Guidelines for Exercise testing and Prescription*. Over the 10 days of exercise intervention, the HIIT group spent 33.33% less time in active exercise than the CMIT group, 96 minutes versus 144 minutes of CMIT. The exercise protocol was modified from the proposed protocol following further review of literature (S2). Exercise sessions were

performed under standard laboratory conditions. The primary investigator documented attendance and ensured that sessions were performed as prescribed.

Post-exercise training protocol

Post-training assessments were conducted at least 24 hours after the last exercise bout, within a 48-hour period. Post-training blood draws were conducted at least 24 hours before $\dot{V}O_2$peak test.

Statistical analysis

Data was analyzed using SPSS version 24 (IBM, USA). Data was tested for normal distribution using Shapiro-Wilk test. Generalized linear model (GLM) was used on all the data for each parameter tested to identify any significant changes within each participant's set of data, between participants and over time using a three-way ANOVA. Where appropriate, pair-wise multiple comparisons were performed using the Bonferroni post-hoc test. The significance level was set at $p < 0.05$. Cohen's (d) effect sizes and 95% confidence intervals (CI) were calculated for all outcome measures. Magnitudes of the standardized effects were interpreted using thresholds of $d \leq 0.2$ (Small), $d \geq 0.5$ (Moderate) and $d \geq 0.8$ (Large) [17]. Effect size was only accepted if the confidence interval did not cross both the positive and negative 0.2 ES thresholds otherwise were considered to be unclear [17].

Results

Ninety-five eligible participants [38 (40%) males and 57 (60%) females] were enrolled into the study. Of these, 88 were black Africans, 5 Asian Indians, 1 Coloured (mixed-race) and 1 Caucasian. Mean age was 23 ± 3.9 years and mean BMI 32.1 ± 5.0 kg/m^2.

Figure 1 outlines the study flow. Of the total study group (n = 95), 60 participants (63%) were IS and 35 (37%) had insulin resistance (IR). When the IS and IR groups were randomised into sub-groups, the CNT and HIIT sub-groups each included 31 participants: IS (n = 20) and IR (n=11); the CMIT sub-group had 33 participants: IS (n = 20) and IR (n = 13).

Sixty (63.2%) participants were included in the pre-versus post-intervention analysis; of these, 31 were from the IS group [CNT (n = 9); HIIT (n = 11); CMIT (n = 11)] and 29 from the insulin resistant group [CNT (n = 5); HIIT (n = 11); CMIT (n = 13)].

Thirty-five (37%) participants were lost to follow-up, of whom 29 were from the IS group [CNT (n = 10); HIIT (n = 8); CMIT (n = 11)], and 6 were from the insulin resistant CNT group (n = 6). Reasons for discontinuing the study were, relocation and busy schedule. There was no statistically significant difference in baseline characteristics between participants who completed the study and those who did not.

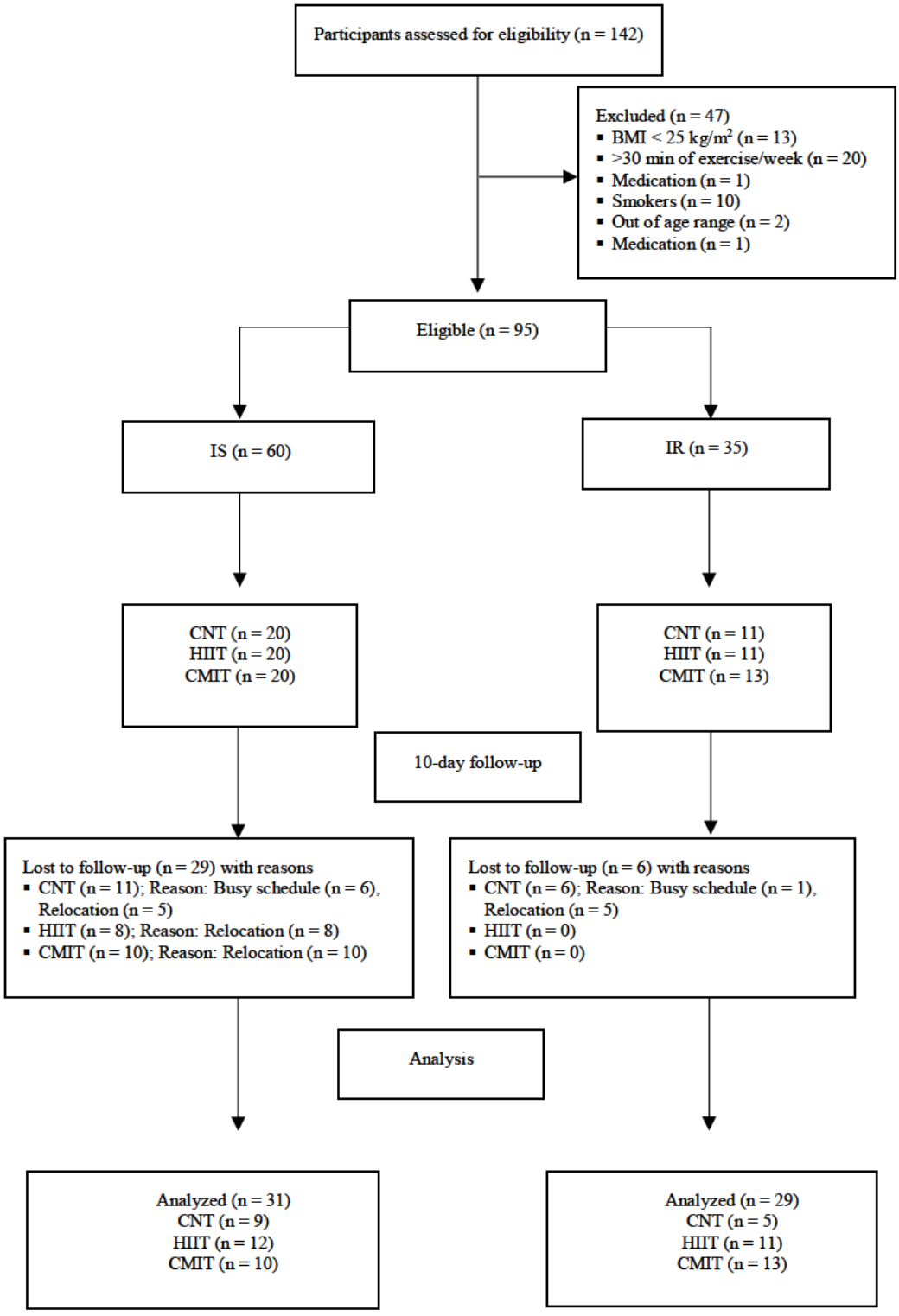

Figure 1. Study Flow chart. IS-insulin sensitive; IR-insulin resistant; CNT-control; HIIT-high intensity interval training; CMIT-continuous moderate intensity training; BMI-body mass index.

Table 1 shows the baseline characteristics of the total study group and sub-group of participants who were followed-up categorized by insulin resistance status. In the total study group, the IR group had significantly higher BMI ($p = 0.00$), BF ($p = 0.00$), HOMA-IR ($p = 0.00$) and CRP ($p = 0.04$). In the sub-group of participants who were followed-up, the IR group had significantly higher HOMA-IR ($p = 0.00$) and lower $\dot{V}O_2max$ ($p = 0.04$).

Table 1: Baseline characteristics of the total study group (n: 95) and sub-group followed-up (n: 60) categorized by insulin resistance (IR) status

Characteristics	Total study group (n: 95)		Sub-group (n: 60)	
	IS	IR	IS	IR
n (M; F)	60 (21; 39)	35 (17; 18)	31 (14; 17)	31 (14; 17)
BMI (kg/m^2)	31.3 (4.6)	37.3 (2.4)***	30.6 (5.0)	34.5 (4.3)
BF (%)	39.0 (8.9)	45.5 (8.0) ***	39.0 (8.4)	45.2 (8.4)
HOMA-IR	1.2 (0.5)	3.1 (0.9) ***	1.4 (0.4)	3.1 (0.9)***
CRP (mg/L)	3.3 (2.3)	5.6 (4.7) *	3.1 (2.8)	6.0 (5.0)
RF (APU)	7.4 (4.1)	6.1 (2.9)	7.0 (4.1)	6.1 (3.0)
PORHmax (APU)	52.9 (27.2)	46.1 (15.8)	52.6 (27.7)	45.3 (17.1)
PORHpeak (APU)	60.3 (29.1)	52.2 (17.0)	59.5 (29.5)	51.3 (18.4)
MV (APU)	6.8 (4.6)	6.0 (3.4)	6.7 (4.5)	9.1 (8.2)
TP (s)	9.9 (6.1)	9.2 (3.6)	9.3 (4.0)	9.4 (3.5)
VT1 (ml/min/kg)	16.0 (3.1)	15.9 (2.9)	16.7 (3.4)	16.0 (2.9)
VT2 (ml/min/kg)	21.6 (4.6)	19.3 (3.0)	22.0 (4.8)	19.3 (2.8)
$\dot{V}O_2$peak (ml/min/kg)	25.2 (5.6)	22.1 (3.7)	26.2 (5.7)	22.6 (3.5)*

Values are expressed as mean (SD).
*p < 0.05; *** p < 0.001.
BMI: Body mass index, BF: Body fat, HOMA-IR: Homeostatic model assessment for insulin resistance, CRP: C-reactive protein, RF: Resting flux, PORHmax: Post occlusive reactive hyperaemia, PORHpeak: Post occlusive reactive hyperaemia peak, APU: Arbitrary perfusion units, MV: Mean velocity, TP: Time to peak, VT1: Ventilatory threshold 1, VT2: Ventilatory threshold 2, $\dot{V}O_2$peak, Peak maximal aerobic capacity.

Table 2 shows the baseline characteristics of the total study group (n: 90) categorized by exercise intensity. When compared with IS subjects, IR participants in the CNT group had significantly higher BMI ($p = 0.04$) and lower MV ($p = 0.02$), Tp ($p = 0.00$), VT1 ($p = 0.04$), VT2 ($p = 0.00$) and $\dot{V}O_2peak$ ($p = 0.01$). IR participants in the HIIT group had significantly higher BMI ($p = 0.03$), %BF ($p = 0.02$), but lower VT1 ($p = 0.04$) and $\dot{V}O_2peak$ ($p = 0.02$). In the CMIT group, IR participants had significantly higher BMI ($p = 0.04$), %BF ($p = 0.01$) when compared with the IS group. There was no significant difference in CRP between the IS and IR groups in the CNT, HIIT and CMIT subgroups.

Table 2: Baseline characteristics of the total study group (n: 95) categorized by exercise intensity

Characteristics	CNT (n = 31)		HIIT (n = 31)		CMIT (n = 33)	
	IS	IR	IS	IR	IS	IR
n (M; F)	20 (10; 10)	11 (4; 7)	20 (6; 14)	11 (5; 6)	20 (5; 15)	13 (7; 6)
BMI (kg/m^2)	31.0 (3.5)	35.9 (5.1)*	29.5 (3.6)	32.7 (3.4)*	30.3 (5.9)	35.2 (4.6)*
BF (%)	39.8 (7.6)	47.6 (6.1)	40.4 (6.5)	43.7 (8.7)*	42.7 (9.5)	45.1 (8.5) *
HOMA-IR	1.3 (0.5)	2.7 (0.6)	1.3 (0.5)	3.4 (1.3)	1.2 (0.5)	3.1 (0.73)
CRP (mg/L)	3.0 (1.8)	6.5 (4.3)	3.3 (4.2)	4.4 (4.0)	2.4 (2.0)	7.2 (6.2)
RF (APU)	7.4 (5.2)	6.1 (4.2)	6.1 (2.2)	6.4 (3.0)	8.6 (4.7)	6.3 (2.8)
PORHmax (APU)	38.3 (11.3)	37.4 (8.3)	50.4 (16.8)	40.7 (13.5)	65.0 (41.3)	51.0 (21.0)
PORHpeak (APU)	45.7 (11.7)	43.5 (6.8)	56.5 (18.0)	47.1 (15.0)	73.6 (44.3)	57.1 (23.0)
MV (APU)	5.5 (1.8)	4.0 (1.8) *	7.0 (5.5)	5.1 (2.5)	7.6 (5.0)	7.0 (4.0)
TP (s)	7.2 (2.2)	11.2 (3.0)***	11.0 (5.1)	9.3 (4.0)	10.0 (3.3)	9.0 (3.4)
VT1 (ml/min/kg)	17.0 (4.2)	15.0 (2.3)*	17.0 (3.6)	15.4 (2.4) *	16.0 (2.4)	17.0 (4.0)
VT2 (ml/min/kg)	23.0 (5.3)	17.0 (1.2)***	21.0 (4.1)	20.0 (3.1)	23 (5.2)	20.0 (2.8)
$\dot{V}O_2$peak (ml/min/kg)	25.1 (6.1)	19.6 (3.4)*	23.6 (3.7)	22.1 (2.9)*	23.0 (5.4)	23.0 (3.9)

Values are expressed as mean (SD).
*p < 0.05; *** p < 0.001
CNT: Control, HIIT: High intensity interval training, CMIT: continuous moderate intensity training, IS: Insulin sensitive, IR: Insulin resistance, BMI: Body mass index, BF: Body fat, HOMA-IR: Homeostatic model assessment for insulin resistance, CRP: C-reactive protein, RF: Resting flux, PORHmax: Post occlusive reactive hyperaemia, PORHpeak: Post occlusive reactive hyperaemia peak, APU: Arbitrary perfusion units, MV: Mean velocity, TP: Time to peak, VT1: Ventilatory threshold 1, VT2: Ventilatory threshold 2, ẆO2peak, Peak maximal aerobic capacity.

Table 3 shows the baseline characteristics of the sub-group of participants who were followed-up (n: 60) categorized by exercise intensity. When compared with IS subjects, IR participants in CNT group had significantly higher HOMA-IR (p = 0.00) and lower Tp (p = 0.03), VT1 (p = 0.01), VT2 (p = 0.00) and $\dot{V}O_2$peak (p = 0.01); in the HIIT and CMIT groups, IR participants had significantly higher HOMA-IR (p = 0.02) and HOMA-IR (p = 0.02), respectively. There was no statistical difference in CRP between the IS and IR groups in the CNT, HIIT and CMIT subgroups.

Table 3: Baseline characteristics of the sub-group that was followed-up (n: 60) categorized by exercise intensity

Characteristics	CNT (n = 14)		HIIT (n = 2 3)		CMIT (n = 23)	
	IS	IR	IS	IR	IS	IR
n (M; F)	9 (4; 5)	5 (2; 3)	12 (6; 6)	11 (5; 6)	10 (4; 6)	13 (7; 6)
BMI (kg/m^2)	34.4 (4.3)	36.4 (4.4)	31.3 (4.6)	32.6 (4.2)	32.0 (8.2)	35.2 (4.6)
BF (%)	38.2 (6.4)	48.7 (8.0)	34.0 (2.6)	35.4 (4.7)	34.6 (14.8)	45.1 (8.5)
HOMA-IR	1.3 (0.4)	2.6 (0.4)***	1.7 (0.1)	3.1 (1.7) *	1.3 (0.3)	3.1 (0.73)*
CRP (mg/L)	1.3 (0.4)	6.5 (4.3)	3.1 (1.7)	5.3 (4.6)	3.0 (2.0)	7.2 (6.2)
RF (APU)	6.2 (5.2)	4.3 (4.2)	5.9 (2.2)	6.2 (2.9)	8.5 (4.7)	6.3 (2.8)
PORHmax (APU))	40.3 (11.4.)	37.7 (13.1)	31.4 (3.3)	39.6 (13.5)	56.7 (41.4.)	51.0 (21.0)
PORHpeak (APU)	46.5 (11.7)	44.3 (6.8)	54.1 (18.0)	45.8 (15.0)	65.3 (44.3)	57.1 (23.0)
MV	5.8 (1.8)	7.5 (6.2)	5.9 (5.5)	4.7 (2.5)	6.6 (4.9)	7.0 (4.0)
TP (s)	7.4 (2.2)	11.0 (2.9) *	11.0 (5.1)	9.6 (4.0)	9.7 (3.3)	9.0 (3.4)
VT1 (ml/min/kg)	17.3 (4.2)	15.5 (2.3) *	17.0 (3.6)	15.4 (2.4)	15.9 (2.4)	17.0 (4.0)
VT2 (ml/min/kg)	23.0 (5.3)	17.0 (1.2)***	21.0 (4.1)	19.5 (2.5)	22.7 (5.2)	20.0 (2.8)
$\dot{V}O_2$peak (ml/min/kg)	26.4 (7.2)	18.5 (1.8)*	23.6 (3.7)	24.2 (4.5)	26.2 (5.6)	23.0 (3.9)

Values are expressed as mean (SD).
*p < 0.05; *** p < 0.001.
CNT: Control, HIIT: High intensity interval training, CMIT: continuous moderate intensity training, IS: Insulin sensitive, IR: Insulin resistance, BMI: Body mass index, BF: Body fat, HOMA-IR: Homeostatic model assessment for insulin resistance, CRP: C-reactive protein, RF: Resting flux, PORHmax: Post occlusive reactive hyperaemia, PORHpeak: Post occlusive reactive hyperaemia peak, APU: Arbitrary perfusion units, MV: Mean velocity, TP: Time to peak, VT1: Ventilatory threshold 1, VT2: Ventilatory threshold 2, V̇O2peak, Peak maximal aerobic capacity.

Table 4 shows the percentage (%) change and Cohen's (*d*) effect sizes (ES) of HIIT and CMIT on primary outcomes of the IR group. HIIT increased PORHmax, PORHpeak and MV by 21.63%, 17.93% and 28.07%, respectively, while CMIT increased PORHmax and PORHpeak by 8.77% and10.21%, respectively, and decreased MV by 20.53%. The ES of HIIT on PORHmax, PORHpeak and MV was large (ES: *d* = 1.2; 95% CI: 0.01, 2.4), (ES: *d* = 1.1; 95% CI: 0.04, 2.3) and (ES: *d* = 1.2; 95% CI: 0.07, 2.3), respectively, while that of CMIT was unclear (ES: *d* = 0.8; 95% CI: -0.1, 1.9), (ES: *d* = 0.8; 95% CI: -0.1, 1.9) and (ES: *d* = 0.7; 95% CI: -0.3, 1.7), respectively.

Table 4: Percentage change and effect sizes of high intensity interval training (HIIT) and continuous moderate intensity training (CMIT) of the insulin resistant group (n = 24)

Variable	HIIT	ES	Interpretation	CMIT	ES	Interpretation	P value
n (M; F)	11 (5; 6)			13 (7; 6)			
BMI (kg/m^2)	-0.14	-1.0 (-2.1, 0.06)	Unclear	1.46	-0.4 (-0.9, 1.0)	Unclear	0.06
BF (%)	0.52	-0.6 (-1.7, 0.4)	Unclear	1.42	-0.4 (-1.5, 0.5)	Unclear	0.63
HOMA-IR	-31.91	-1.0 (-2.1, 0.1)	Unclear	-5.57	-0.4 (-1.4, 0.6)	Unclear	0.16
CRP (mg/L)	43.65	-0.6 (-1.7, 0.3)	Unclear	-22.81	-0.9 (-1.9, 0.1)	Unclear	0.46
RF (APU)	-5.69	-0.04 (-1.0, 1.0)	Unclear	21.75	0.5 (-0.4, 1.6)	Unclear	0.17
PORHmax (APU)	21.63	1.2 (0.01, 2.4)	Large	8.77	0.8 (-0.1, 1.9)	Unclear	0.50
PORHpeak (APU)	17.93	1.1 (-0.04, 2.3)	Large	10.21	0.8 (-0.1, 1.9)	Unclear	0.42
MV (APU)	28.07	1.2 (0.07, 2.3)	Large	-20.53	0.7 (-0.3, 1.7)	Unclear	0.31
TP (s)	-7.84	-0.6 (-1.7, 0.4)	Unclear	64.96	0.3 (-0.7, 1.3)	Unclear	0.07
VT1 (ml/min/kg)	-0.59	0.2 (-0.7, 1.3)	Unclear	-16.67	-0.2 (-1.2, 0.8)	Unclear	0.80
VT2 (ml/min/kg)	-3.24	0.5 (-0.5, 1.6)	Unclear	0.39	0.8 (-0.1, 1.9)	Large	0.12
$\dot{V}O_2$peak (ml/min/kg)	-2.47	0.6 (-0.4, 1.6)	Unclear	1.00	0.7 (-0.3, 1.8)	Unclear	0.07

HIIT: High intensity interval training, CMIT: continuous moderate intensity training, ES:, IR: Insulin resistance, BMI: Body mass index, BF: Body fat, HOMA-IR: Homeostatic model assessment for insulin resistance, CRP: C-reactive protein, RF: Resting flux, PORHmax: Post occlusive reactive hyperaemia, PORHpeak: Post occlusive reactive hyperaemia peak, APU: Arbitrary perfusion units, MV: Mean velocity, TP: Time to peak, VT1: Ventilatory threshold 1, VT2: Ventilatory threshold 2, $\dot{V}O_2$peak, Peak maximal aerobic capacity.

DISCUSSION

This study on physically inactive overweight and obese adults has shown that HIIT or CMIT for 10 consecutive days does not result in statistically significant improvements in beta cell function, insulin sensitivity and insulin resistance. However, Cohen's (*d*) effects sizes (ES) showed that in insulin resistant individuals, HIIT leads to a large (3.66%) increase and a large (31.95%) decrease in insulin sensitivity and insulin resistance, respectively. Thus, although not statistically significant, the effects of HIIT on insulin sensitivity and insulin resistance may be of clinical relevance.

The absence of significant changes (based on p-value statistics) in cardiometabolic parameters assessed in this study, may be due to the occurrence of non-response to exercise which ensues if the mode of exercise training remains unchanged for the duration of the intervention. Bonafiglia et al. reported no change in cardiometabolic parameters following sprint interval training or endurance training [38]. The investigators noted that there was a change in at least one variable when individuals were exposed to both interval and endurance training, suggesting that combining the two modes of training may reduce the incidence of non-response to exercise Some studies however, have reported that short-term HIIT improves HOMA-%β, HOMA%S and HOMA-IR in sedentary, overweight and type 2 diabetes patients. This could be due to the longer duration of intervention in these studies; six [39] and twelve weeks [40] versus 10 day of HIIT training that was used in the present study. Studies of similar duration to the present study have reported positive results. Houmard et al. and Kirwan et al. reported that 7 days of moderate to vigorous training improves insulin sensitivity in previously sedentary, insulin resistant individuals and type 2 diabetes patients [41, 42]. In these studies, however, the duration

of exercise training was 50-60 minutes per session, and insulin sensitivity was measured using a hyperinsulinemic clamp technique, which directly measures insulin sensitivity. Cohen's (*d*) effect sizes indicated that HIIT produced a large (*d* = 0.9) increase in HOMA%S and a large (*d* = -0.9) decrease in HOMA-IR while those of CMIT were unclear. Previous studies have indeed reported that when compared with CMIT, HIIT results in similar improvements in insulin sensitivity and insulin resistance even with the significantly reduced time spent exercising for the HIIT groups [43-45]. In those studies, however, there was concomitant improvement in cardiovascular fitness and decreases in body fat following HIIT. Improved cardiovascular fitness and decreased body fat have been reported to independently improve insulin sensitivity. Consequently, this presents a challenge in drawing conclusions on whether the improvements were from the exercise *per se*. In the present study, the effects of HIIT on HOM%S and HOMA-IR occurred in the absence of changes in body composition and cardiorespiratory fitness, suggesting that the observed improvements may be exclusively related to HIIT training. Possible mechanism for the superior effects of HIIT when compared to CMIT have been attributed to enhanced insulin signaling in skeletal muscle via increased insulin-stimulated phosphorylation of AS160 which regulates GLUT4 translocation [46]. Conversely, Fisher et al. reported that there was no clear advantage in the improvement of insulin sensitivity in sedentary overweight or obese men following HIIT or CMIT. The investigators also reported that, generally, CMIT produced greater improvements in cardiorespiratory fitness [47].

The strengths of this study include the randomized controlled design, identification of participants with insulin resistance, and use of Cohens (*d*) effect sizes to quantify the effects of the exercise intervention. However, the study findings need to be interpreted

with caution. Limitations included the small sample size, high attrition rate, uncontrolled diet and the fact that most participants represented a single ethnic group, black Africans. The high attrition rate attributed to the frequent visits to the testing site, contributed to the small sample size (63% of the estimated sample) which decreased the statistical power of the study.

Study participants were asked to maintain their regular diet, some participants, however, may have been self-encouraged to make healthier dietary choices upon enrolment into the study which may have influenced the results. Future large-scale studies with controlled diet and a broader representation of race groups are necessary to enable a much wider interpretation and application of the findings. Furthermore, the current study explored one of the numerous possible combinations of work to rest ratio during HIIT. The ideal HIIT exercise prescription for individuals with insulin resistance/diabetes remains to be elucidated. Studies to establish the ideal combination of work to rest ratio, frequency and duration during HIIT training are needed.

In conclusion, findings from the present study indicated that HIIT is associated with sizeable percentage changes in insulin sensitivity and insulin resistance, suggesting that HIIT may be an option for individuals when the primary goal is to improve insulin resistance. The significantly brief time required for HIIT may be appealing to individuals whose barrier to exercise is lack of time. Thus, HIIT may be an effective and time-efficient alternative mode of training to be incorporated in therapies for prevention and management of metabolic disorders including insulin resistance. Future large-scale studies to clarify and confirm the effects of CMIT and HIIT in physically inactive overweight/obese adults are needed.

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CHAPTER VI: MANUSCRIPT 5 - CRP MANUSCRIPT

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Elevated C-reactive protein is associated with insulin resistance in overweight African males but not females: A cross-sectional study

Tshidi Thaane[1*], Ayesha A Motala[2], Ekavi N. Georgousopoulou[3,4] and Andrew J McKune[1,4,5]

[1]Discipline of Biokinetics, Exercise and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, South Africa; E-Mail: t.thaane@gmail.com

[2]Department of Diabetes and Endocrinology, School of Clinical Medicine, University of KwaZulu-Natal, South Africa; E-Mail: motala@ukzn.ac.za

[3]School of Medicine, University of Notre Dame Australia, Sydney, Australia; E-Mail: ekavi.georgousopoulou@nd.edu.au

[4]Collaborative Research in Bioactives and Biomarkers (CRIBB) Group, University of Canberra, Australia

[5]Discipline of Sport and Exercise Science, University of Canberra Research Institute for Sport and Exercise Science, Faculty of Health, University of Canberra, Australia; E-Mail: andrew.mckune@canberra.edu.au

*Author to whom correspondence should be addressed: Ms. Tshidi Thaane (TT)

E-Mail: t.thaane@gmail.com

Abstract

Background

Previous studies show that chronic inflammation is associated with insulin resistance (IR). This relationship, however, is inadequately explored among black Africans. Thus, the prognostic value of inflammatory markers in this population remains uncertain. This study, therefore, aimed to determine if there is an association between the inflammatory protein C-reactive protein (CRP) and IR in black African adults.

Methods

A cross-sectional study was conducted on non-smoking overweight/obese participants between 18 and 35 years. The participants were divided into insulin sensitive (IS) and insulin resistant groups based on their homeostatic model assessment of insulin resistance (HOMA-IR) scores. Serum CRP and factors related to IR including body mass index [BMI (kg/m^2)], percentage muscle mass (%MM), endothelial function and aerobic fitness were assessed.

Results

Eighty-eight (66% female) participants were included in the study. The mean (SD) age and BMI were 22.4 (3.72) years and 32.0 (4.90) kg/m^2, respectively. Fifty-nine (67%) participants were IS, and 29 (33%) had IR. When compared with the IS group, the IR group had significantly higher BMI [35.4 (3.90) vs. 30.3 (4.47) kg/m^2 ($p < 0.001$)] and CRP [4.30 (2.55,7.15) vs. 2.80 (1.50, 5.00) mg/L ($p = 0.010$)] but similar %MM [25.3 (5.78) vs. 26.0 (5.03) % ($p = 0.560$)]. Elevated CRP was significantly associated with IR only in males [odds ratio (OR) = 1.53, 95% CI 1.04-2.24]. There was no significant

association observed in females [OR = 1.06, 95% CI 0.847-1.33] after adjustment for muscle mass.

Conclusion

This study demonstrated that CRP is associated with IR in overweight/obese black African males but not females.

Keywords: African, Obesity, C-reactive protein, inflammation, insulin resistance

Introduction

The prevalence of overweight [body mass index (BMI) 25-30 kg/m^2] and obesity (BMI > 30kg/m^2) has almost tripled over the past three decades [1]. In 2016, 39% of the world's adult population were overweight and 13% were obese [1]. The increasing burden of obesity has particularly been observed in developing countries including South Africa which has the highest prevalence (69 % in women and 39% in men) in sub-Saharan Africa [2]. Consequences of obesity include cardio-metabolic disorders such as heart disease and insulin resistance (IR) which is a precursor for type 2 diabetes mellitus (diabetes) [3, 4]. Obesity-induced IR has been found to be partly attributed to chronic inflammation initiated in adipose tissue [5, 6]. Senn et al. reported that pro-inflammatory cytokines particularly, interlukin-6 (IL-6), induce production of suppressors of cytokine signalling proteins (SOCS) that bind to insulin receptors thereby interrupting the insulin signalling pathway [7]. Furthermore, IL-6 has been found to stimulate production of major pro-inflammatory cytokines including interlukin-1 (IL-1) and the acute phase protein C-reactive protein (CRP) [8-10]. Elevated levels of CRP have been found to inhibit endothelial nitric oxide synthase (eNOS) which is responsible for the production of nitric oxide (NO), a vasoactive agent that plays an essential role in the protective effects of the endothelial layer [11]. Impaired NO production therefore, results endothelial damage which is strongly associated with endothelial dysfunction and cardiovascular diseases (CVDs) [12].

With recognition that IR is an inflammatory process, the use of inflammatory markers has been widely investigated as a prognostic tool for IR in high risk populations [13-16]. However, studies exploring the relationship between inflammation and IR among black Africans are sparse [17]. The aim of the current study, therefore, was to determine if there

is an association between the inflammatory marker CRP and IR in overweight/obese black African adults.

Study setting and design

The study was conducted in the Human Performance Laboratory (HPL) of the University of KwaZulu-Natal (UKZN), Durban South Africa, which comprises of a multi-racial student and staff community. Participants were invited to volunteer to partake in the study through poster and social media campaigns. Consenting individuals provided written consent for participation in the study and publication of the research findings. The study protocol was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (reference: BFC098/16). A cross sectional analysis was conducted on a subset of participants who were black Africans and who represented majority (93%) of the total study sample of a clinical trial (reference: PACTR201802003021680) conducted between August 2016 to October 2017.

Methods

Laboratory visits

Study participants presented to the laboratory on 3 occasions. On the first visit, body composition and microvascular function testing were conducted before 12:00 PM. On the second visit, fasting blood samples were collected between 07:00 AM and 09:00 AM. Aerobic fitness testing was conducted on the third visit, 24-48 hours after the second visit.

Body composition assessment

Body composition was measured using a clinically validated bio-electrical impedance analysis machine (BF511 Body Composition Scale Omron®, Japan) [18, 19]. Participants were asked to stand barefoot on the metal sole plates of the machine. Age (years), sex (male/female) and height (cm) details were entered manually into the system. Participants were then asked to hold the device with both arms stretched horizontally in front of the body. Parameters measured included body weight [BW (kg)], body mass index [BMI (kg/m^2)], percentage body fat (%BF), percentage muscle mass (%MM) and visceral fat (VF). Participants were asked to refrain from exercise, food and drink for at least 6 hours prior to the measurement of body composition.

Microvascular function test

A non-invasive laser Doppler flowmetry system (moorVMS-Instruments®, United Kingdom) with computerized software; moorVMS-PC V4.0 (moorVMS-Instruments®, United Kingdom) was used to measure cutaneous blood flow using a protocol adapted from Morales et al. [20]. Briefly, before each measurement, the device was calibrated according to manufacturer instructions (moorVMS-Instruments®, United Kingdom). Participants were then asked to assume a supine position with the right arm measured at a 45°C using a goniometer in a supinated position. The left arm was placed on the chest. Participants were then allowed 15 minutes to acclimatize to laboratory conditions regulated at 22 ± 1°C and 50 ± 5 relative humidity (RH). A blood pressure cuff [(CUFF-ARM RD) moorVMS-Instruments®, United Kingdom] and multi-fibre skin laser probe [(VP1T/7) (moorVMS-Instruments®, United Kingdom] were placed on the distal portion of upper arm and right palmar forearm (4 cm distal to the antecubital crease), respectively.

An additional 15 minutes was allowed before the measurement of baseline cutaneous flux recorded for 1 minute. The cuff was inflated to a pressure of 50 mmHg above resting systolic blood pressure; the occlusion was maintained for 3 minutes. The cuff was rapidly released and reactive hyperemic response was measured using the laser doppler probe. Variables measured included resting flux (RF), amplitude of peak flux (PORHpeak), maximum increase in post-occlusive hyperaemia (PORHmax), time-to-peak (Tp) and mean velocity of the hyperemic response (PORHmax/Tp).

Aerobic fitness test

Study participants completed an individualised incremental cycling test adapted from a previously validated protocol [21]. Briefly, the test was conducted on an electronically braked ergometer (Lode Excalibur Sport, Groningen, The Netherlands) with oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$), minute ventilation (VE), breathing frequency, and tidal volume analysed by an online breath by breath gas collection system (Cortex MetaMax 3b gas analyser, Germany). The initial workload of was set at 50 Watts for 3 minutes. The workload was subsequently increased by 25 watts every 2 minutes until volitional fatigue. The highest $\dot{V}O_2$ achieved during the last stage of the test was recorded as peak aerobic capacity ($\dot{V}O_2$peak). Ventilatory threshold 1 and 2 were automatically detected by the Cortex software (Cortex MetaSoft® Studio, Germany). Criteria used to ensure that maximal effort was achieved included; 1) plateau in $\dot{V}O_2$ defined as an increase of less than 1.5 mL/kg/min despite progressive increases in exercise intensity, 2) a final respiratory exchange ratio (RER) of 1.1 or above and 3) a final heart rate (HR) above 95% of the age-related maximum.

Blood tests

After a 12-hour overnight fast, venous blood samples were drawn from the ante cubital region of the arm for the measurement of serum insulin, serum C-reactive protein (CRP) and plasma glucose. The samples were stored at -80°C until biochemical analysis which were conducted at the Lancet Pathology Laboratory (Durban, South Africa). Serum insulin and serum CRP were measured by radioimmunoassay and in-vitro enzyme-linked immunosorbent assay (ELISA) kit, respectively. Fasting plasma glucose was measured by a glucose hexokinase method on Roche automated analyzer.

Calculations

Fasting serum insulin and plasma glucose were used to calculate homeostatic model assessment of insulin resistance (HOMA-IR) scores using an online calculator (HOMA2 Calculator© The University of Oxford 2013) [22]. In the current study, IR was defined as HOMA-IR score ≥ 2. Participants with HOMA-IR ≥ 1.1 - < 2 were considered insulin sensitive [23]. Risk categories for IR based on CRP levels were as follows; low (CRP < 1.0 mg/L), intermediate (CRP 1.0 - 3.0 mg/L) and high (CRP > 3.0 mg/L) [24].

Statistical analysis

Data were analyzed using the IBM SPSS version 24 (IBM, USA) with statistical significance set at the 95% level ($p < 0.05$). Data were tested for normality using Shapiro-Wilk test. Data that were normally distributed are presented as mean [standard deviation (SD)] while those that were not normally distributed are shown as median [inter quartile range (IQR)]. For logistic regression analyses, data are presented as odds ratio (OR) [95%

confidence interval (CI)]. To ascertain differences in characteristics across sex and IR status, student's *t*-test was conducted. Data that did not meet the criteria for the student's *t*-test [CRP (mg/L) and Tp (s)] were analyzed using the Mann-Whitney test. Binary logistic regression models were used to explore the association between several characteristics [age (years), BMI (kg/m^2), SBP (mmHg), DBP (mmHg), MM (%), CRP (mg/L), Tp (s), VT_2 (mL/min/kg) and $\dot{V}O_2$peak (mL/min/kg)] and IR status, adjusted for important confounders (age, sex, percentage muscle mass). Due to a significant interaction term with sex, the analysis was further stratified by participants' sex.

Results

Complete data were available for 95 participants; of these, 88 (93%) were black African, 5 (5%) Asian Indian, 1 (1%), Coloured (mixed-race) and 1 (1%) white Caucasian. This analysis included only black African participants.

Table 1 shows characteristics of the study participants (n = 88) grouped by sex (M/F; 34/54). The mean (SD) age and BMI were 22.4 (3.72) years and 32.0 (4.90) kg/m^2, respectively. When compared with females, males had significantly higher percentage muscle mass (%MM%) [31.6 (2.81) vs. 22.1 (2.26) % ($p < 0.001$)], aerobic fitness [VT_2; 21.9 (3.64) vs 19.1 (3.01) mL/min/kg ($p < 0.001$), $\dot{V}O_2$peak; 26.01 (4.47) vs. 21.4 (3.43) mL/min/kg ($p < 0.001$)] and systolic blood pressure (SBP) [134 (12.1) vs. 114 (12.9) mmHg ($p < 0.001$)]. Females showed a significantly lower microvascular reactivity [Tp; 49.5 (6.00,14.0) vs. 36.6 (5.75,9.00) s ($p < 0.001$)] when compared with males.

Table 1: Characteristics of the study participants (n=88).

	All (n = 88)	Male (n = 34)	Female (n = 54)	p value
Age (years)	22.4 (3.72)	23.2 (4.72)	21.9 (2.86)	0.108
BMI (kg/m^2)	32.0 (4.90)	33.1 (4.30)	31.3 (5.17)	0.109
SBP (mmHg)	122 (16.0)	134 (12.1)	114 (12.9)	<0.001
DBP (mmHg)	76.5 (13.4)	81.5 (9.00)	73.4 (14.8)	0.005
MM (%)	25.8 (5.26)	31.6 (2.81)	22.1 (2.26)	<0.001
CRP (mg/L)	3.5 (1.80,5.20)	3.4 (1.80,5.60)	3.5 (1.70,5.00)	0.862
Tp (s)	8.0 (6.00,12.0)	7.0 (5.75,9.00)	9.0 (6.00,14.0)	0.021
VT_2 (mL/min/kg)	20.2 (3.51)	21.9 (3.64)	19.1 (3.01)	<0.001
$\dot{V}O_2peak$ (mL/min/kg)	23.2 (4.48)	26.0 (4.47)	21.4 (3.43)	<0.001

Data shown as mean (SD) if normally distributed or median (IQR) if not normally distributed. IR, insulin resistant; IS, insulin sensitive; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; MM, Muscle mass; Tp, time to peak; VT_2, ventilatory threshold2; $\dot{V}O_2peak$, peak maximal aerobic capacity.

When stratified by IR status, 29 (33%) participants were IR and 59 (67%) were IS. When compared with the IS group, the IR group had significantly higher BMI [35.4 (3.90) vs. 30.3 (4.47) kg/m^2 ($p < 0.001$)] and CRP [4.30 (2.55,7.15) vs. 2.80 (1.50,5.00) mg/L ($p < 0.010$)] but similar %MM [25.3 (5.78) vs. 26.0 (5.03) % (p = 0.560)] (Table 2).

Table 2: Characteristics of the study participants (n=88) by insulin resistance status.

	All (n = 88)	IR (n = 29)	IS (n = 59)	p value
Male [n (%)]	34 (38.6)	14 (48.3)	20 (33.9)	0.193
Age (years)	22.4 (3.72)	22.0 (3.54)	22.7 (3.81)	0.413
BMI (kg/m^2)	32.0 (4.90)	35.4 (3.90)	30.3 (4.47)	<0.001
SBP (mmHg)	122 (76.5)	127 (15.3)	120 (16.0)	0.055
DBP (mmHg)	76.5 (13.5)	78.1 (10.3)	75.8 (14.8)	0.449
MM (%)	25.8 (5.26)	25.3 (5.78)	26.0 (5.03)	0.560
CRP (mg/L)	3.5 (1.80,5.20)	4.3 (2.55,7.15)	2.8 (1.50,5.00)	0.010
Tp (s)	8.0 (6.00,12.0)	8.0 (6.00,12.0)	8.0 (6.00,13.0)	0.583
VT_2 (mL/min/kg)	20.2 (3.51)	19.5 (2.81)	20.5 (3.78)	0.171
$\dot{V}O_2$peak (mL/min/kg)	23.2 (4.48)	22.1 (3.63)	23.8 (4.78)	0.114

Data shown as mean (SD) if normally distributed or median (IOR) if not normally distributed. IR, insulin resistant; IS, insulin sensitive; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; MM, Muscle mass; Tp, time to peak; VT_2, ventilatory threshold2; $\dot{V}O_2$peak, peak maximal aerobic capacity

Table 3 shows multi-adjusted logistic regression models for IR status for the study sample stratified by sex. CRP was significantly associated with IR only in males [odds ratio (OR) = 1.53, 95% CI 1.04-2.24]. There was no significant association observed between IR and CRP in females [OR = 1.06, 95% CI 0.847-1.33] after adjustment for muscle mass.

Table 3: Multi-adjusted logistic regression models for presence of IR in the total sample and stratified by sex.

	Total Sample (n = 88)	Males (n = 34)	Females (n = 54)
Model 1: CRP (mg/L)	1.20 (1.02,1.41)	1.53 (1.04,2.24)	1.06 (0.847,1.33)
Model 2: Tp (s)	0.955 (0.863,1.06)	1.00 (0.879,1.14)	0.852 (0.700,1.04)
Model 3: VT_2 (mL/min/kg)	0.851 (0.729,0.995)	0.904 (0.725,1.13)	0.929 (0.704,1.23)
Model 4: $\dot{V}O_2$peak (mL/min/kg)	0.833 (0.723,0.960)	0.869 (0.716,1.05)	0.967 (0.742,1.26)

Data presented as odds ratio (95% CI) for insulin resistance presence. Total sample model was adjusted for age, gender and muscle mass percentage, stratified models adjusted for age and muscle mass percentage. CRP, C-reactive protein; Tp, time to peak; VT_2, ventilatory threshold2; $\dot{V}O_2$peak, peak maximal aerobic capacity.

Discussion

This cross-sectional study shows that CRP is independently associated with IR in overweight/obese black African male adults. To the best of our knowledge this is the first study to show that CRP is strongly associated with IR in black African males but not females.

Previous studies have shown that CRP is a strong predictor of IR in Japanese and Caucasian men [15, 16, 25]. In an 11-year follow-up study, Laaksonen et al. reported that men with supraphysiologic concentrations of CRP (≥ 3 mg/L) had a higher risk of developing IR or diabetes even after further adjustment for confounding lifestyle and IR-related factors including obesity [25]. In the current study, obese participants had significantly high levels of CRP. Literature suggests that this may be attributed to the infiltration of macrophages into subcutaneous adipose tissue which results in hyper secretion of the pro-inflammatory cytokines including IL-6 which induces hepatic synthesis of CRP [9]. Elevated levels of CRP have been linked with endothelial dysfunction characterised by impaired release of nitric oxide (NO), a potent vasodilator with protective effects such as vascular smooth muscle proliferation [11, 26].

Previous studies have shown that oestrogen, a naturally occurring hormone in females, may mitigate endothelial dysfunction and inflammation by regulating angiogenesis, enhancing NO production and expression of cellular adhesion molecules by damaged endothelium [27, 28]. In the current study however, females showed a significantly longer time to peak (Tp) which is an indicator of endothelial dysfunction [29]. Previous studies and meta-analysis have reported strong association between impaired endothelial function and IR even in the absence of overt diabetes [30-32]. Hsueh & Quiñones (2003)

suggested that in the spectrum of IR, endothelial dysfunction-induced atherosclerotic process precedes IR which when uncontrolled, can progresses to diabetes [31].

The World Health Organisation (WHO) recommends changes in behavioural risk factors (tobacco smoking, unhealthy diet and lack of exercise) as the first line of treatment for risk factors of cardiometabolic disorders including obesity, hypetension and IR [33]. Exercise for 30-60 minutes per day, 5 days per week has been shown to improve body composition including percentage body fat and lean muscle mass, factors which have been found to improve insulin sensitivity and may delay progression of IR to overt diabetes [34]. Studies have shown strong inverse relationship between low muscle mass and chronic low-grade inflammation which is also a characteristic of aging (inflammaging) [35]. The precise etiology of age-related inflammation remains unknown due to the ambiguity of the aging process [36]. However, studies have shown that chronic inflammation can result in skeletal muscle protein breakdown, decrease rate of protein synthesis and cause muscle wasting, factors which have been linked to physical disability which can subsequently result in the decline of physical activity [37, 38]. Thus, increased physical activity forms part of interventions that have been proposed to supress, prevent and alter the dynamics of chronic inflammation [39].

Strengths and limitations of the study

The strength of the current study is that it was conducted on a sizeable number of participants of the same race (93% black Africans) which allowed us to build a theory for future in depth studies among this population. A study limitation is that oestrogen was not measured. Our findings, therefore, are to be interpreted with caution due to the lack of hormone measurements. Another limitation is that whilst maximal aerobic capacity

was measured (based on previous research indicating the importance of aerobic capacity for reducing morbidity and mortality), examining skeletal muscle strength would have been worthwhile considering recent reports demonstrating that moderate time strength training may be a beneficial for longevity independent of aerobic activity [40].

Conclusions

In conclusion, the current study shows that CRP is independently associated with IR in overweight/obese black African males but not females. Our findings suggest that serum CRP levels may be an important prognostic tool for IR in overweight/obese black Africans. The sex of the patient, however, must be taken into consideration.

List of abbreviations

CRP	C-reactive protein
HOMA	Homeostatic model assessment
IR	Insulin resistance
IS	Insulin sensitive
NO	Nitric oxide
WHO	World Health Organisation
$\dot{V}O_2peak$	Peak maximal aerobic capacity

Declarations

Ethics approval and consent to participate

The study protocol was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (reference: BFC098/16). Study participants provided written consent to participate in the study and for the publication of the results.

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available to protect the identity of the study participants but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

TT, AAM, AJM conceptualized the study. TT coordinated the data collection process. ENG carried out the statistical analyses. TT, ENG, AJM interpreted the results. TT drafted the manuscript and AAM, ENG, AJM approved the final submission. All authors critically reviewed the manuscript and approved the final manuscript.

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CHAPTER VII: SYNTHESIS

The current research study aimed to explore one of numerous possible combinations of frequency, intensity and duration of a HIIT protocol. In addition to the attempt to study the metabolic effects of exercise in isolation to changes body composition and/aerobic fitness, we envisaged that the 10 days would minimise the high attrition rate associated with longitudinal exercise intervention. Possible practical implications would be to reliably prescribe 10 days of HIIT as the first-line of treatment for resistance states for overweight/obese adults without co-morbidity. Applicability of the 10 days HIIT program in to real-world would possible be addressed by a qualitative approach. The synthesis chapter presents a summary of the study findings. These are followed by the study strengths and limitations, contributions to literature and recommendations for future studies.

7.1. Main findings of the study

7.1.1. Narrative review

Literature review showed that the mode, volume and intensity of exercise are critical components of exercise prescription and must be recommended in accordance with the primary outcome. In individuals with insulin resistance (IR) the primary goal is to improve insulin sensitivity which contributes to glycemic control. Resistance exercise training (RET) appears to be associated with greater improvements in glucose disposal in skeletal muscle. Literature evidence suggests that the improvements may be partly attributed to greater increases in key proteins involved in the insulin signalling pathway such as protein kinase B and protein content of glucose transporter 4 (GLUT-4) in the skeletal muscle following RET [1].

7.1.2. Systematic review of randomized controlled trials examining the effects of short-term exercise training on insulin resistance

A further review of literature in the form of a systematic review, showed that short-term exercise training (≤ 12 weeks) is associated with improved insulin sensitivity independent of changes in body composition and/or aerobic fitness. However, carefully controlled clinical trials were sparse. The review of literature (narrative and systematic) showed that exercise is effective in the prevention and management of metabolic disorders such as insulin resistance in overweight/obese adults. Our findings also showed that 'time efficient' exercise programmes such as HIIT may serve as an addition or alternative to the traditional exercise prescription of 30-60 minutes per day of moderate intensity exercise 5 days per week, given that lack of time is the most commonly cited barrier to physical activity. The systematic review, in particular, showed that there is a lack of randomized controlled trials designed to evaluate and compare the effects of short-term HIIT in overweight and obesity. It was against these finding that we designed a study to evaluate the effects of short-term HIIT versus traditional CMIT in overweight/obese adults with insulin resistance.

7.1.3. Superior effects of high vs. continuous moderate intensity training on insulin resistance in overweight/obese adults: A randomized controlled trial

In light of the reviewed literature, we conducted a randomized controlled clinical trial to examine the effects of two classes of exercise training, high intensity interval training (HIIT) and continuous moderate intensity training (CMIT) on insulin resistance in physically inactive overweight/obese adults. Three questionnaires [physical inactivity physical activity screening questionnaire (PASQ), insulin resistance screening questionnaire (IRSQ) and pre-exercise intervention questionnaire (PEIQ)] administered before the exercise intervention showed that; 1) participants enrolled in the current study did not meet the requirements (assessed by PASQ) for physical activity endorsed by public health guidelines which recommend 30 minutes of physical activity 5 days per week, 2) The participants were at high risk for insulin resistance (i.e. physically inactive, waist circumference greater that 50% of height and family history of type I or type II diabetes), as assessed by IRSQ, and 3) none of the study participants were not at risk for a cardiovascular even associated with exercise testing/intervention, as assessed by PEIQ. The exercise intervention phase of the study (phase 2) showed We found that 10 consecutive days of

HIIT was associated with greater improvements in IR when compared with CMIT. Additionally, the improvements in IR occurred without changes in body fat percentage and aerobic fitness, suggesting that HIIT may be the ideal mode of training when the primary goal is to improve IR in overweight/obese adults. Previous studies with similar findings suggested that the superior effects of HIIT on IR may be attributed to the recruitment of a larger proportion of muscle fibres and greater number
men [9, 10]. In an 11-year follow-up study, Laaksonen et al reported that men with supraphysiologic concentrations of C-reactive protein (≥ 3 mg/L) had a higher risk of developing IR or diabetes even after further adjustment for confounding lifestyle and IR-related factors including obesity [10]. These studies, however, did not include females, and thus the findings could not be inferred across gender.

7.2. Strengths of the study

The current study was conducted in a real-life setting on a sample which was largely black African young adults (18-35 years) [11]. Given that there is limited randomized controlled trials with black South African participants, this exercise intervention study may be of clinical relevance.
Our findings and those of others which showed that HIIT produced superior effects on surrogate markers of IR and endothelial function when compared with CMIT, may be useful in the recommendations for HIIT to be included in the current public health guidelines for physical activity as an alternative or additional form of exercise for the prevention and management of diseases of lifestyle including IR and type 2 diabetes [12].
The strengths of this study also included the identification and distinction of participants who had IR from those who were insulin sensitive, which is not done in most studies.
The short-term (10 days) exercise intervention period allowed us to study the effects of exercise training on IR without changes in body composition and/or aerobic fitness which often occur with chronic (long-term) exercise training. Improvements in body fat or aerobic fitness have been reported to have an independently improve insulin sensitivity and as a result may mask the effects of exercise [13, 14].
We used the gold standard of study designs (randomized controlled trial) which permitted us to make direct comparison between HIIT and CMIT to establish if one form of training was superior than the other.
We complemented our tests of statistical significance with the reporting of Cohens (*d*) effect sizes which allowed us to quantify (unclear, small, moderate or large) the magnitude of the effects of the two forms of exercise training. This allowed us to communicate the practical significance of our findings in a manner that may also be understood by people with no science background.
The cross-sectional analysis (phase 1 of the study) was conducted on a sizable number (93%) of study participants of the same race (black African). Given that majority of studies on inflammation are based on other ethnic groups, our findings allowed may contribute to building a theory for future in-depth studies and recommendations for the use of CRP as a prognostic tool among black Africans.

7.3. Limitations of the study

Study limitations include the use of a single site for the recruitment of study participants. Recruiting from other institutions of higher education would have been worthwhile given that there may be heterogeneity in lifestyle factors for IR (diet and smoking status) among institutions. Diet was not controlled in this study. Study participants were asked to maintain their regular diet, some participants, however, may have been self-encouraged to make healthier dietary choices upon enrollment into the study which may have influenced the results. Analysis of saliva samples and the measurement of inflammatory markers (IL-1, IL-6 and TNF-α) which were of great interest to the study were not measured due to financial constraints, we could only assess one of the markers (plasma CRP). Consequently, we could not establish if there is association between inflammatory markers and laser Doppler markers of endothelial function and whether changes in inflammatory markers parallel to the changes in laser Doppler markers of endothelial function. Efforts were made to retain study participants including reimbursing transport costs. However, special circumstances including frequent visits to the laboratory (for baseline, exercise sessions and post-intervention measures) as well as periods of unrest at the study site (UKZN) resulted in a loss to follow-up of some participants due to relocation. The high attrition rate resulted in a significant reduction of the sample size which may have contributed to the lack of statistical power and reliability of some of the study findings. Conducting post-intervention measures 24-48 hours after the last exercise was among the limitations of the study due to possible acute effects of the last exercise session.

7.4. Contributions to literature

The current study will hopefully contribute to literature in the following ways;

(i) Adding to the growing literature on studies which examine the effects of HIIT vs. CMIT on IR in overweight/obese participants.
(ii) Contributing to the on-going debate on whether HIIT results in greater improvement in inflammation and endothelial function when compared with CMIT
(iii) Presenting that CRP may have prognostic value for systematic inflammation in overweight/obese black South African men but not women.
(iv) Highlighting that exercise intensity is a critical component of exercise prescription and must be recommended based on the primary outcome.
(v) Highlighting the need for more randomized controlled trials examining the effects of short-term HIIT vs. CMIT on IR.

7.5. Recommendations for future studies

The is a need for large-scale studies where diet is controlled. Although controlling diet can be a challenge, it is a critical component of study design due to the heterogeneity of diet among ethnic groups in South Africa.

The current study explored one of the numerous possible combinations of work to rest ratio during HIIT, studies to establish the ideal combination of work to rest ratio, frequency and duration for the management of IR, inflammation and endothelial dysfunction are warranted.

Mechanisms to elucidate superior effects of HIIT on endothelial function have been proposed but have been inadequately explored. The measurement of shear-stress and antioxidant status in future studies may aid in establishing the mechanisms behind vascular protective effects of HIIT. Studies to explore why CRP was associated with IR in black African men but not women are needed to strengthen our findings. Estrogen has been reported to have protective effects against inflammation in females, thus, the measurement of estrogen in future studies could be worthwhile.

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APPENDICES

Appendix I - Ethical approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 June 2016

Ms T Thaane (212515377)
Biokinetics, Exercice and Leisure Science
School of Health Sciences
t.thaane@gmail.com

Dear Ms Thaane

Protocol: Effects of high and moderate intensity exercise on inflammation and endothelial function in insulin resistance.
Degree: PhD
BREC reference number: BFC098/16

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application at a meeting held on 12 April 2016.

The study was provisionally approved by a sub-committee of BREC on 15 June 2016 pending appropriate responses to queries raised. Your responses dated 21 June and 25 May 2016 to queries raised on 21 April and 15 June 2016 have been noted and approved by a sub-committee of the Biomedical Research Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 28 June 2016.

This approval is valid for one year from **28 June 2016.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

Pg. 2/...

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Appendix II - Ethical approval recertification

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

31 July 2017

Ms T Thaane (212515377)
Biokinetics, Exercice and Leisure Science
School of Health Sciences
t.thaane@gmail.com

Dear Ms Thaane

Protocol: Effects of high and moderate intensity exercise on inflammation and endothelial function in insulin resistance.
Degree: PhD
BREC reference number: BFC098/16

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	28 June 2017
Expiration of Ethical Approval:	27 June 2018

I wish to advise you that your application for Recertification dated 10 April 2017 for the above protocol has been noted and approved by the Biomedical Research Ethics Committee (BREC) at a meeting that took place on 11 July 2017 for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

Please note that the meeting was not quorate and the decision taken by the Committee will be ratified at the next BREC meeting to be held on 08 August 2017.

[redacted]

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

cc supervisor: mackunea@ukzn.ac.za
cc postgrad: reneep1@ukzn.ac.za

Appendix III - Approval letter for protocol amendment

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

22 September 2018

Ms T Thaane (212515377)
Biokinetics, Exercice and Leisure Science
School of Health Sciences
t.thaane@gmail.com

Dear Ms Thaane

Protocol: Effects of high and moderate intensity exercise on inflammation and endothelial function in insulin resistance.
Degree: PhD
BREC reference number: BFC098/16

Your request for Amendments dated 13 July 2018 requesting approval of minor Amendments to the Protocol for the above-mentioned study has been noted and approved by the Biomedical Research Ethics Committee at a meeting held on 11 September 2018. BREC has condoned the implementation of the Amendment prior to BREC approval.

[redacted]

Committee

c supervisor:
cc postgrad:

Appendix IV - Gate keeper permission

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

4 May 2016

Tshidi Thaane (SN 212515377)
School of Health Sciences
College of Health Sciences
Westville Campus
UKZN
Email: t.thaane@gmail.com

Dear Ms Thaane

RE: PERMISSION TO CONDUCT RESEARCH

Gatekeeper's permission is hereby granted for you to conduct research at the University of KwaZulu-Natal (UKZN), towards your postgraduate studies, provided Ethical clearance has been obtained. We note the title of your research project is:

"Effects of high and moderate intensity training on inflammation and endothelial function in insulin resistance".

It is noted that you will be constituting your sample by recruiting students, who are willing to participate in this project, on the Westville and Howard College campuses.

Please ensure that the following appears on your questionnaire/attached to your notice:

- Ethical clearance number;
- Research title and details of the research, the researcher and the supervisor;
- Consent form is attached to the notice/questionnaire and to be signed by user before he/she fills in questionnaire;
- gatekeepers approval by the Registrar.

Data collected must be treated with due confidentiality and anonymity.

Yours sincerely

[redacted]

MR SS MOKOENA
REGISTRAR

Appendix V - Information sheet for study participants

Thank you for your interest in participating in this project, details of the project are outlined below.

Project Title

"Effects of high and moderate intensity exercise on inflammation and endothelial function in insulin resistance"

Purpose of project

Current tests for insulin resistance include drawing of blood from the arm. This procedure can be very painful. In this study, we would like to see if saliva can be used as an alternative tool to screen for insulin resistance and diabetes. Also, since the majority of people who do not exercise say that "lack of time" is the main reason they are inactive. This project will investigate short duration high intensity exercise as an alternative to long duration moderate intensity exercise as part insulin resistance and type 2 diabetes prevention and management programmes.

Benefits of the project

The lack of good screening tests for insulin resistance leaves a large number of people undetected until the insulin resistance has progressed to type 2 diabetes which is often accompanied by other disorders such as high blood pressure, heart attack and stroke. Testing saliva could offer an affordable and pain-free alternative to screen for insulin resistance. This could help us diagnose insulin resistance early which would help us provide early treament to prevent or delay the insulin resistance from progressing to type 2 diabetes. High intensity inteval training which only needs atleast 10 minutes of high intensity exercise a day could offer a time-efficent option for the prevention and management of diabetes. Participants in this project will be provided with free health assessments and individualised exercise programmes. On completion of the project, a summary of our findings will be made available to each participant.

Participants

You will only be included in this study if you meet the following criteria;
18-35 year's old
Sedentary or inactive
Overweight or obese
You will be excluded from the study if you;
Physically active
Have a history of smoking; current or chronic smokers
History of gum disease
Are injured or have disability that does not allow you to participate in cycling
Are taking treatment for hypertension, diabetes or human immunodeficiency virus

Voluntary participation

If you meet the inclusion criteria, you may choose to participate in the study. Please note that participating in this study is voluntary, you can withdraw at any stage without consequence.

Participant experience

The following is an outline of what is involved if you choose to take part in this study;

Informed consent
You will be required sign the informed consent form which states that you understand the nature of the project. Administrators will be available to answer any questions you may have regarding the study.

Important guidelines for participants
You will be required to fast for 12 hours prior to saliva and blood sample collection.
You will be required to *not* brush your teeth before saliva sample collection as this may cause the gums to bleed causing contamination of the saliva.
You will be required to avoid any caffeinated drinks and alcohol and any strenuous activity 6 hours prior the blood sample collection and the blood vessel function test.
You will be required to wear light clothing appropriate for exercise during testing.

Testing location
All testing and sample collection will be conducted at the University of KwaZulu-Natal (Westville campus) Human Performance Laboratory, Discipline of Biokinetics, Exercise and Leisure Sciences.

Data and sample collection
Questionnaires
You will be asked to complete 3 short questionnaires which will help us evaluate your current level of physical activity, screen for insulin resistance as well as assess your readiness to take part in a physical exercise programme.

Fasting saliva and blood samples
You will be required to provide 5mL of saliva. A lancet will be used to prick your finger to obtain a small drop of blood. A trained nurse from Ampath laboratories will then to draw approximately 2 tablespoons of blood from your arm.

Physical measurements
You will be asked to step on a scale that will measure your body weight, height and body fat. A tape measure will be used to measure your hip and waist circumference. A digital blood pressure machine will be used to measure your blood pressure and a heart rate monitor with a chest strap with be placed just below your chest to continuously monitor your heart rate.

Blood vessel function test
A pain-free laser Doppler device will be used to test the function of your blood vessels. Prior to testing, you will be asked to lay flat on your back. Two laser Doppler probes and skin warming probes will be placed and secured onto your arm by a double-sided skin tape. You will be given 20 minutes to relax before the test begins. We will then start to record your skin blood flow which will help us determine the health status of your blood vessels.

Exercise tolerance test
This test is designed to determine how well your heart and lungs can tolerate increased physical activity. You will be asked to avoid major meals prior to this test. A banana and

juice will be allowed atleast 1 hour before the test begins. Upon arrival, you will be given 15 minutes to relax following which your weight, height, resting blood pressure and heart rate will be measured. You will then be set-up onto a specialised stationary bike where you will perform the test with the amount of oxygen you use and carbon dioxide produced monitored by an online breath-by-breath gas collection mask. You will be asked to rate the difficulty of the exercise on a scale of 6-20. Your blood pressure and heart rate will be closely monitored during and after the test. You will not be pushed to complete the test and you will be allowed to stop the test at any time.

Exercise sessions

Participants will be randomly assigned to one of two exercise groups, namely high intensity training (HIIT) and continuous moderate intensity training (CMIT) or be asked to be part of the control (no exercise) group which only involves assessment at the beginning and end of the study period. The HIIT and CMIT groups will participate in supervised exercise sessions for 10 consecutive days. HIIT and CMIT sessions are summarised in (Table 1) and (Table 2), below.

Table 1: Summary of high intensity inteval training (HIIT) sessions

HIIT sessions (Days)									
1	2	3	4	5	6	7	8	9	10
Warm-up for 3 minutes									
60 sec of cycling with 30 sec of rest 8 sets				60 sec of cycling with 30 sec of rest 10 sets				60 sec of cycling with 30 sec of rest 12 sets	
Cool-down for 3 minutes									

Table 2: Summary of moderate intensity inteval training (HIIT) sessions

CMIT sessions (Days)									
1	2	3	4	5	6	7	8	9	10
Warm-up for 3 minutes									
12 min of cycling with no rest 1 set				15 min of cycling with no rest 1 set				18 min of cycling with no rest 1 set	
Cool-down for 3 minutes									

In summary, this study will be divided into 3 periods.

Period 1: Baseline measures, approximately 2 hours per participant (all groups)

On the first day of the study we will do the following with you;

1. We will explain the project and related procedures and you will be asked to sign the informed consent form if you are willing to participate
2. We will assist you with completion of the physical activity, insulin resistance and pre-exercise screening questionnaires
3. We will assist you with saliva sample collection
4. A qualified nurse will draw blood sample from your arm
5. We will take your anthropometric measurements
6. We will test your blood vessels function as discussed above
7. We will test your exercise tolerance as discussed above

Period 2: Exercise intervention, approximately 30-40 minutes per participant (HIIT and CMIT groups only)

Participants on HIIT or CMIT groups will undergo supervised exercise sessions for 10 consecutive days as outlined in (**Table 1)** and (**Table 2**) above.

Period 3: Post-intervention measures, approximately 2 hours per participant (all groups)

Measurements conducted on day 1 of the study will be conducted again 24-48 hours after the last day of the exercise programme in all groups.

Potential risks

Precautions will be taken to ensure the safety of participants at all times. There is a risk of bruising, bleeding and infection with blood drawing. To minimise the risks, a qualified nurse from Ampath laboratories will conduct the blood drawing using sterile equipment at all times. Skin warming conducted during the blood vessels function test may cause some degree of discomfort in the small area of the arm that is heated, we will therefore use a method which has been shown in many studies to be harmless and does not cause adverse effects or discomfort. Although rare, there are risks involved in participating in physical activity. Exercise may result in discomfort and muscle soreness. In order to

minimise these risks participants will be supervised by trained professionals including a biokineticists and a physician trained to look for "red flags". Participants will be given many opportunities to rest and will not be forced to carry out the exercise.

Confidentiality

All results will be kept confidential and all data will be kept in the possession of the researchers. If the results of the study are published in a scientific journal, the identity of participants will not be revealed. Participants will not be referred to by name during research reports or study discussions. Participant's anonymity will at all times be safeguarded. The information collected is confidential and will not be disclosed to third parties without consent, except to meet government, legal or other regulatory authority requirements. All records will be stored in a locked filing cabinet in a private office. All computer records will be password protected.

Contacting the investigators

We are happy to answer any questions participants may have. If participants have any queries, you may contact investigators below:

Miss T Thaane	212515377@stu.ukzn.ac.za	(+27) 78 887 1384
Prof AJ McKune	andrew.mckune@canberra.edu.au	(+61) 26 201 2122
Prof AA Motala	motala@ukzn.ac.za	(+27) 31 260 4233

Should you have concerns about the conduct of this study you may contact the ethics committee

This study will commerce following approval by the University of KwaZulu-Natal Biological Research Ethics Committee (BREC), should you have any concerns with respect to the conduct of this study you may contact the University of KwaZulu-Natal Research Committee:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban 4000
KwaZulu-Natal,
SOUTH AFRICA
Tel: (+27) 31 260 4769 Fax: (+27) 31 260 4609
Email: BREC@ukzn.ac.za

Appendix VI - Consent form

Statement of informed consent

General informed consent

I, _____ have been informed about the study entitled "Effects of high and moderate intensity training on inflammation and endothelial function in insulin resistance"

- I understand the purpose and procedures of the study.
- I have had all medical risks explained to me.
- I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.
- I understand that if I decide not to take part in this study or withdraw from the study at any time, there will be no disadvantage of any kind to me including my participation in any national and international competitions.
- I have been informed about any available compensation or medical treatment if injury occurs to me as a result of study-related procedures.
- If I have any further questions/concerns or queries related to the study.
- I understand that I may contact the researcher at the given detail address.
- I recognize my right to a copy of my test results and consent form if I so desire.
- I understand that all information obtained is confidential.
- I recognize that all the test results/data (both soft and hard copies) to be collected from questionnaires, anthropometric, haematological, saliva and exercise tests will be recorded/coded and stored for 5 years in password protected computer in an excel database.
- I have been informed that I will be provided a medical cover if injury occurs to me as a result of study-related procedures.
- I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.
- If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:
  BIOMEDICAL RESEARCH ETIHICS ADMINISTRATION
  Research Office, Westville Campus
  Govan Mbeki Building
  Private Bag X 54001 4000
  KwaZulu-Natal, SOUTH AFRICA
  Tel: 27 31 2604769 - Fax: 27 31 2604609
  Email: BREC@ukzn.ac.za

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

Informed consent for saliva and blood samples

I, _____, hereby authorize the laboratory and the principal investigator to collect saliva samples for C-reactive protein as well as draw a sample of my blood for C-reactive protein, glucose, insulin, cholesterol and triglycerides testing as part of the study entitled "Effects of high and moderate intensity training on inflammation and endothelial function in insulin resistance"

- I understand that my participation in the procedure is entirely voluntary.
- I recognize that blood samples will be drawn two times throughout the study.
- I understand that saliva sample collection procedure requires unstimulated and will be performed two times throughout the study.
- I understand that blood collection procedures involve venipuncture and the risk involved.
- I recognize my right to withdraw from the program at any time.
- I recognize my right to a copy of my test results and consent form if I so desire.
- I understand that all information obtained is confidential.
- I authorize release of serum C-reactive protein, glucose, insulin as well as blood cholesterol and triglycerides testing results only to the principal investigator. Other requests for results will be released only with my signed consent.
- I authorize release of salivary C-reactive protein testing results only to the principal investigator. Other requests for results will be released only with my signed consent.
- I understand that this authorization does not expire unless cancelled in writing by the undersigned.
- I understand I have the right to retain a copy of this authorization. I understand that I have the right to withdraw this authorization at any time by writing to:
  BIOMEDICAL RESEARCH ETIHICS ADMINISTRATION
  Research Office, Westville Campus
  Govan Mbeki Building
  Private Bag X 54001 4000
  KwaZulu-Natal, SOUTH AFRICA
  Tel: 27 31 2604769 - Fax: 27 31 2604609
  Email: BREC@ukzn.ac.za
- My signature below indicates that I have read or have had read to me, the above information and I understand it.
- I have had an opportunity to discuss it and my questions have been answered.

_____ _____

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

Appendix VII - Physical activity screening questionnaire (PASQ)

(Adapted from WHO global physical activity questionnaire (GPAQ))

PHYSICAL ACTIVITY			
The following questions are about the time you spend doing different types of physical activity in a typical week. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, shopping, or seeking employment. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.			
Questions		**Response**	**Code**
Activity at work			
1	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like [carrying or lifting heavy loads, digging or construction work] for at least 10 minutes continuously?	Yes 1 No 2 if **No**, go to P4	P1
2	In a typical week, on how many days do you do vigorous- intensity activities as part of your work?	Number of days:	P2
3	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes	P3
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking [or carrying light loads] for at least 10 minutes continuously?	Yes 1 No 2 if **No**, go to P7	P4
5	In a typical week, on how many days do you do moderate- intensity activities as part of your work?	Number of days:	P5
6	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours: minutes	P6
Travel to and from places			
The next questions exclude the physical activities at work that you have already mentioned. This section is about the usual way you travel to and from places. For example, to work, for shopping, to market, to place of worship.			
7	Do you walk or use a bicycle (pedal cycle) for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 if **No**, go to P10	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days:	P8
9	How much time do you spend walking or bicycling for travel on a typical day?	Hours: minutes	P9
Recreational activities			
The next questions exclude the work and transport activities that you have already mentioned. This section is about sports, fitness and recreational activities (leisure).			
10	Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like [running or soccer] for at least 10 minutes continuously?	Yes 1 No 2 if **No**, go to P16	P10
11	In a typical week, on how many days do you do vigorous-intensity sports, fitness ore recreational (leisure) activities	Number of days:	P11
12	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours: minutes	P12
13	Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that causes a small increase in breathing or heart rate such as brisk walking, (cycling, swimming, volleyball) for at least 10 minutes continuously?	Yes 1 No 2 If **No**, go to P16	P13
14	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities?	Number of days:	P14
15	How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day?	Hours: minutes	P15
Sedentary behaviour			
The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.			
16	How much time do you usually spend sitting or reclining on a typical day?	Hours: minutes	P16

Appendix VIII - Insulin resistance screening questionnaire (IRSQ)

(Adapted from IDF Finnish type 2 diabetes risk assessment form)

Please mark the right option with X

1		**Age**
		Under 25
		25-35
		Over 35
2		**Body-mass index (BMI)**
		Lower than 25 kg/m^2
		25-30 kg/m^2
		Higher than 30 kg/m^2
3		**Waist circumference**
		Men
		Less than 94 cm
		94-102 cm
		More than 120 cm
		Women
		Less than 80 cm
		80-88 cm
		More than 88 cm
4		**Do you usually have atleast 30 minutes of physical activity at work/school and/or during leisure time including normal daily activity?**
		Yes
		No
5		**How often do you eat vegetables, fruit or berries?**
		Every day
		Not every day
6		**Have you ever taken medication for blood pressure on a regular basis?**
		No
		Yes
7		**Have you ever been found to have high blood glucose (e.g. in a health examination)**
		No
		Yes
8		**Have any of the members of your immediate family or other relatives been diagnosed with diabetes (type 1 or type 2)**
		No
		Yes: grandparent, aunt or uncle (but no own parent, brother, sister or child)
		Yes: parent, brother, sister or own child

Appendix IX - Pre-exercise screening questionnaire (PESQ)

(Adapted from ACSM pre-participation screening questionnaire)

Please mark all TRUE statements with an X

History of cardiovascular events		
1		You have had heart attack
2		You have had a heart surgery
3		You have had a cardiac catheterization
4		Coronary angioplasty (PTCA)
5		Pacemaker/implantable
6		Cardiac defibrillator/rhythm disturbance
7		Heart valve disease
8		Heart transplantation
9		Congenital heart disease
History of cardiac symptoms		
1		You experience chest discomfort with exertion
2		You experience unreasonable breathlessness
3		You experience unreasonable breathlessness
4		You experience dizziness, fainting or blackouts
5		You take heart medicine
Other health issues		
1		You have diabetes
2		You have asthma or other lung disease
3		You have burning or cramping sensation in your lower legs when walking short distances
4		You have musculoskeletal problems that limit your physical activity
5		You have concerns about the safety of exercise
6		You take prescription medications
7		You are pregnant
8		You take blood pressure medication
9		You have a close blood relative who had a heart attack or heart surgery: a father or brother before the age of 55, or a mother or sister before the age of 65
10		You are physically inactive (e.g. you get less than 30 minutes of physical activity on at least 3 days per week)
11		You are a smoker
12		**None**

Appendix X - Data recording sheets

Anthropometric, physiological and biochemical variables

Name:_____ Study ID#:_____ Contact number:_____

Next of kin:_____ Intervention:_____

Variable	Pre-intervention	Post-intervention
Height (cm)		
Weight (kg)		
Body mass index (BMI)		
Waist circumference (cm)		
Hip circumference (cm)		
Waist to hip ratio		
Resting heart rate (BPM)		
Age predicted maximum heart rate (BPM)		
Maximum heart rate (BPM)		
Blood pressure (BP)		
Skin blood flow (SkBF)		
Age predicted maximal oxygen consumption (VO_2max)		
Actual maximal oxygen consumption ($\dot{V}O_2max$)		
Salivary CRP		
Serum CRP		
Fasting plasma insulin		
Fasting plasma glucose		
Blood total cholesterol		
Blood triglycerides		
HOMA-IR		
HOMA-%β		
HOMA-%S		

Maximal oxygen consumption ($\dot{V}O_2max$) test recording sheet

Stage	Time (min)	Work load (watts)	Revs per minute (RPM)	Heart rate (BPM)	Respiratory exchange ratio (RER)	Blood Pressure (mmHg)	Rate of perceived exertion (RPE)
Baseline							
1	5	50					
2	1	75					
3	1	100					
4	1	125					
5	1	150					
6	1	175					
7	1	200					
8	1	225					
9	1	250					
10	1	275					
Exhaustion							
Recovery	1						
	1						
	1						
	1						
	1						

Appendix XI - PhD presentation

University of KwaZulu-Natal (UKZN) College of Health Science Symposium October 2018, KRITH Durban (Oral Presentation) – Effects of high versus moderate intensity training on insulin resistance in overweight/obese adults: A randomized controlled trial

SUPERIOR EFFECTS OF SHORT-TERM HIGH VERSUS MODERATE INTENSITY TRAINING ON INSULIN RESISTANCE IN OVERWEIGHT AND OBESE ADULTS: A RANDOMIZED CONTROLLED TRIAL

Thaane, T.*, Motala, A* and McKune, A#

**University of KwaZulu-Natal, # University of Canberra*

Background

In long-term training studies (> 6 weeks), improvements in insulin resistance are amplified by decreased body fat and/or increased cardio-respiratory fitness. Our study purpose was to determine the effects of short-term high intensity interval training (HIIT) and continuous moderate intensity training (CMIT) on insulin resistance (IR) in physically inactive overweight/obese adults.

Methods

Participants were stratified into insulin sensitive (IS) and insulin resistant groups, and randomized into non-exercise control (CNT), HIIT and CMIT sub-groups that underwent baseline and post testing. The duration of exercise sessions was 18-24 minutes for 10 consecutive days. The HIIT group performed 60s of cycling at 90-100% peak oxygen consumption (VO2peak) interspersed with 30s of rest while the CMIT group continuously cycled at 60-70% VO2peak. Results Ninety-five participants (mean age and BMI 23.9 + 3.9 years and 32.1 + 5.0 kg/m2) were enrolled into the study. Of these, 63% were insulin sensitive and 37% had IR. A greater reduction in IR was observed with HIIT compared to CMIT (31.95% vs. 9.40%). Cohen's (d) effect sizes (ES) indicated that the decrease in IR produced by HIIT was large (ES: d = -0.9; 95% CI: -1.7, -0.1) while that of CMIT was unclear (ES: d = -0.2; 95% CI: -1.0, 0.6).

Conclusion

Short-term HIIT was associated with greater improvements in IR without changes in body fat and cardio-respiratory. Our findings suggest that HIIT may be the ideal mode of training when the primary goal is to improve IR in physically inactive overweight/obese adults.

Appendix XII - PhD awards

- National Research Foundation (NRF) Doctoral Innovation PhD scholarship (2015-2018)

- University of KwaZulu-Natal (UKZN) College of Health Sciences Operational Costs scholarship for PhD (2015-2017)

- College of Health Sciences Symposium in PhD student Category National conference travel and book voucher (2018)