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Effects of exercise training on adolescent cardiometabolic health and performance

Abstract

The studies described in this thesis were undertaken to examine the effect of physical fitness, adiposity and acute bouts of ecologically valid exercise on risk factors for cardiometabolic diseases in adolescents. Specifically, the relationship between physical fitness (measured as performance on the multi-stage fitness test (MSFT), the blood lactate response to submaximal exercise and $\dot{V}O_2$ peak) and adiposity with traditional (insulin sensitivity and blood pressure) and novel (pro- and anti-inflammatory cytokine concentration) risk factors for cardiometabolic diseases during adolescence was examined. In addition, a series of studies was undertaken to examine the inflammatory, glycaemic and insulinaemic responses to acute bouts of games-based activity (60 min of basketball) and high intensity intermittent exercise in adolescents. The effect of differing exercise durations (30 vs. 60 min) on the glycaemic and insulinaemic responses to intermittent activity was also examined (Chapter VI). Finally, the effect of continuous training versus remaining inactive on performance on physical capacity tests, $\dot{V}O_2$ peak, adiposity and risk factors for cardiometabolic diseases was examined in adolescents across a 2-year follow-up.

Throughout the present thesis a comprehensive panel of inflammatory cytokines (including IL-1β, IL-6, IL-10, TNF-α) and C-reactive protein was measured alongside blood glucose and plasma insulin concentration. For the epidemiological studies presented in Chapters IV and VII the inflammatory cytokines measured were an indication of low-grade chronic inflammation in the adolescents, whilst the blood glucose and plasma insulin concentrations were used to calculate the homeostatic model assessment of insulin resistance (HOMA-IR). In contrast, in Chapters V and VI, the measurement of pro-inflammatory (IL-1β, TNF-α and CRP) and anti-inflammatory (IL-6 and IL-10) cytokines, blood glucose and plasma insulin concentrations were used to examine the inflammatory, glycaemic and insulinaemic responses to acute bouts of games-based and high intensity intermittent activity.

The first experimental study (Chapter IV) examined the effect of performance on the MSFT, $\dot{V}O_2$ peak and adiposity on risk factors for cardiometabolic diseases in adolescents. Following ethical approval, 121 adolescents (10 - 12 years) were recruited from local secondary schools and sports clubs. Risk factors for cardiometabolic disease (inflammatory cytokines, blood glucose and plasma insulin concentrations) were determined from a fasted capillary blood sample. Participants were separated into quartiles based upon distance run during the MSFT, the blood lactate response to submaximal exercise, $\dot{V}O_2$ peak, and sum of four skinfolds. Data were analysed using two-way between-subjects ANCOVA and multiple linear regression. Participants with the lowest performance on the MSFT had higher blood concentrations of IL-6 (3.25 ± 0.25 pg·mL^{-1}) and IL-1β (4.78 ± 0.54 pg·mL^{-1}) and lower concentrations of IL-10 (1.80 ± 0.27 pg·mL^{-1}) when compared with all other quartiles (all $p < 0.05$). Yet, when categorised into $\dot{V}O_2$ peak quartiles no differences existed for any of the inflammatory mediators (all $p > 0.05$). Adiposity was the only predictor of plasma insulin concentration ($\beta = 0.515$; $p < 0.001$) and blood pressure (diastolic $\beta = 0.259$; $p = 0.042$; mean arterial pressure $\beta = 0.322$; $p = 0.011$). In conclusion, performance on the MSFT, but not $\dot{V}O_2$ peak, was associated with a favourable inflammatory profile in adolescents; whilst adiposity was adversely associated plasma insulin, diastolic and mean arterial blood pressure. These findings demonstrate that enhanced performance on the MSFT and maintenance of a healthy body composition attenuate the presence of risk factors for cardiometabolic diseases in adolescents.

The second experimental chapter (V) aimed to investigate the inflammatory, glycaemic and insulinaemic responses to an acute bout of ecologically valid games-based activity in adolescents. Thirty-nine school children aged 11 - 13 years were recruited to the present study

and completed exercise (E) and rested (R) trial in a counterbalanced, randomised crossover design. Following a standardised breakfast, participants completed 1 h games-based activity (basketball). Capillary blood samples were taken at baseline, immediately and 1 h post-exercise and 30, 60 and 120 min following a standardised lunch. A final fasted capillary blood sample was taken the next morning. Data were analysed using repeated measures ANOVA. IL-6 concentration was higher on day one of the exercise trial (E 3.4 ± 0.4: R 2.7 ± 0.4 $pg{\cdot}mL^{-1}$; $p = 0.006$), as was the anti-inflammatory IL-6: TNF-α ratio (E 5.53 ± 0.93: R 3.75 ± 0.45; $p = 0.027$). Anti-inflammatory cytokine IL-10 increased on day two of the exercise trial (E 2.11 ± 0.23: R 1.66 ± 0.16 $pg{\cdot}mL^{-1}$; $p = 0.032$). Insulin sensitivity was also enhanced on the exercise trial with a reduction in postprandial plasma insulin iAUC (E 2310 ± 834: R 3122 ± 1443 $mU{\cdot}L^{-1}$x120 min; $p < 0.001$). Such findings suggest that games-based activity is an ecologically valid mode of exercise to elicit beneficial effects on risk factors for cardiometabolic diseases in adolescents.

The third experimental chapter (VI) examined the effects of differing durations (30 min *vs.* 60 min) of high intensity intermittent activity on postprandial glycaemic and insulinaemic responses in adolescents. Thirty-one participants (13.6 ± 0.49 years) were recruited and completed a 30 min exercise trial, 60 min exercise trial and rested control trial in a randomised, counter-balanced order. The Loughborough Intermittent Shuttle Test was the chosen mode of high intensity intermittent exercise. Capillary blood samples were taken at baseline, immediately and 1 h post-exercise and 30, 60 and 120 min following a standardised lunch. On day two of the study following the consumption of a standardised breakfast further blood samples were taken at 30 min, 60 min and 120 min to observe the postprandial glycaemic and insulinaemic responses. Data were analysed using a three-way repeated measures ANOVA (trial*time*sex). The pattern of change in blood glucose concentration differed across trials ($p = 0.001$) as postprandial blood glucose concentration was lower 1 h post-exercise during the 30 min (3.8 ± 0.6 $mmol{\cdot}L^{-1}$; $p = 0.022$) and 60 min trials (3.8 ± 0.6 $mmol{\cdot}L^{-1}$; $p = 0.017$) compared to the rested control trial (4.2 ± 0.9 $mmol{\cdot}L^{-1}$). Furthermore, postprandial plasma insulin concentration was lower 1 h following the standardised lunch during the 60 min LIST trial when compared with the rested control trial (60 min LIST: 199.1 ± 125.9 $pmol{\cdot}L^{-1}$: rested trial 259.4 ± 193.7 $pmol{\cdot}L^{-1}$; $p = 0.015$). There was no difference in blood glucose concentration, plasma insulin concentration and HOMA-IR across trials on day two of the study. The present study suggests that 60 min high intensity intermittent running is an ecologically valid mode of exercise that enhances the regulation of blood glucose and insulin sensitivity in adolescents. Furthermore, a shorter bout of high intensity intermittent exercise (30 min) was also as effective in improving the regulation of blood glucose concentration as 60 min of exercise in adolescents. Such findings support the government physical activity guidelines that suggest young people should participate in 60 min of moderate-to-vigorous physical activity per day.

The final experimental study (Chapter VII) longitudinally examined (during a 2-year follow-up) the effect of continued training in comparison to remaining recreationally active during childhood and adolescence on traditional and novel risk factors for cardiometabolic diseases and performance on physical capacity tests. In addition, change in performance and $\dot{V}O_2$ peak and change in risk factors for cardiometabolic diseases were examined to identify whether a relationship existed between training and adolescent health during puberty. From the original cross-sectional sample, 61 adolescents (12 – 14 years) agreed to complete the study. In conjunction with the methods employed in Chapter IV, low-grade chronic inflammation, blood glucose and plasma insulin concentrations were determined from a fasted capillary blood sample. Participants completed a MSFT and a $\dot{V}O_2$ peak test, whilst body composition was

assessed as the sum of four skinfolds and waist circumference. Data were analysed via a mixed methods ANOVA (training group*time*sex). Overall, the trained group had lower concentrations of pro-inflammatory cytokines IL-6 (trained 3.52 ± 1.54 $pg{\cdot}mL^{-1}$: untrained 4.49 ± 1.81 $pg{\cdot}mL^{-1}$; p = 0.005) and IL-1β (trained 3.52 ± 2.11 $pg{\cdot}mL^{-1}$: untrained 5.46 ± 3.95 $pg{\cdot}mL^{-1}$; p = 0.007) than the untrained group, yet had higher concentrations of anti-inflammatory cytokine IL-10 (trained 3.31 ± 2.81 $pg{\cdot}mL^{-1}$: untrained 2.37 ± 1.36 $pg{\cdot}mL^{-1}$; p = 0.008). Overall, the trained group had a lower HOMA-IR than the untrained group (trained 1.4 ± 1.6: untrained 2.7 ± 3.5; p = 0.019). Finally, change in distance run on the MSFT was inversely associated with change in plasma insulin concentration ($r_{(46)}$ = -0.28; p = 0.062) and change in blood lactate concentration during submaximal exercise was negatively correlated with change in HOMA-IR ($r_{(21)}$ = -0.42; p = 0.055); whereas, $\dot{V}O_2$ peak was not related to any of the risk factors for cardiometabolic diseases. The findings of the present study suggest that continued training from childhood into adolescence improves cardiometabolic health, as evidenced by a favourable inflammatory profile and enhanced insulin sensitivity. Furthermore, as the change in performance on distance run on the MSFT and the blood lactate response to submaximal exercise (which are both indicators of training status) was inversely associated with change in risk factors for metabolic health there is further support of a causal relationship between physical fitness and cardiometabolic health in adolescents.

Overall, the findings from the present thesis suggest that regular participation in exercise (of sufficient intensity to enhance performance on the MSFT or to reduce the blood lactate response to submaximal exercise) reduces the presence of both traditional and novel risk factors for cardiometabolic diseases in healthy, normal weight adolescents. Furthermore, intermittent activity (performed as games-based activity and high intensity intermittent running) is an ecologically valid mode of exercise that stimulated an inflammatory, glycaemic and insulinaemic response in adolescents that elicited protective effects for cardiometabolic health, including an anti-inflammatory cascade and enhanced insulin sensitivity. If repeated regularly such exercise has the potential to reduce cardiometabolic risk factors in young people, thus preventing the early development of chronic diseases such as cardiovascular disease and type 2 diabetes. Taken together, the findings of this thesis have important practical implications, emphasising that regular exercise optimises cardiometabolic health during adolescence, which should be considered by Government health policy makers when developing recommendations for lifelong health. In particular, the findings of this thesis suggest that adolescents should participate in intermittent activity on a daily basis, to enhance their cardiometabolic health.

Key Words: Adolescents; Risk Factors for Cardiometabolic Diseases; Intermittent Exercise; Insulin Sensitivity; Inflammation

List of Abbreviations

ANOVA: Analysis of variance
CRP: C-reactive protein
HOMA-IR: Homeostatic model assessment of insulin resistance
iAUC: Incremental area under the curve
IL- : Interleukin
LIST: Loughborough intermittent shuttle test
MSFT: Multi-stage fitness test
S.E.M.: Standard Error of the Mean
tAUC: Total area under the curve
TNF-α: Tumour necrosis factor-alpha
$\dot{V}O_2$ peak: peak oxygen uptake

Acknowledgements

There are a number of people without whom I would not have been able to complete the work presented in this thesis.

Foremost among these is my director of studies, Prof. Mary Nevill and my supervisory team Dr Simon Cooper, Dr John Morris and Dr Caroline Sunderland, who provided me with the opportunity to conduct the work presented in this thesis. In particular, I would like to thank Mary and Simon, who were imperative to my academic development throughout the process as they provided the much needed academic guidance and emotional support.

I would also like to thank Prof. Graham Pockley and Dr Gemma Foulds of the John van Geest Cancer Research Centre. Through allowing us to collaborate with you and in sharing your knowledge and experience, I have been able to conduct novel research as part of my PhD and with your guidance do so with confidence. In addition, I would like to thank Maria Nute for her help with data collection and the analysis of our capillary blood samples. Your expertise was essential – from the beginning to the end. The data collection leading to the work presented would also not have been possible without Ryan Williams and Lorna Hatch. Thank you for giving up your time and keeping spirits elevated throughout testing sessions, you have been an excellent addition to the team both for myself and for the young people that we worked with.

I would also like to thank each of the schools that agreed to participate in the studies and the following members of staff for facilitating the research: Sophie Glover and Raymond Gayne (Trinity Roman Catholic School), Maria Smith (Southwolds Academy), Kelly Grimwood (Chellaston Academy) and Marcus Caton (Long Eaton School). In addition, and most importantly thank you to each of the young people who agreed to participate in the studies presented in this thesis, without you none of this work would have been possible.

Thank you also to my family. To my parents whom taught me to be resilient, to work hard in life and to believe in myself, without you and the traits that you have instilled in me I would not have been able to complete this work – thank you. Finally, to my husband Matthew, thank you for always being there to write down a to-do-list, to listen to me when I needed to get everything off my chest and for giving up your weekends to be our pacemaker during each of the multi-stage fitness tests we conducted. You have made me smile throughout this entire process and have always helped me to see the light at the end of the tunnel. Thank you.

Preface

Unless otherwise indicated by reference to published literature the work presented in the present thesis is that of the author and has not been previously submitted for another degree to this or any other university.

Some of the work presented in this thesis has been published, as follows:

Published Papers

Dring, K.J., Cooper, S.B., Morris, J.G., Sunderland, C. Foulds, G.A., Pockley, A.G. & Nevill, M.E. (2019). Multi-stage Fitness Test Performance, V̇O2 peak & adiposity: effect on risk factors for cardio-metabolic disease in adolescents. Frontiers in Physiology, 10(629). doi: 10.3389/ fphys.2019.00629

Dring, K.J., Cooper, S.B., Morris, J.G., Sunderland, C. Foulds, G.A., Pockley, A.G. & Nevill, M.E. (2019). Cytokine, glycemic, and insulinemic responses to an acute bout of games-based activity in adolescents. Scand J Med Sci Sports, doi: 10.1111/sms.13378.

Published Communications

Dring, K.J., Cooper, S.B., Morris, J.G., Sunderland, C. Foulds, G.A., Pockley, A.G. & Nevill, M.E. Effect of High Intensity Intermittent Games-Based Activity on Adolescent Cardio-Metabolic Health. ACSM Annual Conference: San Diego, CA, USA; May 2016.

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Chapter I

Introduction

Hypokinetic diseases are non-communicable conditions that develop, in part, from insufficient movement or physical activity, and include but are not limited to cardiovascular disease, type 2 diabetes, overweight and obesity (Hardman & Stensel, 2009). The risk factors implicated in the pathophysiology of cardiovascular disease and type 2 diabetes are closely aligned, and as such are often examined together and termed cardiometabolic diseases. The metabolic dysfunction that aligns the two conditions is characterised by hyperglycaemia, insulin resistance, dyslipidaemia, hypertension and low-grade chronic inflammation (Hardman & Stensel, 2009). Cardiovascular disease is the second leading cause of death in the UK, accounting for 168,000 deaths annually (British Heart Foundation, 2018). In contrast, type 2 diabetes is a more recent concern in the UK with the number of people diagnosed with the condition having doubled in the last 20 years (4.6 million people living with type 2 diabetes in 2018), with a further 12.8 million people at risk of developing the condition (Diabetes UK, 2018).

The risk factors for cardiometabolic diseases develop as early as childhood, with children as young as three years presenting with atherosclerotic lesions and impaired insulin sensitivity (Magnussen et al., 2012). In the past two decades, there has been a steady rise in the presence of risk factors for cardiometabolic diseases in young people, which has led to a 7 % increase in the incidence of type 2 diabetes in children (observed between 2000 to 2006) (Chen et al., 2012). Such adverse cardiometabolic health trends, including the increasing prevalence of impaired insulin sensitivity, prediabetes and atherosclerotic plaques, in young people will result in prolonged exposure to the risk factors of cardiometabolic diseases and undoubtedly

lead to the early onset of cardiovascular disease and type 2 diabetes, which will impact the severity of the associated symptoms during adulthood (Pinhas-Hamiel & Zeitler, 2007; TODAY Study Group, 2012). In addition, the presence of risk factors for cardiometabolic diseases are known to track from childhood into adulthood (Nicklas et al., 2002), which also emphasises the need to address the presence of risk factors of cardiometabolic diseases during the earlier stages of life. It is therefore of high importance that effective therapeutic interventions that enhance cardiometabolic health in childhood and adolescence, whilst being age-appropriate and achievable, are developed to counter the adverse health trends observed.

The Framingham Heart Study (1948) was one of the first to identify the risk factors associated with cardiometabolic diseases (O'Donnell & Elosua, 2008). The risk factors were categorised as either non-modifiable, and included sex and family history, or modifiable risk factors, such as lifestyle and environmental risk factors (O'Donnell & Elosua, 2008). As cardiometabolic diseases are defined as hypokinetic, it is not surprising that low physical activity levels, low physical fitness and increased adiposity were identified as modifiable risk factors for cardiovascular disease and type 2 diabetes. Such information led to early epidemiological research, to identify whether an association existed between occupational physical activity levels and risk of cardiovascular disease morbidity and mortality (Morris et al., 1953). The occupational physical activity affected the incidence of coronary heart disease, with bus drivers in their inactive roles at increased risk when compared with the physically active bus conductors. Epidemiological studies in adults have since examined the relationship between different types of physical activity (occupational, household, and recreational) and cardiovascular disease morbidity and mortality, and there is general agreement that an inverse association exists, suggesting that higher physical activity levels are protective against the development of cardiometabolic diseases (Ross et al., 2016).

Whilst there is growing evidence in adults to suggest that physical activity levels are inversely associated with cardiometabolic health, such findings are not directly applicable to children and adolescents. Firstly, the relationship observed in adults was between physical activity, physical fitness and body composition with cardiovascular disease morbidity or mortality (with disease diagnosis being the outcome variable of interest). By contrast, young people do not exhibit cardiometabolic disease morbidity and mortality; and thus the risk factors for cardiometabolic diseases become the outcome variables of interest. Furthermore, adolescence is characterised by several physiological and behavioural changes, which impact on both physical activity and the physiological responses to exercise (Boisseau and Delamarche, 2002). The changes observed during adolescence, which includes transient insulin resistance (Moran et al., 1999), which is not yet fully understood, mean that the responses to exercise might differ between young people and adults; therefore, research specific to young people is high priority.

Early findings of cross-sectional research in children and adolescents suggest that enhanced physical activity and performance on physical capacity tests are associated with a lowering of a select number of risk factors for cardiometabolic diseases, including markers of low-grade chronic inflammation and insulin sensitivity (Buchan et al., 2015; Bugge et al., 2012; Silva et al., 2017). There are though several limitations that should be considered when interpreting the findings of these early studies. Firstly, when selecting the physical capacity test as an indicator of physical fitness there has been limited consideration as to whether the measurement is appropriate in children and adolescents and whether the measurement effectively tracks changes in performance and training status. Of particular concern is the predominant use of $\dot{V}O_2$ peak across previous literature in young people (Bailey et al., 2012; Bugge et al., 2012; Steene-Johannessen et al., 2013). $\dot{V}O_2$ peak is limited by central systems (including cardiovascular and respiratory) which have a strong genetic predisposition (Joyne & Carsten, 2018). Therefore, $\dot{V}O_2$ peak is not that sensitive for tracking peripheral adaptations to training

such as enhanced exercise tolerance and improved efficiency of mitochondrial biogenesis (Joyce & Carsten, 2008). As such, the method of determining physical fitness should be considered, ensuring that the selected measure is sensitive to the peripheral adaptions to training, such as distance run on the multi-stage fitness test (MSFT) or the blood lactate response to submaximal exercise (Edwards, Clark & Macfayden, 2003). Furthermore, previous cross-sectional studies in adolescents have been restricted in the number of outcome variables examined, particularly when assessing inflammatory mediators implicated in the development of low-grade chronic inflammation (Petersen & Pedersen, 2005); consistently these have been limited to pro-inflammatory cytokines interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and C-reactive protein (CRP). To appropriately develop understanding as to the management of low-grade chronic inflammation in young people, a comprehensive panel of inflammatory mediators including each of the inflammatory cytokines involved in the pathogenesis of low-grade chronic inflammation should be measured, including interleukin-1β (IL-1β), IL-6, TNF-α, interleukin-1 receptor antagonist (IL-1ra), interleukin-10 (IL-10) and CRP. Of particular interest is acute phase protein CRP, which has been identified as the best predictor of cardiovascular disease morbidity and mortality in adults (Emerging Risk Factors Collaboration, 2012), yet little is known about the role of CRP in determining cardiometabolic disease risk in children and adolescents.

An inverse relationship has also been observed between adiposity (sum of skinfolds) and several traditional risk factors for cardiometabolic diseases, particularly those implicated in the development of type 2 diabetes (Bugge et al., 2012; Rizzo et al., 2008; Silva et al., 2017). Yet, there is limited understanding of the relationship between adiposity and novel risk factors for cardiometabolic diseases in adolescents, such as the relationship between pro-inflammatory cytokines, associated with low-grade chronic inflammation, and adiposity (Artero et al., 2013; Bugge et al., 2012). Such limited understanding is the result of conflicting findings across

previous research with no relationship (Artero et al., 2013), as well as positive relationships between adiposity and pro-inflammatory cytokines having previously been observed (Bugge et al., 2012). Therefore, to advance understanding into the relationship between adiposity and inflammation there is a need for a holistic analysis of inflammation that includes both pro- and anti-inflammatory cytokines and the moderating effect of pubertal development on the relationship between adiposity and inflammation in an adolescent population.

The physiological responses to acute bouts of physical activity and exercise, particularly the inflammatory, glycaemic and insulinaemic responses, are important because they may reflect the mechanisms which underpin the relationship between physical activity levels and risk factors of cardiometabolic health in young people and adults (Gleeson et al., 2012). The inflammatory response to exercise (which is based on the findings of *in vitro* studies), is stimulated from skeletal muscle contraction, which results in a transient increase in IL-6 concentration in the systemic circulation (Petersen and Pedersen, 2005). As IL-6 concentration increases there is a cascade whereby anti-inflammatory mediators (IL-1ra and IL-10) are stimulated and pro-inflammatory cytokines are inhibited (IL-1β, TNF-α and CRP). These transient changes, if repeated regularly, are hypothesised to reduce low-grade chronic inflammation, which is implicated in the development of atherosclerosis and type 2 diabetes. In conjunction with the inflammatory response, exercise is also suggested to enhance insulin sensitivity by mediating a reduction in blood glucose and plasma insulin concentrations, as a result of increased non-insulin dependent glucose uptake into the skeletal muscle for several hours post-exercise (Mendham et al., 2012; 2013; 2015). Yet, there have been limited studies that have assessed the transient inflammatory, glycaemic and insulinaemic responses to acute bouts of exercise in both young people and adults.

In adults (middle-aged men, 38-48 years), the inflammatory, glycaemic and insulinaemic responses to small-sided games (modified rugby) and traditional endurance activity (40 minutes of stationary cycling at ~ 80 % heart rate max) elicited a protective anti-inflammatory response and reduced glycaemic and insulinaemic responses for up to 4 h post-exercise (Mendham et al., 2012; 2013; 2015). For children and adolescents much less is known about the physiological responses and the inflammatory responses post-exercise, however, IL-6 and anti-inflammatory cytokine IL-1ra have been shown to increase immediately following 90 min wrestling practice in adolescent boys (Nemet et al., 2002). However, the response of a comprehensive panel of inflammatory mediators is yet to be examined, as is the response of these mediators for up to several hours post-exercise (which is especially important given that *in vitro* studies suggest IL-10 and CRP are elevated 24-48 h after physical activity; Gleeson et al., 2012). Research on the glycaemic and insulinaemic responses to exercise in adolescents is also relatively limited, although findings to date examining continuous moderate intensity running for 45 – 60 min in boys aged 9 – 15 years suggest that endurance activity transiently enhances postprandial insulin sensitivity in children and adolescents (improvements between 6 - 13% in plasma insulin total area under the curve) (Cockcroft et al., 2012; Short et al., 2013).

Whilst the early research examining the effect of acute bouts of exercise on the inflammatory, glycaemic and insulinaemic responses in adolescents suggests that acute bouts of continuous moderate intensity exercise increases IL-6 concentration for up to 60 min, it remains unknown whether the increase in IL-6 post-exercise stimulates a protective inflammatory cascade (including the stimulation of anti-inflammatory mediators and inhibition of pro-inflammatory cytokines) and the time scale in which such a response ensues. Furthermore, the inflammatory, glycaemic and insulinaemic responses to exercise in adolescents have consistently been observed following moderate intensity continuous exercise which is of limited ecological validity in adolescent populations (Howe et al., 2010). Typically, adolescents engage with short

bursts of high intensity intermittent activity that is interspersed with periods of rest (Howe et al., 2010), similar to the activity patterns of games-based exercise. Therefore, future research should establish whether or not such exercise mediates the protective inflammatory, glycaemic and insulinaemic responses, as reported in early research in adolescents (Cockcroft et al., 2012; Short et al., 2013). Furthermore, it is important that the physiological responses are observed in conjunction with potential moderating variables that adolescents encounter during their everyday lives, which will include the consumption of ecologically valid meals.

Finally, there are several variables (including frequency, intensity, and duration) relating to the chosen mode of exercise that should be examined, to establish how best to optimise the protective cardiometabolic responses in adolescents, whilst ensuring the exercise is achievable to ensure physical activity recommendations are met. This is a particularly important area for future research as fewer than 20 % of adolescents in the UK currently meet the recommended guidelines of 60 minutes of moderate to vigorous physical activity each day) (NHS England, 2019). Should a lower duration of exercise elicit glycaemic and insulinaemic responses that could be protective against the development of risk factors for cardiometabolic and type 2 diabetes, this could have major implications for future physical activity policies and guidelines.

Given the limited understanding of the relationship between performance on physical capacity tests, adiposity and novel risk factors for cardiometabolic diseases in adolescents and how under-explored the inflammatory, glycaemic and insulinaemic responses following ecologically valid modes of exercise are in adolescents, the present thesis will address such gaps in the literature through the following research questions and thesis objectives.

Research Questions:

1) Does a relationship exist between performance on physical capacity tests, $\dot{V}O_2$ peak and adiposity with traditional and novel risk factors for cardiometabolic health in a heterogeneous sample (in terms of physical fitness) of adolescents? Furthermore, does the chosen physical capacity test mediate the relationship between physical fitness and cardiometabolic health in adolescents?
2) Does an ecologically valid mode of intermittent activity (such as basketball, which is suitable for both boys and girls) stimulate an anti-inflammatory response (transiently increasing IL-6 and IL-10 and reducing IL-1β and TNF-α) and enhance postprandial insulin sensitivity in healthy adolescents?
3) Can a shorter 30 min bout of intermittent activity when compared with a traditional 60 min of intermittent exercise (which achieves the Government guidelines of 60 min moderate-to-vigorous physical activity per day) reduce postprandial blood glucose and plasma insulin concentration in healthy adolescents; thus providing a realistic duration of physical activity for young people to adhere to? Furthermore, it is unknown the timeframe in which the transient glycaemic and insulinaemic responses remain and whether exercise duration effects the length of such responses.
4) What effect does continuous training throughout adolescence *vs.* remaining inactive (during a 2 year follow-up) have on novel and traditional risk factors for cardiometabolic diseases? In addition, which physical capacity tests are most sensitive to tracking changes in training status in the trained and inactive adolescents across time?

Thesis Objectives and Hypotheses:

Therefore, the purpose of the present thesis is to examine the chronic and acute effects of exercise on risk factors for cardiometabolic diseases in adolescents. The objectives are:

1) To examine the relationship between performance on the multi-stage fitness test, $\dot{V}O_2$ peak and adiposity and the cardiometabolic health of adolescents aged 10-12 years; testing the hypothesis that adolescents with a higher distance run on the MSFT or/and a lower blood lactate response to submaximal exercise and to a lesser extent higher $\dot{V}O_2$ peak and adiposity will have lower risk factors for cardiometabolic health.
2) To determine the glycaemic, insulinaemic and inflammatory responses to an acute bout of high intensity intermittent activity (performed as games-based activity) in young people; testing the hypothesis that intermittent games-based activity will stimulate an inflammatory cascade triggered by an increase in IL-6, and enhance insulin sensitivity as observed by reduced postprandial blood glucose and plasma insulin concentration.
3) To investigate whether a shorter 30 min bout of intermittent exercise when compared with a more traditional 60 min bout, improves postprandial insulin sensitivity in young people and the timeframe in which insulin sensitivity remains enhanced; thus testing the hypothesis that 30 min of high intensity intermittent exercise may be adequate to enhance postprandial insulin sensitivity both on the day and the day following exercise in adolescents.
4) To longitudinally examine the effect of continuous training in comparison to being recreationally active during childhood and adolescence on risk factors for cardiometabolic diseases, including novel (inflammatory cytokines) and traditional (fasting blood glucose, plasma insulin, HOMA-IR and blood pressure) risk factors; testing the hypothesis that involvement in training over several years will be protective

in reducing the risk factors for cardiometabolic disease and will continue to be protective during early adolescence.

These thesis objectives were met through a series of four studies. The initial study was cross-sectional and determined whether performance on physical fitness tests and sex had an effect on risk factors for cardiometabolic disease (Chapter IV). The second (Chapter V) and third (Chapter VI) studies of the thesis focused on the effect of acute bouts of intermittent activity on the pro- and anti-inflammatory responses and the glycaemic and insulinaemic responses in adolescents. Specifically, the second study established the inflammatory, glycaemic and insulinaemic responses to a 60 min bout of games-based activity, performed as a basketball training session, up to 24 h post-exercise. Whilst the third study of the thesis investigated the glycaemic and insulinaemic responses to different durations (30 min *vs.* 60 min. *vs.* rested control trial) of high intensity intermittent activity (performed as the Loughborough intermittent shuttle test; LIST) up to 24 h post-exercise. Finally, the participants recruited to the first study were subsequently followed-up two years later to examine whether or not training advantages (in terms of reducing risk for cardiometabolic diseases) were sustained across puberty (Chapter VII).

Chapter II

Review of the Literature

2.1 Overview of the Review of the Literature

The review will begin with a definition of key terms that will be used throughout the thesis (2.1.1) and will then progress to discuss and critically evaluate cross-sectional and longitudinal research that has examined the association between performance on physical capacity tests and adiposity with risk factors for cardiometabolic diseases in children, adolescents and adults (section 2.2). Finally, section 2.3 will review and critically evaluate research that has examined the inflammatory, glycaemic and insulinaemic responses to acute bouts of exercise in children, adolescents and adults.

2.1.1 Physical Activity, Physical Fitness, and Exercise

Physical activity, physical fitness and exercise are terms that whilst inter-related, are distinct. It is widely accepted that physical activity is defined as '*any bodily movement produced by skeletal muscle that results in energy expenditure*' (Caspersen et al., 1985). In contrast, physical fitness is complex and may be separated into subcomponents of functional capacity and health-related physical fitness. Functional capacity pertains to '*an individual's ability to perform daily activities with vigor and alertness*' (Caspersen et al., 1985), whilst health-related physical fitness is the '*demonstration of traits and capacities associated with low risk of premature development of the hypokinetic diseases*' (Pate, 1988). Health-related physical fitness can further be categorised into cardiorespiratory endurance, muscular endurance, body composition and flexibility (Caspersen et al., 1985). Physical fitness is predominantly determined by lifestyle factors, with participation in physical activity/ exercise imperative to the enhancement of fitness (Blair et al., 2001). For clarity, exercise is a '*subset of physical*

activity that is planned, structured and repetitive, done to improve one of more components of physical fitness' (Caspersen et al., 1985).

2.1.2 Health, Hypokinetic Diseases and Cardiometabolic Health

The World Health Organisation (1948) defines health as '*a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity'*. There has been increased interest in the potential for physical activity and physical fitness to be utilised as therapeutic interventions to enhance and maintain health through the prevention of chronic diseases (Lee et al., 2012). Specifically, regular participation in physical activity prevents the development of hypokinetic diseases, which given that hypokinetic refers to insufficient movement or low decreased motor activity, are non-communicable conditions that have an inverse relationship with physical activity and physical fitness (Berryman, 2010). Hypokinetic diseases include, but are not limited to cardiovascular disease, type 2 diabetes, obesity, osteoporosis and some cancers (Berryman, 2010). The present thesis will focus on risk factors for cardiometabolic disease, which are a series of physiological risk factors (hypertension, dyslipidaemia, hyperglycaemia and low-grade chronic inflammation) that increase the risk of atherosclerotic cardiovascular disease and type 2 diabetes (Ruilope et al., 2007).

2.1.3 Cardiovascular Disease

Cardiovascular disease is a collective term given to a group of disorders, which impede the critical functions of the cardiovascular system (Perk et al., 2012; World Health Organisation, 2015). Cardiovascular disease remains the leading cause of mortality worldwide (World Health Organisation, 2015), accounting for 7.1 million deaths annually (Bhatnagar et al., 2015); a figure which is anticipated to rise to 23.6 million by 2030 (Smith et al., 2012).

Cardiovascular disease develops chronically across the lifespan with symptoms typically presenting during the fifth decade of life, when the disease has progressed towards an advanced stage (Magnussen et al., 2012). Atherogenesis is the process through which atherosclerotic lesions develop and increase the risk of developing cardiovascular disease (Magnussen et al., 2012). Atherosclerosis is 'an inflammatory process characterised by the aggregation of lipids, macrophages and smooth muscle cells within the intima layer of epicardial arteries' (Hardman & Stensel, 2009). The 'Response to Injury' hypothesis, as presented in Figure 1, suggests that hemodynamic resistance, dyslipidaemia, hyperglycaemia and low-grade chronic inflammation induce injury to the outer layer of the endothelium and initiates atherogenesis (Hansson, 2005). At the site of the injury macrophages, oxidized lipids and smooth muscle cells aggregate in the intima layer and plaque develops (Sun et al., 2000). Plaque development becomes of clinical concern when blood flow is restricted by ≥ 45 % (the clinical threshold), leading to ischemia of tissues and organs and symptoms associated with cardiovascular disease (Hardman & Stensel, 2009).

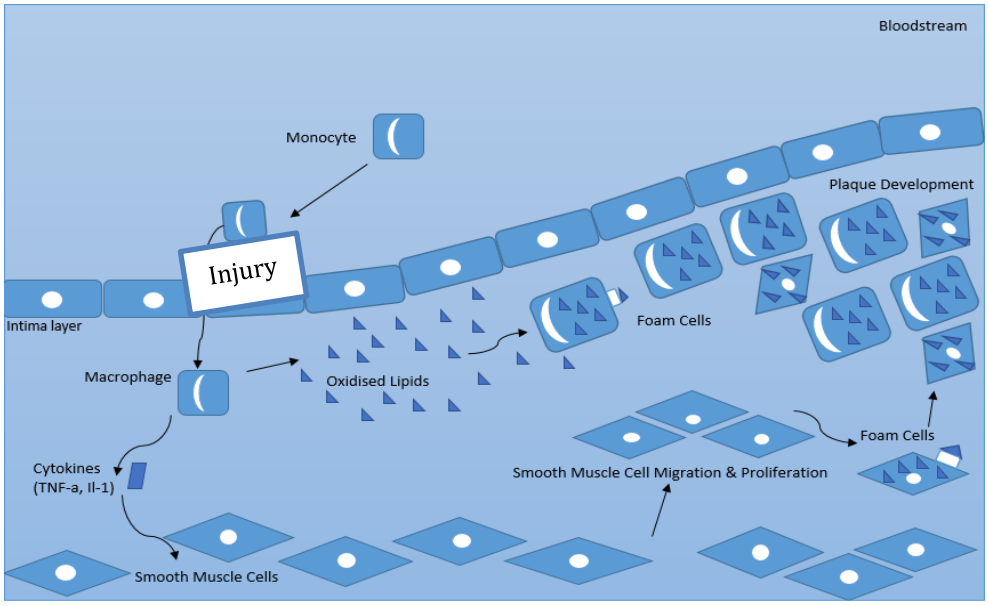

Figure 1. Illustration of the 'Response to Injury' hypothesis of atherogenesis which results in the development of atherosclerotic plaques, adapted from Hardman & Stensel, (2009).

The Framingham Heart Study (1948) was the first to identify risk factors associated with cardiovascular disease and categorised them as non-modifiable or modifiable (O'Donnell & Elosua, 2008). Non-modifiable risk factors include sex, with males at increased risk compared to their female counterparts, and genetic susceptibility, with genetic markers and family history increasing the risk of cardiovascular disease (O'Donnell & Elosua, 2008). Modifiable risk factors for cardiovascular disease include lifestyle and environmental factors, such as physical activity levels, diet, alcohol consumption, and smoking (O'Donnell & Elosua, 2008).

Modifiable and non-modifiable risk factors for cardiovascular disease increase the presence of biochemical and physiological markers in the systemic circulation. Such markers include traditional risk factors such as triglycerides, total cholesterol (LDL-c and HDL-c), hypertension and hyperglycemia. Novel biochemical risk factors include pro-inflammatory cytokines and C-reactive protein (CRP), an acute phase protein (Gleeson et al., 2012). The increase in inflammatory cytokines and CRP in the systemic circulation is referred to as low-grade chronic inflammation, which is defined as '*a chronic two- to three-fold elevation in pro-inflammatory (IL-1β, IL-6, and TNF-a), and anti-inflammatory cytokines (IL-1ra and IL-10)*' (Pedersen & Petersen, 2005). The increasing presence of physiological and biochemical markers increases the likelihood of an injury to the outer endothelium, triggering the response to injury hypothesis and thus is considered a key risk factor for cardiometabolic disease.

2.1.4 Metabolic Diseases: Type 2 Diabetes

Diabetes is a major cause of morbidity and mortality in children, adolescents and adults (Diabetes UK, 2014; Oliveira et al., 2012). There are two forms of the disease: type 1 and type 2 diabetes. Type 1 diabetes typically originates during childhood and is characterised by insulin deficiency, which occurs due to beta-cell autoimmune destruction (Zeitler et al., 2007). Type 2 diabetes is characterised by peripheral insulin resistance, progressive pancreatic beta-cell

failure and chronic hyperglycaemia (American Diabetes Association, 2000; Pinhas-Hamiel & Zeitler, 2007). During the early stages of type 2 diabetes blood glucose homeostasis is maintained through increased insulin synthesis; however, with disease progression hyperinsulinaemia cannot be sustained and pancreatic beta-cell failure ensues (Rhodes, 2005). Risk factors associated with type 2 diabetes include increasing age, obesity, sedentary behaviour, ethnicity and genetic susceptibility (Pinas-Hamiel & Zeitler, 2007). Some of the symptoms associated with chronic hyperglycaemia and the development of type 2 diabetes include glucosuria, and polydipsia (Reinehr, 2013; Scott et al., 1997; Zdravkovic et al. 2004).

Plasma glucose homeostasis in healthy individuals is regulated with a fasting blood glucose concentration of between 4.5 - 6 $mmol.L^{-1}$ (Saltiel & Kahn, 2001) and is regulated through the action of insulin (an anabolic hormone) on hepatic gluconeogenesis, glycogenolysis and the uptake of glucose into peripheral tissues (Bouchard et al., 2007; Seino, 2012). The beneficial effects of physical activity centre around the action of insulin on peripheral tissues, particularly on skeletal muscles, whereby in healthy individuals, insulin binds to receptors on the surface of skeletal muscles to activate an insulin-signalling cascade. The cascade ends with the translocation of GLUT-4 to the plasma membrane to transport glucose into the cell to maintain blood glucose homeostasis (Saltiel & Kahn, 2001).

Peripheral insulin resistance is a key characteristic of hyperglycaemia and the subsequent development of type 2 diabetes and is defined as '*a state of decreased responsiveness of target tissues to normal circulating concentrations of insulin*' (Sesti, 2006). There are many theories that attempt to explain the pathogenesis of peripheral insulin resistance, which include the accumulation of lipids in skeletal muscle impairing the function of insulin; an impairment in the insulin signalling pathway resulting in impaired GLUT-4 translocation and modifications in the insulin receptors (for review see, Sesti, 2006). Chronic insulin resistance leads to

hyperglycaemia and the development of type 2 diabetes, with the compensatory increase in plasma insulin eventually leading to pancreatic beta-cell failure.

2.1.5 Assessment of Cardiometabolic Disease Risk

The present thesis intends to establish the effect of physical activity, physical fitness and exercise on adolescent cardiometabolic health. The present thesis intends to focus on cardiovascular risk factors associated with low-grade chronic inflammation, which include pro-inflammatory mediators IL-1β, IL-6, IL-10, and TNF-α, and acute phase protein CRP (Gleeson et al., 2012). The named inflammatory mediators (interleukins) are small, secreted proteins known as cytokines (Zhang & An, 2009). When assessed chronically the inflammatory mediators are typically pro-inflammatory (IL-1β, IL-6, TNF-α) and contribute towards the presence of low-grade chronic inflammation, which is a novel risk factor for cardiometabolic diseases. However, there are several inflammatory cytokines that have anti-inflammatory properties (IL-1ra, IL-6 and IL-10), which if elevated in the systemic circulation might have the potential to protect against low-grade chronic inflammation.

It is important to note that IL-6 has both pro- and anti-inflammatory properties dependent upon whether the interleukin is raised chronically or transiently, such as following an acute bout of exercise (Pedersen & Petersen, 2005). When IL-6 is increased chronically in the fasted, rested and disease-free state (free from acute infections), the increased concentration of IL-6 contributes towards the development of low-grade chronic inflammation (Pedersen & Petersen, 2005) and thus is a cardiometabolic risk factor that is implicated in the aetiology of several chronic diseases including cardiovascular disease and type 2 diabetes (Gleeson et al., 2012). However, IL-6 also has anti-inflammatory properties when increased transiently, as is the case following an acute bout of exercise that is of sufficient duration and intensity (Pedersen & Petersen, 2005; Gleeson et al. 2012). When increased transiently IL-6 is stimulates an anti-

inflammatory cascade, which mechanistically reduces low-grade chronic inflammation (Gleeson et al. 2012). Therefore, careful consideration must be taken when measuring IL-6 (including whether chronic or acute concentrations are measured) given the dual effects of the inflammatory mediator on cardiometabolic health (Gleeson et al. 2012).

Inflammatory cytokine IL-6 is suggested to increase transiently following acute bouts of prolonged running in adults (> 60 minutes in duration), providing the exercise mode is of sufficient intensity and duration (Gleeson et al., 2012). Furthermore, *in vitro* studies suggest that an increase in IL-6 in the systemic circulation has the potential to increase the concentration of cytokines with anti-inflammatory properties (IL-1ra, and IL-10) (Gleeson et al., 2012). Therefore, the response of these mediators to acute bouts of physical activity are of interest when trying to establish modes of physical activity that reduce chronic cardiometabolic risk factors. However, few human studies, particularly in children and adolescents, have examined the inflammatory responses to exercise.

When determining the inflammatory response to exercise, the timing of measurements is also of importance. *In vitro* studies suggest that the transient inflammatory cascade initiated by IL-6 stimulates the production of inflammatory mediators (IL-10 and CRP) up to 24 - 48 h post the initial stimulus (Petesen & Pedersen, 2005). As such, it is important that the inflammatory response be analysed immediately and up to 24 - 48 h post-exercise, where possible, to ascertain the response of each inflammatory marker accurately. To determine the inflammatory response post-exercise, a baseline measurement is necessary for comparison. It is important that baseline measurements are taken when participants are rested and fasted, as physical activity and food consumption transiently alters concentrations of inflammatory mediators. Similarly, when assessing inflammatory cytokines (IL-1β, IL-6, IL-10, TNF-α) and CRP

chronically, as a measure of low-grade chronic inflammation, the same variables must be controlled to ascertain an accurate representation of cardiometabolic risk.

There are numerous methods available to determine peripheral insulin resistance; however, the method selected depends upon a multitude of factors including patient age, test applicability and availability of resources. The gold standard assessment of diabetes mellitus is the hyperglycaemic clamp technique; however, this relies on intravenous administration of exogenous glucose, which is not ethically acceptable in a non-patient paediatric population (DeFronzo et al., 1979). Simple indices of insulin resistance such as the oral glucose tolerance test (OGTT), the homeostasis model assessment (HOMA-IR) of insulin resistance and biochemical markers (such as leptin, adiponectin, glycated haemoglobin (HbA1c) and inflammatory markers) are desirable alternatives in establishing risk in adolescents (Barr et al., 2014; Stefan et al., 2014). However, the OGTT lacks ecological validity as it does not consist of a mixture of macronutrients, whilst HOMA-IR is a measure of hepatic insulin sensitivity and thus may not be sensitive to the changes in peripheral insulin sensitivity stimulated by exercise. Therefore, the glycaemic and insulinaemic responses to standardised meals are ecologically valid, yet understudied, alternatives to assess peripheral insulin sensitivity.

2.1.6 Adolescents

Adolescence is '*the interval between childhood and the assumption of adult roles and responsibilities, a broad interval of maturation encompassing physical, mental and emotional development*' (Dorn et al., 2006). Following birth, human growth and development can be categorised into five stages of infancy, childhood, juveniles, adolescence and adulthood (Bogin, 1993). Each stage is distinguished by changes in growth, development and sexual maturation, with each progressing towards the 'mature adult state'. The adolescent stage follows the juvenile stage and commences with the onset of puberty, typically commencing at

the chronological age of 10 in girls and 12 in boys (Bogin & Smith, 1996). Adolescents are often categorised, particularly in an academic and sport setting, based on their chronological age. Due to the physiological interaction of puberty with biochemical markers, physical activity levels and physical fitness in young people it is imperative maturation is considered when interpreting findings.

There are several methods to assess maturation including the gold standard X-ray of the left wrist, or field-based alternative assessments of secondary sexual characteristics or age from peak height velocity (APHV) (Bogin & Smith, 1996; Mirwald et al., 2002). Ethically, determining APHV is considered the most appropriate method to assess adolescent maturation. In the present thesis, APHV will not be used to classify participants as children or adolescents as APHV is indicative of maturity timings (the age at which specific maturational events occur) and not maturity status (level of maturation at the time of assessment) (Koziel & Malina, 2018). Therefore, APHV will be utilised as an indication of maturity timing, whilst chronological age will be used as an alternative to classify participants as children or adolescents with individuals aged 11 to 16 classified as 'adolescents' and participants aged under 11 categorised as children.

2.1.7 Cardiometabolic Disease Risk in Adolescence

Whilst cardiometabolic diseases typically present during the fifth decade of life, cardiometabolic risk factors are present during childhood and adolescence (May et al., 2014), with children as young as three years presenting with atherosclerotic lesions (Magnussen et al. 2012). Furthermore, risk factors for cardiometabolic diseases in young people track into adulthood, increasing the risk of early onset cardiovascular disease (Berenson et al., 1980). Similarly, the prevalence of prediabetes in young people, measured by impaired fasting glucose, has increased from 7.0 % in 1999–2000 to 13.1% in 2005–2006 (Chen et al., 2012) and type 2 diabetes (which was once a disease almost exclusive to adults) is now the most

common diagnosis of diabetes in young people (Chen et al., 2012). The increasing prevalence of risk factors for cardiometabolic diseases and type 2 diabetes in youth is of major concern as prolonged exposure will lead to the early onset of such conditions and increased severity of the associated symptoms (Pinhas-Hamiel & Zeitler, 2007; TODAY Study Group, 2012). It is therefore imperative that therapeutic interventions target young people to enhance their cardiometabolic health.

2.1.8 Literature Review Methods

The following literature review consists of a search for key terms (including children, adolescents or adults, physical fitness, physical activity, cardiovascular disease, type 2 diabetes and cardiometabolic risk factors) on PubMed and Google Scholar. To be included in the literature review the paper needed to be an original research article, with systematic reviews and meta-analyses excluded from the present review (due to the inclusion of research that has not been peer-reviewed). Furthermore, examining original research articles ensures that a thorough review of key aspects of study design can be conducted. Finally, research in young people was excluded if the participants had a chronic condition (excluding overweight/ obesity), as the aim of the present thesis was to examine the association between physical fitness and cardiometabolic health in healthy adolescents. These criteria are applicable to all sections of the literature review (sections 2.2, 2.3 and 2.4) as throughout the thesis the aim was to assess the effect of training and acute bouts of exercise on risk factors for cardiometabolic diseases in healthy young people.

2.2 Association between Physical Activity, Physical Fitness & Cardiometabolic Health

The following sections (2.2.1, 2.2.2 and 2.2.3) will review cross-sectional research that has assessed the association between physical activity levels, physical fitness and adiposity with novel and traditional risk factors for cardiometabolic health and cardiometabolic morbidity/mortality in adults, children and adolescents.

2.2.1 Adults

The first observations of inverse relationships between physical activity and cardiovascular disease were reported in cross-sectional studies in adults in the 1950s (Morris et al., 1953). The series of observational studies assessed differences in cardiovascular disease morbidity and mortality across occupations with varying physical activity levels, primarily in middle-aged men (Morris et al., 1953; 1958; 1966). Initial findings revealed that physically active jobs (such as bus conducting) halved the risk of myocardial infarctions and cardiovascular disease mortality in comparison to inactive occupations (such as bus driving) (Morris et al., 1953). Furthermore, the series of studies highlighted that middle-aged men in physically active jobs with cardiovascular disease were diagnosed later in life and had less severe symptoms than their inactive counterparts (Morris et al., 1958) suggesting that physical activity protects against and delays the development of cardiovascular disease.

Since establishing the initial association between occupational physical activity and cardiovascular disease, there has been an abundance of research investigating the potential for different modes of physical activity to prevent and manage cardiovascular disease in adults (Ross et al., 2016). The scope of such research is extensive due to the diversity of physical activity, with potential associations between occupational, leisure-time (including exercise) and household physical activity with cardiovascular disease. In addition, physical activity can

vary by mode, intensity, duration and frequency, with each variable moderating the relationship between physical activity and cardiovascular disease risk. The Harvard Alumni study was the first to explore the diverse nature of physical activity and the relationship with cardiovascular disease (Paffenbarger et al., 1978). Male graduates from Harvard University (1916 – 1950) completed extensive physical activity questionnaires, which detailed sports played during their time at university, the amount of walking/ stairs climbed daily, recreational and sporting activities, and a composite physical activity index (Paffenbarger et al., 1978). Adult estimated energy expenditure derived from physical activity levels was inversely associated with risk of myocardial infarction, with men with the lowest physical activity index (≤ 2,000 kcal of energy expenditure per week) 64 % more likely to suffer a myocardial infarction than men with the highest physical activity index (≥ 3500 kcal per week). In addition, the impact of physical activity on myocardial infarction risk was independent of varsity-athlete status during college. These findings emphasise that to prevent and manage cardiovascular risk physical activity levels should be maintained throughout the entire lifespan.

The most common critique of physical activity research relates to measurement error, which commonly arises from the imprecision of physical activity questionnaires, recall error and limited reliability for select intensities of exercise (Hardman & Stensel, 2009). As such, a preferred method to assess the benefits of prolonged participation in physical activity and exercise is to assess an individual's physical fitness; thus reducing the likelihood of the misclassification of individuals, strengthening the validity of the relationship. Typically, in such research, performance in physical tests categorises participants into quartiles and the prevalence of risk factors for cardiovascular disease, and cardiovascular disease morbidity and mortality is compared across the groups. A review of the most recent literature in adult populations suggests that participants' physical capacity to perform well in exercise tests is inversely associated with cardiovascular disease risk (Ross et al., 2016). Furthermore, the

inverse associations were seen in healthy men and women, cardiovascular disease patients, and those with existing comorbidities such as obesity and type 2 diabetes, with each population benefitting from the protective effects of greater performance on physical capacity tests (Ross et al., 2016).

Inverse relationships have also been observed between, muscular endurance, muscular strength and body composition, and cardiovascular disease risk, morbidity and mortality in adults (Artero et al., 2012). However, a review of such research is beyond the scope of the current thesis and the focus will remain on the prevention of risk factors for cardiometabolic diseases in young people.

2.2.2 Young People

Research to date examining the association between physical activity levels and risk factors for cardiometabolic diseases has predominantly been conducted in mixed populations that consist of children and adolescents without consideration for the physiological and behavioural differences between the two populations (McCabe et al., 2004). Section 2.2.2.2 will review research that focuses on adolescent physical activity levels, physical fitness and body composition and the relationship of each of these variables with risk factors for cardiometabolic diseases.

2.2.2.1 Children

As explained in section 2.1.7, whilst children do not typically suffer from cardiovascular disease unless presenting with congenital heart conditions, it is during childhood that biochemical and physiological markers associated with cardiometabolic diseases present in the systemic circulation (Magnussen et al., 2012). The presence of such risk factors leads to the development of atherosclerotic plaques (Magnussen et al., 2012), which if not managed

appropriately will lead to early onset cardiovascular disease and type 2 diabetes. In the past decade, the incidence of risk factors for cardiometabolic diseases and the diagnosis of type 2 diabetes in children has increased by 14% (May et al., 2012), with such statistics highlighting that conditions which were once associated with adulthood, can present in children if risk is not managed appropriately. Whilst information is available to suggest the presence of these risk factors is increasing, there is little information as to which of these risk factors is most important for determining childhood cardiometabolic health, despite the known predictive potential of CRP for cardiovascular disease morbidity in adulthood (Emerging Risk factors Collaboration, 2012). Furthermore, observational research in adults has suggested that physical activity and enhanced physical fitness can reduce cardiovascular disease morbidity and mortality (Blair et al., 1989; Paffenbarger et al., 1978), but there is very limited research examining the impact of physical activity and enhanced fitness on cardiovascular disease risk factors in children.

The limited number of studies undertaken in children are summarised in Table 1 (note some studies also include the adolescent age-group but data for children only has been presented). Studies have examined similar risk factors for cardiometabolic diseases (e.g. waist circumference, blood pressure, triglycerides, total and HDL-cholesterol), but there are equivocal findings concerning the relationship between objectively (accelerometers) measured physical activity and these risk factors (e.g. positive relationship observed by Cliff et al., 2014 and no relationship observed by Jimenez-Pavon et al., 2013). Such discrepancies could be the result of differences in confounders across studies, including the socio-economic status, education, ethnicity and family history of non-communicable diseases of the participants.

The strongest relationships between physical activity and traditional risk factors for cardiometabolic health in children have been observed when composite or clustered risk scores

were calculated instead of assessing the relationship with individual risk factors (Carson & Janssen, 2011; Cliff et al., 2014; Jiminez-Pavon et al., 2013). It has been reported that moderate-to-vigorous physical activity (MVPA), after adjustment for sedentary behaviour, was independently and inversely associated with cardiometabolic risk score (calculated from traditional risk factors of waist circumference, systolic blood pressure, non-high-density lipoprotein and CRP). The strength of the relationship between MVPA and the cardiometabolic risk score was supported by the dose-response relationship between the two variable which provides some evidence in support of causality (Carson & Janssen, 2011). Yet, despite such relationships and the evidence for causality, other studies in the field have questioned the clinical significance of the relationship between MVPA and clustered scores of cardiometabolic risk factors (Ekelund et al., 2012), given the small inverse associations reported (Ekelund et al., 2012).

The weight of available evidence in the literature also suggests that more vigorous physical activity is most effective in reducing risk factors for cardiometabolic disease. This was recently highlighted by Jimenez-Pavon et al., (2013) when the relationship between MVPA (assessed by accelerometry) and a composite risk score, was strongest in younger boys (with no relationship observed in boys > 6 y or girls) when the intensity of physical activity was vigorous ($\geq$ 1,003 counts·min^{-1}) with no relationship observed with light or moderate physical activity levels). Whilst this study suggests that physical activity must be vigorous in intensity to enhance childhood cardiometabolic health, it is currently a standalone epidemiological study and thus the findings must be viewed with caution until replicated and observed in girls and other age-groups. Future research should continue to investigate the intensity of physical activity necessary to achieve reductions in risk factors for cardiometabolic diseases, as well as progressing to determine the duration and frequency of physical activity that elicits the greatest beneficial effects on children's health.

Research in children that has determined the relationship between performance on endurance capacity tests and risk factors for cardiometabolic diseases is limited (in comparison to physical activity research) and has focused on traditional (rather than novel) risk factors for cardiometabolic diseases (Hosick et al. 2013; Nightingale et al. 2017; Steene-Johannessen et al. 2013) (Table 1.). Nightingale et al., (2017) measured the most comprehensive panel of risk factors for cardiometabolic diseases in children (Table 1 for overview). As part of the study, nine-year old boys and girls completed an 8 min submaximal step test, which estimated peak oxygen consumption from heart rate response during and 1 min after the test. Strong inverse associations between estimated $\dot{V}O_2$ peak and metabolic risk factors existed, with fasting insulin concentration and HOMA-IR reduced by 20% for each interquartile range increase in estimated $\dot{V}O_2$ peak. The strongest relationship existed between estimated $\dot{V}O_2$ peak and CRP (41% reduction in CRP per increase in interquartile range for $\dot{V}O_2$ peak) a finding of significant importance as CRP is considered the best predictor of cardiovascular disease in adults (Karakas & Koenig, 2009). Despite the agreement of the studies in the field that have assessed childhood endurance capacity and markers of cardiometabolic health (Table 1), further research is necessary (that assesses whether a dose-response relationship exists and explores the relationship across different populations) before a causal relationship in children is inferred.

Although the Nightingale et al. (2017) study estimated $\dot{V}O_2$ peak they actually measured endurance capacity or physical fitness using a performance test. Only one study has extended the examination of the relationship between endurance capacity or physical fitness and cardiometabolic disease risk factors by including measures of body composition (Steene-Johannessen et al., 2013). Endurance capacity (measured by a cycle ergometer test to exhaustion) and body composition (measured as waist circumference) were assessed in the 9-year old children. Regression analysis revealed that performance on the exhaustive cycle

ergometer test was inversely associated with CRP, whilst waist circumference was positively associated with CRP (results that are consistent with the findings of Nightingale et al., 2017). Despite these promising findings, which suggest that enhanced endurance capacity and a smaller waist circumference are associated with reduced CRP concentration in children, further research is necessary to ascertain whether physical fitness and body composition enhances a comprehensive range of risk factors for cardiometabolic diseases (inclusive of traditional and novel risk factors for cardiometabolic diseases) in children.

Table 1. A review of the studies examining the effect of physical activity levels, physical fitness and adiposity on risk factors for cardiometabolic diseases in children

Study Details			Methodology			Findings	
Authors	Study n	Age of Participants (y)	Assessment of Physical Activity/ Physical Fitness/ Body Composition	Risk Factors of Cardiometabolic Diseases Assessed	Adjustment for Confounders	Relationship	Direction
Carson & Janssen, (2011)	2527	6 - 19	MVPA assessed by accelerometry	Waist Circumference Systolic Blood Pressure Non-high density lipoprotein CRP	Age Gender Ethnicity Socioeconomic status Diet	Yes	MVPA reduced cardiometabolic risk score Dose-response relationship
Cliff et al., (2014)	120	8.3 ± 1.1	MVPA assessed by accelerometry	Triglycerides HDL-cholesterol Glucose & Insulin Systolic/ Diastolic Blood Pressure Clustered Cardiometabolic Risk (cMet)	Waist Circumference BMI Sedentary Behaviour	Yes	Inverse association with diastolic blood pressure and cMET
Ekelund et al., (2012)	20871	4 – 18 11.3 ± 2.9	MVPA assessed by accelerometry	Waist Circumference Systolic Blood Pressure Fasting Triglycerides HDL-cholesterol Insulin	Sex Age Wear Time Waist Circumference	Yes	Small, inverse association with all risk factors

Hosick et al., (2013)	124	8 - 12	Submaximal cycle ergometer test to predict maximal oxygen consumption	Serum concentrations of IL-6 and TNF-α	Body fat	Yes	IL-6 concentration higher in low fit group than high fit group
Jimenez-Pavon et al., (2013)	3120	2 - 9	MVPA assessed by accelerometry Fitness measured using the 20 m MSFT in children older than 6 y	Composite cardiovascular risk score: Triglycerides Total Cholesterol HOMA-IR Sum of Skinfolds	Age Adjustment for Country	Yes	Vigorous physical activity inversely correlated with composite risk in younger boys
Nettlefold et al., (2012)	102	8 - 11	MVPA assessed by accelerometry	Large & Small Artery Compliance	Body Surface Area BMI Systolic Blood Pressure	Yes	MVPA explained 5.8 % variance in small artery compliance
Nightingale et al., (2017)	1445	9 – 10	Fitness determined by submaximal step test	Blood Pressure Glucose/ Insulin/ HOMA HbA1c CRP Blood Lipids	Sex Age Ethnic Group Month of Measurement Height	Yes	VO_2 inversely associated with fasting insulin, blood glucose, HOMA, CRP, triglyceride, LDL-c & blood pressure. VO_2 positive association with HDL-c

Owen et al., (2010)	2049	9 - 10	MVPA assessed by accelerometry Questionnaire on cycling and swimming	Total Cholesterol Triacylglycerol Glucose & Insulin HOMA CRP	Age Sex Ethnicity Random Effect for School	Yes	Inverse association for physical activity & insulin, HOMA, triacylglycerol, CRP, & BP
Steene-Johannessen et al., (2013)	1467	9 - 10	Performance capacity determined by a cycle ergometer test to exhaustion Muscular fitness by handgrip strength test, standing broad jump and a sit up test	Serum concentrations of adiponectin, leptin, IL-6, and TNF-α	Age Sex Pubertal Stage	Yes	Inverse association for endurance capacity & inflammation
Willis et al., (2015)	395	7.6 ± 0.6	Length of MVPA assessed by accelerometry	BMI Percentile Waist Circumference Blood Pressure Total Cholesterol/ HDL-c Glucose & Insulin Triglycerides	Age Sex BMI Percentile	Yes	MVPA inversely associated with BMI Percentile and Waist Circumference

2.2.2.2 Adolescents

As children reach puberty their bodies undergo several physiological and psychological changes, which alters not only their biochemistry but their behaviour (McCabe et al., 2004). Observational studies report that during puberty, in young girls especially, physical activity levels progressively decline with increasing age (from 9 to 15 y), with almost all children aged 9 y completing the recommended 60 min of MVPA, which declined to only 31 % of adolescents aged 15 y meeting the government guidelines (Nader et al., 2008). Given the changes during maturation (including hormonal changes and body composition, (Ridder et al., 1991), the decline in physical activity in boys and girls between ages 9 and 15 y (Nader et al., 2008), the fact that physical activity during adolescence tracks into adulthood (Telama et al., 2005) and the increasing prevalence of type 2 diabetes and risk factors for cardiometabolic diseases in adolescents in the past decade (May et al., 2012), it is essential that research is conducted specifically in adolescents. Findings from children and adults may not be applicable in the adolescent population and physical activity behaviour during adolescence may impact upon risk factors for cardiometabolic disease in adulthood.

Research in adolescents assessing the relationship between physical activity levels and risk factors for cardiometabolic diseases is limited (Table 2). Of the available evidence, physical activity has consistently been measured objectively using accelerometers (Bailey et al., 2012; Barker et al., 2018; Rizzo et al., 2008) and traditional risk factors for cardiometabolic diseases (such as blood glucose and plasma insulin) have been examined. The measurement of physical activity levels using accelerometers, as well as being objective is also advantageous as it enables the physical activity to be assigned an exercise intensity, based on metabolic equivalent (METs) (ACSM Guidelines, 2014), which is particularly important for exercise prescription for enhanced cardiometabolic health. In brief, the physical activity is categorised as either light (< 3 METs), moderate (3-6 METs) or vigorous (>6 METs). However, across previous

epidemiological research, moderate and vigorous physical activity have been assessed in conjunction rather than separately and is referred to as moderate-to-vigorous physical activity (MVPA).

Despite the consistencies and the advantages of the methods used to assess physical activity levels in adolescents, discrepancies exist when examining the potential for MVPA to predict adolescent cardiometabolic health. Bailey et al., (2012) reported that in 100 adolescents (59 girls) vigorous physical activity was inversely associated with diastolic blood pressure ($r = -0.27$), whilst there were no other relationships between moderate or vigorous physical activity levels with any other traditional risk factors (including waist circumference, systolic blood pressure, blood lipids, and fasting blood glucose). Similarly, Barker et al., (2018) reported an inverse relationship between vigorous physical activity and waist circumference, whereas no relationship was observed between moderate physical activity levels and other traditional risk factors for cardiometabolic diseases. Whilst these studies suggest that moderate physical activity is not associated with cardiometabolic health in youth, a relationship did exist between vigorous physical activities and select risk factors for cardiometabolic diseases. Yet, there is little agreement as to the specific risk factors vigorous physical activity affects in adolescents.

Whilst most research suggests that moderate intensity physical activity is not related to metabolic risk factors, Rizzo et al., (2008) reported that in 613 adolescents (352 girls) moderate, vigorous and total physical activity levels were inversely associated with fasting glucose, insulin and HOMA-IR. Furthermore, the relationship continued to exist when BMI, waist circumference and skinfold thickness were added to the regression analysis, suggesting that physical activity levels were associated with insulin resistance independently of body composition in adolescents. However, these findings oppose those of recent studies stating that

there is no relationship between MVPA and insulin sensitivity in adolescents (Bailey et al., 2012; Barker et al., 2018).

The inconsistent findings might relate to the difficulties of measuring physical activity levels, as even when physical activity is measured objectively by accelerometers some activities such as cycling and swimming cannot be recorded, levels of different activities may vary with season and day of the week and participants may modify their activity while wearing the accelerometers (Strath et al., 2012). In addition, further inconsistencies might exist due to the varying durations of activity measured in the adolescents and the different modes of activity undertaken, which might confound the relationship between physical activity and cardiometabolic health during adolescence (see Table 2 for overview of studies and potential mediating variables relating to physical activity).

Given the limitations associated with the measurement of physical activity levels in adolescents, physical fitness is a preferred method to assess the benefits of prolonged participation in physical activity. Fitness has been poorly defined in earlier studies and often directly measured or estimated $\dot{V}O_2$ peak during graded treadmill tests (e.g. Bugge et al., 2012; Ischander et al., 2007, Silva et al., 2014) or graded cycle ergometer tests (e.g. Bailey et al., 2012) has been used as the fitness measure, but as discussed earlier this has a large genetic component. There has also been a series of studies recently that determined fitness or endurance capacity (accepted as the measure of fitness in this thesis) by performance on the multi-stage fitness test (MSFT) (e.g. Barker et al., 2018; Buchan et al., 2015; Silva et al., 2017). Given the vast array of performance tests used to measure endurance capacity, it is unsurprising that there are discrepancies across previous research relating to which risk factors for cardiometabolic diseases are associated with endurance capacity (see Table 2). For example, Bailey et al., (2012) determined $\dot{V}O_2$ peak using a maximal cycle ergometer test in adolescent boys and girls

and reported that of the comprehensive list of traditional risk factors for cardiometabolic diseases measured, only waist circumference (r= -0.43), diastolic blood pressure (r = -0.26) and triglycerides (r = -0.20) were correlated with $\dot{V}O_2$ peak, whilst no relationship was observed with systolic blood pressure, total cholesterol, HDL-c or blood glucose). In contrast, when endurance capacity was measured by the MSFT, a relationship was observed with body composition (sum of skinfolds) (r = -0.43) and a clustered cardiovascular disease risk score (r = -0.31) (composed of traditional risk factors, Bailey et al., 2012).

Thus far, this review has focused on the relationship between physical fitness and traditional risk factors for cardiometabolic diseases. However, given the suggested role of low-grade chronic inflammation in the development of atherosclerosis and the increasing prevalence of inflammation in young people (Magnussen et al., 2012); it is important to determine whether a relationship exists between physical fitness, body composition and the novel risk factor, low-grade chronic inflammation. The definition of low-grade chronic inflammation encompasses pro-inflammatory (IL-1β, IL-6, and TNF-a) and anti-inflammatory cytokines (IL-1ra and IL-10) (Pedersen & Petersen, 2005). However, the effect of physical activity and fitness on inflammatory mediators (as a risk factor for cardiometabolic diseases) has to date been limited to an examination of pro-inflammatory cytokines IL-6, TNF-α and CRP (Platat et al., 2006, Buchan et al., 2015, Bugge et al., 2012). An inverse association was reported in adolescents aged 14 – 16 y between IL-6, CRP and TNF-a and $\dot{V}O_2$ peak (Buchan et al., 2015, Bugge et al., 2012), muscular fitness (Buchan et al., 2015) and body composition (Platat et al., 2006, Bugge et al., 2012). However, to appropriately determine whether there is a relationship between physical fitness and cardiometabolic health in adolescents an array of pro- and anti-inflammatory cytokines that are implicated in the development of low-grade chronic inflammation must be measured in heterogeneous population of adolescents (Gleeson et al. 2012).

Only one study to date has appropriately assessed the effect of training and body composition on low-grade chronic inflammation, by measuring a comprehensive panel of pro-inflammatory and anti-inflammatory mediators (Jürimäe et al., 2017). Healthy, pubertal girls (n=30, aged 10 – 12 y) who trained in rhythmic gymnastics for 10-12 h per week and competed at national level were recruited. The rhythmic gymnasts were compared against 30 untrained controls, who were healthy but only recreationally active, completing two sessions of physical activity at school each week. Fasted blood samples were analysed for 12 markers of inflammation (which included anti-inflammatory cytokines IL-1ra and IL-10 and a panel of pro-inflammatory cytokines including IL-6) and leptin concentration. Interestingly, there were no differences between the trained gymnasts and the untrained controls for any of the 12 inflammatory mediators measured, nor were the inflammatory markers related to body composition (measured by DEXA) of the rhythmic gymnasts. These findings oppose those of previous research in adolescents whereby select pro-inflammatory markers were related to physical fitness (Platat et al., 2006, Buchan et al., 2015, Bugge et al., 2012). However, the discrepancies might exist as Jürimäe et al. (2017) did not objectively measure any component of physical fitness, and instead compared well-trained and recreationally active young girls. Therefore, the relationship between physical fitness, body composition and low-grade chronic inflammation remains unknown, particularly in young people due to the limitations of previous research; and thus warrants further investigation.

Cross-sectional research that examines the association between physical fitness, adiposity and cardiometabolic health in adolescents that addresses the limitations of previous research is necessary to continue developing understanding of the variables that are most important in determining cardiometabolic health in young people. Such information can be used to inform future health policy and guidelines, emphasising the importance of a physically active lifestyle and providing details relating to the most appropriate performance tests to be conducted in

adolescents (particularly given the potential to track training status and subsequent capability to predict cardiometabolic health). Such tests could subsequently be used in a school-based setting to evaluate the physical fitness of young people and track their cardiometabolic health through puberty via a non-invasive measurement, thus providing the opportunity to target at-risk individuals and develop appropriate goals to reverse the current adverse health trends (Health Survey for England, 2016).

2.2.3 Recommendations for future research in adolescents

From reviewing the literature to date the following recommendations for future research, assessing the relationship between physical fitness and risk factors for cardiometabolic health in young people can be made:

- Studies should be designed so that if a dose-response relationship between physical activity/ physical fitness/ body composition exists, this can be evidenced.
- The method for measuring physical fitness should appropriate for young people and sensitive enough to track the changes in physical fitness that occur with participation in physical activity and structured exercise. This is particularly important given the limitations of directly measured $\dot{V}O_2$ peak due to the large genetic contribution to maximum oxygen uptake, despite this being the gold standard traditional measure of cardio-respiratory fitness.
- A comprehensive panel of inflammatory mediators should be measured when assessing whether a relationship exists between physical fitness and body composition with low-grade chronic inflammation. The list of analytes should include each of the inflammatory mediators defined in low-grade chronic inflammation and include both pro- and anti-inflammatory cytokines.

- The heterogeneity of the population recruited to the study, particularly the heterogeneity of physical fitness/ body composition should be considered, with relationships more easily identified with a diverse population.

Table 2. A review of studies examining the effect of physical activity levels, physical fitness and adiposity on risk factors for cardiometabolic diseases in adolescents

Study Details			Methodology			Findings	
Authors	Study n	Age of Participants (y)	Type and Assessment Physical Fitness/ Body Composition	Risk Factors of Cardiometabolic Diseases Assessed	Adjustment for Confounders	Relationship	Direction
Artero et al., (2013)	639	12.5 – 17.5	Muscular fitness: Handgrip Strength & Standing Long Jump Body Composition: Sum of skinfolds	White Blood Cell Count Complement factors C3 & C4 Leptin CRP	Age Sex Pubertal Status	Muscular fitness: Yes	Muscular fitness inversely associated with inflammation, partly explained by body composition
Bailey et al., (2012)	100	11.8 ± 1.3	MVPA assessed by accelerometry Endurance capacity: Maximal Cycle Ergometer Test	Cardiometabolic risk score: Waist Circumference Triglycerides Total/ HDL-c Glucose Blood Pressure	Sex Age Ethnicity Socio-economic status	MVPA: No Endurance capacity: Yes	Endurance: negatively associated with WC triglycerides, diastolic BP & clustered score VPA: negatively correlated with diastolic BP
Barker et al., (2018)	534	14.7 ± 1.3	MVPA assessed by accelerometry Endurance capacity: MSFT	BMI Waist Circumference Blood Pressure Fasting Triglycerides HDL-cholesterol HOMA-IR Clustered score	Sex Age Tanner Stage	MVPA: No Endurance capacity: Yes	Endurance and Muscular fitness had independent relationship with body composition & clustered score

Buchan et al., (2015)	192	16.7 ± 0.6	Endurance capacity: MSFT	Composite risk: IL-6 PAI-1, CRP and adiponectin	Sex Age Physical Activity	Endurance capacity: Yes	Endurance capacity negatively associated with composite cardiovascular risk
Bugge et al., (2012)	413	13.4 ± 0.3	Endurance capacity: VO_2 peak test (treadmill) Body Composition: Sum of Skinfolds	HOMA-IR CRP IL-1ra IL-6 TNF-α	Sex Pubertal Development	Endurance capacity: Yes Body Composition: Yes	CRP, IL-6 and TNF-α negatively related to VO_2 peak & sum of skinfolds
Ischander et al., 2007)	74	15.5 ± 0.69	Physical Activity Levels: 3 d questionnaire Body Composition: DEXA	HOMA-IR CRP IL-1ra IL-6 TNF-α IGF-1	---	Physical Activity Levels: Yes	Inactive group has higher concentration of inflammatory mediators IL-6, IL-1ra, and TNF-α compared to the physically active group
Jurimae et al., (2017)	60	11.1 ± 0.6	Trained (Rhythmic Gymnasts) vs. Untrained populations	Leptin IL-1a, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α and VEGF	---	No	---

Magnussen et al., (2012)	1642	15	Muscular fitness: Isokinetic Dynamometers/ Standing Long Jump/ 30 s Push-Up Test	Total Cholesterol/ HDL-c Blood Pressure BMI Waist Circumference	Age Sex Body Mass	Muscular fitness: Yes	Muscular strength, power and endurance inversely associated with clustered risk
Rizzo et al., (2008)	613	15.5 ± 0.5	MVPA assessed by accelerometry Body Composition: Waist Circumference and Sum of Skinfolds	Fasting Glucose, Insulin and HOMA-IR	Sex Waist Circumference Pubertal Status	MVPA: Yes Body Composition: Yes	Inverse association between MVPA but not LPA with insulin sensitivity Negative association between body composition and HOMA
Silva et al., (2014)	53	12.3 ± 1.7	Endurance Capacity: VO_2 peak test (treadmill)	Blood Pressure Fasting glucose and Insulin Total/ HDL-c Triglycerides CRP	---	Endurance Capacity: Yes	VO_2 peak test inversely correlated with CRP
Silva et al., (2017)	957	12 - 13	Endurance capacity: MSFT Body Composition: Skinfold Thickness	Waist Circumference Blood Pressure HDL-c Glucose Triglycerides	Age Sex Age from Peak Height Velocity	Endurance Capacity: Yes Body Composition: Yes	Independent inverse associations with cardiovascular risk score

2.3 Physical Activity, Physical Fitness, Body Composition and Risk Factors for Cardiometabolic Health: Longitudinal Studies

Whilst cross-sectional studies are useful for examining the relationship between exposure variables and disease outcomes (such as the association between risk factors for cardiometabolic diseases with physical activity, physical fitness and body composition), the design of cross-sectional research is associated with several limitations. These limitations include the potential for unevenly distributed confounding variables to create a false positive associations between exposure and outcome (including socio-economic status, education, ethnicity), the potential for sampling frames to lead to selection bias and recall bias from the participants, and the nature of the study design meaning cause and effect cannot be distinguished as participants are only assessed at one time point. Longitudinal studies (sometimes referred to as cohort studies), which recruit two groups of participants (a sample group exposed to the mediating variable, typically a physical activity intervention, and a control group, who are not exposed to the intervention) and follow them across time, correct some of the aforementioned limitations of cross-sectional studies. To further understanding of the relationship between physical activity, physical fitness and adiposity with risk factors for cardiometabolic diseases, section 2.3 will review the longitudinal research in the field.

2.3.1 Adults

As per the cross-sectional studies reviewed in section 2.2.1, the early longitudinal studies in the field of exercise and cardiometabolic health in adult populations focused on the relationship between physical activity levels and cardiovascular disease morbidity and mortality (for a thorough review of such research in adults see Blair, Cheng & Holder, 2001). The first cohort study to observe a relationship between physical activity levels and all-cause mortality (part of the Harvard Alumni studies, n = 16,936) was conducted in men aged 35 -

74 years who were followed up between 1962 and 1978. The recruited participants completed physical activity questionnaires which assessed blocks walked, stairs climbed and the sports they participated in, detailing the duration of such activities. Each activity was then expressed as kilocalories of energy expended each week and participants were categorised into three distinct groups based on a physical activity index. The main finding of the study was that men expending < 2000 kcal per week (lowest physical activity index) had a 38 % greater risk of all-cause mortality than men expending > 3500 kcal per week (highest physical activity index) during the 12 -16-year follow-up.

More recent longitudinal research has focused on the relationship between physical activity and cardiometabolic diseases. Consistently across such research, self-reported physical activity levels (assessed by mail-back questionnaires) were inversely associated with cardiovascular disease morbidity and mortality in men and women; as evidenced by the increased relative risk ratios of 1.98 – 3.58 in participants in the lowest physical activity groups compared with the highest (Bijnen et al., 1998; Haapanen et al., 1996; Haapanen et al., 1997). Similarly, Helmrich et al., (1991) reported that in 5,990 male alumni (University of Pennsylvania) leisure time physical activity was inversely related to relative risk of type 2 diabetes in 5990 male university alumni (with reduced relative risk of 0.65 in men participating in moderate and vigorous sporting activity versus no sport) (Helmrich et al., 1991).

A promising finding of this early research is the consistent dose-response relationship observed between physical activity levels and the prevalence of cardiovascular disease and type 2 diabetes. For example, the relative risk of developing type 2 diabetes in 5,990 male Harvard alumni was 0.90 for those originally classified as moderately active, 0.69 for those originally classified as vigorously active and 0.65 for the two groups combined moderate and

vigorous physical activity combined (Helmrich et al., 1991). The dose-response relationships reported support the idea of a causal relationship between physical activity levels and risk of cardiometabolic diseases in adults (for review, see Blair, Cheng & Holder, 2001).

Given the limitations associated with measuring physical activity levels, as discussed throughout section 2.2, the focus of research has progressed to examine the relationship between physical fitness (variously measured) and the risk of cardiometabolic diseases in adults across time (Blair, Cheng & Holder, 2001). The most noted of such research is that of Blair et al., (1989), whereby test duration during a $\dot{V}O_2$ max test on a treadmill and risk of cardiovascular disease mortality was determined in 13,344 participants (10,224 men and 3,120 women) in the United States. Participants were categorised into physical fitness quintiles at baseline (based on test duration during the $\dot{V}O_2$ max test, which is a better indicator of fitness than $\dot{V}O_2$ max itself) and relative risk for cardiovascular disease mortality calculated for each group at follow-up. Average follow-up time was 8 years, during which a total of 283 deaths were identified on national registers. Cardiovascular disease mortality showed a strong gradient across the physical fitness quintiles in men (age-adjusted death rates per 100,000 years: least fit quintile = 25.0, moderate fitness quintile = 7.8, and most fit quintile = 3.1) and women (age-adjusted death rates per 100,000 years: least fit quintile = 7.4, moderate fitness quintile = 2.9, and most fit quintile = 0.8). Interestingly, the greatest reduction in cardiovascular disease mortality existed between the first (lowest fitness) and second quintiles, suggesting that moderate physical fitness, which should be attainable for most adults, protects against cardiovascular disease mortality (Blair et al., 1989).

Further longitudinal research has examined the potential mediating effect of body composition on the relationship between physical fitness and cardiometabolic health (Lee, Blair & Jackson, 1999). This observational cohort study was of a similar design to the earlier

research of Blair et al. (1989), in that 21,295 men were followed-up for 8 years and physical fitness was accepted as $\dot{V}O_2$ max determined from a maximal oxygen uptake treadmill test. In addition, body composition was assessed by hydrostatic weighing and skinfold thickness. Participants were categorised as 'fit' or 'unfit' males who were either lean, normal weight or obese. Relative risk (RR) of cardiovascular disease mortality was positively related to body composition (men categorised as lean and fit were the reference group to which risk comparisons were made). However, physical fitness mediated the relationship between body composition and risk of cardiovascular disease mortality, with a reduced risk in the fit men across each body composition category (for example obese, fit males: RR = 1.35 whereas, obese, unfit males: RR = 4.08). These findings emphasise the importance of considering both physical fitness and body composition in cohort studies when considering exposure variables for cardiometabolic diseases in individuals of all ages.

Finally, it is important to highlight that longitudinal studies facilitate examination of changes in physical activity and physical fitness over time and how such changes affect cardiometabolic disease risk (for review, see Hardman & Stensel, 2009). The first cohort study to assess the changes in physical activity and risk of mortality, again was part of the Harvard Alumni Health Study (Paffenbarger et al., 1993). Participants completed physical activity questionnaires at baseline (1962 – 1966) and completed follow-up questionnaires in 1977. For each of the observations the participants were categorised based on weekly energy expenditure and participation in moderate-to-vigorous intensity sporting activities. By 1977 participants who were originally inactive and increased their physical activity levels between the two observations reported a reduction in RR (0.85) compared to the originally inactive men who did not increase their activity. Furthermore, originally active individuals who reduced physical activity levels during 1962 – 1977, had the same relative risk as their consistently inactive counterparts (RR = 1.1).

A similar design study examining fitness (treadmill maximum oxygen uptake) rather than physical activity (Blair et al., 1995), showed that participants who improved physical fitness between the two observations had a reduced relative risk of mortality (RR = 0.56), in comparison to participants who remained inactive throughout the follow-up of 5 years. Thus, previous activity or fitness alone is not sufficient to define mortality risk in adults and individuals need to sustain or increase physical activity/fitness during adulthood to enhance health.

2.3.2 Children and Adolescents

Cross-sectional studies have provided a preliminary insight into the potential relationships between physical activity levels, physical fitness and body composition with cardiometabolic health in children and adolescents (see section 2.2 for review). This section reviews the longitudinal research in young people (given the cohort study design, children are typically recruited at baseline and followed-up during adolescence, hence the use of the term young people throughout this section), to determine whether a relationship exists between physical activity and/or physical fitness and risk factors for cardiometabolic diseases and whether this is of clinical relevance for future therapeutic interventions.

As evidenced in Table 3, there is general consensus that moderate-to-vigorous physical activity levels in young people, are inversely associated with peripheral insulin resistance, with change in physical activity over time being related to change in peripheral insulin resistance over time. Hendersen et al., (2016), directly examined the relationship between physical activity levels and peripheral insulin resistance (measured through an oral glucose tolerance test; OGTT) in young people. In total, 630 children (8 – 10 years) wore an accelerometer for 7 days to detail time spent in moderate-to-vigorous physical activity. Body composition was measured using dual-energy X-ray absorptiometry (DEXA), which

determined free fat mass and fat mass. Each measurement was completed at baseline and at follow-up two years later. The main finding was that for every 10 min per day increase in moderate-to-vigorous physical activity, insulin sensitivity improved by 4.8 % at follow-up. In addition, adiposity was also a strong predictor of insulin sensitivity across time, with a 1 % increase in fat mass associated with a 3.2 % decrease in insulin sensitivity. These findings are in agreement with those of Hjorth et al., (2014), whereby changes in total physical activity levels and moderate-to-vigorous physical activity levels in young people during a two-year cohort study (children aged 10 years at baseline), were inversely associated with changes in insulin resistance (measured by HOMA-IR). Such corroborating findings suggest that physical activity levels during childhood predict adolescent insulin sensitivity.

Whilst there is agreement that changes in physical activity levels mediate positive effects on insulin sensitivity in young people, there are inconsistencies across longitudinal research as to the relationship between physical activity levels and other risk factors for cardiovascular disease (see Table 3). For example, in the study of Hjorth et al., (2014), a 10 minute increase per day (during the 2 year follow-up) in total and moderate-to-vigorous physical activity levels was positively associated with HDL-c (+ 0.02 $mmol.L^{-1}$ per 10 min increase in moderate–to-vigorous physical activity) and negatively associated with triglyceride concentration (-0.02 $mmol.L^{-1}$ per 10 min increase in moderate–to-vigorous physical activity). However, there was no relationship between total, moderate or vigorous physical activity and blood pressure, BMI z-score or a clustered score of risk factors for cardiometabolic diseases.

In contrast, Carson et al., (2014) stated that baseline vigorous physical activity levels (in adolescents aged 12 years) predicted systolic blood pressure at follow-up 2 years later, with participants performing the most vigorous physical activity presenting with reduced systolic

blood pressure (β = - 2.41) in comparison to the reference group undertaking the least amount of vigorous physical activity. Further inconsistencies are apparent in the study of Telford et al., (2015), whereby physical activity levels in 8-year old children, assessed by pedometers during a 4 year follow-up, were not associated with dyslipidaemia (total cholesterol, HDL-c and triglycerides). Such inconsistencies in part might relate to differences in the measurement of physical activity, the age of the participants at baseline and the different confounding variables adjusted for in the statistical analysis (evidenced in Table 3). Thus, further research is necessary to establish the specific relationship between physical activity (i.e. moderate, vigorous, total physical activity levels or a combination) and risk factors for cardiometabolic diseases, so that the findings can be used to inform health practices in young people.

There have been more consistent findings across the longitudinal studies examining the relationship between fitness (as oppose to physical activity) and risk factors for cardiometabolic disease in young people (see Table 3). A recent study recruited children (aged 6-11 years) from 8 European countries and used performance in the MSFT as a measure of fitness. The selected traditional risk factors for cardiometabolic diseases were blood pressure, triglycerides and HDL-c as a measure of dyslipidaemia, and insulin resistance assessed by HOMA-IR. The measurements were completed at baseline and 2 years later at follow-up. Linear regression analysis (adjusted for sex, age, parental education, BMI and physical activity levels) showed that performance on the MSFT enhanced a composite score of risk factors for cardiometabolic diseases (β = - 0.06). Yet, when examining the relationship between performance on the MSFT with isolated risk factors, only waist circumference was longitudinally predicted by performance on the MSFT (β = - 0.05). Similar findings have been observed in children aged 6 years, during a 2-year cohort study (Andersen et al., 2011), with low $\dot{V}O_2$ peak at baseline moderately predicting a clustered risk score 2 years later, as

calculated from HOMA-IR, total cholesterol, HDL-c, triglycerides and blood pressure (r = 0.49).

Longitudinal studies to date have only focused on the relationship between physical fitness and traditional risk factors for cardiometabolic diseases (see section 2.1.3 for detailed description), with no consideration of the relationship between performance on endurance capacity tests with novel markers (such as the pro- and anti-inflammatory cytokines that are implicated in the pathogenesis of low-grade chronic inflammation) or clinical measures of cardiometabolic health (such as flow-mediated vasodilation). Furthermore, often the statistical analysis did not account for the potential mediating effects of pubertal development, with chronological age the only mediating variable considered in the analysis of previous research (see Table 3). Yet, as discussed in section 2.1.6, pubertal development accounts for physiological and behavioural changes that might influence the relationship between physical fitness and adolescent health, which chronological age cannot. As a result, there is little information relating to the effect of changes in physical fitness across time on changes in traditional and novel risk factors for cardiometabolic health in adolescents during puberty.

Furthermore, to date, only one study has considered the potential for the method for the measurement of body composition and the distribution of fat mass to affect the strength of the association between adiposity and traditional risk factors (with no studies including novel risk factors) for cardiometabolic diseases in young people (Lawlor et al., 2010). The Lawlor et al. (2010) study employed a longitudinal, population-based cohort design, which consisted of 5235 young people. Participants attended three clinics at aged 9-10, 11-12 and 15-16 years and had their body composition assessed by BMI z-score, waist circumference, and a DEXA scan. At the final clinic the adolescents had a fasted blood sample taken, which was subsequently analysed for total cholesterol, HDL-c, LDL-c and triglycerides. All three

measures of childhood adiposity were prospectively associated with adverse cardiovascular profiles in adolescence, with similar magnitudes of association at each age group (increased outcome odds ratios of 1.03 - 1.99 for traditional risk factors for cardiometabolic diseases including blood pressure and insulin sensitivity). Whilst these findings suggest that the measure of adiposity, whether the gold standard or a field measure, does not mediate the relationship between body composition and adolescent cardiometabolic health, it remains unknown which measurement of physical fitness is most appropriate for the prospective prediction of risk factors for cardiometabolic disease in adolescents.

2.3.3 Future Recommendations for Cohort Studies in Young People

The following recommendations are suggested for future cohort studies in young people that directly assess the relationship between physical activity, physical fitness and body composition with risk factors for cardiometabolic health in young people:

- To longitudinally examine how continuous training versus remaining inactive during adolescence effects performance on physical capacity tests, $\dot{V}O_2$ peak, adiposity and traditional and novel risk factors for cardiometabolic diseases. Such information is crucial for the maintenance and enhancement of adolescent cardiometabolic health.
- To longitudinally assess how changes in performance on physical capacity tests, $\dot{V}O_2$ peak and adiposity are related to the changes in risk factors for cardiometabolic diseases in adolescents across time.
- To consider the potential confounding variables that are likely to affect the relationship between physical fitness, body composition and risk factors for cardiometabolic health throughout adolescence, with particular consideration to the effect of pubertal status.
- Future studies should consider the performance test selected to assess physical fitness and the method for measuring body composition to ensure that should a relationship exist, the measurement is sensitive enough and able to detect an association across time.

Furthermore, the measurement should be applicable to children, adolescents and adults to allow for continuation of the study throughout the participant's lifetime.

- As there have been no studies to date that have examined the association between inflammatory mediators with physical fitness and body composition future studies should address this and determine whether a relationship exists across time. The list of analytes should be comprehensive and include each of the inflammatory mediators defined in low-grade chronic inflammation, especially as this is one of the main risk factors implicated in the development of cardiometabolic diseases.
- Whilst early research has suggested that physical activity levels are associated with insulin sensitivity throughout adolescence, there is yet to be a study that has examined whether physical fitness is directly associated with risk of type 2 diabetes in young people (with previous studies assessing cardiovascular and metabolic health collectively within a composite or clustered risk score). Such information is important, given the increasing prevalence of type 2 diabetes in young people in the United Kingdom and the increasing need for therapeutic interventions to reverse current trends.

Table 3. A review of longitudinal studies that have examined the effect of training, performance on physical capacity tests and adiposity on risk factors for cardiometabolic health in adolescents

Study Details			Methodology			Findings	
Authors	Study n	Age of Participants (y)	Type and Assessment Physical Fitness/ Body Composition	Risk Factors of Cardiometabolic Diseases Assessed	Adjustment for Confounders	Relationship	Direction
Andersen et al., (2011)	Baseline: 484 Follow-up: 434	Baseline: 6.8 ± 0.4 Follow-up: 9.5 ± 0.8	MVPA assessed by MTI activity monitor Endurance capacity: Maximal Treadmill Test	Glucose/ Insulin (HOMA-IR) Total Cholesterol/ HDL-c Triglycerides Blood pressure	Age Sex Sum of Skinfolds	MVPA: No Endurance capacity: Yes	Low physical fitness at age 6 predicted later development of clustered CVD risk.
Carson et al., (2014)	Baseline: 315	Baseline: 12.2 ± 0.8 Follow-up: 2 years	MVPA assessed by accelerometry	Waist Circumference BMI z score Systolic Blood Pressure	Sex Age Dietary Intake	MVPA: Vigorous Physical Activity Only	Time spent in VPA at baseline was the primary predictor of cardiometabolic health 2 y later
Hjorth et al., (2014)	Baseline: 723 Follow-up: 632	Baseline: 10.0 ± 0.6 Follow-up: 2 years	MVPA assessed by accelerometry	BMI z score Blood Pressure Glucose/Insulin (HOMA-IR) Fasting Triglycerides HDL-cholesterol Clustered score	Sex Age Pubertal Status Baseline movement behaviour Baseline risk marker of interest	MVPA: Yes	Changes in total PA and MVPA both associated with HDL-c and HOMA-IR. MVPA inversely associated with triglycerides

Henderson et al., (2016)	Baseline: 630 Follow-up: 564	Baseline: 9.6 ± 0.9 Follow-up: 11.7 ± 0.9	MVPA assessed by accelerometry Body Composition: DEXA	Oral Glucose tolerance Test Glucose/Insulin (HOMA-IR) Fasting Triglycerides Total cholesterol/ HDL-c	Season Endurance Capacity	MVPA: Yes Body Composition: Yes	Every 10 min increase in MVPA was associated with a 3.5% decrease in body fat & 4.8% increase in insulin sensitivity.
Klakk et al., (2014)	Baseline: 729 Follow-up: 365	Baseline: 9.4 ± 0.8 Follow-Up: 2 years	Endurance capacity: Andersen Intermittent Test (treadmill) Body Composition: DEXA and waist Circumference	Blood Pressure Composite Risk Score: HOMA-IR, SBP, Total Cholesterol/HDL-c, Triglycerides	Sex Age Pubertal Development	Endurance capacity: Yes Body Composition: Yes	Baseline adiposity and endurance capacity inversely associated with composite risk score.
Lawlor et al., (2010)	Baseline: 5235	Baseline: 9 - 10 Follow-Up: 15 - 16	Body Composition: BMI z score Waist Circumference DEXA	Blood Pressure Insulin/ Glucose/ HOMA-IR, Total Cholesterol/ HDL-c / LDL-c Triglycerides	Household Occupation Parental Education Birth Weight Height Gestational Age Parental BMI Age Pubertal Status	Yes	BMI, Waist Circumference and FM from the DEXA were associated with increased odds of adverse SBP, HDL-c, LDL-c, triglycerides, and insulin.
Telford et al., (2015)	Baseline: 694 First Follow-up: 563 Second Follow-Up: 469	Baseline: 8.1 y First Follow-Up: 10.1 y Second Follow-Up: 12.1 y	Endurance capacity: MSFT Body Composition: DEXA Physical Activity: Pedometer	Total Cholesterol/ HDL-c Triglycerides	Age Height Body Mass Body Surface Area	Endurance capacity: Yes Body Composition: Yes Physical Activity: No	Individual blood lipid markers inversely associated with endurance capacity and body composition

Zaquot et al., (2016)	Baseline: 1635	Baseline: 6 - 11 2 y follow-up	Endurance capacity: MSFT	SBP/ DBP Triglycerides HDL-c Insulin Resistance – HOMA-IR	Sex Age Parental Education Socio-demographic status	Endurance capacity: Yes	Following adjustment endurance capacity was the only significant longitudinal predictor of metabolic syndrome

2.4 Physiological Responses to Acute Bouts of Exercise

The epidemiological studies reviewed in section 2.2 and 2.3 collectively suggest that there is a relationship between physical activity, physical fitness and body composition with traditional risk factors of cardiometabolic diseases in children, adolescents and adults. Yet, as discussed cross-sectional and longitudinal studies only determine associations between exposure and outcome variables, and therefore cannot infer causality. The effect of an acute bout of exercise on traditional and novel risk factors for cardiometabolic diseases has been used to identify the potential mechanisms that in part explain the associations between exposure and outcome variables. Section 2.4 will review the available research that has examined the physiological responses (with a particular focus on the inflammatory, glycaemic and insulinaemic responses) to acute bouts of exercise in young people and adults. Mechanistically, if such responses are repeated regularly it is hypothesised that this would reduce the risk of cardiometabolic diseases (Petersen & Pedersen, 2005; Gleeson et al., 2012).

2.4.1 Adults

Several studies in adults have examined the effect of acute exercise on traditional risk factors for cardiometabolic diseases (such as postprandial lipemia, postprandial glycaemic and insulinemic responses, and endothelial function) (for review, see Freese et al., 2013). Few studies though have examined the moderating effect of the consumption of standardised meals on the exercise response which is important as a large portion of awake time is spent in the postprandial state.

The postprandial state is characterised by elevated triglyceride concentration (regular exposure to elevated triglyceride concentration is a physiological risk factor of cardiovascular disease, see section 2.1.3). In their quantitative review, Freese et al., (2013) reported that an acute bout

of aerobic exercise (in a pooled sample of 574 healthy males and females, taken from 70 studies) had a moderate, inverse effect on triglyceride incremental area under the curve (iAUC) following the consumption of a standardised meal (Cohen's d = - 0.58). Interestingly, the effect of prior exercise was stronger with increased exercise intensity (which was of shorter duration given the increased intensity), as high intermittent exercise was associated with a larger reduction in triglyceride iAUC following the consumption of a standardised meal than moderate intensity exercise (Cohen's d = - 1.49). The collective findings of previous research suggest that an acute bout of exercise successfully reduces postprandial lipemia following the consumption of a high fat meal in healthy adults, with exaggerated effects in women and following high intensity interval exercise.

Hypertension and reduced compliance of blood vasculature (traditional risk factors of cardiometabolic diseases) are risk factors of atherosclerosis, as both can lead to injury to the intima layer of a blood vessel and the response to injury hypothesis (see section 2.1.3). A common method employed to assess the compliance of the blood vasculature and to determine how well accustomed the blood vessels are responding to shear stress, is flow-mediated dilation (FMD) (Gonzales et al., 2010; McClean et al., 2015). It was recently shown that shear stress was significantly higher immediately after high intensity cycling exercise (5 min at 100% $\dot{V}O_2$ max) when compared with exercises of lesser intensity (30 min at 55% $\dot{V}O_2$ max, 20 min at 75% $\dot{V}O_2$ max and 5 min at 100% $\dot{V}O_2$ max) (McClean et al., 2015). The authors suggested that the increased shear stress post-exercise (causing vessels to dilate), if repeated regularly, could explain the relationship between endothelial function and chronic physical activity levels. Such findings have implications for public health recommendations relating to the type of exercise that is best suited for the prevention of risk factors of cardiometabolic diseases in adults. However, more research is required to determine whether the responses to acute bouts

of exercise are responsible for the chronic benefits to cardiometabolic health that result from long-term participation in physical activity.

The glycaemic and insulinaemic responses to acute bout of exercise also have a role in the prevention of traditional risk factors of cardiometabolic diseases in adults (particularly metabolic health, such as the prevention of type 2 diabetes). As discussed in section 2.1.5, there are several methods to assess the glycaemic and insulinaemic response to acute bouts of exercise. The most common of these methods employed across previous research in adults is the change in blood glucose and plasma insulin concentration across time (pre- and up to several hours post-exercise) (Mendham et al., 2012, 2013, 2015). In a series of studies conducted in Australian middle-aged men (38 – 48 years), the glycaemic and insulinaemic responses to small-sided games (modified rugby) and traditional endurance activity (40 min of stationary cycling at ~ 80 % heart rate max) were observed up to 4 h post-exercise (Mendham et al., 2012, 2013, 2015). The main findings of the studies were consistent with previous literature (Fischer, 2006; Kramer and Goodyear, 2007), as the endurance and games-based activity elicited favourable glycaemic and insulinaemic responses (with a reduced plasma insulin from baseline in both trails). Additionally, fasted HOMA-IR (a measure of insulin resistance) measured the morning after the exercise, was also reduced from baseline in each of the exercise trials (baseline: 4.50; cycling trial: 1.76; rugby trial: 1.54 the morning following). Whilst such findings suggest that endurance and games-based activity in middle-aged men elicit transient reductions in fasted blood glucose and plasma insulin concentrations, it remains unknown whether the response differs in magnitude when assessed in the postprandial state. As such, future studies should consider the ecological validity of the study design, considering the consumption of standardised meals to ensure that insulin sensitivity post-exercise remains enhanced when the participants are exposed to additional variables that are incorporated into their everyday lives.

Finally, as described in section 2.1.5, the inflammatory responses to acute bouts of exercise are suggested to have anti-inflammatory properties that could over-time mediate reductions in low-grade chronic inflammation, which is the leading predictor of cardiovascular events in adults (Gleeson et al., 2011). Recently, high intensity intermittent exercise (10 x 60 s cycling at 90% maximum power) (Dorneles et al., 2016) and games-based activity (modified rugby) (Mendham et al., 2015) in middle-aged men induced a transient increase (~ 60% from baseline to 30 min post-exercise) in cytokines with anti-inflammatory properties (IL-6, IL-1ra and IL-10); whereas, moderate intensity exercise (10 x 60 s cycling at 75 % maximum power) did not induce a change in any of the inflammatory mediators measured (including IL-1ra, IL-6, IL-8, IL-10 and IL-17) (Dorneles et al., 2016). Such findings suggest that high intensity exercise and games-based activities might be the most effective potential therapeutic interventions (in comparison with lower intensity activities) to reduce low-grade chronic inflammation and the enhancement of cardiometabolic health in adults. However, it is unknown whether the inflammatory response observed in middle-aged men is applicable to other populations, including women, children, adolescents and older adults. This is particularly important for young people given that childhood health tracks into adult health (Herman et al., 2009).

2.4.2 Children and Adolescents

Given the limited number of studies in children examining the transient response to acute bouts of exercise (only two studies, see Table 4), this section will review previous research in children and adolescents together and will include an evaluation of studies that have examined the response of triacylglycerol, endothelial function, insulin sensitivity and inflammation to acute bouts of exercise.

As mentioned throughout section 2.4.1, regular exposure to elevated triacylglycerol is a key risk factor in the development of cardiometabolic diseases, particularly in the aetiology of

atherosclerosis (Hardman and Stensel, 2009). The consumption of high fat meals, which has become increasingly common in young people in western countries (Taveras et al., 2005), increases postprandial triacylglycerol concentration (Sedgwick et al., 2013), and is therefore of concern for adolescent cardiometabolic health. An acute bout of prior exercise is suggested as a potential intervention to reduce exposure to postprandial triacylglycerol in young people (Bond et al., 2015b; Sedgwick et al., 2013; 2014). Recently, acute bouts of moderate intensity exercise, performed as continuous exercise on a treadmill (Sedgwick et al., 2013) or a cycle ergometer (Bond et al., 2015b), reduced plasma triacylglycerol iAUC following the consumption of a high fat meal by 34 % one hour post-exercise (Bond et al., 2015b) and by 24% the day following exercise (Sedgwick et al., 2013) in comparison to a rested control trial. An acute bout of high intensity cycling at 90 % peak power (a time efficient mode of activity for young people) was also successful in reducing postprandial lipemia in adolescent girls (-38%) following the consumption of a high fat meal (Bond et al., 2015a).

Whilst such findings are promising, such activity is not replicative of the activity patterns of adolescents (Howe et al., 2012), which consist of sporadic, intermittent bouts of activity, similar to the activity patterns observed during games-based exercise. Only one study to date in 15 adolescent boys aged 12 years has examined the effect of games-based activity (small-sided soccer) on postprandial lipaemia in comparison with treadmill exercise (Smallcombe et al., 2018). Interestingly, the games-based activity (48 min) elicited a large reduction in fasting triacylglycerol concentration (-30 %) compared with a rested control trial, which was greater than the effect (-16 %) observed following moderate exercise on a treadmill (48 min at 65 % $\dot{V}O_{2peak}$) (Smallcombe et al., 2018). Although promising, these findings need corroborating and extending before games-based activity can be promoted to reduce postprandial lipemia in adolescents.

In conjunction with the assessment of postprandial lipemia, Sedgwick et al., (2013, 2014) also examined the effect of an acute bout of moderate intensity treadmill walking (at 60% $\dot{V}O_{2peak}$ for 60 min) and high intensity cycling (40 x 6 s maximal sprints on a cycle ergometer) on endothelial function in adolescent boys aged 12-14 years. The consumption of a high fat breakfast and lunch during a rested control trial, reduced FMD by 20 % and 27 %, respectively. A reduction in FMD is a suggested risk factor for cardiometabolic diseases as a reduced ability of the blood vasculature to dilate, increases the risk of an injury occurring to the intima layer of the blood vessel and as such could result in the response to injury hypothesis (see section 2.1.3 for overview). Interestingly, the moderate and high intensity activity undertaken the day prior to the consumption of the high fat meals, reversed the postprandial endothelial dysfunction in the adolescent boys (Sedgwick et al., 2013, 2014). Such findings suggest that prior day exercise, successfully reduces the adverse responses to endothelial function that occur following the consumption of a high fat meal. However, for both postprandial lipemia and vascular function the optimum intensity of exercise to elicit such responses are unknown. Furthermore, there have been no studies to specifically examine the effects of games-based activity on postprandial lipemia or endothelial function in adolescent girls or children aged < 11 years and as such research in these populations should be of high priority.

The glycaemic and insulinaemic responses to acute bouts of exercise in children and adolescents is relatively unexplored to date and studies have not been ecologically valid (see Table 4). As a secondary aim, the studies of Barret et al., (2007) and Sedgwick et al., (2013, 2014) assessed postprandial blood glucose and plasma insulin tAUC to a meal following continuous, moderate intensity and high intensity intermittent activity in adolescent boys. Interestingly, there was no effect of exercise (whether of high or moderate intensity) on blood glucose or plasma insulin tAUC the day following the exercise. Across each of these studies, the standardised breakfast and lunch consumed the day following exercise had a high fat

content (1.25 – 1.5g of fat per kg body mass), as the primary aim of the study was to assess postprandial lipemia. It might be that the high fat content of the meals, which lacked ecological validity, was not appropriate or sensitive to the potential changes in the glycaemic and insulinaemic responses. Furthermore, as the glycaemic and insulinaemic responses were observed the day following exercise it might be that these particular responses are not residual and do not remain beyond 24 h post-exercise. Thus there is a need to determine the time course of the glycaemic and insulinaemic responses to acute exercise bouts to determine the optimum exercise frequency that maintains enhanced insulin sensitivity in young people.

The glycaemic and insulinaemic responses to same-day high intensity intermittent (8 x 1 min sprints at 90% peak power) and moderate intensity cycling (work-matched to the high intensity exercise, performed at 90% of the gas exchange threshold) have recently been observed in children (Cockcroft et al., 2017) and adolescents (Cockcroft et al., 2015). Ten minutes post-exercise, 75 g of glucose in 300 mL of water was consumed (a method commonly used to assess insulin sensitivity, referred to as the oral glucose tolerance test) and the postprandial glycaemic and insulinaemic responses were observed for up to 2 h. In both the children and adolescents, blood glucose and plasma insulin tAUC were reduced following the moderate intensity and high intensity intermittent activity (by 6 - 13%) when compared to a rested trial (Cockcroft et al., 2015).

The glycaemic and insulinaemic responses observed, were concluded to have enhanced insulin sensitivity in the male participants and the high intensity activity was deemed a time efficient alternative to enhance cardiometabolic health. It is important to note that the studies of Cockcroft et al., (2015, 2017) do have several limitations that should be considered when interpreting the findings. The first is that the studies were conducted in a relatively small sample of healthy boys. Given that sex differences exist in the glycaemic and insulinaemic

responses to the consumption of standardised meals (Cooper et al., 2017), which are attributed to pubertal differences between the sexes, it is especially important that findings in adolescent boys are not applied directly to adolescent girls. Furthermore, the oral glucose tolerance test, whilst a commonly used measure of insulin sensitivity in young people, is not ecologically valid given that meals usually contain a mixture of macronutrients, which this specific test does not. Future research should consider such limitations to enhance understanding as to the glycaemic and insulinaemic responses to standardised mixed meals in male and female adolescents, for application in future interventions that aim to enhance insulin sensitivity in young people.

As the physiological responses to acute bouts of exercise are transient in nature, it is also important to determine the time course of the responses, to inform decisions relating to exercise frequency. To date, only one study has considered the acute and residual glycaemic and insulinaemic responses to a standardised meal following 45 min endurance-based activity in adolescents (Short et al., 2013). Participants completed three trials, with the rested control trial on the first week and the two exercise trials (same-day and prior-day exercise) completed in a randomised order thereafter. The exercise consisted of 45 min moderate intensity activity, which included 15 min treadmill walking, 15 min cycling and 15 min boxing on a video game. The mixed meal (which was high in fat) was consumed either 40 min (same-day) or 17 h post-exercise (prior-day). Insulin sensitivity was enhanced on both trials, as evidenced by the reduced glycaemic and insulinaemic response to the high fat meal, however when beta-cell responsivity was examined on the exercise day a greater improvement in insulin sensitivity was observed than when assessed the day following exercise (78 % *vs*. 45 % improvement for same-day and prior-day, respectively).

Whilst these findings enhance understanding of the acute and residual glycaemic and insulinaemic responses to moderate intensity, continuous exercise in adolescents, again there are several limitations. Only endurance exercise was assessed, which has limited application in young people given that adolescents tend to participate in sporadic, intermittent activity (Howe et al., 2010). Therefore, it remains unknown whether games-based activity (an ecologically valid alternative) improves insulin sensitivity in young people. Furthermore, there has previously been no consideration for the optimum duration of exercise (for any mode of activity) for the enhancement of insulin sensitivity in young people. Exercise duration is of high importance considering that young people currently fail to meet current recommendations of 60 min moderate-to-vigorous physical activity per day, and as such it is important to ascertain whether shorter durations of exercise elicit similar benefits to those observed following a 45-60 min bout of continuous activity (Cockcroft et al., 2015; Short et al., 2013).

The response of inflammatory mediators (including anti-inflammatory cytokines IL-1ra, Il-6 and IL-10) to acute bouts of exercise are deemed important for the prevention of low-grade chronic inflammation in young people (Petersen & Pedersen, 2005), as explained in section 2.1.5. Despite the potential role of the inflammatory response to exercise in the prevention of low-grade chronic inflammation, few studies have examined the pro- and anti-inflammatory response to acute bouts of exercise in young people (Table 4). In a series of studies, Nemet et al., (2002, 2003, 2009) assessed the response of select inflammatory mediators (IL-1ra, IL-6, and TNF-α) to different modes of exercise, including 90 min wrestling, 90 min water polo and 60 min cross-country running in adolescent boys and girls. Whilst each of the different modes of exercise transiently increased the concentrations of the inflammatory mediators immediately post-exercise, the magnitude of the response was dependent on the mode of exercise undertaken. For example, wrestling practice increased IL-6 concentration 7-fold, whereas cross-country only elicited a 2-fold increase.

Given the variation in the magnitude of the inflammatory responses to the different types of exercise, it is important to determine the inflammatory response to an ecologically valid mode of exercise to determine whether such activity can enhance cardiometabolic health in adolescents. Furthermore, in previous studies that have assessed the inflammatory response to exercise, only a limited number of cytokines have been measured post-exercise (IL-1ra, IL-6, and TNF-α). Given the findings of *in vitro* studies (as described in section 2.1.5), it is important to determine whether pro-inflammatory mediators are inhibited (IL-1β, TNF-α and CRP) and anti-inflammatory cytokines stimulated (IL-1ra, IL-6, IL-10) post-exercise. Future studies should therefore measure a comprehensive panel of inflammatory mediators following an ecologically valid acute bout of exercise, such as games-based activity.

The series of studies conducted by Nemet et al., (2002, 2003, 2009) specifically examined the inflammatory response pre- and immediately post-exercise in adolescents and did not extend the observation beyond this time point. Whilst the response immediately post-exercise is important, it does not allow for the potential changes that could result up to several hours post-exercise to be observed. Such information is necessary as several inflammatory mediators (e.g. anti-inflammatory cytokine IL-10 and CRP) are suggested to increase 24 - 48 h post-exercise (Petersen & Pedersen, 2005). The longest time post-exercise studied to date is 6 h (600 kcal treadmill running at 65% $\dot{V}O_{2peak}$) in adolescent boys (MacEneaney et al., 2009). Following treadmill exercise, IL-6 concentration increased by 95% from baseline, whereas TNF-α and CRP concentration remained unchanged post-exercise, but the time-course to the 6 h point was not studied and nor were any responses examined beyond this point. Future studies should detail the inflammatory response to acute bouts of exercise across the course of the day to enhance understanding into the response of each of the inflammatory mediators of interest, which should again include a comprehensive panel of inflammatory mediators, (such as, IL-1β, IL-6, IL-10, TNF-α and CRP) and not be limited to a select number of cytokines, as with

previous research. Such studies would therefore become ecologically valid, as the responses to exercise would be observed in conjunction with the daily habits of young people (including the consumption of standardised meals and the diurnal variation of the inflammatory mediators observed).

The physiological responses to acute bouts of exercise are important mechanistically, as these responses (including the inflammatory, glycaemic and insulinaemic responses) when repeated regularly facilitate the relationships observed between physical fitness and risk factors for cardiometabolic health (Gleeson et al. 2012). Knowledge of the inflammatory, glycaemic and insulinaemic responses to exercise and the timescale for which these responses remain are important for the development of physical activity guidelines, particularly when determining the type, intensity, duration and frequency of exercise that should be completed to stimulate a protective response in adolescents. These details are currently under-researched, despite their importance for exercise prescription when aiming to improve cardiometabolic health. It is essential future research examining the physiological responses to acute bouts of exercise continues, whilst addressing the aforementioned limitations and gaps in understanding, to ensure that effective physical activity guidelines, which are ecologically valid and appropriate for adolescents, are developed.

Table 4. A review of the studies examining the cardiometabolic responses to acute bouts of exercise in children and adolescents

Study Details				Outcome			
Authors	Study n	Participant Details	Design and Conditions	Vascular Function	Postprandial Lipemia	Blood glucose/ Plasma insulin/ HOMA	Inflammation
Barrett et al., (2007)	n = 19 Continuous Exercise (CE) = 10 Intermittent Games (IG) = 9	All males Age (CE) = 15.3 ± 0.1 y Age (IG) = 15.4 ± 0.1 y	Randomised, Between Measures Design (separated by 7 d) CE: 4 x 15 min treadmill walking at 59% VO_{2peak} *vs.* rested control trial IG: 4 x 18 min intermittent shuttle running at 69% VO_{2peak} *vs.* rested control trial Test Meal: 1.25g fat, 1.07g CHO, 67 kJ per kg body mass		CE Triacylglycerol tAUC: 14% reduction compared to rested control trial IG Triacylglycerol tAUC: 26% reduction compared to rested control trial No effect of either mode of exercise on iAUC triacylglycerol *vs.* control	CE: No effect on iAUC or tAUC on blood glucose concentration IG: No effect on iAUC or tAUC on blood glucose concentration	
Bond et al., (2015a)	n = 20	10 males, 10 females Age: 14.1 ± 0.3 y	Counterbalanced, within-measure design HIIE: 8 x 1 min at 90% peak power MIE: work-matched cycling at 90% of gas exchange threshold Rested Control Trial	HIIE: FMD decreased immediately post, increased 2 h post from 8.7 – 11.8% MIE: No effect on FMD post-exercise			

Bond et al., (2015b)	n = 19	9 males, 10 females Age: 14.3 ± 0.3 y	Counterbalanced, within-measure design HIIE: 8 x 1 min at 90% peak power MIE: work-matched cycling at 90% of gas exchange threshold High Fat Meal = 1.5g.kg^{-1} fat consumed 1 h post-exercise	No effect of HIIE or MIE on triacylglycerol concentration (tAUC post-exercise) In girls, triacylglycerol iAUC decreased by 38% following HIIE and 34% following MIE	No effect of HIIE or MIE on blood glucose concentration (tAUC or iAUC)
Cockcroft et al., (2015)	n = 9	All males Age: 14.2 ± 0.4 y	Within Measures, Counterbalanced Design HIIE: 8 x 1 min at 90% peak power MIE: work-matched cycling at 90% of gas exchange threshold Oral Glucose Tolerance Test 10 min post-exercise: 75g CHO		HIIE: Blood glucose tAUC decreased (8%); plasma insulin tAUC decreased (13%) MIE: Blood glucose tAUC decreased (6%); plasma insulin tAUC decreased (12%) Insulin Sensitivity 11% & 8% increase in HIIE & MIE, respectively
Cockcroft et al., (2017)	n = 11	All males Age: 8.8 ± 0.8 y	Within Measures, Counterbalanced HIIE: 8 x 1 min at 90% peak power MIE: work-matched cycling at 90% of gas exchange threshold Oral Glucose Tolerance Test 10 min post-exercise: 75g CHO		Insulin sensitivity increased 10% after HIIE and 7% after MIE

Eliakim et al., (2015)	n = 57	Age: 14 - 16 y	Between Subjects Design Inflammatory response to games-based training sessions (water polo, volleyball and wrestling)		Pre- to post-exercise IL-6 concentration increase between 1 - 6-fold Pre- to post-exercise IL-1ra concentration increase up to 92 %
MacEneaney et al., (2009)	n = 10	All males Age: 15.6 ± 0.7 y Normal Weight	600kcal treadmill exercise at 65% VO_2 max Rested Control Trial Oral Fat Tolerance Test consumed the morning following exercise (97g fat, 124g CHO, 1,450kcal per 2 m^2 body surface area)	Total cholesterol increased (5%) during the rested control trial, whereas no change post-exercise	IL-6 concentration increased during the rested control trial (107%) & exercise trial (95%) No changes in TNF-α or CRP on either trial
Nemet et al., (2002)	n = 11	All males Age: 16.5 ± 0.5 y	Within Subjects Design Wrestling Practice 1.5 h – warm up, technique drills and a match		Pre- vs. Post-Exercise IL-6: 700% increase IL-1ra: 92% increase TNF-α: 37% increase IL-1β: 123% increase

Nemet et al., (2003)	n = 10	All girls Age: 14 - 16 y	Water Polo Practice 1.5 h – warm up, technique drills and a match			IL-6 (244%) and IL-1ra (60%) increased post-exercise, whereas TNF-α did not change
Nemet et al., (2009)	n = 8	All girls Age: 16.75 ± 0.5 y	Cross Country Training 1 h – warm up (10 min), continuous run (50 min)			Pre- vs. Post-Exercise IL-6: 100% increase IL-1ra: 48% increase TNF-α: 17% increase
Sedgwick et al., (2013)	n = 13	All males Age: 13.6 ± 0.6 y Normal Weight	Within Measures Design Treadmill walking at 60% VO_{2peak} for 60 min Rested Control Trials High Fat Breakfast & Lunch on Day 2: 1.5g fat and 1.8g CHO	Basal and peak diameter increased following the high fat meals Postprandial FMD was improved following the exercise trial compared to the rested control trial	Plasma triacylglycerol tAUC was 22% lower following the exercise trial compared to the rested trial Plasma triacylglycerol iAUC 24% lower following the exercise trial compared to the rested trial	

Sedgwick et al., (2014)	n = 9	All males Age: 13.1 ± 0.6 y	Within Measures Design 40 x 6 s maximal cycle sprints on Day 1 High Fat Breakfast & Lunch on Day 2: 1.5g fat and 1.8g CHO	No change in fasted FMD following exercise Exercise prevented decline in FMD following consumption of the high fat breakfast and lunch (-20% & -27%, respectively)	Plasma triacylglycerol tAUC was 13% lower following the exercise trial compared to the rested trial Plasma triacylglycerol iAUC 15% lower following the exercise trial compared to the rested trial	No effect on blood glucose or plasma insulin tAUC	
Short et al., (2013)	n = 12	7 Males/ 5 females Age: 14 ± 2 y	Within Measures Design No Exercise Trial Prior Day Exercise Trial and Same Day Exercise Trial (45 min moderate intensity at 75% peak HR – walking cycling, boxing game)			Prior Day Exercise: 45% increase in insulin sensitivity Same Day Exercise: 78% increase in insulin sensitivity	
Timmons et al., (2006)	n = 58	Young Girls (n=14), Young Boys (n=20) aged 12 y Older Girls (n=11), Older Boys (n=13), aged 14 y	Between Subject Design 60 min cycling at 70% VO_2 max				No effect of exercise on TNF-α or IL-8 Increase in IL-6 only in older girls and young boys 60 min post-exercise (284% and 89% increase, respectively)

2.4.3 Recommendations for Future Research Concerning the Inflammatory, Glycaemic and Insulinaemic Responses to Acute Bouts of Exercise in Adolescents

The following recommendations for future research assessing the inflammatory, glycaemic and insulinaemic response to an acute bout of exercise can be made:

- Where possible, studies should employ a counterbalanced, randomised, cross-over study design to control for potential confounding variables affecting the study outcomes.
- Both chronological age and pubertal development of the participants should be recorded, especially given that previous studies assessing the postprandial glycaemic and insulinaemic responses to standardised meals observed sex differences that were attributed to maturation (Cooper et al., 2017).
- When assessing the inflammatory, glycaemic and insulinaemic responses to acute bouts of exercise, the mode of activity employed should be ecologically valid to ensure that young people are likely to adhere to such exercise should it elicit protective benefits for cardiometabolic health. Games-based activity is a suggested mode of exercise that should be explored by future studies given the similarity of such exercise (sporadic, and intermittent in nature) to the activity patterns young people are already engaging with (Howe et al., 2010) and the potential for games-based activity, which is deemed enjoyable by young people, to lead to long term adherence.
- When assessing the inflammatory response to exercise it is important that a comprehensive panel of inflammatory mediators (pro- and anti-inflammatory) be assessed, to ascertain whether there is inhibition of pro-inflammatory cytokines (IL-1β, TNF-α and CRP) and an increase in anti-inflammatory cytokines (IL-1ra, IL-6, IL-10) that will mediate a reduction in low-grade chronic inflammation if repeated regularly.

- Finally, the timescale of the inflammatory, glycaemic and insulinaemic responses to acute bouts of exercise should be examined. To date, information pertaining to pre- and immediately post-exercise comparisons are available and little is known about the delayed effects of exercise, which are important for determining the frequency of exercise necessary to maintain the protective cardiometabolic responses in young people.

In summary, the review of the literature that has identified several limitations and gaps in understanding that future research needs to address; to enhance understanding of the effects of exercise on cardiometabolic health in children and adolescents. The present thesis will aim to address these limitations through a series of studies, which will each require data collection in the target population of adolescents. The general methods (Chapter III) will provide detailed descriptions of the equipment and procedures frequently used throughout the present thesis.

Chapter III

General Methods

The general methods provide an overview of the methodologies undertaken in the studies (Chapters IV - VII) presented in this thesis. The first section (section 3.1) describes the procedures for participant recruitment, including the attainment of participant assent and parental consent. The second section (section 3.3) outlines the preliminary measurements completed during the familiarisation sessions, which included anthropometric measurements and the determination of age from peak height velocity. The following four sections describe the procedures completed during each main experimental trial, including field and laboratory exercise tests, body composition and the measurement of blood pressure (section 3.4), the collection and analysis of capillary blood samples (section 3.5), the standardised meals consumed (section 3.6) and the exercise protocols employed (section 3.7). The final section (3.8) details the statistical analysis conducted.

3.1 Training

Prior to data collection for the experimental chapters, several training courses were required to ensure that the methods presented in this thesis were undertaken safely, accurately and reliably. Initial training included completing First Aid training to ensure that when working in the field (primarily in schools), as lead investigator, there was always someone on site who was first aid trained in case of emergency. In addition, all investigators completed a level 1 accredited course with the International Society for Advanced Kinanthropometry to ensure that all anthropometric measurements (stretched stature and stretched sitting stature), waist circumference and skinfold measurements were accurate and reliable. As part of the training, candidates were assessed for reliability of measurements at each site and only passed once

criterion validity was <5%. Finally, prior to taking capillary blood samples from young people, several hours of practice taking capillary blood samples from colleagues was required to ensure confidence and validity in the measurement.

3.2 Participant Recruitment

Following ethical approval from the Nottingham Trent University Ethical Advisory Committee, adolescents were recruited from local secondary schools, swimming and football clubs in the East Midlands (Nottingham, Derby and Mansfield, UK). The head teacher or the lead coach were contacted and informed of the study aims to ascertain whether they would be interested in their school/ sports club participating. Thereafter, potential participants met with lead researchers and were informed of the study aims and provided with an outline of the experimental procedures. During the meeting participants were able to ask questions and were informed of their right to withdraw from the study at any given time without providing a reason for their withdrawal.

Interested participants were provided with an information booklet, which was taken home to their parents/ guardians. Once participation was agreed, participants and their parent and/or guardian completed the participant assent form (Appendix A), the parental consent form (Appendix B) and a health screen questionnaire (Appendix C). A lead investigator assessed the health screen questionnaires to ensure that any participant with a health condition that would pose undue risk or bias to the findings of the study did not participate. The telephone numbers and email addresses of the research team were provided in the information booklets to ensure parents/ guardians were provided with the opportunity to ask any questions.

3.3 Preliminary Measurements and Familiarisation

For each study presented in this thesis, participants underwent several preliminary measurements, which were completed during familiarisation sessions, including anthropometric measures of stature, body mass, sitting stature and waist circumference. The familiarisation sessions also consisted of a capillary blood sample and for the studies presented in Chapters V and VI participants completed the multi-stage fitness test (see section 3.3.1).

Stretched stature was measured using a Leicester Height Measure (Seca, Hamburg, Germany), accurate to 0.1cm. Stretched stature was measured with the participant's head placed in the Frankfort plane, whilst an investigator applied a gentle upward pressure using their hands (placed on the side of the face, with fingers placed on the mastoid process) to lift their head to obtain maximum height. Body mass was measured using Seca 770 digital scales (Seca, Hamburg, Germany), accurate to 0.1kg. Stretched stature (cm) and body mass (kg) were used to calculate Body Mass Index (BMI) (body mass (kg) / height squared (m^2)). For the measurement of stretched sitting height, participants were seated on an anthropometric measuring box (the sitting height box is a sturdy wooden box (40 cm width x 40 cm depth x 50 cm height); the same box was used throughout all experimental chapters presented in this thesis). To obtain a stretched measurement the same technique was applied as described for stretched stature. The measurement of stretched sitting stature was read from a ruler that had been taped to the wall and aligned using a spirit level. Investigators trained to level two in anthropometric assessment (International Society for the Advancement of Kinanthropometry) completed the anthropometric measurements.

Sitting stature and stretched stature were used to calculate participant leg length (stretched height - sitting height), which is required to predict age from peak height velocity (APHV). APHV is an estimate of adolescent maturity offset (y pre- or post-APHV) and estimates

pubertal development. APHV throughout the present thesis has been calculated in accordance with the equations developed by Mirwald et al. (2002; Eq. 1.1a & 1.1b); as the APHV sex-specific equations have a correlation coefficient of 0.83 with skeletal age offset from chronological age (the gold standard assessment), indicating a strong maturational commonality between the two methodologies (Mirwald et al., 2002). The use of APHV maturity offset is advantageous as it is not personally intrusive to the participant or the adult responsible for the participant (Malina et al., 2012).

Maturity Offset (Girls) =

-16.364 + 0.0002309 * LLSH + 0.006277 *ASH + 0.179 * L:H + 0.0009428 * AW

(Eq. 1.1a)

Maturity Offset (Boys) =

-26.769 + 0.0003007 * LLSH – 0.01177 * ALL + 0.01639 * ASH + 0.445 * L:H

(Eq. 1.1b)

Where LLSH is the leg length and sitting height interaction, ASH is the age and sitting height interaction, L: H is the leg length to height ratio, AW is the age and weight interaction and ALL is the age and leg length interaction (Mirwald et al. 2002).

3.4 Performance and Health Measures

3.4.1 Multi-Stage Fitness Test

Participants completed the MSFT, a field endurance performance test which has also been used to predict maximal oxygen consumption (Leger et al. 1988; Ruiz et al., 2009). Participants completed the MSFT in groups of 10-12, which for the cross-sectional (Chapter IV) and longitudinal study (Chapter VII) were organised based on training status with swimmers, footballers and school children only performing the MSFT with participants of the same cohort. Prior to completing the MSFT, participants were provided with a buffet-style breakfast (which included options of fresh fruit, toast and cereals) and water was allowed *ad libitum*. The breakfast provided was consistent across testing sessions and across time (2 y follow-up), to control for potential effects of diet and hydration. A standardised warm-up led by a member of the research team preceded the test, which consisted of a 2-min jog around the sports hall and a series of full body, dynamic stretches.

Participants were fitted with a heart rate monitor (First Beat Technologies Ltd., Finland) and heart rate was monitored live throughout the MSFT. All MSFT was performed indoors on a wooden sports hall floor for consistency (Ramsbottom et al., 1988). During the MSFT participants, completed progressive 20 m shuttle runs dictated by an audio signal; which starts at a pace of 8.5 $km.h^{-1}$ and increases by 0.5 $km.h^{-1}$ for each 1-min stage completed thereafter. To complete a shuttle run, participants had to place a foot either on or behind the line before, or at the same time as the audio signal. Participants were informed that the aim of the MSFT was to complete as many shuttle runs as possible before either failure to follow the pace of the audio signal for three successive shuttle runs or the point of volitional exhaustion. A well-familiarised member of the research team set the pace of the MSFT, to ensure that participants did not run too fast during the early stages of the test and to encourage maximal performance. Verbal encouragement was provided for each participant as they began to find keeping pace

with the audio signal difficult, thus ensuring they completed the MSFT to the point of volitional exhaustion. The verbal encouragement was provided by the same staff members across testing sessions to ensure consistency between groups. The final shuttle and level achieved was recorded as the criterion measure and for Chapters IV and VII was converted into distance run in metres. In Chapter V an adolescent specific equation (which accounted for the age and sex of the participant) was used to predict $\dot{V}O_2$ peak:

Predicted $\dot{V}O_2$ peak in Adolescents = 25.9 – 2.21 * Gender – 0.8 * Age + 3.4 * Speed

(Barnett et al., 1993)

Where gender represents the sex of the participant (male = 0, female = 1), age is the age on the day of the test and speed is the final speed at the point of volitional exhaustion.

3.4.2 Blood Lactate Response to Submaximal Exercise

In Chapter IV a sub-sample of participants performed a submaximal, incremental treadmill test on a calibrated treadmill (Technogym, Italy). Prior to participation, heart rate monitors were fitted (First Beat Technologies Ltd., Finland) and maximum heart rate during the final minute of each stage was recorded. Participants completed three to six, 4-min runs, interspersed with 1-min rest during which a capillary blood sample was taken (see description in section 3.4). The first stage of the test was completed at an individualised speed that was comfortable for the participant (starting speed varied between 6-10 $km.h^{-1}$), which increased by 1 $km.h^{-1}$ for each stage completed thereafter. The speeds throughout the duration of the test were such that participants worked sub-maximally. The blood lactate concentration at 8.5 $km.h^{-1}$ was the criterion measure in Chapter IV. The blood lactate concentration was calculated by mathematically fitting a curve to the blood lactate-running speed relationship.

3.4.3 $\dot{V}O_2$ Peak Test

Following the measurement of blood lactate response to submaximal exercise, the same sub-sample of participants completed an incremental uphill treadmill test to measure $\dot{V}O_2$ peak ($ml.kg^{-1}.min^{-1}$). The speed of the test was constant and individualised for each participant based on the speed that corresponded with 85% HR_{max} during the submaximal test (described in section 3.3.2). The gradient of the treadmill increased by 1 % each minute of the test completed. Participants were required to run to the point of volitional exhaustion, which was indicated by the participant's rating of perceived exertion on a 6 – 20 Borg scale (Borg, 1998, Appendix D) in conjunction with live monitoring of their heart rate. Prior to the exercise laboratory tests, all participants were shown the Borg scale and given an age appropriate explanation of the information provided from this psychological evaluation of perceived exertion. Participants were instructed to point to the scale to indicate the rating indicating how intense the exercise felt when they were shown the scale. Verbal encouragement was provided as the participant neared predicted maximum heart rate (220-age) and during the final minute of the test to ensure the participant completed the test to the point of volitional exhaustion.

During the final minute of the test, participants expired air into a Douglas Bag, which was later analysed on a Servomex 1440 Gas Analyser (Servomex, USA) to calculate $\dot{V}O_2$ peak ($ml.kg^{-1}.min^{-1}$). Although it is accepted that all young people may not reach maximum oxygen uptake as indicated by the criteria used in adult exercise testing all participants recorded an RPE of 19 / 20 and heart rate was at or above 220 – age at the end of the test. The expired air sample collected during the final minute of exercise was analysed for percentage of oxygen and carbon dioxide, and the volume and temperature of the expired air using a Harvard Dry Gas Meter (Hugo Sachs, Harvard Apparatus, Germany). Barometric pressure on the day of testing was determined using a Fortin barometer (F. D. and Company, Watford, UK). The Haldane

transformation was used to calculate the inspired gas volumes, for oxygen uptake to be calculated thereafter.

3.4.4 Body Composition

Skinfold thickness was the preferred measure of body composition in the present thesis, as it is reported as an effective, valid and reliable method that also meets the ethical constraints in young people (Bugge et al., 2012; Yeung & Hui, 2010). Skinfold thickness was measured using a Harpenden Caliper (Baty International, Burgess, Hill, UK) at four sites (tricep, subscapular, supraspinale, and front thigh). All measurements were taken twice in rotation and on the right-hand side of the body. The mean of the two measurements was taken unless the difference between the two measurements was > 5 %. Under such circumstances, a third measurement was taken and the median value used as the criterion measure. All skinfold measures were completed by trained kinanthropometrists whom adhered to methods described in the International Standards for Anthropometric Assessment manual (2001). The sum of skinfold thickness scores was the preferred assessment of body composition in the present study, as estimating body fat percentage from skinfold thickness is associated with large random error and significant systematic error (Reilly et al., 1995).

3.4.5 Blood Pressure

In Chapters IV and VII, blood pressure was the first measurement taken upon arrival to the exercise laboratory. Participants arrived to the laboratory in a fasted and rested state from 9 pm the previous evening, having been informed only water could be consumed (thus abstaining from caffeine consumption) until all health measurements were complete. In addition, it was emphasised to participants and their parents/ guardians that vigorous physical activity must be avoided on the morning of the blood pressure measurements, with examples provided as a reminder to ensure compliance (such as there should be no running into the building or down

corridors, as this constitutes a short bout of vigorous physical activity). Participants were seated quietly for 5 min prior to two blood pressure measurements from the left arm, which was rested at chest height, using an HBP-1300-UK sphygmomanometer (Omron, Milton Keynes, UK). The mean of the two blood pressure measurements was used as the criterion value, unless systolic blood pressure differed by > 5 mmHg, then a third blood pressure measurement was taken and the median value used as the criterion measure. Mean arterial blood pressure (MAP) was determined using the following calculation as described by Smeltzer et al., (2010):

Diastolic blood pressure + ((0.33 * (systolic blood pressure – diastolic blood pressure)))

3.5 Capillary Blood Sampling

Throughout all experimental chapters capillary blood samples were obtained, treated and analysed for concentrations of blood glucose, plasma insulin, C-reactive protein (CRP) and inflammatory cytokines (IL-1β, IL-6, IL-10 and TNF-α). Capillary blood samples were preferred over venous blood samples in the present thesis due to the ethical constraints of working with young people. Furthermore, when examining postprandial glycaemic and insulinaemic responses in adolescents, capillary blood samples are more sensitive to changes in glycaemic responses and have a lower inter-individual variation than venous blood samples (Wolever et al., 1991, Kuwa et al., 2001).

3.4.1 Collection and Treatment of Capillary Blood Samples

Baseline capillary blood samples were taken in the morning following an overnight fast (from ~ 9 pm the previous evening). To ensure sufficient blood flow for sampling, participants' hands were warmed via submersion in warm water to increase capillary blood flow. The participants' hands were dried and a single-use Unistik lancet (Unistick extra, 21G guage, 2 mm depth,

Owen Mumford LTd., UK) was used to facilitate collection of whole blood into three 300-µl EDTA coated microvettes (Sarstedt LTd., UK).

For the determination of blood glucose (all studies) and blood lactate (Chapter IV), whole blood was collected into a 25-µl plain pre-calibrated glass pipette (Hawslet LTd., UK) and dispensed into a 1.5 ml plastic vial containing 250-µl of 2.5% v/v perchloric acid for deproteinisation (Eppendorph 5415C, Hamburg, Germany). The microvettes and plastic vials were centrifuged at 5000 rev.min^{-1} for 4 min (accuSpin Micro 17R, Fisher Scientific, UK). Plasma was removed from the microvettes and placed into 500-µl plastic vials. All samples were stored at -20 °C until transferred to the -80 °C freezer at Nottingham Trent University within a few hours. All samples remained in the freezer until analysis.

3.5.2 Analysis of Capillary Blood Samples

Blood glucose concentrations were determined in duplicate using a commercially available assay (GOD/PAP method, GL364, Randox, Ireland) and read spectrophotometrically. Plasma insulin concentrations were determined using a commercially available ELISA (Mercodia Ltd., Sweden). Fasted blood glucose and plasma insulin concentration were used to calculate the HOMA-IR index (fasting plasma insulin (µU.mL^{-1}) x fasting blood glucose (mmol·L^{-1})/22.5), as a measure of insulin resistance in adolescents (Keskin et al., 2005). Incremental area under the curve (IAUC) and total area under the curve (tAUC) were calculated for the glycaemic and insulinaemic responses to standardised meals in Chapters V - VI, using the trapezoid methods described by Wolever & Jenkins (1986). Blood lactate concentrations were determined in duplicate using a commercially available assay (PAP method, LC2389, Randox, Ireland) and were analysed spectrophotometrically.

Inflammatory cytokine concentrations (IL-1β, IL-6, IL-10 and TNF-α) were determined using an AimPlex, flow cytometry-based multiplex immunoassay (YSL Bioprocess Development Company, Pomona, USA) and a Beckman Coulter Gallios™ flow cytometer and Kaluza™ acquisition and analysis software (Beckman Coulter, London, United Kingdom). CRP concentrations were determined using the same approach, but on a separate plate.

The coefficients of variation were determined for each of the variables analysed from the capillary blood samples. Ten repeat measurements from a human capillary blood sample were assessed using the methods described above. The coefficient of variation calculation was calculated as:

Coefficient of Variation (CV) = (Standard Deviation / Mean) * 100 %

(Cohen & Holliday, 1982).

Table 5. Intra-assay coefficient of variation based on ten repeat measurements for blood glucose, plasma insulin, inflammatory mediators and blood lactate.

	Coefficient of Variation (%)
Blood Glucose	2.3
Plasma Insulin	3.2
IL-6	15.9
IL-1β	17.4
TNF-α	14.7
IL-10	13.2
CRP	10.4
Blood Lactate	6.7

3.6 Standardised Meals Consumed

In Chapters V and VI, participants arrived at the main trials in a fasted state from ~ 9 pm the previous evening. Participants consumed a standardised breakfast following the fasted capillary blood sample, which consisted of cornflakes, milk, and toast with margarine (Table 6a). For the standardised lunch participants consumed a chicken sandwich (with a cheese alternative provided for vegetarians), baked salted crisps and an apple (Table 6b). Each standardised meal contained 1.5 g carbohydrate per kg body mass. Analysis of the meals was conducted using Microdiet (Microdiet, Downlee Systems Ltd., UK). An example of the food composition for the standardised breakfast and lunch for a 50 kg child is presented in Table 6a and 6b respectively. Participants had 15 min to consume each of the meals. If following the 15 min, food remained it was re-weighed and on the following trial adjustments were made accordingly. Water was allowed *ad libitum*.

Table 6a. Example of a standardised breakfast for a 50 kg participant.

Food Item	Mass (g)
Cornflakes [a]	55
White Bread [b]	42
Margarine [c]	6
1 % fat milk [d]	216
Food Quantity	319

[a] Cornflakes (Kellogs Ltd., UK)
[b] Lightly toasted white bread (Kingsmill soft white thick slice, UK)
[c] Margarine (Flora Original, UK)
[d] 1 % fat milk (Sainsbury's Ltd., UK)

Table 6b. Example of a standardised lunch for a 50 kg participant.

Food Item	Mass (g)	
	Standard	Vegetarian Option
White Bread [a]	70	70
Margarine [b]	8	8
Chicken [c]	115	
Cheese [d]		34
Crisps [e]	35	35
Apple [f]	120	120
Food Quantity	348	267

[a] White bread (Kingsmill soft white thick slice, UK)
[b] Margarine (Flora Original, UK)
[c] Sainsbury's roast chicken slices (Sainsbury's Ltd., UK)
[d] Sainsbury's medium cheddar (Sainsbury's Ltd., UK)
[e] Walkers ready salted baked crisps (Walkers, UK)
[f] Braeburn apple

3.7 Exercise Protocols

In Chapter V during the exercise trial, participants completed a mid-morning bout of exercise, for comparison against the rested control trial. The bout of exercise consisted of 60-min intermittent exercise (a basketball session led by Austin Grant, a Level 4 basketball coach who has over 30 years' experience in coaching basketball to young people and adults in Nottingham; and was recently awarded a Lifetime Contribution Award (Roll of Honour) from Sport Nottinghamshire), which commenced 1-h after breakfast on day 1. An experienced basketball coach delivered the sessions to groups of 10 participants in a school sports hall. The basketball session consisted of a warm-up (5 min of jogging on the court followed by whole body, dynamic stretches), skill-based drills (which included, 30 min of passing, dribbling and shooting drills) and small-sided games (25 min) at the end of the session. Participants were fitted with heart rate monitors (First Beat Technologies Ltd., Finland) at the start of the main trials, and the heart rate system was lapped during the 60 min of exercise to determine average and maximum heart rate as a marker of exercise intensity.

In Chapter VI, participants completed 30 min and 60 min of intermittent activity performed as the Loughborough Intermittent Shuttle Test (LIST) and rested during the control trial. During the LIST, participants ran between two markers, set 20 m apart, at pre-determined speeds dictated by an audio signal. The exercise pattern consisted of three shuttles at walking pace, a 15 m sprint, three shuttles at 95% $\dot{V}O_2$ peak (fast running) and three shuttles at 55% of $\dot{V}O_2$ peak (percentage of $\dot{V}O_2$ peak was determined from performance on the MSFT, described in section 3.3.1). Sprint times were recorded using infrared timing gates (Brower Timing Systems IRD-T173, Utah, USA) and average sprint times for each set was calculated for comparative purposes. Each exercise pattern was repeated eight times to create a block of exercise, which lasted ~12-min (Fig. 2). Exercise blocks were separated by 3 min of active recovery, when participants walked around the sports hall and drank water *ad libitum*.

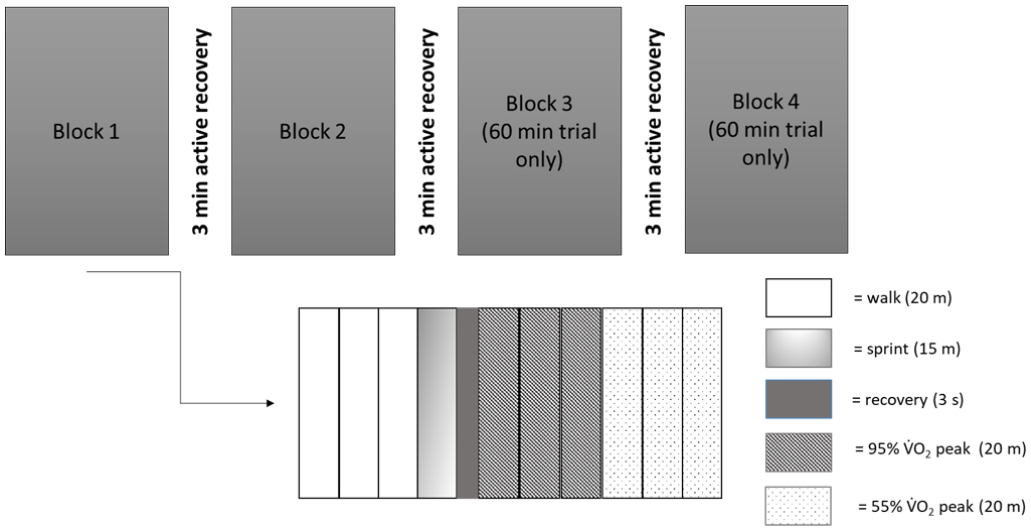

Figure 2. Overview of the Loughborough Intermittent Shuttle Test used during the 30 min and 60 min exercise trial.

3.8 Statistical Analysis

All analysis was completed in SPSS (Version 24, SPSS Inc, Chicago, IL, USA), using a variety of statistical techniques that included; ANOVA, multiple regression, multi-level modelling, and paired and independent sample t-tests. All data were assessed for normality using the Kolmogorov-Smirnov test and for homogeneity of variance using Mauchly's test of Sphericity. For all analysis, significance was accepted as $P < 0.05$. Where significant interactions were observed, post-hoc pairwise comparisons were performed using a Bonferroni correction. Furthermore, where significant effects or trends existed for main effect of trial and/or trial by time interactions, effect sizes were calculated as Cohen's d (small effect sizes d = 0.2, medium effect sizes d = 0.5, large effect sizes d = 0.8) (Cohen, 1988):

$$\textit{Cohen's } d = M_1 - M_2 \,/\, SD_{Pooled}$$

Where M_1 and M_2 are the means for the two comparative groups, and SD_{pooled} is the pooled standard deviation of the groups.

Due to the scope of the analysis conducted, a more detailed overview of each statistical test is provided in each of the relevant chapters.

Chapter IV

Multi-stage Fitness Test Performance, $\dot{V}O_2$ peak & adiposity: effect on risk factors for cardiometabolic disease in adolescents

4.1 Introduction

Low-grade chronic inflammation is a key risk factor in the pathogenesis of cardiometabolic diseases (including hypertension, hyperglycemia and early insulin resistance) and atherosclerotic plaques (Balagopal et al., 2011). The presence of low-grade chronic inflammation is currently the strongest predictor of cardiovascular events in adults, bettering traditional markers of dyslipidemia and hypertension (Petersen and Pedersen, 2005). Although cardiovascular disease typically presents during adulthood, the prevalence of low-grade chronic inflammation in adolescents (Balagopal et al., 2011) is of concern, as early and continued exposure increases the risk of early onset cardiovascular disease and type 2 diabetes (Gleeson et al., 2011).

Low-grade chronic inflammation is defined as a chronic, 2- to 3- fold elevation in the concentrations of inflammatory mediators, including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), tumor necrosis factor-α (TNF-α) and acute phase protein C-reactive protein (CRP) (Petersen and Pedersen, 2005). Acute bouts of physical activity are implicated in the prevention of low-grade chronic inflammation through the anti-inflammatory response that occurs post-exercise (Gleeson et al., 2011). Recently, it has been shown that acute bouts of games-based activity transiently increased concentrations of anti-inflammatory mediators IL-10 and IL-1rα in middle-aged men (Mendham et al., 2015). Increased concentrations of IL-10 and IL-1ra are reported to inhibit the synthesis of pro-inflammatory cytokines (IL-1β and TNF-α) and improve insulin sensitivity when assessed *in*

vitro (Gleeson et al., 2011). Furthermore, regular participation in physical activity prevents excessive adiposity (Van der Heijden et al., 2012) and reduces adiposity in overweight adolescents (Rey et al., 2017) and adults (Alrushud et al., 2017). Although such findings support regular moderate intensity physical activity as a potential therapeutic intervention that protects against the development of risk factors for cardiometabolic disease, the chronic effects of regular training resulting in enhanced physical fitness on low-grade chronic inflammation in adolescents are relatively unknown.

When assessing the effect of physical fitness on low-grade chronic inflammation a comprehensive range of inflammatory mediators (IL-1β, IL-6, TNF-α and CRP) should be measured (Petersen and Pedersen, 2005). Yet, in adolescents and adults, research has focused on the relationship between physical fitness and a limited number of pro-inflammatory mediators (IL-6, TNF-α and CRP) (Platat et al., 2006, Buchan et al., 2015, Bugge et al., 2012, Ischander et al., 2007). In adolescents, the findings of previous studies assessing the relationship between physical fitness and inflammatory mediators IL-6, TNF-α and CRP are inconclusive with no apparent relationship (Platat et al., 2006; Steene-Johannessen et al., 2013), or inverse associations observed (Buchan et al., 2015; Bugge et al., 2012; Silva et al., 2014). Furthermore, the relationship between physical fitness and concentrations of anti-inflammatory mediator IL-10 is unknown despite the potential of IL-10 to reduce low-grade chronic inflammation and improve insulin sensitivity (Petersen and Pedersen, 2005). Increasing adiposity reduces the expression of IL-10 in normal weight and overweight individuals (Esposito and Giugliano, 2004; Utsal et al., 2013), whereas the effect of physical fitness on IL-10 concentration has only been studied once, in healthy, normal weight adults (Jürimäe et al., 2017a) and once in pubertal girls (Jürimäe et al., 2017b). Jürimäe et al. (2017a) reported no relationship between maximal oxygen uptake and IL-10 concentration in well-trained adult rowers. These null findings might relate to the well-trained study population, in

that the variability of fitness among the participants was not diverse enough for a relationship to be established. However, when comparing well-trained, female adolescent rhythmic gymnasts against untrained counterparts, there was still no difference across 12 markers of inflammation, which included anti-inflammatory mediator IL-10 (Jürimäe et al., 2017b). Whilst the inflammatory profiles of the trained gymnasts and the untrained controls were similar, there was no measurement of physical fitness or body composition in the pubertal girls; therefore, the relationship between physical fitness, inflammatory markers and IL-10 concentration remains unknown, particularly in young people.

In previous studies in adolescents and adults, the effect of long-term training on risk factors for cardiometabolic disease has been determined by peak oxygen consumption when using graded treadmill tests (Bugge et al., 2012; Ischander et al., 2007; Silva et al., 2014) and graded cycle ergometer tests (Steene-Johannessen et al., 2013). The discrepant findings of previous research could relate to the limitations of $\dot{V}O_2$ peak as a measure of physical fitness (Coyle et al., 1983), as $\dot{V}O_2$ peak is considered to be relatively insensitive to changes in training status, with up to 50% of an individual's $\dot{V}O_2$ peak being determined by genetics (Bouchard, 2012). Regular participation in moderate-to-vigorous activity moderates an individual's exercise capacity and is the mechanism that stimulates the transient inflammatory response that prevents low-grade chronic inflammation (Gleeson et al., 2011). When focusing on the relationship between physical fitness and risk factors for cardiometabolic disease the measurement of fitness should therefore be sensitive to changes in an individual's ability to perform prolonged exercise (Strasser and Burtscher, 2018). The blood lactate response to submaximal exercise is more sensitive to changes in training status than maximal oxygen uptake in both adults (Edwards, Clark & Macfayden, 2003) and young people (Grant, 2001). Furthermore, the submaximal nature of the test allows the assessment of a heterogeneous population and therefore allows the comparison of individuals from inactive, recreationally active and well-trained backgrounds.

Performance on the MSFT is also a commonly used, reliable and easy to administer, field measure of physical fitness in young people (Ortega et al., 2008) and is sensitive to changes in training status (Aziz et al., 2005). Therefore, the blood lactate response to submaximal exercise and the MSFT are potentially better suited for examining the relationship between physical fitness (capacity to perform prolonged exercise) and risk factors for cardiometabolic disease.

As excessive adiposity mediates an increase in low-grade chronic inflammation, several studies have assessed the relationship between different measures of body composition and levels of the pro-inflammatory mediators IL-6 and TNF-α (Bugge et al., 2012; Utsal et al., 2013; Galcheva et al., 2011; Lopez-Alcaraz et al., 2014). Findings are inconclusive in that adiposity has been reported to have no effect on the pro-inflammatory mediators in several studies (Steene-Johannessen et al., 2013, Lopez-Alcaraz et al., 2014). However, increased adiposity has been associated with higher IL-6 and TNF-α concentration in adolescents in other studies (Bugge et a., 2012; Utsal et al., 2013, Galcheva et al., 2011). Of the studies that have examined adiposity, only one has considered the potential mediating effects of physical fitness (Silva et al., 2014). In the study of Silva et al. (2014) maximal oxygen uptake test was associated with metabolic risk (calculated from traditional risk factors including blood pressure and dyslipidemia). Although these findings suggest that physical fitness is important for the prevention of traditional cardiometabolic risk factors, it remains unknown whether physical fitness or adiposity best predicts, or whether these variables additively predict, risk factors for cardiometabolic disease in adolescents.

Therefore, the aim of the present study was to determine the effect of fitness, as measured by MSFT performance and the blood lactate response to submaximal exercise, $\dot{V}O_2$ peak and adiposity on a comprehensive panel of pro- and anti-inflammatory cytokines in conjunction with traditional cardiometabolic risk factors in adolescents. A secondary aim of the study was

to determine whether peak oxygen uptake (also influenced by genetics), MSFT performance or blood lactate concentration during sub-maximal exercise (better markers of the capacity to perform prolonged exercise) or adiposity better predict risk factors for cardiometabolic disease in adolescents.

4.2 Methods

4.2.1 Participant Characteristics

A cross-sectional sample of 140 adolescents aged 10-12 years were recruited to participate in the present study. Adolescents were recruited from local secondary schools, swimming clubs and football clubs following contact being made with teachers and coaches. A number of existing personal contacts with teachers and coaches were used first; these contacts subsequently provided our research group with contact details of external colleagues, with whom contact was made. Typically, meetings were then held to detail the study protocol and the information that participants would receive relating to their health and performance was discussed. Given that 19 participants withdrew from the study (n = 10 due to illness, n = 5 due to injury which occurred as part of training practice outside the study, and n = 4 due to reluctance to provide a capillary blood sample), 121 young people (61 male, 60 female, age 11.3 ± 0.8 y) participated. All participants underwent anthropometric measures of body mass, height and sitting stature to predict age at peak height velocity (APHV, calculated using the method described in Moore et al., 2015), as the preferred measure of maturation. Body mass was measured using a Seca 770 digital scale which is accurate to 0.1 kg (Seca, Hamburg, Germany), and height was measured using a Leicester Height Measure which is accurate 0.1 cm (Seca, Hamburg, Germany), to allow the determination of body mass index (BMI, (calculated as body mass (kg) / stature $(m)^2$). Participant characteristics were; height 151.9 ± 7.2 cm, body mass 43.1 ± 9.5 kg, BMI Percentile 52.3 ± 29.3; years from peak height velocity 1.9 ± 0.7 y (males -2.0 ± 0.7 y; females -1.9 ± 0.8 y).

4.2.2 Study Design

Ethical approval was received from the Nottingham Trent University's Ethical Advisory Committee (SPOR-400). Participants were recruited from local secondary schools and sports clubs in the East Midlands, United Kingdom. Written parental consent and verbal child assent was obtained during recruitment. Health screen questionnaires were completed by the participants' parent/guardian and checked by a lead investigator to ensure there were no medical conditions that might affect participation in the study.

All trials were separated by a minimum of 7 d (further details of which are provided below). The field measurements (completed during the first trial) consisted of anthropometric measures (body mass, stature and sitting stature), skinfolds and the MSFT, in that order. The health measurements (completed during the second trial, which commenced at ~8.30 am) consisted of resting blood pressure followed by a resting capillary blood sample (fasted from 9 pm the previous evening). Finally, a sub-sample of participants (68 participants, 30 male, age: 11.6 ± 0.6; APHV: -1.9 ± 0.7 y) completed exercise laboratory tests including a submaximal treadmill test and a $\dot{V}O_2$ peak test, which were separated by 20 min passive recovery. Only a sub-sample of participants from the study population volunteered to complete the final part of the study. Those that removed themselves from the exercise laboratory tests did so as they were not willing to take an additional day off school. Prior to all measurements, participants were asked to refrain from moderate-to-vigorous physical activity for 24 h. A telephone call was made to parents/guardians the evening prior to the testing sessions to ensure compliance with the study requirements.

4.2.3 Field Measures

4.2.3.1 Body Composition

Skinfold thickness was measured using a Harpenden Caliper (Baty International, Burgess, Hill, UK) at four sites (tricep, subscapular, supraspinale, front thigh). All measurements were taken

twice in rotation and on the right-hand side of the body as described in the general methods. The use of skinfolds in assessing body composition in young people is reported as an effective, valid and reliable method in young people (Bugge et al., 2012; Yeung & Hui, 2010). Specifically, the sum of the four skinfold thickness scores was the preferred assessment of body composition in the present study, as estimating body fat percentage from skinfold thickness has been associated with large random error and significant systematic error (Reilly et al., 1995).

4.2.3.2 Multi-Stage Fitness Test (MSFT)

During the MSFT, participants completed progressive 20 m shuttle runs until the point of volitional exhaustion (Ramsbottom et al., 1988). The MSFT started at a speed of 8.5 $km \cdot h^{-1}$ and increased by 0.5 $km \cdot h^{-1}$ for each 1-min stage completed. Participants were fitted with a heart rate monitor (First Beat Technologies Ltd., Finland) prior to the start of the test and heart rate was monitored in real-time throughout its duration. Verbal encouragement was provided throughout to ensure participants worked to the point of volitional exhaustion. The distance ran during the MSFT was used as the criterion measure.

4.2.4 Health Measures

4.2.4.1 Blood Pressure

On arrival at the exercise laboratory following an overnight fast, participants were seated quietly for 5 min prior to the measurement of blood pressure which was undertaken as described in the general methods. Mean arterial blood pressure was determined using the following calculation (Smeltzer et al., 2010): *diastolic blood pressure + ((0.33 * (systolic blood pressure – diastolic blood pressure)))*.

4.2.4.2 Capillary Blood Samples

Capillary blood samples were obtained early in the morning following an overnight fast and during the speed lactate treadmill test (baseline and following each progressive stage). Section 3.4.1 details the methods undertaken for the collection of capillary blood samples. In brief, a Unistik single-use lancet (Unistik Extra, 21G gauge, 2.0 mm depth, Owen Mumford Ltd., UK) was used and blood collected into three 300 µl EDTA microvettes (Sarstedt Ltd., UK). A 25 µl whole blood sample was collected and dispensed into 250 µl of ice-cooled 2.5% v/v perchloric acid. The whole blood samples and diluted perchloric acid samples were centrifuged at 1500 g for 5 min (Eppendorf 5415C, Hamburg, Germany). Plasma was pipetted and immediately frozen at -20°C and transferred to a -80°C freezer at the earliest opportunity.

Blood glucose, plasma insulin, cytokine and CRP concentrations were determined using the methods described in section 3.4.2.. Following methodological issues with the analysis of CRP beyond our control (separate instructions and diluent were not sent to our laboratory from the manufacturer resulting in CRP not being detected by the flow cytometer), there was a reduced participant n for this analyte, which led to the analysis being underpowered. For all future analysis of CRP our research group will be aware that the samples need to be diluted before undertaking the assay and will therefore know to check the manufacturer has sent all necessary materials and instructions.

4.2.5 Exercise Laboratory Measures

4.2.5.1 Blood Lactate during Sub-maximal Exercise

A sub-sample of participants (n = 68) completed a submaximal test on a calibrated treadmill (Technogym, Italy) and blood lactate concentration was determined at the end of each 4-min stage as described in the general methods. The blood lactate concentration at 8.5 $km.h^{-1}$ was

used as the criterion measure and was calculated by mathematically fitting a curve to the blood lactate-running speed relationship.

4.2.5.2 $\dot{V}O_2$ Peak Test

A sub-sample of participants (n = 68) completed an uphill treadmill running to determine $\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$) as described in the general methods. During the final minute of the test, participants breathed expired air into a Douglas Bag, which was later analysed on a Servomex 1440 Gas Analyser (Servomex, USA) to calculate $\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$). Verbal encouragement was provided throughout the test to ensure the participant worked to the point of volitional exhaustion.

4.2.6 Statistical Analysis

An *a priori* power calculation was performed using GPower 3.1.9.2 and based on IL-6 data in previous research (Ischander et al., 2007), with an alpha probability level of 0.05, 4 groups and 1 covariate; a total sample size of 107 was required.

Participants were separated into distinct fitness quartiles quantified by distance run on the MSFT, blood lactate concentration at 8.5 $km \cdot h^{-1}$, $\dot{V}O_2$ peak and adiposity quartiles quantified from the sum of skinfolds (Table 7). Quartiles were selected based on the participant n; with quartiles resulting in ~30 participants per quartile (total n = 121), which was deemed suitable for these analyses. The first quartile (which included participants with values ≤ 25% of all values in the present study) included participants with the lowest physical fitness and highest adiposity. For each distinct fitness and adiposity quartile, risk factors for cardiometabolic health were calculated as mean ± S.E.M. and inferential statistics performed.

All analyses were performed in SPSS (Version 24, SPSS Inc, Chicago, Il, USA). The effect of physical fitness and adiposity quartile on each risk factor for cardiometabolic disease was

analysed via two-way (physical fitness or adiposity * sex) between subjects ANCOVA, with maturation (APHV) used as a covariate. When significant interactions were observed, post-hoc comparisons were performed using a least significant difference (LSD) correction. In order to assess differences between boys and girls in each quartile, independent samples t-tests were used. Where significant effects existed, effect sizes were calculated as Cohen's d. Multiple linear regression was used to examine the relationship (adjusted for APHV) between independent variables (distance on the MSFT, $\dot{V}O_2$ peak and adiposity) and each cardiometabolic risk factor (IL-6, IL-1β, IL-10, TNF-α, CRP, fasted blood glucose and plasma insulin, HOMA-IR, systolic, diastolic and mean arterial blood pressure). Blood lactate concentration during sub-maximal exercise was not examined in the multiple linear regression, as the sample size did not meet the minimum criteria necessary for four predictor variables (Vanvoorhis and Morgan, 2007). For all analysis significance was accepted as $P < 0.05$ and data are presented as mean ± S.E.M..

4.3 Results

Quartiles for each variable (distance run on the MSFT, $\dot{V}O_2$ peak, blood lactate concentration at 8.5 $km \cdot h^{-1}$ and adiposity) were separately determined for boys and girls (Table 7). When considering the effect of sex on MSFT performance, boys ran further than their female counterparts across all quartiles (all $p < 0.001$). Similarly, boys in quartiles one to three had a higher peak oxygen consumption, a lower blood lactate concentration at 8.5 $km.h^{-1}$ and lower adiposity when compared with their female counterparts (all $p < 0.001$). There was no difference between boys and girls in $\dot{V}O_2$ peak ($p = 0.970$) or adiposity ($p = 0.086$; Table 7) in quartile four.

Table 7. Performance on the multi-stage fitness test (distance run), $\dot{V}O_2$ peak, Blood lactate at 8.5 $km \cdot h^{-1}$ on the speed lactate test and adiposity from sum of skinfolds separated by sex and into quartiles (Mean ± S.E.M). * denotes significant differences between boys and girls in respective quartiles.

Quartile	MSFT Distance (m)		$\dot{V}O_2$ Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)		Blood Lactate at 8·5 $km \cdot h^{-1}$ on the Speed Lactate Test ($mmol \cdot L^{-1}$)		Adiposity from Sum of Skinfolds (mm)	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
1	860 ± 56	470 ± 48*	40.2 ± 3.2	34.1 ± 1.5*	2.71 ± 0.17	5.20 ± 0.96*	56.4 ± 2.6	97.3 ± 3.1*
2	1300 ± 21	900 ± 31*	49.9 ± 0.6	42.9 ± 0.8*	2.30 ± 0.27	3.62 ± 0.79*	39.5 ± 0.7	54.4 ± 1.7*
3	1500 ± 14	1160 ± 14*	52.7 ± 0.4	48.9 ± 0.7*	1.95 ± 0.38	2.62 ± 0.54*	33.6 ± 0.5	39.0 ± 0.8*
4	1800 ± 41	1540 ± 47*	57.9 ± 1.2	58.0 ± 1.3	1.07 ± 0.22	1.61 ± 0.88	27.2 ± 0.5	28.8 ± 0.8

4.3.1 Inflammation

IL-6: When separating participants into quartiles based on distance run on the MSFT, IL-6 concentration was higher in quartile one when compared with participants in the third ($p = 0.011$, $d = 0.6$) and fourth quartiles ($p = 0.009$, $d = 0.7$; main effect: $F_{(3,90)} = 2.9$, $p = 0.038$; Fig. 3; Table 8). There was no difference in IL-6 concentration when separating participants by $\dot{V}O_2$ peak, blood lactate concentration at 8.5 $km \cdot h^{-1}$ or adiposity (all $p > 0.05$), nor was there any difference between boys and girls (main effect of sex: all $p > 0.05$; interaction effect: all $p > 0.05$). The multiple regression analysis (Table 9) revealed that distance run on the MSFT was the only statistically significant predictor of IL-6 concentration, after adjustment for APHV, with a negative relationship observed between the two variables ($\beta = -0.291$, $p = 0.031$).

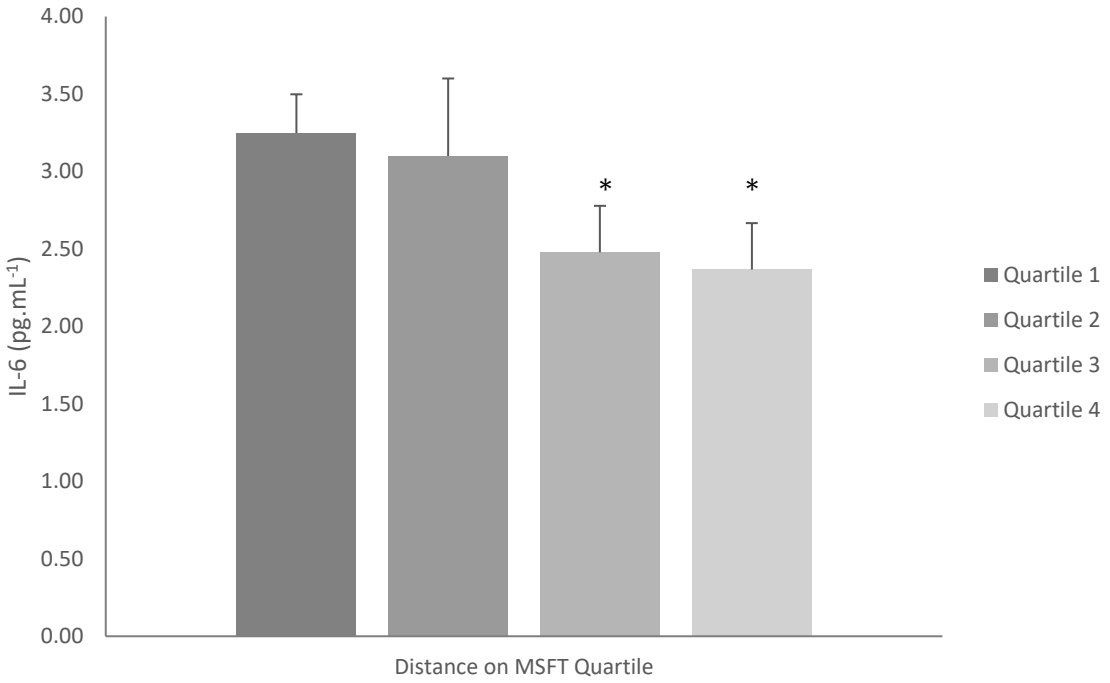

Figure 3. IL-6 concentration ($pg.mL^{-1}$) separated into quartiles by distance run on the multi-stage fitness test (MSFT). Participants in quartile one covered the shortest distance. Mean ± S.E.M., main effect of training status $p = 0.038$. ∗denotes significant difference from quartile one.

IL-1β: When separating participants into quartiles based on distance run on the MSFT, IL-1β concentration was higher in quartile one when compared with participants in the third ($p = 0.039$, $d = 0.6$) and fourth quartiles ($p = 0.008$, $d = 0.8$; main effect: $F_{(3,96)} = 3.1$, $p = 0.032$; Fig. 4; Table 8). There was no difference in IL-1β concentration when separating participants by $\dot{V}O_2$ peak, blood lactate concentration at 8.5 $km \cdot h^{-1}$ or adiposity (all $p > 0.05$). When considering the effect of sex, IL-1β concentration was higher in boys than girls (boys; 4.26 ± 0.44 $pg \cdot mL^{-1}$, girls; 2.94 ± 0.45 $pg \cdot mL^{-1}$; main effect of sex: $F_{(1,96)} = 4.4$, $p = 0.039$, $d = 0.4$). The effect of fitness or adiposity was not different between boys and girls (interaction: all $p > 0.05$). The multiple regression analysis (Table 9) revealed that distance run on the MSFT was the only statistically significant predictor of IL-1β concentration, after adjustment for APHV, with a negative relationship observed between the two variables ($\beta = -0.405$, $p = 0.005$).

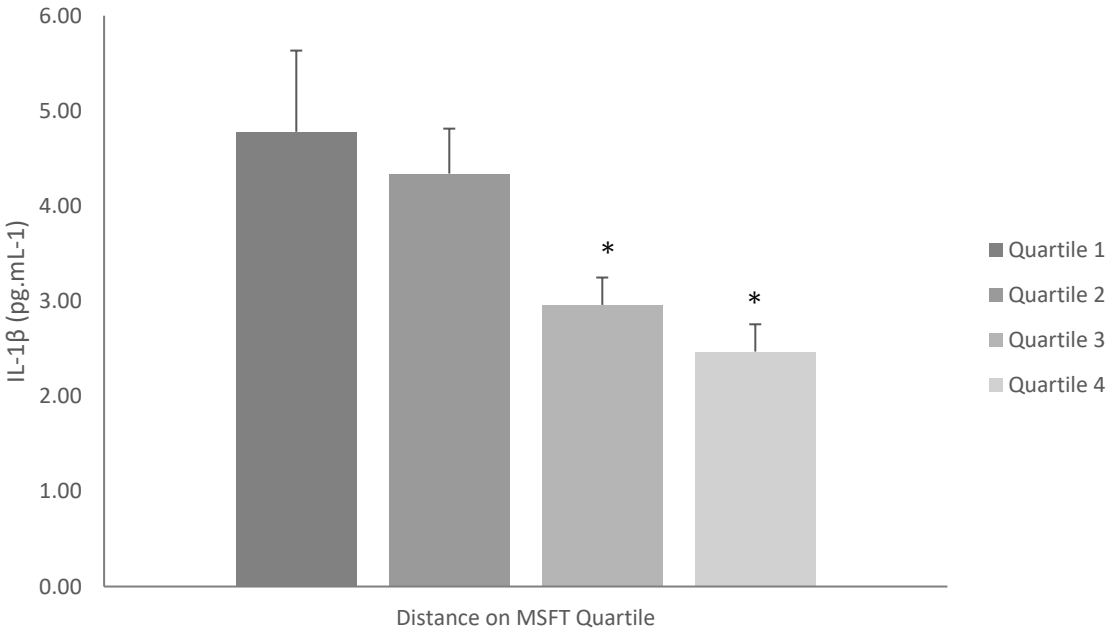

Figure 4. IL-1β concentration ($pg.mL^{-1}$) separated into quartiles by distance run on the multi-stage fitness test. Participants in quartile one covered the shortest distance. Mean ± SEM; main effect of training status $p = 0.032$. *denotes significant difference from quartile one.

IL-10: When separating participants into quartiles determined by blood lactate concentration at 8.5 km·h^{-1}, IL-10 concentration was lower in quartile one when compared with participants in quartile four ($p = 0.006$, $d = 0.9$; main effect: $F_{(3, 27)} = 3.6$, $p = 0.035$, Fig. 5; Table 8). There was no difference in IL-10 concentration when separating participants by distance run on the MSFT, $\dot{V}O_2$ peak or adiposity (all $p > 0.05$), nor was there any difference between boys and girls (main effect of sex: all $p > 0.05$; interaction: all $p > 0.05$). The multiple regression analysis (Table 9) revealed that distance run on the MSFT was the only statistically significant predictor of IL-10 concentration, after adjustment for APHV, with a positive relationship observed between the two variables ($\beta = 0.325$, $p = 0.021$).

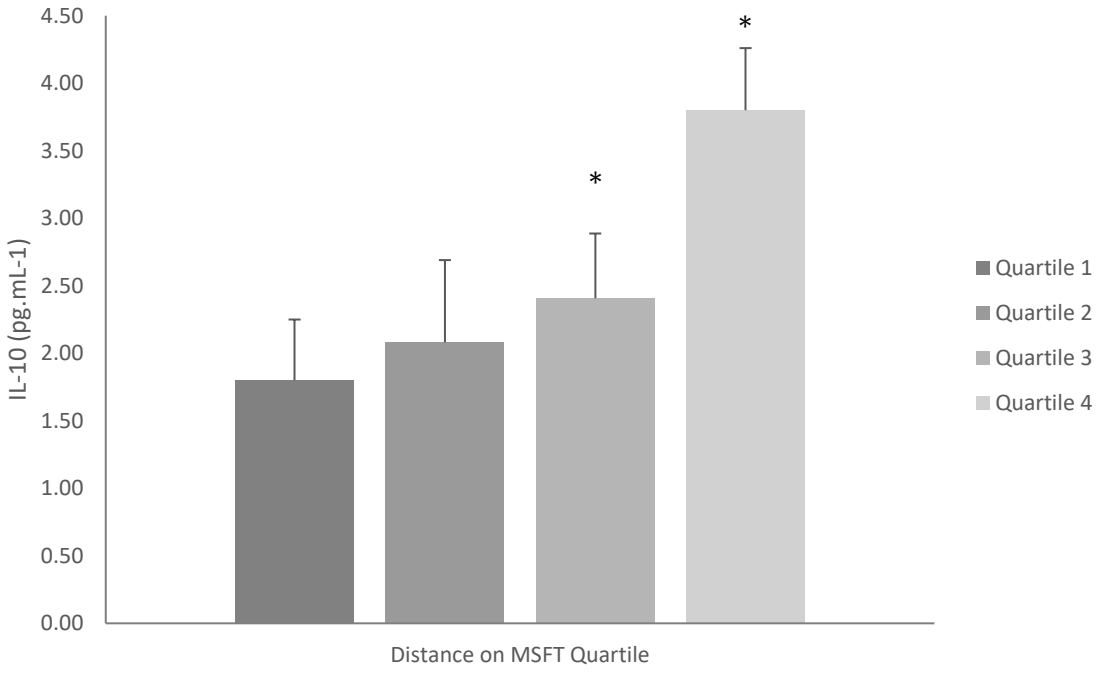

Figure 5. IL-10 concentration (pg·mL^{-1}) separated into quartiles by blood lactate concentration at 8.5 km h^{-1}. Participants in quartile one had the lowest training status. Mean ± SEM, main effect of training status $p = 0.035$. *denotes significant difference from quartile one.

TNF-α and CRP: When separating participants into quartiles by distance covered on the MSFT, $\dot{V}O_2$ peak, blood lactate concentration at 8.5 km·h^{-1} and adiposity there was no difference in TNF-α or CRP concentration across quartiles (all $p > 0.05$, Table 8). Furthermore,

there was no difference between boys and girls (main effect of sex: all $p > 0.05$; interaction: all $p > 0.05$). Multiple regression revealed no statistically significant predictors of TNF-α or CRP concentration (Table 9).

Table 8. Inflammatory cytokines (IL-6, IL-1β, IL-10, TNF-α) and CRP separated into quartiles determined from distance run on the multi-stage fitness test, blood lactate concentration at 8.5 $km \cdot h^{-1}$ during the speed lactate test, $\dot{V}O_2$ peak and adiposity (Mean ± S.E.M). * denotes significantly different from quartile one.

	Distance Run on the MSFT (m)				$\dot{V}O_2$ Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)				Blood Lactate at 8·5 $km \cdot h^{-1}$ during Speed Lactate Test ($mmol \cdot L^{-1}$)				Adiposity from Sum of Skinfolds (mm)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
IL-6 ($pg \cdot mL^{-1}$)	3.25 ± 0.25	3.10 ± 0.50	2.48 ± 0.30*	2.37 ± 0.30*	3.76 ± 0.54	3.47 ± 0.29	2.60 ± 0.37	2.60 ± 0.32	3.57 ± 0.52	3.72 ± 0.57	2.70 ± 0.50	2.88 ± 0.53	3.05 ± 0.26	3.23 ± 0.35	2.95 ± 0.29	2.47 ± 0.29
IL-1β ($pg \cdot mL^{-1}$)	4.78 ± 0.85	4.34 ± 0.47	2.96 ± 0.29*	2.47 ± 0.29*	4.67 ± 1.70	3.14 ± 0.63	3.16 ± 0.62	3.25 ± 0.71	7.35 ± 2.60	3.15 ± 0.65	2.72 ± 1.25	2.82 ± 0.68	5.51 ± 1.06	3.36 ± 0.39	3.58 ± 0.44	2.86 ± 0.34
IL-10 ($pg \cdot mL^{-1}$)	1.80 ± 0.31	2.08 ± 0.19	2.41 ± 0.41	3.80 ± 0.77	2.27 ± 0.43	2.17 ± 0.23	2.18 ± 0.37	3.82 ± 1.23	1.65 ± 0.45	2.61 ± 0.48	2.96 ± 0.61	3.62 ± 0.45*	2.18 ± 0.31	2.11 ± 0.31	2.97 ± 0.76	2.40 ± 0.38
TNF-α ($pg \cdot mL^{-1}$)	1.93 ± 0.53	1.47 ± 0.20	1.47 ± 0.19	1.42 ± 0.24	1.71 ± 0.34	1.24 ± 0.15	1.59 ± 0.23	1.74 ± 0.32	2.32 ± 0.62	2.00 ± 0.57	1.21 ± 1.91	1.86 ± 0.42	1.89 ± 0.17	1.60 ± 0.26	1.65 ± 0.24	1.89 ± 0.54
CRP ($mg \cdot L^{-1}$)	0.52 ± 0.14	0.47 ± 0.21	0.52 ± 0.31	0.35 ± 0.19	0.43 ± 0.14	0.45 ± 0.15	0.41 ± 0.16	0.30 ± 0.10	0.69 ± 0.21	0.68 ± 0.28	0.86 ± 0.39	0.45 ± 0.20	0.52 ± 0.14	0.47 ± 0.14	0.45 ± 0.16	0.38 ± 0.10

4.3.2 Blood Glucose, Plasma Insulin Concentration and HOMA-IR

Fasting Blood Glucose: When separating participants into quartiles determined by distance run on the MSFT, blood glucose concentration was higher in quartile one when compared with quartile two ($p = 0.025$, $d = 0.5$), three ($p < 0.001$, $d = 1.1$) and four ($p < 0.001$, $d = 1$; main effect: $F_{(3,110)} = 7.1$, $p < 0.001$; Table 10). When separating participants into $\dot{V}O_2$ peak quartiles, blood glucose concentration was higher in quartile one when compared with participants in the fourth quartile ($p = 0.001$, $d = 1.1$; main effect: $F_{(3,68)} = 3.9$, $p = 0.013$; Table 10). When considering the effect of sex there was no difference between boys and girls (main effect of sex: all $p > 0.05$; interaction: all $p > 0.05$). The multiple regression analysis (Table 9) revealed that distance run on the MSFT was the only statistically significant predictor of blood glucose concentration, after adjustment for APHV, with a negative relationship observed ($\beta = -0.545$, $p < 0.001$).

When separating participants into adiposity quartiles, blood glucose concentration was higher in quartile one when compared with participants in quartile four ($p = 0.012$, $d = 0.6$; main effect: $F_{(3,115)} = 3.0$, $p = 0.035$; Table 10). Participants in quartile two also had higher blood glucose concentration when compared with quartile four (second quartile; $p = 0.011$, $d = 0.5$). There was no difference in blood glucose concentration across all quartiles between boys and girls (main effect of sex: $p = 0.637$). There was an effect of adiposity on sex (interaction: $F_{(3,115)} = 3.4$, $p = 0.019$), in that girls in the first quartile had higher blood glucose concentration (4.81 ± 0.59 mmol.L^{-1}) when compared with quartiles two (4.12 ± 0.44 mmol.L^{-1}, $p = 0.001$, $d = 1.3$), third (4.23 ± 0.52 mmol.L^{-1}, $p = 0.004$, $d = 1$) and four (4.14 ± 0.46 mmol.L^{-1}, $p = 0.001$, $d = 1.3$). There was no difference in blood glucose concentration across adiposity quartiles in boys (all $p > 0.05$).

Fasting Plasma Insulin: When separating participants into quartiles determined by distance run on the MSFT, plasma insulin concentration was higher in quartile one when compared with

participants in quartiles three ($p = 0.005$, $d = 0.8$) and four ($p < 0.001$, $d = 1$; main effect: $F_{(3,102)} = 5.5$, $p = 0.002$; Table 10). When separating participants into quartiles determined by $\dot{V}O_2$ peak, plasma insulin concentration was higher in participants in quartile one when compared with participants in quartiles three ($p = 0.009$, $d = 0.7$) and four ($p < 0.001$, $d = 1$; main effect: $F_{(3, 62)} = 5.8$, $p = 0.002$; Table 10). Participants in quartile two also had higher plasma insulin concentrations when compared with quartile four ($p = 0.009$, $d = 0.7$). When separating participants into quartiles determined from blood lactate concentration at 8.5 $km{\cdot}h^{-1}$, plasma insulin concentration was higher in quartile one when compared with participants in quartile four ($p = 0.012$, $d = 0.9$; main effect: $F_{(3,28)} = 3.8$, $p = 0.043$ Table 10). When considering the effect of sex, plasma insulin concentration was higher in girls (7.73 ± 0.58 $mU.L^{-1}$) than boys (boys; 6.05 ± 0.55 $mU.L^{-1}$; main effect of sex: $F_{(1,101)} = 4.4$, $p = 0.037$, $d = 0.4$).

When separating participants into quartiles determined by adiposity, plasma insulin concentration was higher in quartile one when compared with participants in quartiles two ($p = 0.003$, $d = 0.9$), three ($p = 0.044$, $d = 0.6$) and four ($p = 0.004$, $d = 0.8$; main effect: $F_{(3,105)} = 4.0$, $p = 0.010$; Table 10). When considering the effect of sex, plasma insulin concentration was higher in girls (7.59 ± 0.56 $mU.L^{-1}$ vs 5.86 ± 0.57 $mU.L^{-1}$; main effect of sex: $F_{(1,105)} = 4.7$, $p = 0.033$, $d = 0.4$). There was an effect of adiposity on sex (interaction: $F_{(3,105)} = 3.5$, $p = 0.018$), in that girls having the highest adiposity had higher plasma insulin concentrations than boys in the same quartile (boys; 6.15 ± 0.82 $mU.L^{-1}$, girls; 11.81 ± 1.67 $mU.L^{-1}$, $F_{(1,97)} = 12.9$, $p < 0.001$, $d = 1$). Girls in quartile one had increased plasma insulin concentration when compared with girls in quartiles two (6.56 ± 0.86 $mU.L^{-1}$, $p < 0.001$, $d = 1.2$) three (6.85 ± 0.57 $mU.L^{-1}$, $p = 0.002$, $d = 1.1$) and four (5.11 ± 0.57 $mU.L^{-1}$, $p < 0.001$, $d = 1.4$). There was no difference in plasma insulin concentration in boys across quartiles (all $p > 0.05$). The multiple regression analysis (Table 9) revealed that adiposity was the only statistically significant

predictor of plasma insulin concentration, after adjustment for APHV, with a positive relationship observed between the two variables ($\beta = .515$, $p < 0.001$).

HOMA-IR: When separating participants into quartiles determined by distance run on the MSFT, HOMA-IR was higher in quartile one when compared with participants in quartiles two ($p = 0.002$, $d = 0.8$), three ($p = 0.002$, $d = 0.8$) and four ($p < 0.001$, $d = 1.4$; main effect: $F_{(3,101)} = 9.4$, $p < 0.001$; Table 10). When separating participants into quartiles based on $\dot{V}O_2$ peak, HOMA-IR was higher in quartile one when compared with participants in quartiles three ($p = 0.003$, $d = 0.8$) and four ($p = 0.001$, $d = 1.1$; main effect: $F_{(3,60)} = 5.7$, $p = 0.002$; Table 10). Participants in quartile two also had increased HOMA-IR when compared with participants in quartile four ($p = 0.019$, $d = 0.7$). When considering the effect of sex, HOMA-IR was higher in girls (1.50 ± 0.13) than boys (1.14 ± 0.12; main effect of sex: $F_{(1,99)} = 4.1$, $p = 0.046$, $d = 0.4$), yet the effect of VO_2 peak on HOMA-IR did not differ between boys and girls (all $p > 0.05$). When separating participants into quartiles by blood lactate concentration at 8.5 $km \cdot h^{-1}$ there was no difference in HOMA-IR across quartiles (all $p > 0.05$, Table 10).

When separating participants into quartiles determined by adiposity, HOMA-IR was higher in quartile one when compared with participants in quartiles two ($p = 0.002$, $d = 0.9$), three ($p = 0.005$, $d = 0.8$) and four ($p < 0.001$, $d = 1$; main effect: $F_{(3,103)} = 5.6$, $p = 0.001$; Table 10). When considering the effect of sex, HOMA-IR was higher in girls (1.52 ± 0.12 vs 1.10 ± 0.12; main effect of sex: $F_{(1,103)} = 5.\ 9$, $p = 0.017$, $d = 0.5$). There was also an effect of adiposity on sex (interaction: $F_{(3,103)} = 4.0$, $p = 0.010$, $d = 1.5$), in that girls in quartile one had higher HOMA-IR (2.58 ± 0.44) than their male counterparts (1.22 ± 0.19). Girls with the highest adiposity also had increased HOMA-IR when compared with girls in quartiles two (1.36 ± 0.19, $p = 0.001$, $d = 0.9$), third (1.18 ± 0.25, $p < 0.001$, $d = 1.1$) and four (0.94 ± 0.15, $p < 0.001$, $d = 1.3$). There was no difference in HOMA-IR across adiposity quartiles in boys (all $p > 0.05$).

The multiple regression analysis (Table 9) revealed that adiposity was the only statistically significant predictor of HOMA-IR, after adjustment for APHV, with a positive relationship observed between the two variables ($\beta = .506$, $p < 0.001$).

4.3.3 Blood Pressure

Systolic Blood Pressure: When separating participants into quartiles based on distance run during the MSFT, $\dot{V}O_2$ peak and blood lactate concentration at 8.5 $km \cdot h^{-1}$ during the speed lactate test or adiposity, there was no difference in systolic blood pressure (all $p > 0.05$, Table 10). When considering the effect of sex there was no difference in systolic blood pressure between boys and girls (main effect of sex: all $p > 0.05$; interaction: all $p > 0.05$). The regression model for systolic blood pressure identified no statistically significant predictors.

Diastolic Blood Pressure: When separating participants into adiposity quartiles, diastolic blood pressure was higher in quartile one when compared with participants in quartiles three ($p = 0.003$, $d = 0.7$) and four ($p = 0.046$, $d = 0.5$; main effect: $F_{(3,116)} = 3.3$, $p = 0.023$; Table 10). There was no difference in diastolic blood pressure across quartiles when participants were separated by distance run during the MSFT, $\dot{V}O_2$ peak and blood lactate concentration at 8.5 $km \cdot h^{-1}$ during the speed lactate test (all $p > 0.05$), nor was there any difference between boys and girls (main effect of sex: all $p > 0.05$; interaction: all $p > 0.05$). The multiple regression analysis (Table 9) revealed that adiposity was the only statistically significant predictor of diastolic blood pressure, after adjustment for APHV, with a positive relationship between the two variables ($\beta = 0.259$, $p = 0.042$).

Mean Arterial Pressure: When separating participants into adiposity quartiles, mean arterial pressure was higher in quartile one when compared with quartile two ($p = 0.021$, $d = 0.6$), three ($p = 0.004$, $d = 0.7$) and four ($p = 0.017$, $d = 0.6$; main effect: $F_{(3,116)} = 3.5$, $p = 0.018$; Table 4). There was no difference in mean arterial pressure when participants were separated by distance

run during the MSFT, $\dot{V}O_2$ peak or blood lactate concentration at 8.5 $km \cdot h^{-1}$ during the speed lactate test (all $p > 0.05$), nor was there any difference between boys and girls (main effect of sex: all $p > 0.05$; interaction: all $p > 0.05$). The multiple regression analysis (Table 9) revealed that adiposity was the only statistically significant predictor of mean arterial pressure, after adjustment for APHV, with a positive relationship observed between the two variables ($\beta = .322$, $p = 0.011$).

Table 9. Standardised regression summary for distance run on the MSFT, $\dot{V}O_2$ peak and sum of skinfolds (adiposity) with individual risk factors.
* denotes significant relationship.

	MSFT Distance (m)			$\dot{V}O_2$ Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)			Sum of Skinfolds (mm)		
	R^2 adj.	β	p	R^2 adj.	β	p	R^2 adj.	β	p
IL-6 ($pg \cdot mL^{-1}$)	.085	-.291	.031*	.035	.060	.800	-.004	-.005	.978
IL-1β ($pg \cdot mL^{-1}$)	.164	-.405	.005*	.244	.313	.106	.004	.004	.981
IL-10 ($pg \cdot mL^{-1}$)	.108	.325	.021*	.134	.151	.419	.118	.173	.419
TNF-α ($pg \cdot mL^{-1}$)	.098	.167	.397	.054	.107	.489	.120	.178	.420
Blood Glucose ($mmol \cdot L^{-1}$)	.297	-.545	< .001*	-.113	-.145	.390	.152	.190	.246
Plasma Insulin ($mU \cdot L^{-1}$)	-.079	-.097	.563	-.150	-.172	.269	.266	.515	< .001*
HOMA-IR	-.096	-.127	.488	-.105	-.122	.450	.256	.506	< .001*
Systolic Blood Pressure (mmHg)	.060	-.091	.666	-.025	-.102	.855	.031	.142	.825
Diastolic Blood Pressure (mmHg)	.094	.135	.472	.000	.000	.998	.067	.259	.042*
Mean Arterial Pressure (mmHg)	.115	.163	.383	-.018	-.023	.892	.088	.332	.011*

Table 10. Cardiometabolic risk factors including blood glucose, plasma insulin, HOMA and blood pressure separated into quartiles determined from distance run on the multi-stage fitness test, blood lactate concentration at 8.5 $km \cdot h^{-1}$ during the speed lactate test, $\dot{V}O_2$ peak and sum of skinfolds (Mean ± S.E.M). * denotes significantly different from quartile one; † significantly different from quartile two.

	MSFT Distance (m)				$\dot{V}O_2$ Peak ($ml \cdot kg^{-1} \cdot min^{-1}$)				Blood Lactate at 8·5 $km \cdot h^{-1}$ during Speed Lactate Test ($mmol \cdot L^{-1}$)				Sum of Skinfolds (mm)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Blood Glucose ($mmol.L^{-1}$)	4.60 ± 0.44	4.35 ± 0.48*	4.08 ± 0.44*	4.11 ± 0.53*	4.63 ± 0.58	4.32 ± 0.57	4.25 ± 0.49	4.00 ± 0.54*	4.48 ± 0.52	4.32 ± 0.65	4.36 ± 0.78	3.82 ± 0.53	4.54 ± 0.68	4.27 ± 0.44	4.19 ± 0.56	4.20 ± 0.44*†
Plasma Insulin ($mU.L^{-1}$)	8.99 ± 1.04	6.71 ± 0.76	5.60 ± 0.54*	4.86 ± 0.82*	8.49 ± 1.24	7.07 ± 0.66	5.18 ± 0.63*	4.02 ± 0.54*†	7.00 ± 0.87	5.60 ± 0.90	6.23 ± 0.67	3.51 ± 0.88*	9.08 ± 1.07	5.55 ± 0.69*	6.68 ± 0.74*	5.70 ± 0.80*
HOMA	2.00 ± 0.68	1.22 ± 0.18*	1.21 ± 0.18*	0.78 ± 0.18*	1.81 ± 0.32	1.48 ± 0.18	0.90 ± 0.09*	0.78 ± 0.13*†	1.35 ± 0.14	1.61 ± 0.17	1.01 ± 0.13	0.60 ± 0.21	1.90 ± 0.27	1.14 ± 0.14*	1.20 ± 0.15*	1.01 ± 0.12*
Systolic Blood Pressure (mmHg)	112 ± 2	112 ± 2	111 ± 2	111 ± 2	111 ± 2	108 ± 2	110 ± 2	110 ± 3	104 ± 2	111 ± 2	106 ± 2	113 ± 3	115 ± 2	109 ± 2	112 ± 2	110 ± 2
Diastolic Blood Pressure (mmHg)	69 ± 2	67 ± 1	70 ± 1	73 ± 1	70 ± 2	72 ± 2	68 ± 1	65 ± 1	74 ± 1	70 ± 2	66 ± 1	68 ± 1	73 ± 1	72 ± 2	67 ± 1*	69 ± 1*
Mean Arterial Pressure (mmHg)	86 ± 2	84 ± 1	82 ± 1	83 ± 1	83 ± 2	84 ± 1	82 ± 1	80 ± 1	84 ± 1	83 ± 2	79 ± 2	83 ± 1	87 ± 1	83 ± 1*	82 ± 1*	83 ± 1*

4.4 Discussion

The primary finding of the present study was that adolescents categorised below the 25th centile for distance run on the MSFT exhibited increased concentrations of pro-inflammatory cytokines IL-6 and IL-1β and reduced concentrations of the anti-inflammatory mediator IL-10 when compared with those categorised above the 25th centile. The present study is the first to report that distance run on the MSFT and the blood lactate response to exercise were the only measures to influence inflammatory cytokine concentrations in adolescents, both of which are deemed more sensitive measures of an individual's endurance fitness - the physical capacity to perform prolonged exercise. In addition, the multiple regression revealed that the MSFT was the only significant predictor of inflammation in adolescents (with no relationship observed for $\dot{V}O_2$ peak or adiposity). Furthermore, adolescents categorised below the 25th percentile with the lowest distance run on the MSFT and $\dot{V}O_2$ peak exhibited increased metabolic risk factors (including fasted blood glucose, plasma insulin and HOMA-IR), whilst adolescents with the highest adiposity also presented with increased diastolic and mean arterial blood pressure compared to adolescents in all other quartiles. These findings emphasise the importance of enhancing the physical capacity to perform prolonged exercise, as evidenced by performance on the MSFT, and maintaining a healthy body composition during adolescence in order to attenuate the risk of developing early onset cardiovascular disease and type 2 diabetes.

Adolescents with the lowest MSFT performance in the present study exhibited increased concentrations of pro-inflammatory cytokines IL-6 and IL-1β, and reduced concentrations of anti-inflammatory mediator IL-10 in comparison to adolescents in all other quartiles. These findings are novel as the present study is the first to measure a range of inflammatory cytokines that are reflective of low-grade chronic inflammation in a heterogeneous population of male and female adolescents (Gleeson et al., 2011). The finding that adolescents with the lowest physical fitness have increased concentrations of pro-inflammatory mediators is consistent

with previous studies in adolescents in that increased concentrations of IL-6 (Buchan et al., 2015, Bugge et al., 2012) and CRP (Buchan et al., 2015) are observed in participants in the lowest quartile for physical fitness. However, the present study is the first to report that participants with the lowest MSFT performance have reduced circulating concentrations of IL-10. These findings are in contrast to those of Jürimäe et al. (2017b) whereby IL-10 concentration was similar in female rhythmic gymnasts and untrained controls. These discrepant findings might relate to the different methods used to categorize participants, as the present study measured the participant's physical capacity to perform prolonged exercise and body composition, whereas Jürimäe et al. (2017b) categorized participants solely based on participation in rhythmic gymnastics or not. Therefore, the present study assessed the objective relationship between performance in submaximal and maximal exercise tests and anti-inflammatory mediator IL-10. Increased concentrations of IL-10 protect against risk factors for cardiometabolic diseases, as *in vitro* studies report that IL-10 inhibits the synthesis of IL-1β and TNF-α which promote the development of low-grade chronic inflammation (Petersen and Pedersen, 2005). As acute bouts of physical activity transiently increase IL-10 concentrations (Petersen and Pedersen, 2005), it is not surprising that participants with the lowest performance on the MSFT had significantly reduced concentrations of the potent anti-inflammatory mediator. Furthermore, there were no differences between pro- or anti-inflammatory cytokine concentrations in adolescents categorised above the 25th percentile, which is consistent with previous research in adolescents (Buchan et al., 2015). These findings suggest enhanced physical capacity to perform prolonged exercise protects against low-grade chronic inflammation in adolescents by reducing exposure to pro-inflammatory mediators and increasing systemic concentrations of the anti-inflammatory cytokine IL-10.

Performance on the MSFT and the blood lactate response to submaximal exercise were the only measures to influence inflammation in adolescents in the present study. This finding was

also observed in the multiple regression model, which revealed that distance run on the MSFT was the only significant predictor of inflammation in adolescents, whilst $\dot{V}O_2$ peak and adiposity were not related to inflammation. Previous studies in adolescents that used $\dot{V}O_2$ peak as an indicator of fitness (Ischander et al., 2007, Steene-Johannessen et al., 2013) also reported no relationship between pro-inflammatory mediators (IL-6, TNF-α and CRP) and physical fitness in adolescents. In contrast, inverse associations between pro-inflammatory mediators (IL-6, TNF-α, CRP) and physical fitness have been observed in adolescents when MSFT performance was the preferred measure of fitness (Buchan et al., 2015; Silva et al., 2014).

To the authors' knowledge, the present study is the first to consider that the methodology used to measure an individual's capacity to perform prolonged exercise influences the relationship between physical fitness and risk factors for cardiometabolic diseases in adolescents. The acute anti-inflammatory response stimulated post-exercise reduces low-grade chronic inflammation in adolescents if repeated regularly (Mendham et al., 2015). Increased engagement with regular physical activity improves exercise tolerance and initiates peripheral adaptations in the muscle, including enhanced efficiency of mitochondrial biogenesis and increased fat oxidation (Joyne and Carsten, 2018). The MSFT performance and blood lactate response to sub-maximal exercise reflect such peripheral changes and are therefore considered to be sensitive to changes in the ability to perform prolonged exercise. In contrast, $\dot{V}O_2$ peak is limited when measuring peripheral adaptations as it is predominantly determined by central systems (cardiovascular and respiratory) that have a strong genetic predisposition (Joyne and Carsten, 2018). Consequently, the MSFT and blood lactate response to sub-maximal exercise are better suited in the measurement of physical fitness specifically for metabolic risk in young people. These findings suggest that adolescents can reduce low-grade chronic inflammation by enhancing distance run on the MSFT, and that improving the capacity to perform prolonged exercise is a

potential therapeutic intervention to prevent the development of risk factors for cardiometabolic diseases.

Adolescents categorised below the 25th centile for distance run on the MSFT, $\dot{V}O_2$ peak and adiposity exhibited increased blood glucose and plasma insulin concentrations, and HOMA-IR when compared with adolescents in all other quartiles. The participants categorised below the 25th centile for HOMA-IR, the chosen measure of insulin resistance, were above the reference cut off values for insulin resistance (> 1.65 in girls and > 1.9 in boys) in healthy adolescents (Rocco et al., 2011). Whereas, participants categorised ≥ 25th centile were below the reference cut off values for insulin resistance. These findings agree with those of Silva et al. (2014) whereby increased adiposity and reduced maximal oxygen uptake increased metabolic risk (calculated from traditional risk factors; blood pressure, blood glucose, triglycerides and HDL cholesterol) in adolescents. The multiple regression model showed that adiposity was the best predictor for metabolic risk factors (plasma insulin and HOMA-IR) and blood pressure in adolescents. This finding is consistent with previous research in adults, which reported the sum of skinfold thickness to be the strongest predictor of insulin resistance (Abate et al., 1995) and adiposity to be associated with systolic and diastolic blood pressure in adolescents (Paradis et al., 2004). Studies in rodents report that increasing adiposity drives an influx of free fatty acids, which deactivate insulin receptors and reduce insulin sensitivity (Capurso and Capurso, 2012). Yet, the mechanisms relating adiposity with higher blood pressure in adolescents are relatively unknown, with disturbances in autonomic function being a potential mechanism (Paradis et al., 2004). These findings have enhanced understanding of the lifestyle factors that are associated with risk factors for metabolic disease in adolescents and emphasise the importance of maintaining a healthy body composition in conjunction with enhancing physical performance (through regular participation in physical activity).

The present study also reports that girls with the highest adiposity had elevated plasma insulin concentration and reduced insulin sensitivity (HOMA-IR) when compared with their male counterparts (boys categorised below the 25th centile for adiposity). These findings may be explained by the significantly increased adiposity of girls in quartile one when compared with boys and girls in all other quartiles (Table 7). These findings also support previous studies, which have reported that girls exhibited reduced postprandial insulin sensitivity when compared with boys of the same chronological age (Cooper et al., 2017). However, there was no difference in APHV between boys and girls in the present study and APHV was a covariate in the analysis to account for the potential confounding effects of maturation. Therefore, it is not feasible to suggest that the increased adiposity and insulin resistance observed in the girls in the present study was the result of differences in pubertal development. However, the potential confounding effect of puberty on the relationship between adiposity and insulin resistance in both males and females does warrant further research. Regardless of the mechanisms involved, it is apparent that adolescent girls with increased adiposity exhibit reduced insulin sensitivity when compared with their male counterparts. As such, future interventions should focus on promoting healthy body composition and physical fitness in adolescent girls, as the findings of the present study report that both variables can mediate improvements in insulin sensitivity.

The present study has several strengths including the measurement of a comprehensive panel of inflammatory cytokines in a heterogeneous sample of adolescents with diverse endurance capacities and adiposity, which allowed for the relationship between these variables and cardiometabolic health to be determined. The heterogeneity of performance capacity (measured using the distance run on the MSFT) in the present study ranged from the 30th-95th percentile for boys and the 20th - 95th percentile for girls, when compared with normative data in European adolescents (Tomkinson et al., 2018). Similarly, the adiposity of the adolescents

in the present study ranged from the 5th to > 95th percentile for BMI, which also supports the heterogeneity of the participants recruited to the present study. The diversity of the adolescents analysed in the present study allows for broad dissemination of the main findings of the study. However, a limitation to the present study was the lack of power for the blood lactate response to submaximal treadmill running and thus its exclusion from the multiple regression model. Future research should determine more fully the effect of the blood lactate response to exercise (as a measure of adolescent training status) on adolescent cardiometabolic health. A further limitation of the present study is the absence of CRP data following the methodological issues that occurred. CRP is reported as the best predictor of cardiovascular events in adults (Gleeson et al., 2012). However, the relationship of CRP with training status, $\dot{V}O_2$ peak and adiposity in adolescents is yet to be examined, despite the importance of reducing CRP across the lifespan in preventing clinical manifestation of cardiometabolic disease in adulthood (for review, see section 2.2.2.2). Further limitations include the absence of measurements pertaining to the ethnicity, daily dietary habits and the typical physical activity levels of the participants. Each of these measures are potential confounders in the relationship between performance tests and the risk factors measured in the present study (Hardman & Stensel., 2009). Nevertheless, given the difficulties of data collection in this age group and population the present study is the most comprehensive yet to examine fitness and the risk factors for cardiometabolic disease in adolescents.

In conclusion, the present study shows that a higher ability to perform prolonged exercise (as indicated by distance run on the MSFT) in adolescents protects against the development of cardiometabolic risk indicators that increase the likelihood of early onset cardiovascular disease and type 2 diabetes. These findings suggest that all young people can benefit from enhancing their ability to perform prolonged exercise as evidenced by the differences across quartiles based on MSFT performance. Furthermore, these findings are particularly important

for those categorised below the 25th centile, as the benefits for metabolic risk factors were observed for those categorized above the 25th centile and for markers of inflammation for those above the 50th centile based on distance run on the MSFT. Although there were also benefits of a high $\dot{V}O_2$ peak and low adiposity, these were not as marked as the benefits of enhanced performance on the MSFT. Thus, enhancing performance on the MSFT is a key factor in successfully reducing cardiometabolic risk in young people and thus, training interventions should be given substantial attention in public policy interventions for young people. Future research should determine the type, duration, intensity and frequency of an ecologically valid mode of exercise that will enhance performance on the MSFT in adolescents and subsequently enhance cardiometabolic health.

Chapter V

Cytokine, glycaemic and insulinaemic responses to an acute bout of games-based activity in adolescents

5.1 Introduction

The findings of Chapter IV suggest that a relationship exists between performance on the MSFT, the blood lactate response to submaximal exercise and novel (inflammatory cytokines) and traditional (markers of insulin resistance) risk factors for cardiometabolic diseases in adolescents. Therefore, the aim of Chapter V was to examine the inflammatory, glycaemic and insulinaemic responses to an acute bout of exercise to suggest some of the potential mechanisms that lead to regular participation in exercise chronically reducing the presence of risk factors for cardiometabolic diseases in young people.

Low-grade chronic inflammation is involved in the pathogenesis of several chronic diseases, including cardiovascular disease and type 2 diabetes (Gleeson et al., 2011; Nassis et al., 2005; Petersen & Pedersen, 2005; Sarzynski et al., 2013). Although such conditions typically present during adulthood, the development of cardiometabolic risk factors for these diseases originate during childhood, with low-grade chronic inflammation, atherosclerotic plaques, and insulin resistance observed in pubertal children (Beresnon et al., 1998; Ehtisham et al., 2000; Warnberg et al., 2008). Low-grade chronic inflammation is defined as a 2-3 fold increase in systemic concentrations of pro-inflammatory cytokines, including IL-6, IL-1β, TNF-α and the acute phase protein CRP (Petersen & Pedersen, 2005). The inflammatory response that follows an acute bout of exercise (reflected by increased IL-6, produced by the contraction of skeletal muscle, subsequently stimulating a systemic increase in the concentration of anti-inflammatory cytokines) is a mechanism that stimulates muscle regeneration and reduces low-grade chronic

inflammation and insulin resistance in adults (Gleeson et al., 2011; Petersen & Pedersen, 2005), with *in vitro* studies reporting that IL-10 inhibits the synthesis of chronic pro-inflammatory mediators TNF-α and IL-1β (Petersen & Pedersen, 2005). Furthermore, in the plantaris muscle of mice increased IL-6 post-exercise increases the expression of glucose transporter-4, which increases glucose uptake (Ikeda et al., 2016), suggesting that a rise in IL-6 post-exercise triggers an anti-inflammatory response and enhances insulin sensitivity.

Despite the presence of low-grade chronic inflammation and insulin resistance in young people (Berenson et al., 1998), information on the inflammatory response to an acute bout of exercise in adolescents is limited. Previous studies have examined the response of inflammatory mediators, IL-6 and TNF-α, to an acute bout of endurance exercise and increases (Nemet et al., 2002; Scheet et al., 1999), decreases (Nemet et al., 2003), and no changes (Timmons et al., 2006; Paltoglou et al., 2017) have been observed. However, young people do not typically participate in endurance exercise, with intermittent activity being both enjoyable (important for long-term adherence) (Howe et al., 2010) and replicative of their physical activity patterns (Rowland et al., 2008). Eccentric exercise is an alternative mode of physical activity that elicits an increase in pro-inflammatory cytokines (IL-6 and IL-1β) in response to the muscle damage incurred (Jamurtas et al., 2012; Paschalis et al., 2010). Furthermore, games-based activities, such as basketball (which is characterised by repeated eccentric muscle contractions), are intermittent in nature and replicate the activity patterns of young people whilst also having previously been reported to induce muscle damage and an inflammatory response (increased IL-6 and IL-1β) in adults (Chatzinikolaou et al., 2014). Therefore, it is important to determine whether intermittent games-based activity elicits a protective anti-inflammatory response beneficial to cardiometabolic health in young people (Gleeson et al., 2011; Petersen & Pedersen, 2005).

Only two studies in young people have assessed the effect of intermittent exercise on inflammatory mediators (Scheet et al., 1999; McMurray et al., 2007). McMurray et al. (2007) reported that 10 x 2 min bouts of high intensity intermittent cycling in pubertal adolescents increased the anti-inflammatory IL-6: TNF-α ratio by 80% 2-h post-exercise. Whereas, Scheet et al. (1999) observed that 90-min of soccer in pre-pubertal children increased IL-6 (125%) and TNF-α (18%) 2-h post-exercise, yet did not affect anti-inflammatory mediator IL-10. The differences in exercise intensity and duration might explain these discrepant findings. Importantly, these studies have only assessed IL-6, TNF-a and IL-10 for 2-h post-exercise, despite *in vitro* studies reporting that inflammatory mediators (IL-10 and CRP) remain elevated for up to 24-h post-exercise (Gleeson et al., 2011; Petersen & Pedersen, 2005). To fully examine the inflammatory response to exercise a complete range of pro-inflammatory (IL-1β, TNF-α and CRP) and anti-inflammatory (IL-6, IL-10 and IL-6: TNF-α) cytokines should be measured up to 24-h post-exercise.

Another important aspect of cardiometabolic health is the glycaemic and insulinaemic response following a meal. Previously, a 45-min bout of aerobic exercise residually enhanced insulin sensitivity in adolescents for up to 17-h, as demonstrated by reduced postprandial glycemic and insulinaemic responses following a high fat meal (Short et al., 2013). Furthermore, high intensity intermittent and moderate intensity cycling in adolescent boys reduced the glycemic and insulinemic responses by 24-29%, following an oral glucose tolerance test, in comparison to a rested control trial (Cockcroft et al., 2014). However, the impact of exercise on the glycemic and insulinemic response to an ecologically valid meal remains unknown and no studies have examined the association between the inflammatory and glycemic/insulinemic responses post-exercise, despite the anti-inflammatory response being associated with insulin sensitivity (Straczkowski et al., 2005).

The present study aims to investigate if an acute bout of intermittent games-based activity stimulates an anti-inflammatory and metabolic response in young people.

5.2 Methods

5.2.1 Study Design

The institutional ethical advisory committee approved all procedures (approval number SST-417). Participants were recruited from secondary schools and written informed parental consent alongside child assent obtained. The following exclusion criteria were applied (a) a medical history of chronic diseases, including but not limited to cardiovascular disease, diabetes and hypertension, (b) prescription of regular medication that may affect participation in the study and (c) any factor that would cause an inability to complete the exercise components of the study. A parent/guardian completed a health screen questionnaire on behalf of the participant to ensure there were no medical conditions affecting participation in the study.

The familiarisation session preceded the main experimental trial by 7 d. During familiarization, the experimental protocol was explained to participants and they were familiarized with the methods included in the main trials. Participants completed the multi-stage fitness test (MSFT) as a performance test of endurance fitness (Ramsbottom et al., 1988) and peak oxygen uptake was estimated from these test results (50.3±4.4 $ml \cdot kg^{-1} \cdot min^{-1}$) using an adolescent specific calculation (Barnett et al., 1993).

5.2.2 Participant Characteristics

Forty-one schoolchildren aged 11-13 y were recruited to the present study. Two participants did not complete the study (as they decided they did not want to miss the required time from school lessons), therefore, 39 participants (20 males, 19 females) were included in the analysis. During familiarization body mass (Seca 770 digital scale, Hamburg, Germany), height and

sitting height (Leicester Height Measure, Seca, Hamburg, Germany) were measured, to determine age at peak height velocity (Moore et al., 2015). Waist circumference and the sum of four skinfold were measured. The participants' anthropometric characteristics were (mean ± SD): age 12.3±0.7 y; height 155.7±7.5 cm; body mass: 46.0±9.5 kg; predicted years from peak height velocity: -0.37±1.1 y; waist circumference: 65.4±5.7 cm and sum of skinfolds: 44.9±19.5 mm.

5.2.3 Main Trials

The study employed a randomised, counterbalanced, crossover design consisting of an exercise and rested (control) trial, separated by 7 d. As one of the main trials incorporated an exercise session, participants were not blinded to the trial condition. The experimental protocol is displayed in Figure 6.

Figure 6. Schematic of protocol for the assessment of an acute bout of games-based activity on inflammatory, glycaemic and insulinaemic responses in adolescents.

Participants recorded their dietary intake for 24 h preceding the first main trial and during evening one of the study; recorded diets were replicated for the subsequent main trial. Participants arrived at school fasted from 9 pm the previous evening on day one and day two of the main trials. Participants were asked to refrain from physical activity 24 h prior to and during all main trials. A telephone call was made to parents/guardians prior to each main trial to ensure compliance.

On arrival at school (8.30 am) participants were fitted with a heart rate monitor (Team Sports System, Firstbeat Technologies Ltd, Finland) which was worn during main trials. A standardized breakfast (cornflakes, milk, toast, margarine) and lunch (chicken sandwich, baked crisps, apple) were provided, each containing 1.5 g carbohydrate per kg body mass, as used in previous research (Cooper et al., 2012). Prior to participation, parents and guardians completed a health questionnaire, which provided them with the opportunity to also state any food allergies and preferences of their child/dependent. In the present study there were no allergies to the foods consumed, however had this been the case suitable alternatives would have been provided. Participants had 15 min to consume each meal.

5.2.4 Capillary Blood Samples

Capillary blood samples were taken at baseline, immediately post-exercise and 60 min post-exercise (Fig 1). Additional blood samples were taken 30 min, 60 min (2 h post-exercise) and 120 min (3 h post exercise) following a standardized lunch. A final fasted capillary blood sample was taken the following morning (day two) using previously described methods (Cooper et al., 2012).

Blood glucose and plasma insulin concentrations were measured in duplicate using commercially available kits (glucose: GOD/PAP method, GL364, Randox, Ireland; insulin: ELISA, Mercodia Ltd, Sweden). Blood glucose and plasma insulin iAUC following the standardized lunch were calculated (Brouns et al., 2005; Wolever & Jenkins, 1986).

Cytokine (IL-1β, IL-6, TNF-α and IL-10) concentrations were determined using a flow cytometry-based multiplex bead approach (AimPlex™ multiplex assay, YSL, Pomona, USA) using a Beckman Coulter Gallios™ flow cytometer and Kaluza™ data acquisition and analysis

software (Beckman Coulter, London, UK). CRP concentrations were determined using the same approach, but in a separate assay due to a greater sample dilution being required.

5.2.5 Exercise Protocol

The exercise trial incorporated a 60 min basketball session, which commenced 1 h after breakfast on day 1 (Fig 1). An experienced basketball coach delivered the sessions to groups of 10 participants in a school sports hall. The basketball session consisted of a warm-up (5 min of jogging on the court followed by dynamic stretches), skill-based drills (30 min passing, dribbling and shooting drills) and small-sided games (25 min) to finish. Immediately post-exercise, participants returned to the classroom and rested quietly for the remainder of the day. During the control trial, participants rested in the classroom throughout the day.

5.2.6 Statistical Analysis

A power calculation was performed using GPower 3.1.9.2 based on the previously reported effects of exercise on IL-6 in young people (Nemet et al., 2002) and an estimated effect size of 0·3 (two-tailed significance), the power analysis yielded a required total sample size of 38.

All data was assessed for normality using the Kolmogorov-Smirnov test prior to statistical analysis. Inflammatory cytokine, blood glucose and plasma insulin concentration data were analyzed in SPSS (Version 24, SPSS Inc, Chicago, USA) via three-way (trial*time*sex) Analysis of Variance (ANOVA) with repeated measures for trial and time. Separate ANOVAs were conducted for day one and between resting measures on day one and day two. Where significant interactions were observed, post-hoc pairwise comparisons were performed using a Bonferonni correction. Blood glucose iAUC, plasma insulin iAUC and heart rate were compared using a paired samples t-test. Where significant effects existed for main effect of trial and trial by time interactions, effect sizes were calculated as Cohen's d. For discussion

purposes, percentage change was calculated for variables that were significantly different between trials (Percentage change = [Trial 1 value / Trial 2 value] * 100). For all analysis, significance was accepted as $p<0.05$ and data are presented as mean ± S.E.M..

5.3 Results

5.3.1 Heart Rate

Mean heart rate during the basketball was 157 ± 11 beats·min^{-1} (76 ± 5 % of maximum heart rate achieved during the MSFT; HRmax) and maximum recorded mean heart rate was 197 ± 9 beats·min^{-1} (96 ± 4 % HRmax). Consequentially, mean heart rate was higher during the exercise trial than the control trial (exercise: 104 ± 14 beats·min^{-1}, 51 ± 7% HRmax; resting: 90 ± 10 beats·min^{-1}, 44 ± 5 % HRmax; $t_{(38)}= -7.2$, $p<0.001$).

5.3.2 Inflammatory and Metabolic Responses

At baseline, there was no difference in inflammatory cytokine, blood glucose or plasma insulin concentration between the exercise and control trials (all $p > 0.05$). When considering the effect of sex there were no differences in inflammatory cytokine concentrations between the boys and girls at baseline or post-exercise (all $p > 0.05$).

5.3.2.1 Inflammatory Variables

The response of the inflammatory variables can be found in Table 11. Overall, IL-6 concentration was higher during day one of the exercise trial when compared with the control trial (exercise: 3.4 ± 0.4, resting: 2.7 ± 0.4 pg·mL^{-1}; main effect trial, $F_{(1,35)}= 8.7$, $p = 0.006$; $d = 0.3$). Furthermore, IL-6 concentration increased across time during both trials on day one (main effect time, $F_{(1,35)}= 11.3$, $p < 0.001$). No trial * time interaction effect was observed for IL-6 concentration on day one ($p = 0.604$), nor were there any differences 24 h post-exercise (main effect trial, $p = 0.422$; trial * time interaction, $p = 0.852$).

Overall, IL-10 concentration did not differ between the exercise and control trial on day one (main effect trial, p = 0.569); nor were there changes across time (main effect time, p = 0.151), nor a trial*time interaction (p = 0.161). However, when considering the response of IL-10 24 h post-exercise, there was a trial * time interaction ($F_{(1,38)}$= 5.9, p = 0.020; d = 0.4, Table 11) whereby IL-10 concentration was higher on day two of the exercise trial than the control trial ($t_{(34)}$= -2.2, p = 0.032).

Overall, there were no differences in TNF-α concentration between the exercise and control trials on day one (main effect trial, p = 0.400), nor were there changes across time (main effect time, p = 0.197). There was a trial * time interaction for TNF-α concentration during day one ($F_{(4,108)}$= 2.5, p = 0.048, Table 11), whereby 2 h post-exercise there was a tendency for TNF-α concentration to increase during the exercise trial in contrast to the control trial ($t_{(27)}$= 2.3, p = 0.076, d = 0.2). When considering the response of TNF-α 24 h post-exercise, there was no difference between trials (main effect trial, p = 0.680; trial * time interaction, p = 0.083).
Overall, IL-1β concentration did not differ between the exercise and control trials on day one (main effect trial, p = 0.220), nor were there any differences in IL-1β concentration across time (main effect time, p = 0.647). Furthermore, the pattern of change in IL-1β concentration was similar between trials during day one (trial * time interaction, p = 0.952). When considering the response of IL-1β 24 h post-exercise, there was no difference between trials (main effect trial, p=0.068; trial * time interaction, p = 0.621).

Overall, CRP concentration did not differ between the exercise and control trials on day one (main effect trial, p = 0.967), nor were there any differences in CRP concentration across time (main effect time, p = 0.190). The pattern of change in CRP concentration was similar between trials during day one (trial*time interaction, p = 0.593). When considering the response of CRP

24 h post-exercise, there was no difference between trials (main effect trial, p = 0.716; trial * time interaction, p = 0.116).

Overall, the IL-6: TNF-α ratio was higher during day one of the exercise trial when compared with the control trial (exercise: 5.53 ± 0.93, resting 3.75 ± 0.45; main effect trial, $F_{(1, 24)}$= 5.5, p = 0.027; d = 0.5). Furthermore, the IL-6:TNF-α ratio increased across time during both trials on day one (main effect time, $F_{(4, 96)}$= 3.3, p = 0.043). There was no trial * time interaction effect observed for the IL-6: TNF-α ratio on day one (p = 0.764), nor was there a difference between trials 24 h post-exercise (main effect trial, p = 0.827; trial * time interaction, p = 0.348).

Table 11. Summary of the inflammatory responses following 60 min high intensity, intermittent, games-based activity and during the rested control trial. Levels of inflammatory mediators reported as Mean ± S.E.M. * Significant differences between trials, $p < 0.05$.

	Rest	Post Exercise	1 h Post Exercise	2 h Post Exercise	3 h Post Exercise	Day 2 Rest
IL-6 ($pg \cdot ml^{-1}$)						
Exercise	2.07 ± 0.34	2.46 ± 0.35	3.51 ± 0.56	4.28 ± 0.49	4.81 ± 0.84	2.30 ± 0.45
Control	1.98 ± 0.33	2.06 ± 0.35	2.88 ± 0.50	3.19 ± 0.70	3.77 ± 0.42	2.14 ± 0.33
IL-10 ($pg \cdot mL^{-1}$)						
Exercise	1.45 ± 0.14	1.63 ± 0.19	1.43 ± 0.14	1.74 ± 0.15	1.75 ± 0.19	2.11 ± 0.23*
Control	1.80 ± 0.17	1.73 ± 0.18	1.44 ± 0.12	1.60 ± 0.14	1.72 ± 0.13	1.66 ± 0.16
TNF-α ($pg \cdot mL^{-1}$)						
Exercise	1.08 ± 0.21	1.00 ± 0.19	0.98 ± 0.20	1.28 ± 0.33*	1.17 ± 0.24	1.18 ± 0.26
Control	1.17 ± 0.19	0.99 ± 0.14	0.93 ± 0.14	0.98 ± 0.17	0.96 ± 0.14	1.02 ± 0.15
IL-1β ($pg \cdot mL^{-1}$)						
Exercise	3.13 ± 0.29	2.95 ± 0.31	3.05 ± 0.31	3.08 ± 0.35	3.02 ± 0.35	3.17 ± 0.34
Control	2.85 ± 0.23	2.68 ± 0.22	2.85 ± 0.25	3.01 ± 0.37	2.94 ± 0.27	2.73 ± 0.23
CRP ($mg \cdot L^{-1}$)						
Exercise	0.24 ± 0.06	0.25 ± 0.06	0.20 ± 0.04	0.21 ± 0.06	0.23 ± 0.06	0.25 ± 0.08
Control	0.26 ± 0.05	0.25 ± 0.05	0.22 ± 0.05	0.20 ± 0.04	0.19 ± 0.04	0.17 ± 0.04
IL-6: TNF-a						
Exercise	2.38 ± 0.30	5.21 ± 1.80	5.48 ± 0.90	7.25 ± 1.83	7.27 ± 2.61	2.66 ± 0.37
Control	2.19 ± 0.28	3.06 ± 0.50	3.95 ± 0.85	4.58 ± 0.68	4.94 ± 0.81	3.15 ± 0.85

5.3.2.2 Metabolic Variables

The response of the metabolic variables can be found in Table 12. Overall blood glucose concentration did not differ between the exercise and control trial (main effect trial, $p = 0.087$), yet did differ over time (main effect time, $F_{(6,210)} = 61.2$, $p < 0.001$). Furthermore, the pattern of change in blood glucose concentration differed between trials (trial*time interaction, $F_{(6,210)} = 8.8$, $p < 0.001$); whereby blood glucose concentration was higher immediately post-exercise during the exercise trial compared to the control trial ($t_{(35)} = 3.1$, $p < 0.001$, $d = 0.9$). Blood glucose concentration was also lower 1-h post-exercise on the exercise trial compared to the control trial ($t_{(35)} = 2.3$, $p < 0.001$, $d = 0.8$). No differences were evident at any other time point on day one (all $p > 0.05$). On day two, fasting blood glucose concentration was lower for the exercise trial compared to the control trial ($t_{(35)} = 3.3$, $p = 0.027$, $d = 0.6$). When considering the effect of sex, blood glucose concentration was higher at baseline and immediately post-exercise in females compared with males (trial * sex interaction, $F_{(1,35)} = 6.5$, $p = 0.016$). Blood glucose iAUC did not differ between the exercise and the control trial (main effect trial, $p = 0.084$), nor was there an effect of sex on blood glucose iAUC following the standardized lunch (trial * sex interaction, $p = 0.083$).

Overall, plasma insulin concentration was lower during the exercise trial than the control trial (exercise: 20.8 ± 2.5, resting: 24.2 ± 1.7 $mU \cdot L^{-1}$; main effect trial, $F_{(1,23)} = 6.7$, $p = 0.016$, $d = 0.3$, Table 12) and changed across time (main effect time, $F_{(6, 138)} = 55.9$, $p < 0.001$). The pattern of change in plasma insulin concentration differed between trials (trial * time interaction, $F_{(6,138)} = 7.9$, $p < 0.001$). Specifically, during the exercise trial plasma insulin concentration reached a higher peak than the control trial immediately post-exercise ($t_{(23)} = -1.33$, $p = 0.011$, $d = 0.5$), whereas plasma insulin concentration 1 h post-exercise was lower on the exercise trial when compared to the control trial ($t_{(23)} = 0.29$, $p = 0.039$, $d = 0.3$). Plasma insulin concentration was lower during the exercise trial compared to the control trial 30-min post-lunch ($t_{(23)} = 1.35$,

$p < 0.001$, $d = 0.7$) and 60 min post-lunch ($t_{(23)} = 0.61$, $p = 0.048$, $d = 0.4$). When considering the effect of sex, the response to exercise did not differ between boys and girls (trial * sex interaction, $p = 0.082$).

Plasma insulin iAUC following the consumption of the standardized lunch was lower during the exercise trial compared to the control trial (exercise: 2310 ± 834, resting: 3122 ± 1443 $mU \cdot L^{-1}$x120 min, $t_{(24)} = 3.0$, $p < 0.001$, $d = 0.7$), but this effect was not different between the sexes (trial * sex interaction, $p = 0.170$).

HOMA-IR was calculated for the fasted blood samples on day one and day two, with no difference between trials (main effect trial, $p = 0.136$), or between day one and day two (main effect time, $p = 0.519$). Furthermore, the change in HOMA-IR between day one and day two was similar between trials (trial * time interaction, $p = 0.439$).

Table 12. Summary of the glycaemic and insulinemic responses following 60 min high intensity, intermittent games based activity and during the rested control trial. Glycaemic and insulinemic responses reported as Mean ± S.E.M. * Significant differences between trials, $p < 0.05$.

	Rest	Post Exercise	1 h Post Exercise	30 min Post Lunch	60 min Post Lunch	120 min Post Lunch	Day 2
Blood Glucose ($mmol \cdot L^{-1}$)							
Exercise	4.38 ± 0.06	5.82 ± 0.16 *	4.25 ± 0.07 *	5.89 ± 0.13	5.01 ± 0.07	4.93 ± 0.08	4.38 ± 0.06
Control	4.54 ± 0.08	5.07 ± 0.10	4.74 ± 0.13	6.07 ± 0.17	5.09 ± 0.12	4.90 ± 0.08	4.72 ± 0.12
Plasma Insulin ($mU \cdot L^{-1}$)							
Exercise	7.26 ± 0.86	35.95 ± 4.03 *	7.83 ± 1.60 *	36.64 ± 3.90 *	28.84 ± 2.31 *	20.11 ± 2.05	7.22 ± 0.72
Control	8.17 ± 0.94	25.98 ± 3.39	10.50 ± 1.98	59.01 ± 7.84	33.26 ± 2.81	26.28 ± 3.19	8.70 ± 1.18
HOMA-IR							
Exercise	1.55 ± 0.63	-	-	-	-	-	1.40 ± 0.75
Control	1.64 ± 0.86	-	-	-	-	-	1.59 0.93

5.4 Discussion

The findings of Chapter IV suggested that distance run on the MSFT and the blood lactate response to submaximal exercise, as performance measures, were inversely related to both novel and traditional risk factors for cardiometabolic diseases in adolescents. Whilst these findings are promising the mechanisms that leads to enhanced performance on such tests predicting cardiometabolic disease risk have previously been unexplored. As such the main aim of Chapter V was to examine the inflammatory, glycaemic and insulinaemic responses to an ecologically valid mode of exercise in adolescents, which if repeated regularly, could result in the chronic relationship between distance run on the MSFT and cardiometabolic health.

The primary finding of the present study was that an acute bout of intermittent games-based activity elicited an anti-inflammatory response with a 132 % increase in IL-6 concentration and a 200 % rise in the anti-inflammatory IL-6: TNF-α ratio 3 h post-exercise when compared with the rested trial. Furthermore, there was a 27 % increase in concentration of anti-inflammatory cytokine IL-10 24 h post-exercise in comparison with the rested trial. The pro-inflammatory cytokine IL-1β and acute phase protein CRP were unaffected by the 60 min bout of games-based activity, whereas the concentration of TNF-α increased following exercise. In addition, the insulinemic response to a standardized lunch was reduced by 35 % following the games-based exercise when compared with the control trial. This is the first study to examine a range of inflammatory cytokines and the glycaemic and insulinemic responses up to 24 h following games-based activity in adolescents.

The response of IL-6 in the present study is consistent with the one other paper examining games-based activity in young people, where a 91 % increase was observed 1 h post-exercise in pre-pubertal boys (Scheet et al., 1999). The present study is novel as it is the first to report that the IL-6 concentration continues to increase in healthy adolescents 3 h post-exercise,

whereas previous studies suggested IL-6 concentration increased transiently and returned to resting levels 1 h post-exercise (Gleeson et al., 2011). The increase in IL-6 concentration stimulated a 2-fold increase in the anti-inflammatory IL-6: TNF-α ratio 3 h post-exercise and a 27 % increase in IL-10 concentration 24 h post-exercise. The 200 % increase in the IL-6: TNF-α ratio was greater than the 80 % increase following 10 x 2 min bouts of high intensity intermittent cycling in adolescents (McMurray et al., 2007), whilst the present study is the first to report an increase IL-10 concentration 24 h post-exercise in young people. The greater inflammatory response observed in the present study may relate to the longer duration of the exercise session compared to previous studies. Alternatively, this might relate to the mode of exercise undertaken, as basketball has an intense eccentric component, which in adults induces muscle damage that stimulates an inflammatory response of similar magnitude to that observed in the present study (Chatzinikolaou et al., 2014). However, these suggestions are speculative and future research should examine the optimum duration and intensity of games-based activity for eliciting an inflammatory response.

The increase in IL-6 concentration post-exercise has both pro- and anti-inflammatory role in exercise induced inflammation (Petersen & Pedersen, 2005), as indicated by the 31 % increase in pro-inflammatory cytokine TNF-α 2 h post-exercise. The increase in TNF-α concentration in the present study is consistent with the ~10 – 30 % increase reported in young people following moderate-to-vigorous exercise (McMurray et al., 2007; Nemet et al., 2002, 2009). Although a chronic increase in TNF-α is suggested to increase cardiometabolic disease risk in adults (Green et al., 2004), the transient increase in TNF-α following exercise in adolescents in the present study may also elicit cardiometabolic health benefits. Previous research has suggested that following damage to skeletal muscle during moderate-to-vigorous exercise, the transient increase in pro-inflammatory cytokine TNF-α advances muscle regeneration and augments glucose uptake with the increased expression of GLUT-1 (Tidball, 2005), potentially

contributing to the enhanced insulin sensitivity associated with regular participation in exercise.

IL-1β and CRP were the only inflammatory markers in the present study to be unaffected by the acute bout of games-based activity. The lack of an effect of exercise on IL-1β concentration is consistent with one previous study that reported no change following 90 min games-based activity in pre-pubertal boys (Scheet et al., 1999). The present study is the first to the authors' knowledge to assess the response of CRP following an acute bout of exercise in adolescents. However, one previous study in adults reported a small increase in CRP concentration the day following a marathon race (Petersen & Pedersen, 2005). It is therefore possible that longer duration bouts of exercise lead to greater increases in the systemic concentration of inflammatory mediators. Further research is required to determine the relationship between exercise intensity, duration and the subsequent inflammatory response. However, it is important to note that the milieu of cytokine responses observed in the present study is likely to arise from the release of IL-6 as a result of the contraction of skeletal muscle during the games-based activity.

In the present study, the glycaemic response to a standardized lunch was similar between trials, with no difference in blood glucose iAUC observed. Yet, postprandial plasma insulin iAUC was 35 % lower and peak plasma insulin was 61% lower following the games-based activity. An acute bout of high intensity intermittent exercise (lasting ~ 22 min) has previously resulted in a 29 % reduction in postprandial blood glucose iAUC and a 24 % reduction in plasma insulin iAUC in adolescent boys (Cockcroft et al., 2014). It is important to note that the present study is the first to report an exercise-induced reduction in peak plasma insulin concentration following an ecologically valid meal; and the reduction in insulin iAUC as a result of games-

based activity was of greater magnitude than in previous studies using different types of exercise.

The greater enhancement in insulin sensitivity may relate to the training status and higher peak oxygen uptake of participants in the present study (Cockcroft et al., 2014; Short et al., 2013) which may have enabled exercise at higher absolute intensities. Although it has previously been proposed that the capacity for insulin sensitivity to change following exercise is reduced in well-trained participants (Cockcroft et al., 2014), the present study suggests that, if higher fit adolescents sustain overall higher absolute exercise intensities they experience a greater enhancement in insulin sensitivity post-exercise. Future research could also explore how relative exercise intensity (perhaps by asking participants to rate their perceived exertion of the exercise) affects these responses. Nonetheless, the enhanced insulin sensitivity observed in the present study reduces the risk of developing chronic diseases such as type 2 diabetes, highlighting the importance of games-based activity for adolescent health.

Finally, the intermittent games-based activity employed in the present study is considered an enjoyable mode of exercise for adolescents (Howe et al., 2010) and can be undertaken during the school day, thereby facilitating participation for all young people. These issues of appropriateness and accessibility of physical activity are particularly important given that only 23 % of adolescents currently meet the recommended guidelines of 60-min moderate-to-vigorous physical activity per day (Rowland et al., 2008).

The present study shows that an acute bout of games-based activity in adolescents elicits anti-inflammatory effects as evidenced by the increase in systemic concentrations of anti-inflammatory cytokines (IL-6, IL-10) and a higher anti-inflammatory IL-6: TNF-α ratio, alongside a reduced insulinemic response to a standardized lunch; demonstrating a beneficial

effect across these cardiometabolic disease risk factors. These findings have important implications for the health of young people, especially given that the anti-inflammatory effects are evident up to 24-h post-exercise, thus if such exercise was repeated regularly it would elicit beneficial effects on cardiometabolic health in adolescents. These findings are of interest to those responsible for designing and implementing physical activity interventions in schools, with information available as to the mode and duration of exercise that will successfully enhance cardiometabolic health in adolescents. Such interventions are particularly important given that less than one in four young people currently meet physical activity guidelines (Rowland et al., 2008) and adherence may be enhanced with games-based activity (Howe et al., 2010). Future research should aim to further quantify the optimum intensity and duration of exercise for cardiometabolic health in adolescents and identify effective interventions for the implementation of this in practice, whilst considering the inclusion of behaviour change models to ensure long-term adherence to the physical activity intervention(s). Collaborative research that examines the physiological responses to exercise to understand details of the optimum type, intensity and duration of exercise to improve health, alongside the psychological constructs to evoke behaviour change, will enhance the likelihood of increasing physical activity levels in adolescents thus reversing current adverse health trends (Sarzynski et al., 2013).

The findings of Chapter V suggest that an anti-inflammatory response is stimulated and insulin sensitivity enhanced following an acute bout of games-based activity in healthy adolescents. These promising findings suggest that an ecologically valid mode of activity, deemed suitable for young people, if repeated regularly will result in enhanced performance and reduced risk factors for cardiometabolic diseases, as observed in Chapter IV. However, the duration of activity required to elicit such beneficial effects is unknown and forms the basis of Chapter VI.

Chapter VI

Effects of Exercise Duration on Acute Glycaemic and Insulinaemic Responses in Adolescents*

6.1 Introduction

The key findings of the thesis thus far suggest that performance on the MSFT is inversely associated with pro-inflammatory cytokines, insulin resistance and blood pressure in adolescent boys and girls (Chapter IV). Furthermore, performance on the MSFT was the best predictor of fasted concentrations of anti-inflammatory mediator IL-10. These novel findings suggest that regular participation in physical activity/exercise, which is of sufficient intensity and duration to enhance performance on the MSFT, is a potential therapeutic intervention to reduce the presence of risk factors for cardiometabolic diseases in otherwise healthy adolescents. The findings of Chapter V further support this hypothesis as an acute bout intermittent activity successfully elicited an anti-inflammatory response and enhanced insulin sensitivity in healthy adolescent boys and girls. Whilst the findings of Chapter V promote intermittent activity as an effective and ecologically valid mode of exercise to enhance cardio-metabolic health in adolescents, there are necessary details relating to the duration of such exercise and the time course in which these protective responses persist, which are essential for exercise prescription for cardiometabolic health in adolescents.

The effect of exercise duration on the glycaemic and insulinaemic responses to intermittent activity is yet to be determined in children, adolescents or adults and will therefore be examined in this Chapter. Recently, 60 min of high intensity intermittent cycling (Cockcroft et al., 2017) and 60 min of games-based activity (Chapter V), which are deemed ecologically valid modes of exercise in adolescents (Howe et al., 2010), both reduced plasma insulin incremental area under the curve (iAUC) by 24 – 30 % in adolescents, which is a greater

response than the 12 – 15 % reduction following 60 min moderate intensity exercise (Cockcroft et al., 2015; Short et al., 2013, 2018). Whilst such findings suggest that intermittent activity of ~60 min in duration can enhance insulin sensitivity, information regarding whether a shorter duration of intermittent activity also elicits these beneficial effects is unknown. Exercise duration is particularly important given that the majority of adolescents in England (~ 80%) do not meet the recommended Government guidelines of 60 min moderate-to-vigorous physical activity per day (Health Survey for England, 2015). Therefore, it is important to determine whether shorter durations of high intensity intermittent activity of ≤ 30 min elicit similar protective glycaemic and insulinaemic responses to those observed following a 60 min bout of intermittent activity. Current data suggest that even those adolescents who are the least active currently achieve ~30 min physical activity per day (Health Survey for England, 2015). However, the effect of this reduced amount of physical activity on cardiometabolic health remain unknown.

In addition, the time course in which the protective glycaemic and insulinaemic responses to acute bouts of intermittent activity persist are yet to be observed and will therefore be examined in the present Chapter. When promoting physical activity as a therapeutic intervention in adolescents it is also important to understand how long the beneficial effects on insulin sensitivity persist, to allow for exercise frequency recommendations to be made. Yet, there is limited information relating to the effect of intermittent activity on insulin sensitivity beyond 1 h post-exercise (Cockcroft et al., 2015, 2018, Chapter V). Furthermore, when insulin sensitivity has been assessed up to 24 h post-exercise, the homeostatic model assessment (HOMA-IR) has been the main measure of insulin resistance (Cockcroft et al., 2015, 2018, Chapter VI). Following high intensity intermittent cycling (Cockcroft et al., 2015, 2018) and games-based activity (Chapter VI) no change in HOMA-IR was observed when compared with a rested control trial. HOMA-IR is a fasted measure of hepatic insulin sensitivity and is

therefore not sensitive to the changes in peripheral insulin sensitivity that are suggested to occur post-exercise (Cockcroft et al., 2017). The oral glucose tolerance test (OGTT) and postprandial glycaemic and insulinaemic response to a standardised meal are more suitable for assessing peripheral insulin sensitivity. To date, only the OGTT has been utilised in research and there was a 13% improvement in insulin sensitivity 24 h post high intensity intermittent cycling (Cockcroft et al., 2018), when compared to a rested trial. However, the OGTT lacks ecological validity and the response to a mixed meal has not been measured in adolescents beyond 1 h post intermittent activity.

Finally, throughout adolescence differences in insulin sensitivity exist between the sexes, with the post-prandial insulinaemic response to a standardised mixed meal being 30-40% greater in adolescent girls (for peak plasma insulin concentrations) when compared with adolescent boys (Cooper et al., 2017). Whilst adolescent girls consistently present with increased insulin resistance throughout puberty when compared with boys of the same chronological age (Moran et al., 1999), there has been no research to date to ascertain whether an acute bout of exercise can enhance insulin sensitivity in adolescent girls, which could if repeated regularly, lessen the differences in insulin sensitivity currently observed between the sexes.

Therefore, the main aim of the present study was to examine the effects of differing durations (30 min *vs.* 60 min) of high intensity intermittent activity on postprandial glycaemic and insulinaemic responses in adolescents. A secondary aim of the study aims was to establish the postprandial glycaemic and insulinaemic responses to a standardised mixed meal up to 24 h post-exercise, to inform the exercise frequency necessary to enhance adolescent metabolic health. Finally, the present study aimed to determine whether intermittent activity could reduce the magnitude of the difference in insulin sensitivity between adolescent boys and girls.

6.2 Methods

6.2.1 Participant Characteristics

Thirty-three participants (13.6 ± 0.5 y) were recruited to participate in the present study. However, based on exclusion criteria two participants were removed from the study due to an inability to undertake the 60 min of high intensity intermittent activity (n = 1) and the presence of a congenital heart condition (n = 1). Therefore, thirty-one participants (12 males and 18 females) completed the study. During familiarisation, body mass (Seca 770 digital scale, Hamburg, Germany), stature and sitting stature (Leicester Height Measure, Seca, Hamburg, Germany) were measured and subsequently used to calculate age at peak height velocity (Moore et al., 2015). For descriptive purposes, four skinfold sites (including tricep, subscapular, supraspinale and front thigh) and waist circumference were measured. The sum of skinfolds was the preferred measure of body composition for the present study. The participants' anthropometric characteristics were (mean ± SD): age 13.5 ± 0.51 y; height 158.4 ± 7.4 cm; body mass: 45.2 ± 7.4 kg; predicted years from peak height velocity: -1.4 ± 0.6 y; waist circumference: 64.8 ± 5.3 cm and sum of skinfolds: 44.4 ± 15.4 mm.

6.2.2 Study Design

The Nottingham Trent University Ethical advisory committee approved all procedures (approval number SST-503). Following recruitment written informed parental/ guardian consent and participant assent were obtained. Parent/guardians also completed a health screen questionnaire on behalf of the participant to ensure there were no medical conditions affecting participation in the study.

Participants completed a familiarisation session and three main trials (each separated by a minimum of 7 d). During familiarisation, participants had the main experimental protocol explained to them and were allowed the opportunity to ask any questions they may have. In groups of eight, the participants then completed the multi-stage fitness test (MSFT) and peak

oxygen consumption (44.5 ± 4.6 $mL.kg^{-1}.min^{-1}$) was predicted using an adolescent specific equation (Barnett et al., 1992). Participants then returned to the classroom and were familiarised with a capillary blood sample. Following 60 min passive recovery, to ensure the participants were able to comply with the high intensity intermittent activity, a 15 min block of the Loughborough Intermittent Shuttle Test (LIST) was performed.

6.2.3 Main Trials

Participants completed three main trials (a 30 min exercise trial, a 60 min exercise trial and a rested control trial) in a randomised, counterbalanced, crossover order (each separated by at least 7 d). Figure 7 displays the experimental protocol. Participants recorded food diaries 24 h prior to the first main trial and during the evening of day one of the study. Recorded diets were repeated for the subsequent experimental trials. Participants refrained from physical activity 24 h prior to and during all experimental trials. Parents/ guardians were contacted the evening before each main trial to ensure compliance with these requirements.

Following an overnight fast (from 9 pm the previous evening on day one and two of the study) participants arrived at school (~ 8.30 am) and were fitted with a heart rate monitor (Team Sports System, Firstbeat Technologies Ltd, Jyvaskyla, Finland), which was worn throughout day one of the experimental trial. Participants consumed a standardised breakfast (cornflakes, milk, and toast with margarine) on day one and two, and a standardised lunch (chicken sandwich, baked salted crisps and an apple) on day one only. Each meal contained 1.5 g carbohydrate per kg body mass. Participants had 15 min to consume the standardised meals and water was allowed *ad libitum*.

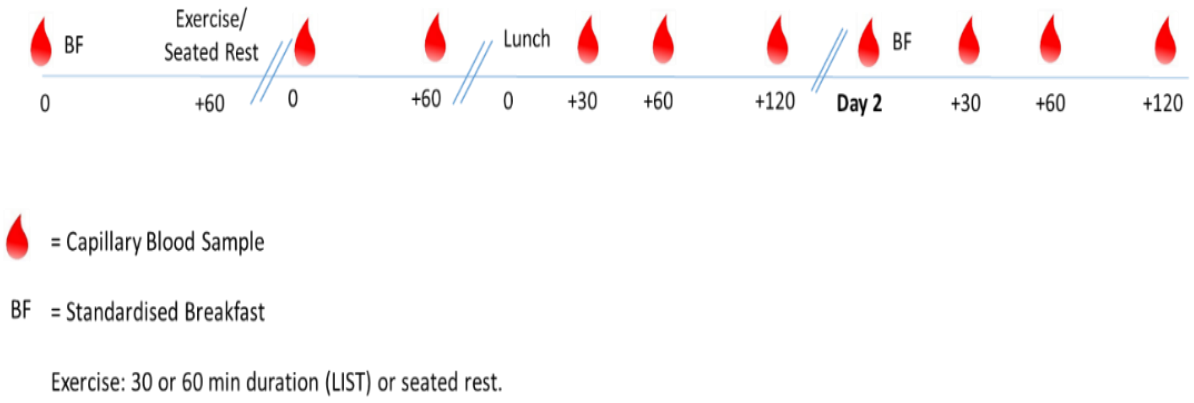

Figure 7. Protocol schematic, detailing consumption of standardised meals and timing of capillary blood samples.

6.2.3.1 Capillary Blood Samples

During day one of the main trials, capillary blood samples were taken at baseline, immediately post-exercise and 60 min post-exercise (Fig. 7). Further capillary blood samples were taken 30 min, 60 min (2 h post-exercise) and 120 min (3 h post-exercise) following the standardised lunch. On day two of the main trials, a fasted capillary blood sample was taken. Following the consumption of the standardised breakfast further blood samples were taken at 30 min, 60 min and 120 min to observe the postprandial glycaemic and insulinaemic responses.

The concentrations of blood glucose and plasma insulin were determined in duplicate using commercially available kits (glucose: GOD/PAP method, GL364, Randox, Crumlin, Ireland; insulin: ELISA, Mercodia Ltd, Uppsala, Sweden). Blood glucose and plasma insulin tAUC following the standardised lunch on day one and the standardised breakfast on day two were calculated (Wolever & Jenkins, 1986). HOMA-IR was calculated as *fasted insulin* ($mU.L^{-1}$) *x fasting glucose* ($nmol.L^{-1}$) */ 22.5.*

6.2.3.2 Exercise Protocol

During the exercise trials, participants completed either 30 min or 60 min of high intensity intermittent activity, in the form of the Loughborough Intermittent Shuttle Test (LIST). During the LIST, participants ran between two markers, separated by 20 m, to pre-determined speeds

dictated by an audio signal. The exercise pattern consisted of three 20 m shuttles at walking pace, a 15-m sprint followed by rest (8 s total duration), three 20 m shuttles at 85% $\dot{V}O_2$ peak and three 20 m shuttles at 55% of $\dot{V}O_2$ peak (percentage of $\dot{V}O_2$ peak determined from performance on the MSFT). Sprint times were recorded using infrared timing gates (Brower Timing Systems IRD-T173, Utah, USA) and average sprint times for each block were calculated. The above pattern was repeated eight times, lasting ~12 min (as presented in Figure 2, Chapter III). The 30 min trial consisted of 2 blocks and the 60 min trial 4 blocks, with 3 min recovery provided between blocks.

6.2.4 Statistical Analysis

All data were analysed using SPSS (Version 24, SPSS Inc, Chicago, IL, USA). Data were assessed for normality using the Shapiro-Wilk test, which revealed that all dependent variables were normally distributed (all $P > 0.05$). Blood glucose, and plasma insulin concentration data were analysed via three-way (trial * time * sex) analysis of variance (ANOVA) with repeated measures for trial and time. Separate ANOVAs were conducted for day one and day two separately. Where significant interactions were observed, post-hoc pairwise comparisons were performed using a Bonferroni correction. Blood glucose tAUC, plasma insulin tAUC, sprint times and heart rate were compared using two-way mixed method ANOVA, with sex as the between subjects factor. Where statistically significant differences existed effect sizes were calculated (Cohen's d). For all analysis, significance was accepted as $P < 0.05$ and data are presented as mean ± S.E.M.

6.3 Results

6.3.1 Performance Variables

Average heart rate during the 30 min LIST trial was 104 ± 12 beats·min^{-1} and maximum recorded heart rate during the LIST exercise was 196 ± 9 beats·min^{-1}; during the 60 min LIST trial average heart rate was 118 ± 8 beats·min^{-1} and maximum recorded heart rate was 199 ± 8

beats·min^{-1} and average heart rate during the rested control trial was 88 ± 10 beats·min^{-1}. Maximum heart rate was higher on the 60 min exercise trials when compared to the 30 min exercise trial ($t_{(29)}$ = -4.2, p = < 0.001). Average sprint times during the 30 min LIST trial (block 1: 3.08 ± 0.29 s; block 2: 3.11 ± 0.29 s) and 60 min LIST trial (block 1: 3.04 ± 0.19 s; block 2: 3.11 ± 0.22 s; block 3: 3.13 ± 0.27 s; block 4: 3.15 ± 0.27 s) were similar across each block of exercise completed (main effect of trial, $F_{(5, 135)}$ = 2.50, p = 0.966).

6.3.2 Glycaemic Response

6.3.2.1: Day One

Overall, blood glucose concentration on day one of the study did not differ between the 30 min LIST trial, 60 min LIST trial and the rested control trial (main effect of trial, p = 0.401), yet did change across time (main effect of time, $F_{(5,120)}$ = 43.8, p < 0.001). The pattern of change in blood glucose concentration differed across trials (trial * time interaction, $F_{(6,168)}$ = 4.2, p = 0.001; Fig. 15); whereby postprandial blood glucose concentration was lower 1 h post-exercise during the 30 min LIST trial (30 min LIST: 3.8 ± 0.6 mmol·L^{-1}, $F_{(2,27)}$ = 4.8, p = 0.022, d = 0.533) and the 60 min LIST trial (60 min LIST: 3.8 ± 0.6 mmol·L^{-1}, $F_{(2,27)}$ = 4.8, p = 0.017, d = 0.536) compared to the rested control trial (rested: 4.2 ± 0.9 mmol·L^{-1}). When considering the effect of sex, the glycaemic response did not differ between males and females (main effect of sex, p = 0.200), nor did the pattern of change in the glycaemic response differ between males and females (trial * sex interaction, p = 0.82; time * sex interaction, p = 0.77, trial * time * sex interaction, p = 0.572).

The tAUC for postprandial blood glucose concentration following the standardised lunch did not differ between trials (main effect of trial, p = 0.216), between the sexes (main effect of sex, p = 0.187), nor was there an interaction between trial and sex (trial * sex interaction, p = 0.705).

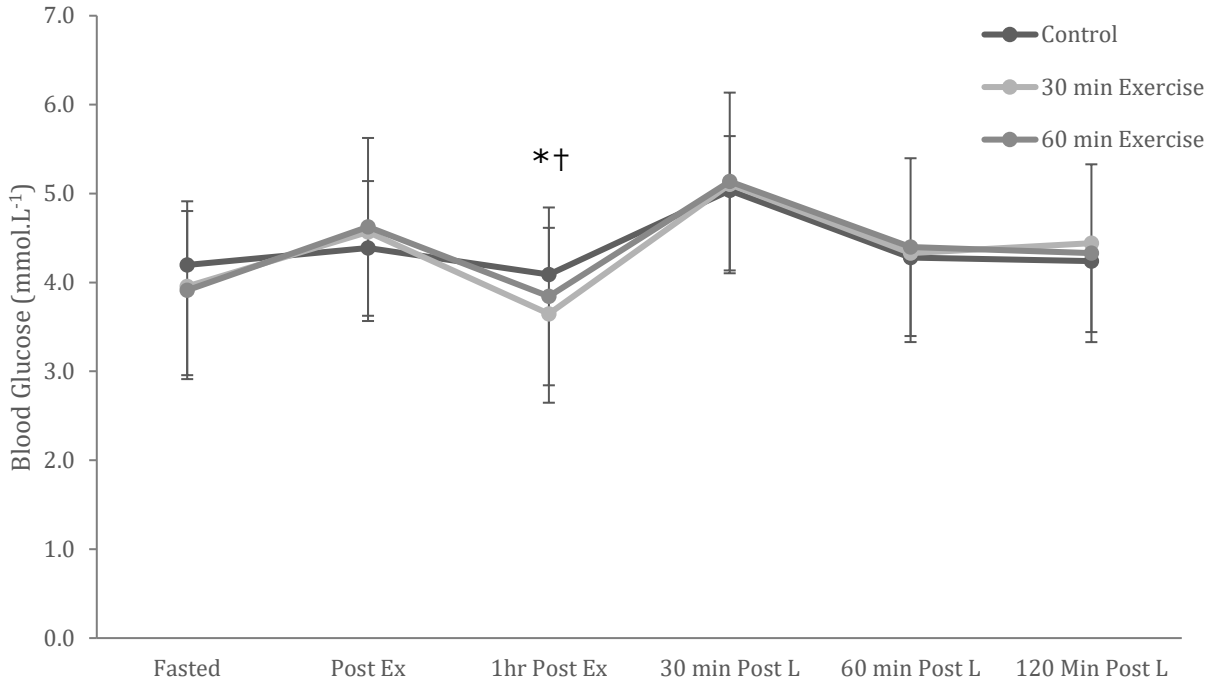

Figure 8. Glycaemic response during the 30 min LIST trial, 60 min LIST trial and rested control trial on day one of the study (Mean ± SD), trial * time interaction, $F_{(6,168)} = 4.2$, $p = 0.001$; * 30 min LIST trial < rested control trial, $p = 0.022$, † 60 min LIST trial < rested control trial, $p = 0.017$).

6.3.2.2: Day Two

Overall, blood glucose concentration following the consumption of the standardised breakfast (day two) did not differ between the 30 min LIST trial, the 60 min LIST trial and the rested control trial (main effect of trial, $p = 0.453$), yet did differ across time (main effect of time, $F_{(3,81)} = 65.7$, $p < 0.001$). Furthermore, the pattern of change in postprandial blood glucose concentration did not differ between trials (trial*time interaction, $p = 0.741$). When considering the effect of sex, the glycaemic response did not differ between males and females (main effect of sex, $p = 0.583$), nor did the pattern of change in the glycaemic response differ between males and females (trial * sex interaction, $p = 0.44$; time*sex interaction, $p = 0.69$, trial * time * sex interaction, $p = 0.858$). Finally, the tAUC for blood glucose concentration following the

standardised breakfast did not differ between trials (main effect of trial, p = 0.751), or sexes (main effect of sex, p = 0.181), nor was there an interaction between trial and sex (trail * sex interaction, p = 0.526).

6.3.3 Insulinaemic Response

6.3.3.1: Day One

Overall, plasma insulin concentration on day one of the study did not differ between the 30 min LIST trial, 60 min LIST trial and the rested control trial (main effect of trial, p = 0.287), yet did change across time (main effect of time, $F_{(5,125)} = 56.1$, $p < 0.001$). The pattern of change in plasma insulin concentration differed across trials (trial*time interaction, $F_{(10,270)} = 3.6$, p = 0.004, Fig. 16), with increased plasma insulin concentration immediately post-exercise during the 60 min LIST trial when compared with the rested control trial (60 min LIST: 177.0 ± 97.7 $pmol \cdot L^{-1}$, rested trial: 131.4 ± 75.9 $pmol \cdot L^{-1}$, $F_{(2,26)} = 4.7$, p = 0.011, d = 0.52). Furthermore, postprandial plasma insulin concentration was lower 1 h following the standardised lunch during the 60 min LIST trial when compared with the rested control trial (60 min LIST: 199.1 ± 125.9 $pmol \cdot L^{-1}$, rested trial: 259.4 ± 193.7 $pmol \cdot L^{-1}$, $F_{(2,26)} = 5.0$, p = 0.015, d = 0.38). When considering the effect of sex, overall plasma insulin concentration was lower in males when compared with females (males: 137.3 ± 15.3 $pmol \cdot L^{-1}$, females: 184.3 ± 15.3 $pmol \cdot L^{-1}$, main effect of sex, $F_{(1,27)} = 5.5$, p = 0.033). However, the pattern of change in plasma insulin concentration did not differ between males and females (trial * sex interaction, p = 0.376, time * sex interaction, p = 0.09, trial * time * sex interaction, p = 0.812).

The tAUC for the insulinaemic response to the standardised lunch was lower on the 60 min LIST trial when compared to the rested control trial (60 min LIST: 23867 ± 10689 pmol·L^{-1} x 120min, resting: 28899 ± 14734 pmol·L^{-1} x 120min, main effect of trial, $F_{(1,28)} = 4.3$, $p = 0.030$, $d = 0.40$, Fig 17). When considering the effect of sex, tAUC was lower in males compared to females (males: 20445 ± 9556 pmol·L^{-1} x 120min, females: 29876 ± 15748 pmol·L^{-1} x 120min, main effect of sex, $F_{(1,28)} = 7.4$, $p = 0.011$, $d = 0.751$), yet the pattern of change did not differ between the sexes across trials (trial * sex interaction, $p = 0.677$).

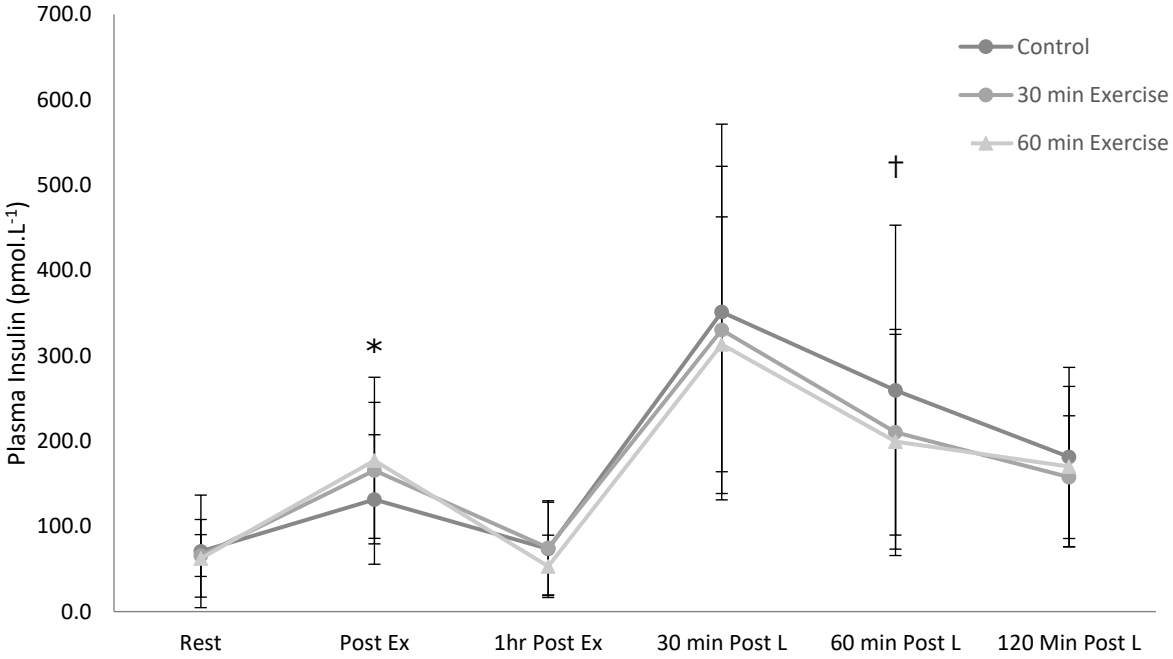

Figure 9. Insulinaemic response during the 30 min LIST trial, 60 min LIST trial and the rested control trial on day one of the study (Mean ± S.E.M), trial*time interaction, $F_{(10,270)} = 3.6$, $p = 0.004$, * 60 min LIST trial > rested control trial, $p = 0.011$, † 60 min LIST trial < rested control trial, $p = 0.015$).

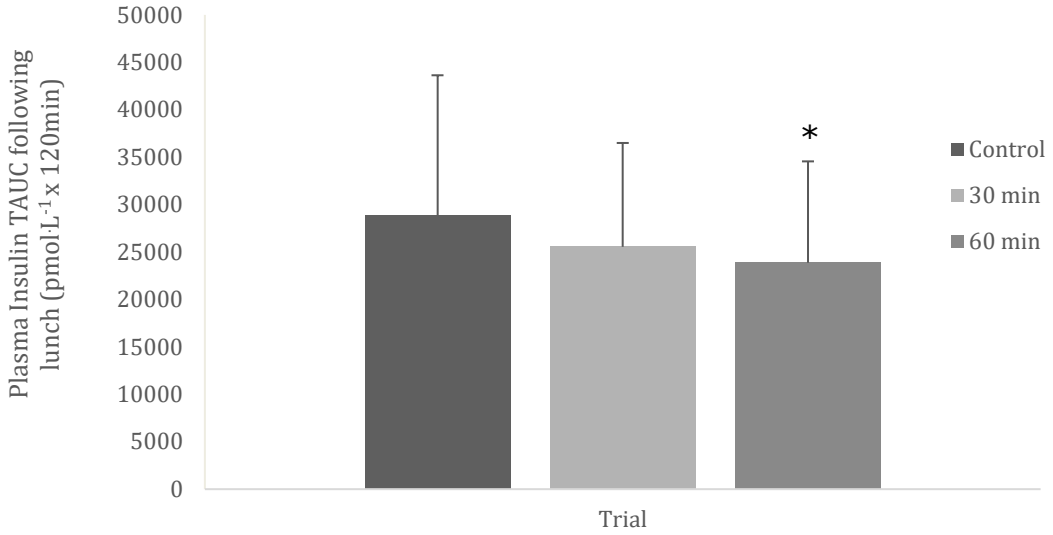

Figure 10. Plasma insulin total area under the curve following the consumption of a standardised lunch on the 30 min LIST trial, 60 min LIST trial and the rested control trial (Mean ± S.E.M), main effect of trial, F= 4.3, p = 0.030, * 60 min LIST trial < rested control trial, p = 0.011).

6.3.3.2: Day Two

Overall, the plasma insulin concentration following the consumption of the standardised breakfast (day two) did not differ between trials (main effect of trial, p = 0.356), yet did change across time (main effect of time, $F_{(3,84)}$ = 104.3, p < 0.001). The pattern of change in the insulinaemic response post-breakfast did not differ across time between the trials (trial*time interaction, p = 0.138). When considering the effect of sex, the postprandial insulinaemic response was lower in males when compared with females (males: 200.7 ± 24.4 pmol·L^{-1}, females: 279.5 ± 19.9 pmol·L^{-1}; main effect of sex, $F_{(1,28)}$ = 6.3, p = 0.029). Furthermore, peak plasma insulin concentrations was lower in males than females (time * sex interaction, $F_{(1,28)}$ = 7.5, p = 0.015), whereby 30 min post-breakfast plasma insulin concentration in females was greater than in males. Finally, the tAUC for the insulinaemic response to a standardised breakfast did not differ across trials (main effect of trial, p = 0.299), but was lower in males compared to females (males: 26684 ± 3291 pmol·L^{-1} x 120min, females: 37050 ± 2688 pmol·L^{-}

[1] x 120min, main effect of sex, $F_{(1,28)} = 6.0$, $p = 0.022$, $d = 0.82$). However, the pattern of change in the tAUC insulinaemic response did not differ across trials between males and females (trial * sex interaction, $p = 0.100$).

6.3.4 HOMA-IR

HOMA-IR was calculated for the fasted blood samples on day one and day two, with no overall difference between trials (main effect trial, $p = 0.231$), or between day one and day two (main effect time, $p = 0.098$). Furthermore, the pattern of change in HOMA-IR between day one and day two was similar between trials (trial*time interaction, $p = 0.842$). When considering the effect of sex there was no difference in HOMA-IR between males and females (main effect of sex, $p = 0.28$), nor did the pattern of change in HOMA-IR differ between the sexes (trial * sex interaction, $p = 0.362$; time * sex interaction, $p = 0.791$, trial * time * sex interaction, $p = 0.874$).

6.4 Discussion

The present study is the first to examine the glycaemic and insulinaemic responses to high intensity intermittent exercise of different durations, for up to 24 h post-exercise, whilst considering the potential moderating effect of sex on these responses during adolescence. The main finding of the study was that 30 min and 60 min of high intensity intermittent exercise (performed as the LIST) reduced postprandial blood glucose concentration by 10 % 1 h post-exercise when compared to a rested control trial. Furthermore, the 60 min bout of high intensity intermittent exercise successfully reduced postprandial plasma insulin concentration, with a 23 % reduction 1 h following the consumption of a standardised lunch. The reduction in plasma insulin concentration was supported by a 17 % decrease in plasma insulin tAUC following the 60 min exercise trial in comparison to the rested control trial. In contrast, fasted and postprandial blood glucose and plasma insulin concentration was not different between trials on day two of the study. Furthermore, when considering the effect of sex on insulin sensitivity

on day one and two of the study, females had consistently higher plasma insulin concentrations in comparison to their male counterparts.

The 10% reduction in blood glucose concentration observed 1 h following 30 min and 60 min of high intensity intermittent exercise is consistent with previous findings, whereby blood glucose concentration was reduced by 11% following 60 min of games-based activity (Chapter VI) and by ~ 15% following 45 min of moderate intensity activity (Short et al., 2013). The findings of the present study are novel as the reduction in blood glucose concentration was consistent across exercise trials of differing durations with a 30 min bout of high intensity intermittent activity proving to be as effective as 60 min for enhancing the regulation of blood glucose concentration in healthy adolescents. Such findings are promising, as the majority of young people in the UK do not currently meet the recommended physical activity guidelines (60 min moderate-to-vigorous physical activity per day) and those that do, do so through shorter accumulated bouts (Rowlands et al., 2008). Therefore, the findings of the present study have important practical implications and suggest that blood glucose homeostasis can be improved through shorter bouts of intermittent activity that are replicative of the activity patterns of young people (Rowlands et al., 2008).

Future work should continue to assess the effect of exercise duration on blood glucose concentration to determine whether accumulative, shorter bouts (< 30 min) of exercise enhance blood glucose homeostasis. Such information is particularly important given the increasing prevalence of type 2 diabetes in adolescents (May et al., 2012), in which hyperglycaemia is a major risk factor. Information pertaining to the regulation of blood glucose concentrations through ecologically valid exercise modes can subsequently be used to inform future therapeutic interventions that are achievable for young people to implement into their daily

lives and to reverse the adverse cardiometabolic health trends currently observed in young people (May et al., 2012).

There were no differences in the postprandial glycaemic response to a standardised meal (tAUC) between trials in the present study. These findings are consistent with those of Chapter VI, whereby 60 min of games-based activity did not reduce the glycaemic response to a standardised meal. Whilst these corroborating findings suggest that intermittent activity in adolescents does not affect postprandial blood glucose concentration, high intensity intermittent cycling (8 x 1 min cycling at 90% peak power) has previously reduced the glycaemic response (8% reduction in tAUC) following an OGTT in adolescent boys (Cockcroft et al., 2015).

The discrepant findings between the present study and the study of Cockcroft et al. (2015) might relate to the different test meals used, in that the high concentration of glucose in the OGTT facilitated a reduced glycaemic response post-exercise (in Cockcroft et al., 2015), whereas the standardised mixed meal did not (in the present study). The discrepancies might also be explained by the different characteristics of the participants, as the $\dot{V}O_2$ peak of the participants in the present study is on the 75th percentile for adolescents (Eisenmann et al., 2011), whereas participants from previous studies are $\leq$ 50th percentile for their chronological age (Cockcroft et al., 2015). Participants with a greater predicted $\dot{V}O_2$ peak might require a more intense exercise stimulus to reduce the postprandial glycaemic response following exercise, whereas individuals with a $\dot{V}O_2$ peak $\leq$ 50th percentile might respond to a lower exercise stimulus (Eisenmann et al., 2011). The moderating effect of $\dot{V}O_2$ peak on the glycaemic response post-exercise is yet to be examined in adolescents, despite such information being essential for the individualisation of physical activity recommendations.

In the present study plasma insulin concentration was reduced by 30% 2 h following 60 min of high intensity intermittent exercise when compared to a rested control trial, which was supported by a 17% reduction in postprandial plasma insulin tAUC on the 60 min exercise trial compared to the rested control trial. The reduction in postprandial plasma insulin in the present study agrees with previous research whereby plasma insulin tAUC decreased by 13% following 8 min of high intensity intermittent cycling (Cockcroft et al., 2015) and 24% following 60 min games-based activity (Chapter V). The reduction in postprandial plasma insulin concentration following high intensity intermittent activity is indicative of enhanced insulin sensitivity in healthy adolescents, with less insulin required to regulate blood glucose homeostasis. Therefore, the findings of the present study suggest that 60 min of high intensity intermittent running, which replicates the activity patterns of young people, is sufficient to enhance insulin sensitivity on the day the exercise was undertaken. In contrast, the 30 min bout of high intensity intermittent running examined in the present study was not sufficient to enhance insulin sensitivity in healthy adolescents. Whilst the findings of the present study suggest that shorter bouts of intermittent activity are not suitable for improving insulin sensitivity, Cockcroft et al., (2015) previously reported that as little as 8 min of high intensity cycling enhances insulin sensitivity (13% reduction in postprandial plasma insulin tAUC). Furthermore, the findings of the present study and trends across previous research suggest that exercise duration mediates the magnitude of the insulinaemic response, with graded improvements in postprandial plasma insulin tAUC with increased exercise duration (Cockcroft et al., 2015; Chapter V). As such, there appears to be a complex relationship between exercise intensity and duration that must be explored to inform the development of successful therapeutic interventions aimed at enhancing insulin sensitivity in adolescents.

The secondary aim of the present study was to examine the residual glycaemic and insulinaemic response to a standardised breakfast the day following exercise in comparison to

a rested control trial. Interestingly, there was no difference in the postprandial glycaemic and insulinaemic responses to the standardised breakfast across trials, which was further supported with HOMA-IR remaining consistent across the exercise trials and rested control trial on day two of the study. The effect of exercise on residual insulin sensitivity (examined primarily using the response to a test meal) has been discrepant across previous research. Intermittent activity has previously had no effect on postprandial blood glucose and plasma insulin tAUC in adolescent boys the day following exercise across several studies (Barrett et al., 2007; Sedgwick et al., 2014); whereas Short et al. (2013) reported a 45% improvement in insulin sensitivity 17 h following moderate intensity exercise. The findings of the present study suggest that exercise only enhances blood glucose homeostasis and insulin sensitivity on the day that the exercise was undertaken. In relation to exercise frequency for the enhancement of cardiometabolic health, such findings would recommend daily physical activity in young people, which is consistent with the government physical activity guidelines. However, it should be acknowledged that inconsistencies across previous research could result from differences in the participant characteristics, the exercise examined (mode, intensity, and duration), and the composition of the macronutrients in the standardised meal provided (with previous studies providing participants with high fat meals). As such, to determine the optimum frequency of exercise, whereby cardiometabolic health is enhanced and the frequency is attainable in a population that are not currently meeting the daily recommendations, future research should examine different exercise models and ascertain whether the reduced glycaemic and insulinaemic responses can remain up to and beyond 24 h post-exercise.

Finally, in the present study plasma insulin concentrations were consistently higher in girls than boys throughout the study, yet the response to exercise did not differ between the sexes. The finding that female adolescents exhibit greater insulin resistance during adolescence is consistent with previous research whereby the insulinaemic response to a mixed meal was 30-

40% higher in girls than boys (Cooper et al., 2017). The present study aimed to examine whether high intensity intermittent running could reduce the insulinaemic differences observed between the sexes and attenuate the insulin resistance observed in adolescent girls. However, on the day the exercise was undertaken and the following day, the insulin response was consistently elevated in girls compared with boys. Future studies should assess whether different modes and intensities of exercise are able to attenuate the difference in insulin sensitivity observed between the sexes during adolescence.

It was also the intended purpose of this study to examine the cytokine responses to different durations of exercise, but the addition of IL-15 to the cytokine plate resulted in none of the assays working. IL-15 is a cytokine that is implicated in reducing adipose tissue (Nielsen & Pedersen, 2007) and has recently been reported to increase following an acute bout of exercise, but only in animal studies (Shamsi et al., 2015). Therefore, the inclusion of IL-15 would have been an additional novel finding that would have advanced understanding of the potential mechanisms that facilitate the relationship between regular participation in physical activity and cardiometabolic health in young people. Unfortunately, despite a second run of the assays on remaining plasma by the manufacturer supplying the plates, the assay was again unsuccessful (we now understand this is due to cross-reactivity of IL-10 and IL-15 within the same assay). Hence this chapter examines only the glycaemic and insulinaemic responses to different durations of exercise.

In conclusion, the findings of the present study suggest that 60 min high intensity intermittent running is an ecologically valid mode of exercise that enhances the regulation of blood glucose and insulin sensitivity in healthy adolescent boys and girls. Furthermore, the main finding of the study suggests that a shorter bout of high intensity intermittent exercise (30 min) is as effective at improving blood glucose homeostasis as 60 min of exercise in adolescents. Yet,

the effect of the exercise on blood glucose and plasma insulin was only maintained on the day the exercise was performed, with no effect on the glycaemic and insulinaemic response to a standardised breakfast the following day. The findings of the present study support the government physical activity guidelines that suggest young people should participate in 60 min of moderate-to-vigorous physical activity per day.

Chapter VII

Protective Effects of Chronic Training on Cardiometabolic Health and Performance in Adolescents: A Longitudinal Study

7.1 Introduction

Physiological risk factors associated with cardiometabolic diseases present as early as childhood (Magnussen et al., 2012); progressing until a clinical threshold develops (typically in adulthood) and cardiometabolic diseases present. Whilst the management of such risk factors in adulthood is commonplace, in the last decade there has been additional emphasis on attenuating risk factors for cardiometabolic diseases in young people, as some cardiometabolic diseases now present in early adulthood and even adolescence (Ayer et al., 2016; Candler et al., 2018). Of major concern is the 36 % increase in the incidence of type 2 diabetes in adolescents (aged < 17 years) in the United Kingdom, as reported in a recent quantitative analysis from 2005 to 2015 (Candler et al., 2018). In adults, acute phase protein CRP is deemed the best predictor of cardiovascular disease and type 2 diabetes morbidity (Emerging Risk Factors Collaboration, 2012), however in young people little is known about the role of specific risk factors in the development of such conditions and how best to manage the prevalence of these specific risk factors in adolescents. Given the limited information available there is concern relating to the future burden that could result from the early onset of cardiometabolic diseases.

In Chapter IV an inverse association was found between endurance capacity (assessed by distance run on the MSFT) and body composition with traditional (blood pressure and insulin resistance) and novel (pro- and anti-inflammatory cytokine concentrations) risk factors of cardiometabolic diseases in children and adolescents aged 10 - 12 years from various sporting

backgrounds. However, cross-sectional studies cannot infer causality and as such the effect of long-term training on risk factors for cardiometabolic diseases throughout adolescence is unknown. To address such limitations, longitudinal studies have been conducted, although only one study to date has prospectively assessed the effect of continued training versus remaining inactive during puberty on risk factors for cardiometabolic diseases in adolescents (Vosoberg et al., 2014). Vosoberg et al., (2014) examined the effect of training (gymnastics) versus remaining untrained across a three-year follow-up in adolescent girls on appetite regulation hormones leptin, ghrelin and adiponectin. When compared with the untrained group, the rhythmic gymnasts had a lower BMI, lower percentage body fat and higher concentrations of adiponectin (implicated in the regulation of blood glucose concentration). Such findings suggest that long-term training during adolescence, enhances aspects of cardiometabolic health, however it remains unknown whether chronic training in adolescent boys and girls is related to traditional (blood glucose, plasma insulin, HOMA-IR and blood pressure) and novel (pro- and anti-inflammatory cytokines) risk factors for cardiometabolic health.

In Chapter IV, performance on the MSFT was adversely associated with pro-inflammatory cytokines (IL-1β and IL-6) and positively associated with anti-inflammatory cytokine IL-10. Whereas, $\dot{V}O_2$ peak (which has a strong genetic component, limiting the capacity to track changes in performance) was not associated with any of the novel markers of low-grade chronic inflammation. Given these findings, distance run on the MSFT is hypothesised to be a more sensitive marker of training status and childhood cardiometabolic health. However, the effect of longer-term training, of several months or years, on MSFT performance (and for comparison $\dot{V}O_2$ peak) has not been examined particularly during the adolescent stage of development.

Therefore, the main aim of the present study was to examine whether the continuation of training elicits protective effects on traditional (blood pressure, blood glucose concentration,

plasma insulin concentration and HOMA-IR) and novel (pro- and anti-inflammatory cytokines) risk factors for cardiometabolic diseases in adolescents during a two-year follow-up. A secondary aim of the study was to determine the performance of young people who were undertaking chronic training in comparison with those remaining recreationally active on endurance capacity tests (distance run on the MSFT and $\dot{V}O_2$ peak) which have previously been reported to be associated to select risk factors of cardiometabolic diseases in adolescents (Chapter IV).

7.2 Methods

7.2.1 Participant Characteristics

From the original cross-sectional sample (Chapter IV), 61 adolescents (from 121 at baseline) aged 12-14 years agreed to complete the follow-up study two years later (baseline analysis completed during 2015/2016, follow-up during 2017/18). Contact was initially made with school teachers and coaches from the relevant schools and sports clubs to contact the participants to enquire whether they would be interested in completing the follow-up study. Where participants had left the school or sports club contact details of parents/guardians were used to determine whether the participant was interested in being recruited to the follow-up. Participants were lost to follow-up for various reasons, including being no longer contactable through the original details provided to the research team (n = 45), choosing to withdraw from the study as they no longer participated in regular exercise and did not feel confident continuing (n = 8), and having suffered a long-term injury (n = 7). As a result of the loss to follow-up there were no instances of participants who were originally trained that had become untrained during the follow-up; similarly verbal confirmation from parents/guardians of the untrained group confirmed that there were no instances whereby untrained adolescents had become trained during the follow-up period. As in Chapter IV, at follow-up all participants underwent anthropometric measures of body mass and stature to calculate body mass index (BMI;

calculated as body mass (kg)/stature $(m)^2$) and also sitting stature to predict age from peak height velocity (APHV; calculated using methods described by Moore et al., 2015). Specifically, body mass was measured using a Seca 770 digital scale, accurate to 0.1 kg (Seca, Hamburg, Germany) and stature was measured using a Leicester Height Stadiometer, accurate to 0.1 cm (Seca, Hamburg, Germany).

Participants were categorised into two groups based on whether they were recruited from a local sports clubs (trained group, consisted of footballers training with local academies training twice weekly and participating in matches at the weekend and swimmers training with the county swimming club participating in 7 training sessions per week) or from local secondary schools (untrained control group, whom verbally confirmed their physical activity consisted only of physical education lessons at school, twice weekly).

7.2.2 Study Design

Ethical approval was received from the Nottingham Trent University Ethical Advisory Committee (Reference: SPOR-400). Details of baseline recruitment are provided in Chapter IV. At follow-up participants were contacted initially through their original school, or sports club, unless the child had since left the school/ club, then contact was made through a telephone call to the participants' parent/ guardian. Written parental consent and verbal assent from the participant was obtained prior to familiarisation. Health screen questionnaires were completed by the participants' parent/ guardian and checked by a lead investigator to ensure there were no medical conditions that might affect participation in the study.

As in Chapter IV, each of the trials were separated by a minimum of 7 d (trial 1: field measures, trial 2: health measures and trial 3: exercise laboratory measures). The field measures consisted of anthropometrics, skinfolds and the MSFT, in that order, and the health measurements

consisted of rested and fasted blood pressure measures and capillary blood samples. Participants that completed the exercise laboratory tests at baseline were also invited to complete the treadmill tests (a submaximal test and a $\dot{V}O_2$ peak test, separated by 20 min passive recovery) at follow-up. Prior to each of the trials, participants were asked to refrain from moderate-to-vigorous physical activity for 24 h. A telephone call was made to parents/guardians the evening prior to the testing sessions to ensure compliance with the study requirements. Detailed descriptions of each of the measurements is provided in Chapters III and IV, and as a result only a brief description of each measurement is provided below.

7.2.3 Field Measures

7.2.3.1 Body Composition

The sum of four skinfolds and waist circumference were used to measure body composition. Skinfold thickness was measured using Harpenden Calipers (Baty International, Burgess, Hill, United Kingdom) at four sites (tricep, subscapular, supraspinale, front thigh). Waist circumference was measured with a tape measure at the narrowest point of the torso between the xiphoid process and the iliac crest, to the nearest 0·1 cm.

7.2.3.2 Multi-Stage Fitness Test (MSFT)

All participants completed the MSFT, which in brief consisted of progressive 20 m shuttle runs to the point of volitional exhaustion (Ramsbottom et al., 1988). Prior to the test participants were fitted with a heart rate monitor (First Beat technologies Ltd., Finland) and heart rate was monitored live throughout the MSFT. Participants were verbally encouraged throughout to ensure the test was completed to the point of volitional exhaustion. Total distance run was used as the criterion measure.

7.2.4 Health Measures

7.2.4.1 Blood Pressure

Following an overnight fast (~ 9 pm the previous evening) participants arrived to the exercise laboratory and were seated quietly for 5 min. Two blood pressure measurements were taken from the left arm, which was rested at chest height, using an HBP-1300-United Kingdom sphygmomanometer (Omron, Milton Keynes, United Kingdom) and a third measurement if necessary as described in the general methods. Mean arterial blood pressure was determined using the following calculation (Smeltzer et al., 2010): diastolic blood pressure + {[0.33∗ (systolic blood pressure – diastolic blood pressure)]}.

7.2.4.2 Capillary Blood Samples

Fasted capillary blood samples were taken following the measurement of blood pressure. Participants' hands were warmed via submersion in warm water to increase capillary blood flow. A Unistik single-use lancet (Unistik Extra, 21G gauge, 2.0 mm depth, Owen Mumford, Ltd., United Kingdom) was used and blood was collected into three 300 µl EDTA microvettes (Sarstedt Ltd., United Kingdom). A 25 µl whole blood sample was collected using a plain pre-calibrated glass pipette (Hawksley Ltd., United Kingdom) and dispensed into 250 µl of cooled 2.5% v/v perchloric acid for deproteinisation. Blood samples were then centrifuged at 15000 x g for 5 min (Eppendorf 5415C, Hamburg, Germany). Plasma was pipetted from the whole blood samples and distributed into three 500 µl plastic vials. All samples were frozen immediately at - 20°C and transferred to a −80°C freezer as soon as was possible.

Blood glucose, plasma insulin, cytokine and CRP concentrations were determined using the methods described in section 3.4.2.

7.2.5 Exercise Laboratory Measures

A total of 27 participants completed the exercise laboratory tests at baseline and follow-up (Males: n = 12, Females: n = 15). Two participants that had completed this aspect of the study at baseline opted not to continue due to apprehension about completing the $\dot{V}O_2$ peak test. Throughout the duration of the test participants wore heart rate monitors (First Beat Technologies Ltd., Finland) and heart rate was monitored live during each test. The $\dot{V}O_2$ peak test and submaximal test were completed on a calibrated treadmill (Technogym, Italy) as described in the general methods. The submaximal test was performed at the same initial speed as conducted at baseline, however progressed up to a maximum of 13 $km \cdot h^{-1}$. Heart rate was measured throughout the submaximal treadmill test and the speed which corresponded with 80% HR_{max} determined the speed that at which the subsequent $\dot{V}O_2$ peak test would be completed.

7.2.6 Statistical Analysis

All data was assessed for normality using the Kolmogorov-Smirnov test prior to statistical analysis. Statistical analysis was conducted in SPSS (Version 24, SPSS Inc., Chicago, Il, United States). Each outcome variable (inflammatory cytokines, blood glucose, plasma insulin, HOMA-IR and blood pressure) was analysed via a mixed methods ANOVA (group * time * sex). Where significant interactions existed, post hoc comparisons were performed using a Bonferroni correction. Where significant effects existed, effect sizes were calculated as Cohen's d. Finally, to determine the relationship between change in distance run on the MSFT, $\dot{V}O_2$ peak and adiposity on the change in risk factors for cardiometabolic diseases, Pearson correlations were calculated. For all analysis significance was accepted as $P < 0.05$ and data are presented as mean ± S.E.M.

7.3 Results

7.3.1 Anthropometry and Pubertal Development

Anthropometric and pubertal development data are shown in Table 13. During the two year follow-up, both groups (trained and untrained) increased in stature (main effect of time, $F_{(1,53)}$ = 641.88, p < 0.001), body mass (main effect of time, $F_{(1,53)}$ = 340.17, p = < 0.001) and BMI (main effect of time, $F_{(1,53)}$ = 24.17, p < 0.001) (Table 13). For stature, there was an interaction effect (group * time interaction, $F_{(1,53)}$ = 6.16, p = 0.016) whereby at baseline there was no difference between the groups but at follow-up there was a tendency for the trained group to be taller than the untrained group (trained: 167.6 ± 9.4 cm, untrained: 162.4 ± 6.2 cm, $F_{(1,53)}$ = 3.51, p = 0.067). Furthermore, there was a tendency at follow-up for boys to be taller than the girls (boys: 171.1 ± 8.2 cm; girls: 161.9 ± 6.1 cm, $F_{(1,53)}$ = 3.18, p = 0.080), which did not exist as baseline causing an interaction effect for stature between time and sex (time * sex interaction, $F_{(1,53)}$ = 5.76, p = 0.020). In contrast, there was no main effect of group, sex or interaction effects for body mass or BMI (all p > 0.05).

Overall, adiposity (measured as the sum of four skinfolds) was greater in the untrained group when compared with the trained group (trained: 39.6 ± 13.8 mm, untrained: 58.4 ± 30.2 mm, main effect of group, $F_{(1,57)}$ = 7.42, p = 0.009, Table 13), yet did not change across time (main effect of time, p = 0. 571). When considering the effect of sex the girls had greater adiposity than the boys (boys: 36.1 ± 21.1 mm, girls: 54.4 ± 29.6 mm, main effect of sex, $F_{(1,57)}$ = 12.06, p = 0.002). However, there were no interaction effects for adiposity (all p > 0.05).

Table 13. Participant anthropometrics, adiposity and maturity offset in trained and untrained adolescents at baseline and at follow-up 2 years later. * group by time interaction ($p < 0.05$); † Change from pre to post within group ($p<0.05$).

	Trained Group		Untrained Group	
	Baseline	Follow-up	Baseline	Follow-up
Stature (cm)	152.8 ± 8.1	167.6 ± 9.4	151.4 ± 6.4	162.9 ± 5.8*
Body Mass (kg)	41.5 ± 6.8	53.1 ± 7.7	45.8 ± 11.2	55.8 ± 12.4
BMI (kg/m^2)	17.7 ± 1.9	18.8 ± 1.5	19.9 ± 4.4	20.6 ± 4.3
Sum of Skinfolds (mm)	41.5 ± 15.0	37.7 ± 12.3†	57.5 ± 35.4	59.4 ± 29.2†
Maturity Offset (y)	-2.5 ± 1.7	0.3 ± 0.8†	-1.6 ± 0.5	-0.2 ± 0.8†*

The pubertal development of the participants, measured by maturity offset, progressed in both groups during the two year follow-up (main effect of time, $F_{(1,109)} = 231.68$, $p < 0.001$, Table 13). When considering the effect of sex, overall girls were more mature than boys (maturity offset: girls: -0.73 ± 1.29 years; boys: -1.21 ± 1.68 years, main effect of sex, $F_{(1,109)} = 11.97$, $p < 0.001$). Furthermore, the pattern of change in maturity offset was different between the trained and untrained group across time (group*time interaction, $F_{(1,109)} = 4.10$, $p = 0.001$), whereby at baseline the trained group were less mature than the untrained group (trained: -2.50 ± 1.69 years, untrained: -1.64 ± 0.53 years, $F_{(1,109)} = 6.17$, $p = 0.046$) and at follow-up the trained group were more mature than the untrained group (trained: 0.28 ± 0.78 years, untrained: -0.23 ± 0.78 years, $F_{(1,109)} = 12.37$, $p = 0.006$).

7.3.2 Inflammation

IL-6: Overall, the trained group had lower concentrations of pro-inflammatory cytokine IL-6 when compared with the untrained group (trained 3.52 ± 1.54 pg·mL^{-1}, untrained: 4.49 ± 1.81 pg·mL^{-1}, main effect of group, $F_{(1,49)} = 8.45$, $p = 0.005$, $d = 0.6$) (Table 14, Figure 11).

Furthermore, concentrations of IL-6 increased in both groups (trained and untrained) during the two year follow-up (main effect of time, $F_{(1,49)} = 50.07$, $p < 0.001$). However, the pattern of change in IL-6 concentration over the two-year follow-up was similar between the trained and untrained groups (group * time interaction, $p = 0.804$). When considering the effect of sex, there was no main effect or interactions for IL-6 concentration (all $p > 0.05$).

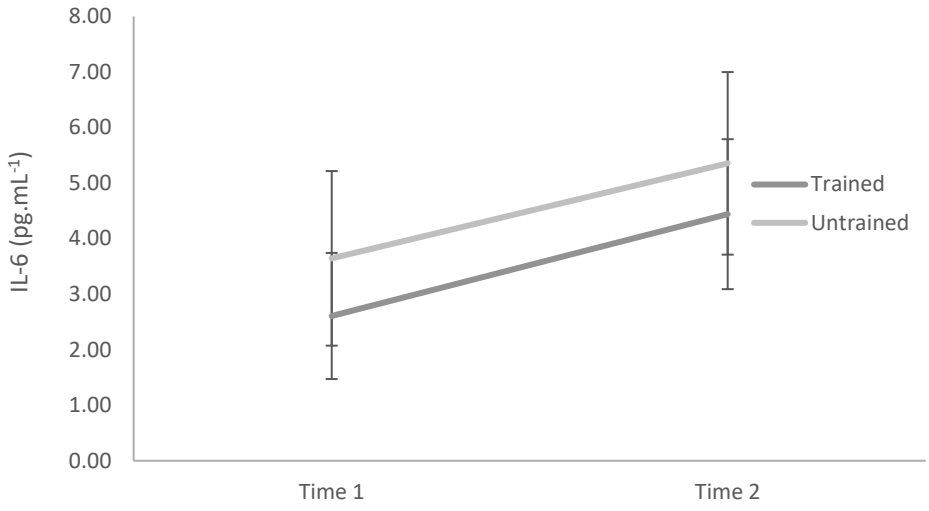

Figure 11. IL-6 concentration ($pg{\cdot}mL^{-1}$) in trained and untrained adolescents at baseline (Time 1) and two years later at follow-up (Time 2). Mean ± SD, main effect of group; $p = 0.005$.

IL-1β: Overall, the trained group had lower concentrations of pro-inflammatory cytokine IL-1β when compared with the untrained group (trained: 3.52 ± 2.11 $pg{\cdot}mL^{-1}$, untrained: 5.46 ± 3.95 $pg{\cdot}mL^{-1}$, main effect of group, $F_{(1,51)} = 7.89$, $p = 0.007$, $d = 0.6$) (Table 14, Figure 12), yet did not change across time (main effect of time, $p = 0.316$). Furthermore, the pattern of change in IL-1β concentration was similar between the groups across time (group * time interaction, $p = 0.992$). When considering the effect of sex, there was no main effect nor were there any interactions for IL-1β concentration (all $p > 0.05$).

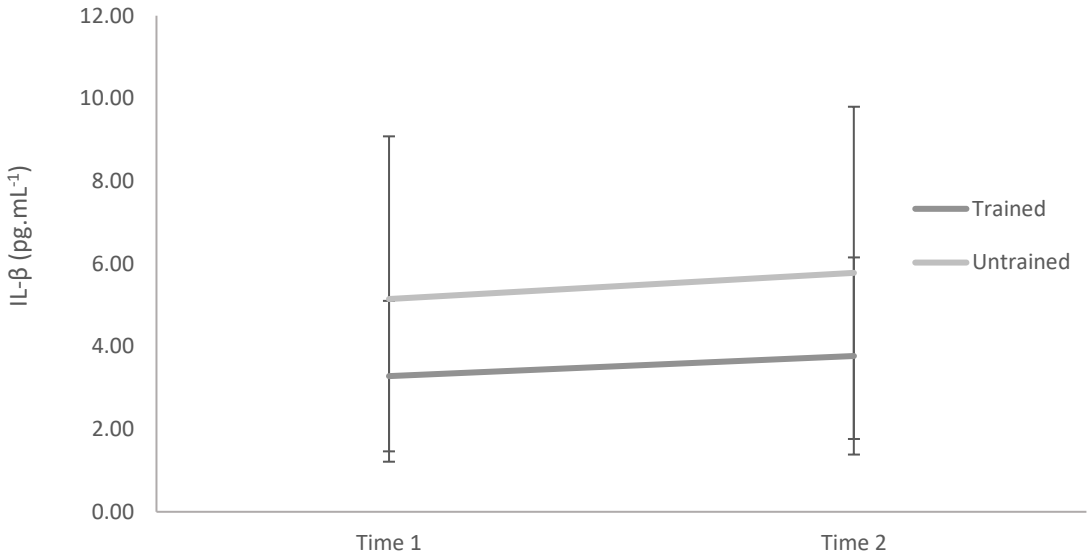

Figure 12. IL-1β concentration (pg.mL-1) in trained and untrained adolescents at baseline (Time 1) and two years later at follow-up (Time 2). Mean ± SD, main effect of group; p = 0.007.

IL-10: Overall, the trained group had higher concentrations of anti-inflammatory cytokine IL-10 when compared with the untrained group (trained: 3.31 ± 2.81 pg·mL-1, untrained: 2.37 ± 1.36 pg·mL^{-1}, main effect of group, $F_{(1,52)} = 6.54$, p = 0.008, d = 0.4) (Table 14, Figure 13), yet did not change across time (main effect of time, p = 0.099). Furthermore, the pattern of change in IL-10 concentration was similar between the groups across time (group * time interaction, p = 0.404). When considering the effect of sex, there was no main effect nor were there any interactions for IL-10 concentration (all p > 0.05).

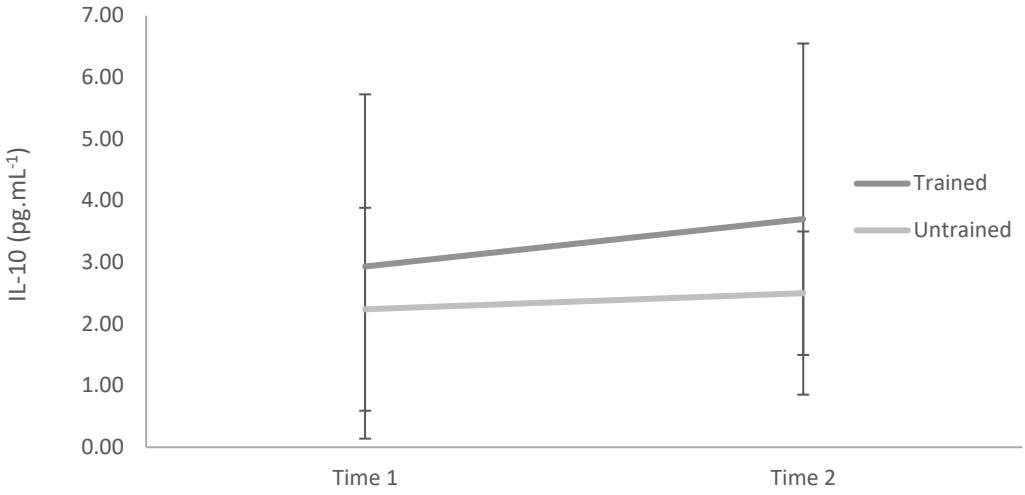

Figure 13. IL-10 concentration ($pg \cdot mL^{-1}$) in trained and untrained adolescents at baseline (Time 1) and two years later at follow-up (Time 2). Mean ± SD, main effect of group; p = 0.008.

TNF-α and CRP: Overall, concentrations of pro-inflammatory mediators TNF-α and CRP were similar between the trained and untrained groups (all $p > 0.05$), yet increased across time in both groups (main effect of time for TNF-α, $F_{(1,45)} = 29.41$, $p < 0.001$; main effect of time for CRP, $F_{(1,40)} = 9.41$, $p < 0.001$). The pattern of change in TNF-α and CRP concentration was similar between the groups across time (all $p > 0.05$). When considering the effect of sex, there was no main effect nor were there any interactions for TNF-α and CRP concentration (all $p > 0.05$).

Table 14. Inflammatory cytokine concentration ($pg \cdot mL^{-1}$) in trained and untrained adolescents at baseline and at follow-up 2 years later. * denotes significant main effect of time and † denotes significant difference between groups, all $p < 0.005$.

	Trained Group		Untrained Group	
	Baseline	Follow-up	Baseline	Follow-up
IL-6 ($pg \cdot ml^{-1}$)†	2.61 ± 1.14	4.44 ± 1.35	3.64 ± 1.57	5.36 ± 1.64
IL-1β ($pg \cdot ml^{-1}$)†	3.28 ± 1.82	3.77 ± 2.38	5.15 ± 3.94	5.78 ± 4.02
IL-10 ($pg \cdot ml^{-1}$)†	2.93 ± 2.79	3.70 ± 2.85	2.24 ± 1.65	2.50 ± 1.00
TNF-α ($pg \cdot ml^{-1}$)	1.59 ± 1.16	9.40 ± 6.41*	1.90 ± 1.89	9.97 ± 9.23*
CRP ($mg \cdot L^{-1}$)	0.31 ± 0.32	0.83 ± 0.68	0.41 ± 0.52	0.78 ± 0.55

7.3.3 Blood Glucose, Plasma Insulin Concentration and HOMA-IR

Blood Glucose: Overall, the trained group had a lower fasted blood glucose concentration than the untrained group (trained 4.19 ± 0.50 $mmoL^{-1}$, untrained: 4.41 ± 0.58 $mmoL^{-1}$, main effect of group, $F_{(1,47)} = 9.51$, $p = 0.003$, $d = 0.4$), yet did not change across time ($p = 0.793$). The pattern of change in blood glucose concentration over the two year follow-up differed between the trained and untrained groups (group * time interaction, $F_{(1,47)} = 10.71$, $p = 0.002$, Table 15, Figure 14), whereby at baseline the untrained group had higher blood glucose concentration than the trained group (trained: 4.06 ± 0.10 $mmol \cdot L^{-1}$, untrained: 4.72 ± 0.11 $mmol \cdot L^{-1}$, $F_{(1,47)} = 7.16$, $p = 0.010$), yet at follow-up blood glucose concentration was similar between the groups ($p = 0.609$). When considering the effect of sex, there was no main effect nor were there any further interaction effects for blood glucose concentration (all $p > 0.05$).

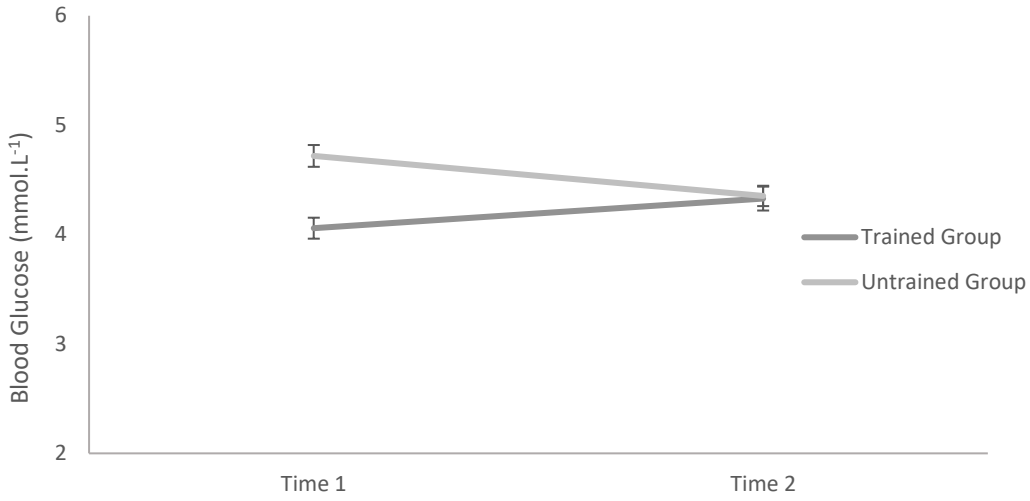

Figure 14. Fasted blood glucose concentration ($mmol \cdot L^{-1}$) in trained and untrained adolescents at baseline (Time 1) and two years later at follow-up (Time 2). Mean ± STDEV, main effect of group; p = 0.003.

Plasma Insulin: Overall, the trained group had a lower fasted plasma insulin concentration than the untrained group (trained 7.18 ± 7.78 $uU.L^{-1}$, untrained: 13.59 ± 16.76 $uU.L^{-1}$, main effect of group, $F_{(1,47)} = 5.58$, p = 0.022, d = 0.5; Table 15, Figure 15). Furthermore, plasma insulin concentration increased in both groups (trained and untrained) during the two year follow-up (main effect of time, $F_{(1,47)} = 6.47$, p = 0.014). However, the pattern of change in plasma insulin concentration over the two-year follow-up was similar across groups (group * time interaction, p = 0.320). When considering the effect of sex, there was no main effect nor were there any further interaction effects for plasma insulin concentration (all p > 0.05).

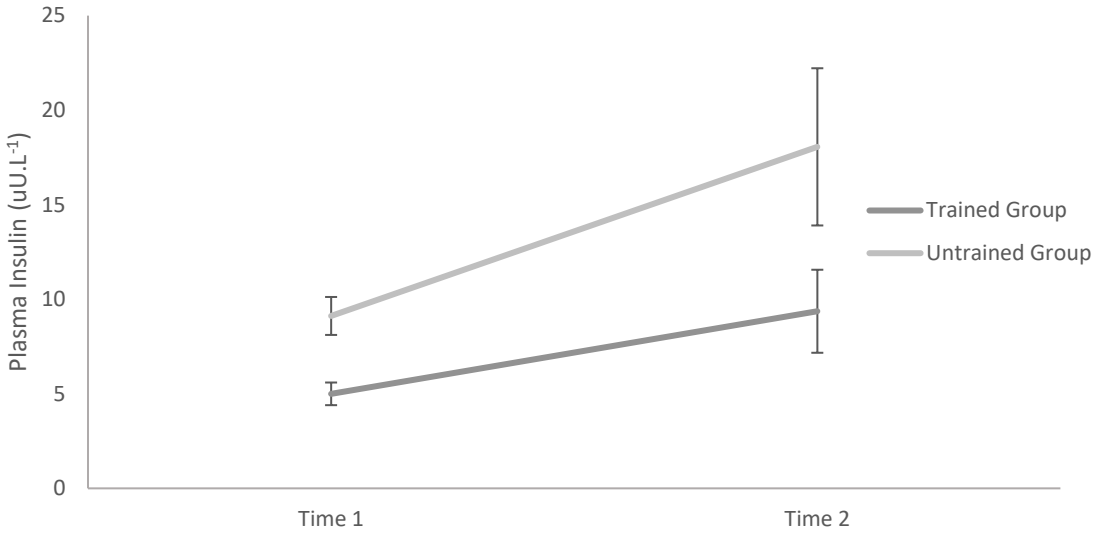

Figure 15. Fasted plasma insulin concentration ($uU{\cdot}L^{-1}$) in trained and untrained adolescents at baseline (Time 1) and two years later at follow-up (Time 2). Mean ± SD, main effect of group; p = 0.022, main effect of time; p = 0.014.

HOMA-IR: Overall, the trained group had a lower HOMA-IR than the untrained group (trained 1.4 ± 1.6, untrained: 2.7 ± 3.5, main effect of group, $F_{(1,47)} = 5.59$, p = 0.019, d = 0.5, Table 15, Figure 16) . Furthermore, HOMA-IR increased in both groups (trained and untrained) during the two year follow-up (main effect of time, $F_{(1,47)} = 5.83$, p = 0.020). However, the pattern of change in HOMA-IR over the two-year follow-up was similar across groups (group * time interaction, p = 0.440). When considering the effect of sex, there was no main effect nor were there any further interaction effects for plasma insulin concentration (all p > 0.05).

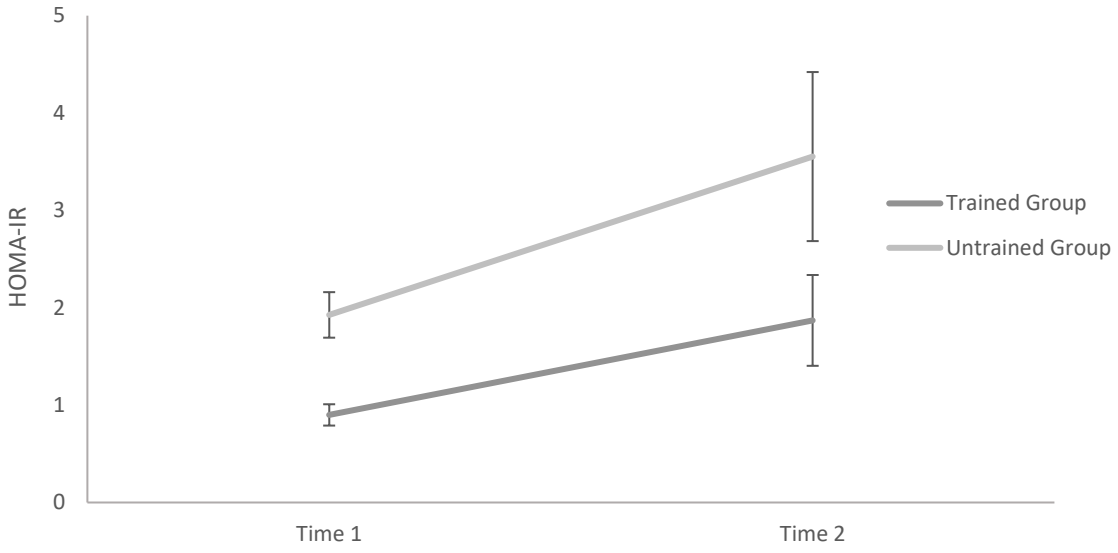

Figure 16. Fasted HOMA-IR in trained and untrained adolescents at baseline (Time 1) and two years later at follow-up (Time 2). Mean ± SD, main effect of group; p = 0.019, main effect of time; p = 0.020.

Table 15. Risk factors for metabolic diseases in trained and untrained adolescents at baseline and at follow-up 2 years later. * denotes significant main effect of time and † denotes significant difference between groups, all p < 0.005.

		Trained Group		Untrained Group	
		Baseline	Follow-up	Baseline	Follow-up
Blood Glucose Concentration ($mmol \cdot L^{-1}$) †	Overall	4.06 ± 0.10	4.33 ± 0.09	4.72 ± 0.10	4.35 ± 0.11
	Boys	3.94 ± 0.17	4.34 ± 0.16	4.62 ± 0.15	4.48 ± 0.19
	Girls	4.16 ± 0.12	4.32 ± 0.11	4.79 ± 0.12	4.27 ± 0.13
Plasma Insulin Concentration ($mU \cdot L^{-1}$) †	Overall	5.00 ± 0.60	9.37 ± 2.20*	9.12 ± 1.00	18.06 ± 4.16*
	Boys	4.13 ± 0.47	4.79 ± 1.18	6.72 ± 0.78	18.45 ± 4.90
	Girls	5.72 ± 1.05	13.18 ± 3.77	10.81 ± 1.51	17.79 ± 3.38
HOMA-IR †	Overall	0.90 ± 0.11	1.87 ± 0.47*	1.93 ± 0.23	3.55 ± 0.87*
	Boys	0.73 ±0.10	0.94 ± 0.23	1.38 ± 0.17	3.99 ± 2.01
	Girls	1.04 ± 0.19	2.65 ± 0.81	2.31 ± 0.36	3.24 ± 0.52

7.3.4 Blood Pressure

Overall, systolic, diastolic and mean arterial blood pressure were similar in both the trained and untrained groups (all $p > 0.05$), yet systolic blood pressure and mean arterial blood pressure increased in both groups (trained and untrained) during the two year follow-up (main effect of time: systolic blood pressure, $F_{(1,53)} = 15.49$, $p < 0.001$; mean arterial blood pressure, $F_{(1,57)} = 5.69$, $p = 0.021$). The pattern of change in systolic blood pressure, diastolic blood pressure and mean arterial pressure over the two-year follow-up was similar across groups (group * time interaction, all $p > 0.05$). When considering the effect of sex, there was no main effect nor were there any further interaction effects for blood pressure (all $p > 0.05$).

Table 16. Blood pressure in trained and untrained adolescents at baseline and at follow-up 2 years later.

		Trained Group		Untrained Group	
		Baseline	Follow-up	Baseline	Follow-up
Systolic Blood Pressure (mmHg)	Overall	110 ± 9	120 ± 13	112 ± 8	114 ± 8
	Boys	113 ± 8	117 ± 14	106 ± 23	109 ± 23
	Girls	109 ± 9	117 ± 9	105 ± 27	111 ± 28
Diastolic Blood Pressure (mmHg)	Overall	69 ± 7	70 ± 9	72 ± 9	71 ± 7
	Boys	69 ± 7	68 ± 9	66 ± 16	67 ± 15
	Girls	71 ± 9	73 ± 6	67 ± 18	67 ± 17
Mean Arterial Blood Pressure (mmHg)	Overall	82 ± 6	87 ± 9	85 ± 7	85 ± 6
	Boys	84 ± 7	84 ± 10	79 ± 17	81 ± 17
	Girls	84 ± 7	87 ± 6	81 ± 20	81 ± 19

7.3.5 Distance Run on the MSFT

Overall, the trained group ran further during the MSFT than the untrained group (trained 1580 ± 460 m, untrained: 800 ± 460 m, main effect of group, $F_{(1,53)} = 63.51$, $p < 0.001$, $d = 1.7$)

(Table 17, Figure 17). Furthermore, distance ran on the MSFT increased in both groups (trained and untrained) during the two year follow-up (main effect of time, $F_{(1,53)} = 55.63$, $p < 0.001$). The pattern of change in distance ran on the MSFT over the two year follow-up differed between the trained and untrained groups (group * time interaction, $F_{(1,53)} = 12.03$, $p = 0.001$), whereby the trained group ran further on the MSFT than the untrained group at baseline (trained: 1400 ± 400 m, untrained: 760 ± 400 m, $F_{(1,53)} = 42.78$, $p < 0.001$, $d = 1.6$) and at follow-up two years later (trained: 1740 ± 460 m, untrained: 860 ± 500 m, $F_{(1,53)} = 68.27$, $p < 0.001$, $d = 1.8$), resulting in a greater increase over the two years in the trained group. When considering the effect of sex boys ran further on the MSFT than girls (boys: 1580 ± 480 m, girls: 940 ± 520 m, main effect of sex, $F_{(1,53)} = 46.36$, $p < 0.001$, $d = 1.2$), yet there were no interaction effects for distance run on the MSFT (all $p > 0.05$).

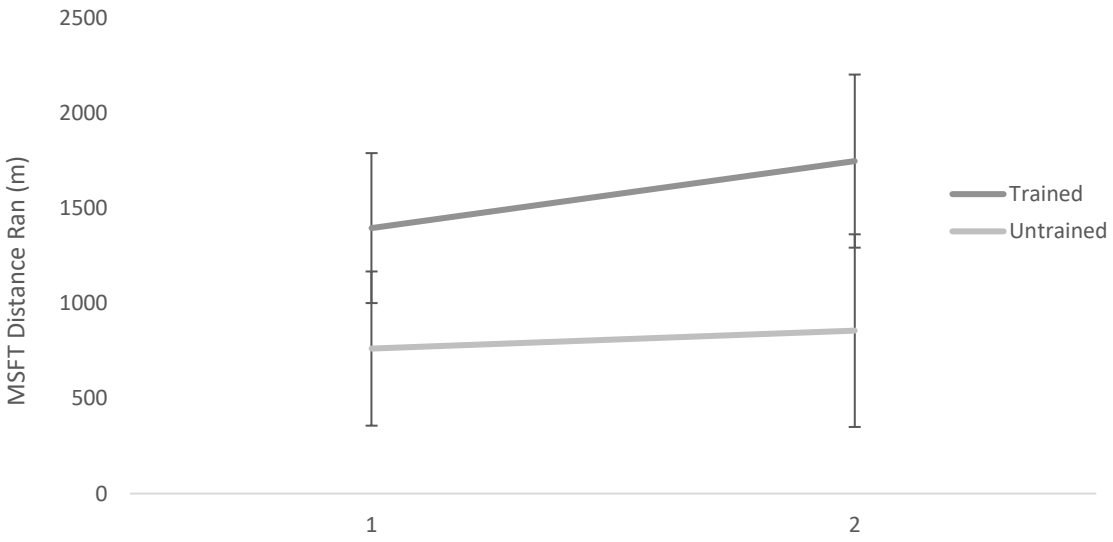

Figure 17. Distance ran on the MSFT in trained and untrained adolescents at baseline (Time 1) and two years later at follow-up (Time 2). Mean ± SD, main effect of group; $p < 0.001$, main effect of time; $p < 0.001$, group by time interaction; $p = 0.001$.

7.3.6 $\dot{V}O_2$ Peak Test

Overall, the trained group had a higher $\dot{V}O_2$ peak than the untrained group (trained: 58.13 ± 7.15 $ml^{-1} \cdot kg^{-1} \cdot min$, untrained: 40.05 ± 9.07 $ml^{-1} \cdot kg^{-1} \cdot min$, main effect of group, $F_{(1,23)} = 34.50$,

$p < 0.001$, $d = 1.3$, Table 17) , yet $\dot{V}O_2$ peak did not change across time (main effect of time, $p = 0.230$). Furthermore, the pattern of change in $\dot{V}O_2$ peak was similar between the groups across time (group * time interaction, $p = 0.714$). When considering the effect of sex, there was no main effect nor were there any interactions for $\dot{V}O_2$ peak (all $p > 0.05$).

Table 17. MSFT performance and $\dot{V}O_2$ peak in trained and untrained adolescents at baseline and at follow-up 2 years later. * denotes significant main effect of time and † denotes significant difference between groups, all $p < 0.005$.

		Trained Group		Untrained Group	
		Baseline	Follow-up	Baseline	Follow-up
Distance Ran on MSFT (m)†	Overall	1400 ± 400	1740 ± 460*	760 ± 400	860 ± 500*
	Boys	1580 ± 360	2020 ± 360	1160 ± 320	1380 ± 380
	Girls	1240 ± 360	1520 ± 400	520 ± 220	560 ± 260
$\dot{V}O_2$ peak	Overall	56.6 ± 6.7	59.7 ± 7.5	39.5 ± 9.5	40.6 ± 8.9
$(ml \cdot kg^{-1} \cdot min^{-1})^{\dagger}$	Boys	54.7 ± 7.8	61.8 ±9.1	43.3 ± 11.2	44.8 ± 11.1
	Girls	59.7 ± 4.8	57.2 ± 4.7	36.7 ± 7.6	37.4 ± 5.7

7.3.7 Relationship between changes in performance, $\dot{V}O_2$ peak and adiposity with changes in risk factors for cardiometabolic diseases

Change in distance run on the MSFT, $\dot{V}O_2$ peak and adiposity (sum of four skinfolds and waist circumference) were not significantly correlated with change in any of the analysed risk factors for cardiometabolic health during the two-year follow-up (all $p > 0.05$). However, there were tendencies for change in distance run on the MSFT to be negatively correlated with change in plasma insulin concentration ($r_{(46)} = -.28$, $p = 0.062$) and for change in blood lactate concentration during submaximal exercise to be negatively correlated with change in HOMA-IR ($r_{(21)} = -.42$, $p = 0.055$). Furthermore, when assessing change in CRP concentration during

the two-year follow-up, a positive correlation was observed with change in diastolic blood pressure ($r_{(33)} = .56$, $p = 0.002$) and mean arterial pressure ($r_{(33)} = .48$, $p = 0.005$).

7.4 Discussion

The main finding of the present study was that continuous training during adolescence compared to remaining recreationally active during a two-year follow-up, reduced concentrations of pro-inflammatory cytokines (30% lower IL-6 and 55% lower IL-1β concentration) and increased concentrations of anti-inflammatory mediator IL-10 (28% higher in trained adolescents compared with untrained). Furthermore, traditional risk factors for cardiometabolic diseases, including metabolic risk factors of blood glucose concentration, plasma insulin concentration and HOMA-IR, were lower in the trained adolescents than their untrained counterparts. A secondary aim of the present study was to prospectively examine changes in the MSFT performance and $\dot{V}O_2$ peak in trained and untrained adolescents. MSFT performance and $\dot{V}O_2$ peak were higher in the trained participants than the untrained controls at baseline and at follow-up, but after the two years of continued training MSFT improved whereas $\dot{V}O_2$ peak was unchanged. Finally, the change in distance run on the MSFT and the blood lactate response to exercise tended to be negatively related to change in plasma insulin concentration and HOMA-IR in the adolescents, whereas change in $\dot{V}O_2$ peak was not related to any of the risk factors for cardiometabolic health.

Overall, trained adolescents exhibited lower concentrations of pro-inflammatory cytokines (IL-6 and IL-1β) and higher concentrations of anti-inflammatory mediator IL-10 during the two-year follow-up, when compared with untrained adolescents. These findings are novel as the present study is the first to longitudinally assess a comprehensive panel of pro- and anti-inflammatory mediators, which are implicated in the development of low-grade chronic inflammation, a major risk factor for cardiometabolic diseases (Gleeson et al., 2011). However,

the finding that trained adolescents present with favourable inflammatory profiles builds upon previous research (presented in Chapter IV), whereby performance on the MSFT was negatively associated with pro-inflammatory cytokines (IL-6 and IL-1β) and positively associated with anti-inflammatory mediator IL-10. Furthermore, rhythmic gymnasts have previously been reported to present with increased concentrations of adipocytokines (predominantly adiponectin) when compared with untrained controls (Vosoberg et al., 2014). Such findings further support those of the present study as adiponectin elicits beneficial metabolic actions (such as suppressing glucose production in the liver and enhancing fatty acid oxidation in skeletal muscle) and reduces inflammation (Wang & Scherer, 2016). Whilst such corroborative findings suggest chronic training during adolescence is a potential therapeutic intervention to regulate inflammation further research is necessary to build on the findings of the present study.

The present study also reported that overall, trained adolescents exhibited lower blood glucose concentration (-6 %), plasma insulin concentration (-89 %) and HOMA-IR (-93 %) when compared with untrained adolescents, findings that suggest the trained group are more insulin sensitive when compared with the untrained counterparts. These findings build on those presented in Chapter IV, whereby adolescents with the lowest distance ran on the MSFT were less insulin sensitive, as measured by HOMA-IR, than adolescents who performed well on the endurance capacity test. Whilst promising, the findings of the present study must be endorsed by future studies, particularly training intervention studies, before training in young people be recommended as a therapeutic intervention that enhances cardiometabolic health in adolescents.

Overall, in the present study distance run on the MSFT and $\dot{V}O_2$ peak test was significantly greater in the trained group than the untrained group. Such findings confirm that long-term

training enhances endurance capacity in young people which previously, and in this study, has been associated with better cardiometabolic health in young people (Klakk et al., 2014; Telford et al., 2015; Zaquot et al., 2016). However, it is also important to consider that the trained group were significantly taller than the untrained group, which is a potential confounding variable (given that typically swimmers and footballers tend to be, in part, recruited to the sport for their stature) affecting performance on the MSFT. Future research should consider the mediating effect of stature on performance on physical capacity tests. Irrespective, only performance on the MSFT tracked improvements in the trained group compared to the untrained group during the two-year follow-up, whereas $\dot{V}O_2$ peak was not sensitive to such changes. Furthermore, changes in distance run on the MSFT and the blood lactate response to submaximal exercise were the only measures of training status that tended to be related to risk factors of cardiometabolic health (change in plasma insulin concentration and HOMA-IR), whilst change in $\dot{V}O_2$ peak was not related to any of risk factor. These findings support those of Chapter IV, which suggests that performance on the MSFT is better suited to assess an individual's capacity to perform prolonged exercise, as the MSFT is a better indicator of the peripheral changes that result from participation in regular training (Joyne & Carsten, 2018) and is therefore a better prediction of adolescent cardiometabolic health. Future studies should progress the findings of the present study to assess whether the MSFT or $\dot{V}O_2$ peak best longitudinally predict cardiometabolic health, as such information will have implications for future policies.

A novel finding of the present study was that change in CRP concentration during adolescence was moderately and positively related to change in diastolic and mean arterial blood pressure. In adult populations CRP has been identified as the best predictor of cardiovascular events (Emerging Risk Factors Collaboration, 2012), yet the presence of this novel risk factor and the relationship with cardiovascular disease risk in young people is unknown. Given the moderate relationship between change in CRP and change in diastolic and mean arterial blood pressure,

which are both major risk factors of hypertension (a form of cardiovascular disease), the present study has identified CRP as also being of high importance for monitoring cardiovascular health throughout childhood and adolescence, as well as during adulthood as suggested by previous research (Emerging Risk Factors Collaboration, 2012).

Finally, the present study observed that trained adolescents had significantly lower adiposity, measured using the sum of four skinfolds, when compared to the untrained adolescents. Furthermore, when considering the effect of sex, the girls exhibited greater adiposity than the boys. Previous research has repeatedly highlighted that adolescent girls are more likely to display reduced physical activity levels, physical fitness and greater adiposity than adolescent boys (for overview, see Hardman & Stensel, 2009). Therefore, such information in conjunction with the findings of the present study suggest that adolescent girls, given their elevated adiposity, warrant particular attention and future interventions should focus specifically on this population. It is therefore essential that behaviour change specialists, in conjunction with exercise and health physiologists, determine effective interventions that adolescent girls will adhere to and also have the desired physiological benefits, as documented in this study.

The findings of the present thesis are novel, as there have been no previous studies to specifically assess the effect of long-term training during adolescence on markers of cardiometabolic health. Future research should however examine how the differences that occur during adolescence manifest as clinically meaningful changes during adulthood. The findings of the present study provide a novel insight into the development of risk factors across the lifespan, highlighting that adolescents as young as 12-14 y not engaging with regular exercise warrant early intervention to prevent the early onset of cardiometabolic diseases. Specifically, the initial findings of the present study suggest that young people can enhance their cardiometabolic health and reduce their risk of type 2 diabetes, which is a current concern

in Western societies with a 36% increase in the number of cases in young people in the last decade (Candler et al., 2018), by engaging with regular training (as participants evidenced by the participants recruited from local swimming and football clubs).

The present study has several strengths including the longitudinal assessment of a comprehensive panel of novel pro- and anti-inflammatory cytokines and traditional risk factors for cardiometabolic diseases in adolescents. Furthermore, the present study is the first to the authors' knowledge to assess the effect of long-term training on different measures of endurance capacity in young people to ascertain which assessment is most appropriate for tracking changes throughout adolescence. However, the present study was limited as no information relating to the ethnicity, dietary habits and typical physical activity levels of the adolescents was collated.

In conclusion, the present study reported that adolescents participating in continuing training during a two-year follow-up study, presented with a favourable inflammatory profile and greater insulin sensitivity than their untrained counterparts. Furthermore, the trained group consistently performed better on performance tests, which measured their ability to perform prolonged exercise, and had lower adiposity. In conjunction, these findings suggest that continued training during adolescence is an effective means to protect against the development of risk factors for cardiometabolic diseases, which is currently of high importance given the increasing incidence of such risk factors in young people in the United Kingdom (Ayer et al., 2016; Candler et al., 2018). Such information can be utilised in the future to develop interventions for adolescents to reverse the current adverse health trends being observed.

Chapter VIII

General Discussion

8.1 Overview of Key Findings

- Performance on the multi-stage fitness test (MSFT distance run) was inversely associated with risk factors for cardiometabolic diseases, including pro-inflammatory cytokines (IL-1β and IL-6), insulin resistance and blood pressure. Furthermore, distance run on the MSFT was a better predictor of inflammation in adolescents than $\dot{V}O_2$ peak, with an inverse relationship between MSFT distance and pro-inflammatory cytokines (IL-1β and IL-6) and a positive relationship with anti-inflammatory cytokine IL-10, but no such relationships with $\dot{V}O_2$ peak (Chapter IV).
- Body composition (measured as sum of skinfolds) was the only predictor of metabolic health (fasted plasma insulin concentration and HOMA-IR) and blood pressure in adolescents, with a positive relationship between the exposure and outcome variables (Chapter IV).
- An acute 60 min bout of games-based activity (basketball) effectively stimulated an anti-inflammatory response in healthy adolescent boys and girls, as evidenced by the increased concentrations of anti-inflammatory mediators (IL-6 and IL-10, deemed anti-inflammatory when transiently increased following exercise given the anti-inflammatory effects), and the anti-inflammatory IL-6: TNF-α ratio up to 24 h post-exercise (Chapter V).
- The acute bout of games-based activity also enhanced peripheral insulin sensitivity following the consumption of a standardised lunch, with reduced plasma insulin iAUC following a standardised lunch on the exercise trial when compared to the rested control trial (Chapter V).

- When considering the duration of high intensity intermittent exercise undertaken, a shorter bout of 30 min high intensity intermittent running (performed as the Loughborough Intermittent Shuttle Test; LIST) was as effective as 60 min for the management of blood glucose concentration on the day of exercise including a standardised lunch (Chapter VI). However, the effect of exercise on blood glucose homeostasis did not remain 24 h post-exercise.
- In contrast, only 60 min of high intensity intermittent running (performed as the LIST), in comparison with 30 min, effectively enhanced postprandial insulin sensitivity in healthy adolescents, with a similar response observed between 30 min of high intensity intermittent running and the rested control trial (Chapter VI).
- When assessed longitudinally, performance on the MSFT (distance run) increased in adolescents who trained continuously during a two-year follow-up whilst $\dot{V}O_2$ peak remained unchanged. However, both MSFT performance and $\dot{V}O_2$ peak were higher at baseline and after two-years of follow-up in the trained in comparison with the control group (Chapter VII).
- Longitudinally, trained adolescents had significantly lower concentrations of pro-inflammatory cytokines (IL-1β and IL-6), higher concentrations of anti-inflammatory cytokine IL-10, and enhanced insulin sensitivity when compared to untrained adolescents during a two-year follow-up (Chapter VII).
- Changes in distance run on the MSFT and blood lactate response to submaximal exercise (across the two-year follow-up period) were inversely related to changes in plasma insulin concentration and HOMA-IR; whilst changes in $\dot{V}O_2$ peak were not related to changes in any of the risk factors for cardiometabolic health throughout adolescence (Chapter VII).

- Finally, changes in CRP concentration throughout adolescence were positively related to changes in diastolic and mean arterial blood pressure, indicative of CRP concentration predicting cardiovascular health in young people as well as in adult populations as evidence by previous research (Chapter VII).

8.2 Effect of MSFT performance, the blood lactate response to sub-maximal exercise and $\dot{V}O_2$ peak on risk factors for cardiometabolic disease in adolescents.

8.2.1 Inflammation

In the studies presented in this thesis the effect of performance on the MSFT (distance run), $\dot{V}O_2$ peak, adiposity and adolescent training status (trained *vs.* untrained) on risk factors for cardiometabolic diseases were examined cross-sectionally (Chapter IV) and longitudinally (Chapter VII). Several inflammatory mediators were analysed as a measure of low-grade chronic inflammation in adolescents, including pro-inflammatory cytokines IL-1β, IL-6, TNF-α and CRP, alongside the anti-inflammatory cytokine IL-10.

Overall, the findings of the experimental studies presented in this thesis suggest that distance run on the MSFT (Chapter IV), the blood lactate response to submaximal exercise (Chapter IV) and chronic participation in regular exercise (Chapter VII) were inversely related to pro-inflammatory cytokines (IL-1β and IL-6) and positively related to anti-inflammatory cytokine IL-10, whereas, distance run on the MSFT and $\dot{V}O_2$ peak were not related to pro-inflammatory cytokine TNF-α or CRP. Furthermore, a regression analysis reported that performance on the MSFT was the best predictor of pro-inflammatory cytokine concentration (IL-1β and IL-6) and the blood lactate response to submaximal exercise was the best predictor of IL-10 (anti-inflammatory cytokine). In contrast, $\dot{V}O_2$ peak did not predict the concentration of any of the inflammatory mediators measured in the present thesis.

The finding in Chapters IV and VII that distance run on the multi-stage fitness test is inversely related to pro-inflammatory cytokines (IL-6, TNF-α and CRP) is consistent with some of the findings, but contradicts other findings, in the just two earlier papers that have examined this relationship in adolescents (Buchan et al., 2015; Bugge et al., 2012). Buchan et al. (2015) used the multi-stage to predict $\dot{V}O_2$ peak in boys and girls (age 16.7 years) and consistent with the present study found that that multi-stage performance (used to predict $\dot{V}O_2$ peak) was inversely related to a panel of inflammatory cytokines. Bugge et al., (2012) reported that $\dot{V}O_2$ peak, directly measured during a treadmill test, was inversely associated with a clustered z-score of inflammatory mediators (including IL-6, TNF-α, CRP and adiponectin) (β = - 0.387) and IL-6 concentration (β = - 0.151) in a cross-sectional sample of adolescents aged 13 – 14 years, which contradicts the findings of the present study where no relationship was found between directly measured $\dot{V}O_2$ peak and inflammatory cytokines. When considering longitudinal changes in markers of inflammation in young people, the present study was the first to examine a comprehensive panel of inflammatory mediators (IL-1β, IL-6, IL-10, TNF-α, CRP) and assess how low-grade chronic inflammation changed across time in trained and untrained adolescents. The present study was also the first to report that untrained adolescents display reduced concentrations of anti-inflammatory mediator IL-10 in comparison to trained adolescents. Jürimäe et al., (2017) is the only other study to date, to the author's knowledge, which has examined IL-10 concentration in trained and untrained adolescents, although these findings contradict those presented in Chapter VII, with no difference observed in IL-10 concentration between the trained and untrained girls. Such discrepancies could be due to several factors, including the physical fitness of the athletes (which was not measured by Jürimäe et al., 2017) and participant characteristics such as age, pubertal development, socio-economic status and ethnicity. For example, age was 11 years in the Jürimäe study, but ranged from 10 -13 across

the studies in this thesis, whereas socio-economic status and ethnicity were not measured by Jürimäe and colleagues or in the present study (Jürimäe et al., (2017).

The effect of training status (as measured by performance on physical capacity tests) on inflammation in adolescents is particularly important given that low-grade chronic inflammation is a key risk factor for cardiometabolic diseases (Gleeson et al., 2011). In western societies young people are increasingly exposed to risk factors for cardiometabolic diseases (Ayer et al., 2016). As such, information relating to the reduction of such risk factors is becoming essential for the enhancement of cardiometabolic health. The novel and corroborative findings of the studies presented in this thesis suggest that enhancing performance on physical capacity tests, particularly the MSFT, and the continuation of training throughout adolescence reduces concentrations of pro-inflammatory cytokines (IL-6 and IL-1β) and increases anti-inflammatory mediator IL-10. The effect of training on adolescent cardiometabolic health is further supported given that performance on the MSFT was the only significant predictor of inflammation (Chapter IV), whereas $\dot{V}O_2$ peak did not predict inflammation. The MSFT was a more sensitive indicator of training status, as evidenced in Chapter VII, as in the trained adolescents distance run on the MSFT improved across time, whereas $\dot{V}O_2$ peak did not change in either group across time, which could potentially be explained by the strong genetic component of $\dot{V}O_2$ peak (Joyne & Carsten, 2018). Such findings are novel, as little consideration has previously been given to the mediating effect of the method for determining physical fitness (which best reflects training status) on the relationship between fitness and metabolic health.

Overall, the studies presented in this thesis suggest that enhancing performance on physical capacity tests (that assess the peripheral changes that occur with regular training) and the continuation with training during adolescence is an effective means to reduce the concentration

of inflammatory cytokines, which are implicated in the development of low-grade chronic inflammation. Furthermore, the findings in the thesis have shown for the first time that training can increase concentrations of IL-10 in the systemic circulation, contributing to an anti-inflammatory environment and an enhancement of cardiometabolic health.

8.2.2 Effect of MSFT performance, the blood lactate response to sub-maximal exercise, $\dot{V}O_2$ peak and adiposity on insulin sensitivity

In the studies presented in Chapters IV and VII, insulin sensitivity was assessed cross-sectionally and longitudinally using fasted blood glucose concentration, fasted plasma insulin concentration and HOMA-IR. Section 8.2.2 will discuss the effect of distance run on the MSFT and the blood lactate response to sub-maximal exercise (sensitive indicators of training status) and $\dot{V}O_2$ peak and adiposity (assessed as sum of skinfolds) on insulin sensitivity in adolescents.

In the present thesis, a cross-sectional analysis of adolescent boys and girls (10 – 12 years) showed that participants categorised below the 25th percentile for distance run on the MSFT, $\dot{V}O_2$ peak and adiposity had increased fasted concentrations of blood glucose and plasma insulin, and were more insulin resistant (with increased HOMA-IR) than adolescents in all other quartiles (Chapter IV). The regression analysis also revealed that adiposity was the best predictor of HOMA-IR in adolescents, with a positive relationship observed between the two variables ($\beta = 0.506$). In addition, the longitudinal analysis revealed that adolescents who trained consistently during the two-year follow-up had lower plasma insulin concentrations and HOMA-IR than their untrained counterparts (Chapter VII). These cumulative findings taken from Chapters IV and VII were further supported by the moderate inverse relationship reported between change in distance run on the MSFT and change in risk factors for metabolic diseases (plasma insulin concentration and HOMA-IR) during adolescence. Such findings support a causal relationship between performance on the MSFT, which is deemed to be more sensitive

(than $\dot{V}O_2$ peak) to changes in the peripheral adaptations that occur with continuous and prolonged training, and adolescent cardiometabolic health.

Consistent with the findings of the present thesis, inverse associations have previously been observed between performance on physical capacity tests (including the MSFT and run time to exhaustion during a $\dot{V}O_2$ peak graded treadmill test) and insulin resistance as measured by HOMA-IR in adolescent boys and girls (Bugge et al., 2012; Ischander et al., 2007). Furthermore, in a two- year longitudinal follow-up study, Zaquot et al., (2016) in adolescents reported that performance on the MSFT significantly predicted risk factors for metabolic syndrome, including HOMA-IR, following adjustment for confounding variables such as sex, age, and the socio-demographic status. As the findings of the present thesis and those of previous research suggest that distance run on the MSFT and adiposity are related to metabolic health, an additional analysis was undertaken on the fasted/ rested data from Chapters IV through VI to provide further support in favour of the argument that enhanced performance on the MSFT and reduced adiposity is important for adolescent cardiometabolic health. A moderate inverse association between distance run on the MSFT and blood glucose concentration ($r_{(171)} = -0.30$, $p < 0.001$) and HOMA-IR ($r_{(181)} = -0.39$, $p = < 0.001$) was observed in of adolescents (total 190, boys 93, girls 97) recruited to the studies presented in Chapters IV - VI. Furthermore, adiposity (defined as sum of four skinfolds) was moderately and positively related to blood glucose concentration ($r_{(171)} = 0.27$, $p < 0.001$) and HOMA-IR ($r_{(171)} = 0.46$, $p < 0.001$) in the adolescents. Taken together, these findings continue to support a major role for participation in regular training of sufficient intensity to enhance distance run on the MSFT in adolescents to help reduce the presence of risk factors for metabolic health during puberty.

Overall, the findings presented in this thesis suggest that regular training, reduced adiposity and enhanced performance on physical capacity tests effectively reduce fasted concentrations

of blood glucose and plasma insulin, and subsequently HOMA-IR. Such findings are particularly important given that the incidence of type 2 diabetes in children and adolescents has increased by 36 % from 2005 to 2015 in adolescents (aged < 17 years) in the United Kingdom (Candler et al., 2018). As such, the prevention and management of insulin resistance, a major risk factor of type 2 diabetes, in young people has become increasingly important in recent years. Therefore, the suggestion that training and reduced adiposity in adolescents, can reduce markers of insulin resistance is particularly important for future policies and interventions that aim to enhance cardiometabolic health in young people.

8.3 Effect of acute bouts of intermittent activity on inflammatory, glycaemic and insulinaemic responses in adolescents

8.3.1 Inflammatory Response

The findings of the experimental studies presented in this thesis suggest that a 60 min bout of intermittent games-based activity (basketball) elicits a transient anti-inflammatory response up to 24 h post-exercise in healthy adolescents (Chapter V). The anti-inflammatory response consisted of increased concentrations of anti-inflammatory cytokines IL-6 and the anti-inflammatory ratio IL-6: TNF-α up to 3 h post-exercise and IL-10 24 h post-exercise. In contrast, the acute bout of games-based activity did not have an effect on acute phase protein CRP with a similar response observed on the exercise and rested control trial.

The inflammatory response of a select number of inflammatory cytokines to acute bouts of exercise has been previously observed following wrestling, water polo and cross-country in adolescent boys and girls up to one hour post-exercise but no study has examined the response of an array of pro- and anti-inflammatory cytokines up to 24 h post-exercise (Eliakim et al., 2015; Nemet et al., 2002, 2003, 2009). Following such exercise between 2- to 7-fold increases in IL-6 concentration have been reported which are consistent with the 2-fold increase observed

following 60 min games-based activity (basketball) in the present thesis. An increase in IL-6 concentration post-exercise is suggested to stimulate a transient anti-inflammatory response to exercise, which if repeated regularly, is hypothesised to reduce low-grade chronic inflammation (Gleeson et al., 2011). Therefore, such promising findings could be implicated in the management of cardiometabolic health in young people. However, it is important to note that the magnitude of the increase in IL-6 concentration varies significantly across previous studies and could potentially be related to the mode, intensity and duration of exercise undertaken. The findings of the present thesis are the first to demonstrate that 60 min of games-based activity, which is an ecological valid mode of activity in young people that is deemed to be practical and enjoyable, stimulates an anti-inflammatory response in healthy adolescents. However, future research should continue to investigate the optimum mode of activity (including the intensity and duration of such activity) that best elicits an increase in IL-6 concentration.

The response of anti-inflammatory cytokine IL-10 has not previously been examined following acute bouts of exercise. As such, the present study advanced understanding by suggesting that an acute bout of games-based activity successfully elicits an increase in potent anti-inflammatory cytokine IL-10 up to 24 h post-exercise, which following *in vitro* studies is suggested to reduce low-grade chronic inflammation if the exercise is repeated regularly (Gleeson et al., 2011). *In vitro* studies suggest that the inflammatory cascade that follows a transient increase in IL-6 concentration remains for up to 24-48 h for select mediators including IL-10 and CRP (Pedersen & Petersen, 2005). Therefore, it is feasible that the response of anti-inflammatory mediator IL-10 remains elevated beyond 24 h post-exercise. However, this is yet to be assessed in human studies. Given the implications of such findings for exercise prescription, such as detailing the frequency of exercise required to chronically reduce low-

grade chronic inflammation, future research should examine the residual effects on inflammation up to 48 h post-exercise in children, adolescents and adults.

In conclusion, the findings of Chapter V suggest that a 60 min bout of games-based activity in healthy adolescents is effective in stimulating an anti-inflammatory response to exercise up for up to 24 h post-exercise. This response was evidenced by increased concentrations of anti-inflammatory mediators IL-6 and anti-inflammatory ratio IL-6: TNF-α up to 3 h post-exercise and IL-10 up to 24 h post-exercise. The present study was novel as it was the first to examine the inflammatory response beyond 1 h post-exercise and was the first to the author's knowledge to measure anti-inflammatory mediator IL-10; therefore, the findings of the present study add to the existing literature by documenting these effects.

8.3.2 Glycaemic and Insulinaemic Responses

The studies presented in this thesis have also examined the glycaemic and insulinaemic responses to acute bouts of intermittent games-based activity (Chapter V) and high intensity intermittent running (Chapter VI). The postprandial blood glucose and plasma insulin response to a standardised lunch was measured in Chapters V and VI, and the response to a standardised breakfast 24 h post-exercise was determined in Chapter VI. Furthermore, Chapter VI progressed current knowledge by examining whether exercise duration had an effect on the glycaemic and insulinaemic responses to ecologically valid standardised meals.

Overall, the findings of the present thesis suggest that 60 min of intermittent games-based activity (Chapter V) and 60 min of high intensity intermittent running (performed as the LIST; Chapter VI) successfully enhanced insulin sensitivity post-exercise, as evidenced by the 23-35 % reduction in postprandial plasma insulin tAUC during the exercise trial when compared with the rested control trial. In contrast, neither study reported an effect of the exercise trial on

HOMA-IR on day two of the study when compared with the rested control trial. Furthermore, when considering the effect of exercise duration (Chapter VI), 30 min of high intensity intermittent running elicited a similar postprandial insulinaemic response following a standardised lunch as the rested control trial in the healthy adolescents on day one and day two of the study; suggesting that 60 min exercise is required to enhance insulin sensitivity.

The findings observed in the present thesis are consistent with previous research, whereby high intensity intermittent cycling (8 x 1 min at 90 % peak power) reduced plasma insulin tAUC following an OGTT by 13 % in healthy adolescent boys (Cockcroft et al., 2015). Short et al., (2013) also reported that an acute bout of moderate intensity exercise (45 min of walking, cycling and boxing) in healthy adolescent boys, enhanced insulin sensitivity by 45 % following the consumption of a high fat meal. Whilst the findings are consistent with those of previous studies, the modes of exercise chosen in the present thesis (games-based activity and the LIST which is replicative of intermittent activity) and the standardised meals consumed are deemed ecologically valid in young people (Howe et al., 2010) and as such have greater application for future physical activity interventions that aim to enhance insulin sensitivity in young people.

The study presented in Chapter VI improved upon limitations of previous studies relating to the measurement of insulin sensitivity the day following exercise, as previously only HOMA-IR has been measured and as an assessment of hepatic insulin sensitivity, is unlikely to detect the peripheral changes in insulin sensitivity that are suggested to occur post-exercise. Therefore, in the present thesis HOMA-IR was assessed in conjunction with the response to a standardised breakfast (a measure of peripheral insulin sensitivity) the day following exercise. In summary, there was no effect of the high intensity intermittent running (on the 30 min or 60 min exercise trials) on HOMA-IR or the glycaemic and insulinaemic response (on the second day of the study) to the consumption of the standardised breakfast when compared with the

rested control trial. Whilst such findings suggest there is no effect of high intensity intermittent running on the postprandial glycaemic and insulinaemic responses the day following exercise, this is the only study to date to examine such responses in young people. Therefore, future studies should continue to investigate whether peripheral insulin sensitivity remains enhanced the day following exercise in adolescents, by examining the effect of different modes, intensities and durations of activity that best elicit and prolong the enhancement in insulin sensitivity post-exercise.

8.4 Mechanisms

The inflammatory response to an acute bout of exercise is one of several mechanisms suggested to mediate the cardiometabolic health benefits reported with regular participation in physical activity in young people and adults (Gleeson et al., 2011; Fiuza-Luces et al., 2013). In brief, at the onset of exercise the contraction of skeletal muscle stimulates the release of IL-6 into the systemic circulation. The increase in IL-6 concentration is suggested to increase with exercise intensity and duration (Gleeson et al., 2011; Petersen & Pedersen, 2005). The acute rise in IL-6 concentration is the suggested mechanism that initiates many of the cardiometabolic health benefits of exercise. One of the proposed mechanisms is that the acute increase in IL-6 concentration post-exercise augments insulin sensitivity. This was recently supported by a study in mice, whereby IL-6 was injected (50-100 $pg{\cdot}mL^{-1}$) into the plantaris muscle and an increase in GLUT4 expression and insulin sensitivity was observed (Ikano et al., 2016).

The acute increase in IL-6 post-exercise is also suggested to mediate the stimulation of anti-inflammatory cytokines (IL-1ra and IL-10) and the inhibition of pro-inflammatory cytokines (IL-1β and TNF-α) through the stimulation of their antagonistic receptors. The stimulation of anti-inflammatory cytokines IL-10 and IL-1ra inhibit the synthesis of further pro-inflammatory cytokines, including IL-1α, IL-1β, and TNF-α (Nimmo et al., 2013). The cascade initiated from

the acute rise in IL-6 concentration suggests a mechanistic link between the contraction of skeletal muscle during exercise and the prevention of low-grade chronic inflammation (a key risk factor for cardiometabolic disease).

Overall, the findings of the present thesis suggest that regular participation in exercise improves glucose tolerance and insulin sensitivity in adolescents. The response of blood glucose and plasma insulin to acute bouts of exercise in adolescents are suggested to mechanistically enhance insulin sensitivity long-term (for review, see Holloszy, 2006). The repeated contraction of skeletal muscle (which occurs during prolonged exercise) stimulates non-insulin dependent glucose uptake through the translocation of GLUT-4 (glucose transporter) to the cell membrane (Hardman & Stensel, 2009). As the acute effect of exercise subsides, it is suggested that insulin receptors on the cell membrane are modified and insulin sensitivity is enhanced. Another proposed mechanism for the chronic enhancement in insulin sensitivity that results from participation in regular exercise is the prevention of glucotoxicity that leads to mitochondrial defects and increased risk of type 2 diabetes (Lowell & Shulman, 2005).

Whilst the determination of the exact mechanisms that reduces risk factors for cardiometabolic diseases in trained adolescents was beyond the direct remit of the present thesis, by examining the inflammatory, glycaemic and insulinaemic responses to acute bouts of exercise speculation has been possible. The acute responses to high intensity intermittent activity in healthy adolescents, whereby an anti-inflammatory response and reduced glycaemic and insulinaemic responses were observed, are in agreement with the previously suggested mechanisms. Future studies should continue to investigate the mechanisms relating to enhanced cardiometabolic health in young people, as such information is important for the development of future physical activity interventions and policies.

8.5 Conclusions

From the studies presented in the present thesis several patterns have emerged relating to the beneficial effects of exercise on risk factors for cardiometabolic diseases in adolescents. Specifically:

- *The importance of training and enhancing performance on physical capacity tests for cardiometabolic health during adolescence*: The results of the cross-sectional analysis (presented in Chapter IV) and longitudinal follow-up (Chapter VII) clearly suggest that participating in regular training and enhancing performance on physical capacity tests during adolescence reduces the presence of pro-inflammatory cytokines (IL-1β and IL-6) in the systemic circulation and also reduces fasted blood glucose and plasma insulin concentrations. Furthermore, training was associated with increased concentrations of potent anti-inflammatory cytokine IL-10. The reduction in the risk factors for cardiometabolic diseases suggest that regular training protects against the early onset of conditions such as type 2 diabetes in young people.
- *The measurement of physical fitness in adolescents*: The cross-sectional analysis (presented in Chapter IV) and longitudinal follow-up (Chapter VII) both suggest that distance run on the MSFT is the best measure of physical fitness in adolescents, when assessing the role of performance on physical capacity tests on cardiometabolic health in young people. Performance on the MSFT consistently throughout the thesis detected significant effects on the concentration of pro-inflammatory cytokines (IL-1β and IL-6) and fasted blood glucose and plasma insulin concentration across a heterogeneous sample of adolescents.

  Furthermore, in the longitudinal follow-up study, after two years of continued training performance in the MSFT improved showing sensitivity to change in training status whereas $\dot{V}O_2$ peak was unchanged. This was further supported by the Pearson

correlations that revealed that longitudinally and across the collated data from Chapters IV - VI that only the MSFT was related to risk factors for metabolic health. Therefore, when assessing physical fitness, as a marker of cardiometabolic health and performance in adolescents, the present thesis suggests that the MSFT is the most suitable measure.

- *The importance of CRP in adolescence as a predictor for cardiometabolic disease risk:* In the present thesis change in CRP concentration during adolescence was related to change in diastolic and mean arterial blood pressure. CRP in adulthood is deemed the strongest predictor of a cardiovascular event, and given the relationship between change in CRP and change in blood pressure (a major risk factor for hypertension which is a form of cardiovascular disease) as observed, it can be suggested that CRP is important throughout the entire lifespan and should be measured consistently from childhood to monitor cardiometabolic disease risk.
- *The inflammatory, glycaemic and insulinaemic responses to acute bouts of intermittent activity*: The findings presented in this thesis suggest that an acute bout of high intensity intermittent exercise (60 min in duration) successfully stimulated an anti-inflammatory response (Chapter V) and reduced the glycaemic and insulinaemic response to a standardised lunch (Chapters V and VI). Such protective responses suggest that intermittent activity, which is an ecologically valid mode of exercise in young people, would be a suitable mode of activity to elicit the inflammatory, glycaemic and insulinaemic responses that are associated with enhanced cardiometabolic health.
- *The time course of the effect:* Finally, the present thesis was novel in that the studies conducted prolonged the time course in which the inflammatory, glycaemic and insulinaemic responses to acute bouts of exercise were observed. Through assessing the inflammatory response up to 24 h post-exercise, an anti-inflammatory response was reported with increased IL-10 concentrations. Furthermore, through determining that

the glycaemic and insulinaemic responses to a standardised breakfast the day following exercise did not differ from a rested control trial, the present thesis suggests that in conjunction with the government physical activity guidelines, young people should participate in 60 min of exercise per day to benefit from the enhanced insulin sensitivity achieved post-exercise.

To ensure the findings reported in the present thesis have impact on adolescents, beyond those that participated in the research, there will be a concerted effort to discuss the main conclusions with key stakeholders such as Nottingham City Council and the Active Partnership Trust (Active Notts and Active Derbyshire), with the ultimate aim of engaging young people with physical activity. The information will be used to emphasise the role of physical activity and exercise as a modifiable lifestyle factor for the enhancement of cardiometabolic health in young people, which is often an oversight with the emphasis frequently placed on nutrition practices in adolescents and their families. The information will be used to detail the potential for intermittent exercise of 30 min in duration to enhance metabolic health in adolescent girls, where overweight, obesity and physical inactivity are of major concern (for overview, see Hardman & Stensel, 2009). The findings of the present thesis are a good foundation for this practice but further research is warranted to elucidate the optimum exercise duration, intensity and frequency to attenuate risk factors for cardiometabolic diseases in adolescents; and how these can be incorporated in to the everyday lives of young people (with consideration of the most appropriate behaviour change models).

8.6 Recommendations for Future Research

To continue to advance knowledge regarding the prevention of risk factors for cardiometabolic diseases in adolescents, the following suggestions are recommended for future research:

- Given the limited longitudinal research available in young people, a series of studies should be undertaken that examine the effect of training on risk factors for cardiometabolic diseases throughout adolescence and, where possible, tracking these changes into adulthood.
- A series of investigations to determine the optimum mode, intensity, duration and frequency of exercise that best elicits the protective post-exercise inflammatory, glycaemic and insulinaemic responses should be undertaken.
- Given young people typically accumulate their daily physical activity through shorter bouts of exercise, a series of studies investigating the effect of accumulated bouts of intermittent activity *vs.* prolonged bouts on the inflammatory, glycaemic and insulinaemic responses in adolescents are recommended.
- Finally, to continue to develop understanding of the relationship between physical activity and exercise with risk factors for cardiometabolic diseases, training/ intervention studies which focus on ecologically valid modes of exercise in young people should be completed to aid with the development of future interventions and policies for the enhancement of cardiometabolic health.

In all future studies it remains important to assess a comprehensive panel of inflammatory cytokines alongside the glycaemic and insulinaemic responses. These studies could progress the findings of the present thesis and assess additional inflammatory cytokines including IL-1ra, IL-4, IL-13 and IL-15, which are additional anti-inflammatory cytokines. Furthermore, the measurement of the risk factors in young people should be ecologically valid (e.g during periods of time that include the consumption of meals) and should accurately measure the metabolic responses in acute studies (such as selecting a measure of peripheral and not hepatic insulin sensitivity).

8.7 Reflections

Throughout the five years in which I have completed my PhD I have developed many new skills, established through the leadership of my supervisory team and the novel experiences that each of the experimental chapters provided. The main learning was that research conducted in the field, particularly when working with children and adolescents, requires a team of researchers that have an array of different skills and copious energy to set up a temporary laboratory in a classroom before conducting a full day of data collection in inquisitive young people. This was a learning curve as I have always been independent in my learning but soon realised that if I continued with this mind-set, data collection for the experimental chapters would not be successful. This realisation coincided with the development of leadership skills during the testing sessions, which were essential as other members of the research team would not know the study protocol in detail and thus relied on my instructions and timings throughout the day to ensure timely measurements were made.

Another reflection that warrants mention is that there is a limit to the number of measurements young people (or participants of any nature) can be expected to complete. Whilst consideration and measurement of all potential confounding variables that might affect the study outcomes is important, it is not feasible to expect participants to complete an infinite number of measurements, whilst maintaining sufficient motivation to complete each of the chosen measurements well. This was a learning curve during my initial experimental chapter, which in addition to all reported measurements included the completion of a physical activity questionnaire (Appendix E). Despite several attempts, the majority of participants did not complete nor return the physical activity diaries and as such the data has not been examined due to incompleteness.

Finally, if I were to be provided with the opportunity to complete the PhD again there are a few changes that I would make to improve the thesis. These include changing the participants that were recruited to experimental Chapters V and VI, thus examining the effects of acute bouts of intermittent exercise on the inflammatory, glycaemic and insulinaemic responses in overweight/obese adolescents. The change to the weight classification of the participants would be made, as following the cross-sectional findings, these individuals present with increased risk factors for cardiometabolic diseases and thus warrant intervention. Advancing understanding of the physiological responses to acute bouts of intermittent exercise in overweight adolescents would provide pertinent information relating to modes and durations of exercise that can be prescribed in this specific population. Furthermore, for experimental Chapters V and VI, I would have collaborated with a psychologist to examine the perceptions of the exercise, to determine whether intermittent activity is deemed engaging and fun by adolescents, as currently this is only speculated throughout the thesis. These reflections provide further scope for future research within the field to advance understanding and also increase the likelihood of the research having impact in wider society.

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Appendix A

Exercise and Health Study

PARTICIPANT ASSENT FORM

- I have read the participant information sheet and understand what I am being asked to do in this study.
- I have talked about this with my parent/guardian/care-giver and they agree that I can take part in the study.
- The purpose and details of the study have been explained to me and I understand that the study involves:

- Completing intermittent running exercise
- Consuming breakfast (cornflakes and toast) and lunch (sandwich, crisps and apple)
- Blood pressure measurements
- Capillary (fingertip) blood samples
- Completing computerised cognitive function tests (short computer tests) and a mood questionnaire

- I have had an opportunity to ask any questions about taking part in the study.
- I understand that there are some risks of taking part in this study but these risks have been minimised and I am not worried about taking part.
- I have been told that I can stop taking part at any time if I change my mind and that I will not have to provide a reason for this.
- If I am worried or want to stop taking part, I just have to talk to Karah Dring (karah.dring@ntu.ac.uk). I can also ask my parent/guardian/care-giver to talk to Karah Dring (karah.dring@ntu.ac.uk) if I am worried but do not want to say so myself.

I agree to take part in this study

Name of participant: ……

Signature of participant: ……

Signature of Researcher: …….....

Date: ……

Appendix B

Parental Consent

1) I, …… *[name of parent/guardian]* agree for my child/dependent, …… *[name of participant] to* partake as a participant in the above study.

2) I understand from the participant information sheet, which I have read in full, and from my discussion(s) with …… *[name of investigator]* that this will involve my child/dependent completing a familiarisation session and three main trials consisting of differing durations of high intensity intermittent running to determine the effect of exercise duration on adolescent health and cognitive function. My child/dependent will undergo health measures including fingertip blood samples, blood pressure, exercise tests and cognitive function tests.

3) It has also been explained to me by …… *[name of investigator]* that the risks and side effects which may result from my child/dependent's participation are as follows: slight bruising on the fingertips from the blood samples, maximal exercise may cause delayed onset muscle soreness and the high intensity exercise may result in risks to health and in extreme cases can be a cause of sudden death. However, in active individuals the risks are minimal and all individuals who wish to take part in this study will complete a health history questionnaire beforehand which will be thoroughly checked by the lead investigator.

4) I confirm that the study has been explained to my child/dependent and that I and my child/dependent have had the opportunity to ask questions about the study. Where we have asked questions, these have been answered to our satisfaction.

5) I undertake to abide by University regulations and the advice of researchers regarding safety.

6) I am aware that I can withdraw my consent for my child/dependent to participate in the procedure at any time and for any reason, without having to explain my withdrawal and their personal data will be destroyed.

7) I understand that any personal information regarding my child/dependent, gained through their participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning my child/dependent appears within published material, their identity will be kept anonymous.

8) I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information provided through my child/dependent's participation in this study, in the form of health screens, questionnaires, blood samples and cognitive function test data will be handled in accordance with this policy.

9) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent my child/dependent from partaking in this research.

If you wish, you can be contacted at the end of the study to be made aware of the results.

To be completed by parent/guardian/care-giver:

Parent/Guardian signature: Date:

Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix C
Health Screen Questionnaire

To be completed by parent/guardian/care-giver

Name or Number .....

Please complete this brief questionnaire to confirm fitness of your child/ dependent to participate:

1. **At present,** do you have any health problem for which you are:

(a)	on medication, prescribed or otherwise	Yes ☐	No ☐
(b)	attending your general practitioner	Yes ☐	No ☐
(c)	on a hospital waiting list	Yes ☐	No ☐

2. **In the past two years,** have you had any illness which require you to:

(a)	consult your GP	Yes ☐	No ☐
(b)	attend a hospital outpatient department	Yes ☐	No ☐
(c)	be admitted to hospital	Yes ☐	No ☐

3. **Have you ever** had any of the following?

(a)	Convulsions/epilepsy	Yes ☐	No ☐
(b)	Asthma	Yes ☐	No ☐
(c)	Eczema	Yes ☐	No ☐
(d)	Diabetes	Yes ☐	No ☐
(e)	A blood disorder	Yes ☐	No ☐
(f)	Head injury	Yes ☐	No ☐
(g)	Digestive problems	Yes ☐	No ☐
(h)	Heart problems	Yes ☐	No ☐
(i)	Problems with bones or joints	Yes ☐	No ☐
(j)	Disturbance of balance / coordination	Yes ☐	No ☐
(k)	Numbness in hands or feet	Yes ☐	No ☐
(l)	Disturbance of vision	Yes ☐	No ☐
(m)	Ear / hearing problems	Yes ☐	No ☐
(n)	Thyroid problems	Yes ☐	No ☐
(o)	Kidney or liver problems	Yes ☐	No ☐
(p)	Allergy to nuts, alcohol etc.	Yes ☐	No ☐

(q) Any problems affecting your nose e.g. recurrent nose bleeds Yes ☐ No ☐

(r) Any nasal fracture or deviated nasal septum Yes ☐ No ☐

4. **Has any,** otherwise healthy, member of your family under the age of 50 died suddenly during or soon after exercise? Yes ☐ No ☐

5. Are there any reasons why blood sampling may be difficult? Yes ☐ No ☐

6. Have you had a blood sample taken previously? Yes ☐ No ☐

7. Have you had a cold, flu or any flu like symptoms in the last Month? Yes ☐ No ☐

8. Does your child/dependent have any dietary requirements we need to be aware of?

Yes ☐ *No* ☐

If YES, please describe briefly the dietary requirements of your child/dependent:

.....
.....
.....
.....
.....
…

Please complete the below contact details so that a member of our research team are able to contact you in the event of an emergency:

Name: _____

Relationship to child/dependent: _____

Contact Telephone Number: _____

Appendix D

RPE Scale (Borg, 1998)

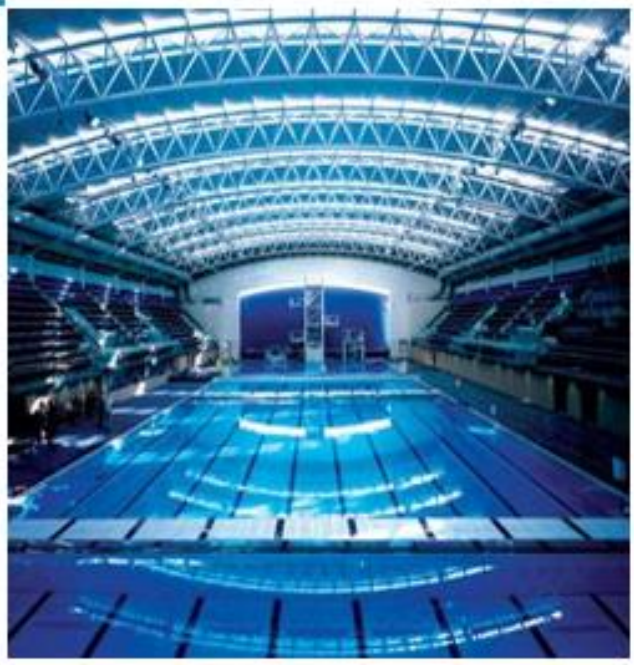
Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix E

Physical Activity Diary Example

Swimmer's Activity Log Book October 2015

Effect of Different Modes of Exercise on Adolescent Health & Fitness Research Project



NOTTINGHAM TRENT UNIVERSITY

Instructions:

NOTTINGHAM TRENT UNIVERSITY

Once you have fully completed this activity diary in the following pages please return to the coach who gave it to you.

You are being asked to complete this diary, detailing a week of training and physical activity during the month of October 2015.

- **In this activity log next to each question or statement there are a series of larger and smaller boxes which need to be completed. For example, the longer boxes require a written response -**

Full Name:	

and smaller boxes require a cross or tick next to the response that best suits your activity or experience -

I am aged …	11 years old		**(tick/cross the box which describes you)**
	12 years old		

If you make a mistake, block out the box and then put a tick in the box you prefer. Try to make your answer as clear as possible.

- **Answer as many questions as honestly as you can.**
- **The activity log is for one full training week in October 2015, all traning detailed must be from the same week, for example from Sunday 4th October to Saturday 10th October.**
- **Try to fill in the diary each day and not at the end of the training week, as it will be difficult to remember the physical activity you completed accurately even from just a few days ago.**
- **All your answers will be kept confidential. The purpose of the study is to determine the effects of different modes of physical activity on the health and fitness of young people like yourself.**
- **Thank you in advance for your time and efforts in competing this activity diary. If you have any questions please do not hesitate to contact Karah Dring, *e-mail:* *karah.dring@ntu.ac.uk***

Example Activity Diary!

NOTTINGHAM TRENT UNIVERSITY

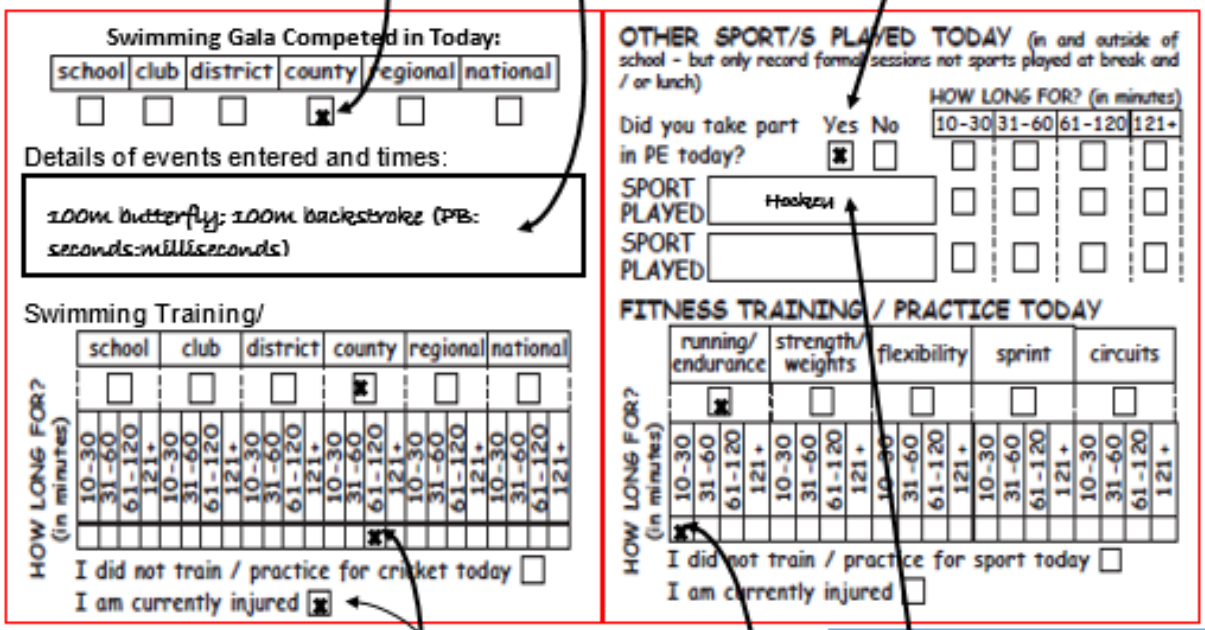
Remember:

Complete as many questions as you can for each day!

5. If you compete in a swimming gala for your county on Friday October 2nd 2015, then put a tick or cross here …

4. Detail the events you entered e.g. 100m Butterfly, 100m Backstroke and whether you achieved a PB…

3. If you took part in PE on Friday 2nd October, indicate YES here and state for how long…



2. If you completed swimming training on Friday October 2nd 2015 state where and for how long with a tick or cross…

1. If you played another sport such as hockey on this day, write this in and indicate how long for …

6. If you are injured and therefore unable to train this day let us know …

7. If you ran for fitness on this day put a cross in the box and indicate how long for …