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Effect of physical activity and fitness on risk factors for cardiometabolic disease and cognitive function in adolescents

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Abstract

Although there is growing evidence that both physical activity and physical fitness are important during adulthood in attenuating the risk factors for cardiometabolic disease, and in enhancing cognitive function, there is limited information on their effects during adolescence which are important years of growth. Therefore, this thesis examines the importance of physical activity and physical fitness for both cardiometabolic health and cognition during these vital adolescent years.

Throughout the thesis, blood glucose and plasma insulin concentrations were measured in the fasted state to calculate the homeostatic model assessment of insulin resistance (HOMA-IR), as well as in the 3 h postprandial window following a standardised, ecologically valid, meal. Furthermore, in Chapters IV and VIII a range of pro- and anti-inflammatory cytokines (IL-6, IL-10, IL-15 and IL-1β) were also measured as an indication of low-grade chronic inflammation. These measures formed the central focus of the risk factors for cardiometabolic disease. The battery of cognitive function tests used in the present thesis was administered via laptop computers. The Stroop test (Chapters IV, VII and VIII; assessing executive function), Sternberg paradigm (Chapters IV, VII and VIII; assessing working memory), the Flanker test (Chapter IV and VIII; assessing executive function) and the visual search test (Chapter IV; assessing visual perception) were used to provide a holistic view of cognitive function. Additionally, brain-derived neurotrophic factor (BDNF) was also measured in Chapters IV, VII and VIII.

The first experimental chapter (Chapter IV) examined the cross-sectional associations between physical fitness (measured by distance covered on the multi-stage fitness test), device measured physical activity and adiposity, and risk factors for cardiometabolic disease and cognitive function in 113 adolescents (63 girls). These associations were examined in the group overall, as well as separately for year 7 (11-12 years, n = 70, 35 girls) and year 10 (14-15 years, n = 43, 27 girls) participants. The main findings of chapter IV were that adolescents with a higher physical fitness had faster response times for several cognitive domains (working memory, executive function, and visual perception), and a higher IL-15 concentration. When considering novel physical activity metrics from accelerometery derived data, greater volume (β = -0.55 mmHg, p = 0.005) and more high-intensity activity (β = 10.92 mmHg, p = 0.006) were associated with lower mean arterial blood pressure; with a stronger association in year 10 participants. Waist circumference was positively associated with systolic blood pressure in the sample overall (β = 0.32 mmHg, p = 0.038), and positively associated with IL-6 concentration in the year 10 participants only (β = 0.03%, p = 0.026). Furthermore, when considering the differences between year group and sex, the key findings were that year 7 girls had higher concentrations of pro-inflammatory cytokine IL-6 (2.59 ± 1.33 pg·ml^{-1}), when compared to boys of the same age (1.70 ± 1.06 pg·ml^{-1}, p < 0.001) and when compared to year 10 girls (1.17 ± 0.55 pg·ml^{-1}, p < 0.001). This study highlights the importance of physical activity, physical fitness and adiposity, for both cardiometabolic health and cognition during adolescence. It also draws attention to the less favourable cardiometabolic health profile in year 7 girls.

The second experimental chapter (Chapter V) examined the determinants of the postprandial glycaemic and insulinaemic response to a standardised meal in a large sample of adolescents (n = 108; 11–13 years). The main findings were that both adiposity (particularly central adiposity, as measured by waist circumference) and physical fitness (measured as distance covered on the MSFT) were important determinants of the postprandial insulinaemic response. Specifically, waist circumference (β = 0.41, p < 0.001) and HOMA-IR (β = 0.37, p < 0.001) were positively associated, and distance run on the MSFT negatively associated (β = -0.16, p = 0.031), with the postprandial insulinaemic response; and collectively they explained 51% of the variance. There was no association between the predictors used and the glycaemic postprandial response (R^2 = 0.03, p = 0.198). This study shows that high physical fitness attenuates, and adiposity exacerbates, the postprandial insulinaemic response to a standardised meal. In addition, physical fitness was an independent determinant (even when adiposity was accounted for) of the postprandial insulinaemic responses.

The third experimental chapter (Chapter VI) examined the effect of football activity and physical fitness (overall effect and moderating effect on the response to exercise) on glycaemic and insulinaemic responses to an ecologically valid, standardised meal in adolescents (n= 36 (16 girls), 11-13 years). The main findings were that postprandial insulin tAUC, following an ecologically valid lunch, was 70% lower in high-fit participants compared to low-fit (high-fit: 3785 pmol·L^{-1} x 120 min vs. low-fit: 6457 pmol·L^{-1} x 120 min, p = 0.002). Furthermore, the acute bout of football led to a 21% reduction in blood glucose concentration pre-lunch (football: 3.8 mmol·L^{-1} vs. control: 4.6 mmol·L^{-1}, p < 0.001). This study demonstrates that an acute bout of football reduces blood glucose concentration transiently following exercise, although the postprandial glycaemic and insulinaemic responses were not affected by exercise. Furthermore, these data support the findings of Chapter V demonstrating that postprandial insulin tAUC is lower in those with a higher physical fitness.

The fourth experimental chapter (Chapter VII) examined the acute effects of the football activity (same participants as Chapter VI) and physical fitness on cognitive function and BDNF concentration in adolescents. The main findings were that response times were consistently quicker in high-fit adolescents, compared to their low-fit counterparts (executive function; high-fit: 960 ± 209 ms vs. low-fit: 1084 ± 243 ms, p < 0.001. working memory; high-fit: 761 ± 151 ms vs. low-fit: 834 ± 207 ms, p < 0.001). Additionally, the exercise-induced improvements in response times on a working memory task were exclusive to high-fit participants (football: 476 ± 85 ms vs. control: 507 ± 120 ms, p = 0.022). The present study was also the first to measure the time-course of changes in BDNF concentrations post-exercise in adolescents, with no effect of exercise observed. Together, chapters VI and VII provide evidence of the potential benefits of games-based activity for both cardiometabolic health and cognition in adolescents.

The final experimental chapter (Chapter VIII) examined the effects of a short-term (2 weeks) sprint-training intervention on risk factors for cardiometabolic disease and cognitive function in adolescent girls (n = 16, 11-12 years). The main findings were that 2 weeks of sprint training improved accuracy on a working memory task (adjusted between group difference; 2.0%, 95%CI [0.02, 3.9%], p = 0.046) and increased BDNF concentration (adjusted between group difference; 17.89 ng·ml^{-1}, 95% CI [10.7, 25.1 ng·ml^{-1}], p < 0.001) in adolescent girls. This is also the first study to assess the effect of chronic exercise training on markers of low-grade chronic inflammation and the postprandial response to a standardised meal in adolescent girls, which were not affected.

In summary, the present thesis provides novel contributions to the literature with the following key findings: i) Physical fitness is crucial in reducing postprandial markers of cardiometabolic health and in enhancing response times during cognitive function tests in adolescents; ii) Adiposity is an important determinant of the magnitude of postprandial markers of metabolic health; iii) An acute bout of football – which is an ecologically valid mode of exercise that reflects the habitual activity patterns of young people – is successful at reducing blood glucose concentrations, as well as selectively improving working memory in those considered high-fit; iv) Although short-term sprint training did not improve markers of cardiometabolic health, the present thesis shows that it can improve cognitive function in adolescent girls; v) The present thesis also demonstrates the utility of new physical activity metrics (representing activity volume and intensity) in paediatric research, which have currently not been used in conjunction with a range of risk factors for cardiometabolic disease and cognitive function; vi) Finally, throughout this thesis physical fitness and adiposity were assessed using accessible, relatively non-invasive, methods (MSFT and waist circumference) and therefore highlight their utility and application in paediatric research. Overall, from the data presented in this thesis it is recommended that every effort is made to enhance physical fitness and to reduce adiposity in adolescents to enhance cardiometabolic health and to optimise cognitive function.

Key Words: Adolescents, Physical Activity, Physical Fitness, Exercise, Cognitive Function, Cardiometabolic Disease

List of abbreviations

ANOVA: Analysis of variance

BDNF: Brain-derived neurotrophic factor

HOMA-IR: Homeostatic model assessment of insulin resistance

IL: Interleukin

SD: Standard deviation

MVPA: Moderate-to-vigorous physical activity

HR: Heart rate

GPS: Global positioning system

tAUC: Total area under the curve

MSFT: Multi-stage fitness test

$V\dot{O}_{2peak}$: Peak oxygen uptake

$V\dot{O}_{2max}$: Maximum oxygen uptake

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Preface

Unless otherwise indicated by reference to published resources, 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 in this thesis has been published:

Published Papers

Williams, R. A., Cooper, S., Dring, K. J., Hatch, L., Morris, J. G., Sunderland, C., & Nevill, M. E. (2021). Effect of acute football activity and physical fitness on glycaemic and insulinaemic responses in adolescents. *Journal of Sports Sciences*, 1–9. doi: 10.1080/02640414.2020.1860362

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Williams, R., Dring, K., Cooper, S., Morris, J., Sunderland, C., & Nevill, M. (2020). Predictors of postprandial glycaemia, insulinaemia and insulin resistance in adolescents. *British Journal of Nutrition, 1-27.* doi: 10.1017/S0007114520003505

Published Communications

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Chapter I

Introduction

Increased physical activity in children, adolescents and adults is important for health across the lifespan and has been associated with the prevention of premature mortality from non-communicable diseases (Lee et al., 2012), reduced all-cause mortality (Ekelund et al., 2019), reduced risk of cardiometabolic diseases (Pedersen & Saltin, 2015) and improved cognition (Hötting & Röder, 2013). Unfortunately, levels of physical activity decline throughout the period of adolescence, at an estimated rate of 7% each year between the ages of 10 – 19 y (Dumith et al., 2011) and physical activity further declines during the transition between adolescence and young adulthood (Corder et al., 2017). Despite these evident declines in physical activity with age, it has been recognised that an active lifestyle can be instilled during childhood and adolescence, with those who are more active as children and adolescents maintaining high activity levels during adult years (Telama et al., 2014). Additionally, girls are consistently shown to be less active than boys at all ages (Dumith et al., 2011; Townsend et al., 2015). Therefore, the monitoring of, and interventions to increase, physical activity during adolescence in boys and in girls, are important for lifelong health, with estimates that 6 – 10% of non-communicable diseases, responsible for 9% of premature mortality worldwide, are attributable to physical inactivity; a similar magnitude of risk to smoking (Lee et al., 2012). Furthermore, interventions to increase physical activity in adolescents, and particularly in girls, are needed and should be designed using modalities of exercise that are ecologically valid, thus improving long-term adherence (Bailey et al., 1995; Howe et al., 2010). Despite the obvious need for increased physical activity in adolescents, there is a dearth of information to inform the design of such school-based interventions and a limited understanding of the underlying mechanisms of adaptation (Bond et al., 2017). There is debate whether it is physical activity per se that is important or the associated improvements in physical fitness (Blair, Cheng & Holder, 2001; Buckley, Thijssen & Lip, 2020). It is therefore important to

know which risk factors for non-communicable diseases are attenuated in adolescents with increases in physical activity or fitness and are these attenuations sustained over time (Balagopal et al., 2011; Nadeau et al., 2015).

It would be beyond the scope of this thesis to cover the impact of physical activity and fitness on a wide range of non-communicable diseases, but cardiovascular disease (CVD), type 2 diabetes and the metabolic syndrome are related conditions, considered as a spectrum under the umbrella term 'cardiometabolic disease', and share related behavioural risk factors such as obesity (Vasuvedan & Ballantyne., 2005) and physical inactivity (Lee et al., 2012; WHO., 2017; Vincent et al., 2017). Thus, this thesis will focus the effect of physical activity and fitness on attenuating the risk factors for cardiometabolic disease in adolescents. A state of insulin resistance is suggested to precede the development of these conditions (Guo et al., 2014; Reaven., 2005) with, in addition, increasing recognition of low-grade chronic inflammation as a mediator of these diseases (Dokken et al., 2008; Van Gaal, Mertens & De Block., 2007). The severity of such conditions has been highlighted recently, with CVD recognised as the leading cause of global deaths (Guo et al., 2014). Additionally, in the UK 90% of deaths were attributable to non-communicable diseases, such as CVD and diabetes (WHO, 2017). The shared behavioural risk factors for these conditions (physical inactivity and adiposity) provide a potential target for interventions aimed at preventing the development of such conditions (WHO, 2017).

Although cardiometabolic diseases present during adult years, the development of conditions such as type 2 diabetes and CVD are known to manifest during childhood and adolescence (Balagopal et al., 2011; Warnberg et al., 2007). In a longitudinal study, where participants were examined at baseline (12 – 18 y) and then 21 y later, cardiometabolic health outcomes during adolescence were predictive of disease risk in adulthood (Laitinen et al., 2012). Specifically, the ideal 'child cardiovascular health index' used in adolescents aged 12 to 18 years was associated with reduced risk of hypertension, high LDL cholesterol and metabolic syndrome in adulthood, thus highlighting the importance of adolescent health with respect to cardiometabolic disease development. Although adolescents are generally a disease-free population, there has been an

increase in the prevalence of risk factors for cardiometabolic disease, and subsequently type 2 diabetes incidence, in young people (WHO, 2019). Therefore, the period of adolescence offers a prime opportunity to investigate the relationships between physical activity and physical fitness and risk factors for cardiometabolic disease, alongside the effectiveness of interventions aimed at ultimately enhancing cardiometabolic health.

Increasingly in adults it is recognised that low grade chronic inflammation (a novel risk factor for cardiometabolic disease), plays a key pathophysiological role in the development of CVD and type 2 diabetes (Dokken et al., 2008; Van Gaal et al., 2007), and the positive benefits of physical activity and fitness on low grade chronic inflammation are becoming recognised (Gleeson et al., 2011; Warnberg et al., 2010). However, the effect of physical activity (habitual, as well as acute and chronic interventions) and physical fitness on novel risk factors for cardiometabolic disease (particularly markers of low-grade chronic inflammation and postprandial glycaemia and insulinaemia) in adolescents is poorly understood. It is important that this understanding is enhanced, because low-grade chronic inflammation increasingly presents during adolescence (Balagopal et al., 2011). In addition, there is much debate as to whether physical activity and physical fitness determine cardiometabolic health in adults and particularly in adolescents or rather is it the associated changes in adiposity that are more impactful (Bird & Hawley, 2017). Thus, there is a need for further studies that examine the related contributions of physical activity, physical fitness and adiposity on cardiometabolic health in adolescents.

In terms of interventions to increase physical activity and to improve fitness during adolescence, whilst the efficacy of short-term interventions warrant investigation, appropriate modalities for the adolescent population and those which can be easily implemented into a school day also requires consideration. Interventions during the school day are important because nearly all adolescents are in school and thus this represents an effective population intervention (Bond et al., 2017). Sprint training may offer an attractive solution to this, as this activity reflects the short, high-intensity bouts of activity that are natural to adolescents (Bailey et al., 1995), as well as requiring very little time; however, such a modality has received limited attention.

In addition to the potential for physical activity and physical fitness to affect cardiometabolic health in adolescents, another important consideration is the effect on cognitive function. Cognitive function refers to a variety of brain-mediated processes that help to store, process and manipulate information which is important for the activities of daily life (Schmitt, Benton & Kallus., 2005). There are many factors suggested to affect cognitive function, such as physical well-being (Schmitt et al., 2005), mood (Schmitt et al., 2005) and nutrition (Isaacs & Oates., 2008). Furthermore, there is increasing evidence that exercise (performed both acutely and chronically) can beneficially affect cognitive function (Chang et al., 2012; Hötting & Röder, 2013) and neuroplasticity (Hötting & Röder, 2013). However, cognitive function is arguably most important during childhood and adolescence, given the role that these brain functions play in determining academic performance (Diamond, 2013). Furthermore, adolescence is a time when many structural and physiological brain changes occur, partly due to maturational processes (Blakemore et al., 2010; Luna., 2009). It has been highlighted that executive function (Diamond, 2013; Luna, 2009) and working memory (Luna, 2009) are two pertinent domains of cognitive function that undergo the greatest development during adolescence. Although there is much evidence demonstrating benefits of physical activity and fitness for cognitive function in children, there is much less information available on adolescents. Therefore, the interaction between physical activity and cognitive function and between physical fitness and cognitive function in adolescents is an important area of research given the developmental processes and scholastic challenges that occur during this period.

The limited research in adolescents has tended to focus on the domain of executive function; thus more work should expand the domains examined to identify the effect of physical activity and physical fitness across a range cognitive function domains. Much like the acute exercise in the cardiometabolic health literature, a lot of previous cognition-based studies have used exercise protocols that lack ecological validity in adolescent populations. Furthermore, despite the potential for physical fitness to moderate the acute effects of exercise on cognitive function (Chang et al., 2012), there is limited empirical evidence of how this may affect adolescents. Of the limited

evidence examining the effect of training interventions on cognition in adolescents, the focus has been on longer duration (6 weeks and above) and moderate intensity exercise, with no study examining the effects of short-term, high-intensity training. Moreover, few studies (both acute and chronic exercise) have measured outcomes that may be mechanistically linked to improvements seen after an acute exercise bout; one of which is systemic concentrations of brain-derived neurotrophic factor (BDNF).

Therefore, the present thesis will examine the effect of physical activity and fitness on risk factors for cardiometabolic disease and cognition in adolescents by addressing the following research questions and testing the associated hypotheses:

1) What are the cross-sectional associations between physical activity, physical fitness and adiposity with novel risk factors for cardiometabolic disease (inflammatory cytokines) and cognitive function amongst adolescents? In addition, are the relationships different in year 7 and year 10 adolescents?

   Hypothesis: higher physical activity and physical fitness are associated with a favourable profile of risk factors for cardiometabolic disease and better cognitive function, whereas higher adiposity will be associated with a worse profile of risk factors and reduced cognitive function in both young and older adolescents.

2) What are the determinants of the postprandial glycaemic and insulinaemic response to a standardised, ecologically valid mixed-meal (a novel risk factor for cardiometabolic disease) in adolescents? What are the independent associations between key participant characteristics (physical fitness and adiposity) and these responses?

   Hypothesis: a higher physical fitness will be associated with lower postprandial glycaemic and insulinaemic responses. Additionally, a higher waist circumference (adiposity) will be associated with large postprandial glycaemic and insulinaemic response.

3) How does an acute, ecologically valid bout of games-based exercise (football) affect glycaemia and insulinaemia in adolescents? Are these responses moderated by physical fitness?

Hypothesis: an acute bout of football will acutely improve glucose regulation following exercise and reduce the postprandial glycaemic and insulinaemic response to a mixed-meal when compared to a resting control trial. Furthermore, those who are considered higher fit will see a larger reduction in these responses.

4) How does an acute, ecologically valid bout of games-based exercise (football) affect cognitive function and BDNF concentrations? Are these responses moderated by physical fitness?

   Hypothesis: an acute bout of football will transiently improve cognitive function and increase BDNF concentrations when compared to a resting control trial and the magnitude of the football-induced improvement in cognitive function will be of a greater magnitude in those considered high-fit.

5) How does a short-term, sprint-training intervention affect novel risk factors for cardiometabolic disease and cognitive function in adolescent girls?

   Hypothesis: short-term sprint will improve novel risk factors for cardiometabolic disease, improve cognitive function and increase BDNF concentration.

Organisation of the thesis

Chapter II will provide a critical evaluation of the literature examining the effect of physical activity, fitness, and adiposity on risk factors for cardiometabolic disease and cognitive function. Chapter III, the general methods, will describe the methods that have been used extensively throughout the studies and are common to several chapters. Chapter IV will examine the cross-sectional associations between physical activity, fitness, and adiposity with risk factors for cardiometabolic disease and cognitive function across adolescence. Chapter V will examine the determinants of the postprandial glycaemic and insulinaemic responses to a mixed-meal in an adolescent population. Chapter VI will examine the effect of an acute bout of games-based exercise (football) on risk factors for cardiometabolic disease whereas Chapter VII examines the effect of the same bout of exercise on cognitive function. Chapter VIII will investigate the effects of a short-term, school-based, high-intensity exercise intervention on risk factors for cardiometabolic disease and

cognitive function in adolescent girls. Finally, in Chapter IX the key findings from the experimental chapters are discussed, overall conclusions are drawn, and practical applications are stated regarding the importance of physical activity and physical fitness for novel risk factors related to cardiometabolic disease and cognitive function in adolescents.

Chapter II

Review of the Literature

2.1 Overview of the Review of the Literature

The review begins in section 2.2 with a definition and explanation of the key concepts that are central to the thesis including ‘adolescence’, ‘physical activity’, ‘physical fitness’, ‘adiposity’, ‘cardiometabolic disease’ and ‘cognitive function’. Following this, in section 2.3 there is a critical evaluation of the cross-sectional literature examining the associations between physical activity, physical fitness and adiposity with risk factors for cardiometabolic disease and cognitive function. In section 2.4 a critical review of the effect of acute bouts of exercise on glycaemia, insulinaemia and cognitive function is undertaken and finally in section 2.5 the chronic effects of exercise training on risk factors for cardiometabolic disease and cognitive function are examined. Each review section will focus predominantly on literature where adolescents are the participants, but a brief evaluation of literature in adults will be included initially to provide some historical context.

2.2 Definitions and Key Concepts

2.2.1 Adolescents

Adolescence is the period of life, typically starting at the chronological ages of 10 and 12 years in girls and boys respectively (Bogin & Smith, 1996), whereby the body begins the transition towards the adult state, encountering many different maturational changes which occur at different rates between individuals (Beunen, Roga & Malina, 2006).

Due to this inherent variability, maturation is not always synonymous with chronological age, which emphasises the importance of examining maturity when investigating adolescent populations. One of the best indicators of maturation, is the assessment of biological maturity, which examines skeletal maturity through X-ray of the hand and wrist (Beunen et al., 2006). Additionally, maturity can be assessed through development of secondary sex characteristics,

which is often done via Tanner stages (Tanner, 1962). However, the invasive nature of these methods raises ethical and practical issues and non-invasive estimates of somatic maturity, using easily obtainable anthropometric measurements, have been developed (Mirwald et al., 2002; Moore et al., 2015). The somatic prediction models produced by Moore et al. (2015) provide an estimate of maturity offset, which identifies the time (in years) before or after peak height velocity.

In this thesis the participants are referred to as adolescents (rather than children) because they are of secondary school age and will largely be in the adolescent stage. In addition, though in all studies an appropriate measure of maturity is included.

2.2.2 Physical Activity

Physical activity is formally defined as "*any bodily movement produced by the skeletal muscles that results in energy expenditure*" (Caspersen et al., 1985) and can be separated into the sub-categories of sleeping, occupational and leisure. Physical activity is a complex, multi-dimensional behaviour which is commonly characterised by intensity, duration and frequency (Miles, 2007) and the quantity of physical activity is typically examined on a daily or weekly basis (Caspersen et al., 1985). Overall, physical activity is considered to encompass all bodily movements that increases the metabolic rate above resting levels (Miles, 2007). The monitoring of daily physical activity in young people has become a central focus for the UK government (Gibson-Moore, 2019) and global (Bull et al., 2020) public health monitoring. The most recent guidelines state that children and adolescents (5 – 17 y) should achieve at least 60 min of moderate-to-vigorous intensity activity per day (consisting of mainly aerobic activities), and vigorous aerobic activity as well as bone and muscle strengthening exercises to be included at least 3 days a week (Bull et al., 2020). Thus, the emphasis of public health guidelines is placed on physical activity; therefore, it is important to understand how physical activity is measured, and the associations between physical activity and outcomes such as cardiometabolic health and cognitive function.

Physical activity is contemporarily assessed via self-report measures, such as questionnaires, or devices, typically in the form of wrist- or hip-worn accelerometers (Miles, 2007). However, among

youth populations the use of self-report measures is often compromised due to recall bias, inability to understand specific terms (such as frequency, intensity and duration) and social desirability (Trost, 2020). Given this, the device-based approach may be more suitable for assessing physical activity in youth populations. Indeed, technological advances over the years have seen the use of device-based methods increase, together with a proliferation of different methods and analytical procedures (Cain et al., 2013), making it increasingly difficult to make comparisons of physical activity across different studies. Some authors have called for a standardisation of methods (Trost, 2020) and newer methods (Migueles et al., 2019) and metrics (Rowlands et al., 2018) for analysing and interpreting device-based accelerometer data have been proposed (see section 3.7).

2.2.3 Exercise

Formally, exercise is defined as "*physical activity that is planned, structured, repetitive, and purposive in the sense that improvement or maintenance of one or more components of physical fitness is an objective"* (Caspersen et al., 1985). Exercise is considered a sub-category of physical activity, specifically leisure time activity (Miles, 2007).

2.2.4 Physical Fitness

Physical fitness is a set of attributes that an individual has, or achieves, that relates to an ability to perform physical activity and exercise (Miles, 2007). In this thesis when we use the term physical fitness we are referring largely to endurance fitness as there is a vast body of literature in adults showing that high endurance fitness is associated with enhanced cardio-metabolic health (Barry et al., 2014; Lamonte et al., 2005; Ross et al., 2016). Physical fitness is typically the product of increased physical activity, although there is a genetic component (Blair, Cheng & Holder, 2001) with the gold standard assessment of cardiorespiratory fitness often referred to as laboratory measured maximum oxygen uptake or $\dot{V}O_{2peak}$ in children and adolescents, although there is growing recognition of field-based alternatives such as the multi-stage fitness test (Tomkinson et al., 2019). The complexity of physical fitness has been acknowledged and is

subsequently categorised into two domains; health-related and functional capacity (Caspersen et al., 1985). Health-related physical fitness is formally defined as "*traits and capacities that are consistent with minimal risk of developing hypokinetic diseases*" (Pate, 1983), whereas functional capacity is "*the ability to carry out daily tasks with vigour and alertness, without undue fatigue"* (Caspersen et al., 1985). In addition, physical fitness may be considered to reflect training status (determined by type, duration and frequency of physical activity) as indicated by the ability to sustain a high percentage of maximum oxygen update for a prolonged period of time, often referred to as endurance capacity (Hardman & Stensel, 2009).

Physical fitness is important because the associations between physical fitness and disease-related outcomes and mortality are often stronger than the associations between physical activity and disease-related outcomes and mortality (Miles, 2007). The endurance aspect of physical fitness in young people is commonly assessed using the multi-stage fitness test (MSFT) (Tomkinson et al., 2019). The MSFT has been shown to be both a good indicator of health-related fitness and functional capacity, whilst also mimicking the typical activity patterns of youth (Tomkinson et al., 2019) and reflects both $\dot{V}O_{2peak}$ and training status, thus while $\dot{V}O_{2peak}$ (assessed by graded treadmill exercise) was not strongly related with any markers of health in adolescents, the distance run during the MSFT was inversely associated with markers of low-grade inflammation and metabolic status (Dring et al., 2019b). Therefore, it is not surprising that the misuse and misinterpretation of predicting $\dot{V}O_{2peak}$ from MSFT performance has been highlighted in detail (Armstrong & Welsman., 2018), with the estimation of $\dot{V}O_{2peak}$ being described as untenable (Armstrong & Welsman., 2018). Thus, in the present thesis the distance covered during the MSFT, rather than any estimation of $\dot{V}O_{2peak}$ from the MSFT results, will be used as the criterion outcome for physical fitness.

2.2.5 Adiposity

Adipose tissue, once considered as just cushion and insulation for the body, is now recognised as an important metabolic and endocrinological tissue (Shuster et al., 2012). Adipose tissue distribution is dependent on several factors, namely sex, age, ethnicity, diet and physical activity

and can be categorised into two main components; subcutaneous and visceral adipose tissue (Shuster et al., 2012). Visceral adiposity, also referred to as central adiposity, has been distinctly linked with conditions such as insulin resistance (Tagi, 2019) as well metabolic syndrome and cardiovascular disease (Schwandt., 2011; Shuster et al., 2012). Visceral adipose tissue is more metabolically active and is known to secrete inflammatory cytokines – particularly IL-6 – that are involved in the development of insulin resistance (Tagi, 2019) and other cardiometabolic diseases (Shuster et al., 2012). The physiological importance of visceral adipose tissue highlights the need to quantify or estimate the amount of visceral adipose issue in adults and adolescents.

Computerised tomography and magnetic resonance imaging techniques are the most precise methods for examining visceral adiposity, but these techniques are expensive, labour intensive and require expert training to perform (Shuster et al., 2012; Klein et al., 2007). Indeed, in a research setting and particularly with younger populations, these methods are not necessarily accessible and feasible. Simple and accessible anthropometric measurements provide the ideal balance between practicality and validity, with waist circumference providing the best non-invasive and simple index of central adiposity (Schwandt, 2011; Klein et al., 2007), but the individual contributions of subcutaneous and visceral adipose tissue to central adiposity cannot be deciphered (Klein et al., 2007). Nonetheless, there is strong evidence for the utility of such a marker when assessing risk factors for cardiometabolic disease (Klein et al., 2007), and as a marker of childhood adiposity it was the best predictor of metabolic syndrome prevalence during early adulthood (Schmidt et al., 2011). Thus, in the present thesis waist circumference will be the primary surrogate for the assessment of adiposity.

2.2.6 Cardiometabolic Disease

Cardiometabolic disease is a spectrum of disorders whereby, initially, insulin resistance develops which leads to subsequent developments of related conditions, such as the metabolic syndrome, type 2 diabetes and cardiovascular disease (Guo et al., 2014). These conditions are grouped together under the term ‘cardiometabolic disease’ as they share many behavioural risk factors, such as obesity (Vasuvedan & Ballantyne, 2005) and physical inactivity (Lee et al., 2012; Vincent

et al., 2017). The consequences of cardiometabolic disease are severe in that an estimated 70% of worldwide deaths are attributable to non-communicable disease (WHO, 2017). Lee et al. (2012) suggest that 6 – 10% of non-communicable diseases are attributable to physical inactivity and are thus responsible for 9% of premature mortality.

2.2.6.1 Type 2 Diabetes

An estimated 422 million adults living with diabetes in 2014 (WHO, 2019), but there are increasing numbers of cases within children and adolescents which rose from 7% in 1999-2000 to 13.1% in 2005-2006 (Chen et al., 2011). Diabetes, which is a group of metabolic disorders, is typically characterised by hyperglycaemia in the absence of treatment (WHO, 2019). The long-term effects of diabetes include retinopathy, nephropathy and neuropathy and predisposes individuals to increased risk of other conditions, such as cardiovascular disease (WHO, 2019). There are two distinct types of diabetes; type 1 diabetes is characterised by β-cell destruction (which is primarily mediated by the immune system) and a lack of insulin production whereas type 2 diabetes is characterised by β-cell dysfunction, resistance to the effects of insulin and is associated with overweight/obesity (WHO, 2019). A common mechanism underpinning the development of both types of diabetes is insulin resistance, which is implicated in β-cell dysfunction and destruction.

Peripheral insulin resistance (muscle and adipose tissue) is a key characteristic in the development of type 2 diabetes (Sesti, 2006). There are many causes of insulin resistance, but it is primarily excess adipose tissue - which exerts pro-inflammatory effects on insulin signalling (Tagi, 2019) – that is thought to be responsible. The chronic presence of insulin resistance ultimately leads to β-cell dysfunction and chronic hyperglycaemia, which subsequently initiates the development of type 2 diabetes and is also related to the development of other diseases, such as cardiovascular disease.

2.2.6.2 Cardiovascular Disease

Cardiovascular disease (CVD) is considered the worldwide leading cause of death, with an estimated 31% of deaths (17.9 million) during 2016 attributable to CVD (WHO, 2017). In the UK,

despite the recent decline of CVD-related mortality, it remains a leading cause of death and is also responsible for 18 – 25% of premature deaths (Bhatnagar et al., 2015). Cardiovascular disease collectively refers to many disorders that are broadly related to the functioning of the heart and circulatory vessels. Traditional risk factors for CVD include, but are not limited to, age, smoking, high LDL cholesterol, hypertension, physical inactivity, diet and dysfunctions to glucose metabolism (Van Gaal, Mertens & De Block, 2007). Endothelial dysfunction is another associated risk factor, which has been identified as an early step in atherogenesis (Hadi, Carr & Suwaidi, 2005).

Atherogenesis is the development of atherosclerotic plaques within arterial walls, which begins with the accumulation of lipids and immune cells, subsequently developing to lesions that are rich with inflammatory cells (Weissberg., 2000). Chronic exposure to this low-grade inflammatory environment leads to an accumulation of lipids which coalesces into a necrotic plaque, reducing vascular function and the ability to provide sufficient nutrient supply to tissues (Libby & Theroux., 2005), thus leading to the development of symptoms associated with cardiovascular disease (Hardman & Stensel, 2009).

2.2.7 Assessment of Cardiometabolic Disease Risk

Among the risk factors associated with cardiometabolic disease, inflammation is becoming increasingly recognised and has been implicated in both type 2 diabetes and CVD development (Dokken et al., 2008; Van Gaal, Mertens & De Block, 2007). The presence of chronic low-grade inflammation is said to precede the onset of diabetes and is partly attributed to the development of insulin resistance, which might provide a link between type 2 diabetes and CVD (Dokken et al., 2008; Esser et al., 2014). Low-grade chronic inflammation is characterised by a 2- to 3-fold increase in systemic concentrations of Tumour Necrosis Factor-α (TNF- α), interleukin (IL)-1, IL-6, IL-1ra and C-Reactive Protein (Pedersen, 2006). Indeed, the presence of chronic low-grade inflammation in adults is typically examined through the assessment of systemic CRP concentrations (Balagopal et al., 2011; Gleeson et al., 2011; Van Gaal, Mertens & De Block,

2007). IL-6 and IL-1β are primarily cited as the main inflammatory mediators in cardiometabolic disease development (Esser et al., 2014).

Cytokines are known for their inflammatory role, but of particular interest is IL-6; which is a pleiotropic cytokine implicated in many physiological roles (Hoffman & Weigert, 2017; Pedersen, 2006; Nielsen & Pedersen, 2007). Visceral adipose tissue secretes IL-6, which is surmised to mediate the development of insulin resistance (Shuster et al., 2012). However, IL-6 is widely regarded as the prototypical exercise factor that is secreted from skeletal muscle in response to contraction and acts both locally and systemically (Gleeson et al., 2011; Hoffman & Weigert., 2017; Pedersen & Febbraio, 2008). When released in response to exercise, IL-6 is suggested to induce an anti-inflammatory cascade – stimulating the production of anti-inflammatory IL-10 and IL-1 receptor antagonist (IL-1ra) – as well as suppressing TNF-α production (Gleeson et al., 2011; Pedersen & Febbraio, 2008). These mechanisms support the notion that regular physical activity can reduce chronic low-grade inflammation (Gleeson et al., 2011; Hoffman & Weigert, 2017; Pedersen & Febbraio, 2008) and potentially insulin resistance (Dokken et al., 2008).

Typically, simple assessments of insulin resistance can be conducted with just a fasting blood sample for the measurement of glucose and insulin, which is subsequently used to derive the homeostatic model assessment of insulin resistance (HOMA-IR) (Matthews et al., 1985). Whilst simple and effective, this method only provides a reflection of hepatic insulin resistance and does not reflect peripheral insulin resistance (Abdul-Ghani et al., 2007; Muniyappa & Madan, 2000). Direct measurements of insulin resistance are obtained through the hyperinsulinaemic euglycemic clamp technique (Muniyappa et al., 2008), but this technique is time consuming, expensive and requires experienced operators and specialist equipment (Muniyappa et al., 2008). An alternative indirect, assessment is the use of an oral glucose tolerance test (OGTT) (Muniyappa et al., 2008). Indeed, the use of post-load (following an OGTT or a test meal) assessments is gaining recognition for the assessment of insulin resistance (DiNicolantonio et al., 2017; Lautt, 2007) and glucose tolerance (Cavalot et al., 2011; Monnier et al., 2007). However, the use of an OGTT is not necessarily ecologically valid – particularly for an adolescent population

– therefore the postprandial responses to mixed-meals, which typically reflect what young people consume, should be examined.

2.2.8 Risk of Cardiometabolic Disease in Adolescents

Whilst young people are generally a disease-free population, the manifestations of diabetes and CVD are known to begin in early life (Balagopal et al., 2011; Warnberg et al., 2007). Furthermore, there is strong evidence that markers of poor cardiometabolic health assessed during childhood track into cardiometabolic disease during adulthood (Laitinen et al., 2012). A state of insulin resistance is common amongst adolescents, due to pubertal effects, but this is also further complicated by the presence of excess adiposity during this period (Tagi, 2019). Additionally, the number of adolescents that are presenting with type 2 diabetes is increasing each year (WHO, 2019). The presence of such risk factors at early ages and thus prolonged exposure will ultimately lead to early onset of related conditions, which is a cause of concern given the associated development of CVD with insulin resistance and type 2 diabetes. Therefore, identifying methods to reduce the severity and prevalence of such risk factors in adolescence is important for lifelong health.

2.2.9 Cognitive Function

Whilst there is no formal definition of cognitive function, it has been summarised as a "*variety of brain-mediated functions and processes that allow us to perceive, evaluate, store and manipulate information*" (Schmitt, Benton & Kallus, 2005). Schmitt et al. (2005) distinctly categorised cognitive functions into six separate domains; memory, psychomotor, attention, perception, executive functions and language skills (Fig 2.1). The six domains can also be further categorised into specific sub-domains, for example attention can be selective, sustained or divided. Despite the separation into separate domains, they are not mutually exclusive, and in fact are partly reliant on one another; the efficiency of one domain can be dependent on the others (Schmitt et al., 2005). For example, new information cannot be stored (memory) unless the appropriate

perception and attention are in place to identify and process that information. The division into domains, in part, makes it easier to objectively examine cognitive function.

Executive function is a domain that encompasses three interconnected sub-domains; inhibitory control, cognitive flexibility and working memory (Diamond, 2013) and refers to top-down mental processes such as reasoning, problem solving, planning, goal-directed behaviour, overriding natural responses, and periods of intense concentration and focus (Diamond, 2013; Jurado & Rosselli, 2007). Importantly, cognitive function can be improved across any age in the life cycle (Diamond, 2013). Indeed, the period of adolescence is crucial for the development of executive functions, which are suggested to be instrumental in everyday learning situations, such as those in a school setting, as well as daily life activities (Diamond, 2013).

Neuropsychological tests, often computer-based to facilitate response capture, have been designed to assess each cognitive function domain with a degree of specificity and can be adapted to suit the population of interest, i.e. children and adolescents (Schmitt et al., 2005). Performance on tests is ideally assessed via both the speed and accuracy of responses; an important consideration to check for the potential for a speed-accuracy trade-off (Schmitt et al., 2005).

Cognitive function is important for the processing of daily mental tasks and for success in the classroom (Diamond, 2013), which becomes particularly apparent during adolescence when public exams loom. The combination of hormonal fluctuations and structural changes to various brain regions during adolescence leads to differential developments of cognitive function (Blakemore et al., 2010). Executive function (Diamond, 2013; Luna, 2009) and working memory (Luna, 2009; Ryan, 2009) are documented as the two domains most pertinent in adolescent development. Thus, identifying interventions to enhance executive function and working memory could enhance the life chances and quality of life of young people. Undertaking more physical activity and enhancing physical fitness present related possibilities by which cognitive function could be improved while at the same time reducing the risk factors for cardiometabolic health and potentially delaying the adult onset of cardiometabolic disease, but at present there are relatively

few well-designed cross-sectional studies and hardly any longitudinal studies examining the relationship between physical activity, physical fitness and cognition and risk factors for cardiometabolic disease in adolescents.

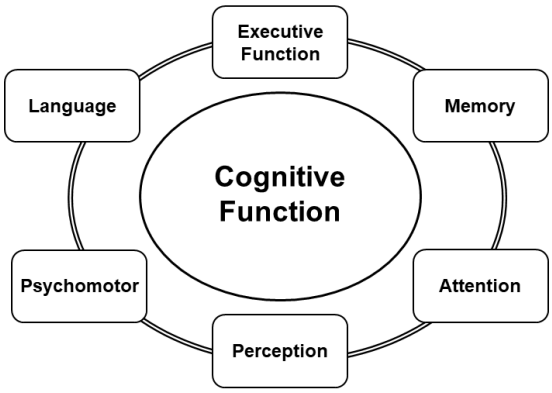

Figure 2.1. A basic schematic representation of the six interconnected domains that comprise cognitive function (adapted from Schmitt et al., 2005)).

2.3 Relationship between Physical activity, Physical Fitness and Adiposity and Risk Factors for Cardiometabolic Disease and Cognitive Function

2.3.1 Relationship between Physical Activity and Risk Factors for Cardiometabolic Disease

Adults

The research-derived benefits of physical activity for health dates back as early as the 1950s, where Morris et al. (1953) showed that bus drivers (light activity occupation) had a greater risk of developing coronary heart disease than bus conductors (heavy activity occupation) (Morris et al., 1953). These findings were later expanded to show that those who participated in more activity outside of work (e.g. cycling, running) had a lower risk of coronary heart disease than those who were less active in their leisure time (Morris et al., 1958; 1966; 1973).

More recently a dose-response relationship between leisure time activity and all-cause mortality in adults has been shown in that those who expended 5 to 10 times more energy in physical activity (MET h·week^{-1}) saw the greatest mortality risk-reduction, although these data were based on a self-report measure of physical activity subject to inaccuracies (Arem et al., 2015). However, a recent harmonised systematic review of accelerometry data also provided evidence of a dose-

response relationship between activity and mortality risk-reduction (Ekelund et al., 2019). These data suggest that, whilst higher intensity activities are more beneficial, lighter intensity activity confers some protective benefit. However, the magnitude of risk-reduction was greater across all categories of activity in the study by Ekelund et al. (2019), when compared to the self-report estimates (Arem et al., 2015), which might reflect the increases sensitivity and precision that device-measured activity offers.

Adolescents

The surveillance of physical activity in adolescents is of great interest from a public health perspective in the UK (Gibson-Moore, 2019), particularly due to the established decline in physical activity (7% each year) throughout adolescence (Dumith et al., 2011). The assessment of free-living physical activity and the relationship with risk factors for cardiometabolic disease in adolescents can be seen in Table 2.1. The relationship between physical activity and health has received little attention, in comparison with the relationship with physical fitness. One reason may be that the preferred method for the measurement of physical activity is with devices, typically accelerometers, which are more costly than field-based assessments of physical fitness. On top of this, the cost-effective and feasible alternative is to use self-report measures, but these are subject to measurement error and bias (Trost, 2020).

A summary of the studies that have examined the relationship between physical activity and risk factors for metabolic disease is presented in two tables in this review. In Table 2.1 the studies have used either traditional device measures, such as accelerometer counts, or self-report measures to determine physical activity, whereas in Table 2.2 the studies have used more recently developed summary measures which reflect total activity and intensity distribution of activity across the whole day.

The relationship between traditional device-measured physical activity and risk factors for cardiometabolic disease is equivocal with 4 studies demonstrating beneficial associations whereas 3 studies found no associations (Table 2.1). For physical activity assessed through self-

report, in 367 16-year-old boys and girls, average energy expenditure (kcal per kg per day) was negatively associated with a composite score for metabolic syndrome (Countryman et al., 2013); with the relationship mediated through physical fitness (Table 2.1). In 456 15 year-old boys and girls when physical activity was assessed with a 3-day recall the prevalence of metabolic syndrome (which was assessed through a clustered score of waist circumference, blood pressure, blood lipids and glucose), was highest in the group accruing < 60 min of MVPA per day (Neto et al., 2011) (Table 2.1). Both studies suggest that greater amounts of physical activity are associated with lower risk of clustered scores for cardiometabolic disease, but the use of and reliance on self-report measures, particularly in an adolescent population, can be problematic due to recall issues, bias, and competition with peers.

For traditional device measured physical activity, light intensity physical activity was shown to be beneficially associated with markers of health (Bailey et al., 2012; Barker et al., 2018; Carson et al., 2013) (Table 2.1). For example, in 1731 13-17-year-old boys and girls a negative, independent association was shown between light-intensity light activity (100 – 799 counts·min^{-1}), as well as high-intensity light activity (800 counts·min^{-1} to < 4 METs) and diastolic blood pressure with a stronger relationship for high-intensity light activity than light-intensity activity (Carson et al., 2013). In contrast, in 100 12-year-old and 450 15-year-old boys and girls high light intensity activity was associated with a high waist circumference suggesting high light activity is positively associated with markers of adiposity (Bailey et al., 2012; Barker et al., 2018) (Table 2.1). These findings may be explained by sedentary activity being included in the light activity category in some studies using accelerometery, dependent on the cut-off points used (see page 25 for criticisms of the methodology).

In line with the results from adults, higher intensity activities, typically reported as MVPA, are beneficially associated with markers of cardiometabolic health in adolescents (Bailey et al., 2012; Barker et al., 2018; Carson et al., 2013; Tarp et al., 2018) (Table 2.1). For example, Tarp et al. (2018) examined the relationship between traditional device-measured activity and risk factors for cardiometabolic health in a mixture of children and adolescents. Activity intensity was inversely

associated with a composite score of cardiometabolic risk, in a dose-response manner, regardless of the activity duration (Tarp et al., 2018) However, it must be noted that the sample consisted of both children and adolescents (9 – 12 y), which reduces the generalisability to adolescents.

Table 2.1. Overview of the studies (2010 onwards) examining the cross-sectional associations between **physical activity, measured by traditional accelerometer counts and by self-report,** and risk factors for cardiometabolic disease in adolescents.

Reference	Sample details	Assessment of Physical Activity	Risk Factors Examined	Analysis and Confounding Variables	Main Findings
Bailey et al., (2012)	n = 100 (59 girls) 11.8 ± 1.3 y	Accelerometry (triaxial) 7-day wear period Categories: SED, LPA, MPA, VPA	Blood pressure Total cholesterol HDL-c Triglycerides Glucose Waist circumference Clustered risk score	Simple correlation with outcomes ANCOVA between activity tertiles adjusted for Age, sex, ethnicity and socioeconomic status	VPA inversely associated with diastolic blood pressure LPA positively associated with waist circumference No further associations No difference between activity tertiles
Barker et al., (2018)	n = 534 (282 girls) 14.6 ± 1.2 y	Accelerometry (uniaxial) 7-day wear period Categories: SED, LPA, MPA, VPA	Blood pressure Total cholesterol HDL-c Triglycerides Glucose Insulin HOMA-IR Waist circumference Sum of skinfolds Clustered risk score	Linear regression on each individual risk factor and clustered risk score Model 1: Adjusted for age, sex, pubertal status Model 2: Further adjusted for physical fitness	VPA inversely associated with blood pressure and TG, model 2 LPA positively associated with waist circumference and skinfolds, model 2
Carson et al., (2013)	n = 1731 (849 girls) 13 – 17 y	Accelerometry; hip (Uniaxial) 7-day wear period Categories: SED, light-intensity LPA, high-intensity LPA, MVPA	Waist circumference Blood pressure HDL-c CRP Glucose Insulin HOMA-%S	Linear regression Model 1: adjusted for age, sex, ethnicity Model 2: adjusted for waist circumference	MVPA negatively associated with waist circumference MVPA negatively associated with systolic blood pressure, insulin and HOMA-%S, model 2 High-intensity LPA negatively associated with diastolic blood pressure, model 2 Light-intensity LPA negatively associated with diastolic blood pressure, model 2

Table 2.1 continued

Countryman et al., (2013)	n = 367 (99 girls) 16.2 ± 0.6 y	Self-report 7-day activity recall Calculated outcome: Kcal per kg per day	Blood pressure Glucose Insulin CRP IL-6 Waist circumference Metabolic syndrome risk score	Linear regression Adjusted for sex and parent education	Inverse association with metabolic syndrome risk Mediated through fitness
Martinez-Gomez et al., (2010)	n = 192 (94 girls) 14.8 ± 1.3 y	Accelerometry; lower back (Uniaxial) 7-day wear period Categories: Total PA, MPA, VPA and MVPA	IL-6 CRP Glucose Insulin HOMA-IR	Partial correlations adjusting for age, sex and pubertal status Linear regression Model 1: adjusted for age, sex, pubertal status and HOMA-IR Model 2: Adjusted for skinfolds and physical fitness added	No observed associations
Martinez-Gomez et al., (2012)	n = 1025 (549 girls) 14.8 ± 1.2 y	Accelerometry; lower back (Uniaxial) 7-day wear period Categories: Total PA, MPA, VPA and MVPA Self-report IPAQ-A Time spent: MPA, VPA, MPVA	IL-6 TNF-α CRP	Linear regression Model 1: adjusted for age and sex Model 2: adjusted for BMI	No association with IL-6, TNF-α or CRP
Neto et al., (2011)	n = 456 (233 girls) 14.5 ± 1.6 y	Self-report 3-day activity recall Calculated total MVPA per day Split into groups: Inactive: < 60 min MVPA Active: 60-90 min MVPA Very active: > 90 min MVPA	Blood pressure HDL-c Triglycerides Glucose Waist circumference Calculated metabolic risk score	Examined prevalence of metabolic syndrome risk within each group Logistic regression between groups and risk prevalence, adjusting for age and sex	Lower prevalence in the very active group (> 90 min MVPA per day) No association with metabolic risk prevalence

Abbreviations: SED = Sedentary Behaviour. LPA = Light Physical Activity. MPA = Moderate Physical Activity. VPA = Vigorous Physical Activity. MVPA = Moderate-to-Vigorous Physical Activity. HDL = High Density Lipoprotein. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. LDL = Low Density Lipoprotein. CRP = C-Reactive Protein. TNF = Tumour Necrosis Factor. IPAQ = International Physical Activity Questionnaire. IL = Interleukin. ANCOVA = Analysis of Covariance.

In purely adolescent samples, vigorous physical activity was negatively associated with diastolic blood pressure in young adolescents (11.8 ± 1.3 y) (Bailey et al., 2012). Similar associations were observed in older adolescents (14.6 ± 1.2 y), whereby vigorous physical activity was negatively associated with systolic blood pressure (Barker et al., 2018), as well as MVPA being negatively associated with systolic blood pressure (Carson et al., 2013). When considering the associations with biomarkers of cardiometabolic disease, the research has focused on traditional markers (such as blood lipids, glucose, insulin), as well as typically creating clustered risk scores (Table 2.1). Carson et al. (2013) found that MVPA was negatively associated with fasting insulin and therefore positively associated with HOMA-%S (an index of insulin sensitivity). Additionally, triglyceride concentrations were also negatively associated with vigorous activity (Barker et al., 2018).

Despite the studies discussed above, there has been little investigation into the associations between physical activity and novel markers of low-grade inflammation, which have an important causal role in the development of cardiovascular disease and type 2 diabetes (Gleeson et al., 2011; Pedersen, 2006). The limited research examining the association between traditional device-measured physical activity and markers of inflammation found no associations with CRP (Carson et al., 2013; Martinez-Gomez et al., 2010) or IL-6 (Martinez-Gomez et al., 2010) in samples of older adolescents (14 – 17 y). Furthermore, these studies used different cut-points to categorise activity and examined different metrics, making comparisons between the two difficult (see next section for criticisms). Thus, it poses an important question to address the associations between physical activity and novel markers of low-grade inflammation in adolescents, with a particular focus on younger participants (i.e. 11 – 13 y).

Issues with the interpretation of traditional device-measured physical activity

Despite being the most commonly used method, and overcoming some of the limitations associated with self-report measures, traditional device-measured physical activity imposes a multitude of decisions for the user during data collection, (i.e. the brand of accelerometer, placement of the device, wear period), and during the processing of the data (i.e. inclusion of

sleep, exclusion of non-wear time, epoch length, processing method) (Cain et al., 2013; Migueles et al., 2017); which significantly impact upon the data collected. Furthermore, the traditional method to assess physical behaviours through these devices is done by categorising behaviour (i.e. sedentary time, light, moderate and vigorous physical activity) through count-based thresholds. Unfortunately, the counts developed by accelerometers are proprietary measures and therefore the thresholds differ across accelerometer brands, device placement sites and populations (Troiano, McClain, Brychta & Chen., 2014), which might lead to activity misclassification and yields significant problems when comparing between studies (Cain et al., 2013; Troiano et al., 2014).

Simpler and more informative metrics from accelerometer counts that summarise the total physical activity and activity intensity distribution across the day

Recently, to overcome the barriers mentioned above, there have been suggestions to use simpler and more informative metrics that encompass the whole physical activity profile (Rowlands et al., 2018b). These metrics are continuous measures that summarise the total physical activity volume (average acceleration) and the activity intensity distribution across the day (intensity gradient) (Rowlands et al., 2018b). Although MVPA is typically of primary focus, given the important associations with health discussed previously, solely focusing on one category of activity intensity does not represent the whole spectrum. The use of such continuous metrics allows for more information about physical activity intensity to be included in analysis, which could provide more resolution on the relationships between physical activity intensity and the outcomes of interest (Rowlands et al., 2018b). Moreover, as these metrics are derived from raw accelerations, and do not rely on proprietary algorithms, they have greater comparability between devices (Rowlands et al., 2016; Rowlands et al., 2017).

Given the recent development of these activity metrics, very few studies have yet been published examining the effects of physical activity on cardiometabolic health in adolescents using these methods (Table 2.2). The limited research to date suggests that average acceleration is negatively associated with markers of cardiometabolic health and thus children and adolescents

who accumulate a higher volume of activity per day have lower risk of cardiometabolic disease (Table 2.2). Furthermore, the intensity gradient was also negatively associated with the same markers (independently of the activity volume; Table 2.2) in 9- to 10-year-old boys and girls, suggesting that the intensity of physical activity, in addition to the volume is an important determinant of the subsequent effects on health (Fairclough et al., 2019). Collectively, these initial results demonstrate the potential utility of the novel metrics for examining the associations between physical activity and cardiometabolic health. In addition, these associations have only been considered with a narrow range of risk factors such as BMI (Buchan et al., 2019; Fairclough et al., 2019; Rowlands et al., 2018) and a composite risk score for metabolic syndrome (Fairclough et al., 2019). Thus, the associations between these metrics and other risk factors of cardiometabolic disease, such as markers of chronic low-grade inflammation and metabolic health (glucose, insulin & HOMA-IR) is an important gap to fill.

Table 2.2. Relationship between **continuous activity metrics (average acceleration and intensity gradient) to measure physical activity** and risk factor for cardiometabolic disease in children and adolescents.

Reference	Sample details	Assessment of Physical Activity	Risk Factors Examined	Analysis and Confounding Variables	Main Findings
Buchan et al., (2019)	n = 246 (138 girls) 9.6 ± 1.4 y	Accelerometry (triaxial) 7-day wear period Non-dominant wrist Traditional metrics: MVPA Inactive Time Novel metrics: Average Acceleration (AvgACC) Intensity Gradient (IG)	BMI z-score	Simple correlations Linear regression Model 1: unadjusted Model 2: adjusted for age and sex Model 3: adjusted for alternate metric	AvgACC correlated with MVPA and inactive time AvgACC negatively associated with BMI z-score (model 2) IG correlated with MVPA and inactive time IG negatively associated with BMI z-score (model 3) MVPA not associated with BMI z-score
Fairclough et al., (2019)	n = 145 (83 girls) 9.6 ± 0.3 y	Accelerometry (triaxial) 7-day wear period Non-dominant wrist Traditional metrics: MVPA Novel metrics: Average Acceleration Intensity Gradient	BMI z-score Metabolic syndrome risk score (waist/height ratio, $V\dot{O}_{2peak}$ and BMI)	Simple correlations Linear regression Model 1: unadjusted Model 2: adjusted for sex, maturation and socioeconomic status Model 3: adjusted for alternate metric	AvgACC correlated with MVPA AvgACC negatively associated with BMI z-score (model 2) IG correlated with MVPA IG negatively associated with BMI z-score and metabolic risk, and positively associated with $V\dot{O}_{2peak}$ (independent of AvgACC)
Rowlands et al., (2018)	n = 1669 (all girls) 12.8 ± 0.8 y	Accelerometry (triaxial) 7-day wear period Non-dominant wrist Traditional metrics: MVPA Novel metrics: Average Acceleration Intensity Gradient	BMI z-score Body fat %	Simple correlations Linear regression Model 1; unadjusted Model 2: adjusted for age, maturity Model 3: alternate metric	AvgACC positively correlated with MVPA and negatively with inactive time AvgACC negatively associated with body fat %, only in model 1 IG positively correlated with MVPA and negatively correlated with inactive time IG negatively associated with BMI z-score and body fat %, in model 3

Abbreviations: AvgACC = Average Acceleration. IG = Intensity Gradient. MVPA = Moderate-to-Vigorous Physical Activity. BMI = Body Mass Index.

2.3.2 Relationship between Physical Activity and Cognition

Adults

It has been clearly shown that adults who engage in more physical activity have an attenuated decline in cognitive abilities during later life and that physical activity during middle age is positively associated with grey matter volume in later life (Hötting & Röder., 2013; Rovio et al., 2010), which might provide an explanation behind the physical activity induced attenuation in cognitive decline with ageing. It is only since around 2000 though that there has been an increasing interest in the role of physical activity in cognitive development in younger populations (Sibley & Etnier., 2003; Donnelly et al., 2016).

Adolescents

Recent meta-analyses suggest that physical activity has a small, beneficial effect on cognitive function (Sibley & Etnier., 2003; Donnelly et al., 2016) and academic achievement (Donnelly et al., 2016) in children and adolescents. However, both reviews acknowledge inconsistencies within the literature, likely due to the variation in methods of measurement of physical activity and cognitive function. Indeed, it has been highlighted that physical activity in these populations is typically measured using self-report methods (Donnelly et al., 2016; Howie & Pate., 2012). Furthermore, both reviews included studies consisting of children and adolescents which may also explain inconsistencies given that age is a strong moderator of the physical activity-cognition relationship, with effects suggested to be more evident in older adolescents (Chang et al., 2012), likely due to the structural brain changes that occur through puberty (Blakemore et al., 2010). Whilst the number of studies utilising device-measured assessments of physical activity has increased over recent years, there are still a mixture of methods being used which may explain the equivocal nature of the results (as seen in Table 2.3).

Cognitive function does not just affect academic performance, but because of the importance of academic performance for adolescents and because it is perceived as relatively easy to measure (from school tests and sometimes teacher assessments for example) it often used as a proxy for

cognitive function in adolescent populations. Cognitive function on the other hand, using carefully controlled paper or computer-based methods is relatively difficult to measure in a school-based setting due to the time-needed to familiarise pupils, the difficulties in controlling the test environment and the accessibility of equipment such as computers. Thus, studies measuring academic performance and cognitive function are critically examined in this review even though cognitive function impacts on all aspects of an adolescent's life, not just on academic performance.

Of the studies employing traditional device-measured assessments of physical activity, academic performance has most typically been the outcome of interest (Cadenas-Sanchez et al., 2020; Kwak et al., 2009; Syväoja et al., 2013). The findings are equivocal with one study suggesting that physical activity is not associated with school grades (Cadenas Sanchez et al., 2020), whilst other studies reported that vigorous physical activity is positively associated with academic performance in girls only (Kwak et al., 2009). Furthermore, Syväoja et al. (2013) found that MVPA was positively associated with school grades, but only for self-report measures and not for MVPA derived from accelerometers. Explanation for the varying findings include the different processing methods and cut points used to quantify physical activity (see page 25) and the different methods for measuring academic performance, including subjective teacher assessment. Thus, the use of robust and objective tests, that measure cognitive function might provide more utility.

The studies examining the associations between physical activity and domains of cognitive function in adolescents can be seen in Table 2.3. In a sample of 697 boys and girls (aged 10 – 11 y), working memory performance (response time on the Digit Span Test) was positively associated with time spent in MVPA, when considering a simple bivariate correlation (Aadland et al., 2017). However, in the full regression model which accounted for other variables (such as age, adiposity, fitness and motor skills) MVPA was no longer associated with working memory performance. Additionally, in 12 – 13-year-old girls and boys (n = 224) no measure of activity classification (sedentary, light, moderate and vigorous) was associated with working memory performance (measured by a Spatial Span test) (Syväoja et al. 2014). In contrast to these findings,

Lee et al. (2014) found that participants (41 girls, 50 boys, aged 16.5 ± 2 y) categorised as regular exercisers (based on a questionnaire) had a superior working memory performance (measured by a Spatial Memory task) when compared to those considered as non-exercisers. However, this evidence comes from a simple comparison between the two groups without accounting for other confounding variables, which the other studies did within their regression analyses (Aadland et al., 2017; Syväoja et al., 2014). Overall, the limited evidence suggests that physical activity is not associated with working memory, when working memory was only assessed by either a Spatial Span or Digit Span tests. Furthermore, only two studies to date have examined this relationship when physical activity was recorded using traditional device-measured accelerometers.

Similarly, the evidence regarding the relationship between physical activity and executive function is equivocal (Table 2.3). In age 16.5 ± 2 y boys and girls, regular exercisers (assessed by self-report) demonstrated better cognitive flexibility and inhibitory control performance (measured by the Wisconsin Sorting Card Task and a Stroop Task, respectively) when compared to non-exercisers (Lee et al., 2014), whilst there was no reported association between physical activity behaviours and cognitive flexibility (measured by the Intra-Extra Dimensional Shift task) in girls and boys aged 12.2 ± 0.6 y (Syväoja et al., 2014) or inhibitory control performance (measured by a Stroop Task) in boys and girls (age 10.2 ± 0.3 y, Aadland et al., 2017; age 10.1 ± 1.1 y; Mora-Gonzalez et al., 2019). MVPA was found to be positively associated with cognitive flexibility performance, which was assessed by verbal fluency (number of animals named in 60 s) and the trail making test (time taken and accuracy) (Aadland et al., 2017) However again, this relationship was non-existent when physical fitness was included in the model. Interestingly, higher amounts of vigorous physical activity were associated with a greater P3 amplitude, assessed by EEG, during the Flanker task which suggests a greater level of attention for each stimulus in the task (Mora-Gonzalez et al., 2019); although this relationship was not as clear once physical fitness was included in the model. As previously mentioned, the liberal analyses (categorising participants as active and non-active from self-report and lack of information on variables controlled for) performed by Lee et al. (2014) may overestimate the role of physical activity and

cognitive function. It is also worth noting that mixtures of older children and young adolescents (10 – 11 y) were included in the previously discussed samples, either considered normally healthy (Aadland et al., 2017) or living with overweight/obesity (Mora-Gonzalez et al., 2019); factors which may further restrict conclusions from these data to the broader population of adolescents. In summary, while this evidence initially appears to support the suggestion that higher physical activity is associated with better executive function, on further analysis the relationship is better explained by physical fitness. However, there is still limited data pertaining to exclusive samples of young adolescents (11 – 12 y), with the previous work focusing on older adolescents (Lee et al., 2014) or on a mixture of children and adolescents (Aadland et al., 2017; Mora-Gonzalez et al., 2019).

The domain of attention has been examined less extensively in previous studies (Syväoja et al., 2014; Vanhelst et al., 2016) (Table 2.3). MVPA in 224 boys and girls (aged 12 – 13 y) was negatively associated with response times during a simple reaction time test, but there was no observed association with a measure of sustained attention (Syväoja et al., 2014). However, more time spent being sedentary was associated with worse performance on the sustained attention task (Syväoja et al., 2014). When splitting activity classifications (sedentary, light, moderate and vigorous) into quartiles, Vanhelst et al. (2016) in age 13- to 15-year-old boys and girls (n = 273) found a linear relationship between moderate physical activity and sustained attention capacity, even when adjusting for physical fitness; whereby those in the highest category of daily moderate physical activity had a better attention score. Despite this, there were no observable differences between quartiles of the other activity behaviours (i.e. sedentary, light and vigorous) and attention score (Vanhelst et al., 2016). The limited research around sustained attention suggests a beneficial role of physical activity, particularly moderate intensity on sustained attention (measured by response time and accuracy). However, there are multiple strands to attention that also warrant further investigation, such as selective attention as for example measured by congruent versions of the Stroop and Flanker tasks (Bates & Lemay, 2004).

Thus overall, the evidence supporting a beneficial role of physical activity and cognition in adolescents is equivocal. In particular, when associations with physical activity and a cognitive function domain were found, these associations were no longer evident after including physical fitness in the models (Aadland et al., 2017; Cadenas-Sanchez et al., 2020). In addition, the differing methods for collecting, processing and categorising accelerometer data also complicate comparisons (see page 25). Whilst the newly proposed continuous physical activity metrics of activity volume and intensity (Rowlands et al., 2018) might provide a step towards broader comparability and harmonisation, no studies to date have examined the associations between these metrics and cognitive function performance.

Table 2.3. Overview of the studies (2009 onwards) examining cross-sectional relationships between **physical activity (as measured by traditional devices and self-report) and academic performance and cognitive function** in adolescents.

Reference	Sample details	Assessment of Physical Activity	Cognitive Function Tests & Other measures (Domains)	Analysis and Confounding Variables	Main Findings
Aadland et al., (2017)	n = 697 (357 girls) 10.2 ± 0.3 y	Accelerometry 7-day wear period (hip) Categories: SED & MVPA	Stroop Test (Selective Attention & Inhibitory Control) Verbal fluency & trail making test (Cognitive Flexibility) Digit Span Test (Working Memory) Created EF composite score Standardised school grades	Linear regression Adjusted for age, skinfolds, pubertal stage, socioeconomic status, fitness	MVPA was positively associated with all cognitive tests, but this disappeared in the full model SED was positively related with composite EF score in girls
Cadenas-Sanchez et al., (2020)	n = 106 (classed as overweight) 10.0 ± 1.1 y	Accelerometry 7-day wear period Categories: LPA, MPA, VPA & MVPA	Standardised Academic Achievement Test School Grades	Linear regression Model 1: adjusted for age, & sex Model 2: adjusted for BMI and fitness	No associations observed
Kwak et al., (2009)	n = 232 (120 girls) 16.0 ± 0.4 y	Accelerometry 4-day wear period (hip) Categories: LPA, MPA & VPA	Academic Performance (School grades)	Linear regression Model 1: adjusted for pubertal status, skinfolds, mother's education Model 2: added the physical activity metrics	VPA was positively associated with school grades for girls only
Lee et al., (2014)	n = 91 (41 girls) 16.5 ± 2 y Split into regular and non-exercisers	Self-report (IPAQ) Calculated average MET's	Stroop Test (Selective Attention & Inhibitory Control) Wisconsin Sorting Card Task (Cognitive Flexibility) Dual Task (Multi-Tasking) Spatial Memory (Memory) BDNF	Linear regression Model 1: participant demographics Model 2: activity and BDNF	Better inhibitory control and cognitive flexibility in the regular exercise group Regular exercisers had lower BDNF

Mora-Gonzalez et al., (2019)	n = 84 (37 girls) (classed as overweight) 10.1 ± 1.1 y	Accelerometry 7-day wear period (hip & wrist) Categories: SED, LPA, MPA, VPA & MVPA	Flanker Test (Selective Attention & Inhibitory Control) EEG (P3 amplitude and latency)	Linear regression Model 1: sex, maturity, BMI and IQ only entered Model 2: Physical activity behaviours added separately Exploratory analysis when controlling for fitness	VPA positively associated with P3 amplitude, but this was attenuated when fitness was included MPA and MVPA positively associated with P3 latency – remained after fitness included No association with cognitive performance
Syväoja et al., (2013)	n = 277 (155 girls) 12.2 ± 0.6 y	Self-report: 7-day recall SED & MVPA Split into tertiles for each Accelerometry: 7-day wear period (hip) Categories: SED & MVPA Split into tertiles for each	School Grades (GPA; 4 – 10)	ANCOVA between activity tertiles Linear regression Model 1: sleep, sex, parent education Model 2: MVPA added	Group with highest MVPA had highest school grades Self-report MVPA was positively associated with school grades Accelerometry-based activity not associated with school grades
Syväoja et al., (2014)	n = 224 (127 girls) 12.2 ± 0.6 y	Accelerometry 7-day wear period (hip) Categories: SED & MVPA Self-report: 7-day recall SED & MVPA	Pattern Recognition Memory Test (Visual Memory) Spatial Span (Working Memory) Intra-Extra Dimensional Shift (Cognitive Flexibility) Simple Reaction Time (Attention) RVIP (Attention)	Linear regression Adjusted for sex, parent education	Accelerometry based MVPA negatively associated with simple reaction time Accelerometry based SED positively associated with RVIP response times
Vanhelst et al., (2016)	n = 273 (147 girls) 14.2 ± 1.1 y	Accelerometry 7-day wear period (lower back) Categories: SED, LPA, MPA, VPA, MVPA	D2 test (Attention)	Linear regression Adjusted for sex, BMI, parent education, physical fitness	MPA and MVPA positively associated with attention capacity

Abbreviations: SED = Sedentary Behaviour. LPA = Light Physical Activity. MPA = Moderate Physical Activity. VPA = Vigorous Physical Activity. MVPA = Moderate-to-Vigorous Physical Activity. EF = Executive Function. BDNF = Brain Derived Neurotrophic Factor. EEG = Electroencephaography. RVIP = Rapid Visual Information Processing

2.3.3 Relationship between Physical Fitness and Risk Factors for Cardiometabolic Disease

The effects of physical activity on risk factors for cardiometabolic disease and, particularly cognitive function, may be mediated through physical fitness. Some have argued that physical fitness is a more sensitive independent variable, when considering risk factors for cardiometabolic disease, as it is indicative of the 'state' of the individual and is therefore more stable and objective (Cadenas-Sanchez et al., 2020), whereas the assessment of physical activity only provides a snapshot at that particular period in time, which introduces more uncertainty, in addition to the aforementioned issues of device-measured physical activity. Thus, physical fitness itself may be the key factor affecting cognitive function with physical activity only contributing beneficially in that it enhances physical fitness.

Adults

Within the literature focusing on adults, there is a strong body of evidence suggesting that a higher physical fitness is beneficial for health both from a disease risk and mortality perspective. A prospective study in adults found that low physical fitness is a strong, independent predictor of metabolic syndrome risk (Lamonte et al., 2005). Furthermore, meta-analytic evidence shows that a higher physical fitness is inversely related with all-cause mortality (Barry et al., 2014; Ross et al., 2016), even in those that are not living with other co-morbidities (Ross et al., 2016). Interestingly, Ross et al. (2016) note that a dose-response relationship is most evident at the lower ends of the physical fitness spectrum, suggesting that individuals do not necessarily need to be 'athletic' in order to benefit from the protective effects of physical fitness.

Adolescents

In adolescents, physical fitness can easily be assessed using field-based measures such as the multi-stage fitness test (MSFT) (Lang et al., 2018; Tomkinson et al., 2019). Indeed, the MSFT has been the most popular method for the assessment of physical fitness within the adolescent literature (Agostinis-Sobrinho et al., 2018; Artero et al., 2011; Barker et al., 2018; Buchan et al., 2015; Dring et al., 2019b; Martinez-Gomez et al., 2010; Neto et al., 2011; Silva et al., 2017).

Laboratory determined $\dot{V}O_{2peak}$ has also been used to measure of cardiorespiratory fitness, either instead of (Bailey et al., 2012; Bugge et al., 2012; Countryman et al., 2013) or in addition to (Dring et al., 2019b) the MSFT. Performance during the MSFT has been recorded as the number of completed shuttles (Artero et al., 2011; Buchan et al., 2015) or the total distance covered (Dring et al., 2019b). Often $\dot{V}O_{2peak}$ has been predicted from MSFT results (Agostinis-Sobrinho et al., 2018; Barker et al., 2018; Martinez-Gomez et al., 2010; Neto et al., 2011; Silva et al., 2017), but this has limited accuracy as MSFT performance reflects training status to a greater extent than $\dot{V}O_{2peak}$ peak which has a large genetic component (Blair et al., 2001).

The weight of the available evidence suggests that the higher the physical fitness of adolescents the lower the composite scores of clustered risk factors for cardiometabolic disease (Artero et al., 2011; Bailey et al., 2012; Barker et al., 2018; Buchan et al., 2015; Bugge et al., 2012; Countryman et al., 2013; Neto et al., 2011; Silva et al., 2018) (Table 2.4). For example, it was shown in over 700 15 year-olds (boys and girls) that MSFT performance was associated with a lower composite risk score that included total cholesterol, HDL-c, triglycerides, insulin, glucose, HOMA-IR, waist circumference and blood pressure (Artero et al., 2011). The relationship between physical fitness and composite risk factors for cardiometabolic disease was sustained when adjustments for potential confounding variables, namely demographic variables and participant characteristics were made (e.g. Artero et al., 2011; Barker et al., 2018) suggesting an independent association between physical fitness and composite risk scores. However, the composite risk scores included different variables in all studies and there is value in teasing out where physical fitness has the major impact in reducing disease risk (Kyung-Song et al., 2013) to enhance understanding of the underlying adaptive mechanisms.

Of the seven studies that have examined *fasting metabolic health* (glucose, insulin and the HOMA-IR index, blood lipids) four found a positive association with physical fitness all measured by MSFT (Agostinis-Sobrinho et al., 2018; Artero et al., 2011; Dring et al., 2019b; Martinez-Gomez et al., 2010). In two studies there was no association (Barker et al., 2018; Bugge et al., 2012) but in one of these laboratory measured $\dot{V}O_{2peak}$, rather than physical fitness, was determined (Bugge

et al., 2012). In one study there was a negative relationship between physical fitness and risk factors for cardiometabolic health (Silva et al., 2016), but this was specific to girls only, which might be due to the suggested increased insulin resistance in girls during puberty (Cooper et al., 2017). Overall, the evidence available suggests that physical fitness is beneficial for fasting insulin sensitivity, which supports evidence from adults (Bird & Hawley, 2017).

However, these estimates of fasting insulin sensitivity only provide part of the picture, as HOMA-IR reflects hepatic insulin sensitivity and does not account for *peripheral insulin sensitivity*, which can be gauged by assessing the postprandial responses to a test meal (Abdul-Ghani et al., 2007; Muniyappa & Madan., 2000). No study to date has examined the role of physical fitness on the postprandial glycaemic and insulinaemic responses (peripheral insulin sensitivity) to an oral glucose tolerance test in adolescents, although one study has examined the effect of $V\dot{O}_{2peak}$ (Maggio et al., 2015). Adolescents (14.0 ± 0.9 y) were split into high- and low-fit groups (based on $V\dot{O}_{2peak}$ during a progressive treadmill test) and OGTT insulin AUC was lower in the group with the higher $V\dot{O}_{2peak}$. However, these adolescents were recruited as they were living with overweight or obesity, thus the applicability of the findings to the broader population of healthy adolescents is unknown. Furthermore, whilst the OGTT is seen as the gold standard laboratory procedure (Muniyappa & Madan, 2000), it does not necessarily reflect a mixed meal that is consumed by adolescents on a daily basis. The importance of postprandial insulinaemia (DiNicolantonio et al., 2017; Lautt, 2007) and glycaemia (Cavalot et al., 2011; Monnier et al., 2007) for metabolic health is of growing interest and the effect of physical fitness on these responses warrants further investigation.

Blood pressure has also been frequently examined within the composite risk factors utilised in the literature. Of the six studies examining the association between physical fitness and blood pressure, four studies found the higher the physical fitness the lower the systolic or diastolic blood pressure in participants aged 11 to 15 y who were of normal body mass (Artero et al., 2011; Bailey et al., 2021; Barker et al., 2018; Silva et al., 2016). Conversely, one study found a positive association in normal weight boys and girls (age 13.4 ± 0.3 y) (Bugge et al., 2012) whilst another

found no observable association (Dring et al., 2019b). Generally, higher levels of physical fitness are associated with lower blood pressure, but perhaps other exposure variables, such as physical activity (reflecting recent activity) and adiposity, may better explain the relationship between physical fitness and blood pressure (Pazin et al., 2020).

The studies examined so far in this section have used traditional risk factors for cardiometabolic disease. The role of *low-grade inflammation* in the development of many chronic diseases is becoming increasingly clear, particularly as low-grade inflammation can be present during adolescence (Balagopal et al., 2011). Several studies have investigated the association between physical fitness and markers of inflammation in adolescents, but these have mainly focused on the measurement of CRP, with CRP found to be inversely associated with physical fitness (Buchan et al., 2015; Bugge et al., 2012; Countryman et al., 2013; Martinez-Gomez et al., 2010 & 2012). Fewer studies have investigated the association between physical fitness and a *range of pro- and anti-inflammatory cytokines* (Bugge et al., 2012; Dring et al., 2019b) which are also involved in the pathophysiology of chronic diseases. In 121 boys and girls (11.3 ± 0.8 y) it was shown that the highest fasting IL-6 concentrations were found in participants from the lowest quartile of physical fitness (assessed by distance run on the MSFT), as well as a negative association between physical fitness and fasting IL-6 concentration (β = -0.291) (Dring et al., 2019b). Bugge et al. (2012) also observed a similar relationship in 413 boys and girls (13.4 ± 0.3 y) between treadmill $\dot{V}O_{2peak}$ and IL-6 concentration (β = -0.151). Physical fitness measured by the MSFT was also negatively (β = -0.405) and positively (β = 0.325) associated with pro- and anti-inflammatory fasting IL-1β and IL-10 concentrations, respectively (Dring et al., 2019b), but there was no relationship between MSFT performance and fasting TNF-α and anti-inflammatory IL-1Ra (Bugge et al., 2012).

Overall, the weight of available suggests that physical fitness measured by MSFT is beneficially related to fasting indices of metabolic health, blood pressure and some markers related to low-grade inflammation (mainly CRP). In addition, in some studies $\dot{V}O_{2peak}$ relates to the same variables, but the relationships are weaker and not so frequently observed. In summary these

studies highlight the importance of physical fitness, most often measured by MSFT, during adolescence, but it remains to be examined how physical fitness is related to the postprandial responses to a mixed meal, which is also an important risk factor. Furthermore, given the recognition of low-grade chronic inflammation in the development of chronic diseases, more information on the relationship between physical fitness and cytokines in adolescents is needed.

Table 2.4. Overview of the studies (2010 onwards) examining the cross-sectional relationship between **physical fitness and risk factors for cardiometabolic disease** in adolescents.

Reference	Sample details	Assessment of Physical Fitness	Risk Factors Examined	Analysis and Confounding Variables	Main Findings
Agostinis-Sobrinho et al., (2018)	n = 529 (267 girls) 14.3 ± 1.7 y	Cardiorespiratory fitness: MSFT (predicted $V\dot{O}_{2\ peak}$) Muscular fitness: Hand grip strength Standing long jump (Composite muscular fitness score created)	Glucose Insulin HOMA-IR CRP Fibrinogen (created a standardised "inflascore" variable)	Linear regression Model 1: unadjusted Model 2: adjusted for age, sex, pubertal status, MSFT and waist circumference ANCOVA with groups from muscular fitness and inflascore	Muscular fitness inversely associated with insulin, model 2 Muscular fitness inversely associated with HOMA-IR, model 2 High inflascore group had lowest MSFT
Artero et al., (2011)	n = 709 (363 girls) 14.9 ± 1.3 y	Cardiorespiratory fitness: MSFT (last completed stage) Muscular fitness: Hand grip strength Standing long jump	Total cholesterol HDL-c Triglycerides Insulin Glucose HOMA-IR Waist circumference Blood pressure Composite risk score	Partial correlations, adjusting for sex, age and pubertal status Linear regression Model 1: adjusted for age, sex, pubertal stage Model 2: adjusted for alternate fitness component	MSFT inversely correlated with all individual risk factors except blood pressure Independent and inversely associated with composite risk score (β = -0.264) Muscular fitness inversely correlated with all risk factors Independent and inversely associated with composite risk (β = -0.249)
Bailey et al., (2012)	n = 100 (59 girls) 11.8 ± 1.3 y	Progressive cycle ergometer test to exhaustion $V\dot{O}_{2\ peak}$ measured Categorised as low- and high-fit	Blood pressure Total cholesterol HDL-c Triglycerides Glucose Waist circumference Clustered risk score	Simple correlations with outcomes ANCOVA of clustered risk by fitness groups Adjusted for age, sex, ethnicity	Negative correlations with waist circumference, diastolic blood pressure, triglycerides and clustered risk score High-fit group had a lower clusters risk score compare to low-fit
Barker et al., (2018)	n = 534 (282 girls) 14.6 ± 1.2 y	Cardiorespiratory fitness: MSFT (predicted $V\dot{O}_{2\ peak}$) Muscular fitness: Hand grip strength	Blood pressure Total cholesterol HDL-c Triglycerides Glucose Insulin HOMA-IR Clustered risk score	Linear regression Model 1: adjusted for age, sex, pubertal status Model 2: included physical activity	MSFT was negatively associated with BMI, waist circumference, skinfolds and clustered risk score – independent of physical activity Muscular fitness was negatively associated with BMI, blood pressure and clustered risk score

Table 2.4 continued.

Buchan et al., (2015)	n = 192 (74 girls) 16.7 ± 0.6 y	Cardiorespiratory fitness: MSFT (number of completed shuttles) Muscular fitness: Countermovement jump	CRP IL-6 Adiponectin Fibrinogen Composite score	Linear regression Model 1: adjusted for age, sex, self-reported physical activity, waist circumference, pubertal status Model 2: included other fitness assessment ANCOVA to examine clustered score across fitness groups	MSFT negatively associated with clustered risk score, independent of muscular fitness Muscular fitness negatively associated with clustered risk score, independent of MSFT Lowest quartile of MSFT had highest composite risk score Lowest quartile of muscular fitness had highest composite risk score
Bugge et al., (2012)	n = 413 13.4 ± 0.3 y	Progressive treadmill run until exhaustion $V\dot{O}_{2\ peak}$ measured	Blood pressure Glucose Insulin HOMA-IR IL-6 IL-1ra TNF-α CRP Composite risk score	Linear regression Adjusted for sex, pubertal status and skinfolds	Negatively and independently associated with composite risk score and IL-6
Countryman et al., (2013)	n = 367 (99 girls) 16.2 ± 0.6 y	Incremental walk/jog test until exhaustion $V\dot{O}_{2\ peak}$ measured	Blood pressure Glucose Insulin HDL-c LDL-c CRP IL-6 Metabolic syndrome risk score	Linear regression Adjusted for sex and parent education	Direct, inverse association with metabolic syndrome risk score
Dring et al., (2019b)	n = 121 (60 girls) 11.3 ± 0.8 y	Progressive treadmill run until exhaustion $V\dot{O}_{2\ peak}$ measured MSFT (Total distance covered) Split into quartiles for both variables	Blood pressure Glucose Insulin HOMA-IR IL-6 IL-1β IL-10 TNF-α	ANCOVA using the fitness quartiles, adjusting for maturity Linear regression Adjusted for maturity	IL-6, IL-1β, glucose, insulin and HOMA-IR all highest in first quartile of MSFT MSFT negatively associated with IL-6 and IL-1β Insulin, glucose HOMA-IR were highest in first quartile of $V\dot{O}_{2\ peak}$

Table 2.4 continued.

Martinez-Gomez et al., (2010)	n = 192 (94 girls) 14.8 ± 1.3 y	MSFT (number of shuttles)	IL-6 CRP Glucose Insulin HOMA-IR	Partial correlations adjusting for age, sex and pubertal status Linear regression Model 1: adjusted for age, sex, pubertal status and HOMA-IR Model 2: adjusted for physical activity or skinfolds Model 3: All	Negative correlation with insulin, HOMA-IR, CRP, C3 and C4 MSFT negatively associated with CRP
Martinez-Gomez e al., (2012)	n = 1025 (549 girls) 14.8 ± 1.2 y	Cardiorespiratory Fitness: MSFT (final completed stage) Muscular fitness: Speed-agility (4 x 10 m shuttle)	IL-6 TNF-α CRP	Linear regression Model 1; adjusted for sex and age Model 2: adjusted for BMI	MSFT independent and negatively associated with CRP Muscular fitness independent and negatively associated with CRP No association with IL-6
Neto et al., (2011)	n = 456 (233 girls) 14.5 ± 1.6 y	MSFT (predicted $V\dot{O}_{2\ peak}$) Split into tertiles	Blood pressure HDL-c Triglycerides Glucose Waist circumference Metabolic syndrome risk score	Prevalence of metabolic syndrome risk in fitness tertiles Logistic regression between fitness tertile and metabolic syndrome risk Adjusted for age and sex	Higher prevalence of metabolic syndrome risk in the lowest fitness group Lowest fitness group had a greater odds ratio for metabolic syndrome risk
Silva et al., (2016)	n = 1037 (601 girls) 12.7 ± 1.4 y	MSFT (predicted $V\dot{O}_{2\ peak}$) Split into sex-specific quartiles	Blood pressure Blood glucose HDL-c Waist circumference Metabolic risk score	Compared metabolic risk across quartiles of MSFT, adjusted for age, BMI and maturity. Linear regression Model 1: unadjusted Model 2: adjusted for age and BMI Model 3: Model 2 + maturity Model 4: Model 3 + physical activity	Inversely associated with metabolic risk score in boys (models 1-4) Inversely associated with metabolic risk score in girls (models 1-3) Highest quartile of MSFT had lowest risk for both boys and girls
Silva et al., (2017)	n = 957 (555 girls) 12 – 13 y	MSFT (predicted $V\dot{O}_{2\ peak}$) Split into sex-specific quartiles	Waist circumference Blood pressure Glucose Triglycerides Metabolic risk score	Linear regression Model 1: unadjusted Model 2: adjusted for age and maturity	MSFT negatively associated with metabolic risk score in boys and girls (model 2)

Abbreviations: MSFT = Multi-Stage Fitness Test. $V\dot{O}_{2\ peak}$ = Peak Oxygen Consumption. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. CRP = C-Reactive Protein. IL = Interleukin. HDL = High Density Lipoprotein. LDL = Low Density Lipoprotein. TNF = Tumour Necrosis Factor.

2.3.4 Relationship between Physical Fitness and Cognition

Adults

There is a growing body of research in adults suggesting a beneficial role of physical fitness for reducing the onset of brain-related diseases (such as Alzheimers), as well as delaying the onset of cognitive decline (Hötting & Röder, 2013). It has been surmised that increased physical fitness enhances neuroplasticity through several mechanisms, primarily neurotrophins – which are important regulators of neural survival, development, and function (Huang & Reichardt, 2001) - although the research on this is still immature (Hötting & Röder, 2013). In addition, some recent empirical evidence in adults demonstrates that physical fitness is beneficially associated with the connective strength of several brain regions that are involved in executive functions (Talukdar et al., 2017).

Adolescents

It has been suggested that physical fitness might have a particularly potent effect on cognitive function during adolescence, which is a crucial period for brain development, thereby rendering the adolescent brain more malleable to the potential effects (Blakemore, Burnett & Dahl, 2010; Spear, 2013). However, many studies examining the effect of physical fitness on cognitive function have included both children and adolescents in their study population (e.g. Donnelly et al., 2016) making interpretation of the results for adolescents only very difficult. Given the documented changes in cognitive function throughout adolescence (Blakemore et al., 2010) and the moderating role of age on the exercise-cognition relationship, whereby stronger effects are seen in older adolescents (Chang et al., 2012; Pontifex et al., 2019), the effect of physical fitness on cognition in adolescents requires consideration.

Both the relationship between academic performance and physical fitness and between cognitive function and physical fitness are summarised in Table 2.5. Academic performance was assessed by writing ability (Cadenas-Sanchez et al., 2020), performance on a maths test (Huang et al., 2015), and most commonly by average grade scores (Aadland et al., 2017; Adelantado-Renau et

al., 2018; Muntaner-Mas et al., 2018; Kwak et al., 2009). Despite the variance in the methods for examining academic performance, particularly as the studies were from different countries, the collective evidence suggests that physical fitness (measured by the Anderson test and MSFT) is positively associated with measures of academic performance.

As summarised in Table 2.5, all four studies examining the cross-sectional relationship between physical fitness and executive function found that higher levels of physical fitness (Andersen test, MSFT) were associated with better performance on tests of executive function which included tests of inhibitory control (Aadland et al., 2017; Huang et al., 2015; Westfall et al., 2018) and cognitive flexibility (Aadland et al., 2017; Westfall et al., 2018). Response times during an incongruent Flanker task were faster in higher-fit (Andersen test, MSFT) healthy and overweight adolescents aged 10 to 15 years (Aadland et al., 2017; Huang et al., 2015; Mora-Gonzalez et al., 2019; Westfall et al., 2018). Furthermore, Westfall et al. (2018) also found that those with a higher physical fitness had greater accuracy on the selective attention and inhibitory control tasks. Only one study examined potential mechanisms underpinning these relationships, whereby P3 amplitude, indicative of enhanced signalling within the brain, was positively associated with physical fitness during the incongruent Flanker task in overweight/obese adolescents (Mora-Gonzalez et al., 2019). Overall, whilst the collective evidence suggests that physical fitness is beneficially associated with performance during executive function tasks, further work should examine other potential mechanisms – such as neurotrophic factors – that may underpin this relationship. Moreover, whilst these studies covered a broad range of adolescents and age was controlled for in the analyses, it remains to be seen if the strength of the relationships, or even the direction, is similar across different stages of adolescence.

In the only study to examine the relationship between physical fitness and working memory, in male adolescents aged 10.2 ± 0.3 y, performance on the digit span test was positively associated with physical fitness (Andersen test) independent of skinfold thickness (Aadland et al., 2017) (Table 2.5). The domain of working memory is crucial during childhood, as it has been established as an important tenet of educational attainment in children and adolescents (Gathercole et al.,

2003). Thus, further work should replicate this solitary study and the observations should be extended to females and to older adolescents.

Additionally, there are no data on the associations between physical fitness and other important domains of cognitive function, such as visual perception. There has also been a lack of measures related to potential mechanisms that are underpinning these associations, with one study examining neuroelectric activity (Mora-Gonzalez et al., 2019). The role of brain-derived neurotrophic factor (BDNF) has been highlighted in many reviews and is associated with physical fitness in adults, yet no study has examined BDNF in adolescents. Finally, given the documented physiological changes that occur throughout adolescence – particularly within the brain – it should be examined whether the associations observed between physical fitness and cognitive outcomes is similar across different adolescent age groups and in boys and girls.

Table 2.5. Overview of the studies (2010 onwards) examining the cross-sectional relationship between **physical fitness and academic performance and cognitive function** in adolescents.

Reference	Sample details	Assessment of Physical Activity	Cognitive Function Tests & Other measures (Domains)	Analysis and Confounding Variables	Main Findings
Aadland et al., (2017)	n = 697 (357 girls) 10.2 ± 0.3 y	Andersen Test (predicted $V\dot{O}_{2peak}$)	Stroop Task (Selective Attention & Inhibitory Control) Trail Making Test (Cognitive Flexibility) Digit Span Test (Working Memory) EF composite score School grades	Linear regression Adjusted for age, skinfolds, maturity, socioeconomic status	Positively associated with all cognitive function test performances and school grades in boys only
Adelantado-Renau et al., (2018)	n = 263 (125 girls) 13.9 ± 0.3 y	Cardiorespiratory Fitness: MSFT (number of completed stages) Muscular Fitness: 4 x 10 m shuttle speed Hand grip strength Standing long jump	School grades (Math, Language, Overall)	Partial correlations, adjusting for sex, maturity, socioeconomic status	MSFT positively associated with Math and overall grades 4 x 10 m, hand grip and long jump positively associated with math
Cadenas-Sanchez et al., (2020)	n = 106 (classed as overweight) 10.0 ± 1.1 y	Cardiorespiratory Fitness: MSFT (number of shuttles) Muscular Fitness: Hand grip strength Standing long jump 4 x 10m shuttle speed Maximal incremental treadmill test (walking) Measured $V\dot{O}_{2max}$	Standardised academic achievement test (Language, writing, math and science) School grades	Linear regression Model 1: adjusted for sex, age and maternal education Model 2: adjusted for BMI and MSFT or 4 x 10 m shuttle speed	MSFT positively associated with overall academic achievement and writing Hand grip positively associated with writing and school grades Treadmill $V\dot{O}_{2max}$ positively associated with writing
Huang et al., (2015)	n = 525 (272 girls) 13.0 ± 0.6 y	Andersen Test (Total distance)	Flanker Task (Selective Attention & Inhibitory Control) Academic Skills (Math)	Linear regression Model 1: adjusted for sex, age and maturity Model 2: adjusted for adiposity	Inversely associated with selective attention and inhibitory control response time (model 2) Positively associated with math score (model 2)

Mora-Gonzalez et al., (2019)	n = 84 (37 girls) (classed as overweight) 10.1 ± 1.1 y	Cardiorespiratory Fitness: MSFT (number of shuttles) Muscular Fitness: Hand grip strength Standing long jump 4 x 10 m shuttle speed	Flanker Task (Selective Attention and Inhibitory Control) Neuroelectric Activity (EEG) (P3 amplitude and latency)	Linear regression Model 1: adjusted for sex, maturity, BMI and IQ Model 2: Fitness variables separately	MSFT performance associated with quicker inhibitory control response time and higher P3 amplitude 4 x 10 m shuttle speed associated with quicker selective attention and inhibitory control response tme Associated with higher P3 amplitude
Muntaner-Mas et al., (2018)	n = 250 (124 girls) 11.0 ± 0.8y	Cardiorespiratory Fitness: MSFT (predicted $V\dot{O}_{2peak}$) Split into high- and low-fit groups Muscular Fitness: Hand grip strength Standing long jump 4 x 10 m shuttle speed	Academic Performance (GPA; 0 – 10)	Linear regression Model 1: adjusted for sex, age, parent occupation Model 2: adjusted for BMI	MSFT and 4 x 10 m shuttle speed positively associated with GPA score (model 2) High fit group had better GPA score compared to low fit
Westfall et al., (2018)	n = 610 (300 girls) 14.2 ± 1.3 y	Andersen Test (Total Distance)	Flanker Test (Selective Attention & Inhibitory Control) Colour-Shape Switch Task (Cognitive Flexibility)	Linear regression Adjusted for sex, age, maturity	Inversely associated with selective attention and inhibitory control response times Positively associated with selective attention and inhibitory control accuracy Positively associated with accuracy during switch task

Abbreviations: EF = Executive Function. BDNF = Brain Derived Neurotrophic Factor. EEG = Electroencephaography. MSFT = Multi-Stage Fitness Test. GPA = Grade Point Average.

2.3.5 Relationship between Adiposity and Risk factors for Cardiometabolic Disease

Adults

There is a strong body of evidence showing that greater deposits of central adipose tissue are associated with increased risk of cardiovascular disease (Van Gaal et al., 2007) and type 2 diabetes (Reaven, 2005) in adults; which is suggested to be mediated through obesity-related insulin resistance (Van Gaal et al., 2007). Waist circumference is often used as a surrogate of central adiposity and is associated with several risk factors related to cardiometabolic disease, such as hypertension, hyperglycaemia, dyslipidaemia, as well as adverse outcomes such as the onset of diabetes, coronary heart disease and death (Klein et al., 2007). In addition, it has been reported that waist circumference is an important predictor of diabetes and cardiovascular disease, independent of traditional risk factors such as hypertension and blood glucose in adults (Klein et al., 2007). Whilst BMI is typically used to assess the status of obesity, recent research found that waist circumference (a chosen surrogate of central adiposity) was associated with risk factors for cardiometabolic disease (glucose intolerance, high cholesterol) even in those with a normal BMI, between 18.5 and 24.9 $kg \cdot m^{-2}$, in a large sample (n = 10, 634) of adults over the age of 19 y (Kim et al., 2019). Clearly adiposity plays a crucial role in the risk of cardiometabolic disease development in adults, but the association between adiposity and risk factors for cardiometabolic disease in adolescents has been little investigated.

Adolescents

An overview of the cross-sectional studies that have examined adiposity in adolescents, in relation to risk factors for cardiometabolic disease, can be seen in Table 2.6. Adiposity was most commonly assessed through the use of waist circumference (Agostinis-Sobrinho et al., 2018; Artero et al., 2011; Bailey et al., 2012; Barker et al., 2018; Buchan et al., 2015; Carson et al., 2013; Isasi et al., 2018; Martinez-Gomez et al., 2010 & 2012; Silva et al., 2016), with other studies using the sum of skinfolds (Bugge et al., 2012; Dring et al., 2019b) and some using both (Barker et al., 2018; Martinez-Gomez et al., 2010; Silva et al., 2017). The use of the adiposity has varied

throughout the literature, with five studies considering adiposity as an exposure variable (Bugge et al., 2012; Dring et al., 2019b; Martinez-Gomez et al., 2010 & 2012; Silva et al., 2017), whereas three studies controlled for adiposity (Agostinis-Sobrinho et al., 2018; Buchan et al., 2015; Carson et al., 2013). Furthermore, five studies used measures of adiposity to compute composite risk scores of cardiometabolic disease (Artero et al., 2011; Bailey et al., 2012; Barker et al., 2018; Silva et al., 2016 & 2017) or on their own as an outcome variable (Barker et al., 2018; Isasi et al., 2018).

Two of the studies examined the association between adiposity and blood pressure (Table 2.6). The sum of skinfolds was positively associated with systolic blood pressure (independent of physical fitness; Bugge et al., 2012), as well as diastolic and mean arterial blood pressure (independent of maturity; Dring et al., 2019b). These observations are supported by recent evidence that demonstrates a greater risk of hypertension in those in the highest quartile of waist circumference, independent of BMI, in Brazilian adolescents (12 – 17 y) (Pazin et al., 2020). Additionally, Pazin et al. (2020) provide evidence that the risk associated with being in the highest quartile is greater in older adolescents (15 – 17 y) than younger adolescents (12 – 14 y). There are currently no published data, to support such a relationship in UK adolescents, which is an interesting area of investigation for future research.

Three studies have examined the association between adiposity and insulin resistance, using the HOMA-IR index, and found that greater adiposity was associated with a higher level of insulin resistance (Bugge et al., 2012; Dring et al., 2019b; Martinez-Gomez et al., 2010). Bugge et al. (2012) found that the sum of four skinfolds (biceps, triceps, subscapular and suprailiac) was positively associated with HOMA-IR, even when adjusting for sex, maturation and physical fitness. Further evidence supports this observation, whereby the sum of four skinfolds (triceps, subscapular, supraspinale and thigh) were positively associated with HOMA-IR, independent of maturation (Dring et al., 2019b). It has also been shown that the sum of five skinfolds (triceps, biceps, subscapular, suprailiac, thigh and calf) is positively associated with HOMA-IR, independent of sex and maturity, although this relationship did not exist when physical fitness

was added to the model (Martinez-Gomez et al., 2010). There are limited data examining the associations between waist circumference and markers of metabolic health in adolescents, with only one study examining this relationship (Martinez-Gomez et al., 2010). Using partial correlations - adjusting for age, sex and pubertal status in 192 adolescents (94 girls) aged 14.8 ± 1.3 y – waist circumference was positively associated with fasting plasma insulin concentration and HOMA-IR (Martinez-Gomez et al., 2010). Thus, further research is required to elucidate the associations between waist circumference and markers of metabolic health in adolescents and to consider how these associations might differ across the years of adolescence (Stevens, Katz & Huxley., 2010).

Table 2.6. An overview of the studies (2010 onwards) examining the cross-sectional associations between **surrogates of adiposity and risk factors for cardiometabolic disease** in adolescents.

Reference	Sample details	Assessment of Adiposity	Risk Factors Examined	Analysis and Confounding Variables	Main Findings
Bugge et al., (2012)	n = 413 13.4 ± 0.3 y	Sum of 4 skinfolds (Biceps, Triceps, Subscapular, Suprailiac)	Blood pressure HOMA-IR IL-6 IL-1ra CRP TNF-α Composite risk score	Linear regression Adjusted for sex, pubertal status and $V\dot{O}_{2peak}$	Positively associated with systolic blood pressure, HOMA-IR, CRP and composite risk score
Dring et al., (2019b)	n = 121 (60 girls) 11.3 ± 0.8 y	Sum of 4 skinfolds (Triceps, Subscapular, Supraspinale, Thigh) Split into quartiles	Blood pressure Glucose Insulin HOMA-IR IL-6 IL-10 IL-1β TNF-α	Linear regression Adjusted for maturity ANCOVA with quartiles, adjusting for maturity	Positively associated with insulin, HOMA-IR, diastolic and mean arterial pressure No association with IL-6, IL-1β, IL-10 or TNF-α Quartile 1 had highest insulin and HOMA-IR Quartile 3 and 4 had lowest mean arterial pressure
Martinez-Gomez et al., (2010)	n = 192 (94 girls) 14.8 ± 1.3 y	Sum of 6 skinfolds (Biceps, Triceps, Subscapular, Suprailiac, Thigh, Calf) Waist Circumference	IL-6 CRP Glucose Insulin HOMA-IR	Partial correlation adjusting for age, sex and pubertal status Linear regression Model 1: Adjusted for age, sex, pubertal status, HOMA-IR Model 2: Adjusted for physical fitness and activity	Positively correlated with HOMA-IR and CRP Positively associated with CRP (model 2) No association in IL-6 for any model
Martinez-Gomez et al., (2012)	n = 1025 (549 girls) 14.8 ± 1.2 y	Waist Circumference	Physical fitness	Partial correlation adjusting for age and sex	Inversely correlated with physical fitness
Silva et al., (2017)	n = 957 (555 girls) 12 – 13 y	Sum of 2 skinfolds (Triceps and Subscapular)	Waist circumference Blood pressure Glucose HDL-c Triglycerides Composite risk score	Linear regression, Model 1: unadjusted Model 2: adjusted for age and maturity	Positively associated with composite risk score with and without waist circumference (model 1 & 2)

Abbreviations: HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. IL = Interleukin. CRP = C-Reactive Protein. TNF = Tumour Necrosis Factor. HDL = High Density Lipoprotein.

The use of postprandial measures, following a test meal, offer additional insight into metabolic function as they reflect peripheral insulin resistance (Muniyappa & Madan, 2000). Despite this, only one study as examined the effect of adiposity on the postprandial insulinaemic and glycaemic response to a mixed meal in adolescents and found that adolescents who were categorised as overweight/obese (based on BMI ≥ 85th percentile) demonstrated a larger insulinaemic response to the test meal (Short et al., 2018). Whilst the use of BMI provides an initial tool to categorise participants, it is suggested that waist circumference is a more reliable surrogate of adiposity (Klein et al., 2007). Furthermore, the effects of other variables on the postprandial response were not considered (such as maturity and fitness). These variables are important to consider, as physical fitness was reported to moderate the response to a test meal in a cohort of overweight/obese adolescents (Maggio et al., 2015). Thus, the associations between measures of adiposity and the postprandial insulinaemic and glycaemic response to a test meal should be further investigated in adolescents, whilst also considering the effects of other related exposure variables.

Adipose tissue is metabolically active and is known to secrete inflammatory mediators that are involved in the pathophysiology of cardiometabolic diseases, providing a rationale for the examination of the associations between adiposity and markers of inflammation in adolescents. Only three studies have examined the relationship between adiposity and markers of chronic low-grade inflammation, with a conclusive finding that inflammation is greater in those with more adiposity (Table 2.6). This is evidenced by the positive association between sum of skinfolds and CRP concentration (Bugge et al., 2012; Martinez-Gomez et al., 2010); which was independent of physical fitness in both studies. Overall, the available evidence suggests that measures of adiposity are positively associated with markers of inflammation. However, there has been limited investigation across a range of markers, with the focus mainly on CRP. Just one study examined the association between the sum of skinfolds and pro- (IL-6, IL-1β) and anti-inflammatory (IL-10) cytokines, but controlling for maturation, no relationship was observed (Dring et al., 2019b). Thus, further work is needed to investigate these relationships, as well as using alternative measures

of adiposity to extend the knowledge base. It would be particularly important to consider the association between waist circumference and pro-inflammatory cytokines, as waist circumference is typically used as a non-invasive measure of visceral adipose tissue (Klein et al., 2007) which is known to be metabolically active and secrete these cytokines (Despres et al., 2008). Furthermore, given that adolescence is a crucial period of growth which may also influence cytokine concentrations (Rubin & Hackney., 2010), these associations should be examined across the range of adolescence.

2.3.6 Relationship between Adiposity and Cognition

Adults

In young adults, BMI and visceral adipose tissue mass (assessed through bioelectrical impedance) was negatively associated with performance on an executive function task in both men and women, with a slower reaction time seen in those with a higher BMI and visceral adipose tissue mass (Huang et al., 2019). Furthermore, a higher BMI assessed during midlife was associated with a greater risk of dementia in late life, highlighting a potential link between adiposity and cognitive decline in adulthood (Anstey et al., 2011). A recent meta-analysis suggests that greater amounts of adiposity are associated with worse cognitive function (only executive function was included), but the participants were not stratified by age making interpretation of the findings difficult (Favieri et al., 2019). Whilst there is strong evidence that weight status is negatively associated with cognitive function in adults, weight status has typically been assessed through BMI and waist circumference may serve as a better marker of adiposity (section 2.2.5).

Adolescents

Although the volume of published literature examining the relationship between adiposity and cognition in adolescents is low, recent reviews have suggested that higher amounts of adiposity are related to poorer cognitive function and academic performance (Mamrot & Hanc, 2019; Martin et al., 2017). However, these reviews included both children and adolescents, with the majority of the studies focusing on pre-adolescent children. Age has been established as a strong

moderator of cognition and therefore, where possible, these populations should be examined separately. Furthermore, most studies have used BMI to categorise participants as overweight or obese, with little consideration of other markers that better reflect adiposity.

Adiposity has been considered to a lesser extent amongst the research investigating solely adolescents. Various surrogate measures of adiposity have been used, including BMI (Cadenas-Sanchez et al., 2020; Mora-Gonzalez et al., 2019; Muntanter-Mas et al., 2018; Vanhelst et al., 2016), skinfold thickness (Aadland et al., 2017; Adelantado-Renau et al., 2018; Kwak et al., 2009), waist circumference (Bugge et al., 2018; Huang et al., 2015; Muntaner-Mas et al., 2018) as well as estimating body fat from bioelectrical impedance (Vanhelst et al., 2016). Within the reviewed studies, the measures of adiposity were mostly used to control for confounding effects in the analyses (Aadland et al., 2017; Adelantado-Renau et al., 2018; Cadenas-Sanchez et al., 2020; Kwak et al., 2009; Mora-Gonzalez et al., 2019; Muntaner-Mas et al., 2018; Vanhelst et al., 2016), with fewer studies using the surrogates as exposure variables (Bugge et al., 2018; Huang et al., 2015).

The studies examining the cross-sectional associations between adiposity and cognitive function in adolescents can be found in Table 2.7. Huang et al. (2015) found that waist circumference was negatively associated with accuracy during an incongruent Flanker task in younger adolescents (12 – 13 y), independent of sex, pubertal status and physical fitness. In addition to this, they also found that waist circumference was positively associated with a large interference score – indicative of worse inhibitory control – although when fitness was entered into the model, this effect was no longer observable. Conversely, waist circumference was not associated with responses times or accuracy during either the congruent or incongruent level of the Flanker task in older (14 – 15 y) adolescents (Bugge et al., 2018). Based on these limited data and the reviews from children, it might be suggested that the effect of adiposity is more pronounced in younger adolescents and children. However, given the limited data within the adolescent population this remains speculative. Additionally, other than for executive function, particularly inhibitory control, the effect of adiposity on most domains of cognitive function has not been investigated in

adolescents. Overall, the association between markers of adiposity and cognitive function in adolescents is unclear and much more research is needed to draw informed conclusions.

Table 2.7. An overview of the studies examining the cross-sectional associations between markers of adiposity and cognitive function in adolescents.

Reference	Sample details	Assessment of Adiposity	Cognitive Function Tests & Other measures (Domains)	Analysis and Confounding Variables	Main Findings
Bugge et al., (2018)	n = 661 (347 girls) 14.2 ± 1.3 y	Waist Circumference	Flanker Task (Selective Attention & Inhibitory Control)	Linear regression Adjusted for sex, maturity, age	No association with response time or accuracy
Huang et al., (2015)	n = 525 (272 girls) 13.0 ± 0.6 y	Waist Circumference BMI	Flanker Task (Selective Attention & Inhibitory Control)	Linear regression Model 1: adjusted for sex, age, ethnicity, maturity Model 2: adjusted for fitness	BMI positively associated with inhibitory control response time but not when fitness added BMI inversely associated with inhibitory control accuracy (model 2) Waist circumference negatively associated with inhibitory control accuracy (model 2) Waist circumference positively associated with inhibitory control response time

Abbreviations: BMI = Body Mass Index.

2.4 The effects of acute bouts of exercise on risk factors for Cardiometabolic Disease and Cognitive Function.

2.4.1 Risk factors for Cardiometabolic Disease

Adults

There is a strong evidence base, in adult populations, that acute bouts of exercise lead to improvements in blood lipid metabolism (Katsanos, 2006), as well as insulin sensitivity up to 72 h post-exercise (Bird & Hawley, 2017), which are involved in the aetiology of cardiometabolic diseases. However, most studies have used pre-diabetic or overweight participants (Bird & Hawley, 2017), thus the applicability to healthy adults is unclear. Whilst the effect of exercise on blood lipid metabolism is strongly related to exercise intensity and total energy expenditure (Katsanos, 2006), the evidence regarding insulin sensitivity is not so clear. Whilst short duration, high intensity exercise leads to improvements in insulin sensitivity among adults, this has not been shown to be more beneficial than moderate-intensity or continuous exercise (Bird & Hawley, 2017). However, the fact that shorter duration higher intensity exercise takes less time to compete may be beneficial for adherence and from a public health perspective. There is also emerging evidence that games-based exercise can elicit favourable biomarker responses in sedentary adult men (Mendham et al., 2012 & 2015). Rugby union small-sided games, lasting approximately 40 min, resulted in similar insulin sensitivity improvements (Mendham et al., 2012) and improved glucose homeostasis (Mendham et al., 2015) when compared to time-matched cycling. Furthermore, IL-6 concentrations were increased following small-sided games (Mendham et al., 2012 & 2015); important in that IL-6 is a cytokine that is believed to initiate the anti-inflammatory cascade following acute bouts of exercise (Gleeson et al., 2011). Overall, the evidence from the literature in adults suggests that acute exercise is beneficial for a number of risk factors related to cardiometabolic disease and also highlights the utility of games-based exercise which is an appealing and applicable modality to be implemented in adolescent research.

Adolescents

Several studies in the adolescent population have examined the effects acute bouts of exercise on blood lipids which are known to play a role in the development of cardiovascular diseases, primarily through atherosclerosis (Katsanos, 2006; Hardman & Stensel, 2009). Non-fasting triacylglycerol concentration may be a better indicator of cardiovascular risk than fasting triacylglycerol concentration (Eberly et al., 2003) and thus most studies have employed postprandial assessments following a high fat meal. The acute exercise bout is most often undertaken ~ 16 h prior to a high fat meal, typically consumed the following morning (Barrett et al., 2007; Sedgwick et al., 2012 & 2013; Smallcombe et al., 2018; Thackray et al., 2018; Tolfrey et al., 2008), with one study examining the effects of exercise on postprandial lipaemia 1 h post-exercise (Bond et al., 2015). The research has utilised a variety of exercise protocols, such as continuous exercise (Barrett et al., 2007; Bond et al., 2015), intermittent exercise (Barrett et al., 2007; Bond et al., 2015; Sedgwick et al., 2013; Smallcombe et al., 2018; Thackray et al., 2018; Tolfrey et al., 2008), accumulation of exercise over a day (Sedgwick et al., 2012) and games-based exercise in the form of football (Smallcombe et al., 2018). In a direct comparison of intermittent exercise and continuous walking, intermittent exercise resulted in a greater reduction (26%), than treadmill walking (14%) when compared to a control trial (Barrett et al., 2007). Interestingly, both high-intensity (6 x 10 min at 75% $\dot{V}O_{2peak}$) and moderate intensity (6 x 10 min at 60% $\dot{V}O_{2peak}$) running led to similar reductions (20-25%) in postprandial lipaemia when compared to a control trial (Tolfrey et al., 2008). Overall, the weight of the evidence suggests that acute bouts of exercise, across a range of protocols and intensities, reduce the postprandial lipaemic response and thus elicit a protective effect against cardiometabolic disease. A full review on postprandial lipaemia following exercise is not within the scope of this thesis, but it has previously been reviewed extensively (Tolfrey, Thackray & Barrett, 2014).

Hyperglycaemia and hyperinsulinaemia following a test meal have also been identified as important risk factors for cardiometabolic disease, particularly for type 2 diabetes in adults (Cavalot et al., 2011; DiNicolantonio et al., 2017; Lautt, 2007; Monnier et al., 2007), but have been

examined to a limited extent in adolescents. Two of the previous studies (Sedgwick et al., 2012 & 2013) also examined the insulinaemic response to the high fat meals, with no difference found between the exercise and control trials. The lack of effect on postprandial glycaemia and insulinaemia may be attributed to the fact that the exercise bouts were performed the day before (~16 h) the test meal, or that the test meals were designed to have a high fat content that might interfere with glycaemic and insulinaemic responses. The postprandial glycaemic and insulinaemic response to high carbohydrate meals has been examined in adolescents using either an OGTT (Cockcroft et al., 2014 & 2017) or a mixed-meal (Dring et al., 2019a & 2020; Short et al., 2013 & 2018), with all studies using a high carbohydrate meal finding a reduction in postprandial insulinaemia following an acute bout of exercise. Four studies also found a reduction in postprandial glycaemia following an acute bout of exercise (Cockcroft et al., 2014 & 2017; Short et al., 2013 & 2018), whilst two studies found no difference between exercise and control trials (Dring et al., 2019a & 2020).

Recently, Dring et al. (2019a) examined the glycaemic and insulinaemic responses in the 1 h period following exercise leading up to the consumption of a mixed meal (providing 1.5g·kg^{-1} body mass of carbohydrate), as well during a 3 h postprandial period in adolescents (11 – 13 y). An ecologically valid games-based exercise protocol was used, whereby participants completed a 60 min session of basketball consisting of skill-drills and small sided games. In the transient period following exercise, blood glucose concentrations were lower 60 min post-exercise when compared to the control trial, which was mirrored by a lower plasma insulin concentration at the same time point. However, despite no difference in the postprandial glucose iAUC, there was a 35% reduction in the postprandial insulin iAUC following basketball. These findings demonstrate that ecologically valid exercise modalities have the potential to improve insulin sensitivity following a mixed meal that is consumed shortly post-exercise. Indeed, the use of games-based exercise (basketball) is likely to offer an accessible and attractive modality for adolescents, given that 85% of adolescents who meet the daily physical activity guidelines do so through team sports (Townsend et al., 2015). Interestingly, however, the most popular form of games-based exercise

within UK adolescent boys and girls, aged 11 – 15 y, is football (DCMS, 2017). To date, only Smallcombe et al. (2018) have investigated the acute effects of football (48 min of small-sided games), whereby fasting glucose and the postprandial lipaemic response to a mixed meal (the following morning) was reduced compared to treadmill running and a resting control trial. Despite the potential superiority of games-based exercise for reducing postprandial lipaemia, no study has examined the effect of football activity on the glycaemic and insulinaemic response to a same day mixed meal in adolescents.

Table 2.8. Overview of the studies (2007 onwards) examining the postprandial physiological and metabolic responses to an acute bout of exercise in adolescents.

Reference	Sample Details	Study Design	Exercise Details	Outcomes & Timing	Main Findings
Barrett et al., (2007)	N = 19 (all boys) Continuous Exercise (CE) 15.3 ± 0.1 y Intermittent Exercise (IE) 15.4 ± 0.1 y	Randomised, control, between-subjects Both groups took part in rested CON	Continuous walking: 4 x 15 min of uphill walking at 60% $\dot{V}O_{2peak}$ 3 min rest between blocks Intermittent games: LIST 4 x 15 min at 60% $\dot{V}O_{2peak}$ 3 min rest between blocks	Total cholesterol, HDL-c, glucose, TAG High fat meal; 1.25g fat and 1.07g CHO·kg^{-1} body mass (following morning ~ 16 h later) Blood 30, 45, 60, 180, 240 and 360 min after	TAG tAUC: Lower after CE vs CON (14%) Lower after IE vs CON (26%) Glucose tAUC No difference between CE and CON No difference between IE and CON
Bond et al., (2015)	N = 20 (10 girls) 14.3 ± 0.3 y	Within-subjects, counterbalanced, control Three conditions: HIIE, MIE, CON	HIIE (cycling): 90% PPO 8 x 1 min, 75 s rest MIE (cycling): 90% GET Duration matched to work during HIIE CON: seated rest	TAG, glucose Fasting, 60 min post-ex and 60, 120, 180 and 240 min post-meal Meal consumed 60 min post-ex High fat meal; 1.5g fat and 1.2g CHO·kg^{-1} body mass	TAG tAUC: No difference between trials TAG iAUC: Lower after HIIE (38%) and MIE (34%) vs CON in girls only Glucose tAUC and iAUC: No difference between trials
Cockcroft et al., (2014)	N = 9 (all boys) 14.2 ± 0.4 y	Within-subjects, crossover, counterbalanced Three conditions: HIIE, MIE and CON	HIIE (cycling): 90% PPO 8 x 1 min, 75 s rest MIE (cycling): Continuous at 90% GET Duration matched to mechanical work done in HIIE CON: Seated rest	Plasma glucose and insulin OGTT (75 g CHO) (consumed 10 min post-exercise) Before and 10, 20, 30, 60, 90 and 120 min-post OGTT Insulin sensitivity (Cederholm Index)	Glucose tAUC Lower after HIIE (7.7%) and MIE (6.2%) vs CON. Insulin tAUC Lower after HIIE (12.7%) and MIE (12.3%) vs CON. HIIE improved insulin sensitivity (11.2%) vs CON.

Table 2.8 continued.

Cockcroft et al., (2017)	N = 8 (all boys) 15.1 ± 0.4 y	Within-subjects, crossover, counterbalanced Three conditions: HIIE, MIE and CON	HIIE (cycling): 90% PPO 8 x 1 min, 75 s rest MIE (cycling): Continuous at 90% GET Duration matched to mechanical work done in HIIE CON: Seated rest	Plasma glucose and insulin OGTT (75 g CHO) (consumed 40 min post-exercise) Before and 10, 20, 30, 60, 90 and 120 min-post OGTT Insulin Sensitivity (Cederholm Index) HOMA-IR Measures repeated 24 h (Day 2) and 48 h (Day 3) later	Glucose tAUC Lower after HIIE (9.2%) and MIE (7.7%) vs CON. Similar over time Insulin tAUC Lower after HIIE (21.4%) vs CON. Similar over time. HIIE (15.3%) and MIE (9.3%) improved insulin sensitivity
Dring et al., (2019a)	N = 39 (19 girls) 12.3 ± 0.7 y	Within-subjects, randomised, counterbalanced, crossover Two conditions: Games-bases exercise and CON	Games-based exercise (basketball): 60 min; skill drills (30 min) and small-sided games (25 min) Intensity; 76% HRmax CON: Seated rest	Fasted glucose and insulin (Day 1 and day 2) Immediately and 60 min post-exercise Mixed meal; 60 min post-exercise 1.5g·kg^{-1} body mass of CHO Samples 30, 60 and 120 min post-meal	Post-exercise glucose: Higher post-exercise vs CON Lower 60 min post-exercise vs CON Post-exercise insulin: Higher post-exercise vs CON Lower 60 min post-exercise vs CON Glucose iAUC: No difference Insulin iAUC: Lower during exercise vs CON (35%)

Table 2.8 continued.

Dring et al., (2020)	N = 39 (23 girls) 12.4 ± 0.4 y	Within-subjects, counterbalanced, randomised, control trial Three conditions: 60 LIST, 30 LIST and CON	60 LIST: 4 x 12 min blocks with 3 min rest between (60 min total) 30 LIST: 2 x 12 min block with 3 min rest between (30 min total) CON: Seated rest	Fasted glucose, insulin and HOMA-IR (day 1 and day 2) Immediately and 60 min post-exercise Mixed meal; 60 min post-exercise 1.5g·kg^{-1} body mass of CHO Blood samples 30, 60 and 120 min post-lunch Breakfast provided on day 2 1.5g·kg^{-1} body mass of CHO Blood samples 30, 60 and 120 min post-lunch	*Day 1* Post-exercise glucose: Lower 60 min post-exercise (14%) for 30 LIST vs CON Post-exercise insulin: Lower 60 min post-exercise (35%) for 60 LIST vs CON Glucose tAUC: No difference between trials Insulin tAUC: Lower during 60 LIST (20%) vs CON Higher in girls vs boys overall *Day 2* Glucose tAUC: No difference between trials Insulin tAUC: No difference between trials HOMA-IR: No difference between trials
Sedgwick et al., (2012)	N = 13 (all boys) 13.6 ± 0.6 y	Within-subjects, control trial Two conditions: Exercise and CON	Exercise (treadmill walking): 60% $\dot{V}O_{2peak}$ 4 x 15 min blocks with 3 min rest Performed in afternoon (16:00) on day 1 CON: Seated rest	TAG, glucose, insulin and FMD Breakfast on day 2(~ 15 h following exercise) 1.5g and 1.8g·kg^{-1} of body mass (fat and CHO respectively) Lunch on day 2 (3 h after breakfast) 1.1g and 1.9·kg^{-1} of body mass (fat and CHO respectively) Blood samples at 30, 60 and 180 min post-breakfast Further samples at 30, 60 and 210 min post-lunch FMD assessed at 3 h post-breakfast and 3.5 h post-lunch	TAG tAUC: Lower in exercise vs CON (22%) TAG iAUC: Lower in exercise vs CON (24%) Glucose tAUC: No difference between trials Glucose iAUC: No difference between trials Insulin tAUC: Lower in exercise vs CON (8%) Insulin iAUC: No difference between trials Postprandial FMD: Lower in CON vs exercise Not associated with postprandial outcomes

Table 2.8 continued.

Sedgwick et al., (2013)	N = 14 (all boys) 12.9 ± 0.7 y	Within-subjects, Two conditions: Exercise and CON	Exercise (treadmill running): 6 x 10 min bouts at 70% $\dot{V}O_{2peak}$ 50 min rest between each bout	TAG, glucose, insulin, FMD, HOMA-IR Breakfast following morning (~15 h after exercise) 1.5g and 1.8g·kg^{-1} of body mass (fat and CHO respectively) Lunch (3 h after breakfast) 1.1g and 1.9·kg^{-1} of body mass (fat and CHO respectively) Blood samples at 30, 60, 180 min post-breakfast Further samples 30, 60 and 180 min post-lunch	TAG tAUC: No difference between trials TAG iAUC: No difference between trials Glucose tAUC: No difference between trials Insulin tAUC: No difference between trials HOMA-IR: No difference between trials Postprandial FMD: Lower in CON vs exercise at 3h and 6h
Short et al., (2013)	N = 12 (7 girls) Physically inactive	Within-subjects, partly randomised, control trial Three conditions: Prior day exercise, same day exercise and CON	Prior day exercise: 15 min walking, 15 min cycling and 15 min video game boxing (45 min total) 75% HRmax Same day exercise: 15 min walking, 15 min cycling and 15 min video game boxing (45 min total) 75% HRmax CON: Seated rest	Glucose, insulin and fatty acids Mixed meal (chocolate shake) 2803 kJ Blood samples 30, 60, 90, 120, 150 and 180 min post-meal	Glucose iAUC: Lower on prior day and same day exercise vs CON Insulin iAUC: Lower on prior day and same day exercise vs CON Insulin sensitivity: Increased on prior (45%) day and same day (78%) vs CON
Short, Pratt & Teague., (2018)	N = 23 NW; N = 11 (6 boys) 15.0 ± 2.0 y OW/Obese; N = 12 (5 boys) 14.0 ± 2.0 y	Between-subjects, partly randomised, control trial Three conditions: Prior day exercise, same day exercise and CON	Prior day exercise: 15 min walking, 15 min cycling and 15 min video game boxing (45 min total) 75% HRmax Same day exercise: 15 min walking, 15 min cycling and 15 min video game boxing (45 min total) 75% HRmax CON: Seated rest	Glucose, insulin, Mixed meal (chocolate shake) 2803 kJ Blood samples 10, 20, 30, 40, 60, 90, 120, 150 and 180 min post-meal	Glucose tAUC Prior day (9%) and same day (6%) lower vs CON for NW Only same day (5%) was lower vs CON for OW/Obese Insulin tAUC: Same day (15%) lower vs CON for OW/Obese OW/Obese 23-36% larger AUC vs NW overall

Table 2.8 continued

Smallcombe et al., (2018)	N = 15 (all boys) 12.6 ± 0.5 y	Within-subjects, counterbalanced, control trial Three conditions: Treadmill exercise (TE), Games-based exercise (GBE) and CON	Games-based exercise (Football): 48 min (6 x 8 min small-sided games) Treadmill exercise: 48 min of running (3 x 16 min blocks with 8 min rest) Ran at 60% $\dot{V}O_{2peak}$ CON: Seated rest	TAG and glucose Fasted blood sample on day 2 Standardised breakfast on the morning after exercise 1.6g and 1.8g·kg^{-1} of body mass (fat and CHO respectively) Standardised lunch (3 hours after breakfast) 1.1g and 1.9g·kg^{-1} of body mass (fat and CHO respectively) Postprandial blood taken 60 min post-breakfast as well as 5 and 60 min post lunch	Fasting TAG: Lower after GBE (30%) and TE (16%) vs CON Lower after GBE (18%) vs TE Fasting glucose: Lower after TE (3%) and GBE (4%) vs CON TAG tAUC: Lower after TE (18%) and GBE (25%) vs CON Lower after GBE (9%) vs TE Glucose tAUC: No difference
Thackray et al., (2018)	N = 31 (16 girls) 11.9 ± 0.5	Within-subjects, crossover Two conditions: HIIE and CON	HIIE (treadmill running): 10 x 1 min at 100% MAS (from $\dot{V}O_{2max}$) 1 min recovery between CON: Seated rest	TAG, glucose Breakfast consumed ~ 15 h after exercise (on day 2) 1.5g and 1.8g·kg^{-1} of body mass (fat and CHO respectively) Blood sampled 30, 60, 180 min after breakfast Lunch consumed 4 h after breakfast 1.2g and 1.9g·kg^{-1} of body mass (fat and CHO respectively) Blood sampled 30, 60 and 180 min after lunch	Fasting TAG Lower after HIIE (12%) vs CON Fasting glucose: No difference between trials TAG tAUC: Lower in boys (27%) vs girls Lower after HIIE (10%) vs CON overall TAG iAUC: No difference between trials or sex Glucose tAUC: No difference between trials

Abbreviations: HIIE = High-Intensity Intermittent Exercise. MIE = Moderate Intensity Exercise. CON = Control. LIST = Loughborough Intermittent Shuttle Test. tAUC = Total Area Under the Curve. iAUC = Incremental Area Under the Curve. HR_{max} = Maximum Heart Rate. GET = Gas Exchange Threshold. OGTT = Oral Glucose Tolerance Test. CHO = Carbohydrate. PPO = Peak Power Output. TAG = Triacylglycerol.

There has been speculation that individuals with a lower physical fitness will be exposed to greater reductions in the postprandial glycaemic and insulinaemic responses following acute exercise (Cockcroft et al., 2014; Short et al., 2013 & 2018). Conversely, others have suggested that those with a higher physical fitness may receive greater benefit due to the ability to sustain a higher absolute exercise intensity during the activity (Dring et al., 2019a); which is particularly important to consider in free-living exercise contexts (such as those encountered by young people on a daily basis). The method of determination of physical fitness in the acute exercise studies has varied, as well as varying participant characteristics (e.g. age and sex), which makes it difficult to decipher if there is indeed a moderating role of physical fitness on the acute physiological and metabolic postprandial responses to exercise. Whilst there are cross-sectional data suggesting that physical fitness moderates the postprandial response (Maggio et al., 2015), no study to date in adolescents, children, or adults has examined if physical fitness moderates the postprandial response to an acute bout of exercise. Indeed, empirical work that compares the postprandial responses between high- and low-fit participants in response to an acute bout of exercise is needed in order to robustly address this question. Furthermore, if a free-living exercise modality (such as games-based exercise) is used, the absolute exercise intensity should be quantified through external load measures (e.g. GPS) as higher-fit individuals may perform more work.

In adults the residual effects of an acute bout of exercise on insulin sensitivity can last up to 72 h (Bird & Hawley, 2017), but in adolescents the evidence has not been so conclusive. Typically, these effects have been examined through fasting indices of insulin resistance, such as HOMA-IR (Cockcroft et al., 2017; Dring et al., 2019a; Dring et al., 2020). Collectively, these results suggest that acute bouts of lab-based cycling (Cockcroft et al., 2017), basketball (Dring et al., 2019a) and intermittent running (Dring et al., 2020) do not affect HOMA-IR the morning after exercise. Additionally, Cockcroft et al. (2017) also measured HOMA-IR 48 h post-exercise, and similarly found there was no effect of exercise. The postprandial glycaemic and insulinaemic response to an OGTT (Cockcroft et al., 2017) and a standardised breakfast (Dring et al., 2020) were also examined 24 h later; and were not affected by the acute bouts of exercise. This

contrasts with the literature examining lipaemia, where there are clear residual effects of exercise, although this is typically attributed to the mechanistic role of lipoprotein lipase, the activity of which peaks around 16 h post exercise (Tolfrey et al., 2014). Interestingly, however, Smallcombe et al. (2018) found that an acute bout of football reduced fasting glucose concentrations the next morning, although insulin was not measured and thus an index of insulin resistance was not presented. Thus, while the acute bouts of exercise used to date do not affect markers of insulin resistance the next day, this evidence is limited and the potential of games-based exercise – particularly football – should be explored further.

Overall, the available evidence suggests that acute bouts of exercise can reduce the glycaemic and insulinaemic response to an OGTT and mixed meals, which are outlined as important risk factors for cardiometabolic disease (Cavalot et al., 2011; DiNicolantonio et al., 2017; Lautt, 2007; Monnier et al., 2007). However, there has been little focus on modalities of exercise that may be more applicable with an adolescent population, such as games-based exercise; particularly football. Additionally, whilst the use of an OGTT is commonplace, the use of mixed meals in this population offers greater ecological validity. Furthermore, physical fitness has been suggested to moderate some of the differential effects seen across the literature, but there is currently no empirical evidence examining this effect.

2.4.2 Cognitive Function

Adults

In adults acute exercise across a range of modalities, from continuous endurance to resistance exercise, can lead to small improvements in cognitive function, with continuous aerobic exercise the most popular modality assessed (Chang et al., 2012; Pontifex et al., 2019). However, the effects of intermittent exercise are poorly understood. In one review the effect of exercise was greatest within the 15 min post-exercise (Chang et al., 2012), but there is limited data on the extended effects of exercise (Pontifex et al., 2019). ‘Very hard’ exercise elicited the largest effect sizes, but only after a delay following the cessation of exercise (Chang et al., 2012). Furthermore,

the post-exercise improvement in cognition was found to be greatest in those with a higher physical fitness (Chang et al., 2012). In most studies (63%), young adults (18 – 34 y) were the participants and adolescents were one of the least represented categories and should be further investigated given that there would be a greater theoretical capacity for improvement in cognitive function (Pontifex et al., 2019).

Adolescents

Table 2.9 provides an overview of studies examining the effects of an acute bout of exercise on cognitive function in adolescents. Most studies examined the domain of executive function (12 of the studies reviewed) and only five studies examined working memory with other domains of cognitive function such as attention and processing speed rarely examined. Running has been the most common modality of exercise (Browne et al., 2016; Budde et al., 2010; Cooper et al., 2012 & 2016; Etnier et al., 2014; Harveson et al., 2016 & 2019), but cycle ergometry (Berse et al., 2015; Hogan et al., 2013), treadmill walking (Soga et al., 2015), resistance exercise (Harveson et al., 2016 & 2019) and coordinative exercise circuits (Budde et al., 2008) have also been implemented. Of the 16 studies reviewed, 14 found an improvement in some aspect of cognitive function following an acute bout of exercise, whereas two found no improvement (Table 2.9). These simple exercise modalities such as running, cycling and walking can be easily used in a research setting and provide greater control over exercise characteristics (intensity and duration), but they are not necessarily ecological valid for an adolescent population. It is known that the physical activity patterns of adolescents are intermittent and high intensity in nature (Bailey et al., 1995; Howe et al., 2010). Therefore, to investigate acute bouts of exercise that are applicable to adolescents, the activity should involve this characteristic.

Team sports, particularly football, are very popular modalities of exercise for adolescent boys and girls within the UK (DCMS, 2017). Games-based exercise (team sports) offers a platform for free-living, intermittent, and high-intensity exercise which best reflects the natural activity patterns of adolescents. However, to date there has been limited research investigating the effects of acute team sports exercise on cognitive function. Cooper et al. (2018) investigated the effects of a 60

min bout of basketball, which consisted of skill drills and small-sided games, on cognitive function (executive function, working memory and information processing speed). Response times were improved immediately post-exercise (executive function and working memory) as well as 45 min post-exercise (executive function) when compared to a rested control trial. Similarly, Lind et al. (2019) found that a brief (2 x 10 min) session of football, in the form of small-sided games, improves response inhibition during a Flanker task in adolescents (11 – 12 y) in comparison to a lower intensity version (walking football) and a resting control trial. This improvement was attributed to greater allocation of attentional resources, determined from the P3 amplitude of the electroencephalography (EEG) measurement. This preliminary evidence suggests that games-based exercise, which is an applicable modality for adolescents, transiently improves cognitive function. However, the only study to date utilising football – the most popular team sports among UK adolescents – used a brief protocol (20 min total) and thus, the effects of longer duration football remain unknown. Furthermore, the use of free-living activity (such as basketball and football) precludes the ability to prescribe exercise intensity, unlike laboratory-based exercise. Therefore, the use of GPS to monitor the external load accrued during such exercise would provide useful insight on exercise intensity and aid comparisons between studies.

The intensity and duration of the exercise bout are also important characteristics in the exercise-cognition relationship (Chang et al., 2012; Williams, Hatch & Cooper, 2019). Brief duration exercise has typically been favoured within the literature, with most protocols consisting of durations lasting 15 min or less (Budde et al., 2008 & 2010; Cooper et al., 2012 & 2016; Ludyga et al., 2018; Soga et al., 2015). Others have examined slightly longer durations, such as 20 min (Harveson et al., 2019; Hogan et al., 2013; Lind et al., 2019; Park & Etnier, 2019), with much less attention on longer durations, i.e. 30 min (Harveson et al., 2016) and 60 min (Cooper et al., 2018). Overall, the evidence suggests that these brief exercise protocols can elicit transient improvements in cognitive function, although it is difficult to compare with longer duration exercise due to the limited scope of research. The exercise intensity, typically recorded as relative exercise intensity, reported as a percentage of maximum heart rate (Budde et al., 2010; Harveson et al.,

2016 & 2019; Hogan et al., 2013; Soga et al., 2014), or the average heart rate attained during the exercise period (Budde et al., 2008; Cooper et al., 2012, 2016 & 2018) is also an important factor, with most studies using intensities between 50 – 85% maximum heart rate, reflecting light to moderate intensity exercise. It is also important how long after a bout of exercise the cognitive tests take place; immediate effects tend to occur with moderate-intensity exercise and benefits that occur after a delay are attributed to higher intensity exercise (Chang et al., 2012; Pontifex et al., 2019). With team sports modalities, the exercise intensity is more difficult to prescribe and control due to the free-living nature of the activity and it is possible higher-fit individuals will perform more work moderating the exercise-cognition relationship (Chang et al., 2012). However, this modality also provides autonomy over the distribution of high-intensity efforts, which may be advantageous and account for individual differences in fitness facilitating individuals in continuing to participate in the exercise (Svensson & Drust., 2005).

There is strong evidence that a higher physical fitness is beneficially associated with cognitive function in children and adults, but as highlighted in section 2.3.4 the evidence focusing solely on adolescents is limited. Despite this, physical fitness has been recognised as a potential moderator of the acute exercise-cognition relationship across a broad age range (Chang et al., 2012; Cooper et al., 2018; Hogan et al., 2013; Pontifex et al., 2019).), although the data are limited. In high- and low-fit participants (split by power output achieved on a maximal cycle ergometry test) 20 min of cycling exercise at 60% of HR_{max} improved response times in a Flanker test (executive function) in the high-fit group only and the low-fit group had a higher error rate (Hogan et al. (2013). Cooper et al. (2018) also found interactive effects between physical fitness and basketball exercise; whereby executive function and working memory performance improved immediately and 45 min post-exercise in the high-fit group only. The heart rate response was deemed similar between the high- and low-fit groups (Cooper et al., 2018), although there was no measure of external load (distance covered) which might have been different between the two fitness groups. Furthermore, these studies only assessed the transient changes up to 45 min post-exercise and there were no data to support potential mechanisms underpinning the moderating role of physical fitness.

Recent reviews suggest that these acute improvements in cognition following acute exercise might be mediated through increased neural activity, cerebral blood flow, arousal or circulating neurotrophic factors (Pontifex et al., 2019). Some attempts have been made to assess neural activity during the completion of the cognitive tasks (Hogan et al., 2013; Lind et al., 2019), with another study examining systemic concentrations of cortisol and testosterone (Budde et al., 2010). Hogan et al. (2013) did not find any evidence to suggest that 20 min of cycling altered neural activity, although they did demonstrate that low-fit participants required a higher amount of neural activity than the high-fit group when completing the same cognitive task at rest. Conversely, Lind et al. (2019) found that a 20 min bout of football led to an increased P3 amplitude – which is indicative of increased neural activity – suggesting there was a greater allocation of attentional resources.

Brain-derived neurotrophic factor (BDNF) is suggested as a potential mechanism that may be responsible for the improvements in cognition following an acute bout of exercise, given the role BDNF plays in neuroplasticity (Huang & Reichardt, 2001; Piepmeier & Etnier., 2015). Recent meta-analyses demonstrate that, in adults, BDNF concentration is increased following acute exercise (Dinoff et al., 2017; Szuhany et al., 2015). The acute increase was augmented following regular exercise training (Szuhany et al., 2015), potentially due to increased physical fitness. In addition, there was a dose-response relationship with exercise duration (Dinoff et al., 2017). Indeed, there is clear evidence that acute exercise increases BDNF concentration, are related to exercise duration and may also be moderated by physical fitness, although this evidence stems solely from adults. No studies have examined BDNF concentration following acute exercise in adolescents. Thus, given the potential mediating role in the exercise-cognition relationship, an examination of the magnitude and the time-course of post-exercise BDNF concentrations in adolescents is an important gap in the literature, particularly given the potential transient nature of the improvements in cognitive function following exercise.

Overall, the weight of available evidence does suggest that acute bouts of exercise improve cognitive function in adolescents (particularly executive function), but there is a lack of research

investigating how ecologically valid modalities of exercise – such as team games, like football – affect cognitive function, which are more applicable for an adolescent population. Despite the domain of executive function receiving much attention, future work should also focus on the effects of exercise on working memory which is, as yet, understudied and an important domain to consider during adolescence (Luna, 2009). Additionally, the effects of acute exercise beyond 60 min post-exercise are currently unknown. Furthermore, there is a lack of empirical evidence investigating the moderating role of physical fitness on the cognitive function responses to acute exercise, as well as no data on BDNF concentration following acute exercise in adolescents.

Table 2.9. An overview of the studies (2008 onwards) examining the effects of acute bouts of exercise on cognitive function and related outcomes in adolescents.

Study	Sample Details	Experimental Design	Exercise Details	Cognitive Tests (Domains) & Related Outcomes	Timing of tests	Main Findings
Berse et al., (2015)	n = 227 14.8 ± 0.9 y	Within-subjects, counterbalanced, randomised, control Two conditions: Exercise and control	Exercise: Incremental cycle to exhaustion 25 W every 10 s Control: Seated rest	Modified Switching Task (Executive Function)	Post-condition	Shifting was improved post-exercise (reduced switch costs)
Browne et al., (2016)	n = 20 (9 girls) 13.0 ± 1.8 y	Within-subjects, crossover, randomised, control Two conditions: Exercise and control	Exercise: 20 min running 65 – 75% HRR Control: Watched a cartoon	Stroop Test (Executive Function)	Pre- and 10 min post-condition	Improved response times on incongruent level 10 min post-exercise
Budde et al., (2008)	n = 99 (19 girls) 15.0 ± 0.8 y	Within-subjects, Two conditions: Exercise and control	Exercise: Coordinative exercises (ball bouncing, throwing, passing) 10 min HR: 122 ± 22 beats·min^{-1} Control: Normal PE lesson HR: 122 ± 27 beats·min^{-1}	d2 (Attention)	Pre- and post-condition	Improved attention and concentration (pre to post) in both groups Greater improvement in coordinative exercise group
Budde et al., (2010)	n = 59 (26 girls) 14.4 ± 0.5 y	Between-subjects, Three conditions: Moderate intensity, high intensity and control	Moderate intensity: Running 12 min 50 – 65% HR_{max} High intensity: Running 70 – 85% HR_{max} Control: Seated rest	Letter Digit Span (Working Memory) Cortisol Testosterone	Pre- and post-condition	Improved working memory in moderate intensity group only Testosterone and cortisol not related to improvements

Table 2.9 continued.

Cooper et al., (2012)	n = 45 (30 girls) 13.3 ± 0.3 y	Within-subjects, randomised, crossover, control Two conditions: Exercise and control	Exercise: 10 bouts of 7 x 20 m shuttles at 8 km·h^{-1} 30 s rest between HR; 172 ± 17 beats·min^{-1} Control: Seated rest	Stroop Test (Executive Function) Sternberg Paradigm (Working Memory) Visual Search Test (Visual Processing)	Pre- and 45 min post-condition	Greater improvement in response times for complex visual processing post-exercise Coupled with a decrease in accuracy Quicker response times on Sternberg post-exercise No difference between trials for Stroop
Cooper et al., (2016)	n = 44 (23 girls) 12.6 ± 0.6 y	Within-subjects, randomised, crossover, control Two conditions: Exercise and control	Exercise: Running 10 x 10 s sprint 50 s rest HR: 181 ± 13 beats·min^{-1} Control: Seated rest	Stroop Test (Executive Function) Digit Symbol (Psychomotor Speed) Corsi Blocks (Working Memory)	Pre-, immediately- and 45 min post-condition	Quicker response times on congruent Stroop 45 min post-exercise vs control Quicker response times on incongruent Stroop immediately post-exercise vs control No effect on digit symbol or corsi blocks
Cooper et al., (2018)	n = 39 (19 girls) 12.3 ± 0.3 y Split into high- and low-fit groups based on MSFT performance	Within- and between-subjects, crossover, control Two conditions: Exercise and control	Exercise: Basketball Skill drills & small games 60 min HR: 158 ± 11 beats·min^{-1} Control: Seated rest	Stroop Test (Executive Function) Sternberg Paradigm (Working Memory) Trail Making Test (Information Processing Speed) d2 (Attention)	Pre-, immediately- and 45 min post-condition	Congruent Stroop response times slower 45 min post-exercise vs control Incongruent Stroop responses times quicker immediately- and 45 min post-exercise vs control Low-fit group slower at 45 min post-exercise on both Stroop levels, whereas high-fit were quicker 5-item Sternberg response times were quicker immediately post-exercise vs control 3-item response times were quicker immediately and 45 min post-exercise in high-fit group Low-fit were quicker during control trial at the same time points No effect on trail making test and d2

Table 2.9 continued.

Etnier et al., (2014)	n = 43 (28 girls) 11 – 12 y	Between-subjects, control Two conditions: Exercise and control	Exercise: Running – MSFT Completed until exhaustion Control: Normal PE lesson	Rey Auditory Verbal Learning Test (Learning and Memory) Recognition Task (Long term Memory)	Post-condition 24 h following (recognition task)	Improved recall ability in exercise group vs control group Improved learning ability in exercise group vs control group Long term memory not affected
Harveson et al., (2016)	n = 94 (46 girls) 15 – 16 y	Within-subjects, randomised, counterbalanced, crossover, Three conditions: Aerobic exercise, resistance exercise and control	Aerobic Exercise: 30 min Walking/jogging 50 – 60% HR_{max} Resistance Exercise: 30 min Weight machines 15 reps of each exercise squat, leg press, bench press, pull down, seated row and overhead press Control: Seated rest	Stroop Test (Executive Function) Trail Making Test (Information Processing Speed)	5 – 40 min post-condition	Aerobic and resistance exercise improved congruent Stroop performance vs control Similar between aerobic and resistance Aerobic and resistance exercise improved incongruent Stroop performance vs control Similar between aerobic and resistance Only aerobic exercise improved trail making test performance
Harveson et al., (2019)	n = 63 (6 girls) 13.7 ± 0.5 y	Within-subjects, randomised, counterbalanced, control Three conditions: Aerobic exercise, resistance exercise, control	Aerobic Exercise: 20 min Walking/jogging 50 – 60% HR_{max} Resistance Exercise: 20 min Weight machines 15 reps of each exercise squat, leg press, bench press Control: Seated rest	Stroop Test (Executive Function) Mathematics Test (Academic Achievement)	5 – 20 min post-condition	Math performance better after resistance exercise vs control Congruent and incongruent Stroop performance better after resistance exercise compared to aerobic exercise and control No difference between aerobic exercise and control

Hogan et al., (2013)	n = 30 14.2 ± 0.5 y Split into high- and low-fit groups	Within- and between-subjects, randomised, control Two conditions: Exercise and control	Exercise: Cycling 20 min 60% HR_{max} Control: Seated rest	Flanker Test (Executive Function) EEG	Post-condition	Response times were quicker post-exercise vs control in the high-fit group only Low-fit group had higher error rates vs high-fit group Higher neural activity in low-fit group vs high-fit group at rest
Lind et al., (2019)	n = 81 (33 girls) 11 – 12 y	Randomised, between-subjects Three conditions: Normal football, walking football and control	Normal football: 2 x 10 min, 5 min break Small games 70 – 100% HRR Walking football: 2 x 10 min, 5 min break Small games 60 – 80% HRR Control: Seated rest (watched football video)	Flanker Test (Executive Function) Visual Memory Task (Declarative Memory) EEG	Pre- and immediately post-condition	Incongruent Flanker accuracy improved after normal football and control trial Congruent response times slower after control trial Real football improved response inhibition Greater P3 amplitude after real football – greater allocation of attentional resources
Ludyga et al., (2018)	n = 94 (all boys) 14 ± 0.8 y	Between-subjects, randomised Three conditions: HIIE,MIIE and Control	HIIE: 16 min Circuit training Skipping, jumping, running, ball dribbling, 60 s work, 30 s rest HR; 154 ± 16 beats·min^{-1} MIIE: 16 min Skipping, jumping, running, ball dribbling 30 s work, 30 s rest HR; 144 ± 20 beats·min^{-1} Control: Seated rest	Flanker Test (Executive Function)	Pre- and immediately, 30 and 60 min post-condition	Reduced response times in MIIE group; pre to immediately post pre to 30 min post pre to 60 min post No effects observed in HIIE or control

Table 2.9 continued.

Park & Etnier., (2019)	n = 22 (11 girls) 15.9 ± 0.3 y	Within-subjects, randomised, counterbalanced Two conditions: Exercise and control	Exercise: Cycling 20 min 64 – 76% HR_{max} Control: Seated rest	Symbol Digit Test (Attention & Psychomotor Speed) Stroop Test (Executive Function) Tower of London (Executive Function)	Immediately post-control 10 min post-exercise	Performance was improved on all tasks following exercise
Soga et al., (2015)[a]	n = 28 (4 girls) 15.6 ± 0.5 y	Within-subjects, crossover, control Two conditions: Exercise and control	Exercise: Treadmill walking 13 min 60% HRmax Control: Seated rest	Flanker Task (Executive Function) Spatial n back (Working Memory)	Pre, during and immediately post-condition	Lower Flanker accuracy during walking compared to after walking No effect on Flanker response time Longer reaction time during walking vs control for spatial n back
Soga et al., (2015)[b]	n = 27 15.8 ± 0.4 y	Within-subjects, crossover, control Two conditions: Exercise and control	Exercise: Treadmill walking 13 min 67% HRmax Control: Seated rest	Flanker Task (Executive Function) Spatial n back (Working Memory)	Pre, during and immediately post-condition	No effect on Flanker accuracy No effect on Flanker response times Lower accuracy for spatial n back during walking vs control Longer spatial n back response time during walking vs control

Abbreviations: HR_{max} = Maximum Heart Rate. HRR = Heart Rate Reserve. HIIE = High-Intensity Intermittent Exercise. MIE = Moderate Intensity Exercise.
[a,b] Two separate experiments from the same study.

2.5 Effect of Exercise Interventions on Risk Factors for Cardiometabolic Disease and Cognitive Function

2.5.1 Effect of High Intensity Exercise Interventions on Risk factors for Cardiometabolic Disease

Adults

High-intensity exercise is emerging as an effective way to accrue physical activity, with less time-commitment but still yielding the same adaptive benefits for physical fitness and health when compared with longer duration, lower intensity exercise (Buchheit & Laursen, 2013; Gibala et al., 2006). Early work demonstrated that low volume, high-intensity exercise elicits similar skeletal muscle and performance adaptations when compared to traditional, high volume training; thus highlighting the time-efficient approach as a suitable alternative to traditional, longer duration protocols (Gibala et al., 2006). Recently, the utility and benefits of high-intensity exercise training for glycaemic control (Cassidy et al., 2017), insulin sensitivity (Bird & Hawley, 2016), physical fitness (Batacan et al., 2016), markers of chronic low-grade inflammation (Cronin et al., 2017) and endothelial function has emerged (Maturana et al., 2020). Overall, there is consistent evidence that regular high intensity, low volume exercise training improves several markers of cardiometabolic disease in adults. Importantly, such protocols involving brief, high-intensity bursts may be applicable for younger populations; given that this better reflects their natural activity patterns compared to continuous exercise and has the potential to be scheduled into breaks within a school day.

Adolescents

An overview of studies examining high-intensity exercise interventions in adolescents can be found in Table 2.10. All reviewed studies were school-based, whereby the training sessions were conducted in school, rather than in a laboratory. This is an important consideration for this population given that a large proportion of the weekdays are spent at school and thus school-based interventions provide greater ecological validity. Ideally, the intervention sessions should

be scheduled in addition to the regular PE lessons in the curriculum, rather than replacing PE (Bond et al., 2017). Interestingly, the reviewed studies consisted of samples containing a mixture of boys and girls (Bond et al., 2015; Buchan et al., 2013; Martin et al., 2015 & 2018; Weston et al., 2016) or just boys (Cockcroft et al., 2019; Logan et al., 2016), supporting the observation that girls are under-represented in exercise training studies amongst adolescents (Bond et al., 2017).

The intervention duration in previous studies has ranged from two weeks to 10 weeks, the majority lasting ~ 7 weeks, with a common frequency of three sessions per week amongst all reviewed studies (Table 2.10). Running-based sessions were used in three studies (Buchan et al., 2013; Martin et al., 2015 & 2018), along with circuit-based exercise in two studies (Logan et al., 2016; Weston et al., 2016) and cycle ergometry in two studies (Bond et al., 2015; Cockcroft et al., 2019). All studies adopted a high-intensity interval approach, with the duration of efforts ranging from 20 s to 60 s (Table 2.10). The intensity of the individual session was prescribed at 90% of peak power output during cycling (Bond et al., 2015; Cockcroft et al., 2019) or ≥ 90% HR_{max} during circuit-based exercise (Logan et al., 2016; Weston et al., 2016). The running-based protocols were all described as 'maximal effort' and resulted in a relative intensity ≥ 90% HR_{max} (Martin et al., 2015 & 2018); although Buchan et al. (2013) did not report the relative exercise intensity. The ecological validity of such approaches is recognised, given that the natural activity patterns of adolescents are typically sporadic and high-intensity (Bailey et al., 1995; Howe et al., 2010). However, the use of cycle ergometry and, to an extent, circuit-based exercise (dependent on the activities included) invokes the necessity for equipment. As well as being a barrier to physical activity amongst adolescents (Robbins et al., 2010), the presence of such equipment cannot be guaranteed at all schools. Thus, if an intervention is to apply to all school-based populations (and potentially be adopted into common practice) then an equipment-free approach should be prioritised.

Five of the included studies (Table 2.10) found improvements in physical fitness (measured by MSFT in three studies and directly measured $\dot{V}O_{2peak}$ in two studies) following the intervention (Buchan et al., 2013; Logan et al., 2016; Martin et al., 2015 & 2018; Weston et al., 2016), although this improvement was only observable in the higher volume (4 and 5 sets of 4 x 20 s circuit

exercises) training groups of Logan et al. (2016). Conversely, two studies found no improvement in physical fitness (when $V\dot{O}_{2peak}$ was directly determined during a cycle ergometer test) following 2 weeks of high-intensity cycling at 90% peak power (Bond et al., 2015; Cockcroft et al., 2019). This could be attributed to either the modality of the exercise, given that the observed improvements occurred within running-based or circuit-based interventions, or the brief intervention length. Indeed, the interventions where improvements were observed consisted of 7-week (Buchan et al., 2013; Martin et al., 2015), 8-week (Logan et al., 2016) and 10-week interventions (Weston et al., 2016). However, the intervention conducted by Martin et al. (2018) was four weeks in length and demonstrated improvements in predicted $V\dot{O}_{2peak}$ from the MSFT, which in effect simply means that distance improved on the MSFT as predicting $V\dot{O}_{2peak}$ in this way is subject to inaccurate estimation (Armstrong & Welsman, 2018). Nonetheless, the effects of short-term (4 weeks) high-intensity training, particularly running, on physical fitness in adolescents is currently unclear.

Aside from physical fitness, a range of outcomes have been examined in relation to risk of cardiometabolic disease, typically consisting of traditional risk factors such as; fasting blood markers of metabolic health (glucose, insulin, lipids), HOMA-IR, blood pressure and body composition (Table 2.10). Waist circumference was reduced following a 10-week circuit-based intervention (Weston et al., 2016) and there was a documented overall reduction (6%) in estimated body fat after 8 weeks of mixed circuit and resistance training (Logan et al., 2016). Buchan et al. (2013) found that waist circumference and body mass were not reduced in the sprint-training group, but these measures did increase in the control group; however, this conclusion was drawn from within-group comparisons which is an inappropriate method of analysis for such experimental designs (Hecksteden et al., 2018). It should be noted however, that the inclusion of a weekly resistance training session may have contributed to the reduction in estimated body fat observed by Logan et al. (2016). Nonetheless, the limited evidence suggests that surrogates of body composition are reduced following high-intensity training in adolescents,

but these changes favour longer duration interventions with the efficacy of shorter duration interventions currently unknown.

The effect of high intensity exercise interventions on fasting blood lipids is mixed, with some studies suggesting no effect (Buchan et al., 2013; Bond et al., 2015; Logan et al., 2016), whereas others provide evidence that fasting triglyceride concentrations (Martin et al., 2018; Weston et al., 2016) and total cholesterol (Martin et al., 2018) are reduced following HIIT in adolescents. Similarly, the evidence pertaining to measures of fasting insulin resistance is equivocal, although more research suggests no effect of training (Bond et al., 2015; Cockcroft et al., 2019; Buchan et al., 2013; Logan et al., 2016; Martin et al., 2015), but fasting insulin concentration and subsequently HOMA-IR index were reduced following 4 weeks of sprint-training (Martin et al., 2018). Cockcroft et al. (2019) suggested that exercise interventions would be more beneficial for those who presented with indexes of higher insulin resistance at baseline. This proposal was built on a negative correlation between baseline insulin resistance (i.e. HOMA-IR) and the change score of this index. However, such a relationship is very likely as the higher the baseline value the larger the expected absolute change in the variable and is indicative of regression to the mean, a well-known statistical phenomenon (Clifton & Clifton, 2019). This does not necessarily suggest an effect of treatment, which is further complicated in this example without a comparator arm (such as a control group). Perhaps in asymptomatic adolescents, like those involved in the reviewed studies, there is less scope to improve fasting markers related to metabolic health. A further consideration is the sample composition, whereby previously a mix of boys and girls were used. Given that there is a puberty-related development of insulin resistance, which may be exacerbated in girls (Cooper et al., 2017), the use of mixed samples (boys and girls) may suppress the effect of exercise on those with the scope for improvement (i.e. girls). Therefore, more work focusing solely on adolescent girls is necessary as they may be more sensitive to the chronic effects of exercise.

Table 2.10. An overview of the studies (2011 onwards) examining the effects of exercise interventions on risk factors for cardiometabolic disease in healthy adolescents.

Reference	Sample Details	Intervention Duration	Exercise Modality	Exercise Frequency	Exercise Intensity & Duration	Outcome Measures and Timing	Main Findings
Bond et al., (2015)	n = 13 (6 girls) 13 – 14 y HIIT; n = 13	2 weeks	Cycling	3 sessions per week	90% PPO, 70-95 rpm 8 x 1 min ; 75 s rest (sessions 1 & 2) 9 x 1 min ; 75 s rest (sessions 3 & 4) 10 x 1 min; 75 s rest (sessions 5 & 6)	Directly measured $\dot{V}O_{2max}$ Glucose, Insulin TAG, TC, HDL-c Blood pressure HRV Endothelial function postprandial response to a test meal (glucose, insulin, TAG) Pre, 1-day and 3-days Post	$\dot{V}O_{2max:}$ No change No effect on any fasting outcomes or postprandial outcomes Improved endotelial function Improved HRV
Buchan et al., (2011)	n = 57 (10 girls) 16.4 ± 0.3 y HIIT; n = 17 MICT; n = 16 CON; n = 24	7 weeks	Running	3 sessions per week	HIIT; 30 s max effort sprint with 30 s rest Weeks 1 & 2: 4 sprints Weeks 3 & 4: 5 sprints Weeks 5 & 6: 6 sprints Week 7: 6 sprints (20 s rest) MICT; 20 min running at 70% $\dot{V}O_{2peak}$	MSFT (number of shuttles) Blood pressure TC, HDL-c, LDL-c CRP, IL-6 Glucose, Insulin Triglycerides Pre & Post (90 h after final session)	Systolic blood pressure reduced in HIIT group, but no difference between groups MSFT improved in both HIIT and MICT Glucose reduced in MICT group No change in IL-6, CRP or lipids
Buchan et al., (2013)	n = 89 (25 girls) 16.7 ± 0.7 y HIIT; n = 42 (12 girls) CON; n = 47 (13 girls)	7 weeks	Running	3 sessions per week	Maximal effort sprints, 30 s Week 1 & 2: 4 x 30 s: 30 s rest Week 3 & 4: 5 x 30 s: 30 s rest Week 5 & 6: 6 x 30 s: 30 s rest Week 7: 6 x 30 s: 20 s rest	MSFT (shuttles) Blood pressure Waist circumference LDL-c, HDL-c, TC Glucose, Insulin CRP, IL-6 Triglycerides Pre & Post	MSFT; Improved in HIIT group SBP; Reduced in HIIT group WC; Increased in CON LDL-c; Reduced in both groups Total Cholesterol; Reduced in CON No change in glucose, insulin, CRP and IL-6

Table 2.10 continued.

Cockroft et al., (2019)	n = 9 boys 14.3 ± 0.3 y HIIT; n = 9	2 weeks	Cycling	3 sessions per week	90% PPO, 70 – 95 rpm 8 x 1 min, 75 s rest (sessions 1 & 2) 9 x 1 min, 75 s rest (sessions 3 & 4) 10 x 1 min, 75 s rest (sessions 5 & 6)	Fasting glucose, insulin, indices of IR and IS Postprandial glucose and insulin following an OGTT Pre, 20 h & 70 h Post	Fasting markers: No change IR & IS indices: No change Postprandial markers: No change
Logan et al., (2015)	n = 26 boys (low activity) 16 ± 1 y Group 1; n = 5 Group 2; n = 6 Group 3; n = 5 Group 4; n = 5 Group 5; n = 5	8 weeks	Choice of; Cycling, running, rowing, cross-trainerm shuttle runs	2 HIIT sessions, 1 resistance session	HIIT; 1 set = 4 x 20 s max effort, 10 s rest ≥ 90% HRmax Group 1; 1 set Group 2; 2 sets Group 3; 3 sets Group 4; 4 sets Group 5; 5 sets Resistance; 8 – 12 reps of 3 exercises	Estimated $\dot{V}O_{2peak}$ from cycle ergometry, body fat percentage, IL-6, LDL-c, HDL-c, triglycerides, glucose, insulin, HOMA-IR Pre & Post (72 h after final session)	$\dot{V}O_{2peak}$; Improvement in groups 4 & 5 Overall improvement of 6% Body fat; Overall reduction of 4% IL-6; Increased in groups 4 & 5 ↑ Glucose, insulin & HOMA; No effect
Martin et al., (2015)	n = 49 (12 girls) 16.9 ± 0.4 y SIT; n = 26 (7 girls) CON; n = 23 (5 girls)	7 weeks	Running Normal PE lessons	3 sessions per week	Maximal effort sprints, 30 s Weeks 1 & 2: 4 x 30 s:30 s Weeks 3 & 4: 5 x 30 s:30 s Weeks 5 - 7: 6 x 30 s:30 s	$\dot{V}O_{2peak}$ (MSFT) Fasting glucose, insulin, HOMA-IR Pre & Post (63 – 84 h after final session)	$\dot{V}O2peak$; Increased in SIT group Reduced in CON group Fasting glucose; Both groups increased Insulin & HOMA-IR No change

Table 2.10 continued.

Martin et al., (2018)	n = 52 (20 girls) 16.5 ± 0.5 yrs SIT; n = 52 (20 girls) CON; n = 30 (11 girls)	4 weeks	Running Normal PE lessons	3 sessions per week	Maximal effort sprints, 30 s Weeks 1&2: 5 x 30 s:30 s Weeks 3&4: 6 x 30 s:30 s	Predicted $\dot{V}O_{2peak}$ (MSFT), blood pressure, fasting glucose, insulin, HOMA-IR, total cholesterol, LDL-c, HDL-c Clustered risk score	Predicted $\dot{V}O_{2peak}$; Larger in SIT group at POST Glucose; No change in either group Insulin & HOMA-IR; Lower in SIT group TG, TC & LDL-c: Lower in SIT group Clustered risk score; Lower in SIT group
Weston et al., (2016)	n = 101 (38 girls) 14.1 ± 0.3 yrs HIIT; n = 41 (8 girls) CON; n = 60 (30 girls)	10 weeks	Boxing, football, basketball, dance Normal PE lessons	3 sessions per week	4-7 bouts of the listed exercises 45 s effort, 90 s rest ≥ 90% HRmax	MSFT (shuttles) Fasting glucose, CRP, WC, total cholesterol, triglycerides Pre and Post	MSFT; Improved in HIIT group WC; Reduced in HIIT group Triglycerides; Reduced in HIIT group

Abbreviations: HIIT = High-Intensity Interval Training. MICT = Moderate-Intensity Continuous Training. SIT = Sprint Interval Training. PE = Physical Education. PPO = Peak Power Output. HRV = Heart Rate Variability. TAG = Triacylglycerol. CON = Control. LDL = Low Density Lipoprotein. HDL = High Density Lipoprotein. TC = Total Cholesterol. CRP = C-Reactive Protein. IL = Interluekin. IR = Insulin Resistance. IS = Insulin Sensitivity.

The assessment of insulin resistance using HOMA-IR, typically reflects hepatic insulin resistance (Muniyappa & Madan., 2000) and the examination of a postprandial response provides additional insight into metabolic health, by offering greater sensitivity in those considered asymptomatic (Lautt, 2007) and providing a measure of peripheral insulin resistance (Muniyappa & Madan, 2000). Currently, the postprandial response to a test meal in healthy adolescents has only been determined in two previous studies (Bond et al., 2015; Cockcroft et al., 2019). Bond et al. (2015) found that three sessions per week (8 x 1 min cycling at 90% peak power output) for two weeks did not affect the postprandial glycaemic, insulinaemic or lipaemic response to a high fat and high sugar meal in a mixed sample (6 girls, 7 boys; 12 – 13 y). In addition, using the same intervention protocol, Cockcroft et al. (2019) found that the postprandial glycaemic and insulinaemic response to an OGTT was not affected in a small sample of boys (n = 9; 14 – 15 y). It could be argued, however, that despite the OGTT being the gold standard method (Muniyappa & Madan, 2000) it lacks ecological validity within an adolescent context. Furthermore, these two studies did not have a comparator arm (such as a control group) which is the recommended structure for trials assessing training efficacy (Hecksteden et al., 2018). Moreover, the effects of training on this marker may be more pronounced in girls (particularly younger, 11 – 13 y), given the established sex differences in insulin resistance (Cooper et al., 2017). Thus, further work is needed regarding the effect of high intensity intermittent training on the postprandial responses to an ecologically valid meal in adolescents, and particularly in girls.

In addition to the aforementioned traditional risk factors for cardiometabolic disease, a two-week high-intensity cycling intervention resulted in improvements in novel risk factors for cardiometabolic disease, whereby endothelial function and heart rate variability were improved (Bond et al., 2015). Inflammatory cytokines are another novel risk factor and can provide additional insight into risk of cardiometabolic disease in an adolescent population (Balagopal et al., 2011). However, only three studies in the reviewed literature have investigated the effects of exercise training on inflammatory cytokines in adolescents (Buchan et al., 2011 & 2013; Logan et al., 2016). IL-6 and CRP concentrations were not affected by 7 weeks of maximal sprint training

(Buchan et al., 2011 & 2013) or moderate intensity running (Buchan et al., 2011). However, there was an increase in resting IL-6 concentration in the groups that received the higher volume of training, consisting of four x 20 s sprints, with a 10 s rest, repeated for four and five sets (Logan et al., 2016). It is interesting that resting IL-6 concentrations were increased, given that regular exercise is hypothesised to reduce resting IL-6 (Ertek et al., 2012; Gleeson et al., 2011). Nonetheless, there are currently limited data regarding the effects of exercise interventions on markers of chronic low-grade inflammation in adolescents, which have important pathophysiological roles in the development of cardiometabolic diseases (Dokken et al., 2008; Esser, 2014).

Overall, the evidence suggests that high-intensity exercise interventions can elicit favourable changes in physical fitness and some risk factors for cardiometabolic disease across a range of intervention durations and exercise doses. However, the research to date has only considered a limited range of largely traditional risk factors related to cardiometabolic disease. Thus, further work examining a range of novel risk factors, particularly in adolescent girls who are presently underrepresented in the literature and less active than adolescent boys, would extend the current evidence base.

2.5.2 Effect of High Intensity Exercise Interventions on Cognitive Function

Adults

The effects of exercise interventions (including high intensity exercise) on cognitive function have received much attention in older adult populations (Levin et al., 2017), largely due to the age-related decline in cognition that occurs and thus highlighting exercise training as a method to attenuate this. It has been reported that regular aerobic exercise training over a 6-month period (three weekly sessions of 60-min walking) in adults has led to simultaneous improvements in the connectivity of neural circuits within the brain and cognitive function performance, across a range of domains of cognition (Hsu et al., 2017). Overall, reviews generally suggest that exercise interventions (including high intensity intermittent exercise), can lead to small improvements in

cognitive function in healthy individuals in adults of all ages (Levin et al., 2017; Ludyga et al., 2020). In the studies that have specifically examined the effect of high intensity intermittent exercise on cognitive function in adults the key findings are; improved performance on a memory interference task in young adults (18 – 30 years) following 6 weeks of high-intensity cycling (three sessions of 10 x 60 s sprint at 90% peak power) (Heisz et al., 2017), and improved information processing speed on a Stroop task in older adults (55 – 75 years) following 16 weeks of high-intensity treadmill walking (three sessions of four x four min walking at 90 – 95% HRmax) (Coetsee & Terblanche, 2017).

Adolescents

The effects of exercise interventions on cognitive function in adolescents has received limited attention (Table 2.11). Due to the sparsity of evidence relating to exercise interventions and cognitive function in adolescents, both continuous lower intensity and high-intensity intermittent protocols are reviewed. The interventions reviewed have lasted from 6 to 12 weeks in length, with exercise frequency ranging from two to five sessions per week. The exercise protocols were mostly continuous in nature (Chen et al., 2016; Jeon & Ho Ha., 2017; Ludyga et al., 2018; Schmidt et al., 2016), with only two studies investigating intermittent exercise (Costigan et al., 2016; Moreau et al., 2017). A range of exercise modalities have been employed from team games (Schmidt et al., 2016), treadmill running (Jeon & Ho Ha, 2017), resistance or combined resistance and aerobic exercise (Costigan et al., 2016) and most commonly, circuit-based aerobic exercise (Chen et al., 2016; Ludyga et al., 2018; Moreau et al., 2017). A range of cognitive function tests were used, but the most common domain assessed was executive function – particularly inhibitory control (Table 2.10); with all studies providing some evidence of improvement in cognitive function for the intervention groups. Only two studies examined an outcome, in the form of BDNF or EEG, that may be mechanistically related to the improvements (Jeon & Ho Ha, 2017; Ludyga et al., 2018).

Interventions utilising continuous exercise protocols over a 12-week period demonstrated improvements in executive function (Chen et al., 2016) and working memory (Jeon & Ho Ha,

2017). Both studies, in boys and girls aged 12 – 13 y (Chen et al., 2016) and 15 - 16 y (Jeon & Ho Ha, 2017), involved four sessions per week, consisting of treadmill running at different intensities (40%, 55% and 70% of $V\dot{O}_{2max}$) until 200 kcal was expended (Jeon & Ho Ha, 2017), or 40 min sessions of walking, skipping and dancing at 60-70% HR_{max} (Chen et al., 2016). Interestingly, all exercise intervention groups improved working memory performance assessed at rest 48 h following the end of the intervention, but the high-intensity group (70% $V\dot{O}_{2max}$) improved to a greater extent (Jeon & Ho Ha, 2017). Other interventions utilising continuous exercise have done so for shorter intervention lengths, consisting of aerobic exercises (tag, relays, ball games for 20 min) five times per week and found that responses times on executive function tasks, which was assessed at rest (but the delay following the end of the intervention was not reported), were improved when compared to a control group (Ludyga et al., 2018). An even shorter intervention length of 6 weeks found that twice weekly sessions of continuous cognitively engaging exercise (45 min) led to greater improvements in executive function, assessed after two morning language lessons (with the delay at the end of the intervention not reported), whereas 45 min of aerobic running had no effect (Schmidt et al., 2016). Overall, the limited evidence suggests that continuous exercise of moderate intensities can lead to improvements in cognitive function after interventions ranging from 6 – 12 weeks in length. However, it could be argued that such continuous protocols are not wholly appropriate for younger populations, given that their natural activity patterns are typically sporadic and high intensity in nature (Bailey et al., 1995; Howe et al., 2010).

Table 2.11. An overview of the studies (2010 onwards) examining the effects of exercise interventions on cognitive function in healthy adolescents.

Reference	Sample Details	Intervention Duration	Modality	Frequency	Intensity & Exercise Duration	Cognitive Tasks (Domain) & Timing	Main Findings
Chen et al., (2016)	n = 50 (22 girls) 12.7 ± 0.7 y (classed as overweight) INT; n = 25 (9 girls) CON; n = 25 (13 girls)	12 weeks	Different exercises (fasting walking, skipping, dancing)	4 sessions per week	40 min session 60-70% HRmax	Wisconsin Sorting Card Test (Executive function; Shifting) Pre & Post	WCST; performance improved in INT and not CON (less errors)
Costigan et al., (2016)	n = 65 (20 girls) 15.8 ± 0.6 y Aerobic; n = 21 Resistance & Aerobic; n = 22 Control; n = 22	8 weeks	Aerobic (shuttle runs, jumping jacks, skipping) Resistance & Aerobic (body weight squats, push ups)	3 sessions per week	Weeks 1-3; 8 min Weeks 4-6; 9 min Weeks 7-8; 10 min 30 s efforts, rest not specified	Trail Making Test (Executive function) (Visual attention, speed, scanning, processing speed) Pre & Post	Resistance & Aerobic: Improved executive function Aerobic; Smaller improvement in a sub-section of EF
Jeon & Ho Ha., (2017)	n = 40 15.3 ± 0.5 y LIE; n = 10 MIE; n = 10 HIE; n = 10 CON; n = 10	12 weeks	Treadmill running	4 sessions per week	LIE; 40% $\dot{V}O_{2max}$ MIE; 55% $\dot{V}O_{2max}$ HIE; 70% $\dot{V}O_{2max}$ Duration set to expend 200 kcal at respective intensities	Wechsler Intelligence scale (Working memory) BDNF Pre & Post	Working memory: HIE group improved to a greater extent than all other groups BDNF; Increased in MIE and HIE
Ludyga et al., (2018)	n = 36 (13 girls) 12.5 ± 0.7 INT; n = 20 (5 girls) CON; n = 16 (8 girls)	8 weeks	Aerobic and co-ordinative exercises (tag, ball games, relays) Played in small teams	5 sessions per week	20 min session 68 ± 5% HRmax	Stroop Task (Executive function; Inhibitory control) Pre & Post	Response Time: INT improved for both levels Accuracy: No change

Table 2.11 continued.

Moreau, Kirk & Waldie., (2017)	n = 305 (187 girls) 7-13 y HIIT; n = 152 (90 girls) CON; n = 153 (97 girls)	6 weeks	Video-based (basic fitness movements) CON: Board games, quizzes	5 sessions per week	5 x 20 s maximal efforts Rest; 30 s, 40 s, 50 s, 60 s, 20 s	Flanker (Executive function; Inhibition) Go/no-go (Inhibitory Control) Stroop (Executive function; Inhibition Backward digit span (working memory) Pre & Post	HIIT group had improved RT on Flanker Go/no-go performance was not affected HIIT group improved response times on Stroop HIIT group improved backward digit span scores
Schmidt et al., (2015)	n = 181 (100 girls) 11.4 ± 0.6 y Team Games; n = 69 (43 girls) Aerobic; n = 57 (29 girls) CON; n = 55 (27 girls)	6 weeks	Team games: Floorball, basketball Cognitively engaging elements included Aerobic: Running CON; Normal PE lesson	2 sessions per week	45 min Mean HR; Team games: 148 beats·min^{-1} Aerobic: 150 beats·min^{-1}	Non-spatial N-back test (Executive function; Updating) Modified Flanker (Executive function; inhibition) Pre & Post	Shifting: Team games group improved shifting No change in aerobic exercise

Abbreviations: HIIT = High Intensity Interval Training. CON = Control Group. HIE = High-Intensity Exercise. MIE = Moderate Intensity Exercise. LIE = Low Intensity Exercise. INT = Intervention Group. HR_{max} = Maximum Heart Rate. BDNF = Brain Derived Neurotrophic Factor. EF = Executive Function.

Therefore, it is also important to consider the effect of high intensity intermittent exercise training interventions in an adolescent population. Costigan et al. (2016) examined the effects of high-intensity, intermittent aerobic activity, compared to combined aerobic and resistance exercise, over a period of 8 weeks in adolescents aged 15 – 16 y. The aerobic exercise group performed 30 s efforts of shuttle runs, jumping jacks and skipping; whereas the combined group performed the same exercises as well as some body weight squats and push ups; these were performed for a total of 8 min (weeks 1 – 3), 9 min (weeks 4 – 6) and 10 min (weeks 7 & 8). The combined group improved their performance on the trail making test (which assesses executive function), with smaller improvements seen in the aerobic only group. A shorter intervention (6 weeks) involving five weekly high-intensity video-based exercise sessions (basic fitness movements, which were not described) led to improvements on inhibitory control and working memory in a mixture of children and adolescents (7 – 13y; Moreau et al., 2017). These two studies provide initial evidence that high-intensity exercise protocols, less than 10 min in total duration, can lead to improvements in cognitive function after 6 – 8 weeks. Indeed, the use of such protocols may be more appropriate for adolescents, but the use of video-based exercise may not necessarily be accessible to all adolescents. Furthermore, it remains to be seen how shorter intervention lengths (such as 4 weeks) of high-intensity exercise would affect cognitive function in a group of adolescents.

Only two studies in adolescents examined the mechanisms that may be related to improvements in cognitive function following exercise training (Jeon & Ho Ha., 2017; Ludyga et al., 2018). Using EEG, there was evidence of increased P300 amplitudes on both the congruent and incongruent levels of the Stroop task following an exercise intervention (Ludyga et al., 2018), indicative of increased attentional resources to the task at hand. Furthermore, in the work of Jeon and Ho Ha (2017), BDNF concentration increased in the moderate (55% $\dot{V}O_{2max}$) and high (70% $\dot{V}O_{2max}$) intensity training groups, when compared to the low intensity (40% $\dot{V}O_{2max}$) and control groups; with a greater magnitude of change in the high-intensity group compared to the moderate-intensity group (4.4 $ng \cdot ml^{-1}$ vs 2.2 $ng \cdot ml^{-1}$, respectively). However, only the high-intensity group had improved working memory performance, which might be related to the greater increase in BDNF;

given the known role BDNF plays in neuroplasticity and memory (Piepmeier & Etnier., 2015). Given that this is the only study that has examined BDNF concentrations following exercise training in adolescents, this point remains speculative and it would be particularly interesting to see if BDNF is increased, simultaneously with cognitive improvements, after short-term interventions (~ 4 weeks).

Thus, early limited findings suggest that exercise interventions of 6 – 12 weeks duration can lead to improvements in cognitive function in adolescents and that high-intensity exercise interventions, which are arguably more applicable for adolescents and ecologically valid given the structure of a school day, could be effective. There is also preliminary evidence that improvements in cognitive function may be underpinned by neuro-electrical mechanisms (increased allocation of attentional resources) or increased release of biomarkers related to neuroplasticity and memory (such as BDNF). However, there is generally a lack of evidence examining the effects of short-term interventions (~4 weeks) and particularly high intensity intermittent exercise interventions which can be included in small breaks in the school day, on cognitive function in adolescents.

2.6 Summary

This literature review has summarised the current evidence base examining the relationship between important characteristics (physical activity, physical fitness and adiposity) with, and the effects of exercise (acute and chronic) on, risk factors for cardiometabolic disease and cognitive function in adolescents. Several limitations and gaps within the literature have been highlighted throughout, which if addressed can help to improve and extend the current understanding of novel risk factors for cardiometabolic disease, and cognitive function, in adolescents. Therefore, the aims of the studies presented within this thesis are:

- To examine the associations between physical activity, physical fitness and adiposity, with risk factors for cardiometabolic diseases and cognitive function across adolescence (chapter IV);

- To examine the determinants of the postprandial glycaemic and insulinaemic responses to an ecologically valid mixed-meal in adolescents (chapter V);
- To examine the effects of an acute bout of football activity on risk factors for cardiometabolic disease (chapter VI) and cognitive function (chapter VII) in adolescents;
- To investigate the effects of a short-term, high-intensity exercise intervention on risk factors for cardiometabolic disease and cognitive function in adolescent girls (chapter VIII).

Chapter III

General Methods

3.1 Introduction

This chapter provides an overall summary of the general procedures used in the methodological approaches employed by the studies presented within this thesis (Chapter IV - VIII) and is split into nine sections. The first section (section 3.2) outlines the participant recruitment process, including the obtainment of participant assent and parent/guardian/care-giver consent. The second section (section 3.3) outlines the preliminary measurements undertaken and describes the familiarisation sessions undertaken before the main experimental trials. The following 6 sections (section 3.4 – 3.8) describe the experimental procedures involved in the main trials of the included studies, such as the assessments of body composition (section 3.4), physical fitness (3.5), cardiometabolic health outcomes (3.6), device-measured physical activity (3.7) and cognitive function (3.8). The final section (section 3.9) outlines the statistical analysis procedures used throughout the thesis.

3.2 Participant Recruitment

Following the ethical approval from the Nottingham Trent University Human Ethics Advisory Committee, adolescents from local secondary schools in the East Midlands (Derby, Nottingham and Melton Mowbray) were approached and invited to participate in the studies. The head of the PE department was contacted and provided with details about the study aims, requirements and experimental procedures involved. Public notices related to the study were posted in each school PE area and young people were asked to let their PE teacher know that they were interested. Following this, a meeting was arranged with interested participants, whereby lead researchers explained the methods involved, the requirements necessary as a participant and they were also made aware of their right to withdraw from participation at any point in the study, without the need

to specify a reason. This also provided the participants with an opportunity to ask any questions they had about the study requirements and methods.

Those that were interested after the initial meeting were provided with an information pack (Appendix A), which was taken home to read along with their parent/guardian/care-giver. Participants were also provided with informed assent (Appendix B) and informed consent (Appendix C) forms, as well as a health screen questionnaire (Appendix D). The consent and health screen were completed by the parent/guardian/care-giver of interested participants and the participants completed the assent. Once returned, a lead investigator checked that the forms had been completed, as well as investigating the health screen to ensure that there were no health conditions present that may pose a risk to the health of the participant - by taking part in the study - or potentially contribute bias to the study outcomes.

3.3 Preliminary Measurements and Familiarisation

For the studies in this thesis (with the exception of Chapter IV), participants attended a familiarisation and preliminary testing session ahead of the main trials. During this session anthropometric measurements (stature, body mass, sitting stature) were administered and the multi-stage fitness test was conducted. In addition, participants undertook a practice capillary blood sample, a practice of the cognitive function test battery, as well as a familiarisation with the acute bout of football (Chapter VI & VII) and sprint training session (Chapter VIII). For the study in Chapter IV, these measurements were administered as part of the first experimental trial. These sessions also provided an opportunity for participants to ask any questions about the protocols, or for the researchers to clarify any elements that were unclear for the participants.

Stretched stature was measured, accurate to the nearest 0.1 cm, with a Leicester Height Measure (Seca, Hamburg, Germany). After the removal of footwear, the participant's head was placed in the Frankfort plane, with gentle upward pressure applied by an investigator with their hands (they were placed on each side of the face, with fingers positioned on the mastoid process), to help lift their head and obtain maximum height. Body mass was measured, accurate to the nearest 0.1

kg, using Seca 770 digital scales (Seca, Hamburg, Germany). This was done following the removal of footwear, thick jumpers and emptying of pockets – to facilitate the most accurate, non-intrusive measurement of body mass. Stretched stature (cm) and body mass (kg) were used to calculated Body Mass Index (BMI; kg·m^2) using the following; (body mass (kg) / height (m^2)). Stretched sitting stature was measured with participants seated on an anthropometric measuring box, using the same stretch technique described above. The quantification of stretched sitting stature was enabled by securing (with electrical tape) a 1 m ruler to a flat surface behind the participant, which was also aligned vertically with a spirit level. This measurement was only conducted by investigators with certified training for the assessment of anthropometric measurements (International Society for the Advancement of Kinanthropometry).

Leg length (stretched height – sitting height) was calculated, as it is used in the prediction of age from peak height velocity (APHV). APHV estimates adolescent maturity offset (MO; y, pre- or post-APHV); a proxy for pubertal development, which has been calculated throughout this thesis in accordance with the re-developed sex-specific regression equations by Moore et al. (2015) (Eq. 1.1a & 1.1b). The non-invasive method and re-developed equations were able to accurately predict 90% of MO cases within ± 1 y in external samples, which is an improvement on the original equation by Mirwald et al., (2002) which achieved 80%.

Maturity Offset (boys) =

$$-8.128741 + (0.0070346 \times (age \times sitting\ height))$$

(Eq. 1.1a)

Maturity Offset (girls) =

$$-7.709133 + (0.0042232 \times (age \times height))$$

(Eq. 1.1b)

N.B. Age = y, height and sitting height = cm.

3.4 Measurement of Body Composition

3.4.1 Waist Circumference

Waist circumference was measured as a surrogate measure for central adiposity, at the narrowest abdominal point between the lower margin of the lowest palpable rib and the iliac crest, to the nearest 0.1 cm, per the World Health Organisation guidelines (WHO, 2008). Measurements were taken twice, with the mean of measurements used as the criterion value. If there was a difference of ≥ 10% between individual measurements, then a third was taken and the median used as the criterion value.

3.4.2 Skinfold Thickness

Body composition was also assessed through skinfold thicknesses (mm), using a Harpenden Caliper (Baty International, Burgess Hill, UK), measured at four sites; triceps, subscapular, supraspinale and front thigh. Only investigators with certified training in kinanthropometry performed these measurements, per the International Society for Advancement of Kinanthropometry guidelines. All measurements were taken twice in rotation (triceps - subscapular - supraspinale - front thigh), on the right hand side of the body. The mean of the two measurements was used as the criterion value, unless the difference between two individual measurements was ≥ 10%, in which case a third measurement was taken and the median was used as the criterion value. The sum of the four skinfold thicknesses (mm) was used as the proxy for body composition, rather than estimating body fat percentage, in line with Dring et al. (2019), due to the large random and systematic errors associated with estimating body fat percentage (Reilly et al., 1995).

3.5 Measurement of Physical Fitness

The multi-stage fitness test (MSFT) was used in all studies presented in this thesis, as a field measure of endurance performance; commonly used within the paediatric field of research (Tomkinson et al., 2019). The total distance covered (m) throughout the MSFT was used as the physical fitness criterion for this test throughout the thesis. This decision is based on previous

work demonstrating that the MSFT performance provides greater sensitivity when assessing the effects of physical fitness in an adolescent population (Dring et al., 2019b), opposed to the previously used prediction equations for estimating $\dot{V}O_{2max}$, or laboratory directly measured $\dot{V}O_{2peak}$ or $\dot{V}O_{2max}$ (e.g., Leger et al., 1988; Barnett et al., 1993).

Prior to starting the MSFT, participants were fitted with a heart rate monitor (First Beat Technologies Ltd, Finland), for the continuous measurement of heart rate throughout the MSFT, and completed a brief warm-up which was led by a member of the research team. In all of the studies within this thesis, the MSFT was performed in the sports hall at the respective schools, in groups of no more than 10 and in accordance with the procedures outlined by Ramsbottom et al. (1988). The test consists of 20 m shuttle runs with increasing speed each stage, whereby the speed was dictated by an audio signal. The test starts at a pace of 8.0 $km \cdot h^{-1}$ (stage 1) and increases to 9.0 $km \cdot h^{-1}$ after 1 min for the next stage (stage 2). Following the second stage, the pace increases by 0.5 $km \cdot h^{-1}$ for each subsequent stage, with each stage lasting approximately 1 min. For a valid shuttle, participants had to place a foot either on or behind the line either before or at the same time as the audio signal. The participants were briefed on this prior to starting the test and it was explained that the aim of the test is to complete as many shuttles as possible, either until volitional exhaustion or until they could no longer match the audio signal for three successive shuttles. An experienced member of the research team ran with the participants, to ensure that the pacing of the participants was appropriate throughout the test, and verbal encouragement was provided. The final shuttle achieved was recorded and then converted into distance run (m). In Chapter IV only, the 15 m version of the MSFT was used for all participants, as several school sport halls were not long enough to permit the 20 m version of the MSFT to be completed. Therefore, distance run for participants in this Chapter IV should not be compared with distance run in the other experimental chapters as participants complete more turns in the 15 m MSFT.

3.6 Measurement of Risk Factors for Cardiometabolic Health

3.6.1 Capillary Blood Sampling

Capillary blood samples were collected in all experimental chapters for the quantification of a variety of biomarkers related to cardiometabolic health and cognition, namely blood glucose, plasma insulin, Interleukins (IL) -6, -10, -15, -1β and Brain-derived Neurotrophic Factor (BDNF). The capillary blood sampling technique was chosen over the venous technique, due to ethical constraints associated with obtaining venous samples from young people. Additionally, capillary blood samples are preferred when calculating postprandial glycaemic and insulinaemic responses (Wolever et al., 1991).

Collection and Treatment of Capillary Blood Samples

In all experimental chapters, baseline blood samples were obtained in the morning following an overnight fast from 9 pm the previous evening. Participants' hands were submerged in warm water to aid capillary blood flow. Following ~ 5 min of submersion, hands were dried and then a single-use lancet (Unistik extra, 21G, 2 mm depth, Owen Mumford Ltd, UK) was used to collect whole blood samples into two 300 µl EDTA microvettes (Sarstedt Ltd, UK) for separation into plasma and a single 300 µl microvette with clotting activator (Sarstedt Ltd, UK) for separation into serum. A single 25 µl whole blood sample was also collected, with a pre-calibrated glass pipette (Hawksley Ltd, UK) and immediately deproteinised in 250 µl ice-cooled perchloric acid (2.5%). The deproteinised whole blood and EDTA coated microvettes were centrifuged at 4000 x g, for 4 min at 4 °C (accuSpin Micro 17R, Fisher Scientific, UK). The microvette with clotting activator was allowed to rest at room temperature for 30 min, before centrifugation at 1000 x g for 15 min. Plasma and serum were removed from the microvettes and aliquoted into 500 µl plastic vials and stored immediately at -20 °C in a portable freezer. The samples were then transferred to -80 °C at the earliest opportunity, where they remained until analysis.

Analysis of Capillary Blood Samples

Plasma insulin concentrations were quantified using a commercially available ELISA (Mercodia Ltd, Sweden) and blood glucose concentrations were determined in duplicate with a commercially available assay procedure (GOD/PAP method, GL364, Randox, Ireland) and read spectrophotometrically. In all chapters, fasting blood glucose and plasma insulin concentrations were used to calculate homeostatic model assessment of insulin resistance (HOMA-IR) index using the equation below (Eq. 1.2), as a surrogate measure of insulin resistance (Matthews et al., 1986). Total area under the curve (tAUC) was calculated as an index of the glycaemic and insulinaemic response to a standardised meal (described in section 3.6.2) for Chapters V, VI and VIII, based on the trapezoid method (Wolever & Jenkins., 1986; Wolever, Jenkins, Jenkins & Josse, 1991) using Graphpad Prism (Graphpad Software, USA). Serum BDNF concentrations were quantified using a commercially available ELISA (Quantikine ELISA, R & D Systems Europe Ltd, UK) in accordance with the manufacturer's instructions.

$$\frac{plasma\ insulin\ concentration\ (\mu U \cdot L^{-1}) \times blood\ glucose\ concentration\ (mmol \cdot L^{-1})}{22.5}$$

Eq. 1.2 Calculation of HOMA-IR.

Plasma concentrations of IL-6, IL-10, IL-15 and IL-1β were determined using the Ella SimplePlex automated immunoassay (ProteinSimple, BioTechne, Oxford, UK), in line with the manufacturer's instructions (Aldo et al., 2016). Samples and wash buffer were added to cartridge inlets, before being loaded into the machine. Briefly, sample is routed through separate microfluidic channels which are coated with analyte-specific antibodies. The channels are washed and then a detection antibody is applied. The detection antibody migrates through the microfluidic channel, into the Glass Nano Reactors (GNRs) whereby the sample is measured in triplicate. Concentrations are generated from the factory-calibrated standard curves that are preloaded into the cartridge. The microfluidic technology used by the Ella system allows for the simultaneous quantification of biomarkers without cross-reactivity (Aldo et al., 2016). Concentrations of IL-6, IL-10 and IL-1β

measured using this method have been strongly correlated with ELISA-based concentrations (Aldo et al., 2016).

For all biomarkers analysed, the coefficients of variation (CV) were calculated to quantify the intra-assay variability. This was done with at least eight repeat measurements, using the methods described above. A breakdown of the CV by biomarker and by study, can be seen in Table 3.1 below.

Table 3.1. Coefficients of variation for each biomarker and each chapter.

	Experimental Chapter				
Biomarker	*Chapter IV*	*Chapter V*	*Chapter VI*	*Chapter VII*	*Chapter VIII*
Serum BDNF	7.3%	NA	NA	6.9%	7.3%
Blood Glucose	5.7%	3.8%	3.8%	3.8%	3.1%
Plasma Insulin	6.5%	4.7%	4.7%	4.7%	6.5%
IL-6	3.8%	NA	NA	NA	3.8%
IL-10	3.9%	NA	NA	NA	3.9%
IL-15	2.9%	NA	NA	NA	2.9%
IL-1β	2.5%	NA	NA	NA	2.5%

BDNF; Brain-Derived Neurotrophic Factor. IL; Interleukin
NA = Not analysed

3.6.2 Standardised Breakfast and Lunch

In Chapters V - VIII, participants were provided with a standardised breakfast. Participants attended the main trials after an overnight fast from 9 pm the previous evening, having only consumed water *ad libitum.* The breakfast was consumed after the initial baseline capillary blood sample and consisted of; cornflakes, milk and white toast with flora, providing 1.5g·kg^{-1} body mass of carbohydrate, as previously used in this population (Cooper et al., 2012). In Chapter VI and VII, participants were also provided with a standardised lunch (which was the test meal used for the postprandial glycaemic and insulinaemic responses), which was consumed ~ 3 h after breakfast. The lunch consisted of a chicken sandwich, baked salted crisps and an apple. Both meals provided 1.5g·kg^{-1} body mass of carbohydrate (Dring et al., 2019a). Participants were given 15 min to consume each of the meals. If there was any food remaining at the end of the 15 min the leftovers were weighed and adjustments were made for the subsequent meals. An example breakfast and lunch composition for a representative 50 kg participant is presented in Table 3.2a and 3.2b, respectively.

Table 3.2a. An example of the standardised breakfast quantities and carbohydrate provision based on a hypothetical 50 kg individual.

Food Item	Mass (g)	Carbohydrate (g)
White Bread [a] *(toasted)*	42	18.8
Flora Original [b]	6	0
Cornflakes [c]	55	46.3
1% Fat Milk [d]	216	9.9
Total Quantity	319	75

[a] Kingsmill soft white thick slice, UK
[b] Flora Original, UK
[c] Kelloggs Ltd, UK
[d] Sainsbury's Ltd, UK

Table 3.2b. An example of the standardised lunch quantities and carbohydrate provision based on a hypothetical 50 kg individual. Examples are provided for the standard and vegetarian option.

Food Item	Standard		Vegetarian	
	Mass (g)	*Carbohydrate (g)*	*Mass (g)*	*Carbohydrate (g)*
White Bread [a]	70	32	70	32
Flora Original [b]	8	0	8	0
Chicken [c]	115	0	--	--
Cheese [d]	--	--	34	0
Baked Crisps [e]	35	26	35	26
Apple [f]	120	13	120	13
Total Quantity	348	71	267	71

[a] Kingsmill soft white thick slice, UK
[b] 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 (Sainsbury's Ltd, UK)

3.7 Measurement of Physical Activity

Typically, accelerometery data are analysed using device specific software that reduces the data to counts and then time use is categorised based on cut-points developed in calibration studies. However, the process of count reduction relies on proprietary algorithms, specific to the brand (Troiano, McClain, Brychta & Chen., 2014), and there are increasing numbers of cut-points for the classification of activity, which makes comparing across studies extremely difficult (Wijndaele et al., 2015). A recommended solution to this problem is through using the open-source R package, GGIR, as this relies on the use of raw accelerations; which is available on all research-grade accelerometers (Migueles et al., 2019). However, there is currently a lack of information on how these two different methods compare when they are supposedly measuring the same outcomes.

The purpose of this section is to assess the extent of agreement and equivalence between estimated physical activity behaviours between the two data processing methods (raw and counts) for hip- worn accelerometery data. There has been some investigation into this area already, demonstrating that estimations of MVPA are not equivalent between methods, but this was limited to children and did not assess sedentary time and light physical activity (Buchan & McLellan., 2019). The data used for this comparison is from Chapter IV contained within this thesis and the results will be used to inform the decision of which physical activity metrics and method will be used in subsequent chapters.

3.7.1 Protocol for Accelerometery Data Collection

Participants wore a hip-mounted accelerometer (Actigraph GT3X+, Actigraph, Pensacola, FL, USA) for the assessment of free-living physical activity, which was set to capture data at 90 Hz. The accelerometer was positioned on the right hip, just above the iliac crest. All participants were fitted with the accelerometer at the end of the first experimental trial to ensure it was appropriately fitted. Participants were handed a diagram upon leaving, depicting how the accelerometer should be worn to ensure further compliance (Appendix E). Participants were instructed to wear the

accelerometer at all times (i.e. 24 hours a day) over the following 7 d period, except for any water-based activities, such as bathing, swimming, based on previous recommendations (Tudor-Locke et al., 2015; Migueles et al., 2017).

3.7.2 Protocol for Accelerometery Data Processing

After the devices were returned, the data were downloaded using Actilife v6.13.4 (Actigraph, Pensacola, FL, USA) and saved in raw format as GT3X+ filetypes. The raw files were converted to .csv files for raw data processing and to AGD format for the counts processing.

3.7.2.1 Raw Processing

The raw (.csv) files were processed in RStudio v 1.2.1335 (Rstudio Team, 2015), www.rstudio.com) using the open-source GGIR package v2.0-0. This package has been developed to allow the processing and analysis of raw acceleration files (Van Hees et al., 2014; Migueles et al., 2019). Initially, GGIR auto-calibrates the raw triaxial signals into a single, omnidirectional measure of acceleration (signal vector magnitude/Euclidean norm minus one (ENMO)); which accounts for the effect of gravity (subtracts 1 g) and rounds negative values to zero (Van Hees et al., 2014). The ENMO was calculated over 5 s epochs and expressed in mg. Following this, valid wear times were estimated using the built-in wear time validation protocol, calculated over 60 min windows with 15 min moving increments (see Van Hees et al. (2013) for details). Data were considered valid if there were at least 4 days (1 weekend day) of at least 10 h of wear time per day. Raw data files were removed prior to analysis if the post calibration error was greater than 0.01 g, in line with the recommendation of Migueles et al. (2019).

3.7.2.2 Counts Processing

The counts files (GT3X+) were processed in Actilife (v6.13.4), with non-wear time identified using the algorithm of Choi et al. (2011) and then removed from the files prior to analysis (Buchan et al., 2019; Migueles et al., 2017). Data were considered valid if there were at least 4 days (1 weekend day) of at least 10 h wear time per day. To enable comparisons between raw and counts methods, sleep periods were removed during the data processing steps (11:00 pm to 06:00 am).

Finally, only those participants meeting the wear time criteria for both methods were maintained for subsequent analysis.

3.7.2.3 Activity Classification

Device, population and location specific cut-points for the raw accelerations were used to determine the estimated time spent in different behaviours. The recommended cut-points for estimating moderate, vigorous and moderate-vigorous physical activity for hip-worn Actigraph monitors are those of Hildebrand et al. (2014). Cut points to classify sedentary time and light physical activity were also developed by the same group (Hildebrand et al., 2016). However, recent work has developed more appropriate cut-points for hip-based sedentary time and light physical activity (Hurter et al., 2018). There are many cut point sets to choose from when processing the counts data (Migueles et al., 2017), but for the purposes of comparison the most popular and recommended cut points for the hip are used (Migueles et al., 2017). An overview of the location specific cut-point thresholds can be seen in Table 3.3.

Table 3.3. Overview of the hip-based activity thresholds used for raw and counts processing.

	Processing Method	
Activity Classification	*Raw (mg)* [a]	*Counts* [b] *(Counts·15s^{-1})*
Sedentary/Inactive Time	≤ 32.6	≤ 180
Light Physical Activity	32.7–142.6	181 – 756
Moderate Physical Activity	142.7– 464.6	757 – 1111
Vigorous Physical Activity	> 464.6	> 1112

[a] Hurter et al. (2018) for Inactive and Light physical activity; Hildebrand et al. (2014) for Moderate and Vigorous
[b] Romanzini et al., (2014)

3.7.3 Statistical Analysis

As the absence of a statistically significant difference between methods does not necessarily mean that they can be deemed equivalent (Dixon et al., 2018; Looney, 2018; Wellek, 2010), a paired equivalence testing approach was used (Dixon et al., 2018). This method tests the hypothesis that the differences between two methods and the associated 90% CI are within a zone of equivalence, and thus considered similar to a difference of zero. Pairwise equivalence

tests were used in the present study, using the two one-sided tests (TOST) method, to examine if the ratio of means between methods and their associated 90% CI, for estimated time in each activity, was within the specified equivalence region (Dixon et al., 2018; Wellek, 2010). Based on previous work in the field, the equivalence region was set as ± 10% (Boddy et al., 2018; Buchan et al., 2019; Rowlands et al., 2017). Equivalence tests were conducted in Minitab v19.

An intraclass correlation coefficient (two-way mixed, absolute agreement, single measures; ICC2) was used to determine the absolute agreement between the two methods (Liljequist., 2019). A second iteration of the intraclass correlation coefficient (two-way mixed, consistency, single measures; ICC3) was used to determine the consistency between the two methods (Liljequist., 2019). The following criteria were used to qualitatively assess the ICC's; < 0.5 (poor), 0.5 – 0.75 (moderate), 0.75 – 0.9 (good), > 0.9 (excellent) (Koo & Li., 2016). Furthermore, the presence of a greater ICC3 relative to ICC2 provides evidence of a systematic bias.

The mean bias and limits of agreement, along with Bland-Altman plots, were calculated to assess agreement at the individual level between processing methods for the estimation of time in each activity (Altman & Bland., 1983; Bland & Altman., 1999), using the "blandr" (v 0.5.1) package in R (Datta, 2017). If the Bland-Altman plots displayed evidence of proportional bias, data were log transformed for the calculation of bias and limits of agreement. The antilog of these values were then reported as percentages (Bland & Altman, 1999). The use of these measures satisfies the recommended criteria for presenting a scaled, unscaled, and visual representation of agreement for method comparisons (Looney, 2018). All descriptive data are presented as mean ± SD, unless otherwise stated.

3.7.4 Results

Data was initially collected from 113 participants, however some were lost due to device malfunction (n = 2), insufficient wear time (n = 8) and providing a value greater than 0.01 mg during auto-calibration in GGIR (n = 2). These losses resulted in a final sample of 101 adolescents. The average percentage of wear time was 82.3 ± 17.1% and 90.0 ± 15.3% for counts

and raw processing, respectively. The average amount of valid wear time, per day between 06:00 am and 11:00 pm, was 15.9 ± 1.4 h and 17 ± 0 h for counts and raw processing, respectively.

Absolute agreement between methods was poor for sedentary time, light, vigorous and moderate-vigorous physical activity and poor to moderate for moderate and physical activity (Table 3.4). Consistency between methods was poor-moderate for sedentary time, light, vigorous and moderate-vigorous physical activity, and moderate-good for moderate physical activity (Table 3.4). Bland-Altman plots depicting the mean bias and limits of agreement between methods, for each behavioural activity, can be seen in Figure 3.1. The point estimates for mean bias, as well as the upper and lower limits of agreement, along with their 95% CI can be found in Table 3.4.

Results from the equivalence tests between the raw and counts processing methods for estimated sedentary time as well as light, moderate, vigorous and moderate-vigorous physical activity demonstrate that equivalence cannot be claimed (all $p = 1.000$). These results were identical with both methods respectively used as the reference. This is graphically represented as the 90% CI for the ratio of means does not sit within the equivalence region, Figure 3.2.

The proportion of adolescents meeting the 60 min·day^{-1} of moderate-vigorous physical activity guidelines was 72.3% of the sample (73/101) for counts processing, whereas only 0.99% (1/101) met this criteria according to raw processing. However, the rank order of estimated daily MVPA was strongly correlated between methods ($r = 0.847$, $p < 0.001$). Finally, there was a strong rank order correlation ($r = 0.84$, $p < 0.001$) between the vector magnitude CPM (765.2 ± 250.8 counts·min^{-1}) and the average acceleration (19.5 ± 5.7 *mg*).

Table 3.4. Summary of absolute agreement, consistency and equivalence between estimated time spent in each behavioural activity using the different processing methods for hip-worn accelerometery.

Activity Classification (min·day^{-1})	Raw Processing Mean ± SD	Counts Processing Mean ± SD	Intraclass Correlation (ICC2) (95% CI)	Intraclass Correlation (ICC3) (95% CI)	Mean Bias [a] (95% CI)	Limits of Agreement (95% CI)		Equivalence (Raw/Counts)[b]	Equivalence (Counts/Raw)[c]
						Lower	Upper		
Sedentary Time	891.7 ± 39.1	674.2 ± 106.1	0.08 (-0.05, 0.26)	0.36 (0.18, 0.52)	217.5 min (199.7, 235.4)	40.3 min (9.7, 70.9)	394.7 min (364.1, 425.3)	N	N
LPA	100.5 ± 29.5	194.8 ± 45.1	0.14 (-0.06, 0.41)	0.55 (0.40, 0.67)	-94.3 min (-101.4, -87.1)	-165.1 min (-177.3, -152.8)	-23.5 min (-35.7, -11.3)	N	N
MPA	25.2 ± 13.1	50.2 ± 16.9	0.30 (-0.07, 0.65)	0.71 (0.60, 0.79)	-25.0 min (-27.3, -22.7)	-47.7 min (-51.6, -43.7)	-2.3 min (-6.2, 1.6)	N	N
VPA	2.5 ± 2.5	34.2 ± 19.1	0.04 (-0.05, 0.15)	0.15 (-0.05, 0.33)	-94.8% ₸ (-95.6, -93.7)	-99.1% (-99.4, -98.8)	-68.5% ₸ (-77.0, -56.9)	N	N
MVPA	27.8 ± 14.6	84.4 ± 33.5	0.18 (-0.06, 0.49)	0.61 (0.47, 0.72)	-69.6% ₸ (-71.7, -67.3)	-85.0% (-86.7, 83.0)	-38.5% ₸ (-45.6, -30.6)	N	N

ICC2; two-way mixed, absolute agreement, single measures, ICC3; two-way mixed, consistency, single measures, LPA = Light Physical Activity, MPA = Moderate Physical Activity, VPA = Vigorous Physical Activity, MVPA = Moderate-Vigorous Physical Activity. N = methods not equivalent.

[a] Calculated using the counts method as reference; ₸ Log-transformed (natural) data with antilog reported as percentages.

[b] Equivalence when counts processing was used as the reference

[c] Equivalence when raw processing was used as the reference

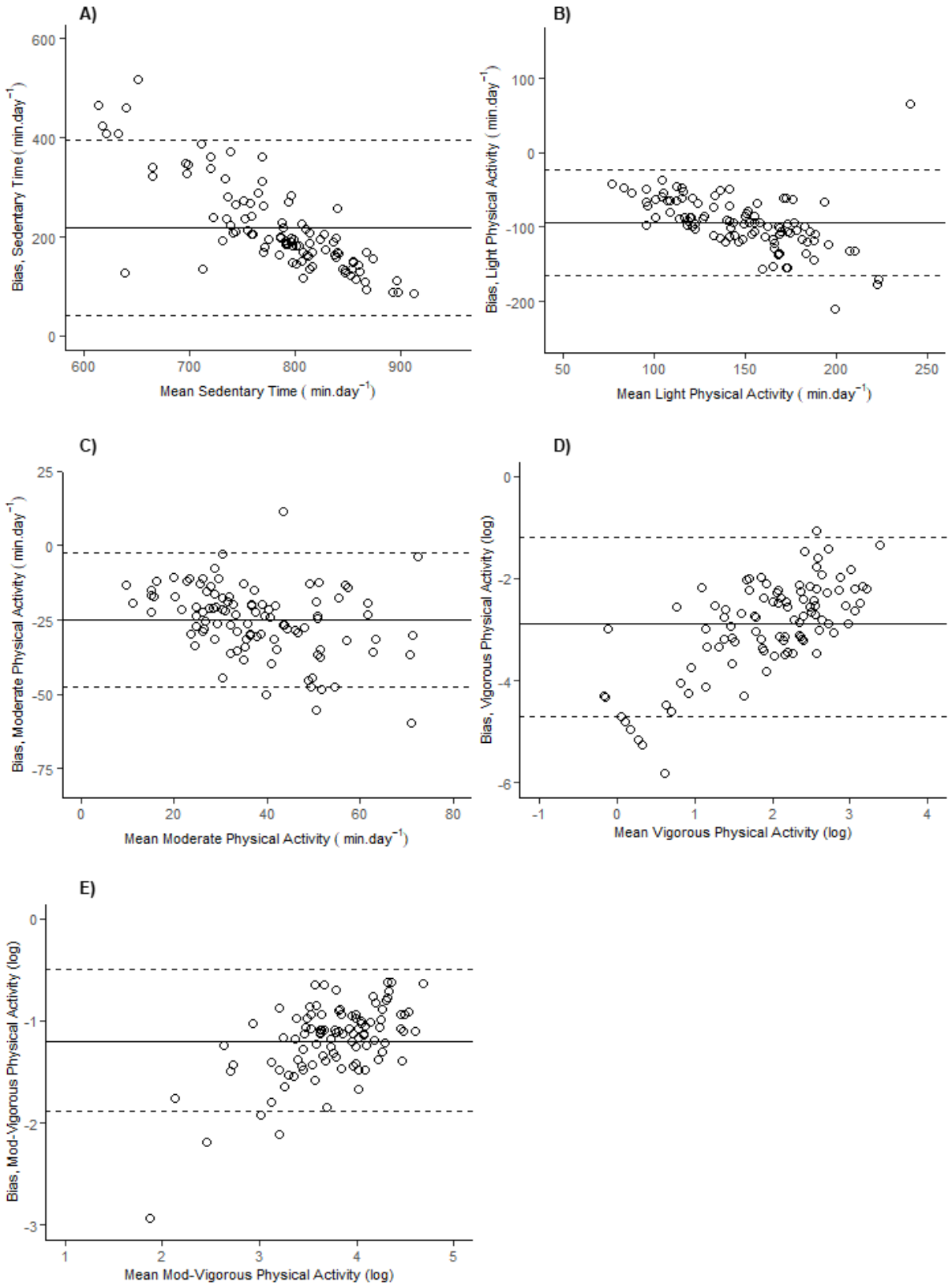

Figure 3.1. Bland-Altman plots to represent agreement between the two methods, with counts processing as the reference, for sedentary time (**A**), light (**B**), moderate (**C**), vigorous (**D**) and moderate-vigorous (**E**) physical activity during hip-worn accelerometery. Solid lines represent the mean bias. Dashed lines represent the 95% limits of agreement.

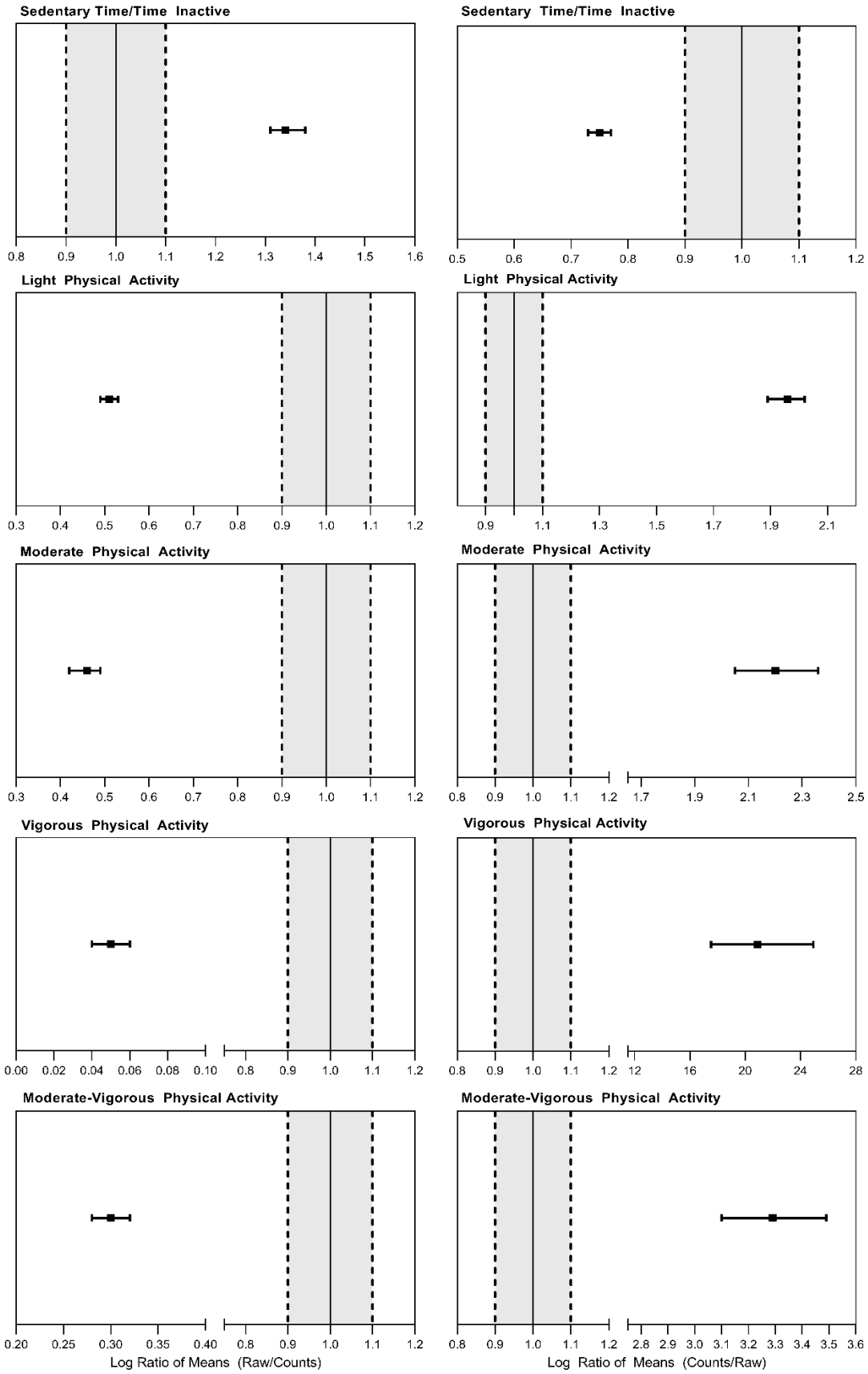

Figure 3.2. Ratio of the means between raw and counts processing methods (left = counts as reference, right = raw as reference) and their associated 90% CI. The shaded area represents the ± 10% zone of equivalence.

3.7.4.1 Hip Worn Accelerometery: Reduced sample with equal wear time

Due to the different methods for detecting non-wear, there was a substantial difference in the available valid wear time between methods. This may contribute for the discrepancies seen between the two methods for classifying activity. Therefore, this section will use data available only for those participants with the maximum amount of valid wear time, between 6 am and 11 pm (1020 min), resulting in a sample of 29 participants.

Absolute agreement between methods was poor for sedentary time, as well as light and vigorous physical activity and poor to moderate for moderate and moderate-vigorous physical activity (Table 3.5). Consistency between methods was poor for vigorous physical activity, poor-moderate for light physical activity, poor to good for sedentary time and moderate-vigorous physical activity and moderate to good for moderate physical activity (Table 3.5). Bland-Altman plots depicting the mean bias and limits of agreement between methods, for each behavioural activity, can be seen in Figure 3.4. The point estimates for mean bias, as well as the upper and lower limits of agreement, along with their 95% CI can be found in Table 3.5.

Results from the equivalence tests between the raw and counts processing methods for estimated sedentary time as well as light, moderate, vigorous and moderate-vigorous physical activity demonstrate that equivalence cannot be claimed (all $p = 1.000$). These results were identical with both methods respectively used as the reference. This is graphically represented as the 90% CI for the ratio of means does not sit within the equivalence region, Figure 3.4.

The proportion of adolescents meeting the 60 min·day^{-1} of moderate-vigorous physical activity guidelines was 69.0% of the sample (20/29) for counts processing, whereas none met this criteria under the raw processing method. However, the rank order of estimated daily MVPA between methods was strongly correlated ($r = 0.866$, $p < .001$). Finally, there was a strong rank order correlation ($r = 0.758$, $p < 0.001$) between the vector magnitude CPM (773.4 ± 220.1 counts·min^{-1}) and the average acceleration (19.8 ± 6.0 *mg*).

Table 3.5. Summary of absolute agreement, consistency and equivalence between estimated time spent in each behavioural activity using the different processing methods for hip-worn accelerometery in the reduced sample with equal valid wear time (n = 29).

Activity Classification (min·day^{-1})	Raw Processing Mean ± SD	Counts Processing Mean ± SD	Intraclass Correlation (ICC2) (95% CI)	Intraclass Correlation (ICC3) (95% CI)	Mean Bias [a] (95% CI)	Limits of Agreement (95% CI)		Equivalence (Raw/Counts)[b]	Equivalence (Counts/Raw)[c]
						Lower	Upper		
Sedentary Time	886.8 ± 36.0	714 ± 67.8	0.11 (-0.03, 0.39)	0.65 (0.38, 0.82)	172.4 min (155.2, 189.6)	83.8 min (54.1, 113.5)	260.9 min (231.2, 290.6)	N	N
LPA	104.0 ± 23.1	214.3 ± 45.5	0.09 (-0.04, 0.34)	0.51 (0.18, 0.74)	-110.2 min (-123.8, -96.6)	-180.4 min (-203.9, -156.8)	-40.1 min (-63.6, -16.5)	N	N
MPA	26.4 ± 14.8	54.6 ± 14.9	0.27 (-0.06, 0.65)	0.76 (0.55, 0.88)	-25.0 (-27.3, -22.7)	-47.7 min (-51.6, -43.7)	-2.3 min (-6.2, -1.6)	N	N
VPA	2.7 ± 2.4	36.2 ± 20.6	0.04 (-0.06, 0.22)	0.16 (-0.22, 0.49)	-94.3% ₸ (-95.8, -92.2)	-98.8% (-99.3, -98.0)	-72.2% ₸ (83.8, -52.2)	N	N
MVPA	29.1 ± 16.6	90.8 ± 32.9	0.18 (-0.05, 0.53)	0.68 (0.43, 0.84)	-71.2% ₸ (-75.0, -66.8)	-40.1% (-53.1, -23.4)	-86.1% ₸ (-89.1, -82.3)	N	N

ICC2; two-way mixed, absolute agreement, single measures, ICC3; two-way mixed, consistency, single measures, LPA = Light Physical Activity, MPA = Moderate Physical Activity, VPA = Vigorous Physical Activity, MVPA = Moderate-Vigorous Physical Activity. N = methods not equivalent.

[a] Calculated using the counts method as reference; ₸ Log-transformed (natural) data with antilog reported as percentages.

[b] Equivalence when counts processing was used as the reference

[c] Equivalence when raw processing was used as the reference

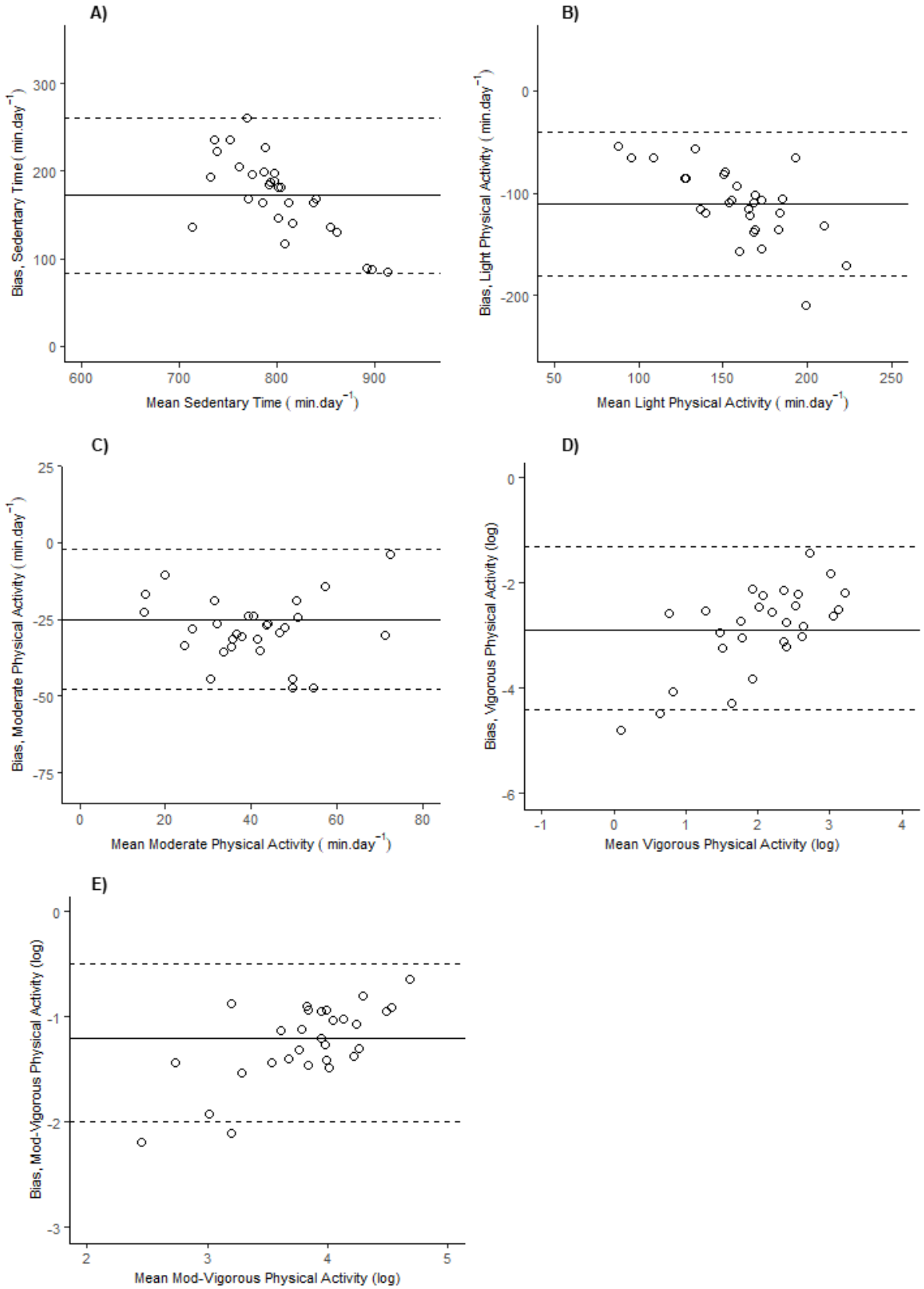

Figure 3.3. Bland-Altman plots to represent agreement between the two methods, with counts processing as the reference, for the reduced hip-worn sample (n = 29). Sedentary time (**A**), light (**B**), moderate (**C**), vigorous (**D**) and moderate-vigorous (**E**) physical activity. Solid lines represent the mean bias. Dashed lines represent the 95% limits of agreement.

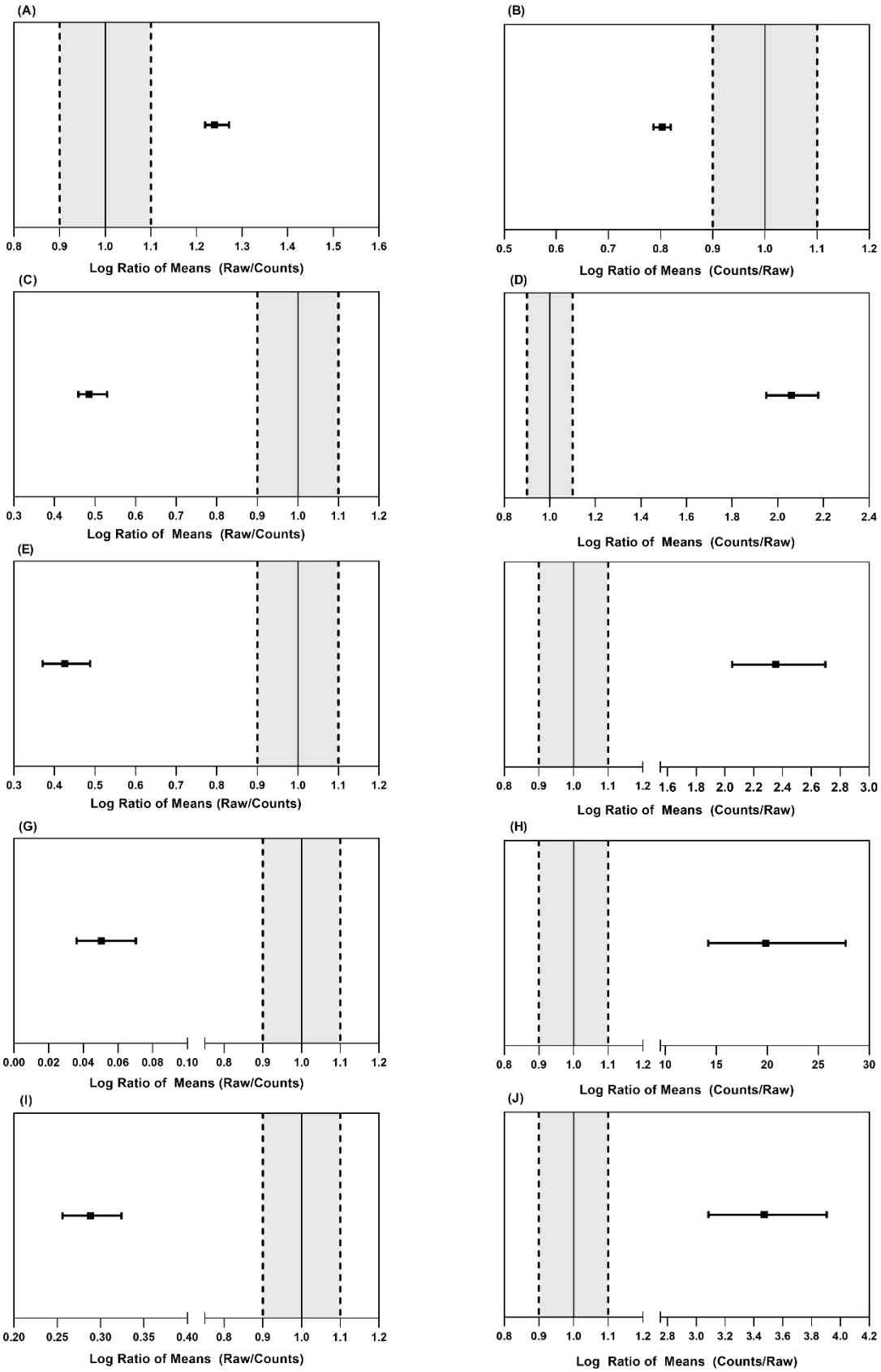

Figure 3.4. Ratio of the means between raw and counts processing methods (left = counts as reference, right = raw as reference) and their associated 90% CI for the reduced hip-worn sample (n = 29). The shaded area represents the ± 10% zone of equivalence.

3.7.5 Interpretation and Conclusions

The collective assessment of agreement, consistency and equivalence between methods for hip-worn accelerometery suggests that the raw and counts processing methods are not comparable in adolescents, for any activity classification category (sedentary, light, moderate and vigorous PA). This is in agreement with previous work (Buchan & McLellan, 2019; Kim et al., 2017), but extends these findings to also apply to sedentary time and light physical activity, as well as older adolescents, and hip-worn data.

The findings presented here demonstrate that there is generally poor agreement between raw and counts processing methods across all activity classifications. The determination of bias and agreement between methods revealed a higher estimated sedentary time, and lower light, moderate, vigorous and moderate-vigorous physical activity for raw processing relative to counts. The disparity in estimated moderate physical activity (bias; -25.0 min·day^{-1}) from hip-worn devices is much less than that of Buchan and McLellan (2019), where a bias of -59 min·day^{-1} was found in their sample of children (age 9-10 y) using the same cut-points. In addition, there was no evidence of equivalence between methods when using an equivalence region of 10%. Due to the differences in validating non-wear time between the methods, there were differences in the available amount of valid time for scoring, which may potentially be one cause of the disparities between methods. However, selecting a sub-sample of participants that had the maximum amount of available wear time between the pre-specified times (06:00 to 23:00), did not improve the estimates of physical activity behaviour.

Irrespective of device placement, a consistent finding from the current data and previous work (Buch & McLellan, 2019; Kim et al., 2017; Migueles et al., 2018) is that the raw processing (using GGIR), compared to counts processing, results in the lowest estimates of those meeting the recommended moderate-vigorous physical activity guidelines of 60 min·day^{-1}. This is of importance, given that this metric is one that is typically used in physical activity surveillance and promotion. However, focusing on just one category of activity (such as MVPA) may be part of the problem when measuring physical activity and comparing between devices (Rowlands, 2018;

Rowlands et al., 2018; Troiano et al., 2014). Indeed, it has been argued that focusing on simple, continuous measures of physical activity might provide greater resolution (Rowlands et al., 2018), and thus a more accurate indicator of total physical activity and intensity. The use of raw processing, through GGIR, can facilitate the quantification of two such measures; the daily average acceleration, which is indicative of physical activity volume, and the intensity gradient (IG), which summarises the time-intensity distribution across a day; reflecting the whole acceleration profile, as opposed to a select fraction such as moderate-vigorous physical activity (Rowlands et al., 2018). Furthermore, these outcomes are derived from raw accelerations, and do not depend on proprietary algorithms, therefore removing the barrier of different device use (Wijndaele et al., 2015; Rowlands et al., 2016).

The simple and continuous measures of physical activity (described above) provide the opportunity to use comparable outcomes in physical activity research, which has been a key barrier for the harmonisation and external comparison of data. Furthermore, there is a need for research examining how the new, continuous metrics associate with outcomes of interest relevant to health and wellbeing. There is currently a small body of work that demonstrates they are useful metrics to examine the role of physical activity volume and intensity for health (e.g., Buchan, McLellan, Donnelly & Arthur, 2019; Fairclough et al., 2019; Rowlands et al., 2018), but more work is required across a broader range of health- and cognitive-related outcomes as well as in older adolescents

Therefore, based on the findings of this method comparison and the recent recommendations in the literature (e.g. Rowlands et al., 2018), the outcomes relating to physical activity behaviours in this thesis (Chapters IV & VIII) will be done so through the use of the average acceleration (indicative of physical activity volume) and intensity gradient metrics (a summary of the time-intensity distribution across a day).

3.8 Cognitive Function Tests

In each study, a combination of cognitive function tests were administered via a laptop computer (Lonovo ThinkPad T450, Lenovo, Hong Kong) which included the Stroop test, Sternberg paradigm, flanker task and visual search test. The particular tests used in each study can be seen in Table 3.6.

Each test was verbally explained to participants by an experienced member of the research team, along with sample instructions provided before each test and test level. The familiarisation trials in Chapters VII & VIII (and the first main trial in Chapter IV) gave the participants an opportunity to practice the tests, as well as to ask any questions about the test. An example of the screen display for each of the cognitive function tests, and test level, can be found in Appendix E. In each study, the battery of tests was completed in silence, with up to 10 participants spread around a classroom. Sound cancelling headphones were worn for all tests, to help minimise external disturbances.

Table 3.6. Summary of the cognitive function test batteries in the experimental chapters.

Order	Chapter IV	Chapter VII	Chapter VIII
1	Visual Search Test	Stroop Test	Stroop Test
2	Stroop Test	Sternberg Paradigm	Sternberg Paradigm
3	Sternberg Paradigm	N/A	Flanker Task
4	Flanker Task	N/A	N/A

3.8.1 Stroop Test

The Stroop test assesses response sensitivity to interference (Stroop, 1935) and is typically used to measure selective attention (van Someren & Brouwer., 1992) and inhibitory control (Miyake, 2000). The test features two different levels; simple (selective attention) and complex (inhibitory control). Both levels involve a test word in the centre of the screen. A target and distractor word are placed on the left and right side, with the target position being counterbalanced between each side for each test level. Participants are required to select their responses, either left or right,

using the appropriate arrow keys on the laptop. Prior to each test level, the participants are presented with six practice stimuli; the data for which are discarded and the participants are provided with visual feedback as to whether or not they were correct.

For the simple level, there were 20 stimuli, with the target, test and distractor words all printed in white ink. In order to choose the correct response, participants had to select the target (either left or right) that matched the central word. The complex level (colour interference) contained 40 stimuli, with the central word now printed in a colour (which was incongruent). The participants had to select the option (either left or right) that matched the colour of the ink that the central word was printed in (i.e. if the central word “Green” was printed in “Red” ink, then the correct response would be “Red”). For both test levels, participants were instructed to respond as quickly and as accurately as possible. Stimuli were interspersed with an interval of 1 s. The variables of interest for this test were the response times of correct responses and the proportion of correct responses made.

3.8.2 Sternberg Paradigm

The Sternberg paradigm is a test of working memory (Sternberg, 1969) which consists of three different levels of increasing difficulty with a different working memory load (one, three or five items). The first (one item) level always used the number “3” as the target and contained 16 stimuli; providing a measure of basic information processing speed. The three- and five-item levels contained 32 stimuli, which were three or five letter strings (i.e. “HDY” or “JCIAF”), respectively. Letters would individually appear on screen, with participants using the right arrow key every time a target appeared (i.e. the number 3 for the one-item or specified letter for the other levels) and using the left arrow key when an item appeared that was not a target. The correct response was counterbalanced between the left and right arrow key for each level, with all stimuli being presented in the centre of the screen with an inter-stimuli interval of 1 s for all levels. Participants were presented with 6 practice stimuli before each level, with the results discarded and visual feedback presented. For this test, the variables of interest were the response times of correct responses and the proportion of correct responses made.

3.8.3 Flanker Task

The Flanker task is a test which assesses aspects of attention and inhibitory control, consisting of two separate levels; congruent and incongruent (Eriksen & Eriksen, 1974). Five horizontal arrows appear on the screen, with all arrows pointing in the same direction (left or right) during the congruent level (attention). Participants were instructed to select the arrow key corresponding to the direction of the arrows on screen.

During the incongruent trial (inhibitory control), the arrows point in different directions (left or right) and the participants were instructed to select the arrow that corresponds to the direction of the central arrow. Both levels were preceded by three practice stimuli, which provided feedback on whether the response was correct. This allowed for the re-familiarisation of the tests. The data from the practice stimuli were discarded and once the test started, no further feedback was provided. For both levels, the arrows were presented on a black background in green ink, after a varied delay of 400 – 4000 ms. The items remained on screen until a response was selected. The variables of interest were the response times of correct responses and the proportion of correct responses.

3.8.4 Visual Search Test

The visual search test measures visual perception and consists of two levels, baseline and complex, each consisting of 21 target stimuli. At the start of each level instructions appeared on screen explaining each test level. Following this, there were three practice stimuli with feedback provided, allowing participants an opportunity to re-familiarise with the test – with the data from these practice attempts being discarded.

During the baseline level, the stimuli were drawn in solid green lines on a black background, providing a measure of visuo-motor speed. During the complex level random green dots were spread over the screen, which were redrawn every 250 ms to induce a flickering background visual effect, acting as a distractor. The dots eventually converged to form a target triangle, gradually becoming clearer until the participant responded, providing a measure of complex visual

processing. Participants were instructed to respond as quickly as possible to the stimuli (triangles) by pressing the spacebar on the keyboard. During both levels, there were 21 different locations for the target stimuli which were randomly allocated. The variables of interest from both levels were the response times of correct responses and the proportion of correct responses.

3.9 Statistical Analysis

For the statistical analysis within this thesis, the main software used was RStudio (RStudio Team, 2020; https://rstudio.com/) which was used to perform multiple linear regression (Chapter IV and V), linear mixed effects models (Chapter VII), both using the 'lme4' package, and analysis of covariance (ANCOVA) using the 'car' package (Chapter VIII). Analysis in Chapter VII was completed using SPSS (Version 25, SPSS Inc, Chicago, IL, USA) and involved mixed-model ANOVA's and independent sample t-tests. For all group comparisons and relationships examined, the level of uncertainty surrounding the point estimates are displayed with 95% confidence intervals (CI).

Due to the variety of statistical procedures involved, further specific details are provided in each subsequent chapter.

Chapter IV

Physical fitness, physical activity and adiposity: associations with risk factors for cardiometabolic disease and cognitive function across adolescence

4.1 Introduction

Although young people do not typically present with cardiometabolic diseases such as cardiovascular disease and type 2 diabetes (Balagopal et al., 2011), the risk factors for these conditions are present during the early years of life (Balagopal et al., 2011; Warnberg et al., 2007) and track into adulthood (Laitinen et al., 2012). This highlights adolescence as a crucial time point to identify the prevalence of risk factors for cardiometabolic disease, as well as associated behaviours and characteristics that can influence them, such as physical activity, physical fitness and adiposity. In addition to cardiometabolic health, the importance of cognition during adolescence has been recognised, as it is related to lifelong physical and mental health (Diamond, 2013) as well as academic performance (Cooper, Dring & Nevill, 2016). Indeed, the cross-sectional relationship between physical fitness, physical activity, and adiposity in relation to risk factors for cardiometabolic disease, as well as cognitive function and academic achievement, during adolescence is relatively unexplored.

The role of physical activity is of particular interest, given that physical activity is central to government and global guidelines for well-being (Gibson-Moore., 2019). Whilst it is generally accepted that physical activity has beneficial effects on traditional risk factors for cardiometabolic disease such as blood pressure (Bailey et al., 2012; Carson et al., 2013) and insulin sensitivity (Carson et al., 2013) in adolescents; the limited studies conducted to date have shown no relationship between physical activity and IL-6 concentration (Martinez-Gomez et al., 2010; 2012). Furthermore, no studies to date have examined other cytokines related to low-grade inflammation (such as IL-1β, IL-10, IL-15). Additionally, the relationship between physical activity and cognitive function during adolescence is unclear, with some evidence suggesting a beneficial relationship with inhibitory control and cognitive flexibility (Aadland et al., 2017; Lee et al., 2014), whilst others

did not find such associations (Cadenas-Sanchez et al., 2020). It is worth noting that the relationships between device-measured MVPA and cognitive function found by Aadland et al. (2017) no longer remained when physical fitness was included, and participants in the study by Lee et al. (2014) categorised participants into regular exercisers based on self-report data.

Furthermore, one of the challenges of assessing physical activity, as discussed in chapter III, is the divergent protocols used and process and categorise accelerometery data (Troiano, McClain, Brychta & Chen., 2014; Wijndaele et al., 2015). To address this, Rowlands et al. (2018) have proposed two new metrics that continuously capture the volume (average acceleration) and intensity (intensity gradient) of physical activity. Initial evidence has demonstrated that these metrics are negatively associated with BMI (Buchan et al., 2019; Fairclough et al., 2019) and a composite score of metabolic syndrome risk (Fairclough et al., 2019), independent of one another. Despite these initial promising findings, there are currently no data on the associations between average acceleration or intensity gradient physical activity metrics and other risk factors for cardiometabolic disease (such as low grade chronic inflammation), nor have they been examined in relation to cognitive function. Thus, the use of these metrics and their associations with health and cognitive function requires further investigation.

There is a strong evidence base that physical fitness is beneficially associated with traditional risk factors for cardiometabolic disease, such as blood lipids (Artero et al., 2011; Bailey et al., 2012), HOMA-IR (Agostinis-Sobrinho et al., 2018; Artero et al., 2011; Martinez-Gomez et al., 2010), blood pressure (Bailey et al., 2012) and adiposity in adolescents (Barker et al., 2018). However, less is known regarding the relationship between physical fitness and novel risk factors for cardiometabolic disease in adolescents, such as inflammatory cytokines. Previous data, although sparse, has shown that lower physical fitness is associated with increased IL-6 (Bugge et al., 2012; Dring et al., 2019b) and IL-1β (Dring et al., 2019b) concentrations, as well as a lower anti-inflammatory IL-10 concentration (Dring et al., 2019b). However, further data on these relationships are required, particularly across adolescence, as well as exploring associations with other cytokines which reflect cardiometabolic disease risk. For example, IL-15 - an anti-

inflammatory cytokine which is involved in adipose tissue regulation (Nielsen & Pedersen, 2007; Walsh et al., 2011) and is linked to improved insulin sensitivity (Nadeau & Aguer, 2018) - has not been examined in association with physical fitness in a sample of adolescents.

Physical fitness is also beneficially associated with a range of cognitive function domains in healthy children (Aadland et al., 2017; Hillman et al., 2005), children living with obesity (Davis & Cooper, 2011) and adults (Fortune et al., 2019). However, there is less known with regards to the relationship between physical fitness and cognitive function during adolescence; but some available evidence suggests that physical fitness is positively associated with academic performance (Adelantado-Renau et al., 2018) and inhibitory control (Westfall et al., 2018) in this population. However, to gain a better understanding of the relationship between physical fitness and cognitive function during adolescence, a more comprehensive overview considering the physical fitness-cognition relationship across a range of domains of cognitive function is required. The development of visual processing speeds throughout childhood and adolescence have been investigated, with development generally peaking at mid-adolescence (Kail & Ferrer, 2007), with evidence also showing positive associations with other domains of cognition, such as attention and memory (Croker & Maratos, 2011), thus rendering it as an important aspect of cognition. However, there is a lack of empirical work investigating how certain characteristics, such as physical fitness, are associated with visual processing speed.

Adiposity is recognised as an important risk factor for the development of cardiovascular disease and type 2 diabetes (Arslanian, 2000). Dring et al. (2019b) provide data showing that adiposity (sum of four skinfolds) is positively related to HOMA-IR and mean arterial pressure, whilst Martinez-Gomez et al. (2010) showed that the sum of six skinfolds was positively related with CRP. Furthermore, there is growing evidence to suggest that adiposity may be detrimental for cognitive function, although there is a lack of work solely focusing on an adolescent population, along with a range of markers, typically BMI, having been used previously (Mamrot & Hanc, 2019). An additional marker of adiposity, waist circumference, is a common proxy of central adiposity, non-invasive, easy to obtain and is the preferred measure when considering risk of

cardiometabolic disease (Klein et al., 2007; World Health Organisation, 2008). Adiposity, measured by waist circumference, has previously been assessed as an outcome related to cardiometabolic disease in adolescents (Artero et al., 2011; Bailey et al., 2012; Barker et al., 2018; Carson et al., 2013), but there has been little consideration as to how waist circumference influences novel risk factors for cardiometabolic disease in adolescents, such as the pro-inflammatory cytokines previously mentioned.

The aforementioned work has highlighted the important associations of lifestyle behaviours and characteristics with risk factors for cardiometabolic disease and cognitive function. However, there is little known about how these outcomes (cardiometabolic disease and cognitive function) and their associations with physical fitness, physical activity and adiposity change during adolescence, which is important given the array physical changes that occur during puberty (Beunen, Rogol & Malina, 2006; Blakemore, Burnett & Dahl, 2010). Moreover, adolescence is an important period of the lifespan for cognition, as the hormonal developments lead to structural and behavioural changes in the brain (Blakemore, Burnett & Dahl, 2010) and age is seen to be an important moderator of cognitive function (Hötting & Röder, 2013).

Therefore, the first purpose of the present study was to compare physical activity, physical fitness, adiposity, risk factors for cardiometabolic disease and cognitive function in year 7 and year 10 boys and girls. The second purpose of the present study was to examine the relationship between the independent variables of interest (physical fitness, physical activity and adiposity) and risk factors for cardiometabolic disease and cognitive function and to examine if these relationships were modified by year group.

4.2 Methods

4.2.1 Experimental Design

The study conformed to the Declaration of Helsinki and was approved by Nottingham Trent University Human Ethics Committee. Written parental consent and adolescent assent were obtained during recruitment, before enrolment onto the study. A health screen was completed by the parent/guardian on behalf of the participant, which was checked by a lead investigator to

ensure there were no medical conditions that would affect the young person's participation, which included any existing neurological and/or health conditions. All participants enrolled were considered healthy.

The study employed a cross-sectional design, consisting of two main experimental trials that took place at the schools, separated by at least 7 d. In the first experimental trial, participants underwent anthropometric measurements (stature, sitting stature, body mass, waist circumference and skinfolds), as well as completing a 15 m multi-stage fitness test and 20 m sprints for the assessment of physical fitness. Following the completion of these measures, participants were then familiarised with the battery of cognitive function tests (described below). At the end of the first trial, Actigraph GT3X+ accelerometers were given to participants to wear for the 7 d period between the two main trials, for the assessment of free-living physical activity. After the 7 d of activity tracking, participants attended the second main trial following an overnight fast (from 10 pm the previous evening). This trial started with a capillary blood sample, following which participants were then fed a standardised breakfast, providing 1.5 g per kg body mass of carbohydrate (Cooper et al., 2011 & 2012), before completing the cognitive function test battery. Finally, participants completed an adolescent appropriate questionnaire for physical activity.

4.2.2 Participants

Seventy participants (35 girls) in year 7 (11.4 ± 0.5 y) and 43 (27 girls) in year 10 (14.3 ± 0.5 y) volunteered to take part in the study[1]. All participants underwent measures of body mass, stature and sitting stature in accordance with the methods outlined in section 3.3 of this thesis. Stature and sitting stature were used to predict maturity offset (year 7 girls: -0.37 ± 0.56 y, year 7 boys: -1.65 ± 0.50 y, year 10 girls: 2.32 ± 0.48 y, year 10 boys: 1.07 ± 0.43 y), following methods described previously (Moore et al., 2015). Body mass and stature were used to determine body mass index (BMI), for which age- and sex-specific centiles were derived based on national reference values (Cole, Bellizzi, Flegal & Dietz, 2000).

[1] The intention was to recruit 100 participants from each year group, however due to the COVID-19 pandemic and school closures the data collection was terminated.

4.2.3 Measurements

4.2.3.1 Physical Fitness

Participants completed the 15 m version of the multi-stage fitness test (MSFT). The 15 m version starts at 6 $km \cdot h^{-1}$ and increases by 0.5 $km \cdot h^{-1}$ for every subsequent stage (approximately 1 min per stage). Participants were fitted with a heart rate monitor (First Beat Technologies Ltd., Finland) prior to the start and heart rate was recorded continuously during the MSFT. Participants were instructed to run until volitional exhaustion and verbal encouragement was provided. The total distance completed (m) was used as the performance criterion for the test.

Participants completed maximum effort 20 m sprints, as an additional assessment of physical performance. After an initial warm up, led by one of the lead investigators, participants were given a practice attempt at the sprint. Infrared timing gates (Brower Timing Systems IRD-T173, Draper, UT, USA) were placed at the start and finish of the 20 m runway, to help the participants identify where the sprint takes place. Cones were set out 0.5 m behind the start timing gate, to standardise the start positions of the participants. Initially, participants completed three efforts, unless their final effort was the fastest in which case they performed an additional sprint, up to a maximum total of five. Participants were instructed to start the sprint in their own time, as the sprint time was automatically recorded by the timing gates. To ensure that participants did not slow before the 20 m gates, a set of 'dummy' gates were placed ~ 2 m beyond, which participants were told were the target gates. The sprints were performed with groups of 10, whereby the first person joined the back of the queue and had their rest period whilst the other participants performed their sprints.

4.2.3.3 Adiposity and Body Composition

Waist circumference and the sum of 4 skinfolds (triceps, subscapular, supraspinale and thigh) were measured in line with descriptions in section 3.4 of this thesis.

4.2.3.4 Blood Pressure

Upon arrival at school, participants were seated quietly for 5 min prior to the measurement of blood pressure. Blood pressure was measured twice on the right arm, which was rested at chest

height, in accordance with guidelines (Frese, Fick & Sadowsky, 2011), using an automated sphygmomanometer (HBP-1300, Omron, Milton Keynes, UK). If systolic blood pressure differed by > 5 mmHg then a third measurement was taken. All blood pressure readings were interspersed by 1 min rest. The average was used if two measures were conducted, whereas the median was selected if a third measurement was required. Mean arterial blood pressure was determined using the following calculation: diastolic blood pressure + ([0.33*(systolic blood pressure – diastolic blood pressure]) (Smeltzer et al., 2010).

4.2.3.5 Capillary Blood Sample

The capillary blood sampling procedure was conducted in line with descriptions in section 3.6 of this thesis. A single 25 µl whole blood sample was also collected, using a pre-calibrated glass pipette (Hawksley Ltd, UK), immediately deproteinised in 250 µl ice-cooled 2.5% perchloric acid in 1.5 ml plastic vials. The whole blood and EDTA coated microvettes were centrifuged at 4000 x g, for 4 min at 4 °C (Eppendorph 541C, Hamburg, Germany). The microvette with clotting activator was allowed to rest at room temperature for 30 min before centrifugation at 1000 x g for 15 minutes. Plasma and serum were extracted into 500 µl vials for subsequent analysis. All samples were frozen immediately at -20 °C and transferred to a -80 °C freezer as soon as possible.

Blood glucose, plasma insulin, serum BDNF and cytokine (IL-6, IL-10, IL-1β & IL-15) concentrations were determined via methods described previously (Section 3.6)

4.2.3.6 Cognitive Function Tests

The cognitive function test battery lasted approximately 15 minutes and consisted of the Visual Search Test, Stroop test, Sternberg Paradigm and a Flanker task which were completed on a laptop computer (Lenovo ThinkPad T450; Lenovo, Hong Kong). Specific test details can be found in section 3.8 of this thesis. The participants completed the tests in a classroom, in silence and separated so that they could not interact during the tests. Sound cancelling headphones were also worn, to minimise external disturbances.

4.2.3.8 Assessment of Physical Activity

Free-living, device-measured physical activity was assessed with ActiGraph GT3X+ triaxial accelerometers (Actigraph, Pensacola, FL, USA). Accelerometer data was collected in accordance with the protocol outlined previously (Section 3.7).

Processing of Accelerometer Data

In line with the processing methods described in section 3.7 of this thesis, the outcome variables from the accelerometer data were the average acceleration and intensity gradient (Rowlands et al., 2018). A total of 20 accelerometry files were excluded from analysis (absent on data collection = 1, insufficient wear time = 16, calibration error > 0.01 g = 3).

Based on the descriptions provided by Rowlands et al. (2018), the total volume of physical activity per day was expressed as the average acceleration (ENMO, *mg*). The intensity gradient was the metric of choice to describe the intensity of physical activity. For detailed information on the derivation of the intensity gradient see Rowlands et al. (2018). Briefly, the intensity gradient describes the relationship between the log values of intensity (represented by intensity bins of 25 *mg* resolution, i.e. 0 – 25 *mg*, 25 – 50 *mg*…3975 – 4000 *mg*) and time (accumulated time in each intensity bin). The intensity gradient is always negative, as this reflects the decrease in time spent at the higher intensity bins (Rowlands et al., 2018). The average intensity gradient over a 24 h period was calculated for each participant, as well as the constant for the linear regression equation and the R^2 value (indicative of the goodness of fit for the model). A higher constant and lower intensity gradient are representative of a steeper intensity distribution, depicting less time spent across higher intensity activities. Conversely, a lower constant and higher intensity gradient represent a shallower intensity distribution, which is indicative of more time spent in higher intensity activities.

4.2.3.9 Self-Reported Physical Activity

Subjective assessment of physical activity was assessed using the Physical Activity Questionnaire for Adolescents (PAQ-A; Kowalski, Crocker & Donen., 2004). This study used a version that has been validated for use in the United Kingdom (Aggio, Fairclough, Knowles &

Graves., 2016). The PAQ-A captures activity levels from the previous week, with participants responding to 10 items such as, “In the last 7 days, what did you normally do at lunch (besides eating lunch)?”. Participants graded their responses on a 5-point Likert-type scale ranging from 1 (*none/low physical activity*) to 5 (*high physical activity*). An overall summary score was calculated, with higher scores representing greater physical activity levels. Typically, an overall score of 2.9 or less indicates an individual who is less physically active, whereas a score of 3 and above indicates an individual who is more active (Kowalski et al., 2004).

4.2.4 Statistical Analysis

All analyses were performed using RStudio (RStudio Team, 2020). To compare the main effects of year group and sex, as well as their interaction (year group*sex), a factorial analysis of variance (ANOVA) for unbalanced designs (type III sum of squares), was conducted using the “car” package (Fox., 2019) . A rank order ANOVA was used for IPAQ data. Residuals for each ANOVA were assessed visually for normality using Quantile-Quantile (QQ) plots and histograms and for homoscedasticity using a plot of residuals versus fit. Where residuals violated these assumptions, the dependent variable was log transformed for the analysis (for ease of interpretation the data are reported in raw format). Descriptive summaries of data are reported as mean ± SD, unless otherwise stated.

Multiple linear regression was performed, using the “lme4” package (Bates et al., 2015), to examine associations between physical fitness (distance run on the MSFT), physical activity (average acceleration and intensity gradient) and adiposity (waist circumference) with risk factors for cardiometabolic disease and cognitive function. The models were first performed whilst adjusting for year group and sex, to determine the overall association of the exposure variable of interest. Following this, an interaction term consisting of year group and the exposure variable of interest (physical fitness, average acceleration, intensity gradient or adiposity) was included, to determine a moderating effect of year group on the relationship. All exposure variables were centred before entry into the models and residuals were assessed for conformity with the underlying assumptions of normality and homoscedasticity. Variance inflation factors (VIF) were

used to assess collinearity (all VIF's were < 5) and therefore satisfied this assumption (Montgomery, Peck & Vining, 2001). Residual analyses were performed and if normality or homoscedasticity were violated, the dependent variable was log transformed and the residuals were checked thereafter. For models where the log version was used, the coefficients and 95% CI are presented as a % change for a 1-unit increase in the exposure variable. Alpha for determining statistical significance was set at $p < 0.05$.

4.3 Results

4.3.1 Comparison between Year Groups and Sex

4.3.1.1 Adiposity

A breakdown by year group and sex of the data for adiposity, physical activity and physical fitness measures can be found in Table 4.1. There was no difference in BMI centile between year groups (main effect of year group; $F_{(1, 109)} = 0.23$, $p = 0.609$) or between boys and girls (main effect of sex; $F_{(1, 109)} = 0.26$, $p = 0.611$), nor did they interact (year group*sex interaction; $F_{(1, 109)} = 0.11$, $p = 0.739$). Year 10 participants had a higher waist circumference than year 7 (main effect of year group; $F_{(1, 105)} = 9.5$, $p = 0.003$, MD = 4.3 cm, 95% CI [1.5 cm, 7.1 cm]) but waist circumference was not different between boys and girls overall (main effect of sex; $F_{(1, 105)} = 1.8$, $p = 0.179$). The difference between boys and girls tended however to be of a greater magnitude in year 10 participants (year group*sex interaction; $F_{(1, 105)} = 3.61$, $p = 0.06$). There was no difference in the sum of skinfolds between year groups (main effect of year group; $F_{(1, 97)} = 0.57$, $p = 0.451$), but a larger sum of skinfolds was evident in girls compared to boys (main effect of sex; $F_{(1, 97)} = 7.89$, $p = 0.006$, MD = 12.0 mm, 95% CI [2.1 mm, 22.0 mm]). However, year group and sex did not interact to affect sum of skinfolds (year group*sex interaction; $F_{(1, 97)} = 1.68$, $p = 0.197$).

4.3.1.2 Physical Fitness

Distance covered during the MSFT was greater in year 10 (main effect of year group; $F_{(1, 108)} = 39.7$, $p < 0.001$, MD = 540 m, 95% CI [360 m, 720 m]), and more distance was covered by boys overall (main effect of sex; $F_{(1, 108)} = 41.3$, $p < 0.001$, MD = 540 m, 95% CI [375 m, 720 m]). The

difference between year groups was dependent on sex (year group*sex interaction; $F_{(1, 108)} = 8.82$, $p = 0.003$), whereby year 10 boys covered a much greater distance than year 7 boys (mean difference = 900 m, 95% CI [570 m, 1245 m]), whereas the difference in the distance covered between year groups for girls was lower (mean difference = 165 m, 95% CI [-120 m, 465 m]).

Year 10 participants were quicker on the 20 m sprint compared to year 7 (main effect of year group; $F_{(1, 106)} = 30.08$, $p < 0.001$, MD = -0.40 s, 95% CI [-0.49 s, -0.30 s]), and boys were also quicker than girls (main effect of sex; $F_{(1, 106)} = 11.7$, $p < 0.001$, MD = -0.17 s, 95% CI [-0.27 s, -0.08 s]). Year group and sex did not interact to affect sprint performance (year group*sex interaction; $F_{(1, 106)} = 2.11$, $p = 0.15$).

4.3.1.3 Physical Activity

Self-reported physical activity was higher in year 7 participants compared to year 10 (main effect of year group; $F_{(1, 103)} = 11.4$, $p = 0.001$, MD = 0.4 AU, 95% CI [0.1 AU, 0.6 AU]), with boys tending to report more physical activity than girls (main effect of sex; $F_{(1, 103)} = 3.92$, $p = 0.051$, MD = 0.3 AU, 95% CI [0.03 AU, 0.5 AU]). Year group and sex did not interact to affect self-reported physical activity (year group*sex interaction; $F_{(1, 103)} = 2.48$, $p = 0.119$).

There was no difference in daily average acceleration between year groups (main effect of year group; $F_{(1, 89)} = 0.57$, $p = 0.454$), between boys and girls (main effect of sex; $F_{(1, 89)} = 1.63$, $p = 0.205$), nor did they interact (year group*sex interaction; $F_{(1, 89)} = 2.76$, $p = 0.100$). There was also no difference in intensity gradient between year 7 and year 10 (main effect of year group; $F_{(1, 89)} = 1.17$, $p = 0.282$), but boys had a smaller intensity gradient compared to girls (main effect of sex; $F_{(1, 89)} = 4.93$, $p = 0.029$, MD = 0.16 AU, 95% CI [0.08 AU, 0.24 AU]). Year group and sex did not interact to affect intensity gradient (year group*sex interaction; $F_{(1, 89)} = 0.24$, $p = 0.624$).

Table 4.1. A summary of the comparison between measures of adiposity, physical activity and physical fitness for boys and girls in year 7 and year 10. Data are mean ± SD and (range).

	Year 7		Year 10	
Variable	*Boys*	*Girls*	*Boys*	*Girls*
BMI Centile	64.3 ± 28.5 (7.9 – 99.8)	63.5 ± 28.1 (4.3 – 99.4)	64.3 ± 29.5 (9.4 – 99.3)	59.7 ± 29.7 (3.1 – 99.7)
Waist Circumference (cm)	65.8 ± 6.9 (56.7 – 84.0)	66.5 ± 7.4 (55.9 – 84.1)	72.8 ± 6.4 (62.8 – 91.6)	68.2 ± 6.4 [a, c] (60.5 – 91.0)
Sum of Skinfolds (mm)	55.5 ± 24.3 (21.7-116.0)	63.7 ± 27.2 (30.8-153.0)	49.3 ± 24.6 (22-107.0)	68.2 ± 24.8 [b] (36.6-128)
MSFT (m)	1395 ± 435 (720-2250)	1215 ± 435 (540-2550)	2295 ± 450 (1650-3240)	1380 ± 405 [a, b, c] (720-2460)
20 m Sprint (s)	3.71 ± 0.23 (3.17 – 4.21)	3.84 ± 0.31 (3.22 – 4.38)	3.22 ± 0.19 (2.94 – 3.66)	3.48 ± 0.18 [a, b] (3.15 – 3.98)
IPAQ (AU; 1-5)	3.2 ± 0.6 (2.0 – 4.3)	2.8 ± 0.7 (1.7 – 4.5)	2.7 ± 0.5 (2.2 – 3.9)	2.6 ± 0.5 (1.4 – 3.8)
Average Acceleration (mg)	17.7 ± 4.6 (11.4 – 29.6)	13.3 ± 3.4 (5.8 – 20.1)	14.2 ± 4 (7.5 – 20.7)	12.5 ± 3.4 (8.1 – 21.9)
Intensity Gradient (AU)	-2.34 ± 0.14 (-2.58 – -2.12)	-2.51 ± 0.19 (-2.83 – -2.13)	-2.43 ± 0.23 (-2.87 – -1.98)	-2.57 ± 0.19 [b] (-2.86 – -2.23)

Abbreviations: BMI; Body Mass Index. MSFT; Multi-Stage Fitness Test. IPAQ; International Physical Activity Questionnaire.
Data are mean ± SD and (range).
[a] Main effect of year group. [b] Main effect of sex. [c] Year group*sex interaction.

4.3.1.4 Risk Factors for Cardiometabolic Disease

A breakdown by year group and sex, for the data of risk factors related to cardiometabolic disease can be found in Table 4.2.

Blood Pressure

Systolic blood pressure was higher in year 10 compared to year 7 (main effect of year group; $F_{(1, 106)} = 8.9$, $p = 0.004$, MD = 10 mmHg, 95% CI [6 mmHg, 15 mmHg]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 1.8$, $p = 0.183$). Year group and sex did not interact to affect systolic blood pressure (year group*sex interaction; $F_{(1, 106)} = 1.1$, $p = 0.292$). Diastolic blood pressure was higher in year 10 compared to year 7 (main effect of year group; $F_{(1, 106)} = 17.6$, $p < 0.001$, MD = 6 mmHg, 95% CI [4 mmHg, 9 mmHg]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 2.9$, $p = 0.090$), and nor did year group and sex interact to affect diastolic blood pressure (year group*sex interaction; $F_{(1, 106)} = 1.2$, $p = 0.273$).

Mean arterial pressure (MAP) was higher in year 10 compared to year 7 (main effect of year group; $F_{(1, 106)} = 17.93$, $p < 0.001$, MD = 8 mmHg, 95% CI [5 mmHg, 10 mmHg]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 0.11$, $p = 0.736$). Year group and sex did not interact to affect MAP (year group*sex interaction; $F_{(1, 106)} = 0.02$, $p = 0.895$).

Metabolic Markers (Fasted Glucose, Insulin and HOMA-IR)

Fasted blood glucose concentration was higher in year 10 participants compared to year 7 (main effect of year group; $F_{(1, 102)} = 7.30$, $p = 0.008$, MD = 0.42 mmol·L^{-1}, 95% CI [0.20, 0.64 mmol·L^{-1}]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 102)} = 0.28$, $p = 0.596$). Year group and sex did not interact to affect fasted blood glucose concentration (year group*sex interaction; $F_{(1, 102)} = 0.00$, $p = 0.964$). There was no difference in fasted plasma insulin concentration between year groups (main effect of year group; $F_{(1, 100)} = 0.91$, $p = 0.342$), between boys and girls (main effect of sex; $F_{(1, 100)} = 0.85$, $p = 0.358$), nor did they interact (year group*sex interaction; $F_{(1, 100)} = 0.12$, $p = 0.736$). There was no difference in HOMA-IR between year groups (main effect of year group; $F_{(1, 98)} = 0.13$, $p = 0.722$), between boys and girls (main effect of sex; $F_{(1, 98)} = 1.16$, $p = 0.285$), nor did they interact (year group*sex interaction; $F_{(1, 98)} = 0.12$, $p = 0.736$).

Cytokines (IL-6, IL-10, IL-1β and IL-15)

IL-6 concentration was higher in year 7 participants compared to year 10 (main effect of year group; $F_{(1, 100)} = 27.81$, $p < 0.001$, MD = 0.89 pg·mL^{-1}, 95% CI [0.49, 1.30 pg·mL^{-1}]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 100)} = 0.92$, $p = 0.339$). However, year group and sex interacted to affect IL-6 concentration (year group*sex interaction; $F_{(1, 100)} = 7.55$, $p = 0.007$). Specifically, year 7 boys had a lower concentration compared to year 7 girls (MD = -35.5%, 95% CI [-55.0%, -7.7%]), whereas boys and girls were similar in year 10.

IL-10 concentration was higher in year 7 participants compared to year 10 (main effect of year group; $F_{(1, 95)} = 11.60$, $p < 0.001$, MD = 0.69 pg·mL^{-1}, 95% CI [0.21, 1.17 pg·mL^{-1}]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 95)} = 0.66$, $p = 0.417$). However, year group and sex interacted to affect IL-10 concentration (year group*sex interaction; $F_{(1, 95)} = 4.33$,

p = 0.040). Specifically, year 7 girls had a higher concentration compared to year 10 girls (mean difference = 41.2%, 95% CI [8.3%, 83.9%]), whereas boys were similar across year groups.

There was no difference in IL-15 concentration between year groups (main effect of year group; $F_{(1, 95)}$ = 1.37, p = 0.244), or between boys and girls (main effect of sex; $F_{(1, 95)}$ = 0.12, p = 0.727). However, year group and sex interacted to affect IL-15 concentration (year group*sex interaction; $F_{(1, 95)}$ = 4.42, p = 0.038). Specifically, year 7 boys had a lower concentration than year 7 girls (mean difference = -27.3%, 95% CI [-41.4%, -9.8%]).

There was no difference in IL-1β concentration between year groups (main effect of year group; $F_{(1, 92)}$ = 1.7, p = 0.195), between boys and girls (main effect of sex; $F_{(1, 92)}$ = 2.23, p = 0.139), nor did they interact (year group*sex interaction; $F_{(1, 92)}$ = 1.71, p = 0.195).

Table 4.2. A summary of the comparison of risk factors for cardiometabolic disease, split by sex and year group. Data are mean ± SD and (range).

	Year 7		Year 10	
Variable	*Boys*	*Girls*	*Boys*	*Girls*
Systolic Blood Pressure (mmHg)	115 ± 12 (95 – 146)	115 ± 11 (96 – 139)	128 ± 13 (107 – 153)	124 ± 8 [a] (105 – 146)
Diastolic Blood Pressure (mmHg)	69 ± 7 (59 – 84)	70 ± 7 (58 – 86)	73 ± 8 (56 – 85)	77 ± 6 [a] (63 – 87)
Mean Arterial Pressure (mmHg)	84 ± 7 (72 – 99)	84 ± 7 (71 – 100)	91 ± 9 (72 – 107)	92 ± 6 [a] (80 – 101)
Blood Glucose Concentration ($mmol \cdot L^{-1}$)	4.02 ± 0.45 (3.10 – 5.0)	4.12 ± 0.59 (3.30 – 5.80)	4.43 ± 0.75 (3.0 – 5.50)	4.52 ± 0.53 [a] (3.60 – 5.50)
Plasma Insulin Concentration ($pmol \cdot L^{-1}$)	54.5 ± 32.3 (14.7 – 123.0)	67.7 ± 29.9 (22.8 – 123.0)	51.2 ± 25.8 (13.3 – 96.7)	60.2 ± 25.8 13.3 – 113.0)
HOMA-IR (AU)	1.58 ± 0.91 (0.39 – 3.40)	2.09 ± 1.02 (0.55 – 4.38)	1.65 ± 0.99 (0.39 – 3.53)	2.00 ± 0.85 (0.35 – 3.82)
IL-6 Concentration ($pg \cdot ml^{-1}$)	1.70 ± 1.06 (0.53 – 4.68)	2.59 ± 1.33 (0.36 – 5.34)	1.38 ± 0.73 (0.59 – 3.39)	1.17 ± 0.55 [a, c] (0.44 – 2.33)
IL-10 Concentration ($pg \cdot ml^{-1}$)	2.97 ± 0.90 (0.69-5.79)	3.88 ± 1.70 (0.97 – 8.27)	2.81 ± 0.59 (1.70 – 3.82)	2.65 ± 1.08 [a, c] (1.25 – 6.09)
IL-15 Concentration ($pg \cdot ml^{-1}$)	2.45 ± 0.63 (1.28 – 3.85)	3.44 ± 1.11 (1.39 – 5.87)	2.94 ± 0.85 (1.78 – 5.4)	3.17 ± 1.17 [c] (1.26 – 5.90)
IL-1β Concentration ($pg \cdot ml^{-1}$)	41.0 ± 25.5 (3.55 – 106.00)	38.4 ± 20.2 (7.37 – 74.80)	43.5 ± 23.6 (6.97 – 80.60)	30.2 ± 16.9 (5.65– 76.60)

Abbreviations: HOMA-IR; Homeostatic Model Assessment of Insulin Resistance. IL; Interleukin.
Data are mean ± SD and (range)
[a] Main effect of year group. [b] Main effect of sex. [c] Year group*sex interaction.

4.3.1.5 Cognitive Function

A breakdown by year group and sex, for the cognitive function data can be found in Table 4.3.

Stroop Task

Year 10 participants were quicker on the congruent Stroop task compared to year 7 (main effect of year group; $F_{(1, 106)} = 31.4$, $p < 0.001$, MD = -180 ms, 95% CI [-225 ms, -137 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 0.39$, $p = 0.529$). Year group and sex did not interact to affect congruent Stroop response time (year group*sex interaction; $F_{(1, 106)} = 1.98$, $p = 0.16$). Year 10 participants were also quicker on the incongruent Stroop task compared to year 7 (main effect of year group; $F_{(1, 105)} = 13.97$, $p < 0.001$, MD = -287 ms, 95% CI [-365 ms, -210 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 105)} = 2.72$, $p = 0.102$). Year group and sex interacted to affect incongruent Stroop response time (year group*sex interaction; $F_{(1, 105)} = 7.21$, $p = 0.008$), whereby boys in year 10 were much quicker than their year 7 counterparts (mean difference = -407 ms, 95% CI [-565 ms, -249 ms]), with a smaller difference seen between year groups for girls (mean difference = -193 ms, 95% CI [-329 ms, -58 ms]). Accuracy on the congruent and incongruent levels of the Stroop were not different between year group and sex, nor did they interact to affect accuracy (all $p > 0.05$).

Flanker Task

Year 10 participants were quicker on the congruent flanker task compared to year 7 (main effect of year group; $F_{(1, 106)} = 25.50$, $p < 0.001$, MD = -123 ms, 95% CI [-167 ms, -78 ms]). There was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 0.38$, $p = 0.540$). Year group and sex did not interact to affect congruent flanker response time (year group*sex interaction; $F_{(1, 106)} = 0.50$, $p = 0.483$). Year 10 participants were quicker on the incongruent flanker task compared to year 7 (main effect of year group; $F_{(1, 106)} = 19.33$, $p < 0.001$, MD = -133 ms, 95% CI [-183 ms, -84 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 0.14$, $p < 0.001$). Year group and sex did not interact to affect incongruent flanker response time (year group*sex interaction; $F_{(1, 106)} = 0.36$, $p = 0.551$). Accuracy on the congruent and incongruent

levels of the Flanker were not different between year group and sex, nor did they interact to affect accuracy (all $p > 0.05$).

Sternberg Paradigm

Year 10 participants were quicker on the one-item level of the Sternberg paradigm compared to year 7 (main effect of year group; $F_{(1, 105)} = 14.85$, $p < 0.001$, MD = -103 ms, 95% CI [-140 ms, -66 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 105)} = 0.01$, $p = 0.932$). Year group and sex did not interact to affect response time (year group*sex interaction; $F_{(1, 105)} = 0.19$, $p = 0.661$). Year 10 participants were quicker on the three-item level of the Sternberg paradigm compared to year 7 (main effect of year group; $F_{(1, 105)} = 16.40$, $p < 0.001$, MD = -172 ms, 95% CI [-226 ms, -117 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 105)} = 0.05$, $p = 0.815$), nor did year group and sex interact to affect response times (year group*sex interaction; $F_{(1, 105)} = 0.73$, $p = 0.396$). Year 10 participants were quicker on the five-item level of the Sternberg paradigm compared to year 7 (main effect of year group; $F_{(1, 105)} = 24.10$, $p < 0.001$, MD = -219 ms, 95% CI [279 ms, -159 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 105)} = 0.00$, $p = 0.995$), nor did year group and sex interact to affect response times (year group*sex interaction; $F_{(1, 105)} = 0.59$, $p = 0.444$). Accuracy on all levels of the Sternberg were not different between year group and sex, nor did they interact to affect accuracy (all $p > 0.05$).

Visual Search Test (VST)

Year 10 participants were quicker on the VST baseline level compared to year 7 (main effect of year group; $F_{(1, 106)} = 6.47$, $p = 0.013$, MD = -58 ms, 95% CI [-84 ms, -34 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 1.47$, $p = 0.229$), nor did they interact to affect response time (year group*sex interaction; $F_{(1, 106)} = 2.18$, $p = 0.143$). Year 10 participants were quicker on the VST complex level compared to year 7 (main effect of year group; $F_{(1, 106)} = 4.35$, $p = 0.039$, MD = -217 ms, 95% CI [-380 ms, -52 ms]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 0.04$, $p = 0.839$), nor did they interact to affect response time (year group*sex interaction; $F_{(1, 106)} = 0.04$, $p = 0.852$). For accuracy on the VST

baseline level there was no difference between year group and sex, nor did they interact to affect accuracy (all $p > 0.05$). Year 10 participants were more accurate on the VST complex level compared to year 7 (main effect of year group; $F_{(1, 106)} = 4.26$, $p = 0.041$; MD = 10.10%, CI [3.80%, 16.40%]), but there was no difference between boys and girls (main effect of sex; $F_{(1, 106)} = 0.00$, $p = 0.982$), nor did they interact to affect accuracy (year group*sex interaction; $F_{(1, 106)} = 0.18$, $p = 0.668$).

BDNF Concentration

For BDNF concentration, there was no difference between year groups (main effect of year group; $F_{(1, 100)} = 1.9$, $p = 0.170$), or between boys and girls (main effect of sex; $F_{(1, 100)} = 0.01$, $p = 0.927$), nor did they interact to affect BDNF concentration (year group by sex interaction; $F_{(1, 100)} = 1.62$, $p = 0.206$).

Table 4.3. A summary of the comparison of risk factors for cardiometabolic disease, split by sex and year group. Data are mean ± SD or median (range).

	Year 7		Year 10	
Variable	*Boys*	*Girls*	*Boys*	*Girls*
Congruent Stroop RT (ms)	856 ± 118 (652-1116)	818 ± 141 (611-1211)	644 ± 68 (515-780)	663 ± 81 [a] (516-846)
Accuracy (%)	96.0 ± 5.2 (75.0 – 100)	97.2 ± 3.9 (85.0 – 100)	97.5 ± 4.10 (85.0 – 100)	96.3 ± 4.4 (85.0 – 100)
Incongruent Stroop RT (ms)	1245 ± 229 (905 – 1788)	1135 ± 216 (799 – 1572)	837 ± 114 (658 – 1010)	942 ± 171 [a, c] (680 – 1340)
Accuracy (%)	89.2 ± 7.8 (62.5 – 97.5)	93.1 ± 6.3 (77.5 – 100)	93.9 ± 4.9 (80.0 – 100)	93.9 ± 7.5 (62.5 – 100)
Congruent Flanker RT (ms)	610 ± 87 (448-801)	631 ± 165 (414-1171)	507 ± 69 (417-687)	491 ± 77 [a] (365-738)
Accuracy (%)	96.9 ± 5.8 (76.7 – 100)	96.5 ± 7.3 (60.0 – 100)	98.1 ± 2.7 (93.3 – 100)	97.8 ± 3.3 (86.7 – 100)
Incongruent Flanker RT (ms)	666 ± 107 (468 – 906)	682 ± 185 476 – 1176)	550 ± 57 (461 – 709)	534 ± 88 [a] (406 – 857)
Accuracy (%)	90.2 ± 14.9 (40.0 – 100)	89.6 ± 16.3 (36.7 – 100)	95.2 ± 4.7 (80.0 – 100)	95.5 ± 3.8 (83.3 – 100)
One Item Sternberg RT (ms)	577 ± 93 (412-826)	563 ± 117 (408-896)	465 ± 62 (364-568)	468 ± 80 [a] (363-730)
Accuracy (%)	91.7 ± 12.6 (50.0 – 100)	95.2 ± 7.1 (75.0 – 100)	94.1 ± 6.2 (75.0 – 100)	96.2 ± 6.8 (75.0 – 100)
Three Item Sternberg RT (ms)	750 ± 160 (377 – 1110)	692 ± 172 (397 – 1257)	556 ± 62 (463 – 662)	546 ± 87 [a] (294 – 767)
Accuracy (%)	92.8 ± 10.4 (43.8 – 100)	91.5 ± 15.5 (12.5 – 100)	95.5 ± 3.6 (87.5 – 100)	92.4 ± 18.9 (3.1 – 100)
Five Item Sternberg RT (ms)	920 ± 171 (445 – 1477)	873 ± 182 (574 – 1594)	677 ± 88 (557 – 882)	677 ± 113 [a] (518 – 1030)
Accuracy (%)	86.7 ± 11.5 (50.0 – 100)	90.7 ± 8.4 (62.5 – 100)	92.8 ± 6.4 (81.2 – 100)	93.9 ± 5.9 (81.2 – 100)
VST Baseline RT (ms)	592 ± 83 (474 – 819)	579 ± 70 (483 – 830)	512 ± 33 (471 – 568)	536 ± 37 [a] (482 – 605)
Accuracy (%)	92.3 ± 10.9 (47.7 – 100)	95.4 ± 6.9 (65.6 – 100)	96.8 ± 4.6 (84.0 – 100)	97.8 ± 3.3 (91.3 – 100)
VST Complex RT (ms)	1573 ± 408 (777 – 2726)	1577 ± 546 (938 – 3130)	1375 ± 244 (927 – 1936)	1348 ± 324 [a] (910 – 2132)
Accuracy (%)	87.2 ± 22.6 (10.1 – 100)	90.1 ± 18.0 (26.9 – 100)	98.7 ± 4.1 (26.9 – 100)	98.8 ± 3.1 [a] (87.5 – 100)
BDNF Concentration ($ng \cdot ml^{-1}$)	30.9 ± 7.3 (12.6 – 45.6)	36.2 ± 11.1 (11.8 – 58.5)	32.4 ± 8.8 (16.8 – 42.6)	32.7 ± 10.2 [a] (13.3 – 47.9)

Abbreviations: RT; Response Time. VST; Visual Search Test. BDNF; Brain-Derived Neurotrophic Factor. Data are mean ± SD and (range)
[a] Main effect of year group. [b] Main effect of sex. [c] Year group*sex interaction.

4.3.2. *Associations between Physical Fitness, Physical Activity and Adiposity with Risk Factors for Cardiometabolic Disease*

The cross-sectional associations, adjusted for year group and sex, between physical fitness (MSFT), physical activity (average acceleration and intensity gradient) and adiposity (waist circumference) are presented in Table 4.4.

Physical fitness was positively associated with IL-15 concentration, whereby a 15 m (1 shuttle) increase in MSFT performance was associated with a 0.3% increase in IL-15 concentration (Table 4.4). There was no moderating effect of year group on this relationship (Year group*MSFT interaction; p = 0.478). There were no clear associations between physical fitness and the remaining risk factors for cardiometabolic disease (Table 4.4), nor were these associations moderated by year group (Year group*MSFT interaction; all $p > 0.05$).

Average acceleration was negatively associated with systolic, diastolic, and mean arterial blood pressure (Table 4.4). Specifically, a 1 mg increase in average acceleration was associated with a 0.8 mmHg (systolic), 0.4 mmHg (diastolic) and 0.5 mmHg (MAP) reductions, respectively. Including the interaction term provided evidence that the association was modified by year group for diastolic blood pressure and mean arterial pressure. Specifically, the association with diastolic blood pressure (β = -0.653, p = 0.036) and MAP (β = -0.71, p = 0.021, Fig 4.1A) was stronger in year 10 participants than in year 7 participants. There were no clear associations between average acceleration and the remaining risk factors for cardiometabolic disease (Table 4.4), nor were these associations moderated by year group (Year group by average acceleration interaction; all $p > 0.05$), with the exception of IL-6 concentration. Though not statistically significant, there was a tendency for an association between average acceleration and IL-6 concentration, dependent on year group (Year group*average acceleration interaction; p = 0.052). Specifically, IL-6 concentration was negatively associated with average acceleration in year 7 participants, whereas it was positively associated in year 10 participants (Figure 4.1C).

Intensity gradient was negatively associated with systolic and mean arterial blood pressure (Table 4.4). Specifically, a 1 AU increase in intensity gradient was associated with a 19 mmHg (systolic)

and 10 mmHg (MAP) reduction respectively. Including the interaction term provided evidence that the associations with systolic, diastolic and mean arterial blood pressure were modified by year group, whereby the association was stronger in year 10 participants (MAP: β = -16.03, p = 0.003, systolic: β = -20.74, p = 0.013, diastolic: β = -13.37, p = 0.013, Fig 4.1B). There were no clear associations between intensity gradient and the remaining risk factors for cardiometabolic disease (Table 4.4), nor were any of these associations moderated by year group (Year group: Intensity gradient interaction; all $p > 0.05$), with the exception of IL-1β concentration. The association between intensity gradient and IL-1β concentration was dependent on year group (Year group*Intensity gradient interaction; p = 0.010). Specifically, IL-1β concentration was negatively associated with intensity gradient for year 7 participants, whereas it was positively associated in year 10 participants (Fig 4.1D).

Table 4.4. Cross-sectional associations between physical fitness, physical activity and waist circumference and risk factors of cardiometabolic disease, when controlling for year group and sex. Coefficients (β) are presented in their unstandardised forms, unless otherwise stated, with 95% confidence intervals

	MSFT		**Average Acceleration**		**Intensity Gradient**		**Waist Circumference**	
Dependent Variables	*β (95% CI)*	*p*	*β (95% CI)*	*p*	*β (95% CI)*	*p*	*β (95% CI)*	*p*
Systolic Blood Pressure	-0.004 (-0.008, 0.008)	0.106	**-0.83 (-1.39, -0.29)**	**0.005**	**-19.10 (-30.68, -7.44)**	**0.002**	**0.32 (0.02, 0.61)**	**0.038**
Diastolic Blood Pressure	-0.002 (-0.004, 0.0003)	0.222	**-0.41 (-0.77, -0.04)**	**0.031**	-6.84 (-14.50, 0.83)	0.084	-0.02 (-0.20, 0.15)	0.815
Mean Arterial Pressure	-0.03 (-0.060, -0.0015)	0.113	**-0.55 (-0.90, -0.19)**	**0.004**	**-10.92 (-18.45, -3.40)**	**0.006**	0.09 (-0.10, 0.27)	0.377
Blood Glucose	-0.0015 (-0.0045, 0.0015)	0.118	-0.01 (-0.04, 0.02)	0.488	-0.10 (-0.74, 0.56)	0.785	0.01 (-0.01, 0.02)	0.649
Plasma Insulin	-0.003 (-0.180, 0.180)	0.971	0.05 (-1.56, 1.66)	0.951	-24.70 (-57.70, 8.28)	0.146	0.70 (-0.19, 1.59)	0.125
HOMA-IR	-0.0015 (-0.001, 0.0003)	0.484	-0.02 (-0.07, 0.04)	0.555	-0.93 (-1.99, 0.13)	0.091	0.02 (-0.01, 0.05)	0.115
IL-6 [a]	0.15 (-0.15, 0.45)	0.367	-0.60 (-11.63, 11.81)	0.712	-6.20 (-50.97, 79.46)	0.846	1.31 (-0.27, 2.91)	0.093
IL-10 [a]	0.09 (-1.455, 0.330)	0.462	0.40 (-1.75, 2.59)	0.711	16.42 (-26.26, 83.80)	0.515	0.80 (-0.18, 1.80)	0.162
IL-15 [a]	**0.30 (0.06, 0.54)**	**0.038**	-0.70 (-2.45, 1.09)	0.421	17.35 (-18.98, 69.97)	0.402	-0.40 (-1.37, 0.58)	0.359
IL-1β [a]	0.45 (-0.135, 1.04)	0.061	2.33 (-1.80, 6.63)	0.280	12.64 (-51.13, 159.60)	0.780	0.60 (-1.39, 2.63)	0.557

Abbreviations: MSFT; Multi-Stage Fitness Test. CI; Confidence Interval. HOMA-IR; Homeostatic Model Assessment of Insulin Resistance. IL; Interleukin
Coefficient and CI are presented as the % change for a 1-unit change in MSFT (15 m), average acceleration (1 mg), intensity gradient (1 AU), waist circumference (1 cm)
Significant associations are highlighted in bold ($p < 0.05$)
[a] Log transformed

Waist circumference was positively associated with systolic blood pressure (Table 4.4). Specifically, a 1 cm increase in waist circumference was associated with a 0.3 mmHg higher systolic blood pressure. There were no clear associations between waist circumference and any of the remaining risk factors for cardiometabolic diseases (Table 4.4), nor were any of these associations moderated by year group (Year group*waist circumference interaction; all $p > 0.05$), with the exception of IL-6 concentration. For year 10 participants, waist circumference was positively associated with IL-6 concentration ($p = 0.026$), whereas there was no clear evidence of an association for year 7 participants ($p = 0.123$, Fig 4.2).

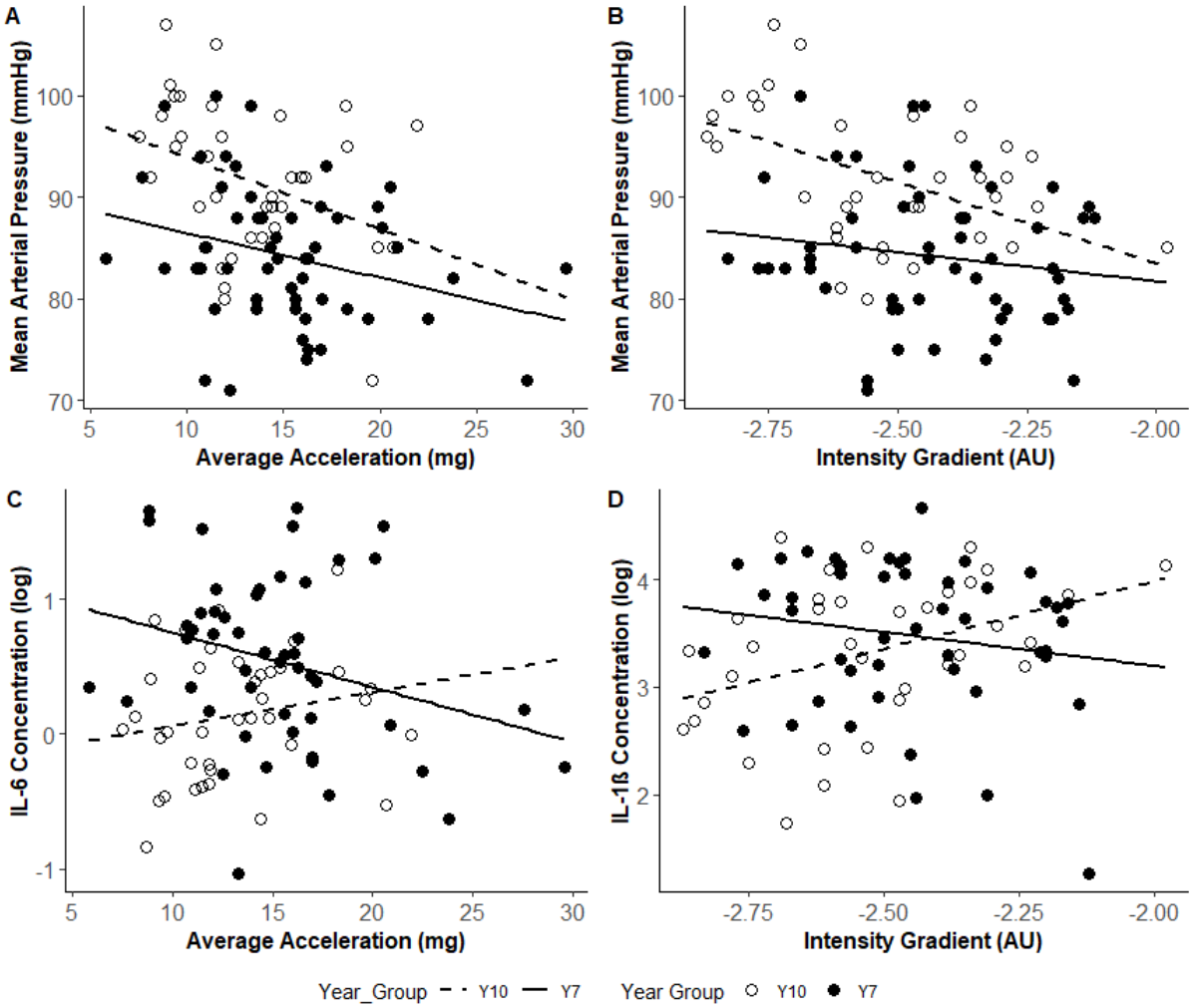

Figure 4.1. (**A**) Association between average acceleration (mg) and mean arterial pressure (mmHg) for year 10 (solid points: β = -0.71 mmHg, $p = 0.021$) and year 7 (open points). (**B**) Association between intensity gradient (AU) and mean arterial pressure (mmHg) for year 10 (solid points: β = -16 mmHg, $p = 0.003$) and year 7 (open points). (**C**) Association between average acceleration (mg) and log-transformed IL-6 concentration for year 10 (solid points: β = -0.06%, $p = 0.052$) and year 7 (open points). (**D**) Association between intensity gradient (AU) and log-transformed IL-1β concentration for year 10 (solid points: β = -2.0%, $p = 0.010$) and year 7 (open points).

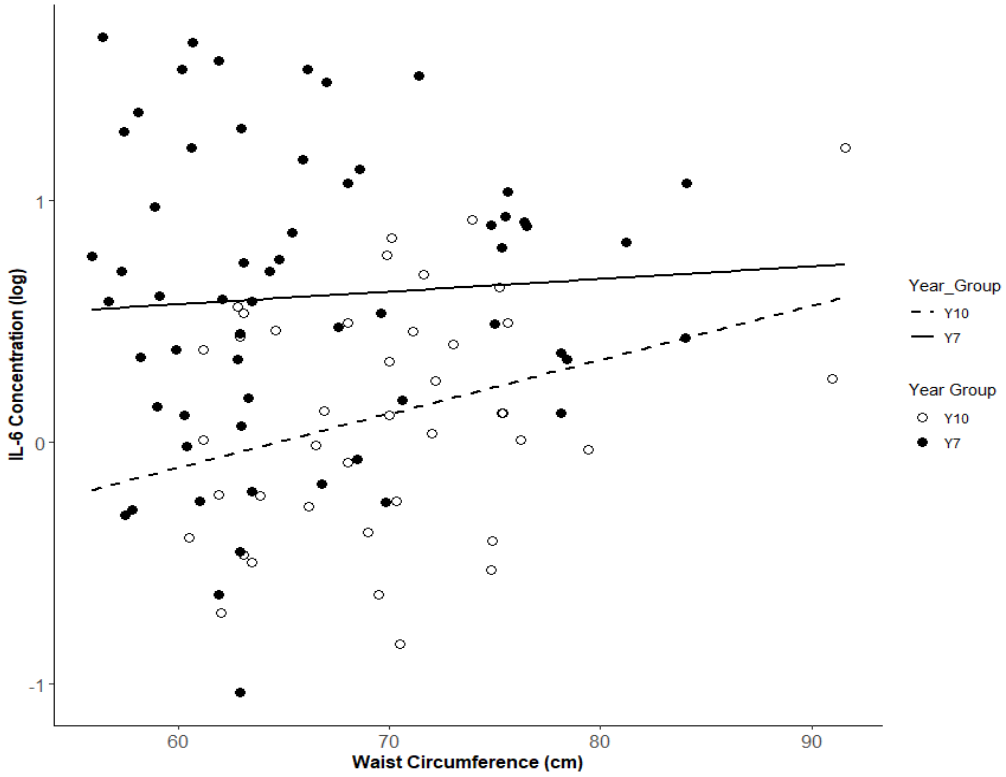

Figure 4.2. Associations between waist circumference (cm) and log-transformed IL-6 concentration for year 10 (solid points: β = 0.03%, *p* = 0.026) and year 7 (open points).

4.3.3. *Associations between Physical Fitness, Physical Activity and Adiposity with Cognitive Function*

The cross-sectional associations, adjusted for year group and sex, between physical fitness (MSFT), physical activity (average acceleration and intensity gradient) and adiposity (waist circumference) with cognitive function outcomes (response times) are presented in Table 4.5.

Physical fitness was negatively associated with response time on the incongruent Stroop task, whereby a 15 m (1 shuttle) increase in MSFT distance was associated with a 0.10 ms reduction in response time (Fig 4.3A). There was no moderating effect of year group on this relationship (Year group*MSFT interaction; *p* = 0.986). Furthermore, physical fitness was positively associated with accuracy on both the congruent (*p* = 0.011) and incongruent (*p* = 0.049) levels of the Stroop task, although these associations were not moderated by year group (congruent; *p* = 0.883. incongruent; *p* = 0.484). Physical fitness was negatively associated with response time on the one-item level of the Sternberg paradigm, whereby a 15 m (1 shuttle) increase in MSFT distance

was associated with a 0.01% reduction in response time (Fig 4.3B). There was no moderating effect of year group on this relationship (Year group*MSFT interaction; $p = 0.241$). Physical fitness was also negatively associated with response times during both levels of the visual search test. Specifically, a 15 m (1 shuttle) increase in MSFT distance was associated with a 0.03 ms reduction in response time on the baseline level and a 0.01% reduction on the complex level (Fig 4.3C & D). There was no moderating effect of year group on these relationships (Year group*MSFT interaction; both $p > 0.05$). For the remaining cognitive function outcomes and BDNF concentration, there were no associations with physical fitness (Table 4.5), nor were these relationships moderated by year group (Year group*MSFT interaction; all $p > 0.05$).

For average acceleration, intensity gradient and waist circumference there were no clear associations with performance on any of the cognitive function tests or BDNF concentration (Table 4.5), nor were any of these relationships moderated by year group (Year group*IV interaction; all $p > 0.05$).

Table 4.5. Cross-sectional associations between physical fitness, physical activity, and waist circumference with cognitive function outcomes and BDNF concentration, when controlling for year group and sex. Coefficients (β) are presented in their unstandardised forms, unless otherwise stated, with 95% confidence intervals.

	MSFT		**Average Acceleration**		**Intensity Gradient**		**Waist Circumference**	
Dependent Variables	*β (95% CI)*	*p*	*β (95% CI)*	*p*	*β (95% CI)*	*p*	*β (95% CI)*	*p*
Congruent Stroop	-0.024 (-0.069, 0.021)	0.303	-2.69 (-8.62, 3.23)	0.375	-60.4 (-183.9, 63.2)	0.341	2.1 (-0.9, 5.1)	0.185
Incongruent Stroop	**-0.10 (-0.177, -0.013)**	**0.025**	-3.92 (-14.30, 6.45)	0.461	-100.5 (-316.7, 115.8)	0.365	2.6 (-2.9, 8.1)	0.356
Congruent Flanker	-0.001 [a] (-0.009, 0.007)	0.828	0.17 (-0.77, 1.12)	0.721	0.90 [a] (-17.4, 23.2)	0.928	0.37 [a] (-0.02, 0.77)	0.121
Incongruent Flanker	-0.001 [a] (-0.009, 0.007)	0.834	-0.11 (-1.05, 0.83)	0.813	-1.10 [a] (-18.9, 20.6)	0.910	0.40 [a] (-0.10, 0.90)	0.109
Sternberg 1 Item	**-0.010** [a] **(-0.014, -0.0001)**	**0.036**	-0.46 [a] (-1.39, 0.48)	0.338	-9.8 [a] (-25.8, 9.8)	0.305	0.40 [a] (-0.05, 0.85)	0.066
Sternberg 3 Item	-0.03 (-0.08, 0.03)	0.387	-3.52 (-10.45, 3.42)	0.323	0.80 [a] (-18.8, 25.1)	0.943	0.21 [a] (-0.34, 0.76)	0.448
Sternberg 5 Item	-0.004 [a] (-0.012, 0.004)	0.246	-0.68 [a] (-1.61, 0.26)	0.160	-13.9 [a] (-29.3, 4.8)	0.138	3.0 (-1.1, 7.1)	0.156
VST Baseline	**-0.029 (-0.054, -0.003)**	**0.032**	-0.004 [a] (-0.532, 0.527)	0.989	-1.8 [a] (-12.0, 9.6)	0.750	1.10 (-0.68, 2.79)	0.236
VST Complex	**-0.012** [a] **(-0.032, 0.008)**	**0.020**	-0.67 [a] (-2.00, 0.68)	0.334	-0.90 [a] (-25.6, 31.9)	0.953	-0.01 [a] (-0.67, 0.66)	0.982
BDNF Concentration	0.003 (-0.001, 0.007)	0.103	0.14 (-0.41, 0.69)	0.613	5.54 (-5.36, 16.44)	0.321	-0.17 (-0.42, 0.10)	0.210

Abbreviations: MSFT; Multi-Stage Fitness Test. CI; Confidence Interval. VST; Visual Search Test. BDNF; Brain-Derived Neurotrophic Factor.
Coefficient and CI are presented as the % change for a 1-unit change in MSFT (15 m), average acceleration (1 mg), intensity gradient (1 AU), waist circumference (1 cm)
Significant associations are highlighted in bold ($p < 0.05$)
[a] Log transformed

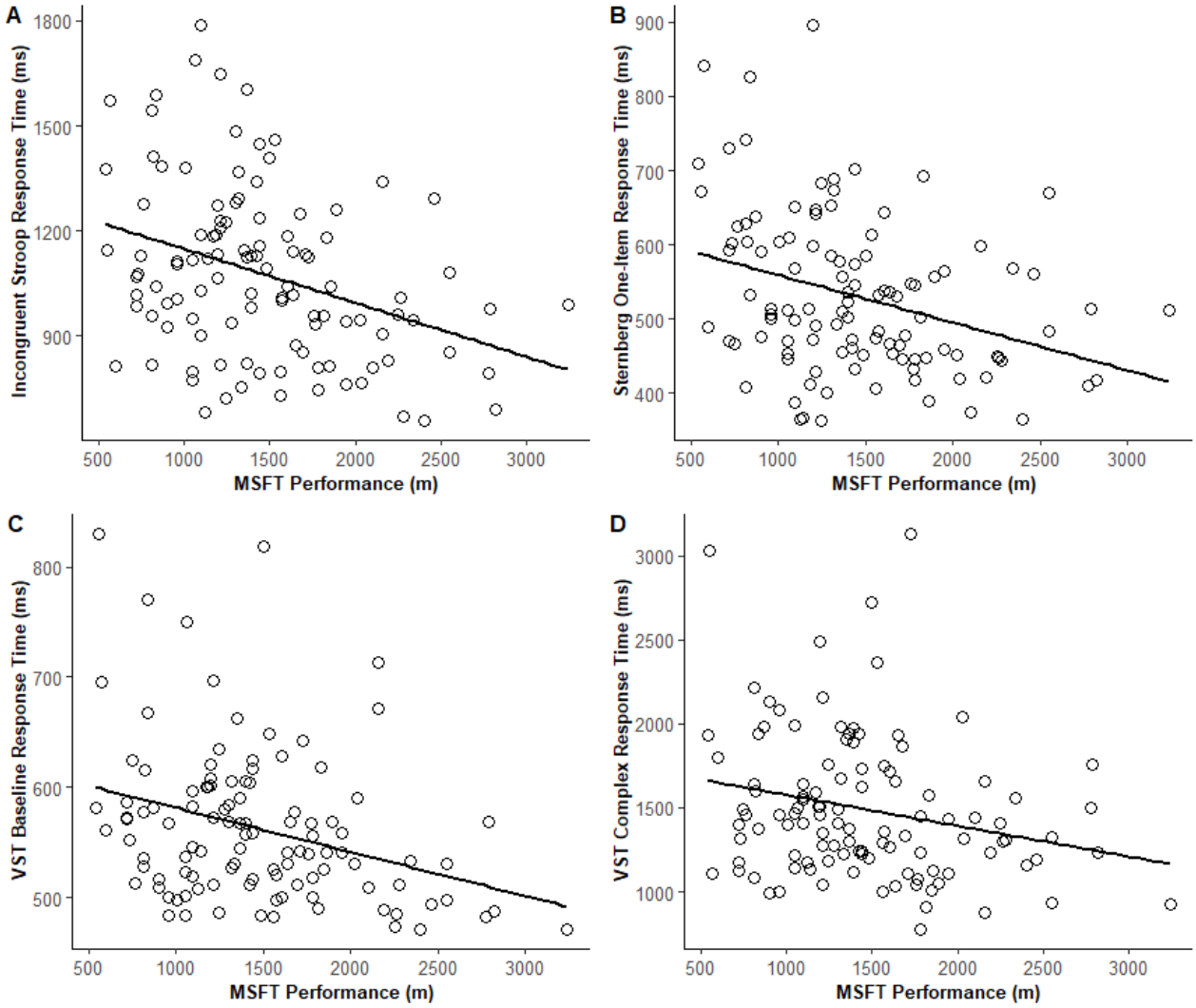

Figure 4.3. (**A**) Association between MSFT performance (m) and response times on the Incongruent Stroop task (β = -0.10 ms, p = 0.025). (**B**) Association between MSFT performance (m) and response times on the One-item Sternberg task (β = -0.01%, p = 0.036). (**C**) Association between MSFT performance (m) and response times on the baseline level of the Visual Search Test (β = -0.029 ms, p = 0.032). (**D**) Association between MSFT performance (m) and response times on the complex level of the Visual Search Test (β = -0.01%, p = 0.020).

4.4 Discussion

With respect to the first aim of the present study, the main findings were that boys had a shallower intensity distribution (more time at higher intensities) compared to girls, and year 7 girls had higher cytokine concentrations (IL-6 and IL-10) in comparison to boys of the same age. Blood pressure and blood glucose concentration were higher in year 10 participants compared to year 7, and year 10 participants were consistently quicker, but similarly accurate, across a range of cognitive function domains (attention, inhibitory control, working memory and visual processing), apart from complex visual processing, whereby year 10 participants were also more accurate. With respect to the second aim of the present study, physical activity volume (average acceleration) and physical activity intensity (intensity gradient) were negatively associated with blood pressure (systolic, diastolic and MAP). MSFT performance was positively associated with (anti-inflammatory) IL-15 concentration and accuracy on the congruent and incongruent Stroop tasks, and negatively associated with response times on working memory, inhibitory control, and visual processing (simple and complex) tasks, so that individuals with a better MSFT performance had a faster response time in these tests. Finally, year group specific associations were identified whereby average acceleration (physical activity volume) was negatively associated with IL-6 concentration (year 7 only), intensity gradient (physical activity intensity) was negatively associated with (pro-inflammatory) IL-1β concentration (year 7 only) and waist circumference was positively associated with IL-6 concentration (year 10 only). Furthermore, whilst the associations between physical activity metrics were evident in both year groups, the associations were stronger in year 10 participants.

The present study provides novel evidence that MSFT performance is positively associated with IL-15 concentration in adolescents (β = 0.02%, 95% CI [0.004%, 0.04%], p = 0.038). IL-15 has a recognised role in adipose tissue regulation (Nielsen et al., 2008) and skeletal muscle insulin sensitivity (Nadaeu & Aguer, 2018), but there is also a suggested role of IL-15 in oxidative metabolism and angiogenesis (Vasoncelos & Salla, 2018). Data from animal models suggest that IL-15 activates PPAR-δ which is responsible for a subsequent improvement in endurance

capacity through regular training (Nadeau & Aguer, 2018). This is further supported by an increased skeletal muscle IL-15 content after 12 weeks of endurance training in adult males (Rinnov et al., 2014). Thus, the current study provides novel data supporting a positive association between endurance capacity (MSFT performance) and IL-15 concentration, which may contribute to chronic training adaptations. However, longitudinal data following a training intervention in adolescents is needed to support this claim.

The present study is the first to demonstrate that physical fitness is beneficially associated with indices of simple and complex visual processing speed (Visual Search Test) in adolescents. The parietofrontal network is recognised as a key component of attentional control in humans (Bisley, 2011), with evidence suggesting that the connectivity of this network is improved after six months of aerobic exercise training, albeit in older adults (Hsu et al., 2017). Nonetheless, this may be a potential mechanism through which a greater physical fitness mediates increased visual processing speed. Physical fitness was also inversely associated with response times across the domain of executive function (specifically inhibitory control and working memory), which is consistent with much of the evidence base in adolescents (Aadland et al., 2017; Mora-Gonzalez et al., 2019; Westfall et al., 2018). It has been stated that adolescents may be more sensitive to the effects of physical fitness on cognition, particularly with regards to executive function, as the associated brain regions are still developing at this stage (Hötting & Röder, 2013). A hypothesised mechanism of these beneficial effects is through the release of neurotrophins, such as brain-derived neurotrophic factor (BDNF) (Hötting & Röder, 2013). However, the present study did not find any positive associations between physical fitness and BDNF. A further step would be to include BDNF in the regression model, to assess the potential independent and additive effects. However, the sample size was not as large as originally intended due to COVID-19 school closures, and thus a parsimonious modelling approach was necessary. Nonetheless, the data from the present study demonstrate a novel, beneficial association between physical fitness and visual processing speed. Future work should seek to replicate this and explore the potential mechanistic links between physical fitness and the parietofrontal network.

The present study is the first to demonstrate that the newly proposed physical activity metrics, both the physical activity volume and intensity of the activity, are negatively associated with mean arterial pressure. It is well known that higher physical activity levels are associated with reductions in blood pressure (Bailey et al., 2012; Barker et al., 2018; Carson et al., 2013; Gopinath et al., 2011) and has protective effects for hypertension development (Diaz & Shimbo, 2013). However, the present study provides novel insight into the associations between the new metrics of physical activity and blood pressure. Furthermore, the association for both physical activity variables was dependent on year group, whereby at stronger negative association was observed in year 10 participants, when compared to year 7 participants. This highlights the importance of physical activity for older adolescents, who are typically less active (Farooq et al., 2018) and are subject to an age-related increase in blood pressure (Shankar et al., 2008). The present study adds further novel insights with regards to the relationship between the activity volume metric and IL-6 concentration, as well as the intensity gradient and IL-1β concentration. Interestingly, the negative association observed between average acceleration and IL-6 concentration, as well as intensity gradient and IL-1β concentration, was exclusive to year 7 participants. It is unclear as to why year group moderates these relationships, particularly as there is currently no other evidence assessing the moderating role of age on similar associations.

The present study is also the first to examine the associations between the newly proposed physical activity metrics, average acceleration and intensity gradient, and cognitive function in adolescents. These data from the present study did not suggest there were any associations between average acceleration and intensity gradient with cognitive function. Whilst some previous studies have reported that physical activity is beneficially associated with measures of cognitive function (Aadland et al., 2017; Lee et al., 2014), others do not (Cadenas-Sanchez et al., 2020). An explanation for this may be the different choice in assessment methods of physical activity, cut points used and the focus on a set category of activity, such as moderate-to-vigorous. Moreover, associations between moderate-to-vigorous physical activity and cognitive function performance that have been previously reported did not hold when physical fitness was accounted

for (Aadland et al., 2017). Indeed, it has been argued that physical fitness, as a state (which to some extent reflects physical activity), is a better measure to use than physical activity itself which varies greatly from day-to-day, when examining the relationship with cognitive function (Cadenas-Sanchez et al., 2020). Thus, there are several possible arguments to consider here: firstly that physical fitness relates more strongly to cognitive function than physical activity because it is a better measure of previous physical activity, thus physical activity remains important, which is supported by the fact that acute bouts of physical activity leads to transient improvements in cognition (Chang et al., 2012; Sibley & Etnier, 2003); secondly that it is physical fitness itself that is important for cognitive function and only some physical activities, of a particular intensity for example, will contribute to improved fitness; and thirdly that both improved fitness and high physical activity are likely to be associated with reduced adiposity which in itself may be the key factor in enhanced cognitive performance. The present study suggests that when considering all the available information, with regards to volume and intensity of physical activity, there is no discernible relationship with cognitive function, leaving the possibility that physical fitness or adiposity may be more important. Given that this is the first study to use the new, continuous metrics, additional work is necessary to replicate these findings.

The present study found that waist circumference was positively associated with IL-6 concentration, although interestingly this was exclusive to year 10 participants. The findings of the present study support the empirical findings of Dring et al. (2019b), whereby sum of skinfolds was positively associated with IL-6 concentration. Indeed, it is generally recognised that adolescents who display larger amounts of adipose tissue have higher concentrations of inflammatory cytokines (Rubin & Hackney, 2010). However, the observation of a year group specific association is an interesting finding in the present study that should be investigated in further work. Waist circumference is a recommended proxy of visceral adipose tissue, which is known as an endocrinological tissue that secretes inflammatory mediators, particularly IL-6 (Eder et al., 2009). It is thought that the adipose tissue-derived IL-6 is mechanistically involved in the development of insulin resistance (Tagi, 2019) and subsequent occurrence of cardiometabolic

diseases, such as cardiovascular disease and type 2 diabetes (Shuster et al., 2012). Thus, the present study provides novel evidence of a positive relationship between waist circumference and IL-6 concentration, which interestingly was exclusive to year 10 participants.

More distance was covered during the MSFT by year 10 participants, with a greater magnitude in boys. Conversely, year 7 participants considered themselves more active based on the IPAQ. Boys overall had a lower intensity gradient, which reflects more daily time spent doing higher intensity activities, in line with earlier observations by Fairclough et al. (2019) and Buchan et al. (2019) – although the total volume of physical activity was similar between year groups and sex. These findings support previous observations that physical fitness, assessed by MSFT performance, increases throughout adolescence (Tomkinson et al., 2016) and is higher in boys compared to girls (Tomkinson et al., 2016), along with boys being more physically active than girls (Dumith et al., 2011; Townsend et al., 2015). These highlight potential target groups of adolescents who may require the most attention for interventions seeking to improve physical activity and physical fitness.

The present study also provides novel information regarding the interaction between age and sex in relation to cytokine concentrations. IL-6 and IL-15 concentrations were lower in year 7 boys compared to year 7 girls, whereas the sex difference was not clear in older adolescents. Furthermore, year 7 girls had a higher IL-6 and IL-10 concentration than their year 10 counterparts. The higher resting concentration of these cytokines observed in girls, especially of younger ages, may reflect maturational changes; as IL-6 has a suggested growth function in humans (Dorn et al., 2016). Furthermore, the hormonal changes of maturation are suggested to occur earlier in females (Beunen, Rogal & Molina, 2006). Indeed, the year 7 girls in this study were close to their predicted peak height velocity (maturity offset -0.37 ± 0.56 y), although inferences about biological maturation using somatic predictions need to be treated with caution (Beunen, Rogal & Molina, 2006). Whilst there is strong evidence that puberty should be controlled for when examining cytokine concentrations (Timmons et al., 2006), the nature of year group

recruitment in the present study led to very high collinearity between maturity offset and year group (r = 0.88), and thus it was not included in analysis.

Although there were no clear sex differences for cognitive function performance or BDNF concentration, there was a clear effect of year group across all levels of cognitive function tests, with older adolescents demonstrating quicker response times (and better accuracy on the Flanker) compared to the younger group. This is consistent with research demonstrating the development of many cognitive functions throughout adolescence (Hartsthorne & Germine, 2015), which is attributed to the hormonal effects of puberty, leading to structural and behavioural changes of the brain (Blakemore, Burnett & Dahl, 2010) and the ability to consistently process and inhibit irrelevant information (Luna, 2009). Additionally, the performance difference between year groups on the incongruent Stroop task was of a greater magnitude in boys, but the direction of the relationship was the same for girls. This is a novel observation for which the mechanisms are currently unclear. It could be related to sex-specific hormone effects such as testosterone, which increases throughout puberty and is associated with higher motivation (Blakemore, Burnett & Dahl, 2009), although the present study did not measure such analytes. Indeed, future work should seek to replicate and investigate the underpinning mechanisms for this finding further.

Whilst the present study has drawn attention to some important associations, the cross-sectional nature of these should be acknowledged which means that causality cannot be inferred. Nonetheless, these were important associations to address as interaction between school year groups were considered, as well as the exploration of newly proposed physical activity metrics. The sample size is also a potential limitation, particularly for the year 10 participants in the study. Unfortunately, data collection was terminated due to school closures because of the COVID-19 pandemic. This meant that the regression models were built to offer parsimony and indeed, some potential confounders were not included in the models. However, the present study demonstrates that the 15 m version of the MSFT can be used to assess associations between physical fitness and cognitive function measure in adolescents, provides additional support for the use of new, continuous physical activity metrics and broadens the scope of the associations between physical

fitness, physical activity and adiposity with novel markers of health status (such as inflammatory cytokines) in adolescents.

The findings of the present study demonstrate important age-related associations between physical activity and novel markers of health status in adolescents (IL-6 and IL-1β), as well as the age-related positive association between adiposity and IL-6 concentration which was exclusive to year 10 participants. Furthermore, the present study provides novel data on the relationship between physical fitness and IL-15 concentration, whereby IL-15 concentrations were greater in those that covered more distance on the MSFT. Along with evidence in adults (Nadaeu & Auger, 2018), this may provide potential mechanistic insight into chronic endurance training adaptations. Additionally, the present study provides evidence that adolescent girls (year 7) had higher concentrations of cytokines (IL-6 and IL-1β), related to chronic low-grade inflammation, than year 7 boys highlighting adolescent girls as potential targets for future interventions. The present study is also the first to document a positive association between physical fitness and visual processing (both simple and complex) and further adds to the evidence base on the importance of physical fitness for cognitive function.

Practical Applications

Based on the findings presented in this chapter, both a higher total volume of physical activity, as well as spending more time doing higher intensity physical activities, are beneficial for risk factors related to cardiometabolic disease. It is therefore suggested that adolescents should make every effort to ensure that physical activity guidelines are being met, particularly older adolescents (14 – 15 y) given that some associations were stronger in this year group. Furthermore, physical fitness should be targeted across adolescence given the consistent and inverse associations observed with responses times on cognitive function tasks. Finally, it is recommended that future physical activity interventions should target younger adolescent girls (11 – 12 y), given that they appear to have a less favourable cardiometabolic risk profile compared to boys and older adolescents.

Chapter V

Determinants of postprandial glycaemia, insulinaemia and fasting insulin resistance in adolescents

5.1 Introduction

The findings from Chapter IV did not display any relationship between physical fitness and fasting indices of metabolic health (blood glucose, plasma insulin and HOMA-IR). However, there is growing interest in the postprandial assessment of these markers, which may serve as more sensitive markers of cardiometabolic risk. Currently, there is little research – particularly in adolescents – on determinants of postprandial glycaemia and insulinaemia following a standardised mixed meal, which is thought to provide a more sensitive measure of peripheral insulin resistance than resting measures (Abdul-Ghani et al., 2007; Muniyappa & Madan, 2000). This is an important outcome to assess, given that peripheral insulin resistance has been highlighted as a key characteristic in the development of cardiometabolic diseases, namely type 2 diabetes (Sesti, 2006).

Insulin resistance and reduced glucose tolerance are typically implicated in the aetiology of type 2 diabetes (Reaven, 2005), with an increasing degree of insulin resistance in young people (Tagi, Giannini, Chiarelli, 2019). Furthermore, the development of insulin resistance and type 2 diabetes in children and adolescents is associated with an increased risk of several co-morbidities, such as cardiovascular disease, in later life (Laitinen et al., 2012; Steinberger et al., 2009). Therefore, due to the potential concern for metabolic health across the lifespan, it is important to understand the factors that affect insulin resistance and glucose tolerance in young people. The postprandial response to an ecologically valid meal is an important marker of cardiometabolic health in young people and favoured over the more typically cited fasting markers (DiNicolantonio et al., 2017; Haffner, 1998; Lautt, 2007). However, the factors that affect the magnitude of the postprandial glycaemic and insulinaemic response in young people are not well understood.

There are many risk factors associated with the development of type 2 diabetes, some of which can be easily modified through lifestyle behaviour change (Tuomilehto et al., 2001). One of the contributing factors to the stark increase in the prevalence of type 2 diabetes is weight status; particularly central adiposity. This can be assessed in various ways (such as, waist circumference, sum of skinfolds and BMI) and is considered an important risk factors for the development of insulin resistance and, subsequently, type 2 diabetes (Arslanian & Suprasongsin, 1996; Arslanian, 2000; Tagi et al., 2019). Sex and pubertal status are also other risk factors during childhood (up to 11 years old) and adolescence (11 – 18 years old), given that there is a degree of pubertal insulin resistance, which may be of greater magnitude in females (Cooper et al., 2017; Kelsey & Zeitler, 2016; Reinehr, 2013); thus it is particularly important to understand the association between risk factors of insulin resistance during adolescence, which has not been explored to date. Low physical activity and physical fitness are risk factors for the development of type 2 diabetes in adults (Aune et al., 2015) and are also linked with poor cardiometabolic health in children and adolescents (Ekelund et al., 2012).

Traditionally, fasting glucose and insulin concentrations are commonly used in models of insulin resistance; the most common being the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) (Tagi et al., 2019). However, it has been argued that the use of such measures do not appropriately screen for related conditions, like type 2 diabetes (DiNicolantonio et al., 2017; Haffner, 1998; Laut, 2007). Furthermore, HOMA-IR typically reflects hepatic insulin sensitivity and does not account for peripheral insulin sensitivity (Abdul-Ghani et al., 2007; Muniyappa & Madan, 2000). Instead, the use of a dynamic assessment of postprandial glycaemia and insulinaemia have been suggested, as a more sensitive marker of cardiometabolic health given that young people spend most of awake time in the postprandial state.

One such method of assessing the postprandial glycaemic and insulinaemic response is the Oral Glucose Tolerance Test (OGTT) (Muniyappa & Madan, 2000), whereby glucose and insulin concentrations are determined at 0, 30, 36 and 120 min following a standard glucose load (75 g), which has been used in adolescents previously (Cockcroft et al., 2015; Cockcroft et al., 2017).

Recent work in adolescents has examined the responses to mixed-meals (Dring et al., 2019a; Dring et al., 2020; Short et al., 2018), providing ecological insights about the responses to regularly consumed meals. Furthermore, assessment of postprandial insulinaemia is an applicable tool for identifying early insulin resistance in healthy, asymptomatic individuals (Lautt, 2007).

Adiposity is a well-known risk factor for the development of insulin resistance and type 2 diabetes (Arslanian & Suprasongsin, 1996; Arslanian, 2000; Tagi et al., 2019), but there is very little known about how adiposity affects postprandial responses in adolescents. A direct comparison of overweight/obese and normal weight adolescents, using BMI, found that those who were overweight/obese had a larger insulinaemic response to a standardised meal (Short et al., 2018). This study, however, only considered BMI as a proxy of adiposity, and did not consider the measure of waist circumference which is the preferred measure of adiposity when considering cardiovascular disease risk (Klein et al., 2007; World Health Organisation, 2008). Future work should consider the discriminatory capabilities of multiple makers of adiposity and how these affect postprandial responses.

It has been suggested that physical fitness and fasting insulin resistance are inversely related in adolescents (Dring et al., 2019b; Haapala et al., 2019; Fraser et al., 2018). In addition, physical fitness is also inversely related to blood lipids and low-grade chronic inflammation in adolescents (Dring et al., 2019b; Zaqout et al., 2016), and metabolic syndrome incidence in adults (LaMonte et al., 2005). It has been reported in one study that higher physical fitness in young people (aged 7 to 15 y), assessed by time taken to complete a 1.6 km run, is inversely related to insulin resistance (assessed via HOMA-IR) in adulthood (Fraser et al., 2018). It is worth noting, however, that this relationship was weaker when adjusting for childhood waist circumference, thus highlighting the importance of adiposity for metabolic health. However, no studies to date have examined whether physical fitness affects postprandial glycaemia and insulinaemia in adolescents, despite the importance of physical fitness for other risk factors for cardiometabolic health (Dring et al., 2019b; Zaqout et al., 2016).

Therefore, the aim of the present study is to explore the factors affecting the postprandial glycaemic and insulinaemic responses in adolescents, including an examination of the interaction between factors known to affect these responses, such as sex and adiposity. In addition, the study will consider how physical fitness influences postprandial responses which is a completely a novel area of enquiry in adolescents.

5.2 Methods

5.2.1 Experimental Design

Data from three separate studies with similar experimental designs (Dring et al., 2019a; Dring et al., 2020; Chapter 6 of this thesis), were pooled to examine the postprandial responses to lunch. Each of the involved studies conformed to the Declaration of Helsinki guidelines and were approved by the Nottingham Trent University Human Ethics Committee. Participants were recruited from secondary schools in the East Midlands area of the UK. Written parental consent and participant assent were obtained during recruitment. A health screen was completed by a parent/guardian of the participant and checked by a lead investigator to ensure there were no medical conditions that would affect the young person's participation. Participants were familiarised with all testing procedures at least 7 d in advance of the main experimental trial. Participants were instructed to refrain from eating or drinking from 9 pm the previous evening. Water was allowed *ad libitum*. Participants were also asked to refrain from physical activity in the 24 h preceding main trials. Participants reported to school at the beginning of the day (between 08:00 am and 08:30 am) and all procedures took place in a classroom at the school.

5.2.2 Participant Characteristics

5.2.2.1 Anthropometric Measurements

In total, the dataset comprised of 108 participants (52 boys) (Table 5.1). Participants underwent anthropometric measurements, consisting of stature (cm), body mass (kg) and sitting height (cm), which were also used to estimate maturity offset. These measurements were conducted in line with descriptions from section 3.3 of this thesis. Waist circumference and the sum of 4 skinfolds

(triceps, subscapular, supraspinale and thigh) were also measured as characteristics of adiposity, as explained in section 3.4 of this thesis. For descriptive purposes, participants are classified as normal weight, overweight or obese based on age- and sex-specific cut-points (Cole et al., 2000).

5.2.2.2 Assessment of Physical Fitness

In each study, assessment of physical fitness was assessed using the multi-stage fitness test (MSFT) (Ramsbottom, Brewer & Williams, 1988). The MSFT was conducted in line with descriptions provided in section 3.5 of this thesis. To ensure maximum effort from the participants, participants were 'paced' by a member of the research team and investigators provided verbal encouragement and maximum heart rate was monitored continuously (Firstbeat Technologies Ltd, Finland). Performance on the test was determined by the total distance covered (m) (Table 5.1). For descriptive purposes the whole sample, as well as boys and girls separately, were characterised based on age- and sex-specific centiles; derived from normative performance data in adolescents (Tomkinson et al., 2016).

5.2.3 Experimental Procedures

5.2.3.1 Standardised Breakfast and Lunch

On the morning of the trials (~9.00 am), a standardised breakfast was provided; which provided 1.5 $g.kg^{-1}$ body mass of carbohydrate (cornflakes, milk, white toast and butter). The standardised lunch (the test meal) was provided 3 h post-breakfast (~12.00 noon) and contained 1.5 $g.kg^{-1}$ body mass of carbohydrate (chicken sandwich, baked crisps and an apple; with a cheese alternative for vegetarians (n = 2 participants had the cheese alternative)). Hypothetical examples of the food quantities can be found in section 3.6 of this thesis. Participants were given 15 min to consume breakfast and lunch. The postprandial period (2 h) started on the first mouthful of lunch (Brouns et al., 2005).

Table 5.1. Participant characteristics and metabolic markers split into boys and girls. Data are presented as mean, standard deviation and range.

Variable	Group					
	Boys (n = 52)			*Girls (n = 56)*		
	M	SD	Range	M	SD	Range
Characteristics						
Age (y)	12.4	0.5	11.4-13.4	12.4	0.6	11.1-13.5
Height (m)	1.59	0.09	1.43-1.81	1.59	0.07	1.45-1.77
Body Mass (kg)	48.4	10.7	31.9-78.1	48.2	9.0	32.6-74.3
BMI ($kg \cdot m^{-2}$)	19.0	2.7	14.0-24.9	19.1	2.7	14.1-28.3
BMI Percentile	61.2	29.8	1.2-98.6	52.4	27.3	0.6-99.5
Maturity Offset (y)	-1.0	0.6	-2.0-0.6	0.6	0.6	-0.8-2.1
Sum of Skinfolds (mm)	48.0	21.8	14.1-102.5	52.4	21.9	24.0-127.0
Waist Circumference (cm)	67.4	6.7	54.5-86.4	66.2	6.8	53.4-92.3
Multi-Stage Fitness Test Distance (m)	1240	420	420-2160	1080	340	360-1740
Metabolic Markers						
Fasting Blood Glucose ($mmol \cdot L^{-1}$)	4.5	0.6	2.6-5.7	4.3	0.7	2.4-6.1
Fasting Plasma Insulin ($pmol \cdot L^{-1}$)	54.1	28.6	11.3-120.0	59.1	27.1	13.8-138.6
HOMA-IR (AU)	1.73	0.92	0.33-3.81	1.93	0.94	0.43-4.01
Insulin tAUC ($pmol \cdot L^{-1}$ x 120 min)	27590	16419	9288-97148	28679	13400	8240-73224
Glucose tAUC ($mmol \cdot L^{-1}$ x 120 min)	587	73	453-791	582	78	443-791

Abbreviations: M = Mean. SD = Standard Deviation. BMI = Body Mass Index. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. tAUC = Total Area Under the Curve.

5.2.3.2 Capillary Blood Samples

A fasting capillary blood sample was taken upon arrival at school. For the postprandial period, a baseline (pre-lunch) blood sample was taken at ~ 12 noon (always exactly 3 h post-breakfast), with additional blood samples at 30, 60 and 120 min post-lunch to represent the postprandial period.

Capillary blood samples were obtained, as described in section 3.6 of this thesis. Blood was collected into two 300 µl EDTA coated microvette (Sarstedt Ltd, UK). A single 25 µl whole blood sample was also collected using a pre-calibrated glass pipette (Hawksley Ltd, UK) and immediately deproteinised in 250 µl ice-cooled 2.5% perchloric acid, in 1.5 ml plastic vials. Both

samples were then centrifuged at 1000 g for 4 min, at 4 °C (Eppendorph 5415C, Hamburg, Germany). Plasma was removed from the microvette and placed into 500 µl plastic vials for subsequent analysis. All samples were frozen immediately at -20 °C and transferred to -80 °C as soon as possible.

Blood glucose and plasma insulin concentrations were determined as described in section 3.6 of this thesis. Blood glucose and plasma insulin total area under the curve (tAUC) following the standardised lunch was calculated (GraphPad Prism 7, GraphPad Software, USA), using methods described previously (Wolever et al., 1986; Wolever et al., 1991). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as an index of insulin resistance (Matthews et al., 1985). For descriptive purposes, participants were classed as “at risk” according to age and sex-specific cut-points (Shashaj et al., 2016).

2.4 Statistical Analyses

All data were analysed using the open-source software RStudio v 1.2.1335 (RStudio Team., 2015). A correlation matrix was created in order to evaluate multicollinearity between independent variables (sex, waist circumference, sum of skinfolds, body mass, body mass index, maturity offset, multi-stage fitness test performance and homeostatic model assessment of insulin resistance). Before analysis, waist circumference, sum of skinfolds, BMI and the MSFT performance were centred to the mean. Simple linear regression was initially conducted for each independent variable on each outcome variable (HOMA-IR, plasma insulin tAUC and blood glucose tAUC). Following this, stepwise hierarchical multiple regression – backwards elimination – was used to develop models for each outcome variable, using the “lme4” package (Bates, Mächler & Bolker., 2015). At each stage, the independent variable that provided the lowest contribution to the model (through evaluation of SE and t-statistic) was removed and then the model was re-run.

5.3 Results

A total of 80 (74.1%) participants were considered normal weight, 18 (16.7%) overweight and 10 (9.3%) obese. Furthermore, 34 (31%) participants were considered "at risk" of insulin resistance, as calculated by HOMA-IR. Regarding MSFT performance, the median centile was 70 for boys (range: 10 – 95) and 80 for girls (range: 20 – 95). When considering the whole sample, fitness was considered "low" for 13.1% (≤ 40th centile), "moderate" for 9.4% (40th – 60th centile), "high" for 32.7% (60th – 80th centile) and very high for 44.9% (≥ 80th centile) of participants.

5.3.1 Multicollinearity between independent variables

Independent variables were assessed for multicollinearity prior to conducting the hierarchical multiple regression, the results of which are shown in Table 5.2. There was a strong correlation between BMI and body mass; which is not surprising given that body mass is used in the calculation of BMI. Therefore, these variables cannot be considered independent and thus, body mass was excluded from subsequent analyses. All other variables did not demonstrate strong correlations (r < .90) and were thus included in the models.

Table 5.2. Correlation matrix for all independent variables.

	Sex	BM	BMI	MO	SumSF	WC	MSFT	HOMA-IR
Sex	.							
BM	.01	.						
BMI	.00	.88**	.					
MO	-.80**	.38**	.21	.				
SumSF	-.09	.64**	.80**	.18	.			
WC	.09	.85**	.88**	.20	.76**	.		
MSFT	.23	-.31*	-.45**	-.14	-.59**	-.38**	.	
HOMA-IR	-.05	.41**	.38**	.21	.26	.35**	-.17	.

Abbreviations: BM = Body Mass. BMI = Body Mass Index. MO = Maturity Offset. SumSF = Sum of Skinfolds. WC = Waist Circumference. MSFT = Distance run on Multi-Stage Fitness Test. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.
Holm correction for multiple testing used. * = p < *.01.* ** = p < .001.

5.3.2 Plasma Insulin Total Area Under the Curve

5.3.2.1 Predictors Individually

Simple linear regression models for insulin tAUC, with each independent variable separately, can be seen in Table 5.3. Waist circumference was the strongest individual predictor, explaining 37.7% of the insulin tAUC variance ($p < .001$). BMI ($p < .001$, Adj. $R^2 = .330$), sum of skinfolds ($p < .001$, Adj. $R^2 = .287$), HOMA-IR ($p < .001$, Adj. $R^2 = .292$) and multi-stage fitness test performance ($p < .001$, Adj. $R^2 = .139$) were all significant individual predictors of plasma insulin tAUC. Sex ($p = .707$, Adj. $R^2 = -.008$) and maturity offset ($p = .079$, Adj. $R^2 = .020$) did not affect plasma insulin tAUC.

Table 5.3. A summary of simple linear regression outputs for each variable predicting plasma insulin tAUC.

Predictor	β_0	β_1	SE	*t*	*p*	R^2	Adj. R^2
Sex	28679	-1088	2886	-0.38	*.707*	.001	-.008
WC	28105	1364	169	8.07	<.001***	.383	.377
SumSF	28138	366	55	6.62	<.001***	.294	.287
BMI	28143	3226	442	7.29	<.001***	.336	.330
MSFT	44969	-14	3	-4.21	<.001***	.148	.139
MO	28524	2538	1431	1.77	.079	.029	.020
HOMA-IR	12268	8780	1324	6.63	<.001***	.299	.292

Abbreviations: β_0 = Intercept. β_1 = Parameter Estimate. SE = Standard Error. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi-Stage Fitness Test. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. MO = Maturity Offset.
* = $p < .05$. ** = $p < .01$. *** $p < .001$.

5.3.2.2 Final Model Development

The hierarchical regression (stepwise, backwards elimination) step-by-step process can be seen in Table 5.4. The final model (step 5) contained waist circumference, multi-stage fitness test performance and HOMA-IR as predictors, explaining 51.5% of the variance in plasma insulin tAUC ($F_{(3, 98)} = 36.78$, $p < .001$, Adj. $R^2 = .515$). The model suggests that: for a 1 cm increase in waist circumference, insulin tAUC would increase by 921 pmol·L^{-1} x 120 min (95% CI; 564, 1278); for a 20 m increase in distance ran during the multi-stage fitness test, insulin tAUC would decrease by 6 pmol·L^{-1} x 120 min (95% CI; -12, -1); and for a 1 AU increase in HOMA-IR, the

model suggests that insulin tAUC would increase by 6046 pmol·L^{-1} x 120 min (95% CI; 3595, 8497).

Table 5.4. Summary of the hierarchical regression (backwards elimination) for variables predicting plasma insulin tAUC. 95% CI are for unstandardized coefficients (B).

Variable	B	SE	*β*	*t*	*p*	95% CI		Adj. R^2
						Lower	Upper	
Step 1 ($F_{(7, 94)}$ = 15.59, *p* = < .001)								.503
Intercept	22269	4826						
Sex	-1855	4276	-0.06	-0.43	.665	-10346	6635	
WC	950	371	0.43	2.56	.012*	214	1687	
SumSF	92	99	0.14	0.93	.356	-105	290	
BMI	-582	948	-0.10	-0.61	.541	-2466	1301	
MSFT	-4	3	-0.12	1.30	.197	-11	2	
MO	-1329	2104	-0.09	-0.63	.529	-5507	2849	
HOMA-IR	6428	1307	0.40	4.92	<.001***	3831	9025	
Step 2 ($F_{(6, 95)}$ = 18.32, *p* < .001)								.507
Intercept	21975	4757						
WC	883	335	0.40	2.63	.009**	217	1550	
SumSF	101	92	0.15	1.04	.299	-91	293	
BMI	-569	944	-0.10	-0.60	.548	-2443	1305	
MSFT	-4	3	-0.13	-1.46	.147	-11	1	
MO	-550	1095	-0.04	-0.50	.616	-2725	1623	
HOMA-IR	6402	1300	0.39	4.92	<.001***	3819	8985	
Step 3 ($F_{(5, 96)}$ = 22.10, *p* < .001)								.511
Intercept	22259	4706						
WC	882	334	0.40	2.64	.009**	218	1546	
SumSF	101	96	0.15	1.05	.299	-90	292	
BMI	-585	940	-0.10	-0.62	.535	-2451	1280	
MSFT	-4	3	-0.13	-1.45	.149	-11	1	
HOMA-IR	6261	1265	0.38	4.95	<.001***	3749	8773	
Step 4 ($F_{(4, 97)}$ = 27.70, *p* < .001)								.514
Intercept	22479	4677						
WC	754	263	0.34	2.87	.005**	231	1277	
SumSF	75	87	0.11	0.87	.388	-98	249	
MSFT	-4	3	-0.13	-1.44	.152	-11	1	
HOMA-IR	6113	1239	0.38	4.94	<.001***	3654	8572	
Step 5 ($F_{(3, 98)}$ = 36.78, *p* < .001)								.515
Intercept	24326	4158						
WC	921	180	0.41	5.12	<.001***	564	1278	
MSFT	-6	3	-0.16	-2.19	.031*	-12	0	
HOMA-IR	6046	1234	0.37	4.90	<.001***	3595	8496	

Abbreviations: B = Regression Coefficient. SE = Standard Error. β = Standardised Coefficient. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi Stage Fitness Test. MO = Maturity Offset. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.
* = *p* < .05. ** = *p* < .01. *** = *p* < .001.
ΔR^2: Step 2 = .004. Step 3 = .004. Step 4 = .003. Step 5 = .001.

5.3.3 Blood Glucose Total Area Under the Curve

5.3.3.1 Predictors Individually

None of the available predictors provided a significant contribution to explaining the variance in blood glucose tAUC, individually (Table 5.5).

5.3.3.2 Final Model Development

The initial model (step 1) including all predictors did not provide sufficient explanation for the variance (3%) in blood glucose tAUC ($F_{(7, 94)}$ = 1.44, p = .198). As no predictors significantly explained any variance in blood glucose tAUC individually, or in the hierarchical model, the backwards elimination process was terminated at step 1.

Table 5.5. A summary of simple linear regression outputs for each variable predicting blood glucose tAUC.

Predictor	β_0	β_1	SE	*t*	*p*	R^2	Adj. R^2
Sex	578	8.2	15.12	0.54	.590	.003	-.007
WC	582	-0.1	1.13	-0.09	.930	.000	-.009
SumSF	582	-0.2	0.34	-0.62	.537	.004	-.006
BMI	582	0.5	2.86	0.17	.862	.000	-.009
MSFT	605	-0.0	0.02	-1.04	.299	.011	.010
MO	581	-6.6	7.58	-0.87	.386	.007	-.002
HOMA-IR	558	13.9	8.26	1.68	.097	.027	.018

Abbreviations: β_0 = Intercept. β_1 = Parameter Estimate. SE = Standard Error. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi-Stage Fitness Test. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance. MO = Maturity Offset.

5.3.4 HOMA-IR

5.3.4.1 Predictors Individually

Simple linear regression models for HOMA-IR, with each independent variable separately, can be seen in Table 5.6. BMI was the strongest predictor for HOMA-IR; explaining 17.5% of the variance (p < .001). Waist circumference (p < .001, Adj. R^2 = .153), sum of skinfolds (p = .008, Adj. R^2 = .057), MSFT performance (p = .033, Adj. R^2 = .035) and maturity offset (p = .004, Adj.

R^2 = .068) also provided a significant contribution to the variance in HOMA-IR. Sex did not significantly explain variance in HOMA-IR (p = .284, Adj. R^2 = .002).

5.3.4.2 Final Model Development

The hierarchical regression (stepwise, backwards elimination) step-by-step process can be seen in Table 5.7. The final model containing BMI and maturity offset as independent variables (step 5; $F_{(2,\ 102)}$ = 14.06, p < .001, Adj R^2 = .201) explaining 20.1% of the variance in HOMA-IR. Specifically, the model suggests that for each additional 1 $kg \cdot m^{-2}$ increase in BMI, HOMA-IR would increase by 0.14 AU; for each 1 y increase in maturity offset, HOMA-IR would increase by 0.17 AU.

Table 5.6. A summary of simple linear regression outputs for each variable predicting HOMA-IR.

Predictor	β_0	β_1	SE	*t*	*p*	R^2	Adj. R^2
Sex	2.02	-0.21	0.19	-1.08	.284	.011	.002
WC	1.83	0.05	0.01	4.44	<.001***	.161	.153
SumSF	1.31	0.01	0.00	2.71	.008**	.066	.057
BMI	1.82	0.15	0.03	4.80	<.001***	.183	.175
MSFT	2.48	-0.00	0.00	-2.16	.033*	.044	.035
MO	1.95	0.28	0.09	2.93	.004**	.076	.068

Abbreviations: β_0 = Intercept. β_1 = Parameter Estimate. SE = Standard Error. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi-Stage Fitness Test. MO = Maturity Offset.
* = p < .05. ** = p < .01. *** p < .001.

Table 5.7. Summary of the hierarchical regression (backwards elimination) for variables predicting HOMA-IR. 95% CI are for unstandardized coefficients (B).

Variable	B	SE	*β*	*t*	*p*	95% CI		Adj. R^2
						Lower	Upper	
Step 1 ($F_{(6, 95)}$ = 4.88, p < .001)								.188
Intercept	1.94	0.32				1.45	2.10	
Sex	0.15	0.33	0.08	0.45	.657	-0.52	0.82	
WC	0.02	0.03	0.17	0.82	.413	-0.03	0.08	
SumSF	-0.01	0.01	-0.22	-1.18	.242	-0.03	0.01	
BMI	0.13	0.07	0.37	1.75	.083	-0.02	0.27	
MSFT	-0.00	0.00	-0.06	-0.50	.616	0.00	0.00	
MO	0.25	0.16	0.27	1.49	.139	-0.08	0.57	
Step 2 ($F_{(5, 96)}$ = 5.87, p < .001)								.194
Intercept	1.97	0.32				1.68	2.01	
WC	0.03	0.03	0.21	1.12	.266	-0.02	0.08	
SumSF	-0.01	0.01	-0.24	-1.31	.194	-0.03	0.01	
BMI	0.13	0.07	0.37	1.75	.083	-0.02	0.27	
MSFT	-0.00	0.00	-0.05	-0.41	.686	-0.00	0.00	
MO	0.18	0.08	0.20	2.16	.033*	0.02	0.35	
Step 3 ($F_{(4, 100)}$ = 7.59, p < .001)								.202
Intercept	1.85	0.08				1.69	2.01	
WC	0.02	0.03	0.15	0.83	.409	-0.03	0.07	
SumSF	-0.01	0.01	-0.21	-1.37	.175	-0.02	0.00	
BMI	0.15	0.07	0.42	2.07	.041*	0.01	0.29	
MO	0.17	0.08	0.19	2.06	.042*	0.01	0.34	
Step 4 ($F_{(3, 101)}$ = 9.92, p < .001)								.204
Intercept	1.85	0.08				1.69	2.01	
SumSF	-0.08	0.01	-0.18	-1.22	.224	-0.02	0.01	
BMI	0.19	0.05	0.53	3.61	<.001***	0.08	0.29	
MO	0.17	0.08	0.19	2.09	.039*	0.01	0.34	
Step 5 ($F_{(2, 102)}$ = 14.06, p < .001)								.201
Intercept	1.85	0.08				1.69	2.01	
BMI	0.14	0.03	0.39	4.34	<.001***	0.07	0.20	
MO	0.17	0.08	0.19	2.09	.039*	0.01	0.34	

Abbreviations: B = Regression Coefficient. SE = Standard Error. β = Standardised Coefficient. WC = Waist Circumference. SumSF = Sum of Skinfolds. BMI = Body Mass Index. MSFT = Distance run on Multi Stage Fitness Test. MO = Maturity Offset.
* = p < .05. ** = p < .01. *** = p < .001.
ΔR^2: Step 2 = .006. Step 3 = .008. Step 4 = .003. Step 5 = -.004.

5.4 Discussion

The main findings of the present study are that in adolescents: i) the combination of waist circumference, performance on the MSFT test and HOMA-IR collectively explained 51.5% of variance in the postprandial insulinaemic response to a standardised mixed-meal; ii) none of the independent variables (BMI, body mass, waist circumference, MSFT, sum of skinfolds, sex, maturity offset and HOMA-IR) explained the variance in the postprandial glycaemic response; iii) BMI and maturity offset collectively explained 20.1% of the variation in HOMA-IR. These findings highlight the importance of body composition, particularly central adiposity, in explaining the insulinaemic response to a standardised mixed meal in adolescents. Furthermore, the present study also highlights that physical fitness is an important and independent explanatory variable when considering the postprandial insulinaemic response in adolescents.

The findings of the present study are novel because no study to date has investigated the factors affecting the postprandial glycaemic and insulinaemic responses in adolescents, which are recognised as important risk factors for cardio-metabolic disease (DiNicolantonio et al., 2017; Haffner, 1998; Lautt, 2007). Furthermore, most waking hours are spent in a postprandial state, therefore it seems logical to examine postprandial responses when evaluating an individual's metabolic function. Although glycaemia has potential clinical use for screening of disease prevalence and risk, there have been some arguments that more attention should be focused on postprandial insulinaemia (DiNicolantonio et al., 2017; Lautt, 2007). Furthermore, we hypothesise that the changes in postprandial insulinaemic responses manifest earlier in the progression of cardiometabolic diseases than the postprandial glycaemic responses and should therefore be examined in young people. The present study provides novel evidence that waist circumference, physical fitness and HOMA-IR are key predictors of this postprandial insulinaemic response in adolescents. These novel findings provide further evidence that more consideration should be given to the assessment of postprandial insulinaemia, alongside glycaemia, as a risk factor for metabolic health (DiNicolantonio et al., 2017; Haffner., 1998; Lautt, 2007), which highlights the utility of this marker for future research.

Out of all the explanatory variables, waist circumference provided the strongest individual explanation of the variance in the postprandial insulinaemic response and was also a strong predictor in the final model. These data are supported by a group comparison of postprandial insulinaemia whereby overweight/obese adolescents (aged 14-15 y) had a greater insulin AUC compared to normal weight adolescents (Short et al., 2018), as well as supporting the relationship between adiposity and insulin sensitivity over a 2 y period in children (aged 9 - 11 years) (Henderson et al., 2016). Whilst previous research has identified differences in postprandial insulinaemia between young people considered overweight and normal weight, the present study offers novel insights into the relationship of adiposity on postprandial responses in adolescents. Furthermore, waist circumference was superior compared to sum of skinfolds, which are also measures of body composition, therefore highlighting the importance and utility of this particular measure. Whilst central adiposity is of great importance for cardiometabolic disease risk, the direct measurement, via dual-energy x-ray absorptiometry for example, requires expensive and specialist radiological imaging equipment (Klein et al., 2007). However, waist circumference is strongly advocated as a surrogate measure of central adiposity and has been associated with cardiometabolic disease risk (Klein et al., 2007; World Health Organisation, 2008). This has important practical implications, given the low-cost and non-invasive nature of such a measuring waist circumference. Collectively, these results demonstrate the importance of adiposity – particularly central adiposity (as measured by waist circumference) – for cardiometabolic health in youth; which is pertinent given that central adiposity is linked to the development of insulin resistance (Arslanian & Suprasongsin, 1996; Arslanian, 2000; Tagi et al., 2019).

Another novel finding of the present study was that physical fitness (assessed by distance covered on the MSFT test) was inversely related to plasma insulin tAUC. Physical fitness is known to be beneficial for many facets of cardiometabolic health in adolescents (Dring et al., 2019b; Zaqout et al., 2016). However, no other studies have examined the relationship between physical fitness and postprandial insulinaemia. The closest comparison comes from evidence in children (aged 6-8 y) where physical fitness was inversely related to fasting insulin resistance (Haapala et

al., 2019). Furthermore, there is evidence of improved beta-cell function in adults with a higher physical fitness (Ramos et al., 2017), which lends support to the result of improved insulin sensitivity in participants with a higher physical fitness in the current dataset. There is also a strong body of evidence that chronic exercise interventions improve insulin sensitivity in obese youth (Lee & Kim, 2013). Whilst there has been suggestion that these improvements might be due to increased capillarisation of skeletal muscle and increased GLUT4 translocation (Bird & Hawley, 2017), others have suggested that the chronic improvements are largely mediated through weight loss (Keshel & Coker, 2015). Identifying a mechanism, through which physical fitness improves postprandial insulinaemia was not in the scope of the present study. However, it is interesting that physical fitness remained in the final model, even in the presence of adiposity, suggesting a strong, independent association. Nonetheless, it is important that future research investigates the mechanisms through which physical fitness leads to better postprandial insulinaemia, and whether this differs from those related to acute and chronic exercise. The present study is the first to show a beneficial relationship between physical fitness and postprandial insulinaemia in adolescents, suggesting that physical fitness may be a key predictor for this outcome even when considering the role of other predictors. This has important practical implications that highlight the need to promote physical fitness in youth, given the strong role it has in metabolic health.

The present study also demonstrates that HOMA-IR provides a significant explanation of the variance in postprandial insulinaemia. These data support and extend previous findings following a standardised breakfast (Cooper et al., 2017) and an OGTT (Cockcroft et al., 2017). Previous work has shown that HOMA-IR is positively correlated (r = .63) with insulin tAUC following an OGTT (Cockcroft et al., 2017). This is of similar magnitude to the present study (r = .53), however the previous association was only applicable to adolescent boys in response to an OGTT (Cockcroft et al., 2017). The present study extends this relationship to a sample of adolescent boys and girls, in response to an ecologically valid mixed-meal. Although the meals provided between the present study and previous work (Cooper et al., 2017) were different, they offered

the same relative energy provision (1.5 $g \cdot kg^{-1}$ body mass of carbohydrate). Collectively, these results suggest that basal metabolic function is important for determining the physiological response to test meals. The results from the present study also suggest that an increase in HOMA-IR (higher basal insulin resistance) will lead to greater postprandial insulinaemic responses, even when other strong predictors such as waist circumference and physical fitness are controlled for.

The present study suggests that when considering fasting metabolic status (using HOMA-IR), BMI and maturity offset were the most informative explanatory variables. Independently, BMI was the stronger explanatory variable which is consistent with previous work in this population stating that adiposity has a strong predictive role in fasting measures of insulin resistance (Barseem & Helwa, 2015; Dring et al., 2019b; Silva et al., 2014) despite using different surrogate measures of adiposity. The current study advances previous work in obese adolescents (Silva et al., 2014) to demonstrate that BMI is strongly related to HOMA-IR in healthy, asymptomatic (from cardiometabolic health conditions) adolescents. Maturity offset was also positively related with HOMA-IR, which is consistent with previous literature stating that there is a degree of pubertal insulin resistance during adolescence (Arslanian, 2000; Cooper et al., 2017; Kelsey & Zeitler, 2016; Reinehr, 2013), which is sometimes more profound in girls (Cooper et al., 2017; Kelsey & Zeitler, 2016). The role of maturity and sex, in the present study, seemed to only be reflected in the fasting proxy of insulin resistance, whereas previously it has been shown that girls are hyperinsulinaemic compared to boys, following the same standard meal (Cooper et al., 2017). This is an interesting observation which may be indicative of potentially differential insulin resistance development during puberty, where fasting hepatic insulin resistance occurs at the earlier stages, with postprandial peripheral insulin resistance developing in the latter stages. However, there are currently no data to support this suggestion which would require the measurement of postprandial insulinaemia in adolescents at different stages of puberty, or a longitudinal follow-up throughout the course of adolescence.

The results of the present study demonstrate that the use of low-cost, non-invasive measures of adiposity and physical fitness provides a much greater explanation of variance in postprandial insulinaemia than the traditional fasting marker of metabolic health, HOMA-IR. This has important practical implications, given the invasive and costly nature of HOMA-IR, and the potential use of these measurements (especially waist circumference) in predicting postprandial insulinaemia. However, there are still other characteristics that might provide additional information about the variance in postprandial insulinaemia. Physical activity is known to attenuate the puberty-related insulin resistance seen in adolescence (Metcalf et al., 2015). Furthermore, in adults matched for $\dot{V}O_{2max}$, those with greater levels of physical activity were more insulin sensitive in response to an OGTT (Laye et al., 2015). Given this evidence, it would be worthwhile including physical activity as an explanatory variable in future work, although it is possible that physical activity may only be important in improving fitness as suggested in explanation of the findings in Chapter IV, which is not fully reflected in a measure of $\dot{V}O_{2max}$, (Laye et al., 2015) which has a large genetic component. In addition, this work could be extended by incorporating participants across the age of adolescence, which would help to identify if the relationships highlighted in the present study exist across different age groups and stages of pubertal development.

The present study has several possible limitations that need consideration. Firstly, a mixed-meal was consumed rather than a traditional OGTT. The OGTT is a valid test meal when examining postprandial responses and the consumption of a solid mixed-meal will have different gastric emptying rates compared to a drink solution, thus comparisons may be limited (Brouns et al., 2005). However, examining the postprandial responses to a mixed-meal has been favoured in recent paediatric research given that young people spend most of awake time in the postprandial state. The present study also used maturity offset as a marker of maturation status (Moore et al., 2015); which is based on predictive modelling using anthropometric measurements. Despite being a prediction of maturation, maturity offset is often favoured in a non-clinical setting over traditional measures (such as the Tanner scale, which examines secondary sex characteristics), which are deemed invasive (Mirwald et al., 2002). Whilst the present study included examined

several relevant predictors of metabolic health, there were also a number of predictors not included (such as the habitual dietary intake and physical activity levels of participants, mode of transport to school and socioeconomic status), which should be examined in future research. Furthermore, as the present study is cross-sectional, causality between the chosen predictors and postprandial responses cannot be inferred. Finally, it is important to consider that the participants in the current study are considered healthy and asymptomatic from cardiometabolic health conditions. Indeed, it might be more appropriate to study the relationships examined in the present study in populations with increased prevalence of risk factors for cardiometabolic diseases, given they would be the target of future interventions. Nonetheless, identifying these relationships in healthy adolescents provides important information, given the role of postprandial hyperinsulinaemia in the pathophysiology of insulin resistance and related cardiometabolic health issues (DiNicolantonio et al., 2017) and the suggested early manifestation of such conditions (Steinberger et al., 2009).

In conclusion, the findings of the present study demonstrate that over half of the variance in postprandial insulinaemia in response to a standard mixed-meal, in adolescents, can be explained by measurements that are frequently employed to characterise participants in paediatric exercise literature; waist circumference, MSFT performance and HOMA-IR. Overall, measures of body composition (particularly waist circumference) were key when explaining the variance in metabolic health in this sample. These data extend previous work using different surrogates of body composition and fasting indices of insulin resistance, thus demonstrating that body composition (particularly waist circumference) is important for postprandial metabolic responses and cardiometabolic health. Future work should investigate additional variables that might help explain the variance in postprandial insulinaemia and glycaemia, such as physical activity, and how the impact of these participant characteristics may change throughout the course of adolescence.

Practical Applications

These findings have important practical implications, as the predictors of postprandial glycaemia and insulinaemia identified are easily measurable in young people and considered modifiable. Therefore, they present attractive targets for interventions. For example, based on the findings of this chapter future interventions aimed at reducing postprandial insulinaemic responses in adolescents should focus on primarily improving physical fitness and reducing waist circumference.

Chapter VI

Effect of physical fitness and acute football activity on glycaemic and insulinaemic responses in adolescents

6.1 Introduction

The previous two chapters examined the cross-sectional relationship between risk factors for cardiometabolic disease and physical activity, physical fitness and adiposity. Importantly it was shown in Chapter 5 that high physical fitness was associated with lower postprandial insulinaemic responses to a mixed meal. However, in the studies reported in Chapters IV and V, all measurements were made while the participants were rested and had not exercised earlier in the day. Whilst there is growing evidence supporting the insulin sensitising effects of acute exercise in adults and, to a limited extent in adolescents, there is a lack of research investigating ecologically valid modalities of exercise such as football, in both adults and in adolescents. Furthermore, given the important established role of physical fitness for cardiometabolic health, and the effect it could have on the external work performed during free living activity such as football, it is also imperative to investigate the potential moderating role of fitness on the effect of acute exercise on the postprandial responses to a mixed meal.

Physical inactivity is associated with poor cardiometabolic health during adolescence (Ekelund et al., 2007; Ekelund et al., 2012) and is considered an important risk factor in the development of most chronic diseases (Booth et al., 2012). It is becoming a highly pertinent issue, highlighted by the increasing prevalence of type 2 diabetes amongst adolescents (Tagi, Giannini & Chiarelli, 2019). Insulin resistance, and reduced glucose tolerance, are involved in the aetiology of type 2 diabetes (Reaven, 2005); which presents them as targets to deal with the increasing prevalence of type 2 diabetes in this population and to enhance lifelong health. The postprandial response to a test meal, particularly the insulinaemic response, is gaining recognition as a key risk factor for the development of insulin resistance and, ultimately, cardiometabolic disease (DiNicolantonio et al., 2017; Lautt, 2007). There are numerous risk factors associated with the development

insulin resistance, some of which can be modified through lifestyle behaviour change (Tuomilehto et al., 2001), such as body composition (particularly central adiposity) (Arslanian & Suprasongsin, 1996; Arslanian, 2000) and physical inactivity (Reinehr, 2013). Of these risk factors, physical activity offers an attractive target for public health initiatives, with participation in physical activity a recommended and cost-effective method to combat the development of metabolic risk factors in young people (Gleeson et al., 2011; Röhling et al., 2016). Indeed, such public health initiatives are needed as it is estimated that only 24% of boys and 18% of girls (aged 5 to 15 y) meet the recommended guidelines of 60 min moderate-vigorous physical activity per day (Scholes & Mindell, 2016).

High-intensity intermittent exercise is an increasingly popular method for implementing physical activity in young people due to the associated health benefits and greater perceived enjoyment (Batacan et al., 2017; Bond et al., 2017). Recent laboratory-based research in adolescents has focused on high-intensity intermittent exercise, using 8 x 1 min bouts at 90% of peak power on a cycle ergometer (Cockcroft et al., 2015; Cockcroft et al., 2017). These findings demonstrate transient improvements in the postprandial insulinaemic and glycaemic response to an oral glucose tolerance test (Cockcroft et al., 2015; Cockcroft et al., 2017), which has important implications for lifelong metabolic health, given their role as a known risk factor for the development of cardiometabolic diseases (DiNicolantonio et al., 2017; Lautt, 2007). Whilst these findings are promising, it could be argued that the modality of exercise, as well as the glucose tolerance test (rather than a test meal), are not ecologically valid. Interestingly, 85% of young people in the UK who met the recommended daily target of 60 minutes moderate-vigorous physical activity do so through informal sports (Townsend et al., 2015). It is worth noting that the most popular sport amongst British adolescents is football, with 45% of 11-15 year olds participating in this activity (DCMS, 2017). The activity patterns seen in team sports like football are known to be intermittent (Svensson & Drust., 2005), much like the natural activity patterns of adolescents (Bailey et al., 1995; Howe et al., 2010). It has thus been suggested that small-sided games could be beneficial for health promotion (Krustrup & Bangsbo, 2015), with empirical

evidence demonstrating that acute small-sided games (Rugby Union) improve metabolic health in sedentary adult men (Mendham, Coutts & Duffield, 2012; Mendham et al., 2015). However, there is little research investigating the effects of team games on risk factors for cardiometabolic disease in adolescents.

Smallcombe et al. (2018) employed a 48 min bout of football (using small-sided games) compared to duration-matched treadmill running in adolescent boys (12.7-13.3 y). The bout of football led to greater improvements in postprandial lipemia in adolescent boys – despite less distance being covered in the small-sided games. However, to the authors' knowledge, only one study has examined the effects of games-based exercise on postprandial glycaemia and insulinaemia in adolescents (Dring et al., 2019a). A 60 min bout of basketball, including skill drills and small-sided games, led to a 35% reduction in the postprandial insulinaemic response to a standardised mixed-meal (Dring et al., 2019a); which is consistent with laboratory findings utilising high-intensity intermittent cycling protocols (Cockcroft et al., 2015). However, it is unknown how an acute bout of football may affect the glycaemic and insulinaemic responses to a standardised mixed meal, in adolescents. This is important gap to fill, given the popularity and ecological validity of football.

Physical fitness is also strongly associated with metabolic health in adolescents. Specifically, higher fit adolescents have a lower degree of fasting insulin resistance, compared to their low fit counterparts (Dring et al., 2019b; Haapala et al., 2019). Furthermore, the findings of Chapter V suggest that physical fitness is also inversely, and independently, associated with the postprandial insulinaemic response to a mixed meal. Despite this, it is unknown if the physical fitness of participants moderates the acute metabolic responses to exercise. It has been suggested that those considered lower fit will see greater metabolic improvements from acute exercise than their higher fit counterparts (Cockcroft et al., 2015; Short et al., 2013; Short, Pratt & Teague., 2018). On the other hand, it has also been suggested that those with a higher physical fitness will benefit more, by virtue of the ability to work at a higher absolute exercise intensity (Dring et al., 2019a). However, these suggestions have been made comparing across studies, with different methods of assessment for physical fitness. Thus, no empirical study to date has

directly investigated the moderating role of physical fitness on the metabolic responses following an acute bout of exercise.

Therefore, the aims of the current study were to examine: i) the acute effects of football activity on the postprandial glycaemic and insulinaemic responses to a standardised meal; ii) to compare the postprandial responses between high- and low-fit adolescents; and iii) how physical fitness may moderate the acute effects of exercise on these postprandial responses. It was hypothesised that the acute bout of football would reduce the postprandial glycaemic and insulinaemic response. It was also hypothesised that low fit adolescents would have greater glycaemic and insulinaemic responses compared to high-fit adolescents, with the acute reduction following football activity being greater in the low-fit participants.

6.2 Methods

6.2.1 Participant Characteristics

Thirty-six adolescents (20 boys, 16 girls; 12-13 years old) volunteered to participate in the study. During familiarisation, all participants underwent anthropometric measurements consisting of height (cm), body mass (kg) and sitting height (cm). These were conducted in line with descriptions in section 3.3 of this thesis and used to calculate age at peak height velocity (Moore et al., 2015). Waist circumference and the sum of four skinfold sites (tricep, subscapular, supraspinale and front thigh) were measured in line with descriptions in section 3.4 of this thesis. Descriptive participant characteristics are presented in Table 6.1.

6.2.2 Experimental Design

The study conformed to the Declaration of Helsinki guidelines and was approved by the Nottingham Trent University Human Ethics Committee. Participants were recruited from local secondary schools in the East Midlands area of the UK. Written parental consent and child assent were obtained during the initial phase of recruitment. A health screen was completed by the participants' parent/guardian and was checked by a lead investigator to ensure there were no medical conditions that would affect the child's participation.

The study employed a randomised, order-balanced, crossover, within-subjects design with two main experimental trials (exercise and resting), separated by at least 7 d. Participants were blind to the trial condition until arrival at school. A familiarisation took place 7 d before the first main trial and allowed participants to be acquainted with all the necessary procedures, including capillary blood sampling and a battery of cognitive function tests (Chapter VII). Participants were also familiarised with a football session, consisting of skill drills and small-sided games.

Table 6.1. Participant characteristics for the group overall, as well as for the high- and low-fit groups. Data are mean ± SD.

Variable	**Overall** (n = 36; 16 girls, 20 boys)	**High-fit** (n = 18; 8 girls, 10 boys)	**Low-fit** (n = 18; 8 girls, 10 boys)	**Independent samples t-test (*p*)**
Age (yrs)	12.6 ± 0.5	12.7 ± 0.5	12.4 ± 0.5	-
Height (cm)	163.1 ± 7.0	163.5 ± 8.0	162.6 ± 6.1	0.709
Body Mass (kg)	53.9 ± 10.0	50.1 ± 8.9	57.6 ± 10.0	0.024
BMI ($kg \cdot m^{-2}$)	20.2 ± 3.0	18.6 ± 2.0	25.7 ± 3.1	0.001
BMI Percentile	63.1 ± 30.0	48.9 ± 26.9	77.3 ± 26.6	0.003
Waist Circumference (cm)	70.0 ± 8.0	66.0 ± 5.4	73.9 ± 8.4	0.002
Sum of 4 skinfolds (mm)	60.3 ± 26.8	42.8 ± 12.8	77.8 ± 25.7	< 0.001
Maturity Offset	0.08 ± 0.94	0.20 ± 0.84	-0.05 ± 1.03	0.449
MSFT Distance (m)	1160 ± 400	1480 ± 300	840 ± 140	< 0.001
Predicted $\dot{V}O_2$ peak ($ml \cdot kg^{-1} \cdot min^{-1}$)[a]	47.9 ± 5.2	52.0 ± 3.7	43.8 ± 2.5	< 0.001

Abbreviations: MSFT = Multi-Stage Fitness Test.
[a] predicted from the multi-stage fitness test using the equations of Barnett et al.

During the familiarisation participants also completed the multi-stage fitness test (MSFT) (Ramsbottom, Brewer & Williams, 1988) for the assessment of physical fitness, in line with descriptions in section 3.5 of this thesis. Prior to the start of the MSFT, participants were fitted with a heart rate monitor (Firstbeat Team Sport System, Firstbeat Technologies Ltd, Finland). Heart rate was monitored throughout the MSFT and maximum heart rate was recorded upon completion. Performance on the test was determined by the total distance covered (m), with participants assigned to high- and low-fitness groups, based on sex-specific median splits of MSFT distance covered. This resulted in 18 low-fit participants (8 girls, 10 boys) and 18 high-fit

participants (8 girls, 10 boys). This criteria was used based on previous work demonstrating that the MSFT is a good indicator of cardiometabolic health in adolescents (Dring et al., 2019b) and it is a valid (Tomkinson et al., 2019) and reliable (Artero et al., 2011) measure of endurance capacity in adolescents. For descriptive purposes, the whole sample was characterised based on age- and sex-specific centiles; derived from normative performance data in adolescents (Tomkinson et al., 2016).

6.2.2.1. Main Trials

Participants were instructed to record their dietary intake for the 24 hours preceding the first experimental trial and during evening one of the first main trial. Recorded diets were then replicated for the second main trial. Participants were asked to refrain from eating or drinking from 9 pm prior to the two main trials. Water was allowed *ad libitum* at all times. Participants were also asked to refrain from physical activity 24 h prior to the main trials and were not permitted to participate in any strenuous physical activity until the final measurement had been taken on the second day of an experimental trial. Parents/guardians were contacted by telephone on the evening prior to each main trial to ensure compliance with these requirements.

On the morning of the main trials, participants reported to school at the beginning of the usual school day (between 8 am and 8:30 am). Upon arrival participants had a fasted capillary blood sample taken and were fitted with a heart rate monitor (Firstbeat Team Sport System, Firstbeat Technologies Ltd, Finland), to be worn throughout the trial. Participants were then given a standardised breakfast and 1 h after the start of breakfast, they either took part in a 60 min exercise session (Football; see section 6.2.3.3) or remained quietly seated in the classroom. Capillary blood samples were taken immediately, 30 and 60 min following the exercise/rest period. Lunch was provided, with additional blood samples at 30, 60 and 120 min post-lunch (1.5, 2 and 3 h post-exercise). Participants returned at the same time on the following morning (Day 2) for an additional fasted capillary blood sample. Both trials followed a time-matched protocol (lasting ~ 5.5 h), with the only difference being the 60 min exercise session. A schematic of the experimental protocol can be seen in Fig 6.1.

6.2.3. Experimental Procedures

6.2.3.1 Standardised Breakfast and Lunch

A standardised breakfast and lunch were provided for participants during the experimental trials. The breakfast provided 1.5 $g \cdot kg^{-1}$ body mass of carbohydrate (cornflakes, milk, white toast and butter). The lunch also provided 1.5 $g \cdot kg^{-1}$ body mass of carbohydrate (chicken sandwich, baked crisps and an apple; with a cheese alternative for vegetarians); which was provided 1 h post-exercise. Participants were given 15 min to consume breakfast and lunch. A hypothetical example of each meal can be found in section 3.6 of this thesis.

6.2.3.2. Capillary Blood Samples

Capillary blood samples were collected in accordance with the description in section 3.6 of this thesis, wtih blood collected into two 300 µl EDTA coated microvettes (Sarstedt Ltd, UK). A single 25 µl whole blood sample was also collected, using a pre-calibrated glass pipette (Hawksley Ltd, UK), and immediately deproteinised in 250 µl ice-cooled 2.5% perchloric acid. Samples were centrifuged at 1000 x g for 4 min at 4 °C (Eppendorph 5415C, Hamburg, Germany). Plasma was removed from the microvettes and placed into 250 µl plastic vials for subsequent analysis. All samples were frozen immediately at −20 °C and transferred to a −80 °C freezer as soon as possible.

Blood glucose concentrations were determined, in duplicate, using a commercially available assay (GOD/PAP method, GL364, Randox, Ireland). Plasma insulin concentrations were determined using a commercially available ELISA (Mercodia Ltd, Sweden). The intra-assay coefficient of variation of the assays can be found in section 3.6 of this thesis. Blood glucose and plasma insulin total area under the curve (tAUC) following the standardised lunch were calculated (GraphPad Prism 7, GraphPad Software, USA), using methods described previously (Wolever et al., 1991).

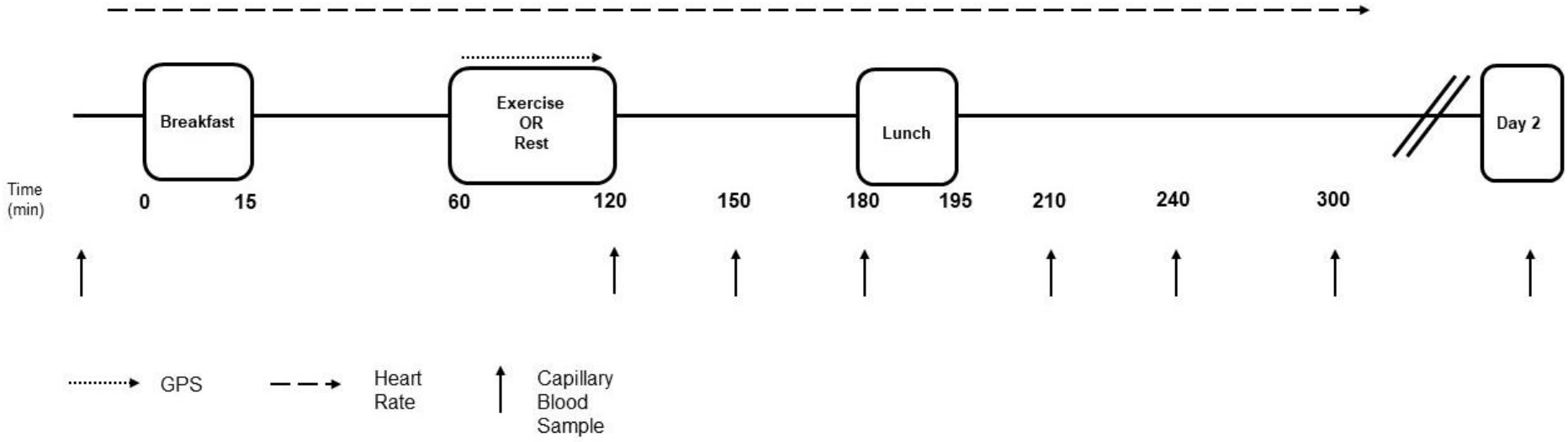

Fig 6.1. Visual representation of the experimental trials. The timeline shown is in cumulative minutes. Vertical arrows denote timing of blood samples, whilst the horizontal arrows represent the duration of heart rate monitoring and GPS tracking.

6.2.3.3 Exercise Protocol

The exercise consisted of a 60 min football session. Football was chosen as it intermittent in nature, which replicates the natural activity patterns of adolescents (Bailey et al., 1995), as well as being an enjoyable and popular form of games-based activity for young people (Taking Part 2017/18, GOV.UK). An experienced football coach delivered the sessions to groups of 10 participants, on outdoor facilities at the respective schools. The session consisted of a warm-up (jogging around the pitch and sprinting between cones; 5 min), skill-based drills (passing, dribbling and ball control; 25 min) and small-sided games (5 vs 5, 4 min games with 1 min break to switch teams; 30 min). At the end of the session, participants walked from the outdoor pitch to the classroom, which acted as a brief cool-down. Heart rate was monitored throughout the session. Maximum heart rate (HRmax), as recorded at the end of the MSFT, and heart rate during the football session were used to calculate the relative exercise intensity (%HRmax). Additionally, Global Positioning System (GPS) devices were worn to quantify the external load during the football session using SPI HPU (15 Hz) portable GPS units (GPSports, Australia). The GPS units were fitted to sit between the scapulae, at the base of the cervical spine, using an elasticated shoulder harness. After each exercise session, the data were downloaded to Team AMS software (Team AMS, GPSports). The variables of interest were total distance covered (m) as well as the distance covered in six different speed categories: standing (≤ 0.4 $km \cdot h^{-1}$), walking (>0.4 to 3.0 $km \cdot h^{-1}$), low-intensity running (>3.0 to 8.0 $km \cdot h^{-1}$), moderate-intensity running (>8.0 to 13.0 $km \cdot h^{-1}$), high-intensity running (>13.0 to 18.0 $km \cdot h^{-1}$) and sprinting (> 18.0 $km \cdot h^{-1}$) (Smallcombe et al., 2018).

6.2.4 Statistical Analysis

All data analysis was conducted using SPSS (Version 24, SPSS Inc, Chicago, IL, USA). Data were assessed for normality using the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots, which revealed that all data (except from insulin) were normally distributed. Insulin data were log-transformed prior to analysis. Heart rate during the 60 min football session was compared between the high- and low-fit groups using an independent samples t-test. The

distance covered across the six speed categories during the football session were analysed between the high- and low-fit group via multivariate analysis of variance (MANOVA). Blood glucose and plasma insulin concentration were analysed via three way (trial*time*fitness) mixed model analysis of variance (ANOVA), with repeated measures for trial and time, and fitness as a between-subjects factor. Blood glucose and plasma insulin tAUC were analysed using a two-way (trial*fitness) mixed model ANOVA, with repeated measures for trial and fitness as a between-subjects factor. Where main effects between fitness groups and trials existed, the mean difference (MD) and the 95% confidence interval (CI) are presented. Data are presented as mean ± SD, unless otherwise stated. For all analyses, statistical significance was accepted as $p < 0.05$.

6.3 Results

For MSFT performance, the median centile for boys was 65 (range: 20 – 95) and for girls was 90 (range: 70 – 95). For the sample overall, performance was classified as "low" for 13.9% (≤ 40th centile), "moderate" for 13.9% (40th – 60th centile), "high" for 27.8% (60th – 80th centile) and "very high" for 44.4% (≥ 80th centile).

6.3.1 Exercise Characteristics

During the 60 min football session, average heart rate was 151 ± 16 beats·min^{-1}, maximum heart rate was 186 ± 13 beats·min^{-1} and relative exercise intensity was 75 ± 8% HRmax (Table 6.2). Average ($t_{(32)} = -2.8$, $p = 0.009$) and maximum ($t_{(32)} = 2.7$, $p = 0.010$) heart rate, as well as relative exercise intensity ($t_{(32)} = -4.1$, $p < 0.001$), were lower in the high-fit group compared to the low-fit group (Table 6.2). Average heart rate across the whole 5 h exercise trial (105 ± 13 beats·min^{-1}) was higher than during the whole 5 h resting trial (84 ± 11 beats·min^{-1}, $t_{(35)} = 11.8$, $p < 0.001$).

During the football session, the average total distance covered by the group overall was 2788 ± 432 m, with the low-fit participants covering a total of 2792 ± 474 m and high-fit participants covering a total of 2786 ± 402 m. There was no significant effect of fitness on the total distance and distance covered across the six different speed categories (Pillai's trace: $p = 0.301$, Table 6.2).

6.3.2 Metabolic Responses

Blood glucose and plasma insulin concentration across the exercise and resting trial, for the group overall as well as the high- and low-fit group, can be seen in Table 6.3.

6.3.2.1 Blood Glucose Concentration

Response to exercise

Blood glucose concentration was similar between the exercise and resting trials (main effect of trial; $p = 0.063$), but changed over time (main effect of time; $F_{(2, 77)} = 29.5$, $p < 0.001$). This pattern of change was different between the exercise and resting trial (trial by time interaction; $F_{(2, 81)} = 11.6$, $p < 0.001$, Fig 6.2A). Specifically, blood glucose concentration was lower 60 min post-exercise compared to 60 min post-rest ($t_{(35)} = -6.3$, $p < 0.001$, MD = -0.80 mmol·L^{-1}, 95% CI [-1.06, -0.55 mmol·L^{-1}], Table 6.3). Blood glucose concentration was similar between high- and low-fit groups (main effect of fitness; $p = 0.596$), and fitness did not moderate the pattern of change (trial by time by fitness interaction; $p = 0.234$).

Table 6.2. Average and maximum heart rate, relative exercise intensity and GPS characteristics for the group overall, as well as the high- and low-fitness splits, during the 60 min Football session. Data are mean ± SD.

Variable	Overall (n = 36; 16 girls, 20 boys)	High-fit (n = 18; 8 girls, 10 boys)	Low-fit (n = 18; 8 girls, 10 boys)
Heart Rate			
Average Heart Rate (beats·min^{-1})	151 ± 16	144 ± 16	158 ± 12*
Maximum Heart Rate (beats·min^{-1})	186 ± 13	180 ± 11	191 ± 11*
Relative Exercise Intensity (% maximum heart rate) [b]	75 ± 8	70 ± 7	80 ± 6*
Distance covered (m) within speed categories			
Standing (≤ 0.4 km·h^{-1})	6 ± 2	7 ± 1	6 ± 2
Walking (>0.4 to 3.0 km·h^{-1})	419 ± 64	398 ± 48	439 ± 72
Low-intensity running (>3.0 to 8.0 km·h^{-1})	1534 ± 234	1521 ± 242	1546 ± 233
Moderate-intensity running (>8.0 to 13.0 km·h^{-1})	648 ± 146	659 ± 133	637 ± 161
High-intensity running (>13.0 to 18.0 km·h^{-1})	160 ± 76	172 ± 58	148 ± 86
Sprinting (> 18.0 km·h^{-1})	22 ± 20	29 ± 21	16 ± 16

Abbreviations: GPS = Global Positioning System. MSFT = Multi-Stage Fitness Test
[b] Relative to the maximum heart rate attained during the MSFT
* Difference between high- and low-fit groups, $p < .01$

Postprandial response to standardised lunch

Following the standardised lunch, blood glucose concentration was lower during the exercise trial compared to the resting trial (main effect of trial; $F_{(1, 34)} = 15.2$, $p < 0.001$, MD = -0.25 mmol·L^{-1}, 95% CI [-0.37, -0.12 mmol·L^{-1}]) as well as changing over time (main effect of time; $F_{(1, 102)} = 56.8$, $p < 0.001$). The pattern of change was different between the exercise and resting trials (trial by time interaction; $F_{(3, 102)} = 10.4$, $p < 0.001$), whereby blood glucose concentration was lower before lunch during the exercise trial compared to the control trial (Table 6.3). Blood glucose concentration following lunch was similar between the high- and low-fit groups (main effect of fitness; $p = 0.373$), and fitness did not moderate the pattern of change (trial by time by fitness interaction; $p = 0.368$).

Blood glucose tAUC following the standardised lunch was similar between the exercise and resting trial (p = 0.124), as well as between the high- and low-fit groups (main effect of fitness; p = 0.551). The difference between trials was similar for the high- and low-fit groups (trial*fitness interaction; p = 0.177).

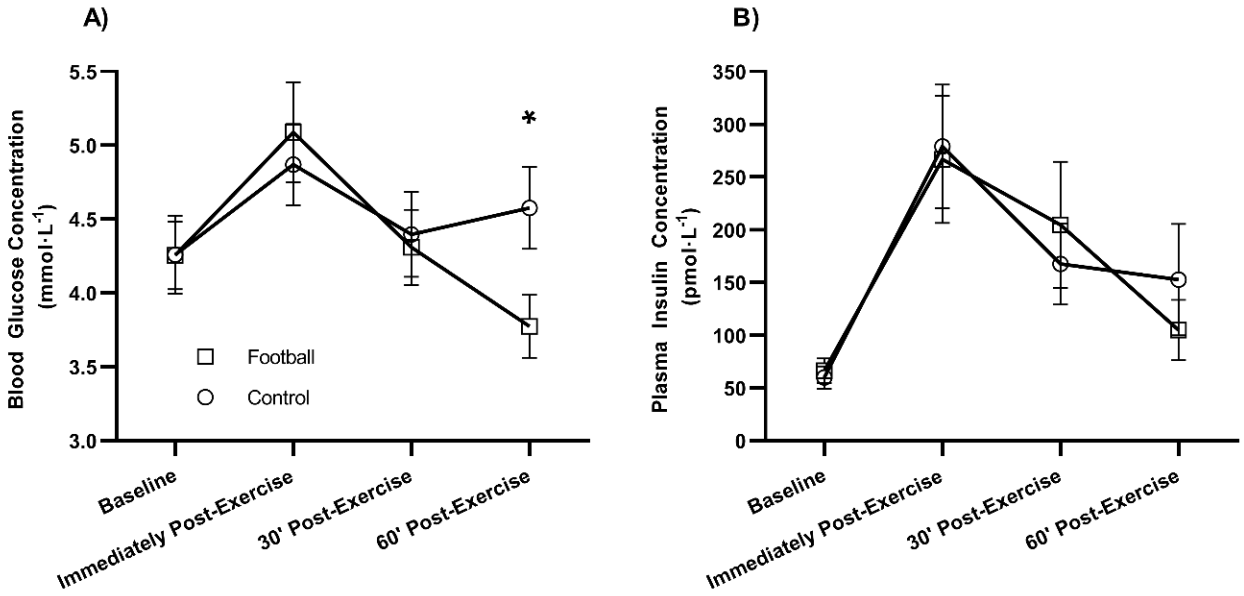

Fig 6.2. Overall post-exercise blood glucose (A) and plasma insulin (B) concentrations for the football and control trials. Data are mean ± 95% CI. *difference between football and control, p < 0.001

Fasted day 1 and day 2 measures

Overall fasted blood glucose concentration was similar between the exercise and resting trials (main effect of trial; p = 0.812) and over time (main effect of time; p = 0.071). The pattern of change between the exercise and resting trial was similar (trial by time interaction; p = 0.663). Fasted blood glucose concentration was similar between the high- and low-fit group (main effect of fitness; p = 0.175) and fitness did not moderate the pattern of change (trial by time by fitness interaction; p = 0.090).

6.3.2.2 Plasma Insulin Concentration

Response to exercise

Plasma insulin concentration was similar between the exercise and resting trial (main effect of trial; p = 0.766), but changed over time (main effect of time; $F_{(2, 80)}$ = 59.1, p < 0.001). The pattern

of change was similar between the exercise and resting trials (trial by time interaction; p = 0.089, Fig 6.2B). Plasma insulin concentration was lower in the high-fit group compared to the low-fit group (main effect of fitness; $F_{(1, 34)}$ = 10.9, p = 0.002, MD = -88.4 pmol·L^{-1}, 95% CI [-142.6, -34.1 pmol·L^{-1}]). Fitness did not moderate the pattern of change (trial by time by fitness interaction; p = 0.186).

Postprandial response to standardised lunch

Following the standardised lunch, overall plasma insulin concentration was similar between the exercise and resting trials (main effect of trial; p = 0.198) but changed over time (main effect of time; $F_{(1, 102)}$ = 46.5, p < 0.001). The pattern of change was similar between the exercise and resting trials (trial by time interaction; p = 0.544). Plasma insulin concentration was lower in the high-fit group compared to the low-fit group (main effect of fitness; $F_{(1, 34)}$ = 11.5, p = 0.002, MD = -133.5 pmol·L^{-1}, 95% CI [-213.5, -53.5 pmol·L^{-1}]). Fitness did not moderate the pattern of change in response to lunch (trial by time by fitness interaction; p = 0.368).

Plasma insulin tAUC following the standardised lunch was similar between the exercise and resting trial (main effect of trial; p = 0.207), but was lower in the high-fit group compared to the low-fit group (main effect of fitness; $F_{(1, 34)}$ = 10.9, p = 0.002, MD = -2672.6 pmol·L^{-1} x 120 min, 95% CI [-4321.1, -1024.0 pmol·L^{-1} x 120 min]; Fig 6.3). However, fitness did not moderate plasma insulin tAUC (trial by fitness interaction; p = 0.952).

Fasted day 1 and day 2 measures

Overall plasma insulin concentration across fasted samples on day 1 and day 2 was similar between the exercise and resting trials (main effect of trial; p = 0.446), and between day 1 and day 2 (main effect of time; p = 0.181). The pattern of change was similar between the exercise and resting trial (trial by time interaction; p = 0.604). Fasted plasma insulin concentration was similar between the high- and low-fit groups (main effect of fitness; p = 0.496), with no moderating effect of fitness on the pattern of change (trial by time by fitness interaction; p = 0.568).

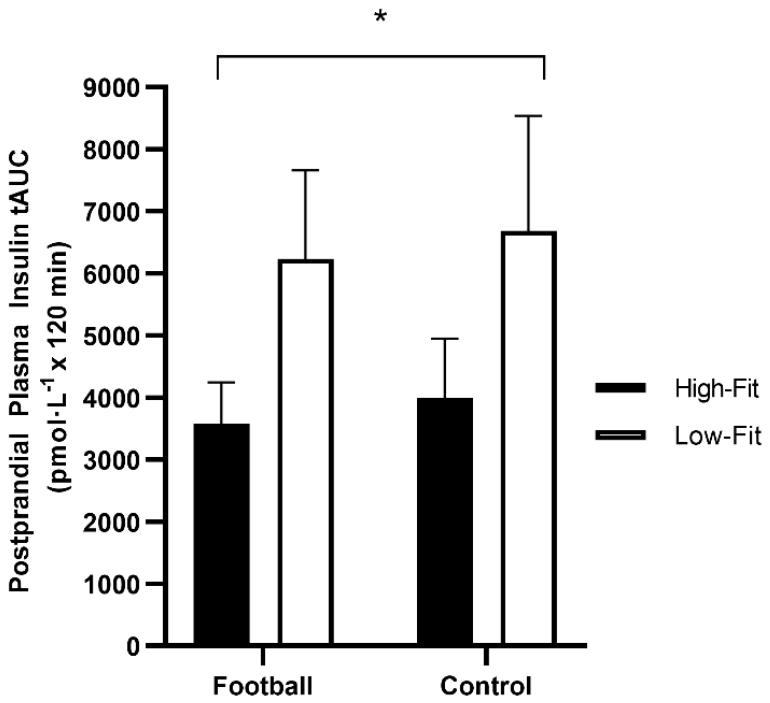

Fig 6.3. Postprandial plasma insulin tAUC following the football and control trials, for the high- (filled bars) and low-fit groups (open bars). Data are mean ± 95% CI. *main effect of fitness, $p < 0.001$

Table 6.3. Blood glucose (mmol·L^{-1}) and plasma insulin (pmol·L^{-1}) concentration across the Football and control trial on day 1 and for the fasted sample on day 2. Data are displayed for the group overall as well as split between the high- and low-fit groups. Data are mean (SEM).

Variable	Group	Trial															
		Football								*Control*							
		Rest	Post-Exercise	30 min post-exercise	60 min post-exercise	30 min post-lunch	60 min post-lunch	120 min post-lunch	Day 2	Rest	Post-Exercise	30 min post-exercise	60 min post-exercise	30 min post-lunch	60 min post-lunch	120 min post-lunch	Day 2
Blood Glucose (mmol·L^{-1})																	
	Overall	4.3 (0.1)	5.1 (0.2)	4.3 (0.1)	3.8* (0.1)	5.7 (0.2)	4.5 (0.1)	4.5 (0.1)	4.4 (0.1)	4.3 (0.1)	4.9 (0.1)	4.4 (0.1)	4.6 (0.1)	5.8 (0.2)	4.4 (0.1)	4.7 (0.2)	4.4 (0.8)
	High-fit	4.3 (0.1)	4.9 (0.2)	4.1 (0.1)	4.0 (0.1)	6.0 (0.2)	4.6 (0.2)	4.5 (0.2)	4.6 (0.2)	4.5 (0.2)	5.0 (0.2)	4.4 (0.1)	4.7 (0.2)	5.8 (0.2)	4.3 (0.1)	4.9 (0.3)	4.5 (0.2)
	Low-fit	4.2 (0.2)	5.3 (0.3)	4.5 (0.2)	3.6 (0.2)	5.5 (0.3)	4.5 (0.2)	4.5 (0.2)	4.2 (0.2)	4.0 (0.2)	4.8 (0.2)	4.4 (0.2)	4.4 (0.2)	5.8 (0.3)	4.5 (0.2)	4.6 (0.2)	4.3 (0.2)
Plasma Insulin (pmol·L^{-1})																	
	Overall	66.3 (5.7)	266.7 (29.8)	204.7 (29.5)	105.0 (14.1)	405.9 (45.7)	233.3 (24.2)	193.7 (19.3)	75.2 (8.3)	59.8 (5.2)	278.9 (28.8)	167.5 (18.9)	152.6 (26.1)	423.4 (44.8)	232.9 (29.1)	218.2 (22.8)	74.0 (7.5)
	High-fit	61.3 (9.2)	197.7 (27.9)	121.8 (28.0)	62.5 (11.9)	305.3 (35.5)	163.2 (13.1)	134.5 (15.9)	78.7 (14.5)	52.0 (6.7)	236.0 (38.8)	129.3 (23.7)	87.6 (16.7)	355.3 (43.9)	152.6 (23.6)	170.0 (25.9)	68.8 (11.6)
	Low-fit	71.4 (6.7)	335.8 (48.1)	287.6 (44.7)	147.4 (21.5)	506.4 (78.3)	303.4 (40.7)	252.9 (29.5)	71.8 (8.7)	67.7 (7.6)	321.9 (41.2)	205.7 (27.3)	217.7 (45.1)	491.4 (76.2)	313.1 (46.6)	266.5 (34.6)	79.2 (9.7)

Data are mean (SEM). *Significantly different compared to control, $p < 0.001$.

6.4. Discussion

The main findings of the present study were that the postprandial insulinaemic response to an ecologically valid meal was 70% lower in the high-fit than in the low-fit participants, demonstrating that higher levels of physical fitness are associated with enhanced metabolic health in adolescents. In addition, whilst postprandial glycaemia and insulinaemia were unaffected by a 60 min bout of football activity, blood glucose concentration was 21% lower 60 min post-exercise.

The present study is the first to provide evidence demonstrating that the postprandial insulinaemic response was, on average, 70% lower in those considered high-fit compared to their low-fit counterparts. This is an important finding, given the role that postprandial insulinaemia plays in the development of insulin resistance and cardiovascular risk (DiNicolantonio et al., 2017; Lautt, 2007). A difference of such magnitude emphasises the crucial role of physical fitness in adolescents for enhancing lifelong health. One possible explanation for this strong effect of fitness might be due to the increased GLUT4 translocation and skeletal muscle capillarisation, which is suggested to be a result of chronic exercise training (Bird & Hawley, 2017). A further possible explanation could be attributed to the body composition of participants, given that body composition is considered a risk factor for insulin resistance (Arslanian & Suprasongsin, 1996; Arslanian, 2000). Unsurprisingly, the characteristics relating to body composition were more favourable in the high-fit group compared to the low-fit group (Table 6.1). A recent study supports these findings by showing that adolescents considered overweight/obese had a higher postprandial insulinaemic response by ~ 36% (Short, Pratt & Teague, 2018). However, $\dot{V}O_{2\ peak}$ in the aforementioned study (Short, Pratt & Teague, 2018) was normalised to lean body mass, rather than total body mass, which makes comparisons with the characteristics in the current study difficult. Whilst the cross-sectional nature of the relationship between physical fitness and metabolic health in the present study prevents the inference of a causal relationship, the present study provides important novel findings that those with a higher physical fitness – non-invasively measured via the MSFT – have a lower postprandial insulinaemic response and also supports

the observations from Chapter V of this thesis, whereby physical fitness was associated with a lower postprandial insulinaemic response, even when considering the potent role of adiposity.

The present study provides novel information regarding the postprandial insulinaemic response to an ecologically valid meal following an acute bout of football; with the current data suggesting that 60 min football activity does not affect postprandial insulinaemic responses. Conversely, previous studies have demonstrated acute reductions in the postprandial insulinaemic response to a test meal (Short et al., 2013; Short, Pratt & Teague, 2018) and an OGTT (Cockcroft et al., 2015; Cockcroft et al., 2017) following high-intensity intermittent cycling exercise (Cockcroft et al., 2015; Cockcroft et al., 2017) and aerobic circuit exercise (Short et al., 2013; Short, Pratt & Teague, 2018). Possible explanations for divergent results might reside in the fact that previous studies were conducted in a tightly-controlled laboratory environment, used an OGTT as the test meal (Cockcroft et al., 2015; Cockcroft et al., 2017), recruited participants with habitually low physical activity (Short, Pratt & Teague, 2018) or were overweight/obese (Short et al., 2013). A previous study has shown that an acute bout of basketball reduces postprandial insulinaemia (by 35%) following a standardised mixed-meal (Dring et al., 2019a), in comparison with a non-statistically significant reduction of 11% in the present study. The present study and the study by Dring et al. (2019a) are the only ones, to the authors' knowledge, that examine postprandial insulinaemia in response to games-based activity. Although the exercise bouts were of similar duration and relative exercise intensity, in the basketball study the participants had a higher predicted $\dot{V}O_{2peak}$ than the participants in the present study (Dring et al., 2019a). Therefore, the absolute exercise intensity may have been higher in the basketball study (Dring et al., 2019a), accounting for the greater reduction in postprandial insulinaemia (35% vs. 11% in the present study).

The present study also provides novel information regarding the transient reduction (21%) in blood glucose 60 min following an acute bout of football in adolescents. This is of greater magnitude compared to the 11% reduction following an acute bout of basketball (Dring et al., 2019a), despite similar duration and relative exercise intensities seen between the studies. A

possible explanation for this may be differences in the movement patterns, muscle fibre recruitment, and active muscle mass, between the two activities, though these suggestions remain speculative at this stage. However, data from the current study and previous work (Dring et al., 2019a) suggest that games-based activity does not affect the postprandial glycaemic response to a standardised meal. This conflicts with previous work using football (Smallcombe et al., 2018) and circuit-based exercise (Short et al., 2012; Short, Pratt & Teague, 2018); although participants were recruited based on low physical activity in previous work (Short, Pratt & Teague, 2018), which may explain these discrepancies. The findings of the present study do however have important ecological application, as acute bouts of games-based exercise incorporated into the daily school routines of adolescents could enhance glucose regulation. Future work should continue to examine the underlying factors that determine the acute glycaemic and insulinaemic responses to different physical activities, particularly with regards to the modality, movement patterns and intensity of the activity.

The variance in physical fitness levels between study participants has been cited as a potential factor which may explain why exercise effects are seen in some previous studies (Cockcroft et al., 2015; Dring et al., 2019a). These suggestions have been made when comparing across separate studies, with no empirical research to date investigating if physical fitness may moderate the response to exercise. The results of the current study provide novel evidence that there was no moderating effect of physical fitness on the postprandial glycaemic and insulinaemic responses, even though fitness was positively related to the overall postprandial insulinaemic response. Despite the metabolic responses being similar between the high- and low-fit groups, the relative exercise intensity of the football session was higher in the low-fit group (80% HRmax) compared to the high-fit group (70% HRmax). Furthermore, the external load – assessed by GPS – was similar between the fitness groups (Table 6.2). This suggests that, in the present study, even though low-fit participants exercised at a higher relative intensity for a similar external load, there was no additional metabolic benefit. Indeed, this is a novel contribution to the literature and future studies should seek to replicate this finding, whilst also increasing the exercise intensity.

The skill level and football experience of the participants was not assessed in the present study, which may be a limitation. Given that there is the potential for all young people to participate in football (or indeed any games-based activity) at school, we did not want to exclude participants based upon their football history or skill level. We also decided to use mixed groups of boys and girls, again because this is often how sessions are run in schools. However, future studies could consider whether factors that may affect the activity patterns of games-based activity (such as skill level and sex) also have a resultant effect upon the potential health benefits.

In summary, although a 60 min bout of football did not affect the postprandial insulinaemic response, the exercise led to a transient reduction in blood glucose concentration 60 min post-exercise, irrespective of physical fitness. This is an important finding, as football is the most popular team sport played by adolescents in England (DCMS, 2018). This highlights the ecological validity of such a modality, with potential to improve adherence in this population and thus, improving glucose regulation. Moreover, the present study provides novel evidence that physical fitness is very important for the postprandial insulinaemic response to a standardised meal, with a ~70% lower postprandial insulinaemic response in high-fit adolescents compared to their low-fit counterparts.

Practical Applications

This chapter demonstrates that a 60 min bout of football – a very popular form of physical activity in adolescents – can transiently improve blood glucose regulation. Such exercise requires minimal equipment and can be easily administered within a school-setting; thus the findings of this thesis would recommend the incorporation of opportunities for such activity within the school day. Additionally, these data further demonstrate the importance of physical fitness for postprandial metabolic health. This further strengthens the argument for promoting improved physical fitness amongst school-aged adolescents, to help combat the development of risk factors for cardiometabolic disease and enhance lifelong health.

Chapter VII

Effect of physical fitness and acute football activity on cognitive function and brain-derived neurotrophic factor in adolescents

7.1 Introduction

Acute bouts of exercise elicit small-moderate beneficial effects on cognitive function in adults (Chang et al., 2012), children (Sibley & Etnier, 2003) and adolescents (Ludyga et al., 2016). However, the exercise-cognition relationship is a complex phenomenon, affected by a number of factors such as the modality, intensity and duration of the exercise bout, age, physical fitness and the cognitive domain assessed (Pontifex et al., 2019; Williams, Hatch & Cooper, 2019). Much of the research in the adolescent population has employed traditional continuous lab-based exercise protocols, examining treadmill running/walking (Browne et al., 2016; Harveson et al., 2016; Soga, Shishido & Nagatomi, 2015) and cycle ergometry (Berse et al., 2015; Budde et al., 2010; Hogan et al., 2013; Stroth et al., 2009). Furthermore, the focus has been primarily on cognitive function immediately post-exercise, including the domains of executive function and working memory (Browne et al., 2016; Berse et al., 2015; Schmidt et al., 2016). There is also some evidence that the benefits of an acute bout of exercise persist for up to 45 min post-exercise (Cooper et al., 2012; Cooper et al., 2016; Cooper et al., 2018); yet the time-course of exercise-induced cognitive effects beyond this are currently unknown. Only one study has demonstrated acute cognitive benefits up to 60 min post-exercise, with improved inhibitory control found 60 min following moderate intensity circuit exercise (Ludyga et al., 2019). However, no study has examined the effect of an acute bout of exercise beyond 1 h post-exercise in adolescents.

Furthermore, many of the acute exercise protocols used in previous studies are difficult to incorporate into a school day due to reliance on specialist equipment, such as motorised treadmills and cycle ergometers, which may not be available in a school setting. This is known to be a prominent barrier to exercise participation in this population (Robbins et al., 2010). Recent research has attempted to address this issue by utilising acute school-based protocols consisting

of sprint intervals (Cooper et al., 2016), shuttle running (Cooper et al., 2012; Etnier et al., 2014), Basketball (Cooper et al., 2018) and cognitively-engaging exercise (Schmidt et al., 2016; Budde et al., 2008). The use of acute games-based activity, such as football, is an attractive modality given that the physical activity patterns of young people are high-intensity and intermittent in nature (Bailey et al., 1995), as seen in team games such as football (Svensson & Drust, 2005). Furthermore, games-based activity is typically a mode of exercise that young people enjoy; a vital consideration for long-term implementation (Howe et al., 2010). Positive effects of such acute school-based protocols have been demonstrated across a range of domains of cognition, including attention (Schmidt et al., 2016; Cooper et al., 2012; Budde et al., 2008), working memory (Cooper et al., 2018) and inhibitory control (Cooper et al., 2016; Cooper et al., 2018). Football is the most popular games-based exercise amongst adolescents (DCMS, 2018), with only one study to date examining the acute effects of football on subsequent cognitive function (Lind et al., 2019). A brief (20 min) bout of high-intensity football improved inhibitory control performance 20 min post-exercise, compared to walking football and a resting control (Lind et al., 2019). However, it is unknown how acute football affects other domains of cognitive function, such as working memory, as well as the duration of the transient improvements post-exercise.

Cross-sectional evidence in adults suggests that those with a higher physical fitness, assessed by $\dot{V}O_{2max}$, have quicker response times on a psychomotor speed task (Fortune et al., 2019). Similar results have been demonstrated in overweight and sedentary children, using $\dot{V}O_{2\ peak}$ as the fitness criterion (Davis & Cooper, 2011). Hillman et al. (2005) found that both high-fit children and adults, assessed by the PACER, a variation of the multi-stage fitness test, performed better on an executive function task than their low-fit counterparts. Whilst there is strong evidence of a positive relationship between physical fitness and cognitive function in children and adults, there is limited knowledge concerning adolescents. Adelantado-Renau et al. (2018) used a battery of fitness tests, including the multi-stage fitness test, in a group of healthy adolescents and found that higher physical fitness was positively associated with academic performance. In addition, higher physical fitness (assessed by the Andersen intermittent test) was associated with a greater

inhibitory control performance in older adolescents (~14 y) (Westfall et al., 2018). However, it is not known how physical fitness affects key cognitive domains such as executive function and working memory in younger adolescents, where they are of particular importance for academic achievement (Cooper, Dring & Nevill, 2016).

Recent reviews suggest that physical fitness moderates the acute exercise-cognition relationship (Chang et al., 2012; Pontifex et al., 2019) – particularly when cognition is measured immediately post-exercise (Chang et al., 2012), or with reference to learning and memory (Pontifex et al., 2019). However, another recent meta-analysis concluded that physical fitness does not moderate the acute exercise response, with respect to aerobic exercise and executive function specifically (Ludyga et al., 2016). It has been shown that adolescents with a higher level of physical fitness, determined by a multi-stage fitness test (Cooper et al., 2018) and a continuous-graded maximal exercise test until exhaustion (Hogan et al., 2013), demonstrate improved response times on an executive function task immediately after cycling (Hogan et al., 2013) and 45 min after basketball exercise (Cooper et al., 2018. Furthermore, error rates were higher (Hogan et al., 2013) and response times were slower (Cooper et al., 2018) following exercise, in low-fit adolescents. In addition, response times on a working memory task were improved, in the high-fit group only, following basketball exercise (Cooper et al., 2018). Overall, the available evidence suggests that higher physical fitness may enhance the post-exercise improvements in cognition. This may also be more applicable to games-based exercise, which has both cognitive and physical demands, whereby those with a higher physical fitness can allocate greater cognitive resources to the activity itself (Ludyga et al., 2016). The underlying mechanisms behind this relationship remain unclear, although it has been surmised that circulating growth factors – particularly brain-derived neurotrophic factor (BDNF) – may have a role to play (Hötting & Röder., 2013; Piepmeier & Etnier, 2015).

BDNF is stated to have an instrumental role in the structural formation and function of the brain (Piepmeier & Etnier, 2015) and plays an important role in the promotion and maintenance of synaptic connectivity (Huang & Reichardt., 2001); which is suggested to be one of the

mechanisms through which BDNF may mediate post-exercise improvements in cognitive function (Pontifex et al., 2019). To date, only resting BDNF has been investigated in relation to objectively measured physical activity in adolescents; whereby physical activity and plasma BDNF were not related (Beltran-Valls et al., 2018). However, mean physical activity and serum BDNF were negatively related in adolescent boys only (Huang et al., 2017). Furthermore, no studies have examined the time-course of BDNF concentrations in the hours following an acute bout of exercise in adolescents – which is an important knowledge gap to fill given the suggested role of BDNF in mediating post-exercise cognitive improvements (Hötting & Röder, 2013; Piepmeier & Etnier, 2015; Pontifex et al., 2019). The response of BDNF to an acute bout of ecologically valid games-based activity in adolescents, and the moderating role of physical fitness in this exercise-BDNF relationship, is currently unknown.

The aim of the present study was to investigate the effect of an acute bout of outdoor Football on executive function, working memory and circulating BDNF concentration in adolescents, for up to 2 hours post-exercise. A secondary aim of the study was to examine whether there were differences in overall cognitive function performance and BDNF concentration between high and low-fit participants, and whether physical fitness moderates cognitive function and BDNF concentrations following exercise.

7.2 Methods

The participants and exercise procedures used in this chapter are the same as the previous chapter (VI). All measures were completed in one experiment, but have been written up as two separate chapters, to facilitate the clear presentation (and full discussion) of all cognitive function data.

7.2.1 Participants

Thirty-six adolescents (20 boys, 16 girls) volunteered to participate in the study. During familiarisation, all participants underwent anthropometric measurements of height (cm), body mass (kg) and sitting height (cm). These were conducted in accordance with descriptions in

section 3.3 of this thesis and were also used to calculate maturity offset (also described in section 3.3). Waist circumference and the sum of our skinfold sites (triceps, subscapular, supraspinale and front thigh) were measured as surrogates of adiposity, in line with descriptions provided in section 3.4 of this thesis. Descriptive participant characteristics are presented in Table 7.1.

Table 7.1 Participant characteristics for the group overall, as well as for the high- and low-fit groups. Data are mean ± SD.

Variable	Overall	High-fit	Low-fit
Age (yrs)	12.6 ± 0.5	12.7 ± 0.5	12.4 ± 0.5
Height (cm)	163.1 ± 7.0	163.5 ± 8.0	162.6 ± 6.1
Body Mass (kg)	53.9 ± 10.0	50.1 ± 8.9 *	57.6 ± 10.0
Waist Circumference (cm)	70.0 ± 8.0	66.0 ± 5.4 *	73.9 ± 8.4
Sum of 4 skinfolds (mm)	60.3 ± 26.8	42.8 ± 12.8	77.8 ± 25.7
Maturity Offset	0.08 ± 0.94	0.20 ± 0.84	-0.05 ± 1.03
MSFT Distance (m)	1160 ± 400	1480 ± 300 **	840 ± 140
Predicted $\dot{V}O_2$peak ($ml \cdot kg^{-1} \cdot min^{-1}$) [a]	47.9 ± 5.2	52.0 ± 3.7 **	43.8 ± 2.5

Abbreviations: MSFT = Multi-Stage Fitness Test.
[a] predicted from the multi-stage fitness test using the equations of Barnett et al., (1993)
* Different compared to low-fit, $p < .05$. ** Different compared to low-fit, $p < .001$

7.2.2 Experimental Design

The study conformed to the Declaration of Helsinki guidelines and was approved by the institution's Human Ethics Committee. Participants were recruited from secondary schools in the East Midlands area of the UK. Written parental consent and participant assent were obtained during the initial phase of recruitment. A health screen was completed by each participant's parent/guardian and was checked by a lead investigator to ensure there were no medical conditions that would affect the child's participation in the study. All participants that enrolled in the study were considered healthy. In particular, any participants that had existing neurological and/or mental health conditions were not eligible to take part. In addition to this, participants with

any physical ailments that could be exacerbated by physical activity were not permitted to take part.

This study employed a randomised, order-balanced, crossover, within-subjects design consisting of two main experimental trials (exercise and resting); separated by at least 7 d. Participants were blind to the trial condition until arrival at school. A familiarisation took place ~ 7 d before the first main trial and allowed participants to be acquainted with all the necessary procedures, including capillary blood sampling and the battery of cognitive function tests. Participants were also familiarised with a Football session, consisting of skill drills and small-sided games to ensure they had the required skills to participate in the Football session as part of the exercise trial.

During the familiarisation, participants also completed the multi-stage fitness test (MSFT) for the determination of physical fitness, as described in section 3.5 of this thesis. Prior to the start of the MSFT, participants were fitted with a heart rate monitor (Firstbeat Team Sport System, Firstbeat Technologies Ltd, Finland). Heart rate was monitored throughout the MSFT and maximum heart rate was recorded upon completion. To encourage maximum effort from the participants, investigators provided verbal encouragement. Performance on the test was determined by the total distance covered (m), with participants assigned to high- and low-fitness groups, based on the median split of the MSFT distance covered for each sex. For descriptive purposes, the whole sample was characterised based on age- and sex-specific centiles; derived from normative performance data in adolescents (Tomkinson et al., 2016).

7.2.2.1 Main Trials

Participants were instructed to record their dietary intake for the 24 hours preceding and during the first experimental trial. Recorded diets were then replicated for the subsequent main trial. Participants were asked to refrain from eating or drinking from 9 pm the previous evening for both days of the two main trials. Water was allowed *ad libitum* at all times. Participants were also asked to refrain from any unusually strenuous physical activity 24 hours prior to the main trials. Parents/guardians were contacted by telephone on the evening prior to each main trial to ensure compliance with these requirements.

On the morning of the main trials, following the overnight, fast participants reported to school (between 8 am and 8:30 am) and were fitted with a heart rate monitor (Firstbeat Team Sport System, Firstbeat Technologies Ltd, Finland). Both trials followed a time matched protocol, with the only difference being the 60 min exercise session. During the exercise trial participants completed 60 min Football (see section 2.3.4); whilst they remained seated in the classroom during the resting control trial.

7.2.3 Experimental Procedures

7.2.3.1 Standardised Breakfast and Lunch

In order to better ascertain the sole effects of exercise, a standardised breakfast and lunch was provided for participants during the experimental trials. The meals were provided in line with descriptions provided earlier in this thesis (section 3.6) and provided 1.5g·kg^{-1} body mass of carbohydrate. Participants were given 15 min to consume each meal. All participants complied with this requirement.

7.2.3.2 Capillary Blood Samples

Capillary blood samples were taken at baseline and immediately, 30 min and 60 min post-exercise. An additional blood sample was taken 60 min post-lunch (2 h post-exercise).

Capillary blood samples were collected in accordance with previous descriptions (section 3.6) and blood was collected into a single 300 µl microvette, with clotting activator (Microvette CB 300 Z, Sarstedt Ltd, UK). The sample was allowed to rest for 30 min at room temperature before undergoing centrifugation at 1000 x g for 15 minutes (Eppendorph 5415C, Hamburg, Germany). Serum was then extracted into 500 µl plastic vials for subsequent analysis. All samples were frozen immediately at −20 °C and transferred to −80 °C as soon as possible.

Brain-derived neurotrophic factor (BDNF) concentrations were determined with a commercially available ELISA (Quantikine ELISA ®, R & D Systems Europe Ltd, UK) as described in section 3.6 of this thesis.

7.2.3.3 Cognitive Function Tests

The cognitive function test battery lasted approximately 8 min and consisted of the Stroop test and the Sternberg Paradigm, completed in this order on a laptop computer (Lenovo ThinkPad T450; Lenovo, Hong Kong). Each test and test level were preceded by instructions on the screen and practice stimuli in order to re-familiarise participants with the test and negate any potential learning effects; the data for the practice stimuli were discarded. Both tests were performed in line with descriptions earlier within this thesis, where specific details of the test protocols can be found (section 3.8). The participants completed the tests in a classroom of 10 participants, in silence and separated so that they could not interact during the tests. Participants were seated 80-100 cm from the screen in a self-selected position that was comfortable. Sound cancelling headphones were worn and the lights in the room dimmed to minimise external disturbances and enhance screen visibility. For each test the variables of interest were the response times (ms) of correct responses (i.e. reaction time + movement time) and the proportion (%) of correct responses made.

7.2.3.4 Exercise Protocol

The exercise consisted of a 60 min football session. Football was chosen because it is high-intensity and intermittent in nature and thus replicates the activity patterns typically observed in this population (Bailey et al., 1995; Howe et al., 2010), as well as being an enjoyable and popular form of games-based activity for young people (DCMS, 2018) and thus has ecological validity. A duration of 60 min was selected to advance on previous work examining the effects of 60 min of Basketball (Cooper et al., 2018), whereas research utilising football protocols is typically shorter in duration (20 min) (Lind et al., 2019). An experienced football coach delivered the sessions to groups of 10 participants, on outdoor facilities at the respective schools. The session consisted of a warm-up (5 min), skill-based drills (25 min) and small-sided games (5 vs 5; 30 min). Heart rate was monitored continuously throughout the session. Maximum heart rate (HRmax), as recorded at the end of the MSFT, and heart rate during the Football session were used to calculate the relative exercise intensity (%HRmax). Additionally, Global Positioning System (GPS) devices

were worn to quantify the external load during the football session using SPI HPU (15 Hz) portable GPS units (GPSports, Australia). The GPS units were fitted to sit between the scapulae, at the base of the cervical spine, using an elasticated shoulder harness. After each exercise session, the data were downloaded to Team AMS software (Team AMS, GPSports). The variables of interest were total distance covered (m) as well as the distance covered in six different speed categories: standing (≤ 0.4 $km \cdot h^{-1}$), walking (>0.4 to 3.0 $km \cdot h^{-1}$), low-intensity running (>3.0 to 8.0 $km \cdot h^{-1}$), moderate-intensity running (>8.0 to 13.0 $km \cdot h^{-1}$), high-intensity running (>13.0 to 18.0 $km \cdot h^{-1}$) and sprinting (> 18.0 $km \cdot h^{-1}$) (Smallcombe et al., 2018).

7.2.4 Statistical Analysis

Data from the Stroop test and Sternberg paradigm were analysed using the open source software, R (www.r-project.org). Response time and accuracy analyses were conducted using Analysis of Variance (ANOVA). Prior to analyses, the response times (of correct responses) were log transformed to exhibit the right-hand skew, typical of human response times. Initially a three-way (trial by time by fitness) ANOVA (with repeated measures for trial and time) was conducted. Where three-way interactions occurred, separate two-way (trial by time) repeated measures ANOVAs for each fitness group were conducted. To explore statistically significant two-way interactions (trial by time), paired samples t-tests with a Bonferroni correction for multiple comparisons were conducted to compare between the trials at each time point. These analyses allow exploration of the effects of the football session, and the moderating effect of fitness, on subsequent cognitive function. For the response time analyses, minimum (<200 ms) and maximum (1500-3000 ms, dependent on task complexity) were applied to eliminate any unreasonably fast and slow responses.

BDNF analysis conducted using SPSS (Version 25; SPSS Inc., Chicago, IL., USA), also adopting a three-way (trial by time by fitness) ANOVA (with repeated measures for trial and time). Heart rate and GPS variables were compared between the high- and low-fitness groups using an independent samples t-test. All data are presented as mean ± SD, unless otherwise stated. Alpha (α) was set at $p < .05$.

7.3. Results

For MSFT performance, the median centile for boys was 65 (range: 20 – 95) and for girls was 90 (range: 70 – 95). For the sample overall, performance was classified as "low" for 13.9% (≤ 40th centile), "moderate" for 13.9% (40th – 60th centile), "high" for 27.8% (60th – 80th centile) and "very high" for 44.4% (≥ 80th centile).

7.3.1 Exercise Characteristics

During the 60 min of football, average heart rate was 151 ± 16 beats·min^{-1}, maximum heart rate was 186 ± 13 beats·min^{-1} and relative exercise intensity was 75 ± 8% (Table 7.2). Furthermore, average ($t_{(32)}$ = -2.8, p = .009) and maximum heart rate ($t_{(32)}$ = 2.7, p = .010), as well as relative exercise intensity ($t_{(32)}$ = -4.1, p < .001), were lower in the high-fit group compared to low-fit (Table 7.2). Average heart rate across the whole 4 h exercise trial (105 ± 13 beats·min^{-1}) was higher than during the whole 4 h resting trial (84 ± 11 beats·min^{-1}, $t_{(35)}$ = 11.8, p < .001).

During the football session, the average total distance covered was 2788 ± 432 m, with the low-fit participants covering a total of 2792 ± 474 m and high-fit participants covering a total of 2786 ± 402 m. There was no significant effect of fitness on the total distance and distance covered across the six different speed categories (p = 0.301, Table 7.2).

Table 7.2. Average and maximum heart rate, relative exercise intensity and GPS characteristics for the group overall, as well as the high- and low-fit groups, during the football session.

Variable	**Overall** (n = 36; 16 girls, 20 boys)	**High-fit** (n = 18; 8 girls, 10 boys)	**Low-fit** (n = 18; 8 girls, 10 boys)
Heart Rate			
Average Heart Rate (beats·min^{-1})	151 ± 16	144 ± 16	158 ± 12*
Maximum Heart Rate (beats·min^{-1})	186 ± 13	180 ± 11	191 ± 11*
Relative Exercise Intensity (% maximum heart rate) [b]	75 ± 8	70 ± 7	80 ± 6*
Distance covered (m) within speed categories			
Standing (≤ 0.4 km·h^{-1})	6 ± 2	7 ± 1	6 ± 2
Walking (>0.4 to 3.0 km·h^{-1})	419 ± 64	398 ± 48	439 ± 72
Low-intensity running (>3.0 to 8.0 km·h^{-1})	1534 ± 234	1521 ± 242	1546 ± 233
Moderate-intensity running (>8.0 to 13.0 km·h^{-1})	648 ± 146	659 ± 133	637 ± 161
High-intensity running (>13.0 to 18.0 km·h^{-1})	160 ± 76	172 ± 58	148 ± 86
Sprinting (> 18.0 km·h^{-1})	22 ± 20	29 ± 21	16 ± 16

Abbreviations: GPS = Global Positioning System. MSFT = Multi-Stage Fitness Test
[b] Relative to the maximum heart rate attained during the MSFT
* Difference between high- and low-fit groups, $p < .01$

7.3.2 Cognitive Function

Data for both cognitive tests, across all time points, for the exercise and control trials are displayed in Table 7.3. Given that there were no differences at baseline between the exercise and resting trials (all $p > .05$) and for ease of interpretation, the figures are displayed as change from baseline.

7.3.2.1 Stroop Test

7.3.2.1.1 Response Times

Congruent level

Overall response times were quicker in the high-fit group compared to the low-fit group (main effect of fitness: high-fit; 719 ± 134 ms, low-fit; 794 ± 164 ms; $F_{(1, 5304)} = 130.2$, $p < .001$). Overall response times were similar between the exercise and resting trial (main effect of trial; $p = .363$) and became quicker across the course of the day (main effect of time; $F_{(3, 5304)} = 18.5$, $p < .001$). The pattern of change between the exercise and resting trial was similar (trial by time interaction:

p = .373). The pattern of change across the exercise and resting trials was, however, different between the high- and low-fit groups (trial by time by fitness interaction; $F_{(3, 5304)}$ = 4.8, p = .002; Fig 7.1). A separate ANOVA revealed a difference in the pattern of change for the low-fit participants (trial by time interaction; $F_{(3, 2619)}$ = 4.10, p = .007, Fig 7.1). Specifically, response times improved to a greater extent 45 min following seated rest compared to 45 min post-exercise (p = .045). However, in the high-fit participants, response times were similar across the morning between the exercise and resting trials (trial by time interaction; p = .231; Fig 7.1).

Incongruent Level

Overall response times were quicker in the high-fit group compared to low-fit (main effect of fitness; high-fit; 960 ± 209 ms, low-fit; 1084 ± 243 ms; $F_{(1, 10668)}$ = 317.1, p < .001). Overall response times were similar between the exercise and resting trial (main effect of trial; p = .994) and became quicker across the course of the day (main effect of time; $F_{(3. 10668)}$ = 22.4, p < .001). The pattern of change was similar between the exercise and resting trial (trial by time interaction: p = .204), as was the pattern of change between the high- and low-fit groups (trial by time by fitness interaction; p = .099).

7.3.2.1.2 Accuracy

Congruent Level

Overall accuracy was similar between the high- and low-fit groups (main effect of fitness; p = .316). Accuracy was also similar between the exercise and resting trial (main effect of trial; p = .324) and across the day (main effect of time; p = .409). The pattern of change across the day was similar between the exercise and resting trial (trial by time interaction; p = .428), as was the pattern of change between the high- and low-fit groups (trial by time by fitness interaction; p = .425).

Incongruent Level

Overall accuracy was similar between the high- and low-fit groups (main effect of fitness; p = .317). Accuracy was also similar between the exercise and resting trial (main effect of trial; p = .317) and across the day (main effect of time; p = .410). The pattern of change across the day

was similar between the exercise and resting trial (trial by time interaction; p = .410), as was the pattern of change between the high- and low-fit group (trial by time by fitness interaction; p = .413).

7.3.2.2 Sternberg Paradigm

7.3.2.2.1 Response Times

One-item

Overall response times were quicker in the high-fit group compared to their low-fit counterparts (main effect of fitness; high-fit; 496 ± 91 ms, low-fit; 529 ± 124 ms, $F_{(1, 4372)}$ = 44.1, p < .001). However, response times were similar between the exercise and resting trials (main effect of trial; p = .639) but became quicker across the course of the day (main effect of time; $F_{(3, 4372)}$ = 11.8, p < .001). The pattern of change in response times was different between the exercise and resting trial (trial by time interaction: $F_{(3, 4372)}$ = 9.2, p < .001) and furthermore, the pattern of change between the exercise and resting trial was different between the high- and low-fit participants (trial by time by fitness interaction; $F_{(3, 4372)}$ = 4.2, p = .006). A separate ANOVA revealed a difference in the pattern of change for high-fit participants (trial by time interaction; $F_{(3, 2225)}$ = 3.0, p = .030, Fig 7.2). Specifically, response times were improved 45 min post-exercise, when compared to 45 min seated rest (p = .022). A separate ANOVA revealed a difference in the pattern of change for low-fit participants (trial by time interaction; $F_{(3, 2147)}$ = 9.7, p < .001, Fig 7.2). Specifically, low-fit participants were quicker 90 min following seated rest, when compared to 90 min post-exercise (p = .020)

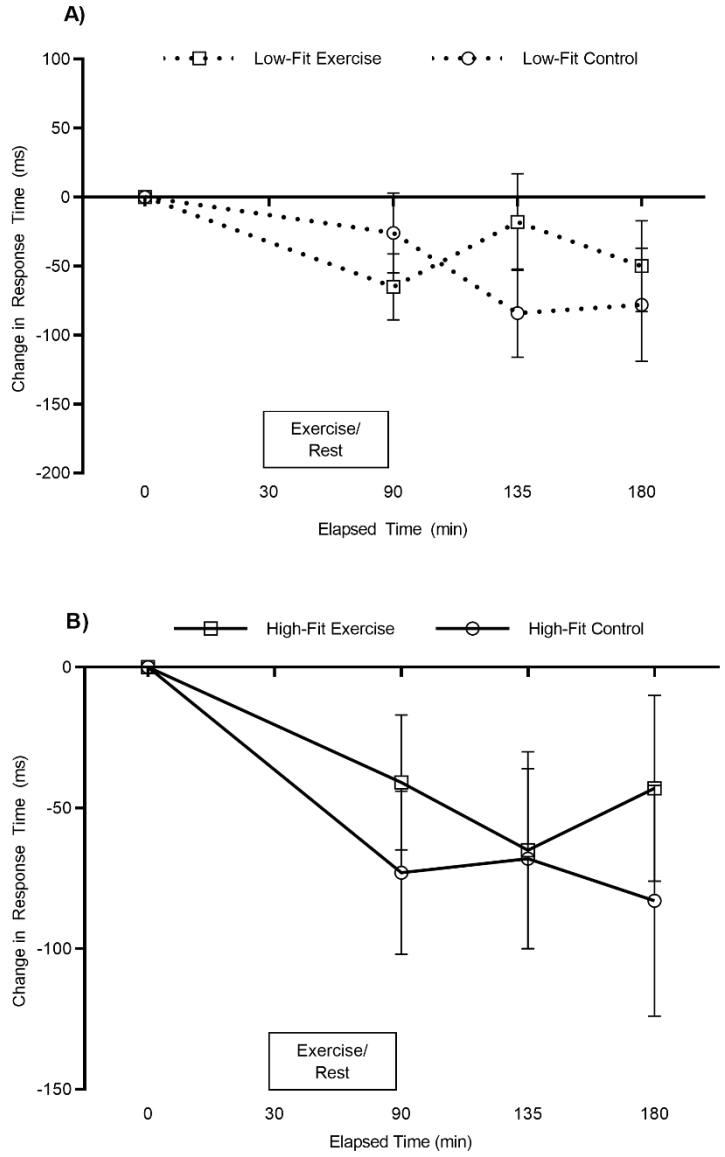

Fig 7.1. Congruent Stroop test response times across the exercise and resting trials for the low-fit (A) and high-fit (B) groups. Data are mean ± SEM.

Three-item

Overall response times were quicker in the high-fit group compared to low-fit (main effect of fitness; high-fit: 628 ± 115 ms, low-fit: 703 ± 160 ms, $F_{(1, 8725)} = 184.4$, $p < .001$). However, response times were similar between the exercise and resting trials (main effect of trial; $p = .327$) but became quicker across the course of the day (main effect of time; $F_{(3, 8725)} = 7.5$, $p < .001$). The pattern of change was different between the exercise and resting trials (trial by time interaction: $F_{(3, 8725)} = 2.7$, $p = .042$), as was the pattern of change across the day between the high- and low-fit groups (trial by time by fitness interaction; $F_{(3, 8725)} = 3.9$, $p = .009$). A separate ANOVA revealed a similar pattern of change between the exercise and resting trial for the low-fit

participants (trial by time interaction; p = .390, Fig 7.2). A separate ANOVA revealed a difference in the pattern of change for the high-fit participants (trial by time interaction; $F_{(3, 4388)}$ = 6.5, p < .001, Fig 7.2). Specifically, response times were quicker 90 min following seated rest, when compared to 90 min post-exercise (p < .001).

Five-item

Overall response times were also quicker in the high-fit group compared to low-fit on the five item level of the Sternberg paradigm (main effect of fitness; high-fit: 761±151 ms, low-fit: 834±207 ms, $F_{(1, 8236)}$ = 99.8, p < .001). Overall response times were quicker in the control trial compared to the exercise trial (main effect of trial; exercise: 803±168 ms, control: 791±200 ms, $F_{(1, 8236)}$ = 4.0, p = .046) and became quicker over the course of the day (main effect of time; $F_{(3, 8236)}$ = 27.1, p < .001). The pattern of change was different between the exercise and resting trial (trial by time interaction: $F_{(3, 8236)}$ = 5.6, p < .001) and furthermore, the pattern of change across the day was different between the high- and low-fit groups (trial by time by fitness interaction; $F_{(3, 8236)}$ = 4.6, p = .003). A separate ANOVA revealed a difference in the pattern of change for the low-fit participants (trial by time interaction; $F_{(3, 4041)}$ = 3.4, p = .018, Fig 7.2). Specifically, response times were slower immediately post-exercise compared to immediately after seated rest (p = .012) as well as 90 min following seated rest (p = .033). A separate ANOVA revealed a difference in the pattern of change for the high-fit participants (trial by time interaction; $F_{(3, 4195)}$ = 7.7, p < .001, Fig 7.2). Specifically, response times were quicker, to a greater extent, 45 min following seated rest compared to 45 min post-exercise (p = .003).

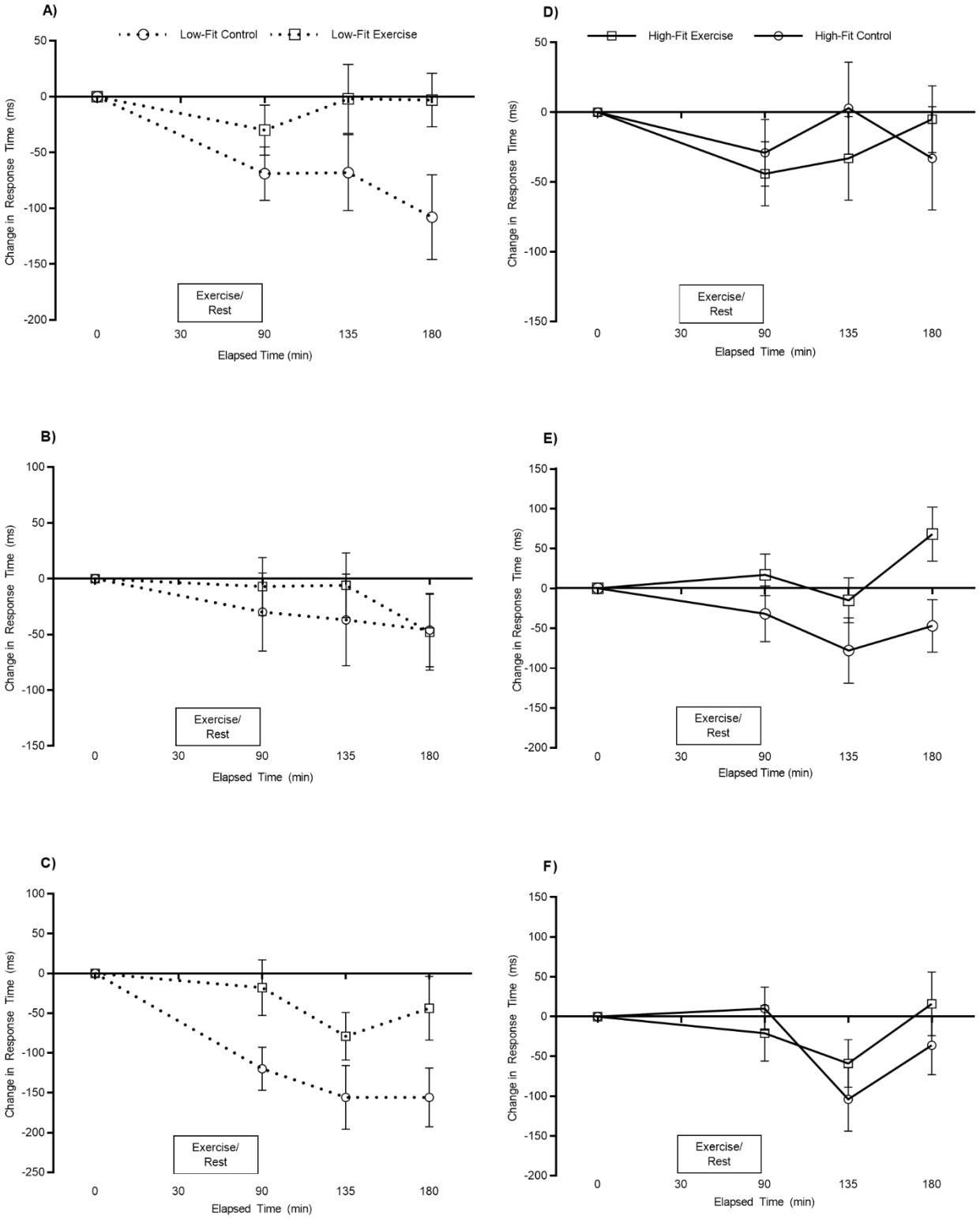

Fig 7.2. Response times, split by fitness group, across the exercise and resting trials for the One Item (A & D), Three Item (B & E) and Five Item (C & F) levels of the Sternberg Paradigm. Data are mean ± SEM.

7.3.2.2.2 Accuracy

One item

Overall accuracy was similar between the high- and low-fit groups (main effect of fitness; p = .314), similar between the exercise and resting control trials (main effect of trial; p = .314) and similar across the course of the day (main effect of time; p = .398). The pattern of change across the day was similar between the exercise and resting trial (trial by time interaction; p = .396) as was the pattern of change between the high- and low-fit participants (trial by time by fitness interaction; p = .399).

Three-item

Overall accuracy was similar between the high- and low-fit groups (main effect of fitness; p = .315), similar between the exercise and resting trials (main effect of trial; p = .317) and similar across the course of the day (main effect of time; p = .398). The pattern of change across the day was similar between the exercise and resting trial (trial by time interaction; p = .394), as was the pattern of change between the high- and low-fit groups (trial by time by fitness interaction; p = .390).

Five-item

Overall accuracy was similar between the high- and low-fit groups (main effect of fitness; p = .321), similar between the exercise and resting trials (main effect of trial; p = .316) and similar across the course of the day (main effect of time; p = .404). The pattern of change across the day was similar between the exercise and resting trial (trial by time interaction; p = .400), as was the pattern of change between the high- and low-fit groups (trial by time by fitness interaction; p = .412).

7.3.3 Brain Derived Neurotrophic Factor

Serum BDNF concentration was similar between the high- and low-fit groups (main effect of fitness; high-fit: 27.1 ± 6.8 $ng \cdot ml^{-1}$, low-fit: 29.1 ± 7.8 $ng \cdot ml^{-1}$, p = .210). Serum BDNF concentrations were also similar between the exercise and resting trial (main effect of trial; p = .082) and were also similar across the course of the day (main effect of time; p = .085). The

pattern of change was similar between the exercise and resting trial (trial by time interaction; p = .167), as was the pattern of change between the high- and low-fit groups (trial by time by fitness interaction; p = .704, Table 7.4).

Table 7.3. Cognitive function data across the exercise and control trials, for the high- and low-fit groups, as well as the group overall. Data are mean ± SD.

Test	Level	Variable	Group	Control Trial				Exercise Trial			
				Pre-rest	*Immediately post-rest*	*45 min post-rest*	*90 min post-rest*	*Pre-exercise*	*Immediately post-exercise*	*45 min post-exercise*	*90 min post-exercise*
Stroop	*Simple*	Response Time (ms)	Low-fit	857 ± 192	812 ± 179	753 ± 136	756 ± 144	828 ± 172	755 ± 161	807 ± 152	785 ± 175
			High-fit	772 ± 170	717 ± 141	723 ± 150	712 ± 149	746 ± 145	712 ± 78	684 ± 85	695 ± 134
			Overall	815 ± 185	764 ± 165	738 ± 141	734 ± 145	787 ± 161	733 ± 127	745 ± 139	740 ± 161
		Accuracy (%)	Low-fit	99.7 ± 1.2	95.8 ± 7.7	94.2 ± 8.4	94.7 ± 9.0	99.4 ± 1.6	96.9 ± 4.2	97.8 ± 3.1	96.7 ± 5.4
			High-fit	97.2 ± 4.0	97.8 ± 3.6	95.8 ± 5.9	96.1 ± 4.4	98.9 ± 2.6	97.8 ± 3.1	95.8 ± 6.3	95.0 ± 5.9
			Overall	98.5 ± 3.1	96.8 ± 6.0	95.0 ± 7.2	95.4 ± 7.0	99.2 ± 2.2	97.4 ± 3.7	96.8 ± 5.0	95.8 ± 5.5
	Complex	Response Time (ms)	Low-fit	1130 ± 232	1074 ± 236	1059 ± 219	1017 ± 285	1168 ± 226	1094 ± 291	1089 ± 242	1042 ± 223
			High-fit	1037 ± 253	939 ± 192	954 ± 202	973 ± 235	957 ± 202	950 ± 206	920 ± 185	953 ± 193
			Overall	1084 ± 243	1006 ± 224	1007 ± 216	995 ± 257	1062 ± 237	1022 ± 260	1005 ± 231	997 ± 214
		Accuracy (%)	Low-fit	96.1 ± 5.4	94.9 ± 6.7	92.7 ± 10.5	92.9 ± 10.0	96.7 ± 4.3	91.4 ± 13.5	95.3 ± 4.9	93.6 ± 11.2
			High-fit	96.3 ± 4.0	94.9 ± 4.6	94.2 ± 5.4	95.1 ± 5.0	96.3 ± 3.9	94.9 ± 5.2	93.6 ± 5.7	91.8 ± 5.4
			Overall	96.2 ± 4.6	94.9 ± 5.6	92.9 ± 8.3	94.0 ± 7.8	96.5 ± 4.0	93.1 ± 10.2	94.4 ± 5.2	92.7 ± 8.7
Sternberg	*One item*	Response Time (ms)	Low-fit	588 ± 164	520 ± 102	531 ± 110	480 ± 118	533 ± 135	502 ± 83	538 ± 135	535 ± 120

		High-fit	516 ± 97	486 ± 87	507 ± 120	482 ± 76	517 ± 111	473 ± 56	476 ± 85	508 ± 91
		Overall	552 ± 138	503 ± 95	519 ± 114	481 ± 98	525 ± 122	488 ± 71	507 ± 115	521 ± 106
	Accuracy (%)	Low-fit	96.5 ± 5.3	97.9 ± 4.3	95.8 ± 5.3	94.8 ± 9.9	96.2 ± 5.3	94.8 ± 7.5	95.5 ± 5.6	93.8 ± 10.9
		High-fit	97.6 ± 3.1	97.2 ± 4.9	96.9 ± 4.4	97.2 ± 4.4	97.9 ± 3.7	97.2 ± 4.4	97.2 ± 3.8	95.8 ± 5.3
		Overall	97.0 ± 4.4	97.6 ± 4.6	96.4 ± 4.8	96.0 ± 7.6	97.0 ± 4.6	96.0 ± 6.2	96.4 ± 4.8	94.8 ± 8.5
Three item	Response Time (ms)	Low-fit	743 ± 185	718 ± 157	691 ± 185	697 ± 153	696 ± 118	705 ± 158	698 ± 146	674 ± 186
		High-fit	660 ± 149	621 ± 113	597 ± 100	613 ± 85	628 ± 111	631 ± 123	606 ± 98	672 ± 128
		Overall	701 ± 171	670 ± 144	644 ± 154	655 ± 129	662 ± 118	668 ± 145	652 ± 131	673 ± 157
	Accuracy (%)	Low-fit	97.1 ± 3.5	96.0 ± 5.1	93.2 ± 8.0	92.9 ± 10.0	96.2 ± 5.1	95.7 ± 6.1	95.8 ± 5.1	95.1 ± 5.1
		High-fit	97.2 ± 2.6	96.2 ± 4.7	95.3 ± 5.4	95.0 ± 4.9	95.3 ± 6.0	95.3 ± 3.8	95.7 ± 4.6	94.8 ± 5.7
		Overall	97.1 ± 3.0	96.1 ± 4.9	94.3 ± 6.8	93.9 ± 7.9	95.7 ± 5.5	95.5 ± 5.0	95.7 ± 4.8	95.0 ± 5.3
Five item	Response Time (ms)	Low-fit	926 ± 205	802 ± 222	762 ± 259	787 ± 182	887 ± 172	878 ± 215	794 ± 189	838 ± 181
		High-fit	799 ± 175	813 ± 194	701 ± 154	745 ± 146	771 ± 128	741 ± 125	727 ± 118	794 ± 147
		Overall	863 ± 199	807 ± 206	732 ± 213	766 ± 164	829 ± 161	810 ± 187	761 ± 159	816 ± 164
	Accuracy (%)	Low-fit	93.8 ± 6.5	89.4 ± 11.9	86.8 ± 16.5	90.5 ± 13.9	91.8 ± 9.5	89.8 ± 9.2	88.7 ± 13.4	86.8 ± 12.8
		High-fit	94.1 ± 6.9	93.2 ± 6.5	92.2 ± 6.7	93.1 ± 7.3	93.2 ± 7.1	94.3 ± 5.8	92.7 ± 6.8	91.7 ± 6.8
		Overall	92.5 ± 8.3	92.0 ± 7.9	90.7 ± 10.6	89.2 ± 10.4	92.5 ± 8.3	92.0 ± 7.9	90.7 ± 10.6	89.2 ± 10.4

Table 7.4. Serum BDNF concentrations ($ng \cdot ml^{-1}$) across the course of the resting and exercise trials, for the high- and low-fit groups, as well as the group overall. Data are mean ± SD

Group	**Control Trial**					**Exercise Trial**				
	Pre-rest	*Immediately post-rest*	*30 min post-rest*	*60 min post-rest*	*120 min post-rest*	*Pre-exercise*	*Immediately post-exercise*	*30 min post-exercise*	*60 min post-exercise*	*120 min post-exercise*
Low-Fit	28.7 ± 6.3	29.2 ± 7.4	28.8 ± 6.2	31.1 ± 6.0	30.2 ± 8.2	28.1 ± 5.5	31.0 ± 10.6	28.4 ± 9.2	27.2 ± 8.2	28.5 ± 9.9
High-Fit	26.1 ± 5.0	26.3 ± 8.4	28.1 ± 6.7	29.3 ± 6.3	31.1 ± 7.3	23.4 ± 4.3	26.3 ± 7.2	26.8 ± 6.8	27.0 ± 5.9	26.3 ± 7.5
Overall	27.4 ± 5.8	27.8 ± 8.0	28.5 ± 6.3	30.2 ± 6.1	30.6 ± 7.7	25.8 ± 5.4	28.6 ± 9.2	27.6 ± 8.0	27.1 ± 7.1	27.4 ± 8.7

7.4. Discussion

The findings of the present study show that acute football activity did not influence subsequent information processing, inhibitory control and working memory response times for this group of adolescents overall. However, response times for the high-fit group were quicker across all levels of cognitive tasks, compared to the low-fit group. When considering the moderating role of fitness on the acute responses to exercise, 60 min of football was beneficial for working memory in the high-fit group, whereas working memory tended to be unaffected by exercise in the low-fit group. The present study is also the first to measure the time course of BDNF post-exercise in an adolescent population, with serum BDNF unaffected by acute football activity and fitness.

The current study demonstrates that response times, during information processing, inhibitory control and working memory tasks, are quicker in adolescents with a higher physical fitness, when compared to their low-fit counterparts. However, this finding is cross-sectional in nature and does not necessarily reflect a causal link. Despite this, recent meta-analyses in children and adolescents demonstrate that chronic exercise interventions – which aim to improve physical fitness – lead to improvements in cognitive function (Ludyga et al., 2020; Xue et al., 2019). The findings of the present study extends previous cross-sectional findings in children (Davis & Cooper, 2011; Hillman et al., 2005; Adelantado-Renau et al., 2018) and adults (Fortune et al., 2019) to three distinct domains of cognitive function (information processing, inhibitory control and working memory) in adolescents. Response times were consistently quicker in the high-fit group across the congruent and incongruent levels of the Stroop Task, as well as across all three levels of the Sternberg Paradigm, compared to the low-fit group. These faster response times in the high fit group for all levels of Stroop and Sternberg, extend the findings from chapter IV, whereby only response times on the one-item level of the Sternberg paradigm were associated with fitness. This enhanced cognition in high-fit adolescents may explain the improved academic performance in high-fit young people that has previously been reported (Lima et al., 2018; Muntaner-Mas et al., 2018). The findings of the current study, and the findings of chapter IV, highlight the importance of high levels of physical fitness for cognitive function in adolescents.

The current study also demonstrates that the acute benefits to working memory following exercise were exclusive to the high-fit group only. This is an important finding, given that physical fitness is suggested as a key moderator of the exercise-cognition relationship (Chang et al., 2012; Pontifex et al., 2019), yet there are few empirical studies directly investigating this, especially in adolescents. Recent work has investigated this through a 60 min basketball session (Cooper et al., 2018) and a 20 min bout of cycling (Hogan et al., 2013). Even though the modality and duration of exercise are vastly different, both studies concluded that the improvement in cognition, following an acute bout of exercise, was enhanced in those considered high-fit; in line with the findings of the present study. An explanation for this may be the differences in relative exercise intensity during the football activity, with the low-fit group working at a higher relative exercise intensity (~80% HRmax) compared to the higher fit children (~70% HRmax). It is possible that for the low-fit children the exercise was of too high an intensity and thus too demanding. A recent review suggests that enhancements in cognitive function, following exercise, tend to occur under moderate intensities with attenuated effects under light- and high-intensities; which is consistent with an inverted-U theory (Pontifex et al., 2019). An additional explanation might be, consistent with the transient hypofrontality hypothesis, that neural activity in the brain particularly in the prefrontal cortex is reduced as a result of very high-intensity exercise (Dietrich, 2006).

The present study also provides novel evidence regarding the effects of football on subsequent cognitive function (particularly working memory) in adolescents, with only one previous study investigating the acute effects of football (Lind et al., 2019). The majority of previous work in adolescents has used traditional forms of exercise; such as continuous running (Browne et al., 2016; Harveson et al., 2016; Etnier et al., 2014; Budde et al., 2008), walking (Soga et al., 2015) and cycling (Berse et al., 2015; Hogan et al., 2013; Stroth et al., 2009). Whilst traditional exercise protocols are easy to control in a laboratory setting, they do not necessarily reflect the physical activity patterns of young people (Bailey et al., 1995). The use of football may provide an attractive model; viable for adolescents and thus has real-world applicability. Whilst the cognitive benefits following football were exclusive to high-fit individuals and the domain of working memory in the

present study, football can still be an attractive modality of exercise for adolescents, given the known health benefits (Smallcombe et al., 2018), the popularity (DCMS, 2018) and the ease of access to the equipment needed.

The present study is the first to examine the time-course of circulating BDNF in adolescents following an acute bout of exercise. This is an important knowledge gap, given the potential mediating role of BDNF in the exercise-cognition relationship (Hötting & Röder, 2013; Piepmeier & Etnier., 2015) and the transient nature of improvements seen in cognitive function following exercise. Whilst peripheral BDNF increases immediately after acute bouts of exercise in adults (Dinoff et al., 2017; Szuhany et al., 2015), data from the current study did not provide evidence of this effect in adolescents. The post-exercise increase in BDNF is positively associated with the intensity and duration of the exercise bout (Dinoff et al., 2017). The exercise bout in the current study was of a sufficient duration, however the intensity may not have been high enough to elicit increases in BDNF post-exercise. The present study did however demonstrate cognitive improvements post-exercise, despite the lack of change in peripheral BDNF. This may be explained by the fact that central BDNF (in the brain) was not measured in the present study, due to the constraints of such an assessment in adolescents, and arguably central BDNF is more important for the cognitive benefits of exercise. Alternatively, another mechanism is mediating these exercise-induced cognitive benefits in the high-fit group.

A potential limitation of the present study is that the socioeconomic status of the participants was not accounted for. It has been reported that socioeconomic status is implicated in the development of attentional processes in young children (Mezzacappa et al., 2004) and executive function throughout childhood and adolescence (Lawson et al., 2018). However, there is evidence suggesting that a lower socioeconomic status is associated with lower levels of physical activity (Stalsberg & Pedersen, 2010) and physical fitness (Pavón et al., 2010) in adolescents; which suggests that the effect of socioeconomic status on cognitive function may, in part, be mediated through physical activity and physical fitness. The relationship between physical fitness and cognitive function in the present study is cross-sectional and thus, causation cannot be attributed.

However, this is still an important finding as this relationship was evident across all test levels for both the Stroop and the Sternberg. Whilst the smaller number of trials used for Stroop and Sternberg in the present study, in comparison with earlier adult studies, could be seen as a limitation it was necessary to facilitate the use of both tests within a realistic timeframe. The choice of control condition (seated rest) in the present study could also be considered a potential limitation, particularly as the exercise session included both physical and cognitive elements. However, it would be difficult to match the social interactions of the exercise session and the use of such a control condition also offers ecological validity.

Overall, the findings of the present study show that high-fit participants performed better across tests of information processing, inhibitory control and all levels of working memory tasks compared to the low-fit group. In addition, the current study also provides novel evidence supporting physical fitness as a moderator of the exercise-cognition relationship. In particular, working memory was improved in the high-fit group 45 min post-exercise, whereas it was unaffected in the low-fit group. The present study also provides novel evidence that a 60 min bout of Football did not alter peripheral BDNF concentration in an adolescent population, nor was BDNF affected by physical fitness. Overall, these findings suggest that physical fitness is an important determinant of cognitive performance in adolescents; and that acute bouts of exercise, appropriate to the fitness levels of the young people, can also enhance subsequent cognition.

Practical Applications

The cognitive benefits were dependent on physical fitness; with only high-fit participants showing improvements in working memory. This suggests that opportunities for exercise within the school day must be appropriate for the young people and, importantly, that a ‘one size fits all’ approach will not elicit cognitive benefits for all young people. Despite these exclusive improvements, the football activity did not lead to worse performance in the low-fit group. Thus, it is still recommended that opportunities for such activity are included within the school day, both given the popularity and lack of specialist facilities required to achieve the health benefits demonstrated in the previous chapter.

Chapter VIII

Effect of two-weeks of school-based sprint training on physical fitness, risk factors for cardiometabolic disease and cognitive function in adolescent girls: a randomised controlled trial

8.1 Introduction

Adolescence is crucial phase of development during the early years of life, with many physiological changes occurring (Beunen, Rogal & Molina, 2006), as well as structural and behavioural changes to the brain (Blakemore, Burnett & Dahl, 2010). Despite this, and the well documented benefits of physical activity, it is alarming that such a small proportion of boys (24%) and girls (18%), between the ages of 5 to 15 years in the UK, are meeting the recommended physical activity guidelines of 60 min of moderate-to-vigorous physical activity per day (Scholes & Mindell, 2016). Furthermore, there is strong evidence showing that physical activity levels decline throughout adolescence, with an average 7% reduction in physical activity each year from 12 to 19 years (Dumith et al., 2011).

Cross-sectional studies have demonstrated that lower levels of physical activity during adolescence are associated with poor cardiometabolic health (Ekelund et al., 2012), which was also evidenced in Chapter IV, although it is not known if this is because of the effect of physical activity on physical fitness and adiposity (Chapter IV). Subsequently, poor cardiometabolic health in childhood is related to poor health in adulthood (Laitinen et al., 2012) and is causally linked with the development of many chronic diseases such as cardiovascular disease and type 2 diabetes (Booth, Roberts & Laye, 2012). Furthermore, cognition is important during adolescence, as it is related to mental and physical health (Diamond, 2013) and it has been suggested that lower levels of physical activity and physical fitness are associated with poorer cognitive function (Sibley & Etnier, 2003; Hötting & Röder, 2013; Chapter IV; Chapter VII). Physical activity interventions provide cost-effective methods to attenuate the development of cardiometabolic risk factors (Gleeson et al., 2011; Röhling et al., 2016) and potentially improve cognitive function,

although the evidence regarding the effectiveness of such interventions in adolescents is less conclusive, with most research focusing on children (Wassenaar et al., 2020).

The use of school-based exercise interventions is receiving greater attention (Bond et al., 2017), as this approach is much more applicable for this population given that a large proportion of the day is spent at school. However, most school-based interventions conducted in adolescents have lasted from ~7 to 10 weeks (Bond et al., 2017), meaning that the efficacy and feasibility of short-term interventions, which may be more realistic in a school setting, are less well understood. Martin et al. (2018) found that four weeks of running-based sprint training (3 sessions per week, 5-6 x 30 s) improved predicted $\dot{V}O_{2peak}$ and reduced a clustered risk score for cardiometabolic disease (waist circumference, systolic blood pressure, fasting glucose, HDL-C and triglycerides) in a sample (n = 52) of boys and girls aged 15 to 16 years. Other research has shown that with two-weeks of cycling at 90% peak power output (3 sessions per week, 8-10 x 1 min with 75 s rest), in a sample of boys and girls (n = 13) aged 13 to 14 years (Bond et al., 2015) and 9 boys aged 13 – 14 years (Cockcroft et al., 2019), that there were improvements in novel markers of health (such as heart rate variability), but not traditional risk factors such as fasting glucose and insulin concentrations (Bond et al., 2015; Cockcroft et al., 2019). Furthermore, in the Cockcroft et al. (2019) study, there were no improvements in fasting or postprandial markers of metabolism.

Whilst both training interventions were conducted at secondary schools (Bond et al., 2015; Cockcroft et al., 2019), the ecological validity of using a cycle ergometer should be questioned. If a school is to adopt high intensity interval physical activity interventions, the required equipment should be minimal so that it can easily be implemented without creating further barriers. Furthermore, previous studies (Bond et al., 2015; Cockcroft et al., 2019) have not included a control group and only compared pre- to post-intervention changes in those undertaking the intervention. In order to ascertain exercise-related effects, along with better precision, a comparator arm (typically a control group or another exercise group) should be included and the outcomes at follow-up, whilst controlling for baseline scores, should be compared between groups (Hecksteden et al., 2018; Ritz et al., 2020). Furthermore, the test meal used previously (Cockcroft

et al., 2019) was an OGTT, which is not necessarily ecologically valid for an adolescent population and thus, mixed meal approaches should be examined.

Exercise interventions implemented to improve cognitive function in adolescents have received less attention (Wassenaar et al., 2020). Of the available evidence to date, most school-based interventions are between 6 to 12 weeks in length and typically use moderate intensity exercise (60-70% HR_{max}), with interventions focusing on coordinative exercise (Ludyga et al., 2018), team games (Schmidt et al., 2015) or circuit based exercise (Chen et al., 2016). These interventions, in 50 boys and girls aged 12 – 13 y (Chen et al., 2016) and 181 boys and girls aged 11 – 12 y (Schmidt et al., 2015), have led to improvements in error rates during a shifting task (Chen et al., 2016; Schmidt et al., 2015) and improved response times during congruent and incongruent levels of a Stroop task in 36 boys and girls aged 12 – 13 y (Ludyga et al., 2018). However, the aforementioned interventions have typically recruited older adolescents (aged 14 – 16 y), mixtures of boys and girls and used intervention durations of 8 – 12 weeks. The one study utilising a high-intensity interval protocol to date employed short duration bouts (8-10 min) of aerobic exercises (shuttle runs, skipping, jumping jacks) and combined aerobic and resistance exercise training (~75% HRmax), in 65 boys and girls aged 15 – 16 y, over a period of 8 weeks (Costigan et al., 2016). Small-moderate improvements in executive function performance were evidenced in both groups in comparison to a control group (Costigan et al., 2016). Whilst a range of moderate intensity school-based interventions of a longer duration (Chen et al., 2016; Ludyga et al., 2018; Schmidt et al., 2015), and an 8 week high-intensity intervention (Costigan et al., 2016), suggest an improvement in cognitive function, the effects of short-term high-intensity interventions, implemented at school, on subsequent cognitive function are poorly understood and require further investigation.

Girls are typically less physically active than boys during adolescence (Dumith et al., 2011; Scholes & Mindell, 2016) and under-represented in the research. In addition, data from a previous study in this thesis (Chapter IV) demonstrate that younger adolescent girls (11 – 12 y) have a poorer cardiometabolic health profile when compared to boys of the same age. In light of this and

given that physical activity levels decline throughout adolescence (Dumith et al., 2011), targeting younger adolescents for exercise interventions provides an opportunity to attenuate this decline and improve physical and mental wellbeing. Therefore, the aims of the present study were to investigate the effects of a two-week, school-based sprint-interval training intervention on risk factors related to cardiometabolic disease, and cognitive function, in adolescent girls.

8.2 Methods

8.2.1 Experimental Design

The study conformed to the Declaration of Helsinki guidelines and was approved by the Nottingham Trent University Human Ethics Committee. Participants were randomised to either the intervention group (sprint training) or a control group. The study consisted of three laboratory-style visits in a school classroom and six training sessions (over 2 weeks) at the school facilities, which took place over a 4-week period[2] (Fig 8.1A). The first visit was an initial familiarisation. The second visit consisted of the pre-intervention measures, which took place 48 h prior to starting the training period. The experimental group then completed six supervised sprint sessions over the subsequent two weeks, whereas the control group continued their normal daily routines; before all participants completed the third experimental visit consisting of the post-intervention measures, which took place 48 h following the final training session. In consequence to the short notice rescheduling of the follow-up measures, due to school closure due to the COVID-19 pandemic, the post-intervention multi-stage fitness test (described below) was performed outside, whereas pre-intervention this was performed indoors. This was because the School could not make indoor testing facilities available within only 24 hours of closure by the UK Government.

8.2.2 Participants

Twenty adolescent girls were recruited from year 7 of a local secondary school. Written parental consent and child assent were obtained prior to enrolment in the study. A health screen was

[2] The original design of the study entailed a 4-week training period, totalling 12 sprint sessions. However, due to the COVID-19 pandemic and the short-notice school closures, the intervention was reduced to 2 weeks so that follow-up measures could be obtained for the thesis prior to lockdown and school closures.

completed on behalf of the participant by the parent/guardian, which was checked by a member of the research team. Groups of participants (10 in each) from two different classes were randomly allocated to the intervention and control group. Initially, ten participants enrolled into each group; however, four participants were not able to complete the study[3]. This resulted in a total of 16 participants (8 in each group) completing the study. A summary of baseline participant characteristics can be seen in Table 8.1.

Table 8.1. Descriptive summary of baseline participant characteristics and physical activity metrics for the intervention and control groups.

Variable	Group			
	Intervention (n = 8)		*Control (n = 8)*	
	Mean ± SD	Range	Mean ± SD	Range
	Participant Characteristics			
Age (y)	11.8 ± 0.2	11.6 – 12.0	11.6 ± 0.4	11.2 – 12.2
Height (cm)	155.3 ± 7.4	142.5 – 165.5	159.7 ± 7.4	147.9 – 171.7
Maturity Offset (y)	0.01 ± 0.32	-0.49 – 0.42	0.13 ± 0.52	-0.59 – 1.14
Body Mass (kg)	43.2 ± 8.6	33.8 – 57.4	47.6 ± 9.9	33.8 – 60.5
BMI ($kg \cdot m^{-2}$)	17.8 ± 2.2	15.0 – 21.6	18.5 ± 3.0	15.3 – 23.2
BMI Centile	44.4 ± 31.7	5.3 – 89.4	52.9 ± 36.0	9.3 – 94.9
Sum of Skinfolds (mm)	50.0 ± 17.3	30.2 – 83.5	58.6 ± 30.0	23.4 – 117.5
	Physical Activity Variables			
Average Acceleration (mg)	22.5 ± 5.3	17.4 – 33.2	21.5 ± 3.1	18.7 – 25.6
Intensity Gradient (AU)	-2.32 ± 0.08	-2.44 – -2.22	-2.39 ± 0.12	-2.53 – -2.26

Abbreviations: SD; Standard Deviation. BMI; Body Mass Index. AU; Arbitrary Unit.
The R^2 for the intensity gradient represents the goodness of fit between the log-linear relationship of time and intensity (Rowlands et al., 2018).

[3] These participants were required to self-isolate, again due to the COVID-19 pandemic, and thus could no longer participate.

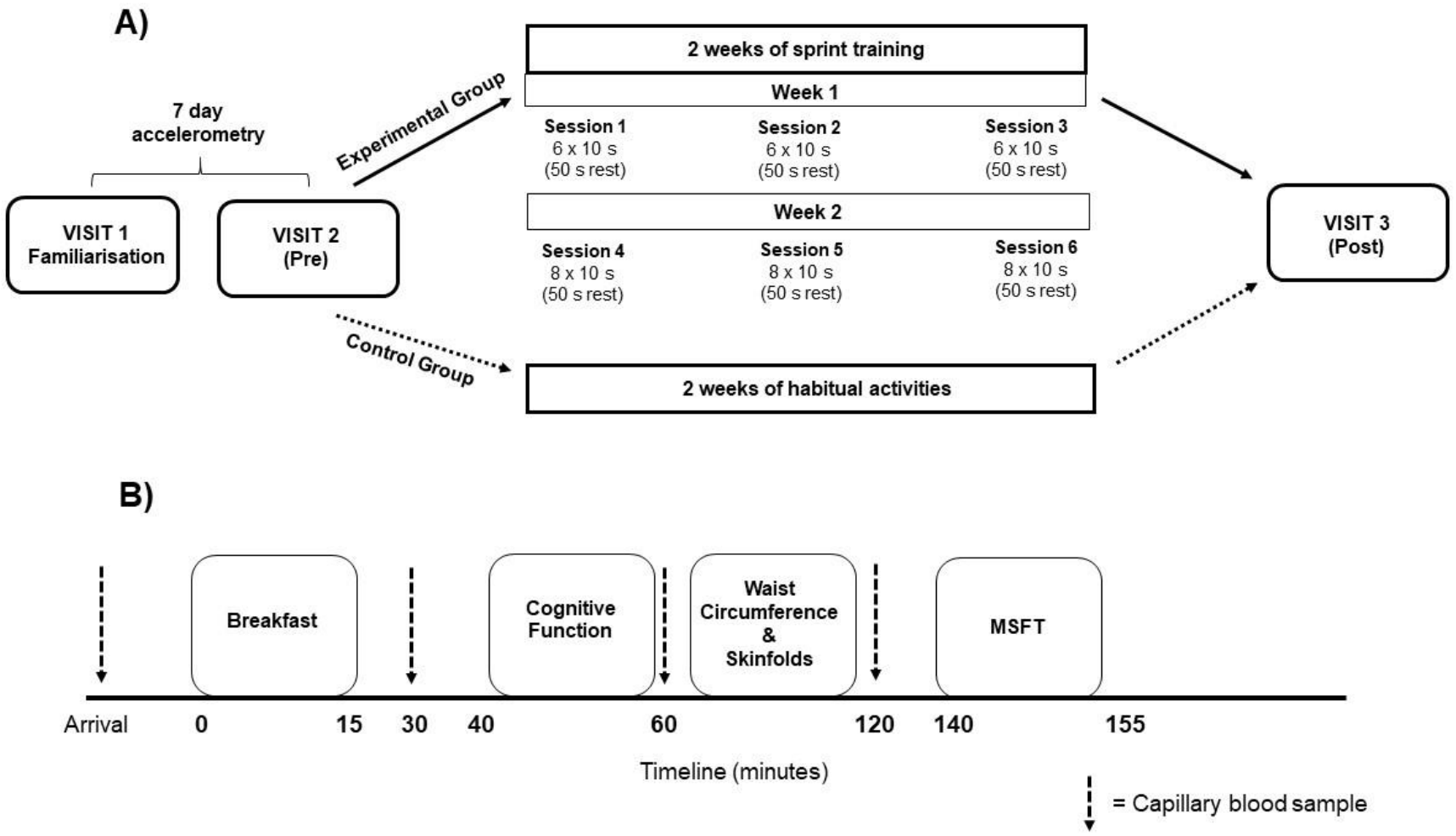

Fig 8.1. (**A**) Schematic representation of the overall study design. Training session details indicate the number of sprints performed. (**B**) Visual representation for the timeline of measurements during experimental visit's 2 and 3. Thick dashed lines represent the timing of capillary blood samples.

8.2.3 Experimental Visits

8.2.3.1 Visit One: Familiarisation

During the familiarisation participants underwent anthropometric measurements of height (cm), sitting height (cm) and body mass (kg). Participants were given a full practice of the cognitive function test battery, as well as being familiarised with a capillary blood sample. The experimental group also practiced the sprint protocol to be used during the training sessions. At the end of the familiarisation participants were given accelerometers to wear for the next 7 d, to measure physical activity prior to the pre-intervention measures.

8.2.3.2 Visits Two and Three: Pre- and post-intervention testing measures

Participants were required to avoid any strenuous physical activity and record their dietary intake for 24 h prior to the main experimental visits; dietary intake prior to the pre-intervention measures was then replicated prior to the post-intervention measures. Participants attended the classroom where the laboratory was set up at ~ 08:30 after a 12 h overnight fast, with *ad libitum* water consumption allowed at all times. Participants provided a fasted capillary blood sample, after which they were provided with a standardised breakfast that provided 1.5 $g \cdot kg^{-1}$ body mass of carbohydrate (more details of which can be found in section 3.6.2). The meal was consumed over a 15 min period, then subsequent capillary blood samples were taken at 30-, 60- and 120-min post-breakfast for the assessment of postprandial blood glucose and plasma insulin concentrations. No other food or drink (only water) was allowed during this period. Participants completed the cognitive function test battery (which lasted ~ 10 min) 45 min post-breakfast. Participants also underwent waist circumference and skinfold thickness measurements. The multi-stage fitness test was performed ~ 20-min after the final blood sample (Fig 8.1B).

8.2.3.3 Sprint Training Sessions

Participants in the experimental group completed a two-week sprint-based training programme. The training sessions took place on an outdoor court at the school, with 3 sessions scheduled per week (Monday, Wednesday and Friday). The sessions were conducted during morning form time

(08:45 – 09:15), so that it did not interfere with the school day or detract from the participant's usual Physical Education lessons. Upon arrival to the classroom, participants were fitted with a heart rate monitor (Firstbeat Technologies Ltd, Finland) and a global positioning (GPS) unit (Catapult Sports, Melbourne, Australia) to monitor the internal and external load of the sessions. The training sessions consisted of 10 s maximum effort sprints, with 50 s of passive (walking) recovery. Sprints were performed as shuttles between two cones which were placed 40 m apart. Participants were encouraged to cover as much distance as possible within the 10 s. The session started with a brief warm up, consisting of 2 shuttles at a self-selected pace. A custom audio file was used to administer the sprint session, with a beep signalling the start and end of the sprint, as well as audio notifications half-way through the rest period and 5 s before the start of the next sprint. The first 3 training sessions consisted of 6 x 10 s sprints, leading to a total commitment of 6 min per session and a weekly total of 18 min. The final 3 sessions consisted of 8 x 10 s sprints, leading to a total commitment of 8 min per session and a weekly total of 24 min. The total training time over the intervention (excluding warm up and cool downs) was 42 min (over 2 weeks). Participants were given verbal encouragement for every sprint.

8.2.4 Experimental Procedures and Measurements

8.2.4.1 Anthropometric and Body Composition Measurements

Anthropometric measurements consisting of height, body mass, sitting height, BMI and somatic maturity (predicted) were measured in accordance with descriptions in the General Methods of this thesis (Section 3.3). The sum of four skinfold sites (triceps, subscapular, supraspinale and front thigh) and waist circumference were measured as surrogates of adiposity, in line with descriptions provided earlier in this thesis (Section 3.4).

8.2.4.2 Standardised Breakfast

An ecologically valid breakfast was used as a test meal to assess postprandial responses, which has been used successfully before in this population (Dring et al., 2019a & 2020). The breakfast was standardised in order to provide 1.5g·kg^{-1} body mass of carbohydrate and consisted of;

cornflakes, milk, white toast and butter. Participants were given 15 min to consume the meal. If any leftovers remained during visit 2, these were weighed and subtracted from the visit 3 breakfast to ensure identical meal provision for pre- and post-measurements.

8.2.4.3 Assessment of Physical Activity

Device-measured physical activity was assessed with an Actigraph GT3X+ triaxial accelerometer (Actigraph, Pensacola, FL, USA), which was conducted in line with descriptions provided earlier in this thesis (Section 3.7). The main variables of interest were average acceleration (mg) (used to describe the average physical activity volume over the recording period) and the intensity gradient (used to describe the average intensity distribution over the recording period), in line with the recommendations of Rowlands et al. (2018) (see section 3.7 for full details). Participants' data were excluded if the post-calibration error was greater than 0.01 g or if there were less than 3 valid days (including 1 weekend day) of wear time (defined as ≥ 16 h per day) (Rowlands et al., 2018).

8.2.4.4 Capillary Blood Samples

A fasted capillary blood sample was taken for the determination of a range of pro- and anti-inflammatory cytokines (IL-6, IL-1β, IL10 & IL-15), serum brain-derived neurotrophic factor (BDNF) as well as plasma insulin and blood glucose concentrations. Three additional samples were collected at 30-, 60- and 120-min post-breakfast for determination of plasma insulin and blood glucose concentrations. Capillary blood samples were collected in accordance with the protocol outlined earlier in this thesis (Section 3.6.1). All samples were frozen immediately at -20 °C and transferred to -80 °C as soon as possible.

8.2.4.5 Blood Analyses

Blood glucose concentrations were determined in duplicate using a commercially available assay (GOD/PAP method, GL364, Randox, Ireland). Plasma insulin concentrations were determined using a commercially available ELISA, in line with the manufacturer's instructions (ELISA; Mercodia Ltd, Sweden). Blood glucose and plasma insulin total area under the curve (tAUC)

following the standardised breakfast was calculated (GraphPad Prism 7, GraphPad Software, USA), using methods described previously (Wolever et al., 1991). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as an index of insulin resistance (Matthews et al., 1985). Plasma IL-6, -10, -15 and 1β concentrations were determined simultaneously with an automated SimplePlex immunoassay, using the anti-body-based Ella system (ProteinSimple, BioTechne, Oxford, UK), per the manufacturer's instructions (Aldo et al., 2016). All analytes were measured in triplicate and concentrations were determined using preloaded calibration curve. Serum BDNF concentrations were determined using commercially available methods, in accordance with the manufacturer's instructions (Quantikine ELISA, R & D Systems Europe Ltd, UK).

8.2.4.6 Cognitive Function Test Battery

The cognitive function test battery lasted approximately 12 min and consisted of the Stroop test, Sternberg paradigm and the Flanker task. A brief description of the tests can be found below and are explained in full detail earlier in the thesis (Section 3.8). All tests were completed on a laptop computer (Lenovo ThinkPad T450; Lenovo, Hong Kong). Each test, and test level, were preceded by practice stimuli in order to re-familiarise participants (the data for these practice stimuli were discarded). The tests were conducted with 10 participants at a time, separated and in silence, in a darkened room with noise-cancelling headphones to minimise external disturbances.

The Stroop test consists of two levels, congruent and incongruent, assessing selective attention and executive function (specifically the domain of inhibitory control), respectively (Miyake, 2000). The Sternberg paradigm measures working memory (Sternberg, 1969) and consists of three levels of ascending difficulty (one-, three- and five-item). The Flanker task consists of two levels (congruent and incongruent) and assesses attention and inhibitory control (Eriksen & Eriksen, 1974).

8.2.4.7 Physical Fitness (Multi-Stage Fitness Test)

Physical fitness was assessed using the multi-stage fitness test (MSFT), which has been described in detail previously in this thesis (Section 3.5). The MSFT was performed indoors during experimental visit 2 (pre-intervention), but due to the short notice rescheduling, that was explained earlier, the MSFT during experimental visit 3 (post-intervention) was performed outdoors. Participants were 'paced' by a member of the research team, to ensure they were able to stick to the designated speed. Verbal encouragement was provided by the research team and heart rate (Firstbeat Technologies Ltd, Finland) was monitored continuously throughout to ensure maximum effort. Performance on the test was determined by the total distance covered (m). For descriptive purposes, age- and sex-specific centiles were derived, for both groups, from normative MSFT performance data (Tomkinson et al., 2016).

8.2.4.8 Training Load Monitoring

Heart Rate (Internal Load)

Heart rate was measured continuously throughout each training session (Firstbeat Team Sport System, Firstbeat Technologies Ltd, Finland) to provide internal load characteristics. Average and maximum heart rate were extracted from each training session. The maximum heart rate (HR_{max}) achieved during MSFT was used to calculate the average and maximum relative exercise intensity ($\%HR_{max}$) of each session. The internal load of the training sessions can be seen in Table 8.2.

Global Positioning System (GPS) (External Load)

GPS was used to quantify the external workload of each training session using PlayerTek units (Catapult Sports, Melbourne, Australia). The units were fitted to sit between the scapulae, using an elasticated shoulder harness. After each training session, the data were downloaded to the PlayerTek software. The variables of interest were top speed ($m \cdot s^{-1}$) and total distance covered across all 10 s sprints (m). The data from each session can be seen in Table 8.2.

8.2.5 Statistical Analysis

Statistical analyses were performed using RStudio (RStudio Team., 2020) and are presented as mean ± SD, unless otherwise stated. Analysis of covariance (ANCOVA) was used for all outcomes of interest, to examine the between group (intervention vs control) differences at follow-up, while controlling for the baseline score (covariate) of that outcome; which is the recommended approach for such experimental designs (Hecksteden et al., 2018; Ritz., 2020; Vickers & Altman, 2001). For each comparison, the mean difference and associated 95% confidence interval (CI) are presented to provide a measure of uncertainty. The associated R package to perform this analysis was the "car" package (Fox, 2019). Residual analysis was performed for each model to assess distribution (histograms) and the spread of residuals, as well as homogeneity of regression slopes. The assumptions for each model did not display any extreme deviations from normality, therefore no variables were log transformed. The adjusted mean and 95% CI at follow-up for each group were calculated for all variables (R package "effects" (Fox & Weisberg, 2019)), but the original mean ± SD are also presented. The alpha for determining statistical significance was set at $p < 0.05$.

8.3 Results

At baseline, the MSFT median centile for the intervention group was 70 (range: 10 – 95); with 1 classed as "low" (≤ 40th centile), 2 as "moderate" (40th – 60th centile), 3 as "high" (60th – 80th centile) and 2 as "very high" (≥ 80th centile). For the control group the median centile was 65 (range: 10 – 95); with 2 classed as "low", 2 as "moderate", 2 as "high" and 2 as "very high".

At follow-up, the MSFT median centile for the intervention group was 88 (range: 50 – 95); with 0 classed as "low", 2 as "moderate", 2 as "high" and 4 as "very high". For the control group, the median centile was 75 (range: 20 – 95); with 1 classed as "low", 1 as "moderate", 3 as "high" and 3 as "very high".

8.3.1 Compliance and Training Load

Seven participants attended all 6 training sessions, with one participant attending 5 sessions (due to being absent from school for one session). A descriptive overview of the average external and internal training load (GPS and HR) for each session across the intervention is presented in Table 8.2.

Table 8.2. Average external and internal training load for each session across the intervention period. Data are mean ± SD (range).

Session	Total Distance Covered (m)	Distance Covered per Sprint (m)	Average Top Speed ($m \cdot s^{-1}$)	Average HR ($beats \cdot min^{-1}$)	Average Relative Exercise Intensity (%HRMax)[b]	Maximum HR ($beats \cdot min^{-1}$)	%HRMax[b] during each training session
			6 x 10 s Sprints				
1	280 ± 17 (263 – 316)	47 ± 3 (44 – 53)	6.0 ± 0.4 (5.5 – 6.8)	181 ± 7 (171 – 191)	88 ± 4 (83 – 94)	194 ± 5 (186 – 201)	94 ± 3 (91 – 100)
2	252 ± 21 (215 – 287)	42 ± 3 (36 – 48)	5.8 ± 0.3 (5.3 – 6.4)	179 ± 9 (168 – 193)	86 ± 3 (82 – 91)	193 ± 6 (185 – 203)	93 ± 2 (90 – 94)
3[+]	269 ± 19 (248 – 300)	45 ± 3 (41 – 50)	5.8 ± 0.4 (5.3 – 6.4)	177 ± 8 (163 – 189)	86 ± 4 (79 – 88)	192 ± 4 (186 – 199)	93 ± 3 (89 – 97)
			8 x 10 s Sprints				
4	337 ± 15 (323 – 367)	42 ± 2 (40 – 46)	5.7 ± 0.4 (5.3 – 6.5)	182 ± 7 (171 – 191)	88 ± 3 (82 – 93)	195 ± 5 (185 – 203)	94 ± 3 (91 – 99)
5[++]	327 ± 41 (242 – 373)	42 ± 2 (40 – 47)	5.7 ± 0.5 (5.2 – 6.5)	179 ± 6 (172 – 187)	87 ± 3 (82 – 89)	191 ± 5 (183 – 197)	92 ± 2 (90 – 94)
6	329 ± 26 (302 – 376)	42 ± 3 (38 – 47)	6.3 ± 0.5 (5.6 – 7.0)	181 ± 9 (167 – 194)	88 ± 5 (82 – 96)	194 ± 9 (181 – 210)	94 ± 4 (90 – 100)

Abbreviations: HR; Heart Rate. HRMax; Maximum Heart Rate.
[b] Maximum Heart Rate achieved during the Multi-Stage Fitness Test.
[+] n = 5 due to technical issues with GPS units. [++] n = 7 due to absence from school.

8.3.2 Differences in Physical Fitness, Risk Factors for Cardiometabolic Disease and Cognitive Function

Physical fitness and cardiometabolic health outcomes can be found in Table 8.3, and cognitive function outcomes in Table 8.4.

For MSFT performance, there was no statistically significant difference between the intervention and control group at follow-up controlling for baseline performance ($p = 0.204$, Fig 8.2). For waist

circumference, there was no statistically significant difference between the intervention and control group at follow-up, when controlling for baseline waist circumference (p = 0.579, Fig 8.2).

There was no statistically significant difference between the intervention and control group at follow-up for fasting blood glucose concentration (p = 0.516), fasting plasma insulin concentration (p = 0.171), HOMA-IR (p = 0.181, Fig 8.3), postprandial plasma insulin tAUC (p = 0.434, Fig 8.3) and postprandial blood glucose tAUC (p = 0.898, Fig 8.3), when controlling for the respective baseline measurements. There was no statistically significant difference between the intervention and control group at follow-up for plasma IL-6 (p = 0.526), IL-10 (p = 0.102), IL-15 (p = 0.393) and IL- 1β concentrations (p = 0.921, Fig 8.4), when controlling for the respective baseline measurements.

There was no statistically significant difference between the intervention and control group at follow-up for response times or accuracy on any of the cognitive function tests (all $p > 0.05$, Fig 8.5 to 8.7), when controlling for the respective baseline measurements. However, there was a statistically significant difference between the intervention and control group at follow-up for accuracy during the three-item Sternberg paradigm ($F_{(1, 13)} = 4.9$, $p = 0.046$), when controlling for baseline accuracy. Specifically, the intervention group (99.6%, CI [98.3%, 100%]) was more accurate than the control group (97.6%, CI [96.2%, 99.0%]). For serum BDNF concentration, there was a statistically significant difference between the intervention and control group at follow-up, when controlling for baseline BDNF concentration ($F_{(1, 13)} = 38.4$, $p < 0.001$ (Fig 8.8). Specifically, the intervention group (42.9 $ng \cdot ml^{-1}$, CI [37.4 $ng \cdot ml^{-1}$, 48.3 $ng \cdot ml^{-1}$]) had a higher concentration compared to the control group (19.2 $ng \cdot ml^{-1}$, CI [13.8 $ng \cdot ml^{-1}$, 24.7 $ng \cdot ml^{-1}$], Fig 8.8).

Table 8.3. Baseline and follow-up results of physical fitness (MSFT), 20 m sprint performance and cardiometabolic health outcomes, with data presented as mean ± SD. Follow-up and between-group differences adjusted for baseline results are also presented as mean ± 95% CI.

Outcome	Intervention (n = 8)			Control (n = 8)			Adjusted Between-Group Difference Mean (95% CI) [a] [*p value*]
	Baseline (Mean ± SD)	*Follow-Up (Mean ± SD)*	*Adjusted Follow-Up Mean (95% CI)* [a]	*Baseline (Mean ± SD)*	*Follow-Up (Mean ± SD)*	*Adjusted Follow-Up Mean (95% CI)* [a]	
MSFT (m)	850 ± 323	1025 ± 350	999 (884, 1114)	798 ± 367	873 ± 388	898 (783, 1013)	100 (-62, 263) [p = 0.204]
Waist Circumference (cm)	61.8 ± 5.3	60.9 ± 6.0	62.3 (60.9, 63.6)	64.8 ± 6.2	64.2 ± 5.3	62.8 (61.5, 64.1)	-0.5 (-2.3, 1.4) [p = 0.579]
Fasting Blood Glucose ($mmol \cdot L^{-1}$)	4.40 ± 0.36	4.58 ± 0.45	4.68 (4.37, 4.92)	4.60 ± 0.23	4.84 ± 0.32	4.77 (4.49, 5.04)	-0.11 (-0.49, 0.27) [p = 0.516]
Fasting Plasma Insulin ($pmol \cdot L^{-1}$)	59.8 ± 33.9	46.4 ± 21.1	52.9 (43.6, 62.2)	70.0 ± 22.6	49.7 ± 14.0	44.0 (35.4, 52.7)	8.9 (-4.4, 22.1) [p = 0.171]
HOMA-IR (AU)	1.96 ± 1.14	1.62 ± 0.81	1.91 (1.52, 2.29)	2.39 ± 0.80	1.79 ± 0.58	1.54 (1.19, 1.90)	0.36 (-0.19, 0.92) [p = 0.181]
Blood Glucose tAUC ($mmol \cdot L^{-1}$ x 120 min)	726 ± 68	711 ± 115	722 (664, 779)	750 ± 61	728 ± 64	717 (659, 774)	5 (-75, 85) [p = 0.898]
Plasma Insulin tAUC ($pmol \cdot L^{-1}$ x 120 min)	21659 ± 6890	23685 ± 8660	28119 (22537, 33702)	32525 ± 11059	29370 ± 10996	24935 (19352, 30517)	2279 (-4931, 9490) [p = 0.434]
IL-6 ($pg \cdot ml^{-1}$)	1.76 ± 1.90	1.02 ± 0.53	1.05 (0.61, 1.48)	1.41 ± 1.38	0.82 ± 0.26	0.85 (0.33, 1.36)	0.20 (-0.49, 0.89) [p = 0.526]
IL-10 ($pg \cdot ml^{-1}$)	2.54 ± 0.86	2.55 ± 0.48	2.57 (2.02, 3.11)	3.33 ± 1.72	2.02 ± 0.80	1.95 (1.44, 2.46)	0.62 (-0.14, 1.38) [p = 0.102]
IL-15 ($pg \cdot ml^{-1}$)	2.23 ± 0.65	2.14 ± 0.69	2.24 (1.48, 3.00)	2.65 ± 0.61	2.82 ± 1.20	2.71 (1.89, 3.53)	-0.47 (-1.63, 0.69) [p = 0.393]
IL-1β ($pg \cdot ml^{-1}$)	45.47 ± 37.20	36.6 ± 16.14	45.79 (29.23, 86.38)	60.83 ± 39.10	52.10 ± 75.93	42.56 (16.84, 83.67)	3.23 (-67.04, 73.49) [p = 0.921]

Abbreviations: SD; Standard Deviation. CI; Confidence Interval. MSFT; Multi-Stage Fitness Test. HOMA-IR; Homeostatic Model Assessment of Insulin Resistance. tAUC; Total area under the curve. IL; Interleukin.

[a] Adjusted for baseline score. * Statistically significant difference between groups, $p < 0.05$.

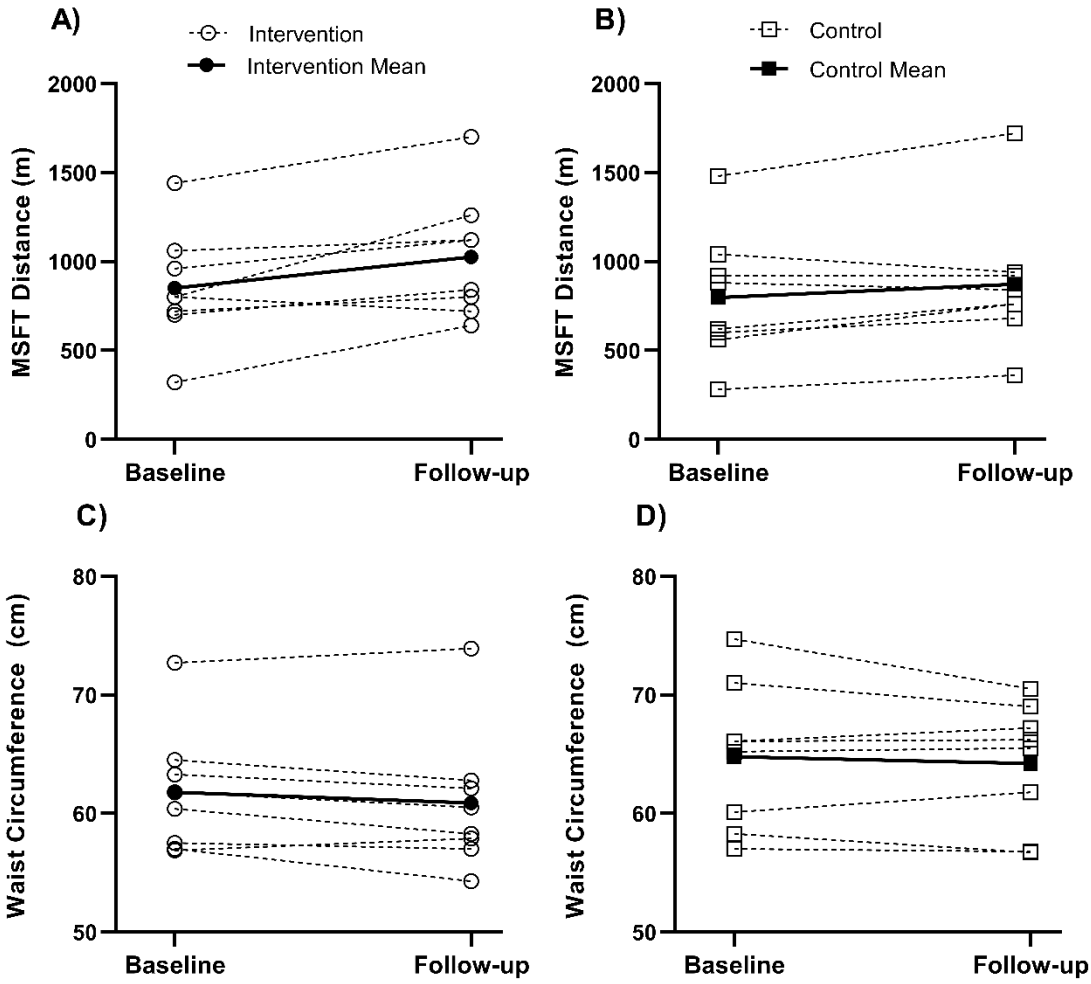

Fig 8.2. Individual (dashed) and mean (solid) baseline and follow-up measurements for the experimental group (circles) and control group (square) for MSFT (**A & B**) and waist circumference (**C & D**).

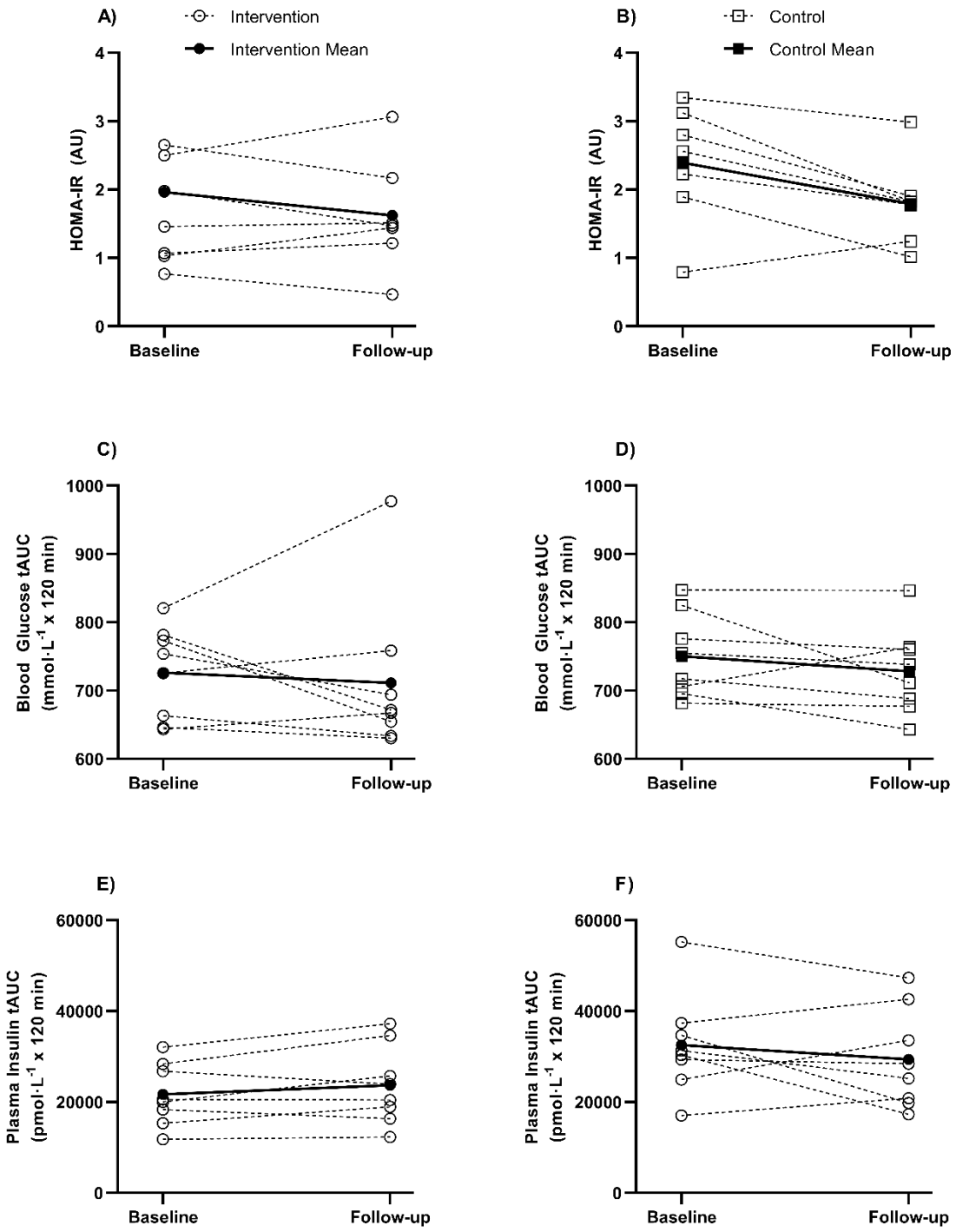

Fig 8.3. Individual (dashed) and mean (solid) baseline and follow-up measurements for the experimental group (circles) and control group (square) for HOMA-IR (**A & B**), postprandial blood glucose tAUC (**C & D**) and postprandial plasma insulin tAUC (**E & F**).

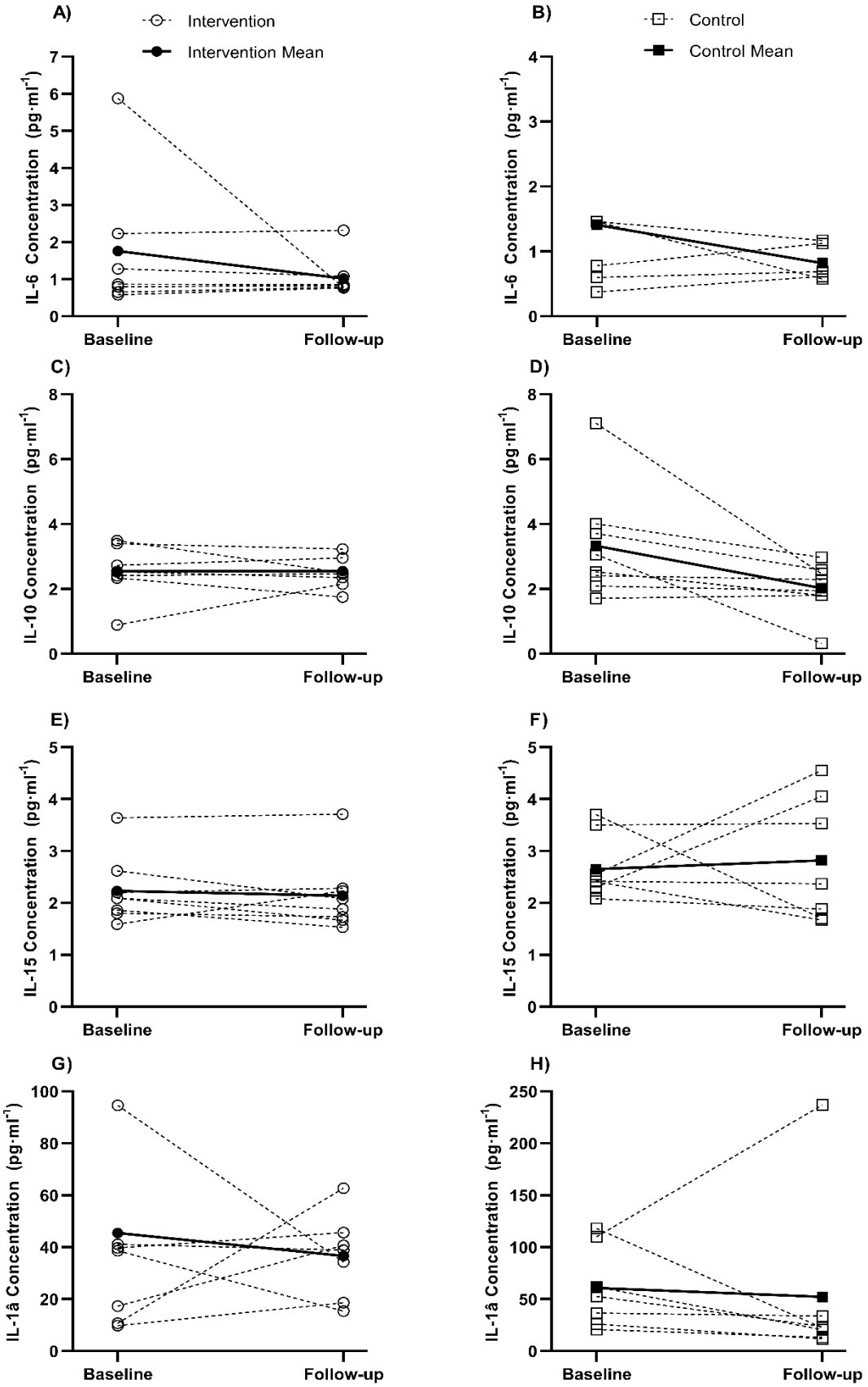

Fig 8.4. Individual (dashed) and mean (solid) baseline and follow-up measurements for the experimental group (circles) and control group (square) for IL-6 (**A & B**), IL-10 (**C & D**), IL-15 (**E & F**) and IL-1β (**G & H**) concentrations.

Table 8.4. Baseline and follow-up results of cognitive function outcomes, with data presented as mean ± SD. Follow-up and between-group differences adjusted for baseline results are also presented as mean ± 95% CI.

Outcome	Intervention (n = 8)			Control (n = 8)			Adjusted Between-Group Difference Mean (95% CI) [a] [*p value*]
	Baseline (Mean ± SD)	*Follow-Up (Mean ± SD)*	*Adjusted Follow-Up Mean (95% CI)* [a]	*Baseline (Mean ± SD)*	*Follow-Up (Mean ± SD)*	*Adjusted Follow-Up Mean (95% CI)* [a]	
				Response Times (ms)			
Congruent Stroop	880 ± 150	778 ± 131	764 (691, 837)	833 ± 143	798 ± 113	811 (738, 884)	-45.7 (-148.3, 56.9) [p = 0.347]
Incongruent Stroop	1224 ± 173	1118 ± 194	1102 (1030, 1175)	1193 ± 239	1104 ± 249	1119 (1048, 1191)	-16.5 (-118, 85) [p = 0.731]
Sternberg One-Item	633 ± 112	629 ± 150	600 (520, 680)	551 ± 53	553 ± 61	583 (502, 663)	14 (-93, 121) [p = 0.761]
Sternberg Three-Item	819 ± 105	766 ± 158	736 (660, 813)	746 ± 86	721 ± 65	751 (675, 827)	-12 (-116, 91) [p = 0.784]
Sternberg Five-Item	974 ± 132	869 ± 152	831 (720, 942)	883 ± 166	882 ± 214	920 (809, 1031)	-81 (-234, 72) [p = 0.254]
Congruent Flanker	664 ± 145	580 ± 109	565 (520, 611)	616 ± 128	568 ± 91	583 (537, 628)	-17 (-81, 47) [p = 0.576]
Incongruent Flanker	705 ± 147	620 ± 138	612 (547, 677)	681 ± 149	633 ± 115	641 (576, 705)	-28 (-120, 64) [p = 0.515]
				Accuracy (%)			
Congruent Stroop	97.8 ± 2.5	97.5 ± 3.8	97.7 (95.1, 100.0)	100 ± 0	99.4 ± 3.5	99.2 (96.6, 100.0)	-1.1 (-4.4, 2.2) [p = 0.411]
Incongruent Stroop	97.6 ± 2.9	95.3 ± 4.1	95.1 (92.8, 97.4)	97.2 ± 2.5	95.9 ± 3.5	96.2 (93.8, 98.5)	-1.0 (-4.0, 2.2) [p = 0.499]
Sternberg One-Item	98.1 ± 2.9	97.7 ± 3.2	97.6 (95.1, 100.0)	98.4 ± 2.9	97.7 ± 3.2	97.7 (95.1, 100.0)	-0.02 (-3.6, 3.6) [p = 0.988]
Sternberg Three-Item	96.7 ± 2.7	99.6 ± 1.1	99.6 (98.3, 100.0)	97.3 ± 2.0	97.7 ± 2.2	97.6 (96.2, 99.0)	**2.0 (0.02, 3.9) * [p = 0.046]**

Table 8.4. *Continued*

Sternberg Five-Item	94.7 ± 6.2	96.5 ± 3.5	96.6 (92.7, 100.0)	95.7 ± 6.0	93.8 ± 6.3	93.7 (89.7, 97.6)	2.9 (-2.6, 8.4) [p = 0.295]
Congruent Flanker	99.6 ± 1.2	99.6 ± 1.2	99.5 (98.6, 100.0)	99.2 ± 1.5	99.6 ± 1.2	99.6 (98.7, 100.0)	-0.1 (-1.4, 1.1) [p = 0.855]
Incongruent Flanker	99.2 ± 1.54	96.6 ± 3.7	96.6 (93.2, 100.0)	97.5 ± 3.9	96.7 ± 4.7	96.7 (93.2, 100.0)	-0.04 (-4.8, 4.7) [p = 0.986]
BDNF ($ng \cdot ml^{-1}$)	18.47 ± 7.51	39.12 ± 9.88	42.9 (37.4, 48.3)	28.69 ± 11.36	22.95 ± 9.13	19.2 (13.8, 24.7)	**17.89 (10.7, 25.1) ** [p < 0.001]**

Abbreviations: SD; Standard Deviation. CI; Confidence Interval. BDNF; Brain-Derived Neurotrophic Factor.
[a] Adjusted for baseline score. * Statistically significant difference between groups, $p < 0.05$. ** Statistically significant difference between groups, $p < 0.001$.

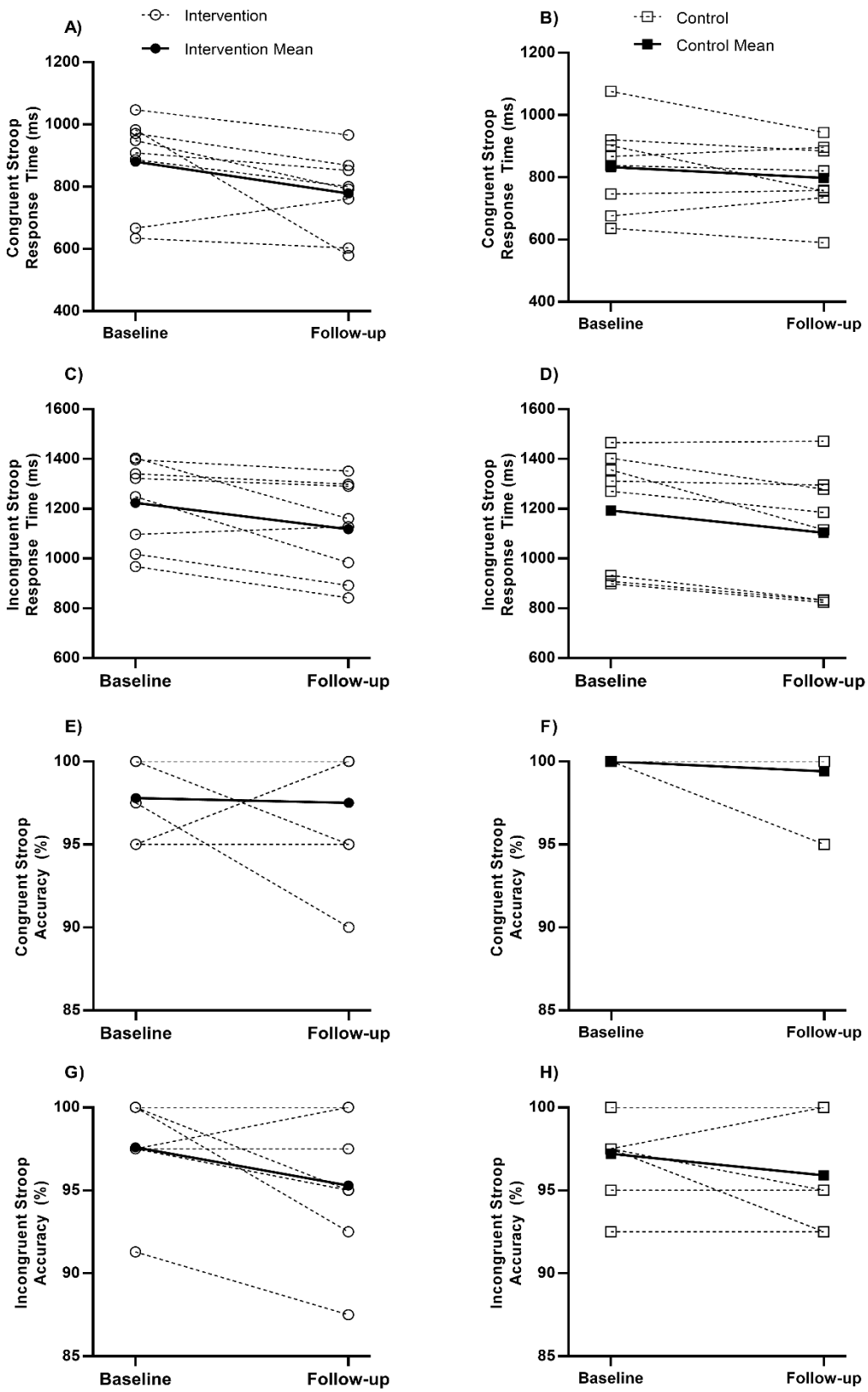

Fig 8.5. Individual (dashed) and mean (solid) baseline and follow-up measurements for the experimental group (circles) and control group (square) for response times during the congruent (**A & B**) and incongruent levels of the Stroop task (**C & D**) and accuracy during the congruent (**E & F**) and incongruent levels (**G & H**).

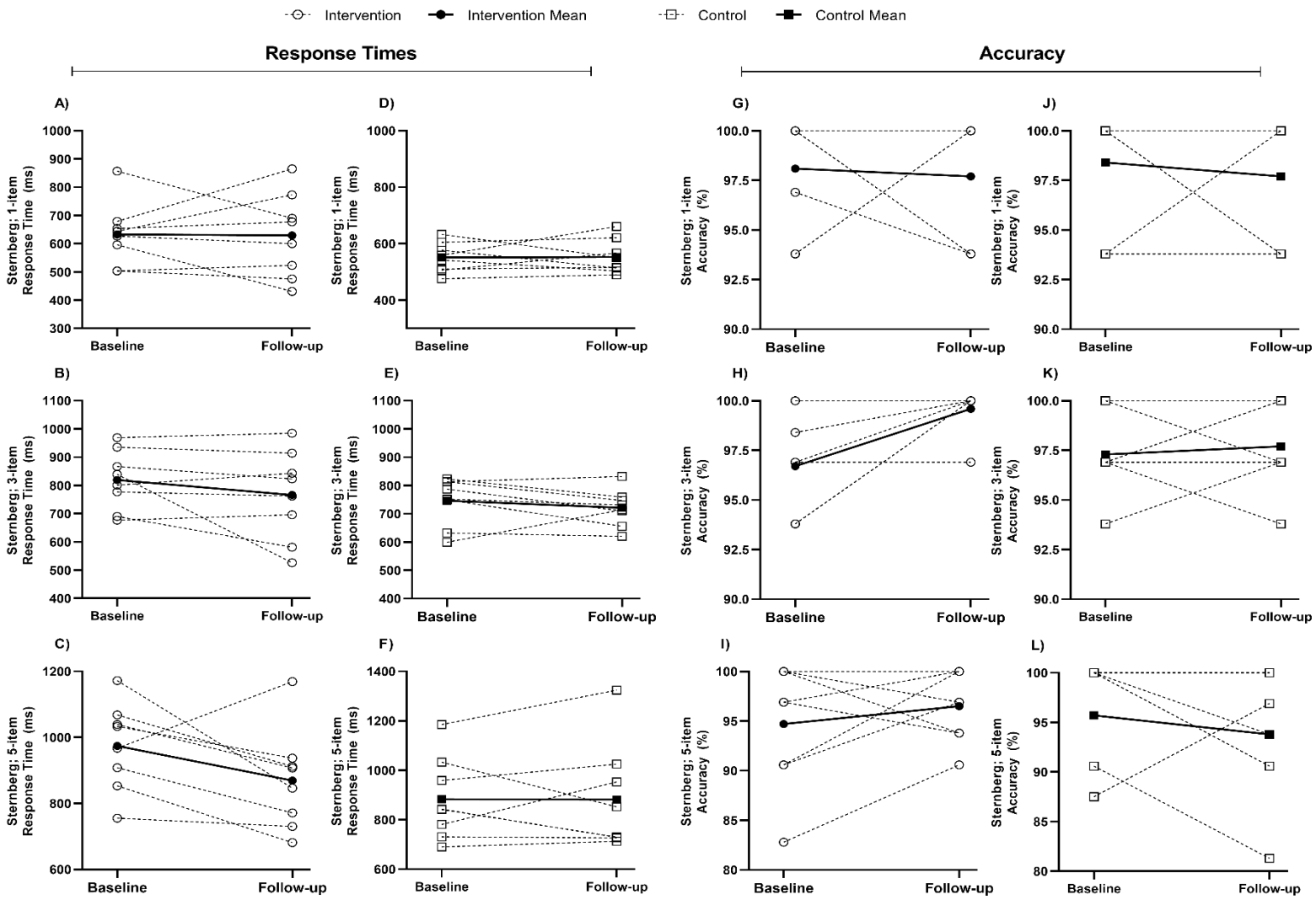

Fig 8.6. Individual (dashed) and mean (solid) baseline and follow-up measurements for the experimental group (circles) and control group (square) for response times and accuracy during the one-item (Response Times: **A & D,** Accuracy: **G & J**), three-item (Response Times: **B & E,** Accuracy: **H & K**) and five-item (Response Times: **C & F,** Accuracy: **I & L**) levels of the Sternberg Paradigm.

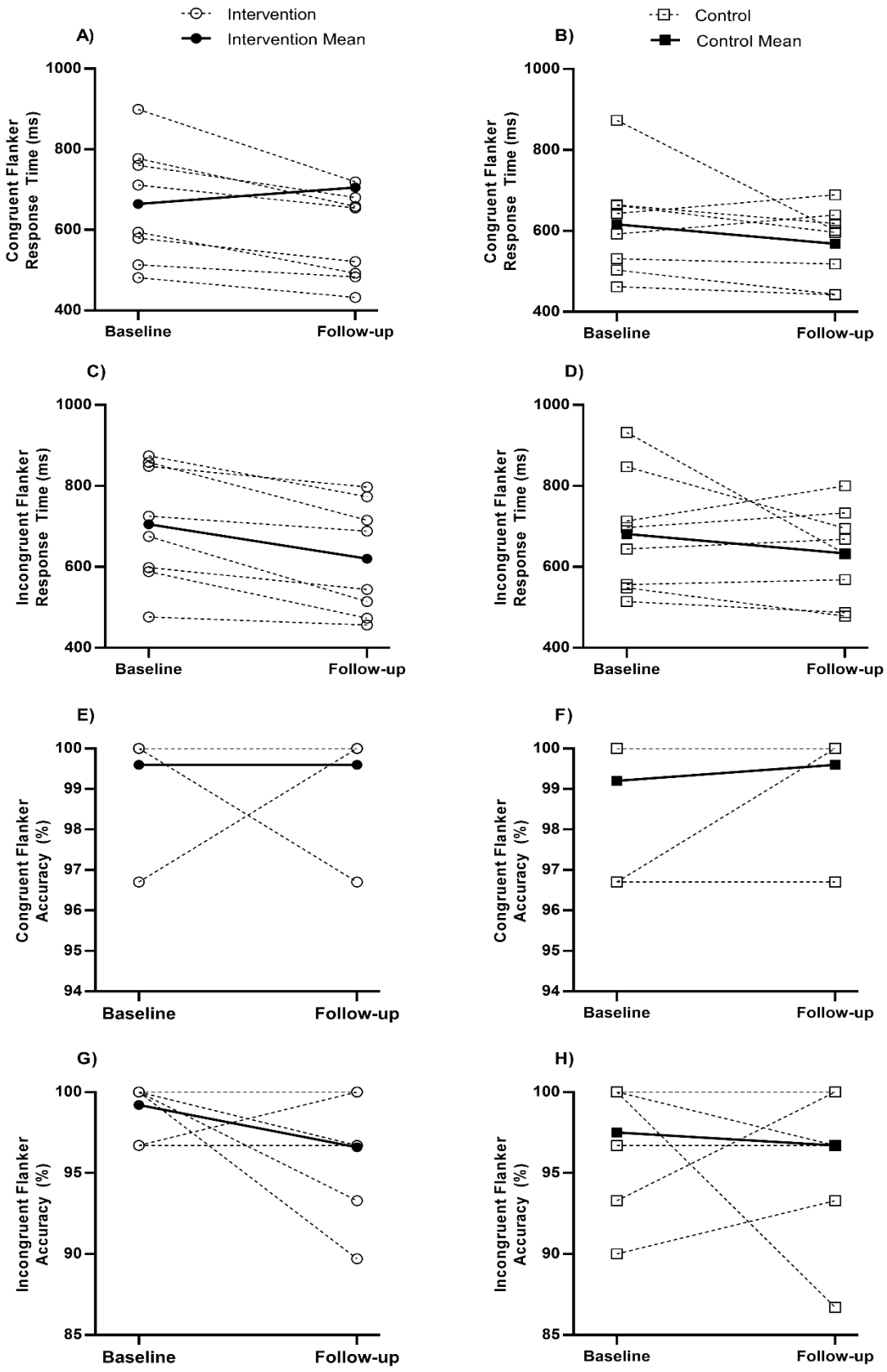

Fig 8.7. Individual (dashed) and mean (solid) baseline and follow-up measurements for the experimental group (circles) and control group (square) for response times during the congruent (**A & B**) and incongruent levels of the Flanker task (**C & D**) and accuracy during the congruent (**E & F**) and incongruent levels (**G & H**).

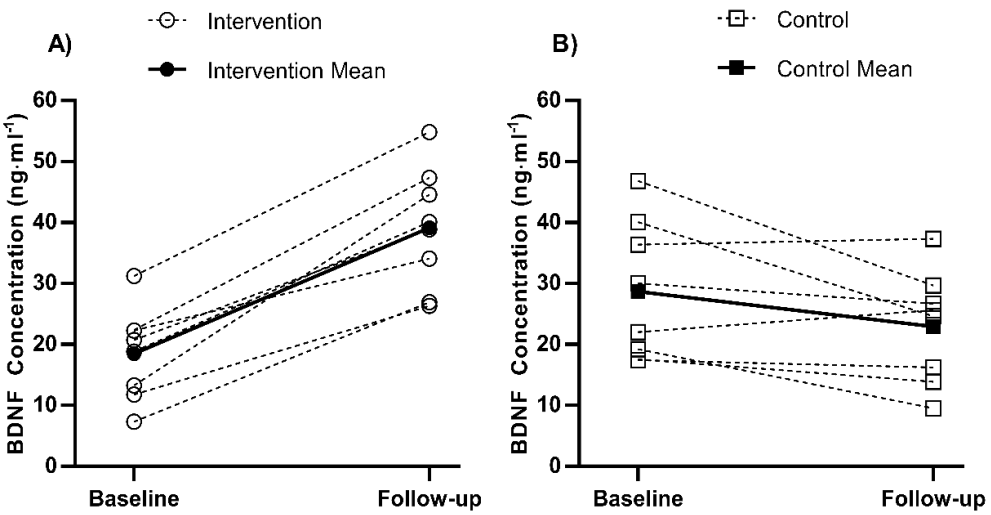

Fig 8.8. Individual (dashed) and mean (solid) baseline and follow-up measurements for the experimental group (**A**) and control group (**B**) for BDNF concentration.

8.4 Discussion

The main findings of the present study are that two weeks of a school-based, sprint training intervention improved accuracy during a working memory test and increased fasting BDNF concentrations, but did not affect other aspects of cognitive performance, MSFT performance or markers of health related to cardiometabolic disease. The present study provides novel contributions to the literature by demonstrating that the postprandial response to a standardised breakfast, a range of cytokines related to low-grade inflammation, as well as the domain of executive function are not affected by two weeks of sprint training. Moreover, this is the first study to examine the effects of an ecologically valid, school-based, sprint-interval intervention in a group of adolescent girls.

The main finding of the present study was that two weeks of school-based sprint training led to greater accuracy (mean difference = 2.0%, CI [0.02%, 3.9%]) on the three-item level of the Sternberg paradigm (working memory). This is supported by previous work demonstrating that exercise training can lead to less errors on the Wisconsin Sorting Card Test assessing executive function (Chen et al., 2015), as well as general improvements in working memory capacity (Jeon & Ho Ha, 2017). Importantly, this improvement accuracy was not at the expense of increased

response times, which indicates an actual improvement rather than a speed-accuracy trade-off. Although short-term memory ability is suggested to improve throughout adolescence (Ryan, 2009), these data suggest that additional benefits can be achieved by regular exercise training. This could have important implications, given that working memory is an important domain of cognition related to learning during adolescence and is linked to academic performance (Gathercole et al., 2003).

The present study is the first to demonstrates that a short-term school-based intervention increased resting BDNF concentration in adolescent girls. There are currently limited and equivocal data on resting BDNF concentration following exercise interventions in adolescents (Azevedo et al., 2020). The magnitude of difference in the present study is much larger than that reported after an 8-week treadmill running intervention (Jeon & Ho Ha, 2017), which also only reported within-group differences, thus making comparisons difficult. Interestingly, Jeon and Ho Ha (2017) provide evidence that the BDNF increase may be exercise intensity-dependent, which lends support to an increase seen after sprint training in the present study. Furthermore, Jeon and Ho Ha (2017) also found improvements in working memory performance. Given the sparse data on chronic training and BDNF concentration in adolescents, such a large increase should be interpreted tentatively until more data can replicate this. Nonetheless, the present study provides novel evidence that two weeks of a school-based sprint training intervention increased BDNF concentration in adolescent girls, alongside a concomitant increase in accuracy on a working memory task.

For the remaining cognitive function outcomes there was no difference between groups after two weeks of school-based sprint interval training. It is worth noting, however, that the mean difference in response times suggested the intervention group were quicker, but due to the small sample size these estimates were surrounded by large windows of uncertainty (Table 8.4). Much of the previous evidence has demonstrated improvements in cognitive function outcomes across a range of domains after a period of exercise training in adolescents (Chen et al., 2016; Costigan et al., 2016; Jeon & Ho Ha, 2017; Ludyga et al., 2018; Schmidt et al., 2015). These interventions

were generally performed for a longer duration (ranging from 6 to 12 weeks), more frequently (from 2 to 5 sessions per week) and at a lower intensity (ranging from 60% - 70% HR_{max} and 40% - 70% $\dot{V}O_{2max}$) than the present study. Collectively, these results suggest that cognitive function will benefit from a higher volume and longer duration exercise intervention of a moderate intensity in adolescents. However, the present study, to the authors' knowledge, is the first examining the effects of short-term high-intensity exercise interventions on cognitive function in adolescents. To provide a more robust evidence base on the efficacy of short-term interventions, more work is needed, particularly to explore the manipulation of training volume and intensity over a short period.

Data from the present study demonstrate that cytokine concentrations (IL-6, IL-10, IL-15 and IL-1β) were not affected by the short-term intervention. Logan et al. (2015) found that IL-6 concentration was increased in the groups performing higher volume (16 x 20 s and 20 x 20 s sprints) high-intensity exercise over an 8 week intervention, in low-active boys (16 ± 1 y). In addition to this, an 8-week sprinting intervention did not elicit reductions in IL-6 concentration in older adolescents (Buchan et al., 2013). Interestingly, these data collectively oppose the evidence in adults that regular physical activity is negatively associated with IL-6 concentration (Pedersen and Febbraio, 2008) and exercise interventions reduce basal IL-6 concentration (Cronin et al., 2017; Raimondo et al., 2016) in adults. IL-6 is a pleiotropic cytokine, secreted from several sources and is involved in many physiological processes (Hoffman & Weigert, 2017; Pedersen & Febbraio, 2008). Therefore, the exact origin of the systemic IL-6 concentrations might explain the abundance, or lack thereof, in response to exercise interventions (Raimondo et al., 2016). Nonetheless, this is the first study to examine the effects of school-based sprint-training on markers of inflammation in adolescent girls; who may be prone to higher basal concentrations, evidenced by results from a previous study within this thesis (Chapter IV). Future work should examine different exercise interventions (manipulating the intensity, volume and frequency of the exercise) and the effects on chronic low-grade inflammation in adolescent girls.

In the present study, two weeks of school-based sprint training did not lead to improvements in fasting markers of metabolic health (fasting glucose, insulin and HOMA-IR) in adolescent girls. These data, collectively with previous findings (Bond et al., 2015; Buchan et al., 2013; Cockcroft et al., 2019; Logan et al., 2015; Martin et al., 2015), suggest that short-term exercise interventions generally do not improve markers of fasting metabolic health, in healthy adolescents. Exercise interventions conducted with obese or overweight adolescents have, however, demonstrated improved fasting insulin concentration and HOMA-IR (de Araujo et al., 2012) and reduced fasting glucose concentration (Tjonna et al., 2009). Additionally, the postprandial glycaemic and insulinaemic response to a mixed meal was also not affected by two weeks of school-based sprint training in the present study. To the authors' knowledge, there are only two previous investigations examining the postprandial response to a test meal following an exercise intervention in healthy adolescents (Bond et al., 2015; Cockcroft et al., 2019), whereby responses were not affected by the interventions. There is evidence however that postprandial glycaemic and insulinaemic responses to an OGTT are affected by exercise training in overweight/obese adolescents (Tjonna et al., 2009). Overall, it seems that exercise interventions may be more effective at improving metabolic health in those who have greater scope for improvement (i.e. overweight/obese). Nonetheless this area of research is novel, and more work is needed to ascertain the effects of prolonged exercise interventions (longer than 2 weeks) on the postprandial responses to a mixed meal in healthy adolescents, as well as further work in those who are living with overweight and obesity.

The MSFT performance was unaffected by 2 weeks of sprint training in these young adolescent girls. It is worth noting that the adjusted mean difference between groups suggests that more distance was covered by the intervention group compared to the control group (100 m), however the large window of uncertainty surrounding this (95% CI [-63 m, 263 m]) also needs to be taken into consideration. Previous interventions utilising sprint-training in older adolescents (Buchan et al., 2013; Martin et al., 2015; Martin et al., 2018) and circuit-based exercise (Weston et al., 2016) have resulted in improved MSFT-derived indices of fitness. However, the duration of these

interventions were considerably longer, i.e. 7 weeks (Buchan et al., 2013; Martin et al., 2015) and 10 weeks (Weston et al., 2016). Despite this, an intervention as short as 4 weeks improved predicted $\dot{V}O_{2peak}$ (Martin et al., 2018). Interventions of a similar duration to the present study, utilising 8 x 1 min cycling repetitions (90% PPO), found no improvements in physical fitness (measured by $\dot{V}O_{2max}$) when assessed with an incremental cycle ergometer test (Bond et al., 2015; Cockcroft et al., 2019). Generally, the collective evidence suggests that longer interventions are generally better for improving physical fitness. Indeed, if the present study had been extended to 4 weeks (original planned duration), then it could be reasoned that improvements in MSFT performance may have been evident. Nonetheless, this remains speculative and the effect of increased duration of interventions (e.g. 4 – 6 weeks), using sprint-training in adolescent girls, should be examined.

The present study has several strengths; it is the first to assess the effect of an ecologically valid short-term, school-based exercise intervention on novel risk factors for cardiometabolic disease, and cognitive function, in healthy adolescent girls. Despite the field-based investigation, every attempt was made to ensure that all possible confounding factors were controlled for, such as the monitoring of physical activity and replication of dietary intake. Furthermore, the present study recruited only adolescent girls which is important as they are generally less active (Townsend et al., 2015) and are represented less in research. Finally, the present study also included a control group which was not a feature of previous short-term exercise interventions (Bond et al., 2015; Cockcroft et al., 2019) and also assessed the effect of the intervention through direct between-group comparisons, controlling for baseline measures (Hecksteden et al., 2018; Ritz., 2020). The major limitation of the present study was that the intervention had to be reduced to 2 weeks (down from 4) due to the COVID-19 pandemic; which also led to a much smaller than intended sample size.

The present study is the first to demonstrate that two weeks of school-based sprint training improved accuracy during a working memory task; which was complemented by an increased in resting BDNF concentration. There were though, no effects on other cognitive function outcomes

or across a range of markers related to cardiometabolic health in adolescent girls. The potential efficacy of short-term, school-based exercise interventions is unclear and much more evidence is needed to establish a robust evidence base. Indeed, future work should continue to investigate the effect of short-term interventions and training volume and intensity in adolescent girls, who are a population that require directed attention. Furthermore, the optimal training dose may to differ for health- and cognitive function-related outcomes.

Practical Applications

This chapter demonstrates that a brief, short-term sprint intervention is feasible in adolescent girls. Although the intervention was terminated early due to the Covid-19 pandemic, improvements in cognitive function accuracy were observed. Anecdotally, the participants seemed to enjoy the intervention and there were no major issues with adherence to the sessions, nor the logistics of conducting the training sessions first thing in the school morning. These are important considerations given that young adolescent girls are less active compared to adolescent boys, and under-represented in research to date. Therefore, future efforts to target this population should be made – whilst also utilising protocols, such as the one employed in the present study, that can be implemented in the school day that does not detract from scheduled PE or other curricular activities.

Chapter IX

General Discussion

9.1 Summary of key findings

The studies presented within this thesis have examined the effects of physical activity, physical fitness and adiposity on risk factors for cardiometabolic disease and cognitive function in adolescents. The main findings are summarised below:

Chapter IV

- Higher levels of physical activity and physical fitness had protective effects on risk factors for cardiometabolic disease in adolescents. Specifically, physical activity (assessed with novel accelerometer metrics) was inversely associated with blood pressure and some pro-inflammatory cytokines (IL-6 & IL-1β) in year 7 (age 11-12 y) participants. Furthermore, physical fitness was positively associated with the anti-inflammatory cytokine IL-15. Adiposity was associated with a poorer inflammatory profile, evidenced by the positive association between waist circumference and IL-6 concentration.
- Physical fitness was inversely associated with response times (higher physical fitness, faster response times) on executive function, working memory and visual perception tasks, while accuracy was unaffected. There were no observed associations between other exposure variables (adiposity and physical activity) and cognitive function outcomes.

Chapter V

- Adiposity (waist circumference), physical fitness (MSFT distance) and HOMA-IR additively explained the most variance (51%) in the postprandial insulinaemic response to a mixed-meal. However, the available independent variables were not able to explain a significant proportion of the variance in the postprandial glycaemic response.

- When considering an index of fasting insulin resistance (HOMA-IR) as the outcome, both BMI and maturity were positively associated with HOMA-IR and additively explained the most variance (20.1%).

Chapter VI

- The 60 min bout of football did not affect the postprandial insulinaemic or glycaemic response to a mixed-meal, when compared to a resting control trial. These responses were similar between the high- and low-fit groups.
- Three hours post breakfast, the 60 min bout of football reduced (by 21%) the blood glucose concentration at 60 min following the cessation of exercise when compared to a resting control trial. Plasma insulin concentration was not affected transiently following exercise. These responses were similar between the high- and low-fit groups.
- Postprandial insulin tAUC was substantially lower (~ 70%) in high-fit, compared to low-fit participants; confirming the importance of fitness in determining the postprandial insulinaemic response (Chapter V).

Chapter VII

- The 60 min bout of football did not affect cognitive function performance, or BDNF concentration, when compared to a resting control trial.
- However, when considering the moderating role of physical fitness, high-fit participants were quicker on a working memory task 45 min post-exercise, when compared to a resting control trial, while accuracy was unaffected. The 60 min bout of football did not improve cognitive function for the low-fit participants.
- During the cognitive function tests high-fit participants were consistently quicker than the low-fit group, with quicker response times evident on all levels of every cognitive task that was performed.

Chapter VIII

- Two weeks of sprint training did not affect any of the risk factors related to cardiometabolic disease, compared to a control group, in a small sample of adolescent girls.
- Despite the small sample, there was a mean difference of 100 m for MSFT performance at follow-up (not statistically significant p = 0.214), indicating that the intervention group may have shown an improvement had a larger sample size been recruited.
- The two-week sprint intervention improved accuracy on the three-item level of the Sternberg Paradigm (working memory), in comparison to the control group, but the response times remained unchanged; an effect that may be mediated by higher BDNF concentrations which were increased in the intervention group. However, there was no effect of the intervention on any of the other cognitive function outcomes.

9.2 Risk factors of Cardiometabolic Disease

9.2.1 Cytokines (IL-6, IL-10, IL-1β and IL-15)

In the studies presented in this thesis, several inflammatory cytokines related to chronic low-grade inflammation were examined cross-sectionally (Chapter IV) and in response to short-term exercise training (Chapter VIII). These included pro-inflammatory cytokines IL-6 and IL-1β and anti-inflammatory cytokines IL-10 and IL-15.

The overall positive, but small, association between a marker of physical fitness (MSFT distance) and IL-15 concentration is a novel contribution to the literature. There are currently no other data in adolescents to compare with, but there are suggestions that IL-15 is related to adipose tissue regulation (Nielsen et al., 2008), as well as oxidative metabolism and angiogenesis (Vasoconcelos & Salla, 2018). Furthermore, data from animal studies suggest that IL-15 subsequently activates Peroxisome proliferator-activated receptor-delta (PPAR-δ), which is a potential mechanism for improved endurance capacity through regular training, along with data showing increased skeletal muscle IL-15 content after 12 weeks of exercise training in adults (Rinnov et al., 2014). However, after a short-term (2 weeks) sprint training programme (Chapter

VIII) there were no increases in systemic IL-15 concentration in adolescent girls. Therefore, the current study and the previous observations suggest that physical fitness is associated with systemic IL-15 concentration, but to elicit an increase in IL-15 concentration a 2-week intervention (of brief, high-intensity sprinting) was not sufficient; indeed future work should examine the optimisation of training intensity, type and duration to increase IL-15 concentration.

Furthermore, this thesis assessed physical activity using newly proposed, continuous metrics of physical activity (Rowlands et al., 2018) which had not been previously examined in association with a range of markers for health. The negative associations between the physical activity metrics and cytokine concentrations (IL-6 and IL-1β) were only observable in year 7 participants (Chapter IV). These are interesting observations and, to the author's knowledge, are the first associations between the newly developed physical activity metrics and markers of low-grade chronic inflammation of any population. Given the potential relationship between puberty and markers of inflammation discussed above, it could be reasoned that older adolescents may be less susceptible to the beneficial effects of physical activity on inflammatory cytokines. However, data from Chapter VIII do not show reductions in pro-inflammatory cytokines after two weeks of sprint training in year 7 girls. The inverse association between physical activity and pro-inflammatory cytokines supports the notion that through regular exercise resting markers of inflammation are reduced (Gleeson et al., 2011), which is hypothesised to be a result of the regular increase in IL-6 after bouts of activity, leading to a subsequent cascade of anti-inflammatory cytokine activation and pro-inflammatory cytokine down-regulation (Gleeson et al., 2011; Pedersen, 2006; Petersen & Pedersen, 2005).

There was a differential association between waist circumference and IL-6 concentration, with a positive relationship in year 10 participants only. This highlights the important, negative contribution that adiposity has with regards to risk for cardiometabolic disease, as IL-6 is one of the main proponents of low-grade inflammation (Gleeson et al., 2011; Pedersen, 2006), with the findings of this thesis suggesting this is particularly the case in older adolescents. Whilst there is a strong evidence base of the positive associations between markers of adiposity and

inflammation (Dring et al., 2019b; Rubin & Hackney, 2010), this is the first study to report age-related associations in different age groups of adolescents.

The findings of this thesis also suggest that year 7 girls (aged 11 – 12 y) had a markedly different inflammatory milieu when compared to boys of the same age, as well as in comparison with older girls (year 10; 14 – 15 y). Indeed, year 7 girls had higher resting concentrations of pro-inflammatory IL-6 compared to year 7 boys and year 10 girls along with higher concentrations of anti-inflammatory IL-15 (compared to year 7 boys) and IL-10 (compared to year 10 girls). Although the role of pubertal development on inflammation has been acknowledged (Rubin & Hackney, 2010), there has been no study to date that has quantified age- and sex-related differences in cytokines, thus highlighting the novelty of these findings. These findings might be explained by pubertal development, as cytokines (particularly IL-6) have a suggested role in growth (Dorn et al., 2016).

9.2.2 Markers of metabolic health (Glucose, Insulin, HOMA-IR)

Fasted markers of metabolic health

In the studies presented in Chapter IV and V, metabolic health was assessed cross-sectionally via fasted blood glucose and plasma insulin concentrations, and subsequently HOMA-IR. Blood glucose and plasma insulin concentrations were examined 24 h following an acute bout of exercise (Chapter VI). They were also assessed, in a fasted state, following a two-week high-intensity exercise intervention (Chapter VIII).

Although the data from Chapter IV suggest that physical fitness, physical activity or adiposity are not associated with fasting markers of metabolic health, stepwise multiple regression (Chapter V) found that HOMA-IR was best explained by the combination of BMI and maturity offset (R^2 = 0.20). This is consonant with other research, where measures of adiposity (Barseem & Helwa, 2015; Dring et al., 2019b) and maturation (Cooper et al., 2017) are positively associated with fasting measures of insulin resistance. The use of stepwise regression enabled the best (most variance explained) model to be derived from an initial set of explanatory variables (including

physical fitness, waist circumference, sum of skinfolds and sex). Thus, these data along with previous observations suggest that, for fasting measures of metabolic health in adolescents, adiposity and maturation are the most important determinants. However, additional data from this thesis suggest that acute (Chapter VI) and short-term (two weeks) exercise training (Chapter VIII) do not affect fasting markers of metabolic health, although it is possible that had training been increased in duration an effect may have been seen consistent with other longer duration studies in the literature (Bird & Hawley, 2017).

Collectively, the findings presented in this thesis suggest that BMI and maturation are positively related to a fasting index of insulin resistance. This emphasises the need to consider maturity status when examining insulin resistance in adolescents, and also provides a potential target (BMI) for interventions looking to reduce the prevalence and development of type 2 diabetes in adolescents, which has increased in recent years (Candler et al., 2018).

Post-exercise markers of metabolic health

The acute exercise in Chapter VI (60 min of football) led to a reduction in blood glucose concentration, by 21%, 60 min following the cessation of exercise (3 h post-breakfast); which confirms previous observations utilising an acute basketball protocol of similar duration (Dring et al., 2019a). Despite this reduction, there was however no effect on plasma insulin concentrations following football activity, which may be attributed to the lower absolute intensity of the football session due to almost half of the session (~ 20 min) spent doing skill-drills, in comparison with the basketball (Dring et al., 2019a). Whilst the relative exercise intensity of both studies were similar (Football: 75% ± 8% HRmax vs Basketball: 76 ± 5% HRmax), the participants in the basketball study (Dring et al., 2019) were of a higher physical fitness, thus leading to an increased absolute exercise intensity. The assimilation of these observations suggests that games-based exercise can elicit beneficial, transient responses with respect to post-exercise blood glucose concentrations, which could be important for the implementation within a school setting.

Postprandial markers of metabolic health

In the studies presented in this thesis, the postprandial glycaemic and insulinaemic response to a mixed meal (the same meal across all chapters) was examined cross-sectionally in relation to a number participant characteristics (Chapter V), in response to a 60 min bout of football activity (Chapter VI) and after a two-week high-intensity exercise intervention (Chapter VIII).

A consistent and novel finding from the studies in this thesis (Chapter V and VI), is that plasma insulin tAUC in response to a test meal, is lower in those with a higher physical fitness (MSFT distance). No previous study has examined the influence of physical fitness on the postprandial response to a meal, with much of the focus towards the role of adiposity. When split into high- and low-fit groups (Chapter VI) the plasma insulin tAUC was, on average, 70% lower in the hight-fit group compared to low-fit. The high-fit participants had considerably lower surrogates of adiposity (sum of skinfolds, waist circumference) than the low-fit group, which might confound this difference. Adiposity (or markers of) are known to be a risk factor for insulin resistance (Arslanian, 2000) and those considered overweight/obese demonstrate a higher postprandial insulinaemic response (Short, Pratt & Teague, 2018). To further address the potential confounding role of adiposity on this relationship the cross-sectional association was examined in a large sample, whilst controlling for waist circumference (Chapter V). There was a negative association between physical fitness and plasma insulin tAUC, independent of waist circumference and fasting HOMA-IR (Chapter V), thus highlighting the importance of physical fitness with regards to postprandial metabolic health.

The final model from the stepwise regression contained waist circumference, MSFT and HOMA-IR, explaining 51.5% of the variance in plasma insulin tAUC. Waist circumference provided the strongest association individually with plasma insulin tAUC ($\beta = 0.41$, $p < 0.001$), whilst also providing the strongest explanatory contribution, independent of physical fitness and HOMA-IR, in the final model. The importance of waist circumference is supported by previous empirical work assessing the postprandial response to a mixed meal in overweight/obese adolescents (Short, Pratt and Teague, 2018) and a two-year longitudinal study examining the relationship between adiposity and insulin sensitivity (Henderson et al., 2016). The present thesis adds to this by

demonstrating the strong association between waist circumference and postprandial insulin tAUC, even when considering the role of other related variables. Furthermore, this thesis provides evidence that a large proportion of the variance in postprandial insulin tAUC can be explained by waist circumference, physical fitness and HOMA-IR.

It has been suggested that the pro-inflammatory environment seen within adipose tissue leads to a reduction in insulin sensitivity (Marson et al., 2016). Indeed, IL-6 concentration has been shown to be positively associated with the degree of adipose tissue insulin resistance in obese adolescents (Hagman et al., 2019). The weight reduction (or maintenance as adolescents grow) resulting from regular exercise is one mechanism commonly cited that may explain the improvements seen in insulin sensitivity (Bird & Hawley, 2017; Keshel & Coker, 2015; Marson et al., 2016). However, there is also recognition that insulin sensitivity can improve independent of weight loss which may be mediated by improved physical fitness (Bird & Hawley, 2017). There is evidence that the relationship between physical fitness and insulin sensitivity is mediated through improved β-cell function (Bird & Hawley, 2017; Ramos et al., 2016), increased GLUT4 translocation (Bird & Hawley., 2017) and increased skeletal muscle capillarisation (Bird & Hawley, 2017). Examining the postprandial response typically reflects assessments of peripheral insulin sensitivity (Abdul-Ghani et al., 2007; Muniyappa & Madan, 2000), thus the local muscle adaptations, such as improved β-cell function and increased skeletal muscle capillarisation, provide the most plausible explanation for the association.

Although previous work has demonstrated that acute bouts of games-based exercise (Dring et al., 2019a; Smallcombe et al., 2018) and cycling (Cockcroft et al., 2015; 2017) affect the postprandial glycaemic and insulinaemic response, there was no effect of 60 min of football in Chapter VI. Half of the football session used in the present thesis consisted of skill-based drills, which would likely reduce the overall intensity of the session, whereas participants in the study of Smallcombe et al. (2018) performed small-sided games for the entirety of the 48 min session. Furthermore, a two-week sprint training intervention did not affect the postprandial glycaemic and insulinaemic response to a mixed meal. Perhaps such brief and high-intensity exercise sessions,

like those used in chapter VIII, would need to be repeated for a longer duration (e.g. 4-6 weeks) to elicit any benefit; although there are currently no data exploring this.

Collectively, the findings from this thesis demonstrate the important beneficial role of physical fitness and adiposity in determining the postprandial insulinaemic response to a mixed meal. Therefore, these results demonstrate that to reduce the postprandial insulinaemic response to a meal, and enhance cardiometabolic health, interventions could target improvements in physical fitness, reductions in waist circumference, or a combination of these.

9.2.3 Blood Pressure

Blood pressure was examined cross-sectionally in Chapter IV of this thesis, with respect to age- and sex-related differences, as well as age-specific associations with physical fitness, physical activity and adiposity.

Physical activity volume was inversely associated with systolic (β = -0.83), diastolic (β = -0.41) and mean arterial (β = -0.55) blood pressure. Furthermore, physical activity intensity was inversely associated with systolic (β = -19.10) and mean arterial pressure (β = -10.92), thus demonstrating consistent beneficial associations of activity volume and intensity with blood pressure, regardless of age or sex. Whilst there is consistent previous evidence demonstrating similar associations (Bailey et al., 2012; Barker et al., 2018; Carson et al., 2013), this is the first time such associations have been demonstrated with the novel activity metrics. This thesis also extends the findings with regards to the novel activity metrics, which have only previously been shown to be associated with BMI z-score (Buchan et al., 2019; Fairclough et al., 2019; Rowlands et al., 2018) and a composite score of metabolic syndrome risk (Fairclough et al., 2019). Systolic, diastolic and mean arterial blood pressure were higher in year 10 participants compared to year 7, which is consistent with previous observations (Shankar et al., 2005). When further considering interaction between year group and average acceleration/intensity gradient on mean arterial pressure, there were different associations for year 7 and year 10. The associations were inverse for both year groups, but the slope was steeper for year 10 participants, suggesting a more sensitive role of physical

activity with blood pressure in older adolescents. These different associations for the year groups are an interesting and novel contribution to the literature, but they should be replicated in further cross-sectional work as well as examining such relationships by tracking the same adolescents over time in a longitudinal study design.

Overall, the findings from this thesis support previous findings that individuals who are more physically active are associated with a lower blood pressure, extending this to novel activity metrics reflecting volume and intensity. Furthermore, an important finding was the strength of the associations was dependent on year groups, with a stronger association observed in older adolescents (year 10).

9.3 Cognitive Function

9.3.1 Executive Function

In the studies presented in this thesis, executive function was examined by the Stroop and Flanker tasks. Specifically, it was examined cross-sectionally in relation to age, physical fitness, physical activity and adiposity (Chapter IV), in response to a 60 min bout of football (Chapter VII; Stroop only) and following a two-week exercise intervention (Chapter VIII).

The findings within this thesis provide consistent evidence that a higher physical fitness is associated with better performance (i.e. quicker response times) on executive function tasks (Chapters IV & VII). This supports previous findings in children (Aadland et al., 2017), younger overweight adolescents (Mora-Gonzalez et al., 2019) and older adolescents (14 y; Westfall et al., 2018). The present findings extend earlier observations, by demonstrating that this association is similar across adolescence (as year group did not interact with MSFT to affect the relationship). However, executive function (when assessed via the Stroop test) was not affected by an acute bout (60 min) of football (Chapter VII). Previous research has found that acute sprint exercise (Cooper et al., 2016), an acute bout of basketball (Cooper et al., 2018), and a short (20 min) bout of football (Lind et al., 2019) all improved executive function performance. The bout of football used in Chapter VII was of similar duration, and relative exercise intensity to that seen in the

basketball of Cooper et al. (2018). The participants in Cooper et al. (2018) had a higher predicted $\dot{V}O_{2peak}$, which means that they will have performed more absolute work for the same relative exercise intensity, potentially explaining the improvements seen following exercise in that study. However, this is difficult to directly compare as there was no quantification of external load during the basketball session in the previous work (Cooper et al., 2018). Finally, when considering the effects of regular exercise, the short-term intervention (two weeks) did not improve executive function response times or accuracy.

9.3.3 Working Memory

In the studies presented in this thesis, working memory was examined by the Sternberg Paradigm. Specifically, it was examined cross-sectionally in relation to age, physical fitness, physical activity and adiposity (Chapter IV), in response to a 60 min bout of football (Chapter VII) and following a two-week exercise intervention (Chapter VIII).

The findings within this thesis consistently demonstrate that physical fitness positively affects working memory. The findings of this thesis demonstrate an inverse relationship between physical fitness and working memory response times (Chapter IV), as well as high-fit participants having a quicker response time during working memory tasks compared to low-fit (Chapter VII). This supports the only other study that has examined the association between physical fitness and working memory ability in adolescents, which also found that performance on the digit span test was better in those with a higher physical fitness (Aadland et al., 2017).

Although the acute bout of football (Chapter VII) did not affect working memory performance overall, there was evidence of a moderating role of physical fitness. Specifically, the high-fit group saw an improvement in working memory at 45 min post-exercise, an effect not seen in low-fit participants. In addition, accuracy during the three-item level of the Sternberg paradigm was greater following the two-week training intervention (Chapter VIII). This suggests that even two weeks of sprint training can improve accuracy on a working memory task without detrimentally affecting response times. Future work should continue to examine the moderating effect of fitness

during different modalities and intensities of exercise, as well as investigating the effects of other short-term (~ 4 weeks) training interventions on working memory performance.

Collectively, these results demonstrate the importance of physical fitness for working memory in adolescents. Not only does it improve baseline working memory ability, but working memory was also improved following 60 min of football in high-fit participants and some aspects of working memory were enhanced following the two-week sprint training intervention.

9.3.4 Visual Perception

Visual perception was examined by the Visual Search Test and it was examined cross-sectionally, in relation to age, physical fitness, physical activity and adiposity (Chapter IV).

The main finding was that physical fitness was inversely associated with response times on both levels of the visual search test (regardless of age and sex) without any impact on accuracy. There are no other data to compare to, as visual perception is not as frequently assessed in the literature; thus, this thesis provides novel evidence of the importance of physical fitness for perception. The consistent finding across both levels of the visual search test provides more rigour to the finding that fitness is related to visual processing ability, although replications of this association are warranted. There is evidence that six months of aerobic training, in older adults, improves the connectivity of the parietofrontal network (Hsu et al., 2017), which is a key component of visual perception (Bisley, 2011), which may mechanistically explain the findings of this thesis.

9.3.5 Brain-Derived Neurotrophic Factor

In the studies presented in this thesis, brain-derived neurotrophic factor (BDNF) concentration was examined to enhance understanding of the mechanisms underpinning the effects of physical activity and physical fitness on cognitive function. It was examined cross-sectionally with respect to age, physical fitness, physical activity and adiposity (Chapter IV), following an acute (60 min) bout of football activity (Chapter VII) and after a short-term (two weeks) exercise intervention (Chapter VIII).

From the findings of Chapter IV and VII, there was no evidence of an association between physical fitness, physical activity or adiposity with BDNF concentration (Chapter IV). Furthermore, there was no effect of acute exercise on BDNF concentration (Chapter VII). There is evidence from adults that physical fitness and physical activity are inversely associated with BDNF (Currie et al., 2009), although others have found no association with physical activity and BDNF in adolescents (Beltran-Valls et al., 2018). On top of this, a recent meta-analysis found that acute exercise transiently increases BDNF concentration (Szuhany et al., 2015). However, it was suggested this is dependent on intensity, for which the acute bout of football in this thesis may not have been intense enough to elicit this increase.

Interestingly, BDNF concentration was increased after the short-term exercise intervention (Chapter VIII). There are currently limited, unclear data on BDNF concentrations following exercise interventions (Azevedo et al., 2020). The magnitude of the difference at follow-up in the present study is much greater than the effect seen by the other studies in the meta-analysis by Azevedo et al. (2020), even though the included interventions were all eight weeks or longer in duration. Given the low sample size in the training study of this thesis, and such a large change, the interpretation should be treated with caution. Indeed, more data is needed on BDNF concentrations following exercise interventions in adolescents.

9.4 Strengths and Limitations

The work presented in this thesis has a number of strengths. Firstly, the studies utilise methods that provide an ecologically valid approach to paediatric exercise research; such as the use of football, a mixed-meal and conducting all measurements on-site at the schools. Although this field-based approach made it difficult to use more complex laboratory-based measurements (e.g. direct determination of maximal oxygen uptake via indirect calorimetry), appropriate and valid alternatives were used to provide robust estimates of the measures of interest (e.g. the MSFT). Furthermore, throughout the thesis, novel risk factors related to cardiometabolic disease (namely inflammatory cytokines) were examined, in conjunction with traditional risk factors to broaden the scope of the research. The participants recruited to each study also contained an even mixture

of boys and girls, or focused solely on girls (chapter VIII), extending previous work which has tended to focus more on male adolescents.

However, the work presented in this thesis is not without limitations. One of the main limitations is the smaller than intended sample sizes for chapters IV and VIII, as well as the reduced duration of the intervention in chapter VIII. These were enforced due to the enforced school closures at the start of the Covid-19 pandemic and are therefore unavoidable. Nonetheless, every effort was made to recruit as many participants as possible (chapters IV and VIII) and to ensure that the intervention was completed, and follow-up measures were obtained; hence the two-week duration instead of four (chapter VIII). A further limitation of the work presented in the work of this thesis is the use of a mixed-meal instead of the often used OGTT. The combination of macronutrients is likely to alter absorption of glucose into the bloodstream. However, the mixed-meal was chosen as this reflects a typical meal that adolescents would normally eat, and thus has greater ecological validity (as mentioned above).

9.5 Conclusions and Practical Applications

Throughout this thesis several patterns have emerged relating to the importance of physical activity, physical fitness and adiposity for risk factors associated with cardiometabolic disease and cognitive function in adolescents. Specifically, these are:

- *The relative importance of physical fitness for cardiometabolic health and cognitive function in adolescents:* The consistent, beneficial association between physical fitness and risk factors for cardiometabolic health, as well as cognitive function, highlights the importance of, and therefore the need to promote, physical fitness in adolescents. Furthermore, physical fitness was examined using performance on the MSFT – which is an easy to administer field-based fitness test. Indeed, interventions in school-aged children should target the improvement of physical fitness which can be monitored with the MSFT in a field-based setting. Taken together, these findings highlight the practical utility of tracking physical fitness in adolescents with a feasible test.

- *The associations of adiposity with risk factors for cardiometabolic health:* In addition to the benefits of physical fitness, the present thesis also demonstrated the negative associations between adiposity (assessed by waist circumference) and novel risk factors for cardiometabolic disease; namely postprandial insulinaemia and IL-6 concentration. Again, a simple and non-invasive measure such as waist circumference provides the opportunity to track measures of adiposity within an adolescent population, and also provides a target for interventions aiming to improve health and wellbeing in young people.
- *The utility of novel physical activity metrics and their association with markers of health in adolescents:* The newly proposed metrics, which have several advantages over the current cut-point approach, were positively associated with a number of risk factors for cardiometabolic disease. This expands the utility of such metrics, having previously only been associated with BMI z-scores. Hopefully, this brings more attention to the use of these metrics and they can be adopted by future research, aiding the comparability and harmonisation amongst physical activity data in adolescents.
- *The potential utility of short-term exercise interventions to improve physical fitness and cognitive function in adolescent girls:* The short-term intervention improved accuracy on a working memory task, as well as increasing BDNF concentration. Indeed, these are positive findings after such a short intervention duration (2 weeks). Although physical fitness and markers of health were not affected, a longer duration (as was intended) may be more suitable for these markers. Furthermore, the intervention was conducted in adolescent girls – who are undoubtedly under-represented in research and are known to be less physically active than boys. Anecdotally, there were no issues with the exercise; with all participants seeming to enjoy the short duration, high-intensity sessions. These were performed before morning form, which also shows the potential utility for such exercise sessions to be scheduled within a school day, without affecting curricular activities.

9.6 Directions for Future Research

To advance the knowledge regarding the importance of physical activity, physical fitness and adiposity for the reduction of risk factors related to cardiometabolic disease and the improvement in cognition, during adolescence, the following suggestions are recommended for future research:

- Longitudinal assessments of postprandial responses to a mixed meal, with measurements of physical fitness, adiposity and other related characteristics to examine how these relationships may change and develop throughout adolescence. It would also be useful to track cognitive function throughout adolescence and similarly assess how the associations with physical activity, physical fitness and adiposity develop.
- Investigations that use games-based exercise, manipulating the intensity and duration, and how they affect markers of health and cognition; so that the optimum dose that could be used within a school day can be identified. Investigations should be targeted (where possible) to those who would benefit most, i.e. those living with overweight/obesity, or adolescent girls.
- Whilst there is an established role for IL-6 as the prototypical myokine related to exercise-induced health benefits, future work should implement proteomic analysis to identify novel markers that might also be related. This could be done in response to exercise, but cross-sectional work with participants of different ages (maybe through adolescence) and physical fitness abilities might also provide a useful starting point.
- Further development of short-term physical activity interventions that can be feasibly implemented within a school-setting, with a particular focus on adolescent girls who should be a priority target population.

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Appendix A

Participant Information:

What does the study involve?

- Your child will complete two sessions of data collection each lasting no longer than 3 hours, separated by 7 days in which physical activity will be measured by wearing accelerometers around their waist. These will take place on **Monday 4th & Monday 11th May**. PE kit (*with shorts*) is required for both sessions.
- Trial 1 will include height, weight and body composition measurements, a bleep test, 20 m sprints and practise attempts at the cognitive function tests (which will measure attention, memory and perception) as well as a practice at completing the mood, sleep and physical activity questionnaires.
- At the end of trial 1, your child will be given an accelerometer to be worn on their waist at all times (except for when in contact with water, i.e. Shower, bath, swimming) during the following 7 days.
- During the trial 2 your child will complete a battery of cognitive function tests, a mood, sleep and physical activity questionnaire. In addition, they will also have health measurements which include blood pressure and markers of cardiovascular health from a fingertip blood sample (see over for details). Breakfast (cornflakes, milk, toast and

Information on measurements to be taken:

Finger Prick Blood Sample:

Small fingertip blood samples will be taken during main trial, then analysed for a variety of health markers. Such samples are similar to those taken by diabetics wanting to check their

Cognitive Function Tests:

The cognitive function test battery will be conducted on a laptop and take approximately 12 minutes to complete. The battery will include the following tests;

- ***Stroop Test (attention):*** A word will appear (this word is always a colour) in the centre of a laptop screen. The colour of the font the word is written in will differ to the word itself. Your child must select the colour the word has been written in rather than the word itself.
- ***Sternberg Paradigm (working memory):*** Letters will appear on screen and participants must select whether it is one of their "target" letters (assigned before) by pressing the right arrow key, or whether it is a distractor by pressing the left arrow key.
- ***Flanker (executive function):*** Arrows will appear on screen (in groups of 5) and participants must select the direction of the middle arrow.

- ***Visual Search Test (perception):*** Triangles will appear on screen with participants pressing the space bar when they see them. On the difficult level, dots will form the

Accelerometry:

Your child will wear an accelerometer – which are very small devices - on their waist at all times (except when in contact with water) for the 7 day period between both trials. This helps us to measure how physically active they have been during this period. During this 7 day period they should just continue

Exercise:

1. The 'Bleep' test is a 20 metre shuttle run performed to the sound of bleeps from a CD player. The exercise is designed to be physically challenging but when your child cannot keep pace with the bleeps they are to stop and walk to cool down.

2. Your child will perform 3 x 20-metre sprints with plenty of rest between, and after a sufficient warm up. Your child will be able to start the sprint in their own time as the sprints will be timed automatically by timing gates. If their 3rd sprint is the fastest, they will be asked to do another sprint until they no longer improve

Important Notes:

- While your child's school has agreed to take part in this study, it is **NOT** compulsory your child takes part.
- Your child's school has agreed to participate in this project, thus taking time off normal lessons to participate has been cleared with the school.
- Your child can withdraw at any time without providing a reason and your child's data will be destroyed.
- Although it is extremely unlikely, high intensity exercise has been known to reveal unsuspected heart or circulation problems and very rarely these have had serious or fatal consequences.
- All staff involved in the study undertake training in the measures involved and undergo a DBS check to clear them to work with children.
- Your child will be supervised on all occasions and will only leave the testing session once they feel comfortable to do so.
- All information will be stored anonymously and your child's individual data will not be reported in the findings of the study.
- The study has been approved by Nottingham Trent University's Ethical Advisory

What is requested of you and your child next?

What to do next?

If you are willing for your child to participate in this study **please complete the enclosed consent form and health screen questionnaire and return them to your child's school as soon as possible.**

Contact Details: If you have any questions you wish to ask - please do not hesitate in contacting Ryan Williams, Dr Simon Cooper, Dr John Morris or Professor Mary Nevill on the details below:

Email:

ryan.williams2013@my.ntu.ac.uk

simon.cooper@ntu.ac.uk

john.morris@ntu.ac.uk

mary.nevill@ntu.ac.uk

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Appendix B

Physical Activity, Health and Cognitive Function 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:

- Consuming breakfast (cornflakes, with milk, and toast with butter)
- A bleep test and 20 metre sprints
- Blood pressure measurements
- Capillary (fingertip) blood samples
- Completing computerised cognitive function tests (short computer tests) and a mood, sleep, physical activity questionnaire and a 24 hour food recall diary
- Wear a waist-mounted accelerometer for a 7 day period

- 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 Ryan Williams (ryan.williams2013@my.ntu.ac.uk) or Dr Simon Cooper (simon.cooper@ntu.ac.uk). I can also ask my parent/guardian/care-giver to talk to Ryan Williams (ryan.williams2013@my.ntu.ac.uk) or Dr Simon Cooper (simon.cooper@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 C

Parent/Guardian Statement of Consent for Child/Dependent to Participate in the Investigation Entitled:

A comparison of the physical activity, physical fitness, cognitive function and cardiometabolic health of young people in the United Kingdom and Hong Kong.

1) I, *[name of parent/guardian]* agree for my child/dependent, *[name of participant] to* partake as a participant in the above study on Monday 4th May and Monday 11th May.

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 two main trials each lasting approximately 2-3 hours. During these sessions my child/dependent will undergo various health measures, such as; fingertip blood sample, blood pressure and anthropometric measurements as well as some maximal exercise tests and laptop based cognitive function tests.

3) It has also been explained to me by *[name of investigator]* that the risks and side effects that may result from my child/dependent's participation are as follows: slight bruising of the fingertips from blood samples, maximal exercise may cause delayed onset muscle soreness. 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, anthropometric measurements, exercise performance, physical activity 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.

Parent/Guardian signature: Date:

Contact Number:

Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix D

NTU Exercise and Health Study - Health screen

To be completed by parent/guardian/caregiver

Name or Number .....

Please complete this brief questionnaire to confirm fitness 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 Yes ☐ No ☐ Month?

8. *Does your child/dependent have any dietary requirements we need to be aware of (i.e. intolerances, preferences)?*

The breakfast being provided consists of; *cornflakes, semi-skimmed milk, white bread (toasted) and flora*

Yes ☐ **No** ☐

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

.....
.....
.....
.....
.....

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

Name: _____

Relationship to child/dependent:_____

Contact Number:_____

Appendix E

Accelerometer Instructions

- Place accelerometer around your waist (like the picture below). The accelerometer should be secure, but not too tight so that it is uncomfortable.

Fasten the accelerometer firmly around the waist, with the red device sitting above the right hip bone (as seen in the picture left). The accelerometer should be worn **underneath clothing.**

- Ensure that the accelerometer is positioned correctly (use the pictures below to help with this)

Front view of the accelerometer.

View from above (black circle and number should be on the top)

- The accelerometer **MUST** be worn at **all times** during the day.

- The only times when it can be removed are as follows;

. During any water-based activities (i.e. baths, shower, swimming)
. When sleeping (feel free to leave it on overnight, if you want to)

- Please complete the included compliance log by ticking the days that you wore the accelerometer and, where possible, include the time that you put it on (in the morning) and take it off for bed.

- There is also a notes section for you to record any additional comments (i.e. times when the accelerometer was removed for baths, showers, swimming etc) – only if you remember!

If you have any queries, please get in touch;

Ryan Williams
ryan.williams2013@my.ntu.ac.uk

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